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 : Identifying the role of cholinergic signaling in the aversive brain

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

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

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED158-Cerveau, cognition, comportem**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : Dania JUNDI (50%, 2018 - current), Maroun ABI YOUNES (100%, 2019- current)

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Unité de Recherche :
Intitulé : Laboratoire Jean Perrin
Code *(ex. UMR xxxx)* : UMR 8237

Ecole Doctorale de rattachement : **ED564-Physique en IdF** Ou si ED non Alliance 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) : Benjamin Gallois (100%, 2017-current), Guillaume Le Goc (50%, 2017 - current)

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

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en lien avec le projet.
Préciser le profil d'étudiant(e) recherché.

Summary : The main goal of this project is to understand how cholinergic signaling (activation of endogenous receptors for acetylcholine, a major neurotransmitter/ modulator) play a role in regulating the activity and function of neural networks in vivo. The habenulo-interpeduncular nucleus (Hb-IPN) pathway contains a large number of cholinergic neurons and has recently been shown to play a crucial role in regulating aversive emotions including fear, stress and nicotine addiction. This project will focus on understanding how altering cholinergic signaling in the Hb-IPN affects 1) neuronal activity within the Hb-IPN pathway, 2) whole brain activity and 3) how the animal responds to aversive stimuli. We will use genetic tools such as CRISPR-generated mutants, calcium imaging and optogenetics to analyze and manipulate neuronal activity in zebrafish larvae with altered cholinergic signaling. Finally, the response to aversive stimuli will be analyzed to understand how cholinergic signaling in the Hb-IPN pathway regulate behavior.

Working Hypothesis and State of Art : Epidemiological studies on children whose mothers smoked during pregnancy show neurobehavioral defects, such as attention-deficit hyperactivity disorder, learning disorder and increased risk of substance abuse as adults(1). Prenatal nicotine exposure in mice lead to similar behavioral outcomes(2), suggesting that the adverse influence of smoking on fetal development is largely due to nicotine(3), which interferes with the endogenous neurotransmitter, acetylcholine (ACh). However, how central nervous system cholinergic signaling (ACh binding to its receptors including the nicotinic acetylcholine receptors (nAChRs)) participate in neuronal network activity and behavior during development has not been studied in vivo.

This project focuses on a highly conserved, major cholinergic area in vertebrates; the medial habenulo-interpeduncular nucleus (Hb-IPN) pathway. In mammals, the medial Hb (mHb), bilateral clusters of dorsal forebrain neurons extend axon tracts to the interpeduncular nucleus (IPN) in the ventral midbrain. The mHb is composed of two subnuclei containing ACh or substance P-releasing neurons that innervate the IPN(4) . The downstream targets of the IPN include the serotonergic raphe nucleus and dorsal tegmental nucleus(5) (which project to the dopaminergic ventral tegmental area), two crucial areas that determine animal behavior by controlling mood and motivation. Recent studies have identified the Hb-IPN pathway as an important regulator of aversive emotions, such as fear, anxiety or withdrawal symptoms associated with nicotine addiction(6).

In larval zebrafish, the dorsal Hb (the mHb in mammals) exhibit prominent left-right (L-R) differences in gene expression and connectivity(7, 8). However, despite this asymmetry, our and other recent works demonstrate conservation in the neurotransmitter identity and function between zebrafish and mammals (8–11). Nevertheless, the asymmetry of the two Hb-IPN subnuclei (RHb: mostly cholinergic, LHb: mostly non-cholinergic) combined with the advantages of the zebrafish (see below) offer a unique opportunity to understand how cholinergic signaling regulate the activity of the Hb-IPN pathway, whole brain activity, to finally influence the animals' response to aversive stimuli.

Justification of work strategy : Due to advancements in microscopy and genetic tools, neural dynamics can be analyzed from a single cell to the whole larval zebrafish brain expressing GCaMP by functional Ca2+ imaging using light-sheet microscopy (12, 13). This approach will enable the identification of single neuron cellular dynamics in the Hb-IPN pathway as well as functionally connected neural networks throughout the whole brain. Furthermore, technical developments using

CRISPR technology to invalidate specific gene expression and optogenetics to alter neural activity will facilitate manipulation of neurotransmission and neural activity during development to understand how they play a role in network formation. Finally, various behavioral paradigms have been developed in the larvae to dissect distinct behaviors. The larval zebrafish provides the ideal system to take advantage of emerging technological advances for a comprehensive understanding of how neural networks contribute to specific behaviors, ranging from the cellular to neural network levels. The expertise from the Hong team (NPS) in developmental neuroscience, zebrafish genetics and physiology combined with that from the Candelier (LJP) team in statistical physics will allow this strong interdisciplinary project to be achieved in the best possible conditions and perfectly fit the objective of the i-Bio PhD fellowship.

Preliminary data and Experimental approaches : To understand the role of cholinergic signaling on Hb activity, we carried out calcium imaging of spontaneous neuronal activity (SNA; without any external stimuli) on nicotine-treated larvae during early development (Zaupa et al., manuscript in prep.). Interestingly, we found a decrease in the percentage of active neurons, which exhibited fewer number of calcium events (decreased frequency). As the Hb-IPN pathway is activated upon aversive external stimuli, we analyzed the calcium response of Hb terminals at the IPN to consecutive mild electric shocks. While in control animals, the amplitude of the calcium response decreased after the first electric shock, the nicotine-treated larvae did not show this 'habituation' response. Finally, analysis of their locomotive behavior upon dark-to-light changes revealed that nicotine-treated larvae also showed less 'habituation' in their startle response. As nicotine treatment affects all nAChRs in the larva, the Hong team generated CRISPR mutants for the vesicular acetylcholine transporter (vacht-b), which is only expressed in a very few distinct nuclei in the brain including the Hb. Unlike other species, there are two alleles for the vacht gene enabling the precise targeting of the few vacht-b expressing cholinergic neurons only in the brain. Importantly, it is not expressed in motoneurons (where vacht-a is expressed), allowing the analysis of locomotive behavior in the mutants. Preliminary data suggests that these vacht-b mutants show decreased Hb neuron activity and defects in their startle response.

Aim 1. Identifying the role of cholinergic signaling on habenular activity

We will carry out calcium imaging of Hb neurons using spinning disk and light-sheet microscopy in vacht-b mutants and compare their activity with that in wild-type siblings. We will analyze neuronal activity during different developmental stages, 5-, 6- and 7-days post fertilization (dpf). In addition, we will compare the activity of Hb neurons and their terminals at the IPN upon consecutive electric shocks. Both the calcium imaging of Hb neuron soma during spontaneous activity and the terminals upon electric shock on the spinning disk or light-sheet microscopy have already been carried out by previous members of the team. We already have Matlab scripts written to analyze spontaneous and shock-induced activity.

Aim2. Elucidating the role of cholinergic signaling on whole brain activity

In the retina, SNA propagate to activate downstream pathways(14). Therefore, analyzing brain areas that exhibit correlated activity with the Hb during SNA would allow the identification of circuits that are up- or downstream of this pathway. We propose to first identify efferent and afferent targets of the Hb-IPN pathway by analyzing SNA that exhibit correlated neuronal activity with the Hb in zebrafish larval brain using light-sheet microscopy. We will use a transgenic line that co-

express the genetically-encoded calcium indicator, GCaMP6f in all neurons, [Tg(elavl3:GCaMP6f)] and different neurotransmitter populations that are labeled with RFP; such as glutamatergic(15), GABAergic(16), cholinergic neurons, etc. Next, we will compare the activity of the whole brain between vacht-b mutants with their wild-type siblings to elucidate the impact of cholinergic signaling on whole brain activity. Since vacht-b is also expressed in other nuclei in the brain, we will test whether the change in whole brain activity is specific to inhibition of cholinergic signaling in the Hb-IPN pathway. This will be achieved by using optogenetics to activate Hb neurons in a specific pattern to see whether it could partially "rescue" the whole brain activity. Channelrhodopsin2 (ChR2) will be expressed in the Hb-IPN pathway using the Tg(gng8:gal4;UAS:ChR2)(8) line and blue light stimulation will be used to activate ChR2-expressing Hb neurons. The R. Candelier is an expert in whole brain imaging and analyses.

Aim3. Understanding the role of cholinergic signaling on startle responses from external aversive stimuli

We will subject larval or adult vacht-b mutant fish to behavioral paradigms to assay for their response to aversive external stimuli, including electric shock and light-dark changes (17, 18). These assays have already been set up and recordings analyzed in the Hong team using the Zebrabox (Viewpoint Life Sciences). In addition, the Candelier team have developed a setup to record freely moving zebrafish larvae and adults in a quasi-borderless arena, in addition to creating a software to analyze locomotive behavior in large number of animals.

Reference: (1) Bruin et al., Toxicol. Sci. 116, 364–374 (2010); (2) Dwyer et al., Pharmacol. Ther. 122, 125–39 (2009); (3) Wickstrom Curr. Neuropharmacol. 5, 213–22 (2007); (4) Cuello et al., Brain Res. 149, 413–429 (1978); (5) Sutherland. Neurosci. Biobehav. Rev. 6, 1–13 (1982); (6) Viswanath et al., Front. Hum. Neurosci. 7 (2014); (7) Gamse et al., Development. 132, 4869–4881 (2005); (8) Hong et al., PNAS 110, 21171–6 (2013); (9) deCarvalho et al., Genesis. 52, 636–655 (2014); (10) Parker et al., Front. Neural Circuits. 7, 63 (2013); (11) Okamoto et al., Dev. Neurobiol. 72, 386–394 (2012); (12) Panier et al., Front. Neural Circuits. 7, 65 (2013); (13) Ahrens et al., Nat. Methods. 10, 413–20 (2013); (14) Ackman and Crair Curr. Opin. Neurobiol. 24, 166–75 (2014); (15) Miyasaka et al., J. Neurosci. 29, 4756–4767 (2009); (16) Satou et al., Development. 140, 3927–31 (2013); (17) Blaser et al., Behav. Brain Res. 208, 56–62 (2010); (18) Stewart et al., Rev. Neurosci. 22, 95–105 (2011)

Publications relevant to project : Left Habenular Activity Attenuates Fear Responses in Larval Zebrafish. Duboué ER, Hong E, Eldred KC, Halpern ME. Curr Biol. 27(14):2154-2162 (2017) ; Cholinergic left-right asymmetry in the habenulo-interpeduncular pathway. Hong E, Santhakumar K, Akitake CA, Ahn SJ, Thisse C, Thisse B, Wyart C, Mangin JM, Halpern ME. PNAS. 110, 21171–6 (2013) ; From behavior to circuit modeling of light-seeking navigation in zebrafish larvae. Karpenko S, Wolf S, Lafaye J, Le Goc G, Panier T, Bormuth V, Candelier R, Debrégeas G. Elife. 9:e52882 (2020) ; Whole-brain functional imaging with two-photon light-sheet microscopy. Wolf S, Supatto W, Debrégeas G, Mahou P, Kruglik SG, Sintes JM, Beaurepaire E, Candelier R. Nat Methods. 12(5):379-80 (2015)

PhD candidate profile: The candidate should have a background in developmental neuroscience with experience using confocal or spinning disk microscopy. Previous experience with zebrafish genetics with some background in Matlab coding is preferable. The candidate should have strong work ethics and willingness to explore new techniques.

Merci de nommer votre fichier pdf :
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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.