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 : Serotonin in the postnatal instruction of microglia and its functional consequences.

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

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Unité de Recherche :
Intitulé : Institut du Fer à Moulin
Code *(ex. UMR xxxx)* : UMR-S 1270

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED394-Physiologie,Physiopathologie The**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 coencadrement à 20%, 1ère année d'inscription 2018

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Unité de Recherche :
Intitulé : Equipe "Learning, Fuzzy and Intelligent systems", département DAPA (Données et APprentissage Artificiel), Laboratoire d'Informatique de Paris 6
Code *(ex. UMR xxxx)* : LIP6

Ecole Doctorale de rattachement : **ED130-EDITE**
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) : 3 : 1 direction thèse cifre, quotité 50%, 1ère année d'inscription 2018; 1 coencadrement thèse cifre, quotité 50%, 1ère année d'inscription 2019 ; 1 codirection, quotité 50%, 1ère année d'inscription 2019

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.

Serotonin in the postnatal instruction of microglia and its functional consequences.

Scientific context

In recent years, it appeared that cells of the innate immune system, like macrophages, –and not only lymphocytes–, are capable of memory. It means that a first immune stimulus can elicit reprogramming of these cells, or "priming", such that even after months, the same cells show enhanced or reduced responsiveness to a second stimulus. Microglia, the brain resident macrophages, are important players in the elaboration of neuronal circuits, through regulation of neuronal migration, pruning or induction of synapses. These highly plastic cells are also known to be primed by various insults (inflammation, chemicals, mutations), resulting in amplified or reduced responses to a second stimulus.

Epigenetic changes,i.e., alterations that affect gene activity without altering the nucleotide sequence of DNA, are essential determinants of the identity and phenotype of a cell. Such changes can include modifications of the DNA itself (such as methylation), and changes in DNA packaging proteins (i.e., histones). With the description of immune priming in microglia, it became apparent that microglia may be programmed or reprogrammed by various events through epigenetic modifications. Given the key role of microglia throughout life, priming of microglia could impact on hallmarks of neurological disease and sensitize to mental disorders. It is thus important to understand the mechanisms by which events occurring during a defined period can shape the phenotype of microglia and have an enduring impact on their function and reactivity.

Furthermore, microglia can respond to neuromodulators like acetylcholine, noradrenaline and serotonin (5-HT) (Albertini et al, 2020, Neurosc Letters, in press). Neuromodulators are released by specific neurons with broad projections that could allow to control microglia disseminated in the parenchyma. Among neuromodulators, serotonin is particularly interesting, as we have shown that its local application on acute mouse brain slices induces a rapid extension of microglial processes. This effect is mediated by the microglial 5-HT2B receptors, the main receptor for serotonin in microglia (Kolodziejczak et al., 2015, ACS Chem Neurosci 6 1219-1230, Etienne et al., 2019, JoVE 143 e58788).

Serotonin, in addition of its role as neuromodulator, is known as a developmental factor for several brain structures, and modifying its level in the neonatal period induces long term effects on brain wiring and behavior. For example, neonatal exposure to selective serotonin reuptake inhibitors (SSRI), which increases serotonin level, favors anxiety in adulthood, while early tryptophan depletion decreases social interactions.

Here, we propose that serotonin also regulates the maturation of microglia during a critical period of the neonatal life.

Justification of the scientific approach and objectives of the project

The critical role of serotonin in the maturation of sensory and emotional circuits (P2-P12) overlaps in part with synaptogenesis. Noteworthy, transcriptomic analyses indicate that this neonatal period is also critical for microglia development. Indeed, they undergo several stages of maturation, with differences in particular between the neonatal (<P10) and the adult (>P30) periods. Importantly we found that two phases could be also distinguished for the expression of 5-HT2B receptor in microglia: an early phase from P1-P8 with a low 5-HT2BR level and a later phase from P15-P30 with a two-fold higher expression.

In an submitted study (Béchade et al), we showed that the absence of Htr2b, the 5-HT2B receptor gene, is associated to enhanced weight loss, increased microglial activation and prolonged neuroinflammation upon a peripheral immune challenge. The use of conditional knock-out mice allowed us to precisely point out that the lack of Htr2b specifically in microglia at the neonatal period, and not during adulthood, is responsible for these effects. In addition, our preliminary results show that the early invalidation (at birth), only in microglia, of 5-HT2B receptors, causes a deficit of social interactions and of cognitive flexibility. Interestingly, we observe none of the mentioned phenotype when invalidation is performed at adult stage. Altogether, this indicates that a lack of serotonin receptor in microglia during a critical neonatal period induces an abnormal priming of these cells. This has also many consequences in adulthood, including an exacerbated responsiveness to peripheral inflammatory stimuli. Noteworthy, preliminary analyses revealed few basal differences in gene expression in microglia in basal condition. It is thus likely that the neonatal activation of 5-HT2B receptor rather impacts microglia on the long-term through epigenetic marks, a mode of regulation already implicated in the control of microglia reactivity.

Our preliminary results suggest that early changes in serotonin levels may alter microglia development and impact on later response to peripheral inflammation. We propose to test the hypothesis that the action of serotonin on microglia during a critical neonatal period is necessary for microglia instruction through epigenetic modifications. Alterations of this instruction would thus prime or misprogram microglia, and thereby compromise normal brain development and function.

Project description

Invalidation of Htr2b in microglia has a different impact depending on the developmental stage indicating that the 5-HT2B receptor has different roles in the neonatal period and in adulthood. We hypothesize that 5-HT2BR stimulation or absence during the neonatal period impacts on the epigenetic landscape of microglia. This project will thus aim to document how serotonin instructs microglia during the neonatal period, from its mode of action to the consequences of its disruption on microglial fate and on the functional response to a peripheral immune challenge.

Due to limited amount of material, classical epigenetic studies are difficult to performed on native cells. However, the recently developed technique of Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq), is a fast and sensitive alternative method for assaying nucleosome positions in accessible regions of the genome, and thus mapping chromatin accessibility genome-wide. This technique is compatible with cell sorting and therefore is a

robust and sensitive method for epigenomic profiling that can provide a multidimensional portrait of gene regulation in a small population of purified cells such as microglia.

In order to decipher the control exerted by serotonin on neonatal microglia, we propose to compare three groups of animals, all wild-type but exposed to different treatments, daily from P2 (postnatal day 2) to P14. In the control group, 1, the pups will receive a saline solution. In group 2, the pups will be treated with the SSRI (selective serotonin reuptake inhibitor) fluoxetine, to mimic an excess of 5-HT. In group 3, the pups will be treated with RS127445 a 5-HT2B receptor antagonists, to mimic a lack of 5-HT stimulation.

At P15, cortical microglia of these mice will be purified and analyzed in parallel by RNASeq and ATACSeq, to gain information on RNA expression and accessible chromatin, respectively. We will focus on cortex in order to limit regional variability meanwhile purifying a number of microglia sufficient to combine both analyses for each individual. At this stage, the collaboration with the informatician will be essential to conduct a fine analysis of the three groups and two kinds of analyses. Based on proper algorithms for feature selection from these multivariate quantitative data, we expect to identify signatures, at the chromatin accessibility and at the transcriptional level, specific of the effect of a lack or excess of serotonin to microglia signal.

In parallel, we will test if these treatments led to a functional imprinting of microglia. To this aim, other mice having received the same three treatments will be tested in adulthood (6 weeks) for their responsiveness to a peripheral immune challenge. Our preliminary results showed that genetic-based elimination of 5-HT2BR in microglia enhanced the behavioral and neuroinflammatory response to a peripheral injection of LPS, a bacterial wall component. We will thus compare the response to LPS or polyIC (to mimick a viral infection) in the three groups of mice, in terms of weight loss, change of microglia morphology and regulation of specific brain inflammatory genes. We expect to see an exacerbated response in mice treated neonatally with the 5HT2B antagonist (group 2), and maybe a decreased response in mice treated with SSRI (group 3). Note that an increase could also happen in group 3, as 5-HT dose-dependent effects often follow a U shape.

Beside this model of inflammation, activation of microglia is observed in neurodevelopmental, neuropsychiatric, and neurodegenerative diseases. The results should thus open perspectives on whether control of microglia by serotonin during the neonatal period could prevent some psychiatric disorders or modify the progression of neurodegenerative diseases.

Suitability for the initiative; Role of each supervisor and skills; Required profile

This project is transdisciplinary. On one hand, the PhD student will be trained in biology at the IFM (note that for ATACseq experiments, we will be in contact with Dr Marion-Poll, post-doc in the team of Edith Heard in EMBL Heidelberg). On the other hand, for the analysis of the ATACSeq and RNASeq data, he/she will be supervised by Dr Lesot from the LIP6 (Informatics). Indeed, it is obvious that to avoid pitfalls and to extract the most meaningful information of "big data", it's necessary to collaborate with experts. Dr Lesot is a specialist in analysis and clustering of quantitative data (list of her publications: https://webia.lip6.fr/~lesot/publications.html). With the PhD student, they will thus perform cutting-edge analyses, testing different algorithms, including machine

learning, to compare the samples and identify critical variables ("feature selection") in each experimental condition. Importantly, we may also work in companionship with the ARTBio platform of the IBPS, which can provide advice on programs available for biological data analysis.

In summary, the candidate will perform the biological experiments AND the analysis of RNASeq and ATACSeq results. Thus, although this last part will be done under an expert supervision, a real motivation both for biology and bioinformatics is required.

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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.