Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2019-2020

1. Host team :

Research Unit (e.g. Department or Institute) : ICM
Research Unit Director : Alexis Brice
Research Team Director : Catherine Lubetzki and Bruno Stankoff
Team name : La remyélinisation dans la sclérose en plaques: de la biologie à la translation clinique

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2. Internship project title:

Determination of (de)myelination of neuronal subtypes in multiple sclerosis hippocampi

3. Internship Description :

Multiple sclerosis (MS) is a disseminated, chronic, inflammatory demyelinating disease, with a variety of neurological symptoms. In addition, cognitive deficit occurs in up to 70% of cases, beginning in early phase of the disease. Several studies indicate that hippocampal demyelinated lesions are frequent and extensive in MS, suggesting that hippocampus take part in cognitive dysfunction in MS patients.
Neuronal circuits comprise two major neuronal classes : excitatory pyramidal cells and inhibitory interneurons, which release the neurotransmitters glutamate and GABA, respectively. Myelination of axons ensures fast propagation of action potentials, optimizes information processing by adjusting the timing of impulse processing and decreases metabolic cost. Recent works highlighted that both excitatory and inhibitory neurons are myelinated in the cortex and hippocampus of rodents and humans (Stedehouder et al., 2017). However, excitatory and inhibitory myelinated axons have distinct molecular and structural organization that may underlie differences in their vulnerability to injuries (Micheva et al., 2018).
Our recent work on myelination mechanisms and node of Ranvier assembly has revealed a particular mode of **node of Ranvier formation prior to myelination** (or "prenodes") in GABAergic hippocampal neurons, induced by oligodendroglial secreted factors and correlated with increased axonal conduction velocity (Freeman et al., 2015; Dubessy et al., under review).

This project aims at investigating **hippocampal neuron dysfunction in MS** and will address (1) what are the demyelinated neurons in MS hippocampus, among the subpopulations of excitatory neurons and/or GABAergic neurons (Parv, SST); (2) Is there a selective vulnerability of different subpopulations of hippocampal neurons (3) Is there a regional vulnerability in the hippocampus.
Human brain (CRB Neuro-CEB, Pr Duyckaerts and Seilhean, Hôpital Pitié-Sapétrière) from MS patients and controls will be used. The hippocampus is a well-studied brain region with important sub-regional

specialization (i.e., dentate gyrus, cornu ammonis (CA) CA1, CA2 and CA3). Our study will be focusing on demyelinated lesions and myelinated area in different identified sub-regions of the hippocampus.
Hippocampal sections (30µm) will be cut and characterized for (de)myelination by immunostaining using proteolipid protein (PLP). This will be followed by collection of subsequent sections to carry out further immunohistological analyses. The student will associate myelin staining with either pan-neuronal marker (Smi32) or specific markers for GABAergic neurons (i.e. GAD staining) and GABAergic subtypes (i.e. Parv and SST). Moreover, he (she) will examine neuroaxonal damages (i.e. neuronal loss, neurite transection and synapse loss). He (she) will determine the inflammatory status of the lesions (recruitment of macrophages with CD68 staining, and activation with HLA-DR staining) and measure the density of microglia/macrophages, oligodendrocytes, astrocytes and neurons with immunostainings for Iba1, CC1, GFAP and Smi32, respectively.
Array-scan acquisition with 20x or 40x objectives will be done associated to automated counting of immuno-stained sections. Confocal microscopy using 63x objective will be done for more detailed analysis when necessary.

These analyses will allow to specify whether there is a preferential demyelination of pyramidal excitatory axons or inhibitory interneurons in the hippocampus, and whether different sub-regions are preferentially targeted. In addition, they will provide information about inflammatory status and glial or neuronal loss or enrichment in MS demyelinated lesions compare to MS non-demyelinated area and control.

This will be the **first step of a single nucleus RNA-seq based approach** to reveal the gene expression profile of neuron subpopulations in the hippocampus of control and MS patients. The student will also participate to the single nucleus RNA-seq analysis. We expect to simultaneously clarifies complementary aspects of molecular, cellular and circuit properties of neurons, and their alteration in MS hippocampus.

Bibliography

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