Title of the PhD project:

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

PhD Supervisor

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Title of the team : Development of Spinal Cord Organization
Team leader (*if different*) *:* Pascal Legendre/Jean-Marie Mangin
Doctoral School : ED3C

Overview of the scientific projects of the team

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.

Main publications since January 1er, 2016

1. Rima M, Lattouf Y, Abi Younes M, Bullier E, Legendre P, **Mangin JM**, Hong E (2020). Dynamic regulation of the cholinergic system in the spinal central nervous system. *Scientific Reports* **10**, 15338.

2. Pham C, Moro DH, Mouffle C, Didienne S, Hepp R, Pfrieger FW, **Mangin JM**, Legendre P, Martin C, Luquet S, Cauli B, Li D (2020) Mapping astrocyte activity domains by light sheet imaging and spatio-temporal correlation screening. *Neuroimage* **220**, 117069.

3. Angelim MKSC, Maia LMSS, Mouffle C, Ginhoux F, Low D, Amancio-Dos-Santos A, Makhoul J, Le Corronc H, **Mangin JM**, Legendre P (2018). Embryonic macrophages and microglia ablation alter the development of dorsal root ganglion sensory neurons in mouse embryos. *Glia* **66**, 2470-2486.

4. Boeri J, Le Corronc H, Lejeune FX, Le Bras B, Mouffle C, Angelim MKSC, **Mangin JM**, Branchereau P, Legendre P, Czarnecki A (2018). Persistent Sodium Current Drives Excitability of Immature Renshaw Cells in Early Embryonic Spinal Networks. *Journal of Neuroscience* 38(35):7667-7682

5. Osterstock G, Le Bras B, Arulkandarajah KH, Le Corronc H, Czarnecki A, Mouffle C, Bullier E, Legendre P, **Mangin JM** (2018). Axoglial synapses are formed onto pioneer oligodendrocyte precursor cells at the onset of spinal cord gliogenesis. *Glia* **6**:1678-1694.

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Title of the team : Imagerie calcique et comportement du poisson zèbre
Team leader *(if different) : Georges Debrégeas / Raphaël Candelier*
Doctoral School : EDPIF

Overview of the scientific projects of the team

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.

Main publications since January 1er, 2016

1. S. Karpenko , S. Wolf , J. Lafaye , G. Le Goc , T. Panier , V. Bormuth , R. Candelier , **G. Debrégeas** (2020). From behavior to circuit modeling of light-seeking navigation in zebrafish larvae. *eLife* 2020;9:e52882
2. J. Tubiana, S. Wolf, T. Panier, G. Debrégeas (2020) Blind deconvolution for spike inference from fluorescence recordings. *Journal of Neuroscience Methods*, **342**, 108763

3. G. Migault, T.L. van der Plas, H. Trentesaux, T. Panier, R. Candelier, R. Proville, B. Englitz, **G. Debrégeas**, V. Bormuth (2018). Whole-Brain Calcium Imaging during Physiological Vestibular Stimulation in Larval Zebrafish. *Current Biology* **28**, 1-13.
4. S. Wolf, A. Dubreuil, T. Bertoni, U.L. Böhm, V. Bormuth, R. Candelier, S. Karpenko, D.G.C. Hildebrand, I. H. Bianco , R. Monasson , **G. Debrégeas** (2017). Sensorimotor computation underlying phototaxis in zebrafish. *Nature Communication* **8**, 651.
5. R. Olive , S. Wolf , A. Dubreuil, V. Bormuth, **G. Debregeas** , R. Candelier (2016). Rheotaxis of Larval Zebrafish: Behavioral Study of a Multi-Sensory Process. *Frontiers in System Neuroscience*, **10**, UNSP 14. (2016)

Doctoral Project

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

Abstract :

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. To address this question, we will use an all-optical method allowing us to both manipulate and visualize electrical activity in ependymal cells and spinal neurons during fictive locomotion in spinal cord explants. More specifically, this project will combine transgenic mice allowing the specific expression of optogenetic actuators and calcium sensors in the spinal cord with a next-generation 3D imaging and optogenetic system recently developed with the physicists at Laboratoire Jean Perrin. If successful, the proposed project will be a major milestone in understanding the spinal CPG in mammals and how it could be manipulated to restore locomotor function in various pathological conditions.

Context and objective :

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 (*1*). 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 (*1*, *2*). 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 (*2*). 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 (*3*). 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 (*4*). Second, preliminary observations indicate that embryonic 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 (*2*). Moreover, rhythmic activity of the CPG can still be observed in the presence of tetrodotoxin, a blocker of conventional sodium action potential in neurons (*5*). 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 (*6*). 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 (*7*). Accordingly, T-type calcium channel are found in floor plate-derived cells until at least the perinatal stage (*8*) and the calcium activated potassium channels $K_{Ca}3.1$ is highly and specifically express in ependymal cells surrounding the central canal (*9*). 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 postnatal spinal cord support the hypothesis that these ependymal cells could be the missing component of the locomotor CPG. Therefore, in the present project, we propose to use a state-of-art all-optical neurophysiological approach to investigate this hypothesis by:

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

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

<u>Experimental plan</u>

Experimental model: All experiments will be performed using whole spinal cord preparations from perinatal mice (*10*), a well-established model to study the spinal circuitry generating locomotor rhythms in rodents (*1*, *2*). 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 (*3*, *11*), 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. 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.

Aim 1a. To first validate our optical approach and model at perinatal stages, we will combine 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. We will determine whether they can generate rhythmic sodium and/or calcium action potentials in response 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 markers (Sox 2, Vimentin).

Aim 1b. In collaboration with the group of G. Debrégeas at Laboratoire Jean Perrin (SU/CNRS), we will use the imaging system and deconvolution algorithms they recently designed to perform and analyze 3D light-sheet calcium imaging (*12*, *13*) on whole spinal cord preparations from transgenic mice expressing the genetically-encoded calcium sensor in ependymal cells.

Combined with pharmacology, this will allow us to visualize where the ependymal cells generating action potentials are located and how activity propagates (chemical transmission/gap junctions) between different segments of the spinal cord during fictive locomotion. 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.

Aim 2. We will use the all-optical neurophysiological system developed at LJP to combine calcium imaging of spinal neurons with optogenetic stimulation or inhibition of ependymal cells in order to determine whether ependymal cells drive spinal cord activity during fictive locomotion. Using pharmacology, we will determine whether gliotransmitters and/or electrical synapses are involves in this process. This approach has recently been validated at fetal stages.

1. M. Goulding, Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* **10**, 507–518 (2009).
2. O. Kiehn, K. J. Dougherty, M. Hägglund, L. Borgius, A. Talpalar, C. E. Restrepo, Probing spinal circuits controlling walking in mammals. *Biochem. Biophys. Res. Commun.* **396**, 11–18 (2010).
3. K. Hervé Arulkandarajah, G. Osterstock, A. Lafont, H. Le Corronc, N. Escalas, S. Corsini, B. Le Bras, J. Boeri, A. Czarnecki, C. Mouffle, E. Bullier, E. Hong, C. Soula, P. Legendre, J.-M. Mangin, *bioRxiv*, in press, doi:10.1101/2020.05.23.111955.
4. O. Kjaerulff, I. Barajon, O. Kiehn, Sulphorhodamine-labelled cells in the neonatal rat spinal cord following chemically induced locomotor activity in vitro. *J. Physiol.* **478 (Pt 2)**, 265–73 (1994).
5. S. Hochman, L. M. Jordan, J. F. MacDonald, N-methyl-D-aspartate receptor-mediated voltage oscillations in neurons surrounding the central canal in slices of rat spinal cord. *J. Neurophysiol.* **72**, 565–577 (1994).
6. T. M. Anderson, M. D. Abbinanti, J. H. Peck, M. Gilmour, R. M. Brownstone, M. A. Masino, Low-threshold calcium currents contribute to locomotor-like activity in neonatal mice. *J. Neurophysiol.* **107**, 103–113 (2012).
7. S. M. Cain, T. P. Snutch, T-type calcium channels in burst-firing, network synchrony, and epilepsy. *Biochim. Biophys. Acta - Biomembr.* **1828**, 1572–1578 (2013).
8. F. Frischknecht, A. D. Randall, Voltage- and ligand-gated ion channels in floor plate neuroepithelia of the rat. *Neuroscience*. **85**, 1135–49 (1998).
9. R. Lu, C. Flauaus, L. Kennel, J. Petersen, O. Drees, W. Kallenborn-Gerhardt, P. Ruth, R. Lukowski, A. Schmidtko, KCa3.1 channels modulate the processing of noxious chemical stimuli in mice. *Neuropharmacology*. **125**, 386–395 (2017).
10. J.-R. Cazalets, M. Gardette, G. Hilaire, Locomotor Network Maturation Is Transiently Delayed in the MAOA-Deficient Mouse. *J. Neurophysiol.* **83**, 2468–2470 (2000).
11. G. Osterstock, B. Le Bras, K. H. Arulkandarajah, H. Le Corronc, A. Czarnecki, C. Mouffle, E. Bullier, P. Legendre, J.-M. Mangin, Axoglial synapses are formed onto pioneer oligodendrocyte precursor cells at the onset of spinal cord gliogenesis. *Glia* (2018), doi:10.1002/glia.23331.
12. T. Panier, S. A. Romano, R. Olive, T. Pietri, G. Sumbre, R. Candelier, G. Debrégeas, Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy. *Front. Neural Circuits*. **7**, 65 (2013).
13. J. Tubiana, S. Wolf, T. Panier, G. Debregeas, Blind deconvolution for spike inference from fluorescence recordings. *J. Neurosci. Methods*. **342**, 108763 (2020).

Justification of suitability for *i-Bio*: The present project is an interdisciplinary project at the interface between Biology and Physics bringing together two of the core groups of the I-bio initiative (Débregeas at LJP & Legendre/Mangin at NPS). The project will investigate at a single-cell resolution the large-scale functional dynamic of the neuro-ependymal spinal network during rhythmic locomotion. It will combine state-of-the-art neurophysiological approaches developed by the Legendre/Mangin group of neurobiologists with the new imaging, optical stimulation and data analysis tools developed by the Debrégeas group of physicists. The project will further benefit from the possibility to combine these new optical techniques with electrophysiology in order to further develop collaboration between both teams.

Role of each supervisor / skills provided: J.M. Mangin will provide the expertise in spinal and ependymal neurophysiology as well as the transgenic mouse models necessary for the project. G. Debrégeas will provide the expertise in large-scale/high resolution functional imaging techniques and optical stimulation approaches (digital mirror device), analytical and deconvolution tools as well the system they developed to perform all-optical control and monitoring of electrical activity in large sample of nervous tissues.

Profile of the desired student: The candidate should hold a Master degree or equivalent in Biology or Physics. Additionally, they should have received training in at least two of the following domains: physiology, neuroscience, genetics, optical microscopy and/or image processing/analysis.