Neuroinflammatory processes induced during EAE also affect the hippocampus and its associated cognitive processes

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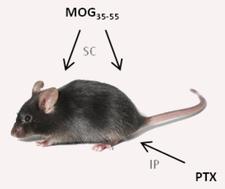
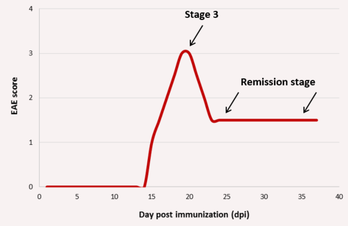
Introduction

Scientific advances have clearly showed the important role of the immune system and glia on many neuronal functions like synaptic plasticity and cognition. In the healthy brain, a complex neuroimmune crosstalk takes place between neurons, glia and infiltrating immune cells to maintain CNS homeostasis and ensure the remodeling of synaptic circuits contributing to neural plasticity and memory. However, under diseased conditions, the delicate balance between neuroprotective and neurotoxic effects of immune responses can be rapidly disrupted due to an excessive or prolonged activation of immune and glial cells and can lead to neuronal damages inducing synaptic plasticity alterations and cognitive impairments. These deficits are very common in many neuroinflammatory diseases like multiple sclerosis but the mechanisms involved are still poorly understood. This project aims to study the effects of neuroinflammation on neuronal network activity and synaptic plasticity in mouse hippocampus and to highlight the inflammatory actors related to cognitive disorders. We are particularly interested in immune mechanisms developed during experimental autoimmune encephalomyelitis (EAE), a model of MS that we use in our study like a model of CNS chronic neuroinflammatory disease.

Experimental autoimmune encephalomyelitis



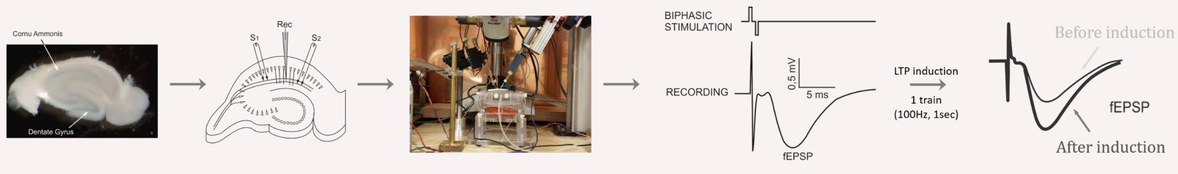
EAE score	Motor symptoms
0	No motor problems
1	Limp tail
2	Weakness of hind legs
3	Complete paralysis of hind legs
4	Complete hind leg and partial front leg paralysis
5	Severe paralysis, euthanasia



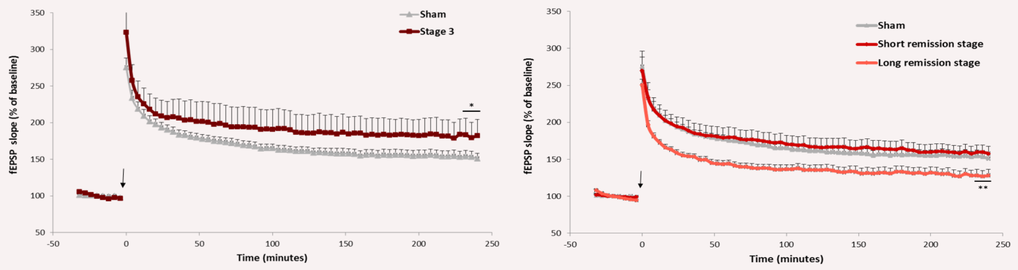
EAE is induced in C57BL/6 mice by immunization with a CNS antigen (MOG_{35-55} peptide) in an emulsion with Freund's adjuvant followed by administration of pertussis toxin (PTX). According to motor symptoms, disease progression is assessed daily using the standard EAE grading scale, ranging from 0 to 5.

Classical EAE onset is 10 to 15 days after immunization, with peak of disease 3 to 5 days after onset (stage 3) followed by partial recovery of motor skills (remission stage).

A. Hippocampal synaptic plasticity is modified during EAE

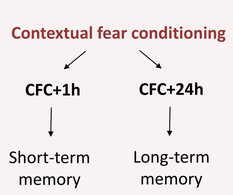


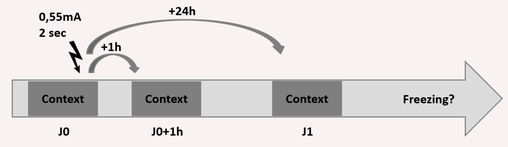
Hippocampal slice | Electrodes positioning | Recording chamber | fEPSP recording | LTP induction

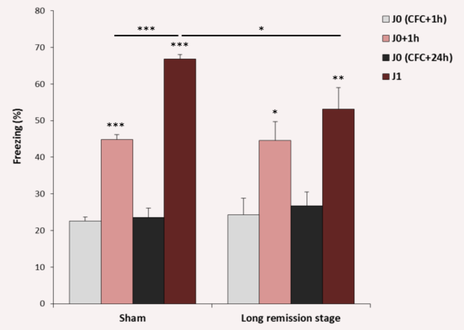


Hippocampal synaptic plasticity of EAE mice was analysed by electrophysiological recordings (LTP) made on acute hippocampal slices. fEPSP slope was measured before and after LTP induction (arrow). The level of potentiation enhances at the peak of EAE (stage 3) but progressively decreases during the remission stage to reach a level significatively lower than the control one.

B. Remitting mice present a cognitive impairment

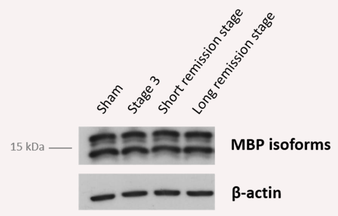




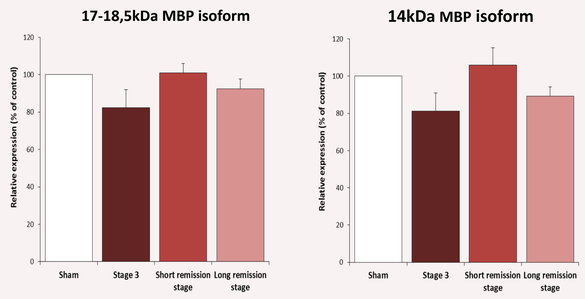


Spatial learning of remitting mice was evaluated by the contextual fear conditioning (CFC). CFC is a hippocampus-dependent learning paradigm used to assess the capacity of mammals to associate environmental cues with a mild aversive stimulus. CFC exhibits short- and long-term forms. Similar freezing behavior was obtained with the CFC+1h between sham and EAE mice. However remitting mice present a lower freezing 24h after the learning episode suggesting that a cognitive impairment develops during the remission stage of EAE and implies long-term but not short-term memory.

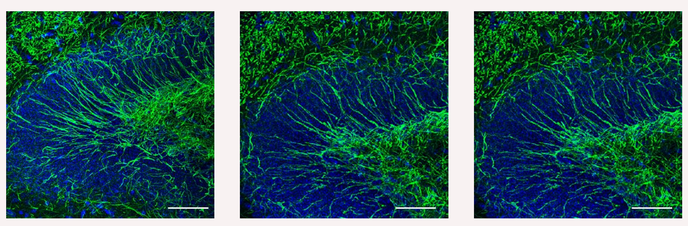
C. No demyelination occurs in the hippocampus of EAE mice



Representative western-blot of MBP expression in the hippocampus of sham and EAE mice.



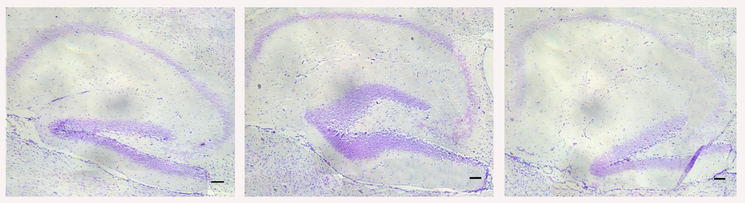
Relative quantification of MBP isoforms expression. Two MBP isoforms were detected by WB in the hippocampus of sham and EAE mice and were quantified separately. No modification of MBP expression was found at any stage of EAE suggesting no demyelination occurs in the hippocampus of EAE mice. So hippocampal synaptic plasticity modifications can not be explained by a disrupted neurotransmission linked to demyelination.



Sham | Stage 3 | Remission stage

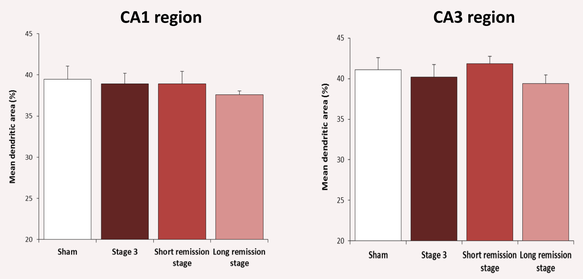
Confocal microscopy images of myelin in the gyrus dente of the hippocampus. Myelination in the hippocampus of sham and EAE mice was analysed by fluorescent immunostainings of MBP made on free-floating hippocampal sections (50µm). No alteration of hippocampal myelin was observed during both the stage 3 and the remission phase of EAE compared to sham. Scale bars: 50µm.

D. Structural integrity of the hippocampus is preserved during EAE



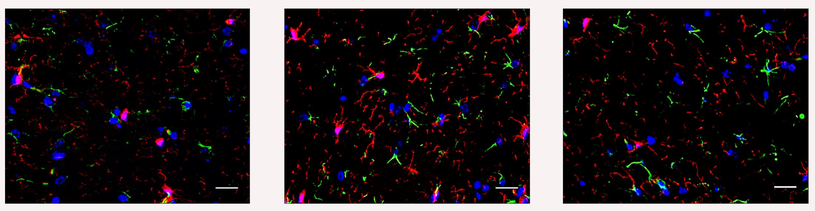
Sham | Stage 3 | Remission stage

Cresyl violet staining of hippocampal sections (5µm) from sham and EAE mice. The architecture of the hippocampus is preserved during EAE. No atrophy, neurodegeneration or inflammatory infiltrates were observed in the hippocampus at any stage of EAE. Scale bars: 100µm.



Analysis of the dendritic surface (MAP2+) in the CA1 and CA3 regions of the hippocampus of sham and EAE mice. Quantification of MAP2-immunoreactive dendrites revealed no modification of dendritic area suggesting the preservation of the structural integrity of the hippocampus in EAE mice.

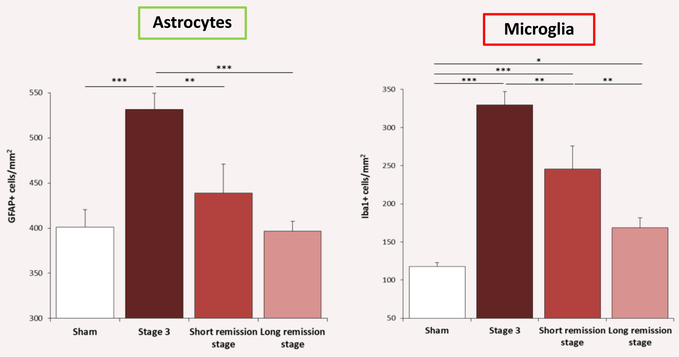
E. Astrocytes and microglia are activated in the hippocampus of EAE mice



Sham | Stage 3 | Remission stage

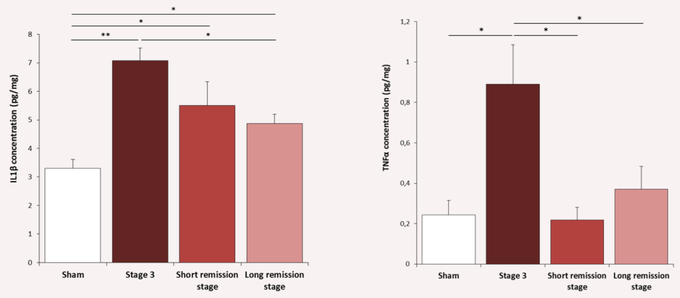
DAPI → Nuclei
GFAP → Astrocytes
Iba1 → Microglia

Fluorescent immunostainings of GFAP (astrocytes, green), Iba1 (microglia, red) and DAPI (blue) made on hippocampal sections (5µm) from sham and EAE mice. Scale bars: 25µm.



Quantification of astrocytes (GFAP+ cells, left) and microgliocytes (Iba1+ cells, right) in the hippocampus of sham and EAE mice. The number of both GFAP+ and Iba1+ cells follows the disease progression as it enhances at the peak of EAE and then decreases during the remission stage. The activation profile is similar between astrocytes and microglia during EAE but microglial proliferation is more important than astrocytes at the peak of EAE and remains high during the remission stage compared to shamed mice.

F. Proinflammatory cytokines are produced in the hippocampus during EAE



Quantification of IL1β and TNFα concentration in the hippocampus of sham and EAE mice. ELISA experiments revealed a production of IL1β (left) and TNFα (right) during EAE which follows the disease progression. The level of these cytokines is higher at the peak of EAE and then decreases during the remission stage suggesting the development of an inflammatory reaction in the hippocampus of EAE mice.

Conclusion

Our study demonstrates that although motor impairments are the main symptoms of EAE, immune responses and neuroinflammation developed during EAE can also affect cognitive structures like hippocampus and can lead to cognitive impairments. Different modifications of hippocampal synaptic plasticity were observed during the course of the disease and despite the lack of demyelination and any structural alterations, an inflammatory state marked by activated astrocytes and microglia along with the production of inflammatory cytokines (IL1β, TNFα) develops in the hippocampus of EAE mice. Future plans will consist on a more detailed analyse of the role of the NFκB signaling pathway, activated microglia and the inflammasome in the hippocampus during EAE to investigate their potential implication in cognitive disorders associated to neuroinflammation.