ICN PhD Program

Aix – Marseille University

LIST OF SELECTED PhD RESEARCH PROJECTS

Research project #1

Title: Treatment of KCNQ2 encephalopathies using pre-clinical models

Supervisor: Laurent VILLARD

Laboratory: UMR_S 910 Génétique Médicale et Génomique Fonctionnelle, http://umr910.timone.univ-mrs.fr

Summary:

State of the art: Nearly 50 % of children with intellectual disability have epilepsy. The causal relationship between epilepsy and the deterioration of brain function has been defined by the term "epileptic encephalopathy". Our laboratory studies Early Onset Epileptic Encephalopathies (EOEE) that are characterized by frequent seizures starting before third month of age and a pathological interictal EEG activity. The phenotype of EOEE is severe with an absence of language, an autistic behavior, impaired fine motor skills and abnormal movements. In order to better understand this disease, we have established a cohort of 350 EOEE patients and found (with others) that KCNQ2 (potassium channel, voltage-gated, KQT-like subfamily, member 2) is a major gene involved in EOEE. Functional KCNQ channels are composed of homo- or hetero-meric assemblies of 4 subunits including KCNQ2/KCNQ3 that give rise to the M current (I_M).

Objective: The aims of our project is to understand the mechanisms by which KCNQ2 mutations cause epileptic encephalopathy and to develop pharmacological approaches to prevent this severe disease.

Methods: We will use a combination of genetic, electrophysiological, biochemical and cellular analyses *in vivo* and *in vitro*. The biophysical characteristics of the I_M channel will be analyzed using patch-clamp recordings (in collaboration) and a morpho-functional analysis of neurons will also be performed to determine if KCNQ2 mutations affect the surface expression and trafficking of the channel. We are currently creating a knock-in mouse (available Summer 2015) that will constitutively express the KCNQ2 mutation found in the patient with the most severe clinical presentation in our cohort. Heterozygous animals will be used as a model for electrophysiological analysis, phenotypic evaluation and as a pre-clinical model for pharmacological intervention. We have also generated induced pluripotent stem cells (iPSC) from EOEE patients and will differentiate these cells to obtain human neurons that will be used to study the mutant channels.

Relevance to integrative and clinical neuroscience: Our project starts from one of the largest cohort of EOEE patients in the world to end with the pre-clinical evaluation of candidate drugs to treat this devastating disease. Meanwhile, our experiments will contribute to improve the knowledge of normal and mutant potassium channels biology and to understand the mechanisms leading to epilepsy and to the severe neurological dysfunction caused by KCNQ2 mutations. This is a unique opportunity to simultaneously provide new insights into basic mechanisms of neuronal excitability and the neurobiology of potassium channels, and to test therapeutic strategies in a domain where not much is offered to the affected families.

Expected results: Our ultimate objective will be to determine if I_M activators are effective in improving the electrophysiological consequences of the KCNQ2 mutations. We believe that a rapid restoration of I_M may improve the neurological development of the patients. There is currently no efficient treatment available. Beyond the clinical and translational aspects of our project, the results obtained will improve our knowledge on the regulation of KCNQ channel, their function during brain development and synaptic plasticity. Hopefully, this project will ultimately translate into a clinical trial for KCNQ2-related EOEE patients.

Feasibility: Our laboratory holds all the necessary reagents and platforms to perform the proposed experiments. The knock-in mouse model is being produced for us and will be available in Summer 2015, two iPS cell lines are already obtained, the differentiation towards neurons is used in our laboratory. Our team already has a strong background in translational research for rare diseases with two patents and two phase IIa clinical trials originating from our work. Part of the proposed work will be performed in collaboration with L.Anikstejn (Inmed, Marseille) and J.Devaux (CRN2M, Marseille) for electrophysiology and neuronal morphology analysis. This research program is funded by ANR (plan d'action 2014).

Research project #2

Title: Toward a common therapy for Alzheimer's and Parkinson's diseases
Supervisor: Jacques FANTINI
Laboratory: PPSN EA-4674
Summary:

State of the art:
Ca^{2+}-permeable amyloid pore channels are currently considered as the most neurotoxic species at work in various neurodegenerative diseases, including Alzheimer's and Parkinson's. These oligomeric structures are formed under the control of plasma membrane gangliosides, explaining the prominent role played by brain gangliosides in the pathogenesis of these diseases. Recently we deciphered the ganglioside-recognition code controlling specific ganglioside binding to Alzheimer's β-amyloid (Aβ1-42) peptide and Parkinson's disease-associated protein α-synuclein. Cracking this code allowed us to engineer a short chimeric Aβ/α-synuclein peptide that recognizes all brain gangliosides and inhibits, at nanomolar concentrations, the formation of any amyloid pore (patent # EP14305353.6). The main objectives of the project are: i) to evaluate the activity of the chimeric peptide against amyloid pores assembled from the mutated amyloid proteins (Aβ1-42, α-synuclein) that are associated with inherited forms of Alzheimer's and Parkinson's diseases, ii) to engineer neural cell lines secreting the neuroprotective peptide and evaluate their resistance to amyloid pore formation (pre-clinical gene therapy approach), and iii) to test various pharmaceutic formulations of the peptide in order to start its evaluation in animal models of Alzheimer's and Parkinson's diseases.

Methods:
Cell culture, single-cell Ca^{2+} fluorescence microscopy, molecular dynamics simulations, lipid-protein interactions with Langmuir-Blodgett techniques, DNA transfection, ELISA, *in vitro* blood-brain barrier permeability studies.

Expected results:
Our ambition is to validate the use of the chimeric peptide designed in our team as a universal therapeutic compound for all neurodegenerative diseases caused by amyloid pores. We expect decisive results pertaining to the evaluation of our chimeric peptide against amyloid pores assembled from mutated proteins and to the determination of the best possible peptide formulations (including gene therapy approaches) to be used in animals.

Relevance to either Integrative or Clinical Neuroscience:
Our data indicate that a common therapeutic approach for both Alzheimer's and Parkinson's diseases, targeting the gangliosides recognized by amyloid proteins, is possible. Our project is thus deeply linked to clinical neuroscience.

Feasibility:
All the techniques that are necessary for the project are routinely used in our research team. All preliminary studies have been performed, so that the research program can begin without any delay. Several formulations of the chimeric peptide are already available in our team (multi-branched peptide, peptide encapsulated into liposomes, peptide coupled with nanoparticles). A first attempt of transfection of neural cells with a peptide-designed vector has been successful. The funding for animal studies has been already obtained (patent-linked grant from the SATT-Sud-Est). These studies will be conducted in partnership with international laboratories. All wild-type and mutated proteins have already been received. A rapid glance at PubMed will help the readers of this proposal to assess the publication activity of our research team. Finally, Jacques Fantini (PhD supervisor) and Nouara Yahi (team leader) have recently co-written a book on '*Brain Lipids in Synaptic Function and Neurological Disease: Clues to Innovative Therapeutic Strategies for Brain Disorders*' (Elsevier/Academic Press, available in July 2014). The ideas developed in this book are directly linked to the current proposal.

Research project # 3

Title: The effect of learning on the synaptic strength distribution in the cerebellar cortex

Supervisors: Païkan MARCAGGI, Valérie CREPEL

Laboratory: INMED, http://www.inmed.fr/

Summary:

State of the art: The cerebellar cortex contains more than half the total number of brain neurons. It is made of a repetitive and geometrical circuit evoking a conserved and optimized "learning machine". Its general role in learning, beyond its established role in motor learning, is increasingly accepted. However, it remains unclear whether memory is actually encoded in the cerebellar cortex. Early modeling work predicted that plasticity at granule cell (GC) to Purkinje cell (PC) synapses might underlie supervised learning. This prediction has been supported by the discovery of long-term plasticity mechanisms, the physiological relevance of which has remained questioned. A remarkable paradox manifested by the GC to PC synapses is that more than 85% of them appear to be electrically "silent". This extreme synaptic strength distribution has been suggested to enable maximized information storage capacity. However, it remains to be shown that it results from learning-driven synaptic plasticity mechanisms. The role of silent synapses in the strategy used for cerebellar learning therefore requires in depth study.

Objectives: 1. Establish a novel optogenetic-based approach enabling ready estimate of the fraction of silent GC to PC synapses and confirm that the majority of them are electrically silent. 2. Determine how the fraction of silent synapses changes along postnatal development. 3. Investigate the causal link between cerebellar learning and the synaptic strength distribution to obtain evidence for a memory trace in the cerebellar cortex.

Methods: Optogenetic. A mouse line expressing the fluorescent calcium indicator protein GCaMP6f selectively in cerebellar granule cells will be generated. ***Patch-clamp***. Purkinje cell will be recorded by patch-clamp in acute mouse cerebellar slices. ***Calcium imaging***. Calcium transients evoked by the stimulation of the granule cell axons will be imaged by epifluorescence and two-photon microscopy**.** ***Mouse behavior***. Enriched environment promoting mouse agility learning will be set up and monitored. Cerebellar learning will be assessed by specific motor learning paradigms.

Expected results : Only two experimental reports have supported that a majority of GC to PC synapses are electrically silent. With a novel approach (see Feasibility), the student will confirm this proposal. He/she will further provide the most accurate estimate of the fraction of silent synapses and map how it varies amongst cerebellar microzones. The student will apply the same experimental method to determine how the fraction of silent synapses varies along postnatal development. This will provide useful hints in the quest for the plasticity mechanisms at work during postnatal development. The student will examine how learning paradigms impact on the fraction of silent synapses to establish a causal link between cerebellar learning and the synaptic strength distribution.

Relevance to either Integrative or Clinical Neuroscience: This research will advance our knowledge of (i) physiological processes underlying learning and memory in brain tissue, and (ii) the function of the cerebellum. Indirectly, this project will be useful to human health. Pathologies involving cerebellar dysfunction range from ataxia to dystonia and autism spectrum disorders. Without any doubt, research targeting these pathologies will benefit from a better understanding of how memory is encoded in the cerebellar cortex.

Feasibility : Driver and reporter mice are available at INMED. The project relies on a novel method taking advantage of recent advance in optogenetic to image activated inputs and the highly geometrical arrangement of the GC to PC connection. Using mice expressing GCaMP2, we set up the method and checked that the fraction of silent synapses on a given recorded PC could be readily estimated. With a 20-fold higher signal to noise ratio, GCaMP6 indicators will tremendously facilitate the project. Mouse behaviour investigation will benefit from collaboration with D. Robbe at INMED.

Research project #4

Title: Cell-type specific plasticity of striatal projection neurons and their modulation by cholinergic interneurons in parkinsonism

Supervisor: Corinne BEURRIER

Laboratory: IBDM, http://www.ibdm.univ-mrs.fr/

The dorsal striatum is part of the basal ganglia circuitry and integrates information about sensory, motivational and motor state conveyed by cortical and thalamic neurons, facilitating the selection of actions that achieve desirable outcomes and avoid undesirable ones. Excitatory connections from the cortex and thalamus synapse onto projection neurons in the striatum called medium spiny neurons (MSNs). There are two main types of MSNs which express either the dopamine D1 receptors (D1 MSNs) or the dopamine D2 receptors (D2 MSNs). The tuning of these two populations is a critical process of basal ganglia functioning that impacts motor control. Dopamine (DA), coming from the substantia nigra compacta (SNc), and acetylcholine (ACh), originating from the striatal cholinergic interneurons (CINs), are two major players that interact to modulate MSN output. In Parkinson's disease (PD), the progressive loss of SNc DA neurons leads to a variety of biochemical changes in the striatum. However, the intrinsic and synaptic adaptations occurring in MSNs in PD are not well understood. The development of transgenic mice in which the expression of D1 or D2 receptors is reported by fluorophores is a useful tool to understand MSN properties. A second major technical advance that could greatly deepen our understanding of striatal function and dysfunction comes from optogenetics that allow the selective manipulation of microcircuits. The goals of this project are to use state of the art techniques to study the *in vitro* physiology of D1 and D2 MSNs and their pathological functioning in a mice model of PD. ***Aim 1***. Most of the *in vitro* electrophysiological studies on PD mice models are done in unidentified MSNs and/or after acute DA lesion (5 days) which is far from mimicking the chronic loss of DA in PD. ***Objectives:*** we will study the impact of long-term (2-3 weeks) DA depletion induced by 6-hydroxydopamine (6-OHDA) on D1 and D2 MSN physiology (intrinsic and synaptic properties). ***Method and feasibility***: whole-cell patch-clamp recordings of D1 and D2 MSNs will be performed in control and 6-OHDA slices from transgenic mice expressing the fluorescent reporter tomato in DA D1 receptor-containing neurons. Patch-clamp recordings in slices and DA lesion by stereotaxic injection of 6-OHDA are perfectly mastered in the lab and D1 tomato transgenic mice are housed in our animal facility. ***Aim 2.*** Long-lasting changes in synaptic efficacy at MSN corticostriatal synapses are thought to be the cellular basis of motor learning and are severely disrupted in PD. In the healthy striatum, ACh released by striatal CINs is a key modulator of corticostriatal synaptic plasticity. In PD, the loss of DA and the consequent perturbation in ACh signaling suggest that changes in these neuromodulators will profoundly impact MSN plasticity. ***Objectives:*** we will study the impact of optogenetic manipulation of CIN activity on the synaptic plasticity of D1 and D2 MSNs in control and 6-OHDA mice. ***Method and feasibility:*** *in vitro* electrophysiology: see aim 1. To simultaneously distinguish D1 and D2 MSNs and manipulate CIN activity, we will use transgenic mice expressing light-activated opsins in CINs and tomato in DA D1 receptor-containing neurons. We have already successfully used this approach to demonstrate the impact of optogenetic inhibition of CINs on D1 and D2 MSN excitability in 6-OHDA mice (paper submitted). ***Expected results:*** This study will fill a major gap in the literature by dissecting the synaptic and intrinsic adaptative changes occurring in D1 and D2 MSNs after long-term DA depletion and their modulation by CINs. ***Relevance to Integrative or Clinical Neuroscience:*** This project has both fundamental and clinically-relevant aspects as it will improve our knowledge of PD pathophysiology and provide unique data concerning the possible development of therapeutic strategies targeting striatal CIN activity in PD.

Research project #5

Title: Dynamic visuomotor integration from visual to motor cortex
Supervisors: Thomas BROCHIER, Bjørg KILAVIK
Laboratory: INT, http://www.int.univ-amu.fr/
Summary:

Motor cortex is considered the final cortical output in a large network binding vision and action. Lately it has become clear that visuomotor processes do not follow a simple serial processing from visual to motor areas. Rather, they occur in parallel in different areas, as evidenced from motor selectivity being observed in visual areas (Mirabella, Bertini, Samengo, Kilavik et al. (2007) Neuron 54:303) and premature visual cue anticipation and processing being observed in motor areas (Confais et al. (2012) J Neurosci 32:15359; Kilavik et al. (2010) J Neurophysiol 104:2338). Anatomical connections possibly underlying this parallel visuomotor processing are largely known. However, while the functional specializations of either sensory or motor areas have been extensively studied in isolation, only few studies have aimed at studying the complex interactions between areas. This prevents understanding the functions of the premature visual processing in motor areas, and the motor selectivity in visual areas.

In this project we aim at describing cortico-cortical oscillatory interactions during dynamic visuomotor behaviour. The student will be involved in recording intra- cortical neuronal activity simultaneously from visual, parietal and motor areas in behaving macaque monkeys. Furthermore, he will contribute to extend this multi-area, yet local intra-cortical description of task-related oscillatory interactions by recording also the electrocorticogram (ECoG) with chronically implanted transcranial micro-wires, covering the entire visuomotor pathway. Analysis of neuronal spiking activity, considered local 'output' and of local field potentials (LFPs) and ECoG, considered 'input', will allow determining task-related functional specializations in different areas. Functional connectivity measures such as linear correlation, phase coherence and Granger causality between spikes and LFPs/ECoG will be used to infer inter-areal coupling between motor, parietal and visual areas coupling during visuomotor behaviours (Brovelli (2012) Comput Math Meth Med 2012: 697610; Brovelli et al. (2004) PNAS 101: 9849). The results will greatly improve our understanding of the specific roles and dynamical interactions between motor, parietal and visual areas during visuomotor behaviour, permitting a better understanding of their complementary activations during cue anticipation and processing through movement monitoring.

Relevance to integrative neuroscience and project feasibility:

This project lies at the interface between behavioural, systems and cognitive neuroscience, linking cortical function and cognition. The approach of recording local intra-cortical and large-scale ECoG signals simultaneously allows bridging between macro-scale studies, currently mainly done with whole-brain imaging methods in humans (EEG/MEG), and micro/meso-scale studies mainly performed by local intra-cortical recordings from one or a few areas in animal models. Macaques are the choice model for better understanding human cortical sensorimotor functions, due to their very similar projection pattern to the spinal cord, and the relative expansion of supragranular cortical layers that dominate cortico-cortical projections, compared to rodents. The larger research program entailing this PhD project is ongoing, and all needed equipment is in place. When the student starts, one monkey will already be involved in intra-cortical recordings and a second monkey undergoing behavioural training. This assures that the student can complete this project within the 3 year PhD period. The CoMCo team has the required expertise for both the experimental and analytical parts of the research and can provide a broader view of the project and results in the context of human and non-human primate research in sensorimotor functions.

Research project #6

Title: Functional role of oligodendrocyte progenitors in physiological conditions and in neurodegenerative diseases

Supervisor: Myriam CAYRE

Laboratory: IBDM, http://www.ibdm.univ-mrs.fr/

Summary:

State of the art: Oligodendrocyte precursor cells (OPCs) generated during development mature into myelinating oligodendrocytes after birth and contribute to form functional white matter tracts underlying the "brain connectome". White matter occupies around 50% of the human brain, a proportion that strongly decreases during aging. Interestingly, after the end of the myelination program, some OPCs are maintained as quiescent progenitors in the adult brain; they are disseminated throughout brain parenchyma where they represent 5-8% of all neural cells. OPCs continue to divide and differentiate at a slow rate to contribute to myelin remodelling during adulthood (Rivers), a process important for motor skill learning (McKenzie et al., , Science 2014). However their physiological functions, notably during aging, is far from being completely understood. After demyelination insult, adult OPCs can be re-activated to produce new oligodendrocytes and regenerate myelin sheaths. Beside, recent studies outlined that white mater lesions are detected in early stages in several neurodegenerative pathologies such as Alzheimer's disease (AD)(for review see Verkhratsky Neuroscientist 2014). Furthermore, in AD patients white matter loss correlates with cognitive impairment. Transgenic mice models of AD allowed to show that myelin alterations even precede Aß plaques and tangles formation (Desai et al., Glia 2009). Thus, although Alzheimer's disease (AD) is commonly regarded as a disease of grey matter, oligodendrocytes and their precursors (OPC) may also contribute to AD pathogenesis.

Objectives and methods: Our goal is double**:** 1- to better characterize OPCs function in physiological conditions and during aging; and 2- to examine the potential role of OPCs in the pathogenesis of AD. We are currently generating a transgenic mouse model ("Olig2-PDGFRa-DTR mice ") which will allow us to specifically kill OPC in a timely controlled manner (i.e. in adult mice). Olig2-PDGFRa-DTR mice will be characterized at the cellular (immunohistology) and behavioural levels to assess sensory-motor and cognitive functions of these animals. In order to determine whether OPC ablation make mice more susceptible to develop AD, we will perform streptozocin ICV injection (a non genetic model of AD, see Chen et al 2013) in our Olig2-PDGFRa-DTR mice 2 months after OPC ablation, and examine the time course of apparition and the severity of AD symptoms and histological hallmarks

Expected results: Why are so many progenitors maintained in the brain when development is over? If they only serve as a pool of progenitors for repair, then their deletion should not trigger any deleterious phenotype. By contrast, if they have additional functions, then these will be revealed after OPC ablation. Beside, this project could unveil new players involved in AD pathogenesis. Indeed, the current research on AD is biased by the neuron-centered approach and the systematic use of mouse models carrying specific familial mutations that only represent 5% of the cases and may not cover the whole spectrum of the disease pathogenesis. This is a break for the discovery of other mechanisms involved in this disease. It is important to search for other potential players and OPCs may be good candidates.

Feasibility: Our laboratory is equipped with all technical platforms necessary for the achievement of this project: molecular biology, imaging facility, animal house with a battery of behavioural tests. We already have obtained recombined ES cells to generate our transgenic mouse line. However, in case it failed to be developed, we could still perform the planned experiments using PDGFRaCre^{ERT2} mice crossed with Rosa-DTR mice (we have both mouse lines in our animal house). The only disadvantage of this approach is that tamoxifen-induced recombination is not 100% efficient, therefore OPC ablation would be incomplete.

Research project #7

Title: Synaptic zinc: role in the pathophysiology of Parkinson's disease
Supervisor: Abdel-Mouttalib Ouagazzal
Laboratory: Laboratoire de Neurosciences Cognitives (UMR7291), http://federation3c.com
Summary:

Parkinson's disease (PD) is the most common neurodegenerative movement disorder in the elderly and, as yet, does not have satisfying treatment. The clinical motor features, tremor, rigidity and bradykinesia, primarily arise from the loss of the dopaminergic nigrostriatal tract and the dramatic reduction of striatal dopamine (DA). PD patients also experience a range of non-motor symptoms including depression, cognitive impairment and autonomic disturbances, which can appear in the early, often premotor, phase of the disease. Up till now, there is a lack of full understanding of the underlying causes and molecular mechanisms leading to PD. PD can be inheritable, but most forms are sporadic and are presumably triggered and promoted by environmental risk factors.

Zinc has long been associated to the pathogenesis of PD because excessive zinc deposits have been found in the substantia nigra and striatum of PD patients. Animal studies showed that cytosolic accumulation of ionic zinc (Zn^{2+}) is a component of the processes leading to dopamine neuron death. While most zinc in the brain is tightly bound to metallobinding proteins, a substantial amount exits in ionic or chelatable form concentrated almost exclusively inside the synaptic vesicles of glutamatergic terminals in the forebrain (olfactory bulb, neocortex, hippocampus, striatum and amygdala). The physiological function of synaptically released zinc is to modulate the activation of several neurotransmitter receptors and voltage-gated ion channels. Accordingly, we recently showed that synaptic Zn^{2+} modulates pain (Nozaki et al., 2011, *Nat Neurosci*.14:1017-22) and emotional processing (Ouagazzal et al. *in preparation*) by dampening GluN2A-NMDAR activity. However, under pathological conditions (excitotoxicity), excess synaptic zinc can also gain entry into postsynaptic neurons through a variety of ion channels and participates to neuronal injury. The aim of this project is to investigate the contribution of synaptic zinc to basal ganglia functions and its role in the pathophysiology of PD. We will use rodent models mimicking early and late stages of PD and a combination of approaches (pharmacological and genetically modified mice) and tools (behavioural, optogenetic, immunohistochemistry, western blot and quantitative real-time PCR techniques) to explore how synaptic zinc contributes to the development of motor and non-motor features. The present project will help to shed new light on the function of synaptic zinc and may open novel avenues for development of new therapies for PD.

This project will be conducted in the team "**Basal Ganglia Cognition and Physiopathology**" led by Dr. M. Amalric. The research team has broad-based expertise that spans neuropharmacology, biochemistry, optogenetic and behavioural genetics. During his (her) PhD work, the student will acquire strong expertise in behavioural analysis (battery of tests for cognitive, emotional and motor functions) and neuropharmacology (e.g., stereotaxic implantation and intracerebral injections). He/she will also acquire biochemical and cellular biology techniques to measure protein kinase activities *in vivo* (western blotting) and map gene and protein expression (quantitative real-time PCR and immunohistochemistry techniques). The student will benefit from a rich scientific and technical environment, in the FR3C Research Federation (FR3C:3512) platforms, that will let him/her to develop his/her researcher potential and communication skills.

Research project #8

Title: Reactive neurogenesis and restoration of balance and posture following vestibular insult
Supervisor: Brahim Tighilet
Laboratory: Laboratoire de Neurosciences Intégratives et Adaptatives UMR 7260, http://federation3c.com
Summary:

Reactive neurogenesis occurs in the vestibular nuclei (VN) of the adult mammal after unilateral vestibular neurectomy (UVN; Tighilet et al. 2007) and contributes to the restoration of balance and posture (Dutheil et al. 2009). Modifications of the deafferented microenvironment (neurotransmitter systems, neurohormones, neurotrophic, angiogenic and inflammatory factors) induced by UVN are believed to regulate the reactive neurogenesis induction. This is supported by data showing that such factors modulate neural stem cell proliferation, survival and their integration in neural networks (Dutheil et al. 2013). The aim of the research project is to study the ability of these factors to regulate the different steps of adult neurogenesis observed in the VN of the adult rat, after UVN. Using histological (immunocytochemistry) and electrophysiological approaches (patch-clamp on VN slices), we will investigate the effects of intracerebral infusion of these factors and their antagonists on the different steps of neurogenesis in brain structures affected by UVN. At the behavioural level, we will determine the impact of such pharmacological treatments on the vestibular functional recovery. Though these combined approaches, we expect identifying the main cellular pathways that control reactive neurogenesis in VN. This is a prerequisite for further developments of pharmacological approaches to stimulate the restoration of balance and posture in patients that undergo unilateral vestibular impairments.

References :

TIGHILET B., BREZUN JM., GUSTAVE DIT DUFLO S., GAUBERT C and LACOUR M. (2007). New neurons in the vestibular nuclei complex after unilateral vestibular neurectomy in the adult cat. **Eur J of Neuroscience** 1:47-58.

DUTHEIL S, BREZUN JM, LEONARD J, LACOUR M, **TIGHILET B** (2009) Neurogenesis and astrogenesis contribution to recovery of vestibular functions in the adult cat following unilateral vestibular neurectomy: cellular and behavioral evidence. **Neuroscience** 164: 1444-56.

DUTHEIL S, WATABE I, ESCOFFIER G, GHARBI A, **TIGHILET B**. (2013). GABAA Receptors agonist and antagonist alter vestibular compensation and the different steps of reactive neurogenesis in the deafferented vestibular nuclei of adult cats. **J Neurosci**. 2013 Sep 25;33(39):15555-66. doi: 10.1523/JNEUROSCI.5691-12.2013.

Research project #9

Title: hiPSC-based transplantation therapy for Parkinson's disease in animal models
Supervisor: Rosanna Dono
Laboratory: IBDM, http://www.ibdm.univ-mrs.fr/
Summary:

State of the art: Recent breakthroughs in stem cell (SC) research have generated tremendous hope for the therapeutic potential of human induced pluripotent SCs (hiPSCs). However, the clinical application of hiPSCs requires the development of strategies that enable to exacerbate their therapeutic potential while minimizing side risks. This project is based on the discovery that downregulation of the morphogen regulator Glypican4 (Gpc4) in mouse embryonic SCs (mESC) and hiPSCs confers to them a unique biological state, defined as a "safe-iPSC state", characterized by: **1)** maintenance of self-renewal/pluripotency in stemness conditions, **2)** oriented/accelerated cell lineage entry in differentiation conditions, **3)** loss of tumorigenicity after xenografts (***Stem Cells 2012***). mESCs in a "safe-iPSC state" rescue motor defects, without causing tumours when transplanted into brains of Parkinson disease (PD) rats (***J. Neuroscience 2014***). Our findings show that $Gpc4^{mut}$ hiPSCs can provide a therapeutic advantage for cell-based therapy in PD.

Objectives & Methodologies: This PhD research proposal is based on three aims:

Aim 1: Intriguing results from our studies indicate that Gpc4 downregulation in hiPSCs a) switches fate of neural cells from dorsal to ventral; b) promotes generation of dopaminergic versus serotonergic neurons. The applicant will study $Gpc4^{mut}$ hiPSC cultures compared to controls to explore: 1) the kinetic of enhanced neuronal differentiation; 2) whether/how Gpc4 modulation can be used to trigger a neuronal cell fate switch. Studies will involve *in vitro* neuronal differentiation of hiPSCs, molecular analysis with qRT-PCR, western blots, and immunocytochemistry.

Aim 2: Following our studies published in ***J. Neuroscience***, the applicant will explore the potential of $Gpc4^{mut}$ hiPSC transplantation as a therapeutic approach for PD. This aim has been designed to provide a framework in which the applicant will use PD rats transplanted with hiPSCs (by Syncrosome company) to acquire knowledge on 1) graft properties overtime, such as re-establishment of dopamine levels, by preclinical imaging using NMR/PET-scan (collaboration with CERIMED, Marseille, within DHU Neurosciences & Aging consortium); 2) monitor functional recovery through complementary behavioural tests (by Syncrosome company in collaboration with L. Kerkerian, IBDM, and within DHU Neurosciences & Aging consortium). The applicant will then complement all these parameters by assessing the acquisition of distinct neuronal identity of grafted cells and by evaluating the tumour side risk through neuroanatomical, biochemical, and molecular analyses in dissected brains.

Aim 3: Ongoing gene expression profile studies on $Gpc4^{mut}$ SCs indicate that threshold levels of distinct molecular components relevant to self-renewal/differentiation and tumorigenicity underlie a "safe-iPSC state". The applicant will determine the functional relevance of candidate genes through qRT-PCR, biochemistry, gain-/loss-of-function approaches combined with differentiation or signalling luciferase reporters.

Expected results & Relevance to either Integrative or Clinical Neuroscience: Outcomes from the proposed PhD project are expected to fill knowledge gaps in hiPSCs biology related to neural/neuronal differentiation and on mechanisms preventing hiPSCs tumour side effects. The acquired knowledge may have a direct impact on strategies to challenge human neurological disorders related to aging and long-term care.

Feasibility: Tools and expertise are well established in the host lab and through the established collaborative setting.

Research project #10

Title: The neural bases of path integration

Supervisor: Etienne SAVE

Laboratory: Laboratory of Cognitive Neuroscience UMR 7291, http://federation3c.com

Summary:

State of the art: Considerable interest in the neural bases of spatial navigation in mammals arose from the discovery of neurons with spatial activity in a number of brain structures. Current theories assume that spatial navigation involves the processing of both environmental information (allothetic) and self-motion cues (idiothetic). In the absence of allothetic cues, mammals can navigate using idiothetic cues, a strategy referred to as path integration (PI). There is evidence that the functional circuit underlying the processing of idiothetic cues and PI involves a large network of cortical and subcortical structures. Although some structures have been identified to be important for PI in the rat (e.g. entorhinal cortex, retrosplenial cortex, anterodorsal thalamus), the extent of the network and the specific function and interaction of the regions involved are poorly known. Interestingly, these structures contain head-direction cells, i.e. neurons that increase their firing when the animal's head is facing a particular direction. Because the head-direction processing pathway is suggested to play a major role in conveying idiothetic information, in particular vestibular, we hypothesize that it may underlie PI and allow combination of idiothetic and allothetic cues for accurate navigation.

Objectives: The overall purpose of the thesis is to improve our knowledge of the functional network involved in the processing of idiothetic cues and PI in the rat. More specifically the objectives are to 1) Identify the structures belonging to the functional network involved in PI, and 2) Determine the specific contribution of these structures to PI. It is hypothesized that the structures involved in PI (with a particular focus on those belonging to the head-direction cell network) will be identified using immediate-early gene (IEG) imaging in rats that perform a PI task. Next, the impact of damaging or inactivating these regions in PI tasks will be examined. Because PI requires the processing of both angular and linear acceleration during movement, two behavioural tasks that tax the two components will be used in both IEG and lesion/inactivation studies.

Methods: The techniques include: 1) IEG imaging. IEGs (e;g. c-fos, Arc,zif268) are markers of cognitive-related neuronal activation that are rapidly induced after behavioural experience. Detection of IEG mRNA or protein will allow us to identify the structures activated during a PI test phase. 2) Lesions and reversible inactivation will be performed using intra-cerebral infusion of NMDA or ibotenic acid (lesion), muscimol, a GABA A receptor agonist or lidocaine (inactivation). 3) Behavioural testing using various tasks taxing PI.

Expected results: Using IEG imaging to identify the structures that are activated during PI has never been performed before. We therefore expect to determine whether the regions within the head-direction cell network are involved in PI. By testing the effect of lesions or inactivation of these structures during different kinds of PI tasks, we expect to elucidate their specific role in PI, i.e. integration of angular vs. linear movements.

Relevance to either Integrative or Clinical Neurosciences: This project is relevant to the field of integrative neuroscience as it aims at understanding how different sources of information are integrated to produce complex behaviour. In addition, although fundamental in nature, the project has clinical relevance as it may help to better understand the path integration deficits and their neural cause in aged patients and patients suffering from vestibular syndrome. It may also contribute to shed some light on the compensatory abilities used by visually deficient persons to navigate in their environment.

Feasibility: The IEG imaging technique is available in the Federation 3C in St Charles and will involve collaboration with an engineer. Lesion and inactivation approaches are routinely performed in the team. All path integration tasks have been used in previous published studies. The project is thus feasible.

Research project #11

Title: Natural scene statistics and visual motion processing in non-human primates
Supervisor: Guillaume MASSON
Laboratory: INT UMR7289 CNRS & AMU, http://www.int.univ-amu.fr/
Summary:

State of the art: The mammalian visual system is extremely well adapted to process natural scenes. Indeed, visual neurons are more accurate and more temporally precise when analysing natural images than the impoverished stimuli such as dots, bars or gratings that have been used for decades. A timely challenge is to understand how information embedded in the complex statistics of natural scenes is extracted to form visual motion or shape perception and to control behavioural responses. This new perspective about sensory processing faces several exciting questions. A theoretical challenge is to understand how cortical networks optimally encodes the dimensions of the statistics that are relevant for a given behaviour. Experimentally, the challenge is to design behavioural tasks and neuronal recording techniques that can probe these mechanisms. We[1], and others[2], have designed novel naturalistic stimuli called dynamical textures that mimic the statistics of natural images. We have recently shown in *Nature Neuroscience*[3] that these naturalistic textures drive with greater accuracy tracking eye movements in humans. This was the first demonstration that natural movies processing can be probed using simple sensorimotor tasks. The next step is to push forward this research in macaque monkeys to link together behavioural and neuronal dynamics. ***Objectives:*** Using behavioural and physiological methods, the objective is to understand in macaque monkeys how visual motion information is encoded from natural scenes. First, we will extend our previous work[3] by studying how the statistics of naturalistic moving textures impact reflexive tracking eye movements. Second, using multi-electrode arrays recordings, we will investigate the neuronal dynamics at single-cell and population levels in response to the same moving textures to understand how natural scene statistics impact neural coding in the macaque primary visual cortex. ***Methods:*** Ocular following responses are reflexive tracking eye movements in both humans and non-human primates. Over the last 15 years we and others demonstrated that they can be used to behaviourally map the properties of low-level motion mechanisms[4]. In macaque monkeys, eye movements will be recorded with the scleral search coil technique. Single-cell and population neuronal responses will be recorded from area V1 using 10x10 electrode arrays that are chronically implanted[5]. ***Expected results:*** Two scientific advancements are expected. First, we aim at better understanding how visual motion information in extracted from natural images in order to control eye movements. Second, we will show how direction and speed of moving textures are encoded at single-cell (spiking activity) and population (LFP, spatio-temporal correlations) levels in area V1 of behaving macaques. These two sets of results will unveil how natural scenes are encoded for controlling the animal's behaviour. ***Relevance to either Integrative or Clinical Neuroscience:*** Elucidating the dynamics of sensory processing for natural scenes and its relevance for behaviour is a hot topic in integrative neuroscience. Training includes human and monkey behavioural psychophysics and population recordings in behaving monkeys, stimulus design and state-of-the-art data neuronal data analysis. This will be highly valuable for a post-doctoral training in both integrative and clinical neurosciences. ***Feasibility:*** INT is the largest institution for monkey brain research in France. The project is supported by the SPEED ANR project (2013-2017). The PhD student will work in close collaboration with a post-doctoral fellow working on human ocular following responses to natural scenes. A monkey set-up is available with state-of-the-art systems for eye movements and multi-electrode arrays recordings in behaving macaques. The InViBe team has strong expertise in stimulus generation and modelling (L. Perrinet) and monkey electrophysiology (F. Chavane) and the doctoral project will be conduced in close collaboration with them.

[1] Sanz-Léon, Vanzetta, **Masson** & Perrinet (2012) *Journal of Neurophysiology* 107 : 3217-3226
[2] Freeman et al. (2013) *Nature Neuroscience*
[3] Simoncini, Perrinet, Montagnini, Mamassian & **Masson** (2012) *Nature Neuroscience* 15: 1596-1603
[4] **Masson** & Perrinet (2012) *Neuroscience and Biobehavioral Reviews* 36 :1-25
[5] Benvenutti, Chemla, Boonman, **Masson** & Chavane (2015) *VisionScience Society Meeting* (in press)

Research project #12

Title: Tackling spasticity by gene therapy
Supervisors: Frédéric BROCARD, Hélène BRAS
Laboratory: INT, UMR 7289, http://www.int.univ-amu.fr/
Summary:

State of the art: Spasticity is one of the most disabling motor deficit affecting more than 12 million people worldwide. Spasticity is commonly caused by stroke, multiple sclerosis, cerebral palsy, cerebral infection and/or cerebrospinal trauma. Spasticity results from excessive motoneuron excitation, leading to involuntary muscle contraction that interferes with movement and locomotion. Clinical manifestations of spasticity are hypertonicity, clonus, muscle spasm and pain for which there is currently no cure. We recently demonstrated that spasticity following a spinal cord injury (SCI) is associated with an overactivity of motoneurons, resulting from an excitatory/inhibitory imbalance. This imbalance results, on one side, from an upregulation of the persistent sodium current (I_{NaP}) mediated by Nav1.6 channels and, on the other side, by a downregulation of KCC2 cotransporters which causes a net disinhibition of motoneurons. **In sum, our studies point out two potential targets, Nav1.6 channels and KCC2 cotransporters, for exploring gene-based treatment of spasticity.**

Methods and Objectives: The PhD student will be responsible for developing a retrograde axonal transport of recombinant adenoviral vectors (AAV) to deliver specific genes to motoneurons after intramuscular injection of the vectors into spastic muscles. There are several components to the project. First we will test whether an AAV carrying the gene for KCC2 would restore the inhibition of motoneurons after SCI. Second, we will investigate whether the post-translational gene silencing of Nav1.6 channels by AAV encoding specific short hairpin RNA (shRNA) prevents the upregulation of I_{NaP} in motoneurons after SCI. Finally, we will determine whether the co-infection of AAV-KCC2 with AAV-shRNA-Nav1.6 alleviate spasticity. The cellular and molecular mechanisms will be investigated at the motoneuronal level by means of intracellular patch clamp recordings *in vitro* and immunohistochemical techniques, while the functional outcomes of spasticity will be quantified by electromyographic exploration *in vivo*.

Expected results: The gene therapy should restore a normal expression level of KCC2 and Nav1.6 channels in sublesional motoneurons and thereby reduce spasticity in SCI animals.

***Relevance to integrative and clinical neuroscience*:** Existing symptomatic therapies of spasticity face a variety of limitations. Although there are a number of oral medications available to treat spasticity, none are effective without serious side effects. Furthermore tolerance to treatments develops in most patients. Thus, spasticity imposes significant burdens on health services and society. In the absence of treatments to reverse the neurological damage and restore motor function, the present project aims at improving the quality of life of patients by reducing spasticity in different disorders with an original, effective, tolerable and minimally invasive treatment designed by gene therapy.

Feasibility: Our laboratory holds cutting edge technical platforms including a biosafety containment level (L2 laboratory) for manipulation of virus to carry out the project. We routinely perform *in vitro* and *in vivo* recordings, molecular biology and confocal microscopy for the analysis of immunolabellings.

Research project #13

Title: Dynamics of Functional Connectivity during epileptogenesis
Supervisors: Christophe BERNARD, Viktor JIRSA
Laboratory: Institut de Neuroscience des Systèmes, http://ins.univ-amu.fr/
Summary:

State of the art: Resting state fMRI (rsfMRI) provides invaluable information regarding brain function and dysfunction (in neurological disorders). One hallmark of brain dynamics is its non-stationary nature. This can be reproduced *in silico* within The Virtual Brain (TVB), a simulation platform located at INS. This platform allows the virtualization of human brains, using tractography information (DTI), and to generate fMRI and electrophysiological activity. We have virtualized the brains of >15 patients with refractory epilepsy in Marseille and demonstrated that the mutual use of personalized connectivity and modelling has predictive value for the seizure propagation. This demonstration provides the neurosurgeons for the first time with quantitative guidelines for the presurgical evaluation of individual patients. One major question is to understand how the anatomical reorganization, which characterizes epilepsies, is causally linked to seizure genesis and propagation. This issue is difficult to assess in patients for two reasons: tractography provides a very incomplete estimate of the real connectome and patients are evaluated after a long history of seizures and heavy treatments.

Objectives: Our objectives are 1) to assess the validity of using DTI versus the "true" connectome to study whole brain dynamics and 2) to study how brain dynamics evolve in time as a function of changes in connectivity, during epileptogenesis, the process transforming a "normal" brain into an "epileptic" one.

Methods: The Allen institute has published the full true connectome of the mouse brain derived from enhanced green fluorescent protein (EGFP)-expressing adeno-associated viral vectors to trace axonal projections. Recently, we virtualized the mouse brain in the TVB based on the true connectome and modelled rsfMRI, with results similar to those found *in vivo* (paper in preparation for Nature Methods).

In order to fulfill the first objective, we will obtain DTI and rsfMRI from a series of control mice, in collaboration with Dr. Angèle Viola (CRMBM) in La Timone. We will use the 7T and 11.7T MRI machines. Each mouse will be virtualized in TVB using its DTI. We will compare the simulations obtained with DTI with those obtained with the true connectome.

In order to fulfill the second objective, each control mouse will receive an injection of pilorcapine to induce status epilepticus, which triggers epileptogenesis. The same acquisition sequences will be obtained at different time points during the progression of epilepsy. Seizures will be generated *in silico* and we will study their underlying properties (genesis and propagation) as a function of the changes occurring at the functional connectivity level.

Expected results: We will provide the first validation of DTI to study whole brain dynamics. This information will constitute a cornerstone in the imaging field, with a direct clinical impact.

We will provide the first longitudinal evaluation of the evolution of epilepsy, using a combined experimental and theoretical approach, leading to key predictions on the mechanisms of seizure genesis and propagation.

Relevance to either Integrative or Clinical Neuroscience: The project is clearly integrative and fully embedded in a clinical question. Its results could directly be transferred to the clinic to improve the interpretation of patient's data. It is multidisciplinary, making use of state-of-the-art basic and theoretical neuroscience, with a translational outcome.

Feasibility: All techniques are mastered on site.

Research project #14

Title: Genetic modelling of FSHD-like neuromuscular FATopathies

Supervisor: Françoise HELMBACHER

Laboratory: IBDM, CNRS UMR 7288, http://www.ibdm.univ-mrs.fr/

Summary:

State of the art: Facioscapulohumeral dystrophy (FSHD) is a hereditary human muscular dystrophy affecting groups of muscles in the face and shoulders. FSHD is caused in most cases by chromosomal abnormalities at 4q35, leading to excess production of a transcription factor, DUX4, thus de-regulating its target genes. However, *DUX4* activation is not sufficient to trigger the symptoms on its own, implying the existence of disease modifiers. My team started working on FSHD after having discovered that 1) disruption of the *Fat1* cadherin gene in mice caused muscular and non-muscular symptoms, resembling those of FSHD, 2) alterations of the *FAT1* locus in humans, located near the FSHD critical region on chromosome 4q35, were associated with FSHD and 3) FAT1 ias a modifier gene in FSHD and a key player of muscular pathologies [Caruso et al., PloS Genetics 2013] and [Puppo et al., Human Mutation, 2015]. *Fat1* ablation in mice causes abnormalities in shape of selective groups of muscles and leads to regionalized muscle wasting at postnatal stages, the map of affected muscles being highly similar to the map of muscles affected in FSHD [Caruso 2013]. The possibility of a link between FAT1 alterations and FSHD was investigated. a) We found a reduced *FAT1* expression levels in fetal FSHD muscles [Caruso 2013] but also in adult FSHD1 and FSHD2 muscles [Mariot et al., Annals of Neurology, in press]. b) We identified human mutations in the *FAT1* locus segregating with FSHD: i) Heterozygous deletions of a putative regulatory enhancer, predicted to cause tissue-specific depletion of *FAT1*, co-segregate with FSHD [Caruso 2013]; ii) heterozygous point mutations, either perturbing splicing of *FAT1*, or leading to deleterious amino-acid changes, were found in FSHD-like patients carrying neither pathogenic 4q35 alterations nor mutations in the other FSHD modifier gene SMCHD1 [Puppo et al., Human Mutation 2015]. Thus, FAT1 is a compelling novel FSHD modifier gene, which partial loss-of-function (tissue-specific or /function-specific) is sufficient to recapitulate FSHD-like symptoms and which deregulation was found to co-occur with FSHD. ***Objectives:*** The proposed PhD project aims to determine how alterations of *FAT1* found in FSHD-like patients contribute to FSHD symptoms, and whether *FAT1* is a relevant therapeutic target. The lab is currently developing preclinical mouse models carrying some of these mutations. One model uses CRISPR/Cas9 technology to reproduce a mutation that perturbs *FAT1* RNA splicing and leads to exon skipping. These mice will be phenotypically compared to the already available constitutive and conditional knockouts for *Fat1*. As such exon-skipping mutations can be corrected with antisense oligonucleotides (AON), which mask target sequences for splicing factors, we will screen for AONs effective at restoring *FAT1* RNA and functions *in vitro* and *in vivo*, and aim to determine their therapeutic benefit to ameliorate muscle regeneration. ***Methods:*** Mice will be produced using CRISPR/Cas9 technology (outsourced). Phenotype characterization will involve mouse genetics and various histology techniques, on embryos or adult mice. AON screening will be done using cell culture assays, in collaboration with M. Bartoli. *In vivo* efficiency will be tested by performing acute muscle lesions in mutant mice to trigger the natural process of regeneration, with or without the therapeutic AONs. ***Expected results:*** Since the selected mutations were found in absence of any other mutations in FSHD-like patients, we expect mice carrying the corresponding mutation to exhibit FSHD-like symptoms, including alterations of muscle shape and muscle wasting in adults, as well as deficient regeneration upon muscle injury. We have already identified therapeutic AONs efficient *in vitro*, and expect them (or variants) to correct the consequences of the mutation *in vitro* and *in vivo* and alleviate the symptoms. ***Relevance to either Integrative or Clinical Neuroscience:*** FSHD is the second most frequent myopathy after Duchenne Muscular Dystrophy and represents a major challenge in terms of clinical management and care. Although clinical expression is variable in severity, 20% of cases are wheel-chair bound. There is so far no cure for this disease. ***Feasibility:*** This project, already based on solid preliminary results, will benefit from the complementary expertise of the Helmbacher lab in mouse genetics and the Bartoli/Levy labs in translational myology, thus guaranteeing high feasibility.

Research project #15

Title: Shaping the axon initial segment
Supervisors: B. DARGENT, C. LETERRIER
Laboratory: CRN2M, UMR7286 CNRS-AMU, http://crn2m.univ-mrs.fr/
Summary:

States of the art: The axonal initial segment (AIS) is a unique domain that plays a central role in the physiology of the neuron, as it orchestrates both electrogenesis and the maintenance of neuronal polarity. The voltage-gated ion channels responsible for generating action potentials are concentrated at the AIS through interactions with the scaffolding protein ankyrin G. The latter also binds to adhesion proteins, and links membrane proteins to actin cytoskeleton through b4 spectrin and to the microtubules through End Binding Proteins EB3 and EB1. The precise location and length of the AIS influence neuronal functions. Recent findings highlighted that the entire AIS is a dynamic structure, adapting its length and its position in response to electrical activity.

Objectives: The mechanisms underlying the activity-induced changes in AIS location remain to be unravelled. It should be emphasized that basic questions related to the morphology of the AIS remain also open. How is defined AIS length? How is defined AIS position along the proximal axon? The overall goal of this proposal is to obtain a comprehensive understanding of the molecular mechanism accounting for AIS positioning along the proximal axon.

Methods: For the accomplishment of the proposed project, a multilevel analysis from nano scale level to brain structure will be developed. A wide range of approaches will be used: protein/protein interactions (proteomic, pull-down, immunoprecipitation, surface plasmonic resonance,), *in vivo* and *in vitro* neuronal transfection/transduction, immunocyto- and histo-chemistry, state-of-the art imaging, including super resolution microscopy and single particle tracking. This will be complemented by the use of conditional mice. The multi-technical expertise of the team members in biochemistry, molecular biology and cellular imaging will guarantee the feasibility of the project and, more importantly, a very nourishing environment for the PhD student. Finally, this project will benefit from collaborations and well-established platforms.

Expected results: This project is based on solid unpublished results. The completion of the project should bring a major breakthrough into the mechanisms governing AIS positioning and shaping with high impacts in the field of the homeostatic regulation of neuronal excitability. It will allow us to reveal novel protein-protein interactions at the AIS, providing important information for mutation screening in human brain, heart and muscle pathologies. It will also enhance the development of potential targets for new pharmacological agents and of conditional mice. This project will be highly interesting to a large community of scientific researchers (e.g. molecular and cellular neuroscience, biology, development, psychiatry...). It will also have a strong impact in the field of the cell biology of the AIS, in which Dargent's team is an active member.

Relevance to either Integrative or Clinical Neuroscience: The development of novel concepts stemming from basic neuroscience research will allow greater development of neuropathological studies, thus allowing for progress of knowledge in the areas of brain function and dysfunction. This proposal should shed new light on the physiology of AIS and nodes of Ranvier (which possess a similar molecular organization) both in the normal situation and in the context of mental disorders such as bipolar disease and autism, demyelinating diseases like multiple sclerosis, and epilepsies.

Feasibility: This original and innovative project is feasible because of our recent, solid and exciting results supporting the achievement of the thesis project. We recently discovered several unidentified molecular components of the AIS involved in AIS positioning in hippocampal neurons. The novel findings further deserve *in vivo* validation using mice models and/or *in vivo* viral transduction, as well as the analysis of the underlying mechanisms at the nano-scale, molecular and brain levels.

Research project #16

Title: Investigation of the human spinal cord pathologies at ultra-high field (7T)
Supervisor: Virginie CALLOT
Laboratory: CRMB, https://crmbm.univ-amu.fr/
Summary:

State of the art:
Spinal cord MRI has been identified as a major tool to characterize spinal pathologies, including Multiple Sclerosis, Amyotrophic Lateral Sclerosis (ASL), Trauma or Cervical Spondylotic Myelopathy (CSM). Conventional anatomic MRI is nonetheless insufficient to fully characterize the pathologies and to predict the evolution of the patients. Numerous studies are thus conducted worldwide in order to develop or evaluate multi-parametric MR techniques and their advantages toward prognosis and better management of the patients (Diffusion Tensor Imaging (DTI), Magnetization Transfer (MT), Spectroscopy (MRS)). The emerging field of ultra-high field MRI (3T<) now opens new perspectives in terms of SC investigations, with new contrasts, higher spatial resolution and spectral enlargement.

Objectives:
Our objectives are to take advantages of the newly installed 7T whole body system at CRMBM-CEMEREM to improve the spinal cord tissue characterization by investigating physiological aspects so far hardly achievable at 1.5 and 3T, notably vascular and metabolic aspects of the pathologies.

Methods:
The vascular aspects will be investigated through "conventional" perfusion and susceptibility-weighted imaging whereas the metabolic aspects will rely on MR 1H spectroscopy and chemical exchange saturation transfer (CEST) techniques.
MR methods will first be developed and optimized on healthy subjects and then applied to the investigation of ALS and CSM patients.

Expected results:
Along with the techniques existing or being already in-development in the lab (including DTI and atlas-based post-processing tools), the developed techniques will offer the possibilities to better understand and describe various aspects of the SC pathologies (and more particularly ALS and myopathy) that are currently not accessible with conventional *in vivo* MR systems. In the long run, this may open new perspectives for patients' management.

Relevance to either Integrative or Clinical Neuroscience:
Although playing a very important role in the degenerative SC tissue process, vascular and metabolic aspects are not well described, nor accessible through conventional MR. Investigations at 7T will thus open new perspectives to understand the SC dysfunctions, which perfectly match with Clinical Neuroscience.
Moreover, one part of the "global" SC project, within which this phD proposition is included, is conducted within the "BSIP" International Associated Lab in "Biomechanics of Spine Injury and Pathologies", thus opening great perspectives for neurobiomechanical developments, relevant for Integrative Neuroscience.

Feasibility:
The CRMBM-CEMEREM has been equipped with a new generation whole-body 7T MR system in 2014. A dedicated 8Tx-8Rx spinal coil RF coil has been designed for the SC project. The SC team is fully integrated within the CNS team and works closely with clinicians from the Timone and North Hospitals. The team has a transversal experience from animal to human studies, from technological developments to pathological applications.
More details related to the SC project can be found here: http://crmbm.univ-amu.fr/CALLOT-Virginie?lang=en

Research project #17

Title: Role of KCC2 in dendritic spine remodelling after temporal lobe epilepsy
Supervisor: Claudio RIVERA
Laboratory: INMED, http://www.inmed.fr/
Summary:

State of the art:

Temporal Lobe Epilepsy (TLE) is a severe intractable form of epilepsy that originates in the limbic structures. Only some aspects of the transformation of a naïve network into an epileptic one are known. As such, the inaugurating status epilepticus generates a cascade of events associated with cell loss, aberrant neurite sprouting and formation of synapses on targets that normally are not innervated. Severe deterioration of cognitive performance is a well-known consequence of epilepsy, and its link with dendritic spines is well documented for other cognitive disorders. However, only a few studies have addressed the pathological changes in morphology and stability of dendritic spines in TLE model. Our on going work has revealed an important role of KCC2 in the mechanisms controlling neuronal circuits in epilepsy and after brain trauma. KCC2 expression is known to be strongly reduced in different pathological conditions including TLE and correlates with decrease of fast inhibition through changes in chloride homeostasis.

Objectives:

According to our recently published data, on the structural role of KCC2 in the formation of spines and synapses during development, we propose that KCC2 could play a critical role in the formation of aberrant synapses in epileptic conditions. Our preliminary data show the dramatic change in the density and morphology of dendritic spines, a few days after status epilepticus, together with steep decrease of KCC2 expression in epileptic hippocampus. The goal of this project is to establish a causal link between KCC2 downregulation, spine malformation and reactive plasticity in epileptic hippocampus.

Methods:

To achieve the goals of the project, we will **combine expertise in KCC2, cellular neurobiology and physiology/epilepsy**. During the last years, we developed a number of tools and concepts, instrumental for analysing spine and synapse formation (Hotulainen et al J. Cell Biol. 2009, Llano et al J. Cell Biol. 2015 and Saarikangas et al Dev. Cell 2015). Importantly, we have been working in the field of chloride homeostasis and related transporters for more than fifteen years. In addition, during recent years, we also developed **a number of novel optogenetic tools to monitor and manipulate KCC2 expression**. In addition, our laboratory has a long record in studying epilepsy and has profound expertise in both *in vitro* and *in vivo* electrophysiology. To study formation of aberrant synapses we will use two models of epilepsy: *in vitro* long-term hippocampal organotypic cultures for proof of concept experiments and *in vivo* dentate gyrus of pilocarpine-treated mice, including transgenic KCC2 floxed mice suitable for light-inducible KCC2 knock-out. Then, we will utilize optogenetic tools to manipulate KCC2 expression in combination with long-term telemetric EEG recordings as well as cognitive behavioural tests in order to establish a causal link between KCC2 levels, reactive plasticity and cognitive performance in hippocampal circuits.

Expected results and Relevance to either Integrative or Clinical Neurosciences:

Severe deterioration of cognitive performance is a well-known consequence of epilepsy. The link between cognitive performance and characteristics of dendritic spines is well documented for other cognitive disorders [33]. However, very few studies have addressed the pathological changes in dendritic spines and mechanisms affecting spine stability in epilepsy models of TLE. The present work will enlighten this unattended area of research. **It has the potential to find novel mechanisms of regulation of aberrant glutamatergic synapses and transmission at an early stage of reactive plasticity**.

Feasibility:

We have a long experience in this research area and we are already working with novel tools in a project that has established momentum.

Research project #18

Title: The mysterious medullo-spinal CSF-contacting neurons: a further step towards understanding their function.

Supervisors: Nicolas WANAVERBECQ, Jérôme TROUSLARD

Laboratory: PPSN - EA 4674

State of the art: The presence of neurons in contact with the cerebrospinal fluid (CSF) had been known for decades but their physiological role(s) still remains elusive. At the level of the medullo-spinal central canal (cc), CSF-contacting neurons (CSF-cNs) represent a unique neuronal population conserved from lower vertebrates up to primates. These neurons are characterized by a typical morphology and selective expression of the polycystic isoform of the "transient receptor potential (TRP)". This channel also known as Polycystin Kidney Disease 2-Like 1 (PKD2L1) is calcium permeable, has a large conductance and its trafficking to the plasma membrane and activity are regulated by protein partners, such as members of the Polycystin 1 family and RACK1, a scaffolding protein. Because, its activity is modulated by various stimuli, notably protons and osmolarity, PKD2L1 was suggested to act as a sensory receptor.

Our laboratory recently demonstrated that medullo-spinal CSF-cNs are GABAergic, receive exclusively GABA- and glycinergic synaptic inputs and exhibit spontaneous unitary PKD2L1 channel activity. This activity is modulated by changes in the composition of extracellular medium with a direct consequence on CSF-cNs excitability. We further indicate that PKD2L1 acts as a spike generator in CSF-cNs and participates in the setting of their excitability. Preliminary results from the laboratory also suggested that axons from spinal CSF-cNs form fibers bundle in the ventro-median region of the spinal cord.

Objectives: The proposed project is relevant to integrative neuroscience but demonstrating the function(s) of these peculiar neurons will have repercussions in the field of clinical neuroscience. To reach this goal, one would first need to pursue the characterization of CSF-cNs properties at the cellular level. Second, a crucial step in understanding their physiological role will be the identification of the network CSF-cNs are inserted in.

Methods: The project relies on the Cre(Lox)-dependent technology and PKD2L1:CRE transgenic mice (hosted in our laboratory) that allows selective manipulation of CSF-cNs. In particular, we will express EGFP or GCamp6 (a Genetically Encoded Calcium Probe) in CSF-cNs by cross-breeding PKD2L1:CRE transgenic mice with LacZ-EGFP reporter or Floxed-GCamP6 mice, respectively. To conduct this study the PhD Student will benefit from a large panel of techniques largely mastered in the laboratory (classical brain slice patch-clamp recordings, calcium imaging, immunohistochemistry and molecular biology).

Using classical biochemical, molecular biology and *in situ* hybridization techniques on primary cultures from dissociated CSF-cNs or spinal cord acute slices, the protein co-assembling with PKD2L1 and the nature of this interaction will be determined. We will then manipulate the expression of PKD2L1 and its identified partners by transfecting CSF-cNs obtained from PKD2L1:GCamP6 animals with a liposome-mediated technique (developed in collaboration with Precision-nanosystems©). Finally, the functional consequences on CSF-cNs physiology of such manipulations will be determined with the whole-cell patch-clamp technic combined with calcium imaging. Finally, PKD2L1 was suggested to be modulated by calcium and by intracellular cascades involving the phospholipase C pathways. This cellular approach will also allow demonstrating the presence and the nature of such mechanisms in CSF-cNs.

In a second axis of the project, we will conduct immunohistochemical studies from whole spinal cord preparations or longitudinal sections to identify the synaptic partners and the axonal projection of CSF-cNs based on the selective EGFP expression. We will subsequently carry out the functional analysis in preparations from PKD2L1:GCamP6 animals and combine calcium imaging s and/or whole-cell patch-clamp recording with extracellular electrical stimulation. Local presynaptic partners will be identified and characterized pharmacologically in spinal cord acute coronal or longitudinal slices. While, the direction of CSF-cNs projections and their direction (*i.e.* ascending or descending) will be determined in spinal cord hemisections using targeted extracellular electrical stimulation.

Expected results: We are confident with the results generated through this study to demonstrate the mechanisms by which PKD2L1 channel activity is regulated and the repercussion on CSF-cNs physiology, excitability and calcium homeostasis. Further, the morphofunctional study will allow us to identify the synaptic connections and projections of CSF-cNs that represent the first crucial step towards the identification and comprehension of the physiological role for CSF-cNs in the spinal cord.

Research project #19

Title: Consequences of *Tshz3* haploinsufficiency in the fear/defense circuitry of a new model of neurodevelopmental disorder

Supervisor: Laurence HAD-AISSOUNI

Laboratory: IBDM UMR7288 CNRS-AMU

Summary:

State of the art. Teashirt3 (Tshz3 in rodents, TSHZ3 in human) is a zinc finger transcription factor found recently in both mouse and human to be important to control the molecular identity of deep layers cortical neurons during embryonic development. While studying the exploratory behavior of *Tshz3*$^{+/lacZ}$ heterozygous (het) mice as compared to wild-type (wt), it was observed that het mice showed more marked escape/avoidance behaviors when confined in lit- or placed on elevated-unfamiliar spaces. Our working hypothesis is that the marked escape/avoidance behaviors of het mice are indicative of higher activity in the fear/defense circuitry of this new mouse model of neurodevelopmental disorder. **Objectives.** The project aims primarily to determine the cellular and molecular consequences of *Tshz3* haploinsufficiency in the fear/defense circuitry in het mice. Preliminary data obtained from X-gal staining of brain slices from het mice suggest that *Tshz3* is expressed both in the amygdala (laterobasal complex and olfactory part) and the dorsal periaqueductal grey (PAG), two important structures in mediating responses to threatening stimuli. It is also expressed in structures projecting to amygdala (cortex, thalamus, olfactory bulbs and tracts), in the hypothalamus (that may be a relay between amygdala and dorsal PAG for fight/flight behavior as well as a control center for physiological responses to threatening stimuli) and finally in different sensory and motor parts of the brain stem and spinal cord. This suggests a critical role of *Tshz3* in the fear/defense circuitry. **Methods.** - We will use functional MRI on adult awake wt and het mice to study the differences in activity in the fear/defense circuitry induced by the fact of being confined in a lit-unfamiliar space (the MRI apparatus). - We will determine if the fear/defense circuitry is overactive from birth in het as compared to wt pups by using the behavioral test of cliff avoidance and measuring overactivity of key structures by analysis of c-fos expression (c-fos is an immediate early gene which expression level has been correlated with neuronal activity).- We will determine change in key structure size and/or connectivity in new born and adult het mice as compared to wt using MRI (anatomic MRI and tractography, respectively) and/or histological techniques. Transgenic mice, such as the Golli-GFP or Fezf2-GFP mice that respectively label L6 or L5 projections neurons as well as amygdala projections during development, will be also used to assess developmental neuronal defects in different mice models of *Tshz3* deletion.- We will iontoporate *Tshz3*, or known target genes or partners, in key structures of het mice or in different mice models of *Tshz3* deletion at post natal stages and evaluate the targeted neurons for changes in c-fos expression, morphology and activity. **Expected results.** In human, *TSHZ3* is part of a transcription module thought to be important for human cortical development as it contains *FEZF2* and *TBR1,* both being associated with autism (Kang et al., 2011; Kwan, 2013) and the latter controlling amygdala connectivity (Huang et al., 2014) and *Fezf2* its size (Hirata-Fukae & Hirata, 2014). The proposed experiments could demonstrate that *Tshz3* is required in the fear/defense circuitry for adaptative plasticity of the amygdala and that absence of a single copy of the gene may lock the fear/defense circuitry in an active mode explaining the marked escape/avoidance behaviors of het mice. **Relevance to either Integrative or Clinical Neurosciences.** The activity of the fear/defense circuitry has not been investigated in this new mouse model of neurodevelopmental disorder. To note, this circuit could be overactive in autistic patients as specific phobia is the main comorbidity in autism, even if patients do not show any of the physiological signs of fear (Settipani et al., 2012). Interestingly, when testing the fear to be enclosed in an lit confined space (cylinder test), het mice showed marked escape behavior (jumps) that was not associated with increased physiological signs of anxiety (urination, defecation) nor decrease with repeated exposure (no habituation) suggesting that these mice could be a proper model to study amygdala dysfunction that may be important for autism as well as phasic fear. This may eventually lead to identification of new therapeutic targets. **Feasibility.** The institute is equipped to perform all the experiments but MRI experiments that will be done on the MRI facility of the Federation 3C with the help of N. Baril (St. Charles Campus, Marseille). We are in contact with D. Jabaudon in Geneva that developed iontoporation at birth to reprogram post-mitotic cortical neurons. His lab will assess the feasibility of amygdala iontoporation.

Research project #20

Title: Synuclein–Microtubule interaction and neurodegenerative diseases
Supervisors: François Devred, Vincent Peyrot
Laboratory: Inserm, CRO2 UMR_S 911, http://www.cro2-timone.fr/
Summary:

State of the art:
Synuclein family consists of small acidic neuronal proteins (α, β, and γ synuclein), which have been found to be aggregated in several neurodegenerative diseases along with tau protein or beta-amyloid. Mutations or changes in synucleins level of expression have been observed in Alzheimer's and Parkinson's diseases as swell as amyotrophic lateral sclerosis. Although the molecular events leading to synuclein aggregation are still unclear, it is known that an insoluble complex of α-synuclein and tubulin (the main protein of microtubular cytoskeleton) can progressively accumulate in neurons, leading to their degeneration. Recently, several members of the synuclein family have been shown to bind to tubulin and stabilize microtubules in axons. This stabilization of intrinsically very dynamic microtubules is necessary to guaranty axon structure and efficient axonal transport. Thus, dysregulation of microtubules is likely to lead to neurodegenerative processes observed in all mentioned above diseases. In spite of the importance of tubulin interaction with synucleins and its potential implication in neurodegenerative diseases, this process is still poorly studied on molecular level.
Objectives: The goal of this project is to characterize the role of synucleins in microtubule regulation, in order to understand their implication in the development and progression of neurodegenerative diseases.***Methods:*** We will study the interaction of different synucleins with tubulin as well as their impact on tubulin polymerization into microtubules. Consequences of mutations and level of expression of these proteins will also be addressed. This study will be conducted at the molecular level both *in vitro* (using purified tubulin and synucleins) and in cells. Tubulin-synucleins binding will be studied by microcalorimetry (isothermal titration calorimetry), analytical ultracentrifugation, circular dichroism as well as common biochemical methods. The influence of synucleins on the dynamics of microtubule network will be analyzed by turbidimetry, electron microscopy and FRET in neuronal cells.
Expected results: By characterizing microtubule regulation by synucleins we will be able to explain the consequences of synuclein mutations and expression level changes found in neurodegenerative pathologies.
Relevance to either Integrative or Clinical Neuroscience: A better knowledge of axon microtubule physiology and their regulation by synucleins will help to find new pathways or strategies to improve neurodegenerative disease therapies.
Feasibility: The approach and all the methods mentioned above have been set up and successfully used over the years in our lab to study other microtubule associated proteins (tau, stathmin, EB1). Our team is known for its expertise on microtubule interacting partners and its ability to characterize interactions at the molecular level, both *in vitro* and in cells. In addition, this project will be conducted in close collaboration with Natalia Ninkina from Cardiff University, who has been studying γ-synuclein in amyotrophic lateral sclerosis mice models.