École des
Neurosciences
Paris Île-de-France

CHILEAN INTERNS

ENP INTERNSHIP PROPOSALS

2016-2017

CONTENTS

Cendra AGULHON's team

Team: Glia-Glia & Glia-Neuron Interaction group

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Investigating the role of astrocytic signaling in neurodevelopmental disorders

Supervisor: Cendra Agulhon

Severe mental disorders such as schizophrenia, bipolar disorder, autism and age-related neurodegenerative diseases are leading contributors to cognitive illness, imposing emotional burdens 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 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, biochemistry and electrophysiology. The student interested to join our laboratory will contribute to one of the aims of this ambitious project.

Period: Anytime from January 2017 to June 2017

Maria Cecilia ANGULO's team

Team: Physiology of NG2 cells

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Impact of the activity of interneurons on cortical oligodendrogenesis

Supervisor: Maria Cecilia Angulo

In the brain, myelination is a critical developmental process necessary to speed up the conduction of action potentials. Major developmental brain disorders induce irreversible myelination defects. One possibility to overcome myelination impairment is to stimulate the production of mature oligodendrocytes from endogenous oligodendrocyte precursor cells (OPCs). To reach this aim, however, it is first necessary to understand the extrinsic and intrinsic signals that control OPC proliferation and differentiation during postnatal development.

In the last decade, it has been demonstrated the existence of functional synaptic connections between neurons and OPCs which role remains elusive. We recently demonstrated that synaptic inputs from GABAergic interneurons onto cortical OPCs is higher when the production of oligodendrocytes reaches a peak and disappears after (Velez-Fort et al., 2010; Orduz et al., 2015). This correlation between transient synaptic innervation and oligodendrogenesis suggests that GABAergic synaptic inputs influence OPC proliferation/differentiation and thus myelination. Hence, synaptic neurotransmitter release onto OPCs constitutes a suited mechanism to control the fate of these progenitors.

The student will participate in a project that aims at unraveling whether the activity of GABAergic interneurons has an impact on OPC proliferation and differentiation in the developing somatosensory cortex. He/She will evaluate the regulation of activity-dependent oligodendrogenesis by combining an optogenetic approach in brain slices and *in vivo* with electrophysiology, immunohistochemistry and confocal microscopy. Using this multidisciplinary approach, we expect to demonstrate that cortical oligodendrogenesis depends on the activity of interneurons.

References:

1- Wake H*, Ortiz FC*, Woo DH, Lee P, Angulo MC, Fields D (2015) "Non-synaptic junctions on myelinating glia promote preferential myelination of electrically-active axons". *Nat Commun* 6:7844 (*Co-first authors)

2- Orduz D*, Maldonado PP*, Balia M, Vélez-Fort M, de Sars V, Yanagawa Y, Emiliani V, Angulo MC (2015) Interneurons and oligodendrocyte progenitors form a structured synaptic network in the developing neocortex. e*Life* 4:e06953 (*Co-first authors)

3- Balia M*, Vélez-Fort M*, Passlick S, Schäfer C, Audinat E, Steinhäuser C, Seifert G, Angulo MC (2015) Postnatal down-regulation of the $GABA_A$ receptor gamma2 subunit in neocortical NG2 cells accompanies synaptic-to-extrasynaptic switch in GABAergic transmission mode. *Cereb Cortex*, 25(4):1114-23 (*Co-first authors)

Period: Anytime from January 2017 to June 2017

Angelo ARLEO's team

Team: Aging in Vision and Action (AVA)

Fields of research: Cognitive neurosciences / neuropsychology

Internship project:

Visual aging and spatial cognition: coupling behavioral and neuroimaging analysis in human experiments

Supervisors: Angelo ARLEO (Vision Institute) & Ricardo CHAVARRIAGA (EPFL, Switzerland)

Aging progressively alters sensory and cognitive functions. We study the physiological and pathogenic mechanisms underlying visual aging in humans, and we focus on their impact on perceptual and cognitive processes. This project will combine behavioral observations and neuroimaging techniques to assess age-related changes in spatial orientation and navigation (Moffat, 2009).

We will use the Streetlab research platform (www.streetlab-vision.com) to reconstruct an urban environment and examine how old versus young adults actively explore their world to learn a mental representation of space. The subject behavior will be analyzed through biometric sensors. In particular, the kinetics of the body will be recorded by a motion capture system (VICON), and the eye movement signatures will be recorded through a portable eye tracker. Also, brain activity recordings will be performed through an ambulatory wireless electroencephalography (EEG) system. The signals from these sensors will be synchronized and recorded in real time while the subjects will be moving in the artificial street.

We will analyze how aging impacts the encoding of visual information (e.g., landmark configuration in the environment) during active spatial exploration. The evoked brain responses (recorded by EEG) will be used to get an insight in the time course of visual recognition and spatial learning. The modulation of visual evoked potentials may indeed reflect cortical activity related to cognitive visual tasks (Gerson et al., 2005; Polich, 2007; Uscumlic et al., 2013.). Kamienkowski et al. (2012) showed differences in evoked potentials after fixations on target stimuli with respect to distractor stimuli. Similar differences were observed in more complex tasks including visual exploration in virtual environments (Jangraw et al., 2014). However, none of these studies have focused on the effects of aging on these correlates or on their relation to subsequent spatial behavior in real 'ecological' setups such as the Streetlab. This project aims at fulfilling this gap by combining the expertise at Vision Institute on behavioral analysis of aging-related visual processes with the EEG analysis techniques developed at EPFL.

References:

- Gerson AD, Parra LC, Sajda P (2005) Cortical origins of response time variability during rapid discrimination of visual objects. Neuroimage, 28:342-53.
- Jangraw DC, Wang J, Lance BJ, Chang SF, Sajda P (2014) Neurally and ocularly informed graph-based models for searching 3D environments. J Neural Eng, 11:046003.
- Kamienkowski JE, Ison MJ, Quiroga RQ, Sigman M (2012) Fixation-related potentials in visual search: a combined EEG and eye tracking study. J Vis, 12(7):4.
- Moffat SD (2009) Aging and spatial navigation: what do we know and where do we go? Neuropsychol Rev, 19(4):478-89.
- Polich J (2007) Updating P300: An integrative theory of P3a and P3b. Clin Neurophysiol, 118:2128-48.
- Uscumlic M, Chavarriaga R, Millán JdR (2013) An iterative framework for EEG-based image search: robust retrieval with weak classifiers. PLoS ONE, 8:e72018.

Period: Anytime from January 2017 to June 2017

Thierry BAL's team

Team: Physiology of NG2 cells

Fields of research: Neurophysiology/systems neuroscience

Internship project:

The role of dendritic synaptic bombardment using single-neuron voltage-sensitive dye imaging

Supervisor: Thierry Bal

This experimental project is based on imaging rapid voltage changes in subparts of neurons in slices in vitro. Traditional whole cell approach has been limited to recording from only the largest portions of neurons – the soma and large dendritic branches. By using a recent improvement in voltage-sensitive dye imaging technique that provided exceptional spatial (up to 1 µm2) and temporal (up to 0.05 - 0.1 ms) resolution, it is now possible to follow the propagation of fast electrical events such as action potentials into axon collaterals [1], and in axons and tiny dendrites of interneurons [2]. We are currently installing the technique in the UNIC lab.

In vivo, neurons are constantly exposed to background barrages of synaptic inputs, called "synaptic noise," which likely interact with their membrane properties and impact on their response to sensory synaptic input [3]. Likewise, in thalamocortical neurons we proposed a theory according to which the modulation of integrative properties by top-down synaptic noise provides a mechanism for selective attention in thalamic neurons [4].

We propose to study experimentally in slices in vitro (and possibly using modeling by collaborating with other teams at UNIC) the impact of synaptic bombardment in dendritic integration in cortical and thalamic neurons.

Two solutions can be used to realize in vitro a synaptic bombardment in the dendrites of neurons. We will first use slices of mouse neocortex that spontaneously generate population activities such as the so-called up- and down-states, which consist of recurrent synaptic bombardment activities generated within the cortical network and that closely mimic the natural slow activities recorded in the intact brain [5]. Second we can mimic a synaptic bombardment via conductance injection in the soma of neurons using dynamic-clamp (see[4]).

Depending on the evolution of the project at the time of the internship, we may also study the impact of (TMS-like) magnetic stimulation on neuronal activities. Transcranial Magnetic Stimulation (TMS) is used to stimulate brain functions and to address pathologies such as severe depression. The effects of this non-invasive stimulation is neither understood at the cellular (neurons types and glia) nor at the subcellular levels (dendrites and axons). The combination of magnetic stimulation with up- and down states in vitro and optical imaging of membrane potential in neurons may provide a unique opportunity to understand at the local scale the mechanisms underlying TMS.

References :

1- Foust et al., Journal of Neuroscience 30, 6891–6902 (2010).
2- Casale et al., Journal of Neuroscience 35, 15555–15567 (2015).
3- Destexhe et al., Nat Rev Neurosci 4, 739–751 (2003).
4- Behuret et al., Front Neural Circuits 9, 11633 (2015).
5- Tahvildari et al., Journal of Neuroscience 32, 12165–12179 (2012).

Period: Anytime from January 2017 to June 2017

Brice BATHELLIER's team

Team: Cortical dynamics and multisensory processing

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Uncovering the cortical bases of auditory perception and learning using large scale imaging with cellular resolution and optogenetics

Supervisor: Brice Bathellier

The student will work in Brice Bathellier's team at UNIC (Unit for Neuroscience, Information and Complexity, CNRS), offering an interdisciplinary environment for the multiscale study of brain dynamics.
How does the brain of humans or animals build the complex perceptions necessary for efficient interaction with the environment? A large amount of research suggest that complex representations of the sensory stimuli are implemented the large circuitry of sensory cortical areas. However, because of the sparsity of traditional electrophysiology method, the neuronal organisation of these representations and the circuit mechanisms underlying their emergence remains highly elusive. Moreover, it is still poorly known how these circuits are shaped by behaviour and experience, allowing a flexible interaction with the environment.

To start addressing these questions, the Bathellier team has recently established several experimental protocols allowing (i) to record from more than a thousand of neuron simultaneously in the auditory cortex and follow their activity over weeks using GCAMP6 based on two-photon calcium imaging in awake transgenic mice, (ii) to train mice to discriminate complex auditory stimuli in order to assess their perception, (iii) to manipulate cortical activity during behaviour with sculpted light patterns to show necessity (loss of function) and sufficiency (gain of function) of cortical activity for auditory perception and learning.

Using these tools, the goal of the internship will be to identify the cortical activity patterns emerging when a mouse learns to associate complex sounds with different behavioural outcomes. The student will learn to train mice to perform a cortex-dependent auditory task, as demonstrated with optogenetics, while imaging from large neuronal populations in auditory cortex. He/she will then analyse the large data sets with state-of-the-art computational methods, which for the most part are already implemented in the lab in order to reveal the changes in the dynamics of cortical circuits that accompany a sensory motor association.

References:

- Thomas Deneux, Alexandre Kempf, Aurélie Daret, Emmanuel Ponsot and Brice Bathellier, Temporal asymmetries in auditory coding and perception reflect multi-layered nonlinearities, Nature Communications: in press, (2016)
- Yves Frégnac and Brice Bathellier, Cortical Correlates of Low-Level Perception: From Neural Circuits to Percepts, Neuron 88(1): 110-126, (2015)

Period: Anytime from January 2017 to June 2017

Gilles BONVENTO's team

Team: Cell-cell interactions in neurodegenerative diseases

Fields of research: Neurological and psychiatric diseases

Internship project:

Heterogeneity of reactive astrocytes in neurodegenerative diseases

Supervisor: Carole Escartin

Astrocytes have long been neglected in the field of Neuroscience. Now that their multiple roles in brain physiology are recognized, the next challenge is to understand their implications in diseases. Astrocytes become reactive in virtually all pathological situations affecting the brain (Ceyzeriat et al., 2016). Astrocyte reactivity was initially defined by morphological criteria (hypertrophy and overexpression of intermediate filament proteins). These two cardinal features of reactive astrocytes do not provide much information about how these cells function (Ben Haim et al., 2015a). In fact, astrocyte reactivity appears as a very heterogeneous response, encompassing various functional states. Not only reactive astrocytes display heterogeneity between disease conditions but also over time or between sub-populations of cells observed within the same animal (Anderson et al., 2014; Khakh and Sofroniew, 2015). Such functional diversity may in fact explain why it is so challenging to establish the impact of reactive astrocytes especially for neurodegenerative diseases (ND) (Ben Haim et al., 2015a; 2015b).

We hypothesize that various classes of reactive astrocytes appear in each disease context, having a specific effect on surrounding neurons and disease progression. The goal of this project is to identify and characterize these different classes of reactive astrocytes in selected mouse models of ND (Alzheimer's and Huntington's diseases), based on their transcriptional profiles, the signaling cascades involved, and their functional features. For that, we will use viral gene transfer of reporter systems, immunostainings, cell-sorting of astrocytes and transcriptomic analysis.This multidisciplinary project aims at disentangling the complexity of astrocyte reactivity, thanks to cell-specific and novel techniques applied to relevant in vivo models of ND. It will provide key insight into astrocyte reactivity and address the important issue of the roles of reactive astrocytes in diseases.

References:

- Anderson MA, Ao Y, Sofroniew MV (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565:23-29.
- Ben Haim L, Carrillo-de Sauvage MA, Ceyzeriat K, Escartin C (2015a) Elusive roles for reactive astrocytes in neurodegenerative diseases. Frontiers in cellular neuroscience 9:278.
- Ben Haim L, Ceyzeriat K, Carrillo-de Sauvage MA, Aubry F, Auregan G, Guillermier M, Ruiz M, Petit F, Houitte D, Faivre E, Vandesquille M, Aron-Badin R, Dhenain M, Deglon N, Hantraye P, Brouillet E, Bonvento G, Escartin C (2015b) The JAK/STAT3 Pathway Is a Common Inducer of Astrocyte Reactivity in Alzheimer's and Huntington's Diseases. J Neurosci 35:2817-2829.
- Ceyzeriat K, Abjean L, Carrillo-de Sauvage MA, Ben Haim L, Escartin C (2016) The complex STATes of astrocyte reactivity: How are they controlled by the JAK-STAT3 pathway? Neuroscience 330:205-218.Khakh BS, Sofroniew MV (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18:942-952.

Period: Anytime from January 2017 to June 2017

Jocelyne CABOCHE & Peter VANHOUTTE's team

Team: Neuronal Signaling and Gene Regulation

Fields of research: Neuropharmacology/cell signaling

Internship project:

Role of MAPkinase/ERK in miRNAs processing and striatal responses to cocaine

Supervisor: Jocelyne Caboche

Drug addiction is a clinically devastating neuropsychiatric disorder resulting from neural adaptations at the molecular, cellular, and circuit levels following repeated drug exposure[1]. Drugs of abuse affect neuronal plasticity in key regions of the brain's reward circuitry, including the ventral part of the striatum (nucleus accumbens, NAc), which receive dense dopaminergic (DA) inputs from the mesencephalon. The persistent and experience-dependent aspects of the addicted phenotype have suggested a key role for epigenetic modifications in drug-induced gene expression, as epigenetic mechanisms can durably maintain transcriptional states. These epigenetic mechanisms comprise miRNAs, a category of 21-25 nucleotides non-coding RNAs, involved in multiple neuronal functions and altered in addiction[2]. miRNAs modulate gene expression by binding to complementary sequences in the 3' untranslated regions (3'UTRs) of up to hundreds of target mRNAs per miRNA, thereby inducing mRNA degradation and/or repressing mRNA translation[3]. The biogenesis of mature miRNA in the brain begins with a sequential cleavage of pri-miRNA by the nuclear RNase III DROSHA into a shorter pre-miRNA hairpin that is then exported from the nucleus, cleaved in the cytoplasm by a second RNase III called DICER, which is associated with *TRBP and Argonaute 2 protein (AGO2) to constitute a catalytically active RNA-induced silencing complex (RISC) containing a mature miRNA.* Accumulating identifies canonical miRNA biogenesis as a regulatory hub in response to a plethora of physiological and pathological stimuli and subsequent signaling cascades[4]. In particular, the *ERK pathway – which responds to addictive drugs and mediates drug-triggered long-term striatal plasticity*[5]*– has been proposed to modulate miRNA biogenesis by phosphorylating and stabilizing TRBP at the RISC complex in an in vitro model*[6].

The aim of the present project will be to decipher the role of ERK signaling in miRNA processing in response to cocaine. After chronic administration of cocaine (IP, 20mg/kg), mice will be euthanized by deep anesthesia, at different times after the last administration, and kinetics of TRBP phosphorylation will be studied owing to phosphor-specific antibodies by immunocytochemistry on striatal slices or western blots. Pharmacological (interfering ERK/TRBP peptides) and genetic (conditional knock-out of TRBP in specific striatal populations) approaches will be used to interfere with TRBP functions. Molecular (miRNAs production and corresponding targeted mRNAs) and behavioral responses will be studied in response to chronic cocaine. Altogether, these studies will provide new insigths about inter-relationship between intracellular signaling pathway and miRNA production produced by prolonged cocaine exposure.

References:

1- Lüscher and Malenka (2011). Neuron *69*, 650–663.
2- Nestler (2014). Neuropharmacology *76 Pt B*, 259–268.
3- Kim, Han and Siomi (2009). Nat. Rev. Mol. Cell Biol. *10*, 126–139.
4- Blahna and Hata (2013). Curr. Opin. Cell Biol. *25*, 233–240.
5- Pascoli et al (2011). Biol. Psychiatry *69*, 218–227.
6- Paroo et al (2009). Cell *139*, 112–122.

Period: Anytime from January 2017 to June 2017

Serge CHARPAK's team

Team: From sensory processing to functional hyperaemia

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Optical control of brain activity in naive animals

Supervisor: Serge Charpak

Over the past 10 years the use of optogenetics to drive genetically distinct populations of brain cells has profoundly increased our understanding of neural circuitry and brain function in health and disease. Optogenetics is now regularly integrated with functional brain mapping techniques such as blood oxygenation level-dependent (BOLD) fMRI in order to generate brain-wide maps of connectivity generated by activation of specific populations of cells. In a recent study, we have tested whether light stimulation protocols similar to those commonly used in vivo modulate brain activity in mice that do not express light sensitive proteins. Combining two-photon laser scanning microscopy and ultrafast functional ultrasound imaging, we found that in the naïve brain of anesthetized mice, light per se modifies specific brain outputs. These results impose careful consideration on the use of photo-activation in studies requiring repetitive stimulations to correct cellular defects in pathological models. Since these data were acquired in anesthetized animals, the candidate will investigate, using two-photon imaging, the extent the effects of light are maintained in awake mice.

Period: Anytime from January 2017 to June 2017

Filippo DEL BENE's team

Team: Neuronal Circuit Development Group

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Role of the secreted proteins Meteorin and Meteorin-like in the developement of the visual system

Supervisors: Filippo DEL BENE & Flavia DE SANTIS

Meteorin and Meteorin-like are newly discovered secreted proteins involved in both glia and neuronal cell differentiation (Jorgensen et al., 2012; Nishino et al., 2004). More recently, they have also been implicated in axonal growth in in vitro systems. We have cloned the zebrafish orthologues gene (Meteorin, Metrnl1 and Metrnl2) and determined their expression during CNS development in larval zebrafish. We have also created null alleles of all three genes via CRISPR/Cas9 technology. Although the mutant fish are viable they show very penetrant and specific axonal growth defect in the case of Metrnl1. Preliminary work has demonstrated that these axons fail to innervate the proper brain areas and establish the correct synaptic connectivity. The student will characterize in detail these defects in retina ganglion cell axons using transgenic lines, in vivo imaging and immunohistochemical analysis. He/she will as well analyze double and triple mutants for these genes that have already been generated, to unmask new phenotypes, including abnormalities in commissural axon crossing and visual perception defects via calcium imaging of behaving animals. A biochemical screen has also been initiated to identify the receptor of Metrorin proteins in vivo and the best candidates from the screen will be functionally validated in vivo. In parallel we have generated a transgenic line to overexpress a GFP-tagged Meteorin protein in various part of the central nervous system. The analysis of axonal growth and development in this transgenic model will also be performed. This work will be the first characterization of the role of Meteorin in CNS development via loss-of-function analysis in a vertebrate model.

References:

- Jorgensen, J. R., Fransson, A., Fjord-Larsen, L., Thompson, L. H., Houchins, J. P., Andrade, N., Torp, M., Kalkkinen, N., Andersson, E., Lindvall, O., et al. (2012). Cometin is a novel neurotrophic factor that promotes neurite outgrowth and neuroblast migration in vitro and supports survival of spiral ganglion neurons in vivo. Exp Neurol 233, 172-181.
- Nishino, J., Yamashita, K., Hashiguchi, H., Fujii, H., Shimazaki, T. and Hamada, H. (2004). Meteorin: a secreted protein that regulates glial cell differentiation and promotes axonal extension. The EMBO journal 23, 1998-2008.

Period: Anytime from January 2017 to June 2017

Alexander FLEISCHMANN's team

Team: Neural circuits and Behaviour

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Neural identity and odor coding in the olfactory cortex

Supervisor: Alexander Fleischmann

Our laboratory is interested in the neural circuits that underlie the perception of smell. We use a combination of (opto)genetic, *in vivo* imaging, behavior and computational approaches 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. 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 determine the functions of different neural cell types and circuit elements in the olfactory cortex, and to understand their contributions to sensorydriven cortical activity, memory and behavior. The olfactory cortex provides an ideal model system to study cortical functions. Compared to visual or auditory cortex, the olfactory cortex is simple and in close proximity to the sensory input from the nose. Furthermore, animals heavily 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.

References:

- Odor concentration-invariant subnetworks in the mouse olfactory cortex. Roland B, Deneux T, Franks K, Bathellier B, Fleischmann A. *submitted*
- Molecular signatures of neural connectivity in the olfactory cortex. Diodato A, Ruinart de Brimont M, Yim YS, Derian N, Perrin S, Pouch J, Klatzmann D, Garel S, Choi G, Fleischmann A. (2016) *Nat. Comm. 2016*
- Massive normalization of olfactory bulb output in mice with a 'monoclonal nose'.
- Roland B, Jordan R, Sosulski DL, Diodato A, Fukunaga I, Wickersham I, Franks KM, Schaefer AT, Fleischmann A. *Elife. 2016*

Period: Anytime from January 2017 to June 2017

Bruno GASNIER's team

Team: Membrane Transport

Fields of research: Neuropharmacology/cell signaling

Internship project:

Cellular pathology of neuronal ceroid lipofuscinoses

Supervisors: Corinne Sagné & Bruno Gasnier

Recently, lysosomes have regained considerable attention owing to their role as signaling platforms for activation of the master growth regulator mTORC1, their participation in a transcriptional feedback loop which adapt them to the cell's degradative needs and, last but not least, their implication in numerous pathologies, including neurodegeneration. A clear example linking lysosomes and neurodegeneration is a group of rare, childhood-onset diseases known as neuronal ceroid lipofuscinoses (NCLs). NCLs share similar clinical features (blindness, seizures, motor and cognitive decline) and a characteristic accumulation of autofluorescent material in lysosomes. These fatal disorders are genetically heterogenous, with at least 13 genes, out of which 4 are lysosomal hydrolases involved in protein degradation. The function of the other CLN proteins, including the lysosomal membrane protein CLN3, is unknown.

In a preliminary metabolomic study of purified lysosomes from CLN3-defective mice, we found a specific signature suggesting that CLN3 modulates lysosomal proteolysis. These metabolomics findings were confirmed by biochemical measurements and fluorescence microscopy analysis of wild-type and CLN3-defective cellular models. Defective lysosomal proteolysis may thus has a wider occurrence than previously anticipated among NCLs. Moreover, it might represent a convergent mechanism in the pathological cascade of NCLs, opening the way for novel treatments which can address several genetic forms simultaneously.

During this internship, the student will examine the biochemical selectivity of the hydrolysis defect and test whether enhancement of lysosomal proteases can rescue the consequences of CLN3 dysfunction. A large variety of experimental approaches will be used in molecular biology (plasmid construction, PCR), cell biology (cell culture and transfection, genome editing with the CRISPR/Cas9 approach, FACS, fluorescence imaging), biochemistry (subcellular fractionation, western-blot) and mouse KO strain management (mice production for biochemical experiments).

References:

- Kollmann, K., Uusi-Rauva, K., Scifo, E., Tyynelä, J., Jalanko, A., & Braulke, T. (2013). Cell biology and function of neuronal ceroid lipofuscinosis-related proteins. *Biochimica et Biophysica Acta*, *1832*, 1866–81.
- Sharifi, A., Kousi, M., Sagné, C., Bellenchi, G. C., Morel, L., Darmon M, Hulková H, Ruivo R, Debacker C, El Mestikawy S, Elleder M, Lehesjoki AE, Jalanko A, Gasnier* B, Kyttälä*, A. (2010). Expression and lysosomal targeting of CLN7, a major facilitator superfamily transporter associated with variant late-infantile neuronal ceroid lipofuscinosis. *Human Molecular Genetics*, *19*, 4497–4514. (*, corresponding authors).

Period: Anytime from January 2017 to June 2017

Patricia GASPAR & Christine METIN's team

Team: Developmental mechanisms of brain disorders

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Control of cortical development by the Sonic hedgehog signaling pathway: cellular and molecular mechanisms controlling interneurons migration

Supervisors: Christine Métin & Christine Laclef

The adult cerebral cortex contains excitatory principal neurons and inhibitory interneurons (INs), which play essential role for computation in cortical circuits. Abnormal development or function of INs is now considered as a major factor in psychiatric diseases such as schizophrenia as well as in epilepsy.

Cortical INs are generated in the basal forebrain in a region that strongly expresses Sonic Hedgehog. After cell proliferation, they migrate a long distance to reach the cerebral cortex where they settle among excitatory neurons. Defects in INs migration can lead to altered positioning, density and morphology of INS. Those defaults are responsible of functional defects at adult stage.

Migrating INs are highly polarized cells with a long leading process at the cell front that explores the environment and adheres to the substrate. We have shown that the centrosome, which organizes the microtubule cytoskeleton, can moreover assemble a primary cilium at the surface of INs. In mammals, the primary cilium is an organelle required for transcriptional outcome of Shh signals. INs with a genetic ablation of the primary cilium exhibit morphology and trajectory defects in the developing cortex.

Our objective is to understand by which mechanisms (transcriptional mechanisms, local signaling) Shh and the primary cilium control the migration of INs and the formation of inhibitory circuits in the cortex.

We have generated several mouse models with abnormal ciliogenesis in cortical INs or with abnormal Shh signaling, which moreover express fluorescent proteins in INS, primary cilium and/or centrosome. We analyze the role of Shh and the primary cilium in INs migration by studying in vivo during development their morphology and distribution along migratory routes and in cortical targets. In parallel, dynamics analyzes are performed on organotypic slices of embryonic cortex by live cell imaging. Detailed morphological and dynamic analyses are performed in culture and co-culture models to characterize the responses of control and mutant INs to Shh and to adhesive signals.

During internship, the student will be trained to primary cell culture techniques, live cell imaging and super-resolution microscopy. He/She will study the migratory behavior of future cortical interneurons with a mutation in the Shh signaling pathway.

References:

- Laclef, C., et al. (2015) The role of primary cilia in corpus callosum formation is mediated by production of the gli3 repressor. Hum Mol Genet. pii: ddv221
- Métin C., Pedraza M. (2014) Cilia : traffic directors along the road of cortical development. The Neuroscientist. 20(5):468-82
- Luccardini C et al. (2013) N-cadherin sustains motility and polarity of future cortical interneurons during tangential migration. J. Neurosci. 33(46):18149-18160
- Baudoin§ JP, Viou§ L, et al. (2012) Tangentially migrating neurons assemble a primary cilium that promotes their re-orientation to the cortical plate. Neuron, 76(6):1108-1122

Period: Anytime from January 2017 to June 2017

Carsten JANKE's team

Team: Regulation of Microtubule Dynamics and Function

Fields of research: Neurogenetics/neurodevelopment

Internship project:

Regulation of neuronal microtubule-based transport by polyglutamylation

Supervisors: Carsten Janke & Maria Magiera & Satish Bodakuntla

The integrity of the microtubule cytoskeleton in neurons is essential for their development, function and survival. Various populations of microtubules, all composed of alpha- and beta-tubulin dimers, interact with different MAPs (Microtubule -Associated Proteins) and molecular motors. These differential interactions confer diverse functions to microtubules. One of the mechanisms of microtubule specialisation are the posttranslational modifications of microtubules (Janke, 2014), for instance polyglutamylation. Polyglutamylation levels can influence MAP binding to microtubules and molecular motors processivity, both being essential for neuronal survival. Our hypothesis links the levels of tubulin polyglutamylation to the survival of neuronal cells. We have already shown that in a mouse model with hyperglutamylated tubulin some neuronal population indeed degenerate (Rogowski et al, 2010).

The aim of the present project is to analyse the molecular mechanisms behind tubulin polyglutamylation-linked neurodegeneration. We have generated mouse KO models for several deglutamylases, which now allow studying the effects of hyperglutamylation in the whole brain. The present project will involve work with those mouse models and their histological analysis in order to decipher the phenotypes associated with hyperglutamylation. The successful candidate will also participate in the study of the mechanism behind the polyglutamylation –dependent neurodegeneration, which could imply axonal transport abnormalities. This part of the project will require the use of primary neuronal cultures from transgenic mice and the analysis of neuronal development and axonal transport parameters (see for example Nirschl et al, 2016). This last aspect will be addressed using the microfluidic chambers and life microscopy (Taylor et al, 2005).

References:

- Janke C (2014) The tubulin code: Molecular components, readout mechanisms, and functions. *J Cell Biol* 206: 461-472 *(a general review on tubulin posttranslational modifications)*
- Nirschl JJ, Magiera MM, Lazarus JE, Janke C, Holzbaur ELF (2016) alpha-Tubulin Tyrosination and CLIP-170 Phosphorylation Regulate the Initiation of Dynein-Driven Transport in Neurons. *Cell Rep*: celrep 2509 *(this paper demonstrates how neuronal transport is measured by a combination of in-cellulo and in vitro approaches – in this case for another tubulin modification, detyrosination)*
- Rogowski K, van Dijk J, Magiera MM, Bosc C, Deloulme J-C, Bosson A, Peris L, Gold ND, Lacroix B, Bosch Grau M, Bec N, Larroque C, Desagher S, Holzer M, Andrieux A, Moutin M-J, Janke C (2010) A family of protein-deglutamylating enzymes associated with neurodegeneration. *Cell* 143: 564-578 *(this publication describes for the first time the link between tubulin polylgutamylation and neurodegeneration in a mouse model)*
- Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL (2005) A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat Methods* 2: 599-605 *(this publication provides the methodological background of the microfluidic technique we use)*

Period: Anytime from January 2017 to June 2017

Sophie NICOLE & Bertrand FONTAINE's team

Team: NeuroGenetics & Physiology

Fields of research: Neurological and psychiatric diseases

Internship project:

Role of meningeal lymphatic vessels and associated meningeal myeloid cells in Multiple Sclerosis disease progression.

Supervisor: Mohamed El Behi

Multiple Sclerosis (MS) is an incurable and disabling immune-mediated neurodegenerative disease of the central nervous system (CNS) characterized by inflammation, demyelination, neuron-axonal injury and gliosis. The etiology and exact pathogenesis of MS are unknown however it is widely believed that unidentified environmental risk factor(s) in genetically susceptible individuals promotes the activation of myelin-specific T cells, which normally reside in the periphery in a tolerant state. If these cells are able to cross the blood brain barriers and enter the CNS, they are reactivated by infiltrating or resident antigen presenting cells loaded with myelin antigens. Once reactivated, these T cells orchestrate localized inflammation leading to myelin and axonal damage. MS was originally thought to be predominantly a white matter disease but recent pathological and MRI studies have shown early and widespread involvement of cerebral grey matter (GM) as well as a substantial meningeal inflammation, both previously underappreciated. Indeed, the meninges are gaining appreciation as non-lymphoid sites of active immune responses during CNS inflammation as several line of evidence suggests direct relationships between the magnitude of early meningeal inflammation and the extent of CNS damage and disease outcome in MS. In addition, the recent description of functional lymphatic vessels linking meningeal compartment with peripheral cervical lymph nodes raises several questions regarding the role of these vessels in the immune surveillance of the CNS. In this proposal, we intend to study whether immune response development within the meninges drives CNS inflammatory disease and is responsible for GM damages by using different rodent model of MS like disease (aim 1), what are the roles played by meningeal lymphatic vessels in development and progression of CNS inflammation (aim 2), and what are the contributions of different population of meningeal resident myeloid cells (aim 3). These studies address important questions regarding triggers of MS and its progression as a chronic disease. Better understanding of these mechanisms can open opportunities for designing new therapeutic approaches in MS as well as in others neurological disease in which CNS lymphatic vessels and/or meningeal inflammatory cells may play a role.

In this project several experimental techniques will be used including immune cell isolation and culture, tissue microdissection, stereotaxic injections, and immune cells analysis using flow cytometry.

Period: Anytime from January 2017 to June 2017

Serge LAROCHE & Cyrille VAILLEND's team

Team: Cellular and molecular mechanisms of plasticity and memory

Fields of research: Neurophysiology / systems neuroscience

Internship project:

Hypothyroidism and Alzheimer's disease.

Supervisor: Valérie Enderlin

Factors impacting the risk of developing sporadic forms of Alzheimer's disease (AD), which accounts for over 99% of the cases, remain poorly understood. Thyroid dysfunction is a risk factor for Alzheimer's disease (AD) [1]. We showed that hypothyroidism in rats leads to early brain changes reminiscent of AD, notably hippocampal amyloïd beta (Aβ) and proinflammatory cytokine production, and Tau phosphorylation associated with memory deficits [2-3]. Our recent findings indicate that thyroid hormone (TH) supplementation rescues most of these alterations. Our general objective is now to gain new insights into the relationships between localized hypothyroidism and AD-related pathological hallmarks. Recent evidence suggests a detrimental impact of hypothyroidism on (1) synaptic transmission and memory, (2) neuronal and astrocytes survival [4]. TH is also considered as an important signaling factor that affects glial changes [5]. Changes of microglia and astrocytes functions may contribute to the early inflammatory response and modulate the level of Aβ peptide and Tau phosphorylation [6]. It is therefore of prime importance to finely characterize the inflammatory response and determine its role in the sequence of events that leads to a favorable environment for the development of AD pathology under conditions of hypothyroidism.

Experimental approach

Experiments will be assayed on control, hypothyroid rats and hypothyroid rats administrated with TH. To assess the inflammatory status, we will quantify markers of microglial and astrocyte activation, inflammatory elements such as cytokines and ROS; glial morphology will be evaluated. Various markers of CaM/MAPK signaling pathways, key regulators of synaptic plasticity and astrogliosis, will be quantified [7]. This project will be conducted using biochemical approaches.

References:

[1] Davis et al, 2008, Curr Aging Sci, 1:175–81.
[2] Ghenimi et al, 2010, J Neuroendocrinol, 22:951.
[3] Chaalal et al, 2014, Hippocampus, 24:1381.
[4] Cortes et al, 2012, Thyroid, 22:9.
[5] Noda, 2015, Front Cell Neurosci, 9:194.
[6] Morales et al, 2015, Front Cell Neurosci, 8:112.
[7] Sticozzi et al, 2013, Neurosci, 252:367.

Period: Anytime from January 2017 to June 2017

Serge LAROCHE & Cyrille VAILLEND's team

Team: Cellular and molecular mechanisms of plasticity and memory

Fields of research: Neurophysiology / systems neuroscience

Internship project:

Regulation of quiescence entry/exit of normal and pathological stem cells

Supervisor: Jean-Vianney Barnier

Normal and cancer stem cells share the unique property to proliferate or to remain in quiescence. They can also differentiate to several specific lineages in response to intrinsic genetic programs and regulatory external cues such as growth factors, hormone, extracellular matrix. Several signaling pathways that control stem cell activation and reentry into quiescence have been described, but some molecular mechanisms and effectors remain unknown (1). A better understanding of the biological features that govern stem cell fate is pivotal for the improvement of cancer therapy and to further develop regenerative medicine (2).

We have recently observed that the expression of the *pak3* gene, which is involved in the proneural embryonic pathway and is also a proneural marker in glioblastoma (GBM), is strongly increased during differentiation of mouse neural stem cells and human GBM stem cells (3). However PAK3 controls cell cycle exit during primary embryogenesis (4). We hypothesize that the transcriptional level of *pak3* may control stem cell fate in normal and pathological conditions regulating cell cycle exit, quiescence, and first steps of differentiation.

The successful candidate will participate in a program that aims to analyze the effects of modulating *pak3* expression on quiescence, proliferation, specification and differentiation of adult mouse neural stem cells and in human GBM stem cells. He/she will develop modified versions of the CRISPR/dCas9 system, in order to specifically target the *pak3* promoter, and express a dCas9 enzyme fused to the transcriptional inhibitory domain KRAB or the transcriptional activator VPR, respectively, in order to inhibit or activate *pak3* transcription (5). Stem cells will be transduced by lentivirus and cell quiescence, proliferation, specification and differentiation will be appraised using immunofluorescence, western blot and real-time PCR. The resulting data may present pak3 as a suitable therapeutical target, where several lead compounds that regulate its activity exist (6).

References:

(1) Homem et al., 2016, Nat. Rev. Neurosci., 16, 647
(2) Takeishi & Nakayama, 2016 Cancer Science, 107, 875
(3) Domenichini F, thesis, 2014; Magne N, thesis 2016.
(4) Souopgui et al., 2002, Embo J., 21, 6429
(5) Gilbert et al., 2014, Cell, 154, 442
(6) Senapedis et al., 2016. Anti-Cancer Agents in Medicinal Chemistry, 2016, 16, 75

Period: Anytime from January 2017 to June 2017

Richard LEVY's team

Team: FRONTlab: the frontal systems, functions and dysfunctions

Fields of research: Cognitive neurosciences / neuropsychology

Internship project:

Implicit categorization in healthy subjects: a behavioral and electrophysiological study

Supervisors: Emmanuelle VOLLE & Béatrice GARCIN

Semantic Categorization is a fundamental process that allows us to classify our knowledge about object and events. The ability to categorize information has an impact in virtually all domains of cognition and behavior, from learning (children learn new concepts by categorizing items that have similar properties or aspect) to survival (to recognize an animal as dangerous by categorizing it as similar to a previously encountered dangerous animal). Research in this field mainly has focused on explicit semantic categorization, but whether semantic categorization can occur implicitly or automatically, allowing fast responses, remains an open question.

Objective: This project aims at determining if semantic categorization can occur implicitly in healthy subjects and at exploring its neurophysiological correlates using EEG.

Method: We will record response time and accuracy during a double semantic priming paradigm called IMPLICAT: two primes will be briefly presented, followed by a target word, for which participants will have to perform a lexical decision task ("is it a word or non-word?"). We will use four different conditions:

1- *Categorization priming*: 2 prime words related to the target (RR-T), eg: *table* (*prime 1*) + *chair* (*prime 2*) - FURNITURE (TARGET)
2- *Priming without categorization*: 1 prime related and 1 prime unrelated to the target (RU-T), eg: *table* + *banana* - FURNITURE
3- *Priming without categorization:* Same but primes in the reverse order (UR-T), eg: *orange* + *chair* - FURNITURE
4- *No priming*: 2 primes unrelated to the target (UU-T), eg: *banana* + *window* - FURNITURE.

We will record high-resolution EEG with 256 channels for evoked potential analysis during the experimental task.

Hypotheses:

1- There is an implicit categorization process that can be evidenced by a stronger priming effect in the *categorization priming* (RR-T) condition (ie a shorter reaction time as compared to UU-T condition) than for both the UR-T and RU-T conditions.
2- The priming effect can be related to spatial and temporal electrophysiological markers, such as for instance the amplitude of the N400 that is usually associated with priming. The N400 component is a negative wave registered at the vertex 200 to 500 ms after the presentation of a stimulus, like a word.

Why it is important: The IMPLICAT paradigm will help determine the processes underlying implicit categorization in normal conditions, and could be used in neurological patients who have categorization difficulties to better understand these difficulties.

Feasibility:

- A pilot study showed the expected priming effect in 16 healthy subjects.
- The project has a funding and legal authorizations (Inserm ANALOG, C14-17)
- We will collaborate with the EEG facility (Dr. Georges) for EEG recording and analysis, and with the PICNIC Team (Pr. Naccache) for their expertise in semantic priming.

Period: Anytime from January 2017 to June 2017

Jean LIVET's team

Team: Development of Neuronal Circuits

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Dissecting neural development with new transgenic approaches

Supervisors: Jean Livet and colleagues

The vast majority of the neurons of the vertebrate central nervous system are generated by neural progenitor cells (NPCs) located in the embryonic neuroepithelium. How NPCs share the task of building nervous tissue and what is the exact sequence of their development are questions still debated. Probing these aspects requires tracking individual NPC development in intact nervous tissue over the entire course of neurogenesis. It is not possible to achieve this by live imaging in most species including mice, due to nervous tissue thickness, prolonged development and poor accessibility. Instead, one can use transgenic reporter systems to track the development of NPCs and their clonal progeny over the long term.
Our laboratory has developed new methods to mark individual NPCs with color labels encoded by genome-integrated transgenes and to image the clones of neuronal cells that they produce in intact nervous tissue (Loulier et al. Neuron 2014). Approaches that provide additional information are however required to further inform on NPC behavior, in particular by making it possible to dissect the sequence of development of neural clones, i.e. the order in which the cells that compose them are produced. The student will participate in a project aimed at developing such system with members of the laboratory. The proposed internship will consist in building the required transgenes by molecular biology approaches, validating these transgenes in cell culture, applying them in electroporated embryos to dissect neural clone development, and assaying the result by epifluorescence and confocal imaging. This rotation will allow the student to get exposed to diverse approaches in developmental neurobiology including molecular biology, embryonic electroporation, histology and microscopy.

Period: Anytime from January 2017 to June 2017

Muriel PERRON's team

Team: SCaNR : Stem Cells and Neurogenesis in the Retina

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Neural stem cell proliferation in the *Xenopus* retina: role of reactive oxygen species

Supervisor: Morgane Locker

Reactive oxygen species (ROS) were long considered as harmful and detrimental molecules, which when overproduced can affect a wide range of cellular functions. However, it is now widely accepted that ROS also play physiological roles at basal levels as modulators of key cell behaviours. Although they recently emerged as crucial regulators of both pluripotent embryonic and adult stem cell biology[(1)], only a few studies addressed so far their functions in adult neural stem cells[(2)]. The main objective of the project is thus to seek for their potential requirement in the control of neural stem cell activity *in vivo*, using the *Xenopus* retina as a model system. In contrast to mammals, this species has the tremendous advantage to bear active retinal stem cells (RSC) in a region called the ciliary marginal zone (CMZ). This region sustains the continuous growth of the retina and contributes to regeneration following lesion. Specifically we will address how ROS production imbalance affects RSC proliferative behaviour in physiological or degenerative conditions as described below.

1. Impact of NOX-dependent ROS production on RSC proliferation. The NADPH oxidase enzymes (NOX) constitute one of the main sources of endogenous ROS production within cells. We recently set-up optimal conditions allowing for their inhibition *in vivo* using the drug DPI. Our preliminary data revealed that interfering with NOX activity leads to decreased EdU incorporation in RSC. Interestingly, progenitor cells do not seem affected, suggesting distinct sensitivities of these proliferating populations to ROS levels.

These data must first be confirmed. We will next determine what causes such decreased EdU incorporation. Hypothesis to be tested are: (i) RSC irreversibly exit the cell cycle, (ii) they become quiescent, (iii) they exhibit altered cell cycle kinetics. Of note, all methods needed for *in vivo* exploration of cell cycle dynamics are mastered in the lab.

2. Seeking NOX-dependent signalling pathways in RSC. We will then seek which intracellular targets might be regulated downstream NOX in RSC. A candidate approach will be undertaken, focusing on Wnt signalling since we already characterized its function in retinal stem cell maintenance and set-up all tools needed to perturb or visualize its activity[(3)].

3. ROS requirement during retinal regeneration. ROS requirement during retinal regeneration will be investigated in a *Xenopus* transgenic line we developed, allowing for conditional ablation of photoreceptors. Following NOX inhibition, we will assess retinal regeneration by analysing proliferation (EdU incorporation assays) and neurogenesis (EdU pulse-chase coupled to photoreceptor labelling).
Main technics to be used: *In vitro* fertilization ; Pharmacological treatments ; Microinjection ; Immunofluorescence

References:

(1) Bigarella, C. L. *et al. Development* 4206–4218 (2014).
(2) Le Belle, J. E. *et al. Cell Stem Cell* 8, 59–71 (2011).
(3) Borday, C *et al*. Development 139(19): 3499-509

Period: Anytime from January 2017 to June 2017

Serge PICAUD's team

Team: Retinal information processing: pharmacology and pathologies

Fields of research: Neurophysiology / systems neuroscience

Internship project:

Decomposing the retinal circuit by population recording and single cell stimulation

Supervisor: Olivier Marre

The retina is an ideal system to understand how neuronal networks are able to process neuronal information, thanks to its crystalline and layered structure. The signal flows from photoreceptors through a few layers of neurons to the retinal ganglion cells, which send spikes down the optic nerve to the brain. In addition to the vertical transmission through bipolar cells, classes of interneurons called horizontal cells and amacrine cells spread signals laterally across the plane of the retina. Although recent studies have identified different types of information processing within the inner plexiform layer, the precise implication of bipolar cells in this processing remains unsolved. There is currently no model able to predict the response of retinal ganglion cells to complex stimuli. It seems that bipolar cells already perform complex computations on visual information, but these cells are hard to access with standard physiological techniques.

We have combined several state of the art tools to measure, and stimulate, several bipolar cells, while recording a large fraction of the ganglion cells that will receive the bipolar output. For this we have combined several techniques: 1) multi-electrode array (MEA) recordings allowing simultaneous recording of large ensembles of ganglion cells (100+ cells) in a patch of the retina; 2) Two-photon stimulation and imaging at very high spatial resolution, 3) expression of optogenetic tools allowing cell-specific activation. We combine these techniques to precisely stimulate the retinal network and simultaneously measure the impact of these stimulations on the retinal output.

The aim of this project is to use these tools to decompose the retinal circuit, and understand how the visual information is processed and transferred between two successive layers, the bipolar and ganglion cells. We will record ganglion cells, the retinal output, with MEA and stimulate bipolar cells expressing optogenetic proteins using two-photon laser holographic stimulation. This dual approach will enable us to define how a single optogenetically activated bipolar cell can influence ganglion cells, and to reconstruct the functional connectivity map between two essential layers of the retina.

The candidate will be trained to use this combination of techniques, to perform its own experiments, and will analyze the resulting data. Please don't hesitate to contact us for more information.

Period: Anytime from January 2017 to June 2017

Alessandra PIERANI's team

Team: Genetics and Development of the Cerebral Cortex

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Programmed cell death of Cajal-Retzius neurons in the construction of functional and dysfunctional cortical circuits

Supervisors: Alessandra Pierani & Eva Coppola

The assembly of cortical circuits begins during embryogenesis through a precise orchestration of proliferation, spatio-temporal generation of distinct cell types and control of their migration. During this integrated process, a specific population of early-born and transient neurons, Cajal-Reztius cells, has been shown to play essential roles.

Cajal-Retzius cells (CRs) are among the first neurons generated in the embryonic neocortex. They are located in the superficial layer (marginal zone (MZ)/layer I) until the first postnatal weeks, when they are progressively eliminated. Notably, persistence of CRs during postnatal life has been detected in pathological conditions such as cases of Temporal lobe epilepsy (TLE), Ammon's horn sclerosis (AHS) and polymicrogyria, thereby opening the intriguing possibility that their failure to disappear might contribute to dysfunction of cortical circuits.

We have generated a mouse line preventing the death of CRs. The project will involve 1) a histological approach to analyze the development of cortical layers and functional areas; and 2) a phenotypical approach, which is already underway and show defects including in the susceptibility to epileptic seizures, to assess behaviours in these mice. Our results are now providing the first mouse model for the persistence of CRs in the postnatal animal allowing for testing the role of programmed cell death (PCD) in cortical development and dysfunction. This will permit to study whether and how survival of CRs in adults recapitulates pathological phenotypes.

Period: Anytime from January 2017 to June 2017

Jean Christophe PONCER & Sabine LEVI's team

Team: Lab Plasticity of Cortical Networks and Epilepsy

Fields of research: Neuropharmacology / cell signaling

Internship project:

The WNK signaling pathway as a novel target for the treatment of epilepsy

Supervisors: Sabine Lévi & Jean Christophe Poncer

GABA is the main inhibitory neurotransmitter in the central nervous system. As GABAA receptors (GABAAR) are permeable to chloride ions, the efficacy and polarity of responses to GABA depend on the intracellular chloride concentration ([Cl-]i). In mature neurons where GABA is hyperpolarizing/inhibitory, this concentration is maintained low by the neuronal chloride extruder K+/Cl- co-transporter KCC2. A reduced membrane expression and/or stability of KCC2 and thereby a shift from hyperpolarizing/inhibitory to depolarizing/excitatory action of GABA has been associated with several neurological and psychiatric disorders (e.g. epilepsy, neuropathic pain, schizophrenia).

Pathological conditions involving hyperexcitability rapidly alter KCC2 membrane stability and function. We have recently discovered an activity-dependent regulation of KCC2 membrane dynamics, clustering and function via GABAAR-mediated inhibition and the chloride-sensing With No lysine Kinase WNK1 signaling pathway that directly phosphorylate KCC2 on key Threonine 906 and 1007 residues. Our results suggest WNK and downstream effectors SPAK/OSR1 constitute novel and promising targets to enhance KCC2 membrane stability and function and to prevent the emergence of epileptic seizures.

The aim of this project is to test the hypothesis that inhibition of the WNK/SPAK signaling pathway will reduce/abolish seizures in an *in vivo* model of temporal lobe epilepsy. We will test whether reduced WNK1 expression in heterozygous mice reduces seizures (by EEG and video recordings) in models of temporal lobe epilepsies. We will compare onset latency of the first seizure, seizure severity and latency to epileptic seizure in WNK1 heterozygous mice as compared to +/+ animals upon injection of either the GABAAR antagonist pentylenetetrazole or the muscarinic agonist pilocarpine. WNK1 has been shown to phosphorylate KCC2 either directly or via intermediate kinases SPAK/OSR1 In parallel, we will evaluate the impact of a pharmacological blockade of SPAK on seizure susceptibility using Closantel, an efficient inhibitor of SPAK both *in vitro* and *in vivo*. These experiments will be coupled to a biochemical analysis of the hippocampus of WNK1 +/- vs +/+ animals. We will determine whether epilepsy increases Thr906 and 1007 phosphorylation of KCC2 and lead to reduced membrane expression of KCC2 in WT but not in WNK1 +/- animals or in WT animals exposed to closantel.

These experiments may help to validate the WNK signaling pathway as a therapeutic target in the treatment of epilepsy and probably other pathologies associated with dysregulation of KCC2.

Period: Anytime from January 2017 to June 2017

Brahim NAIT OUMESMAR's team

Team: Molecular and cellular approaches of Myelin repair

Fields of research: Neurological and psychiatric diseases

Internship project:

Role of the Sox17 Transcription Factor in Myelination

Supervisor: Brahim Nait Oumesmar

In the central nervous system (CNS), myelination is a critical process, timely regulated by oligodendroglial cell lineage progression. The HMG-box transcription factors are key regulators of oligodendroglial cell proliferation and differentiation. Among these factors, Sox17 was previously identified as a regulator of oligodendrocyte development. In the developing CNS, Sox17 expression is detected in oligodendroglial cells and is highest in differentiating oligodendrocytes. In oligodendrocyte progenitor cell (OPC) cultures, Sox17 expression correlates with the transition between OPC proliferation and differentiation. To investigate the function of Sox17 *in vivo*, we recently generated a transgenic mouse model overexpressing Sox17 and the EGFP reporter in oligodendroglial cells, in a doxycycline (Dox)-inducible. We showed that Sox17 gain-of-function increased of the number of OPCs and down-regulated the proportion of differentiated oligodendrocytes, leading to a severe CNS hypomyelination during post-natal development. These data reveal critical functions of Sox17 in OPC development and lineage progression. The main goal of this internship is to characterize the down-stream targets of Sox17 and to decipher their functions in oligodendrocyte development and myelination. Various technics will be used during this project, including mouse transgenesis, lentiviral-mediated gene know-down, glial cell cultures, immunofluorescence histochemistry and electron microscopy.

References:

- de la Fuente AG, Errea O, van Wijngaarden P, Gonzalez GA, Kerninon C, Jarjour AA, Lewis HJ, Jones CA, Nait-Oumesmar B, Zhao C, Huang JK, ffrench-Constant C, Franklin RJ (2015) Vitamin D receptor-retinoid X receptor heterodimer signaling regulates oligodendrocyte progenitor cell differentiation. J Cell Biol 211(5):975-85.
- Weider M, Wegener A, Schmitt C, Küspert M, Hillgärtner S, Bösl MR, Hermans-Borgmeyer I, Nait-Oumesmar B, Wegner M. (2015) Elevated in vivo levels of a single transcription factor directly convert satellite glia into oligodendrocyte-like cells. PLoS Genet 11(2):e1005008.
- Wegener A, Deboux C, Bachelin C, Frah M, Kerninon C, Seilhean D, Weider M, Wegner M, Nait-Oumesmar B (2015) Gain of Olig2 function in oligodendrocyte progenitors promotes remyelination. Brain 138(Pt 1):120-35.
- Blanchard B, Heurtaux T, Garcia C, Moll NM, Caillava C, Grandbarbe L, Klosptein A, Kerninon C, Frah M, Coowar D, Baron-Van Evercooren A, Morga E, Heuschling P, Nait Oumesmar B (2013) Tocopherol derivative TFA-12 promotes myelin repair in experimental models of multiple sclerosis. J Neurosci, 33(28):11633-42.
- Moll NM, Hong E, Fauveau M, Naruse M, Kerninon C, Tepavcevic V, Klopstein A, Seilhean D, Chew LJ, Gallo V, Nait Oumesmar B (2013) SOX17 is expressed in regenerating oligodendrocytes in experimental models of demyelination and in multiple sclerosis. Glia (10):1659-72.
- Sohn J, Natale J, Chew LJ, Belachew S, Cheng Y, Aguirre A, Lytle J, Nait-Oumesmar B, Kerninon C, Kanai-Azuma M, Kanai Y, Gallo V (2006) Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. J Neurosci. 26(38):9722-35.

Period: Anytime from January 2017 to June 2017

François ROUYER's team

Team: Molecular genetics of circadian rhythms

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Neuronal circuits that control sleep-wake cycles 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 have selected transgenic lines that express Gal4/LexA transgenes in specific populatins of neurons whose anatomical location in the brain suggests possible connections with clock neurons. These transgenes allow the considered neurons to be analyzed anatomically and functionally. The project will focus on neurons that might be downstream targets of the clock neurons that contribute to the evening peak of activity in light:dark cycles.

We will use molecular-genetic tools to anatomically and functionally verify whether and how these putative downstream neurons are connected different types of evening clock neurons. The behavioral contribution of these neurons will be analyzed by manipulating their activity (hyperexcitation or silencing) in different environmental conditions. The experiments will involve Drosophila genetics, behavioral analysis (sleep-wake cycles), as well as anatomical and functional imaging of the clock/target neurons.

Period: Anytime from January 2017 to June 2017

Daniel SHULZ's team

Team: Neural Processing, Neuromodulation and sensory plasticity

Fields of research: Neurophysiology / systems neuroscience

Internship project:

Fribroscopy for large scale imaging of cortical dynamics during active sensing in freely behaving mice

Supervisor: Isabelle Ferezou & Daniel Shulz

The team is specialized in integrative neuroscience, using the tactile somatosensory pathway from whiskers to cortex in rodents as model system. Our research is centered on the study of neuronal processes responsible for the coding of tactile sensory information and perception, as well as their regulation through the interaction of the animal with the environment.
By means of electrophysiology and functional imaging in anesthetized rats and mice, we have shown that the primary somatosensory cortex can extract emergent properties of a complex multiwhisker tactile stimulation, such as the global direction of a multiwhisker stimulus generating an apparent motion across the whiskerpad (Jacob et al., 2008, Vilarchao et al., in preparation). To understand the involvement of such integration mechanisms and their functional importance, it is essential to link the recording of cortical dynamics and the animal's behavior in an experimental paradigm involving the extraction of global properties of an object by the animal.

Nowadays, neurophysiologists generally address this type of questions by working with awake "head-fixed" mice, in other words with animals that have been accustomed to be held by the head (with implants fixed on the skull) under conventional optics. It is indeed possible, in this configuration, to train animals to perform simple behavioral tasks, while imaging large assemblies of neurons. These experiments, however, remain highly restrictive and limit the behavioral repertoire that can be studied.

On the contrary, we aim to work with freely moving animals in conditions allowing the development of an operant conditioning task where the mice have to extract the global properties of an object through the use of their whiskers. We will study the cortical dynamics during the task at the mesoscopic scale (with a field of view covering the cortical representation of all the macrovibrissae within the primary somatosensory cortex) by using voltage sensitive dye imaging, which offers excellent spatial and temporal resolution. This will be made possible through the use of a fiber bundle interface, which provides sufficient flexibility and lightweight properties to disrupt minimally the behavior of the animal without sacrificing the optical qualities of imaging system (Ferezou et al., 2006).

These experiments will allow analyzing the cortical dynamics governing the extraction of global properties of an object from complex sequences of multivibrissal contacts.

References:

- Jacob V, Le Cam J, Ego-Stengel V, Shulz DE. Emergent properties of tactile scenes selectively activate barrel cortex neurons. Neuron. 2008 Dec 26;60(6):1112-25.
- Vilarchao ME, Shulz DE, Férézou I. Spatial organization of global direction selectivity in the mouse barrel cortex (in preparation).
- Ferezou I, Bolea S, Petersen CC. Visualizing the cortical representation of whisker touch: voltage-sensitive dye imaging in freely moving mice. Neuron. 2006 May 18;50(4):617-29.

Period: Anytime from January 2017 to June 2017

German SUMBRE's team

Team: Zebrafish neuroethology

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Neuronal circuit dynamics and behavior in zebrafish larvae

Supervisor: German Sumbre

The lab uses optogenetics, and two-photon or light-sheet microscopy to monitor whole-brain dynamics in behaving zebrafish larvae. We propose two projects:

Perceptual bi-stability

The brain can't perceive two percepts simultaneously, instead perception switches from one percept to the other. The reasons and mechanisms for the switch between percepts still remains a mystery.
The student will study the neuronal patterns and mechanisms underlying the switch of one visual percept to the other, by examining the ongoing spontaneous activity preceding the perceptual switch.

Role of glia in sensory processing

Astrocytes play an important role in plastic adaptations of neuronal circuits. Using genetic and optogenetic methods, the student will investigate the fine tuned dialogue between neurons and glia for the proper development and function of neural circuits and sensory processing. More precisely, the student will measure the pairwise correlations between glia and neurons, during periods of ongoing spontaneous activity to learn about their functional connectivity, and monitor glia responses during visual stimulation. Visually induced responses will also be measured in the neuronal population after ablation of glia cells.
** Note that notions of computer programming will be an advantage for these projects.*

References:

1- Candelier R*, Murmu MS*, Romano SA, Jouary A, Debregeas G*, Sumbre G.*. (2015) A microfluidic device to study neuronal and motor responses to acute chemical stimuli in zebrafish. *Scientific Reports*. 5(12196) doi: 10.1038/srep12196
2- Romano SA, Pietri T, Pérez-Schuster V, Jouary A, Haudrechy M and Sumbre G. (2015) Spontaneous neuronal network dynamics reveal circuit's functional adaptations for behavior. *Neuron*. 85(5):1070–1085.
3- 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
4- Panier T, Romano S, Olive R, Pietri T, Sumbre G, Candelier R and Debrégeas. (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
5- Sumbre G, Muto A, Baier H and Poo MM. (2008) Entrained rhythmic activities of neuronal ensembles
6- *as perceptual memory of time interval. Nature. 456(7218):102-106.*

Period: Anytime from January 2017 to June 2017

Laurent VENANCE's team

Team: Dynamic and Pathophysiology of Neuronal Networks

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Dopaminergic control of cortical and thalamic interplay for striatal synaptic plasticity

Supervisors: Laurent Venanc & Marie Vandecasteele

Basal ganglia are involved in adaptive control of the movement and procedural learning and memory. Striatum, the main inputs nucleus of basal ganglia, acts as a coincidence detector of cortical and thalamic glutamatergic inputs: striatum extracts from background noise information judged as pertinent depending on the context. Dopamine tunes this striatal detection threshold.

We have pioneered the field of spike-timing dependent plasticity (STDP), a synaptic Hebbian learning rule, at the level of basal ganglia (1-4). In the dorsal striatum, we have recently characterized a new form of LTP mediated by the endocannabinoid system, which is induced by a very low numbers of spikes (5, 6) and could account for the fast learning.

The project is to explore the impact on striatal synaptic plasticity (mediated by endocannabinoids) of cortical and thalamic activities timed with dopamine opto-stimulation or opto-inhibition (in DAT-ChR3 and DAT-ArCh2 mice available in the lab). Striatal output neurons will be recorded, with patch-clamp techniques, and subjected to STDP paradigm while manipulating (optogenetics) the dopamine release.
This project aims at better understanding (1) how cortical and thalamic activity together with dopamine concurred to striatal synaptic plasticity and the engram of procedural learning and (2) the distal reward problem (Pavlovian and instrumental conditioning).

Experimental approaches:
electrophysiology (in vivo and ex vivo patch-clamp), optognetics, pharmacology, 2-photon microscopy.

References:
(1) Fino et al., (2005) J Neurosci
(2) Fino et al., (2010) J Physiol
(3) Puente et al., (2011) Nat Neurosci
(4) Paillé et al., (2013) J Neurosci
(5) Cui et al., (2015) J Physiol
(6) Cui et al., (2016) eLife

Period: Anytime from January 2017 to June 2017

Claire WYART's team

Team: Lights-on Locomotion: optogenetic dissection of spinal circuits underlying locomotion in Vertebrates

Fields of research: Computational neurosciences / neural theory

Internship project:

Functional and genetic identification of the Mesencephalic Locomotor Region (MLR) in zebrafish larva

Supervisor: Claire Wyart

Shik, Severin and Orlovskii discovered in cats that electrical stimulation of a locus at the junction between the midbrain and hindbrain elicited controlled walking and running (1). They called this region the Mesencephalic Locomotor Region or MLR. This locomotor center has been shown since to control locomotion in various vertebrate species (lamprey, salamander, stingray, rat, guinea-pig, rabbit or monkey). Recently Karachi et al. showed that in human subjects asked to imagine they are walking, there is an increased MRI activity in the pedunculopontine, cuneiform and subcuneiform nuclei, brainstem nuclei corresponding to the human MLR (2). Clinicians are now performing deep brain stimulation of the MLR to alleviate locomotor symptoms of patients with Parkinson's disease.

Results from stimulation indicate that the MLR is more complex than a simple relay in a serial descending pathway activating the spinal locomotor circuits: it can have multiple functions. In all species studied so far, the circuit organization of the MLR still remains a matter of debate.

We propose here to probe the MLR in a genetically accessible model, the zebrafish larva, by taking advantage of its transparency to combine optical sensors of neuronal activity with actuators to activate neuronal populations while recording fictive locomotion at the level of the ventral nerve roots (3, 4). We will use transgenic lines to target specific types of neurons (GABAergic, cholinergic or glutamatergic) and digital holography to pattern the light in 3D at the junction between midbrain and hindbrain while monitoring behavior as previously established in our lab (5-10).

References:

(1) Shik, Severin and Orlovskii, Dokl Akad Nauk SSSR. 1966.
(2) Karachi, André, Bertasi, Bardinet, Lehéricy, Bernard. J. Neurosc. 2012.
(3) Portugues, Severi, Wyart, Ahrens. Curr Opin Neurobiol. 2013;
(4) Fidelin and Wyart. Curr Opin Neurobiol. 2014.
(5) Fidelin et al., Current Biology 2015.
(6) Böhm, Prendergast et al., Nature Communications 2016.
(7) Hernandez et al., Nature Communications 2016.
(8) Sternberg, Severi, et al., Current Biology 2016.
(9) Hubbard *et al., in revision.*
(10) Oldfield *et al., in revision.*

Period: Anytime from January 2017 to June 2017

Jean-Léon THOMAS & Bernard ZALC's team

Team: Development of oligodendrocytes and Neurovascular Interactions

Fields of research: Neurogenetics / neurodevelopment

Internship project:

Live Imaging of inflammatory response during demyelination and either spontaneous or pharmacologically-induced myelin repair in the central nervous system

Supervisor: Bernard Zalc

Multiple sclerosis **(MS)** affects about 2.5 Million individuals worldwide, half of which develop a permanent disability after 15 years of evolution. MS combines inflammation, demyelination and neurodegeneration. Microglia is a key player in pathophysiology of MS and depending on its stage of activation, microglia favors either myelin sheath destruction or repair. **Microglia/oligodendrocyte interactions** remain to be deciphered to develop **better-targeted therapeutic** strategies. Whether microglia and macrophages are beneficial or harmful in MS, is currently under debate. In rodent, live imaging technologies are limited to superficial myelinated fibers (0.5mm in depth). To circumvent this limitation, the aim of our project is to develop experimental models allowing **visualization of microglia behavior during demyelination and remyelination**. These simple models will be used to **screen *in vivo* for molecules favoring remyelination** whether acting on oligodendrocyte, or microglia/macrophages, or both.

1) Live imaging of microglia during demyelination and repair in Xenopus tadpoles

We have generated a ***Xenopus* transgenic line**, *MBP-GFP-NTR,* allowing **conditional ablation of myelin-forming oligodendrocytes.** In this line, demyelination induced by oligodendrocytes ablation is followed by spontaneous **remyelination,** a process that can be impressively accelerated by **pharmacological treatments.** Thanks to the transparency of tadpoles, demyelination and remyelination can be quantitatively monitored by 2-photon imaging on living animals. To visualize both oligodendrocytes and microglia, we will generate double transgenic by knock-in *tdTomato* reporter into a macrophage/microglia specific locus, in *MBP-GFP-NTR* embryos using the Crispr/Cas9 technology.

2) Microglia interactions with axonal domains during demyelination and repair

Axonal initial segment and **nodes of Ranvier** allow generation and rapid propagation of nerve impulse along myelinated fibers. To monitor the modifications of nodes of Ranvier during the process of demyelination and remyelination we have already generated a double transgenic *MBP-GFP-NTR/NbetaT-beta1NavGFP.* To investigate by live imaging the dynamic **interactions of nodes of Ranvier and microglia** during demyelination and remyelination we will generate a **triple Xenopus transgenic** *MBP-GFP-NTR/NbetaT-beta1NavGFP/microglia-tdTomato*, by inserting *microglia-tdTomato* into the genome of *MBP-GFP-NTR/NbetaT-beta1NavGFP* Xenopus embryo.

3) Comparison of RNA profiles of resting microglia and microglia interacting with axonal domains. To identify changes in RNA profile of microglia when they contact axonal domains during the remyelination process, we will use single-cell RNA-seq to investigate the transcriptome of microglia both in living tadpole and in mouse organotypic slice cultures

All together, we propose to **monitor by live imaging, microglia behavior during demyelination and remyelination** and **to identify molecules favoring myelin repair** acting on either oligodendrocytes, or microglial cells or both**.**

References:

- Kaya F, et al. (2012) Live imaging of targeted cell ablation in *Xenopus*: a new model to study demyelination and repair. *J Neurosci.* Sep 12;32(37):12885-95.
- Sekizar S, et al., (2015) Remyelination by Resident Oligodendrocyte Precursor Cells in a Xenopus laevis Inducible Model of Demyelination. *Dev Neurosci.* 37(3):232-42.

Period: Anytime from January 2017 to June 2017