Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral : Elucidating the role of intrinsic electrical gradient during the differentiation of neural stem cells

Directeur de Thèse porteur du projet (titulaire d’une HDR) :

NOM : MANGIN  Prénom : Jean-Marie
Titre : Chargé de Recherche ou e-mail : jean-marie.mangin@inserm.fr
Adresse professionnelle : Sorbonne Université, Institut de Biologie Paris Seine, Laboratoire (site, adresse, bât., bureau) Neuroscience Paris Seine, batiment B, porte B210, 7-9 Quai Saint Bernard, 75005 Paris

Unité de Recherche : Intitulé : Neuroscience Paris Seine
Code (ex. UMR xxxx) : UMR 8246

Ecole Doctorale de rattachement de l’équipe & d’inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ère inscription et la quotité d’encadrement) :

2 doctorants: Hervé Kalamalikan Arulkandarajah (2016, 100%), Agathe Lafont (2019, 100%)

Co-encadrant :

NOM : SORRE  Prénom : Benoît
Titre : Chargé de Recherche ou HDR e-mail : benoit.sorre@univ-paris-diderot.fr

Unité de Recherche : Intitulé : Laboratoire Physico-Chimie Curie
Code (ex. UMR xxxx) : UMR 168

Ecole Doctorale de rattachement :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ère inscription et la quotité d’encadrement) :

2 doctorants :

Gabriel Thon, 2018 (co-encadrement JM di Meglio, Laboratoire Matieres et Systemes Complexes)

Gael Simon, 2019 (50%, encadrant principal Jerome Collignon, Institut Jacques Monod)

Cotutelle internationale : Non Oui, précisez Pays et Université :

Description du projet de recherche doctoral (en français ou en anglais)
Summary of the proposal: Understanding how neural stem cells (NSC) acquire the molecular and physiological traits necessary for the function of adult neuronal networks is a major goal of developmental neurobiology. Bioelectric signals have emerged as potential cues orchestrating this process. To overcome the difficulties of working in vivo with developing embryos, we will test this hypothesis in spinal organoids derived from pluripotent stem cells. Micropatterning and microfluidic techniques will allow fine tuning of NSC cellular diversity and organization while state-to-the-art electrophysiological and imaging approaches will allow precise measurement and modulation of their electrical activity. By combining physiological, microfluidics and microfabrication approaches, the project will elucidate how bioelectrical gradients participate to the patterning and differentiation program of mouse and human NSCs.

Context of the proposal (* indicates PhD advisors’ relevant publications): In the central nervous system (CNS) of vertebrates, the formation of functional neuronal networks during development depends on the emergence of distinct domains of neural stem cells (NSCs) able to generate different neuronal and glial cell subtypes in a highly specific manner, both temporally and spatially [1,2]. Elucidating how NSC domains emerge and differentiate is one the major goal of developmental neurobiology. From mouse and chick model systems, we have learned that two anti-parallel signalling gradients along the dorso-ventral axis of the embryonic neural tube participate to the elaboration of 11 distinct pools of NSC that will produce each of the neuronal subtype composing the sensorimotor networks of the spinal cord [2,3,4,5*,6,7]. Secreted from the dorsal roof plate, BMPs trigger a dorsal to ventral graded activity of their downstream effectors the Smads [6]. Conversely, Shh emanating from the notochord and the ventral floor plate (FP) induces a ventral to dorsal gradient of Gli transcription factor activity [7]. In addition to these classical cues, recent evidence supports the idea that bioelectric signals act as another morphogenetic forces within NSCs [8,9*]. Indeed, the embryonic spinal cord has long been known to generate spontaneous electrical activity at early development stages [10,11*,12*]. More importantly, the team of Jean-Marie Mangin has recently discovered that the ventral floor plate (FP) has the unexpected ability to generate action potentials and establishes a depolarization gradient in NSC domains that parallels Shh signalling gradient [13*]. FP excitability precedes neuronal excitability and differs from it as it relies on T-type voltage-gated calcium channels instead of sodium channels and propagates through the floor plate and NSC via gap junction instead of chemical synapses. The importance of T-type calcium channel during early neural tube formation is supported by a recent discovery made in tunicates - a proto-vertebrate with a single gene coding for T-type channels in its genome - where its mutation leads to severe neural tube defects [14]. Yet, whether T-type calcium and FP excitability regulates NSC differentiation remains to be investigated. Important roadblocks are (i) the functional redundancy in T-type calcium channels (ii) the non-specific targeting of pharmacological approaches to manipulate T-type calcium channel in vivo. Moreover, acute neural tube and spinal explants used by JM Mangin’s team are not suitable to the long-term and large-scale studies needed
to elucidate the role of electrical gradient in NSC. To overcome these issues, we will take advantage of recent advances made by the team of Benoît Sorre in developing organoids able to recapitulate in vitro spinal cord development [15*,16*,17*]. Indeed, the accessibility and scalability of organoids makes them particularly well suited to quickly implement complex and highly-reproducible manipulations over several days while being amenable to the use of multiple complementary approaches specifically in NSCs (Genetic, optogenetic, pharmacological, electrophysiological). Based on their preliminary data and the complementary expertise acquired by the 2 PhD advisors, the present project will elucidate how bioelectrical gradients participate to the patterning and differentiation program of mouse and human NSCs. By combining approaches from biology (electrophysiology, functional imaging) with material and complex system physics (micro-patterning, microfluidics, modelling), the proposed project fits with the objective of the ISVI initiative (I-Bio) to promote interdisciplinary approaches applied to the study of biological systems.

Experimental plan and Methodology

Aim 1. Floor plate cells and NSCs electrophysiological properties in PSC derived organoids: We will survey the electrical activity of cells in 2D spinal organoids recently developed by Benoît Sorre (“SpineOnChip”) where the diversity and the organization of NSC along the D-V neural tube axis is recapitulated thanks to the combination of micropatterned substrates and graded morphogen gradients established by microfluidic. We will assess levels and distribution of T-type calcium channels in NSCs using in situ hybridization and qRT-PCR and recorded their activity by patch-clamp electrophysiological recordings and functional calcium imaging (developed by Jean-Marie Mangin). The application of the specific T-type calcium channel blocker TTA-P2 will be used to confirm their contribution to NSC electrical activity. The identity of the NSC recorded will be characterized using post-hoc immunohistochemistry or thanks to domain specific fluorescent reporters (developed by Benoît Sorre). All these approaches have already been validated by the two PhD advisors’ teams. This first aim will allow us to determine (i) the minimal neuro-epithelial structure able to generate electrical activity in vitro, (ii) the relationship between electric gradient and the diversity of NSC domains generated, (iii) the evolution of NSC electrical activity over time as neurons appear and become excitable, (iv) the steepness and amplitude of gradient in relation to organoid size, (v) potential differences between mouse and human NSCs. In the unlikely event that our organoid systems do not express T-type calcium channels or do not generate any electrical activity, it will indicate that they need to be modified to fully reproduce their counterparts in vivo. Gain of function experiments described in Aim 2 will help address this issue.

Aim 2. Implication of electrical signals during NSC patterning and differentiation: Here we will test how floor plate and NSC excitability in 2D organoids impacts on the specification of NSC and the generation of spinal neurons using electrophysiological and imaging approaches mastered by the Mangin team [9*,10*,11*,13*]. Gain in excitability will be obtained by depolarizing NSCs using chemical (high potassium/calcium), optogenetic (Channelrhodopsin) or genetic manipulations (T-type calcium overexpression). Conversely, decrease in excitability will be generated using chemical (low potassium/calcium) pharmacological (T-type channel blocker TTA-P2, gap junction blocker), optogenetic (Halorhodopsin) and genetic (potassium Kir2.1 channels) manipulations. Importantly, some of these approaches will allow fine temporal tuning of the levels of depolarization of the cells. The molecular identity of
NSC or neurons will be evaluated by either immunohistochemistry using antibodies against classic markers of NSC lineages and spinal neuron subtypes or using reporters for the activity of enhancers active in specific NSC lineages (developed by Benoît Sorre). The latter will allow the visualization of NSC patterning in real time using time-lapse imaging. We will also pay attention to levels of Shh signalling, as in vivo the gradient of Shh signalling matches that of the electric flux triggered by the FP activity. We will use cells lines carrying a reporter for Gli activity or assess for the levels of expression of Shh downstream targets. Furthermore, we will compare the rate of proliferation, survival and differentiation of the NSC. Taken together these experiments will uncover (i) cellular and molecular mechanisms by which depolarization of NSCs influence their differentiation (ii) their relation to Shh dependent specification of NSC domains.

- Profile of the PhD student: The PhD candidate should have a Master degree including a training in either Developmental Biology, Stem Cell Biology, Neurosciences, Physiology or Physics/Engineering. Double cursus in Biology and Physics are strongly encouraged to apply. Past experience or a strong motivation to learn electrophysiology, image processing and/or microfluidics will also be required.


Merci de nommer votre fichier pdf :
«ACRONYME de l’institut/initiative_2 NOM Porteur Projet_2020 »
à envoyer simultanément par e-mail à l’ED de rattachement et au programme :
cd_instituts_et_initiatives@listes.upmc.fr avant le 30 mars.