Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : Dissecting how excitable ependymal cells participate to locomotor rhythms using an all-optical approach

Directeur de Thèse porteur du projet (titulaire d'une HDR) :
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Unité de Recherche :
Intitulé : Laboratoire Neuroscience Paris Seine
Code : UMR 8246

Equipe de Recherche (au sein de l'unité) :
Intitulé : Development of Spinal Cord Organization

Thématique de recherche : The DSCO team scientific projects are focused along a single unifying theme: Elucidating the mechanism(s) and role(s) of functional interactions between neurons and glial cells during the development of the vertebrate nervous system, using the mouse spinal cord as our main model of study. More specifically our project aims at 1) understanding how electrical signals are generated and patterned by neurons and glial cells during development, 2) determining how electrical activity influence neuronal and glial development and 3) define how neuro-glial interactions participate to generate rhythmic electrical activity. To address these questions, our team has developed state-of-the-art neurophysiological approaches and various transgenic models in order to record (patch-clamp recordings, calcium imaging) and manipulate (pharmacology, optogenetics) the electrical activity of specific sub-populations of neurons and glial cells in the developing mouse spinal cord.

Responsable d'équipe :
NOM : Legendre Prénom : Pascal
NOM : MANGIN Prénom : Jean-Marie

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : ED 158 Cerveau-Cognition-Comportement (ED3C)

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 doctorant SU , 01/10/2019, 100% / 1 Doctorant FRM, 01/10/2016, 100% (soutenance en Avril 2021, décalée pour cause de COVID)

<u>CO-DIRECTION</u> (obligatoire)
Co-Directeur de Thèse (titulaire d'une HDR) :
NOM : Georges Prénom : *DEBRÉGEAS*
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Unité de Recherche :
Intitulé : Laboratoire Jean Perrin
Code: UMR 8237
Equipe de Recherche (au sein de l'unité) **:**
Intitulé : Imagerie calcique et comportement du poisson zèbre

Thématique de recherche : Nous utilisons l'imagerie fonctionnelle et l'analyse comportementales chez les larves de poisson-zèbre pour comprendre les mécanismes neuronaux qui sous-tendent l'intégration sensorimotrice complexe chez les vertébrés. Nous nous intéressons en particulier aux comportements de taxis (phototaxis, chemotaxis, rheotaxis, thermotaxis). Notre approche pluridisciplinaire combine des développements en optique et en microfluidique, avec des méthodes théoriques inspirées de la physique statistique. Le groupe a été en particulier pionnier dans l'utilisation de l'imagerie par feuille de lumière, en régime mono- et biphotonique, pour réaliser l'imagerie du cerveau entier.

Responsable d'équipe :
NOM : *DEBRÉGEAS* Prénom : *Georges*
NOM : *CANDELIER* Prénom : *Raphaël*
Ecole Doctorale de rattachement : ED 564 Physique en Ile-de-France (EDPIF)

Ou si ED non SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 3eme année : 1 (co-encadrement) , 4eme année : 1+ 1 en co-encadrement , 5eme année : 1 (soutenance en décembre, décalée pour cause de COVID)

Cotutelle internationale : ☐ Non ☐ Oui, précisez Pays et Université :

Joindre en annexe un descriptif du PRD avec références au format pdf

(« NOM_2_IPV_2021 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :

AVIS et VALIDATION de l'ECOLE DOCTORALE :

AVIS FAVORABLE

ECOLE DOCTORALE 3C
CERVEAU COGNITION COMPORTEMENT
Sorbonne Université
Université de Paris
PSL
Directeur : Prof. Alain Trembleau

à envoyer simultanément par e-mail à l'ED de rattachement et au programme : interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 février minuit.

Résumé (2 000 caractères maximum) :

From peristaltic crawling in earthworms to bipedal walking in humans, animal locomotion relies on repeated sequences of muscle contractions triggered by a rhythmic central pattern generator (CPG) located in the ventromedial spinal cord. Despite decades of research, the identity of the spinal neurons constituting the CPG remains unclear. In the present project, we will investigate the original hypothesis that the spinal CPG is not exclusively made of neurons but also rely on a unique group of ventromedial ependymal cells recently found by our team to generate non-neuronal action potentials. We will use an all-optical method to both manipulate and visualize electrical activity in ependymal cells and spinal neurons during bouts of fictive locomotion. This project will use transgenic mice to specifically express genetically-encoded optogenetic actuators and calcium sensors in ependymal cells and spinal neurons. this state-of-art neurophysiological approach will be combined to a next-generation functional imaging and optogenetic system developed at Laboratoire Jean Perrin. It will allow us to image large field of mouse spinal cord explants in 3D at a high spatiotemporal resolution using light-sheet microscopy (LSM) while performing controlled optogenetic stimulation using a Digital Micromirror Device (DMD). This will allow us to manipulate and visualize electrical activity with the resolution necessary to resolve the location, cellular identity and dynamic of the pacemaker underlying the spinal CPG. If successful, the proposed project will be a major milestone in understanding the spinal CPG and how it could be manipulated to restore locomotor function in various pathological conditions.

In summary, in the present project, we propose to use a state-of-art all-optical neurophysiological approach to:

Aim 1. Determine whether and how floor-plate derived ependymal cells generate action potentials and rhythmic oscillations during fictive locomotion et perinatal stages.

Aim 2. Investigate whether and how specific activation of floor-plate derived ependymocytes could activate spinal motor neurons triggering muscle contraction.

IPV PhD Project 2021 (MANGIN/DEBREGEAS)

Title: Dissecting how excitable ependymal cells participate to locomotor rhythms using an all-optical approach

Context of the project

Despite decades of scientific research, the cellular nature of the spinal central pattern generator (CPG) controlling rhythmic locomotion in vertebrates has yet to be fully deciphered. The spinal circuitry composing the CPG is thought to be exclusively made by interneurons located in the ventromedial spinal cord (Goulding, 2009). Numerous electrophysiological and genetic ablation studies have provided invaluable information on how specific populations of spinal interneurons and their connections to other spinal interneurons or motor neurons contribute to generate locomotor patterns such as left-right alternation (Goulding, 2009; Kiehn et al., 2010). However, none of the many interneuron subtypes studied until now has been found to possess all the required intrinsic features to act as the rhythm generator of the CPG. To explain this, it has been proposed that the rhythm generator is either a distributed and redundant circuit made of several types of spinal interneurons or is made of a group of pacemaker interneurons that has yet to be discovered (Kiehn et al., 2010). We propose here a radical alternative that has never been considered; namely that the core rhythm generator of the CPG is not only made of neurons but also rely on a uniquely excitable ependymal structure surrounding the central canal of the spinal cord. More specifically, we hypothesize that the CPG pacemaker may correspond to a sub-population of ependymal cells surrounding the central canal and derived from an embryonic structure called the floor plate. Indeed, we recently demonstrated that floor plate cells have the unique ability to generate action potential in a rhythmic manner (Hervé Arulkandarajah et al., 2020). However, in order to dissect the spinal mouse CPG network at a functional level, it is necessary to record and analyze the activity of numerous individual neurons and ependymal cells at a single cell level and over a large area of neural tissue. This technical challenge has recently become tractable thanks to recent advances in large-scale 3D functional Ca^{++} imaging and analytical tools made developed by the group of Georges Debrégeas (Panier et al., 2013; Tubiana et al., 2020).

Although unorthodox, previous work supports the hypothesis that floor-plate derived ependymocytes surrounding the central canal could indeed participate to the adult CPG. First, it has long been thought that the core elements of the CPG are located in the vicinity of the central canal (Kjaerulff et al., 1994). Second, preliminary observations indicate that floor plate activity can be triggered by the same compounds - NMDA and serotonin - known to trigger CPG rhythmic activity and fictive locomotion in spinal cord explants from neonatal mice (Kiehn et al., 2010). Moreover, rhythmic activity of the CPG can still be observed in the presence of tetrodotoxin, a blocker of conventional sodium action potential in neurons (Hochman et al., 1994). We found that – unlike most spinal neurons – embryonic floor plate cells spontaneously generate mixed sodium and calcium action potentials where the calcium component is resistant to tetrodotoxin and rely on the expression of T-type voltage gated calcium channels. Strikingly, T-type calcium channels are already known to play an important role in CPG rhythmic activity (Anderson et al., 2012). More generally, T-type calcium channel have unique activation properties making them particularly suited to generate intrinsic rhythmic oscillations, especially when combined with calcium-activated potassium channels (Cain and Snutch, 2013). Accordingly, T-type calcium channel are found in floor plate cells at perinatal stages (Frischknecht and Randall, 1998) and the calcium activated potassium channels $K_{Ca}3.1$ is highly and specifically express in ependymal cells surrounding the central canal (Lu et al., 2017).

Objectives

Taken together, our discovery that floor plate ependymal cells can generate a rhythmic electrical activity at fetal stages and our current knowledge about the location and properties of the CPG in the perinatal spinal cord support the hypothesis that these ependymal cells could be the missing

component of the locomotor CPG. Therefore, in this interdisciplinary PhD project, we propose to use a state-of-art all-optical neurophysiological approach to:

Aim 1. Determine whether and how floor-plate derived ependymal cells generate action potentials and rhythmic oscillations during fictive locomotion et perinatal stages.

Aim 2. Investigate whether and how specific activation of floor-plate derived ependymocytes could activate spinal motor neurons.

Methodology

All experiments will be performed using whole spinal cord preparations from perinatal mice (Cazalets et al., 2000), a well-established model to study the spinal circuitry generating locomotor rhythms in rodents (Goulding, 2009; Kiehn et al., 2010). We have routinely used this approach in our laboratory to perform optogenetic stimulation together with calcium imaging and electrophysiological recordings of ependymal and other glial cells at fetal stages (Hervé Arulkandarajah et al., 2020; Osterstock et al., 2018), and establish the protocol to use these preparations at perinatal stages. Preparations will be obtained from wild-type mice and transgenic mouse models (Glast-CreER) already set up in our team and allowing the specific expression of the genetically-encoded calcium sensor Gcamp6, the optogenetic activator channelrhodopsin 2 or the optogenetic inhibitor halorhodopsin in ependymal cells derived from the floorplate. Alternatively, we are setting up other mouse model (FoxA2-CreER, HoxA1-Cre, Shh-Cre) able to drive expression of calcium sensor and optogenetic actuator (ChR2 and NpHR) in floorplate-derived ependymal cells. We have also established and validated two approaches to visualize electrical activity in motoneurons (Islet:Cre x Gcamp6f) and in all spinal neurons (Rhod2-AM calcium probes) using calcium imaging.

Together with members of laboratoire Jean Perrin (Georges Debrégeas & Thomas Panier), we have set up and adapted their "Braintegration" system in order to combine electrophysiological recordings, optogenetic stimulation and light-sheet imaging of mouse spinal cord explants. We have acquired preliminary data demonstrating that each of the 3 modules of the system (electrophysiology, imaging, optogenetic) is functional. We are currently finalizing the integration of the different modules (Electric noise reduction, sample recording chamber design, etc).

Aim 1. Determining whether and how floor-plate derived ependymal cells generate action potentials and rhythmic oscillations during fictive locomotion et perinatal stages.

Aim 1a. The PhD student will first validate the experimental approach and model at perinatal stages by combining optogenetic and patch-clamp recordings of ependymal cells with pharmacological manipulation to analyze which voltage-gated channel are functionally expressed in perinatal floor plate cells. The PhD student will determine whether ependymal cells can generate rhythmic sodium and/or calcium action potentials in response to optogenetic stimulation and to compounds known to induce fictive locomotion (Ex: serotonin, NMDA and dopamine). Their ependymal identity will be confirmed by filling the recorded cells with neurobiotin and by performing post-hoc immunostaining for ependymal (Sox 2, Vimentin) and floor-plate markers (FoxA2)(Hervé Arulkandarajah et al., 2020).

Aim 1b. With the group of G. Debrégeas at Laboratoire Jean Perrin (SU/CNRS), The PhD student will use the Braintegration imaging system and deconvolution algorithms they recently designed to perform and analyze 3D light-sheet calcium imaging (Panier et al., 2013; Tubiana et al., 2020) on whole spinal cord preparations from transgenic mice expressing the genetically-encoded calcium sensor in floor-plate derived ependymal cells. Combined with pharmacology, this will allow to visualize where ependymal cells generating action potentials are located in ependymal cells between different segments of the lumbar spinal cord during fictive locomotion. Using pharmacological blockers, the student will also determine whether and where gliotransmitters, electrical synapses, T-type voltage-gated channels and calcium activated potassium channels are involved in ependymal activity. We have previously used this approach at fetal stages to show that calcium spikes propagate through gap junctions (electrical synapses) between floor plate cells along the entire length of the fetal spinal cord (Hervé Arulkandarajah et al., 2020).

Aim 2. Investigating whether and how specific activation of floor-plate derived ependymocytes could activate spinal motor neurons.

The PhD student will use the all-optical Braintegration system to combine large-scale calcium imaging of spinal neurons (Rhod2-AM) with localized optogenetic stimulation or inhibition (Digital Mirror Device) of ependymal cells (Glast-CreER x Floxed-Chr2 or NphR) in order to determine whether ependymal cells drive spinal cord activity during fictive locomotion induced by a classic cocktail of serotonin and NMDA. Using algorithms developed at Laboratoire Jean Perrin, the student will analyze how activity correlate between these different spinal cell population and determine the most likely sequence of cell activation during cycles of fictive locomotion and in response to optogenetic stimulation or silencing of ependymal cells. Using pharmacological blockers, the student will also determine whether and where gliotransmitters, electrical synapses, T-type voltage-gated channels and calcium activated potassium channels affect the activity of these different spinal cell populations during fictive locomotion. This approach has already been validated at fetal stages.

Interdisciplinarity of the proposed project

The present PhD project is an interdisciplinary project at the interface between Biology and Physics. It will investigate at a single-cell resolution the large-scale functional dynamic of the neuro-ependymal spinal network during rhythmic locomotion. J.M. Mangin will provide the expertise and training in spinal and ependymal neurophysiology as well as the transgenic mouse models necessary for the project. G. Debrégeas will provide the expertise and training in large-scale/high resolution functional imaging techniques and optical stimulation approaches (digital mirror device), analytical and deconvolution tools as well the BRAINTEGRATION system they developed to perform all-optical control and monitoring of electrical activity in large sample of nervous tissues.

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