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Title of the research project :

Role of serotonin in postnatal microglial maturation

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Code *(ex. UMR xxxx)* :

U1270

Doctorate School

Thesis supervisor's doctorate school (candidate's futur doctoral school) : ED 394

PhD student currently supervised by the thesis supervisor (number, year of the first inscription) :

0

PhD project: Role of serotonin in microglial maturation

Scientific context:

Microglia, the resident macrophages of the central nervous system, play a crucial role in the brain throughout life: during development, they are involved in modulating neuronal migration, synapse formation and elimination, and later, in maintaining CNS homeostasis (Tay et al., 2017). The morphology and phagocytic capacity of microglia change during development, with an increase in the length and complexity of their ramified processes, and a decrease of phagocytic markers. Importantly, recent transcriptomic analyzes allowed to define a step-wise maturation, with at least three successive stages: "early microglia" (until embryonic day 14 (E14)), "pre-microglia" (from E14 to postnatal day 9 (P9)) and "adult microglia" (> 8 weeks) (Matcovitch-Natan et al., 2016). Unfortunately, microglia between P9 and 8 weeks were not analyzed in this study, although it as a period of intense synaptic remodeling necessary to achieve proper brain wiring. Each of these stages is characterized by a specific gene expression program. The changes in gene expression during the maturation of microglia probably explain that the functional state of these cells varies according to the stages of cerebral development, passing from a proliferative phase to the regulation of neuronal circuits formation and then to immune surveillance. Intriguingly, genetic or environmental disturbances during microglia development can lead to impaired "phase-specific" functions. For example, in young mice having undergone maternal immune activation, microglia that should have corresponded to the pre-microglia stage are transcriptionally shifted to a more advanced stage of maturation (Matcovitch-Natan et al., 2016). Such a decorrelation between the development of microglia and that of the brain probably contributes to increase the risk of neurodevelopmental disorders. However, little is known to date about the factors regulating the pace of microglial maturation and in particular, there are few studies investigating the maturation of microglia in the neonatal period.

The laboratory hypothesized that the serotonergic system would help regulate the transition between the pre-microglia and adult microglia phases of maturation. Indeed, serotonin (5-HT) is known to be a regulator of embryonic and postnatal development: in rodents, an imbalance in the level of 5-HT during the perinatal period leads to wiring defects and behavioral disorders such as sociability defects and increased anxiety (Tanaka et al., 2018). In addition, the laboratory has shown that a local application of 5-HT on mouse brain slices induces an oriented extension of microglial processes towards the source of 5-HT, in particular during the early postnatal period, and that this effect is mediated by the 5-HT2B receptor (R-5-HT2B), which is the main 5-HT receptor expressed in microglia (Kolodziejczak et al., 2015). In another study, the laboratory looked at the effect of disabling *Htr2b* gene (encoding R-5-HT2B) from birth and only in microglia, on the inflammatory response. This study showed that this invalidation was sufficient to lead to prolonged neuroinflammation in mice after a peripheral immune challenge, compared to non-invalidated mice. Surprisingly, this prolonged neuroinflammation was not found in mice disabled for R-5-HT2B in microglia only since P30 (Béchade et al., 2020). Thus, all of these results suggest that serotonin could play a determining role in the maturation of microglia during a critical period comprised between birth and P30.

Objectives:

The global aim of this project is to define how the maturation of microglia is regulated by serotonin. To do so, we will compare the effects on microglia maturation of increasing (Part I) or inhibiting (Part II) serotonin to microglia signaling in the early postnatal

period. The effects on microglia will be assessed at different levels: gene expression and epigenetic modification (RNA Seq, ChIP Seq), morphology and structural plasticity (confocal microscopy, live 2-photon imaging). Lastly (part III), we will study the impact of a lack of serotonin on microglia signaling on microglial sub-populations and other cell types (single-cell RNA Seq). The project is summarized in Figure 1 below.

Methodology:

Part I: Impact of an excess of postnatal serotonin on microglial transcriptome and epigenome

Increasing serotonergic signaling will be achieved through a pharmacological approach: wild-type pups that will be treated from P2 to P14 with a saline solution or with Fluoxetine, a serotonin specific reuptake inhibitor (SSRI, used as antidepressants in humans) which increases extracellular 5-HT in the brain.

To determine the impact of this chronically increased 5-HT level on microglia maturation, we will first assess at P15 their morphology and motility, which are both crucial for microglia functions at this age, and can reveal general alterations. This will be carried out by confocal and 2 photon live imaging, respectively. Then, to gain insight into the molecular pathways altered, microglia will be purified from the brain of individual mice of both experimental groups. Cells will be processed in parallel for RNA extraction and chromatin immunoprecipitation for specific histone modifications. In collaboration with a local bioinformatics platform (https://www.artbio.fr/), we will perform a differential gene expression analysis to spot genes, gene networks and gene ontology most affected by serotonin changes. Then, integration of ChIP with RNASeq data will provide a multidimensional portrait of gene regulation. Candidate genes and pathways will be confirmed by additional approaches depending on the tools available (e.g.: antibodies, functional tests).

Part II: Impact of a lack of postnatal serotonin to microglia signaling on microglial transcriptome and epigenome

Inhibition of serotonin to microglia signaling will be achieved with a genetic approach. Indeed, we will compare, following the same workflow as in Part I, microglia from mice with the invalidation of the *Htr2b* gene in microglia only, since birth (*Htr2b* cKO$^{\mu g\text{-}birth}$ *: Cx3cr1*$^{creERT2/+}$; *Htr2b*$^{fl/fl}$ mice treated with tamoxifen at birth), with microglia from the control genotype (cWT: *Cx3cr1*$^{creERT2/+}$; *Htr2b*$^{+/+}$).

Integration of the results from the pharmacological model, which enhances serotonin signaling, and of the results from the genetic model, which decreases it in microglia, will strengthen the validity of our 5-HT-dependent candidate genes and pathways.

Part III: Impact of a lack of postnatal serotonin to microglia signaling on microglial sub-populations and other cell types

Recent studies have unraveled that different microglial sub-populations, characterized by specific transcriptional markers and functions, can be distinguished along development and brain regions. Thus, only a subpopulation of microglia may be affected by challenging serotonin signaling. Moreover, preliminary data of the lab show that neuronal properties are altered in the *Htr2b* cKO$^{\mu g\text{-}birth}$ mice at P15. Therefore, our interest will be focused on exploring, through single-cell RNA sequencing, the changes that occur in the brain of *Htr2b* cKO$^{\mu g\text{-}birth}$ mice at P15, in the microglial sub-populations and other cell types. The scRNA library will be prepared using the Chromium 10X platform. Then, the processed data will be clusteried to identify sub-populations. To visualize them we will use the Uniform Manifold

Approximation and Projection (UMAP) while their cell identity will be defined by detecting known cell-specific markers. At this level, it will be possible to spot the different microglial sub-population and compare the changes in their features and their abundance, also carrying out the single-cell trajectory analysis of the microglial sub-populations to study their differentiation.

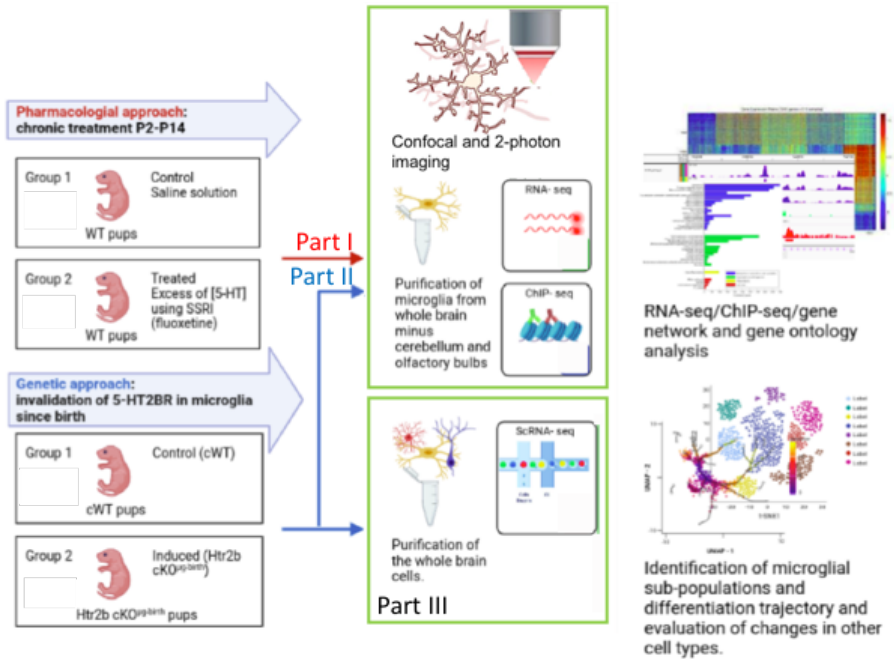

Figure 1: Different approaches (part I, II, III) will allow us to address the effects of 5-HT on microglia postnatal maturation.

Expected results:

This set of experiments will allow to determine the microglial genes, pathways and functions which are regulated by serotonin during development. Underneath is the hypothesis that mutations or perinatal exposure to treatments or toxics affecting the serotonergic system can perturb microglial development and indirectly contribute to other cell types impairment and the emergence of behavioral alterations. Alternatively, this may also open new opportunities for pharmacological intervention to prevent or revert neurodevelopmental disorders.

Candidate's profile:

interest for neuroimmunology and experiments with mice, skills or interest for bioinformatics and/or image analysis.

References (articles from the lab are highlighted)

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