IMAGING BIOMARKER AND PATHOPHYSIOLOGY OF EARLY MEMORY IMPAIRMENT IN MULTIPLE SCLEROSIS: A PRE-CLINICAL STUDY WITH DIFFUSION-TENSOR IMAGING OF HIPPOCAMPAL LAYERS.

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Target audience: Scientists interested in pre clinical models of multiple sclerosis and in imaging biomarkers.

Background and purpose: Although hippocampal atrophy has been correlated to memory impairment in multiple sclerosis (MS), the early cellular modifications and pathophysiological mechanisms leading to hippocampal degeneration are poorly understood and there is no *in vivo* method to capture these features. We aimed at validating MRI biomarkers and identifying pathophysiology mechanisms of early memory impairment in MS.

Methods: Mice with experimental autoimmune encephalomyelitis (EAE), the most widely used mouse model of MS, were studied at the early stage of the disease, 20 days after immunization, and compared to control mice receiving injection of the complete Freund's adjuvant (CFA) only. Mice were explored with *(i)* a dedicated and unbiased hippocampal-dependent task of contextual fear conditioning, *(ii)* MRI hippocampal volumetry, *(iii)* a dedicated high-resolution *in vivo* diffusion-tensor imaging (DTI) sequence for layer-by-layer analysis of the hippocampus using 80x80x200µm resolution at b=2000s/mm², *(iv)* histology, and *(v)* pharmacological experiments.

Results: Using *in vivo* MRI, we have demonstrated a selective decrease of the fractional anisotropy (FA) and axial diffusivity (AD) in the molecular layer (ML) of the dentate gyrus of EAE-mice exhibiting significant memory impairment at the early stage of the disease, prior to hippocampal atrophy (**Fig1**). While there was diffuse hippocampal microglia activation, a selective dendritic loss and neuronal death in the dentate gyrus of EAE-mice were the main correlate of the low FA and AD (**Fig2**). After a backward stepwise selection of all the histological metrics, the final model of MR-to-histology correlation amounted to a univariate relationship between FA (or AD) and dendritic loss with r=0.56 (and r=0.47). To test whether microglia could be the primary neurotoxic agent leading to the early disruption of the ML of the dentate gyrus, EAE-mice were treated with the microglial inhibitor minocycline from day 7 to day 20. We showed a neuroprotective effect of minocycline that was measurable *in vivo* as an increase of FA and that protected EAE-mice against memory impairment (**Fig3**). These data confirm the causal link between microglial activation, dentate gyrus degeneration and early memory impairment.

Discussion and conclusion: We have demonstrated: *(i)* a selective and early neurodegeneration in the ML of the dentate gyrus of EAE-mice with memory impairment, *(ii)* that activated microglia caused dendritic loss in the ML of the dentate gyrus, *(iii)* that *in vivo* DTI parameters were correlated with these early microstructural changes, *(iv)* that DTI of hippocampal layers was more sensitive than MRI-derived atrophy, *(v)* that DTI of hippocampal layers could be used as an *in vivo* marker of therapeutic response and *(vi)* that minocycline was a potential neuroprotective treatment which prevented memory impairment in EAE-mice. Considering that there is still no treatment for memory impairment in MS, high resolution layer by layer analysis of the hippocampus using DTI could become an exciting surrounding biomarker to screen promising molecules in clinical trials.

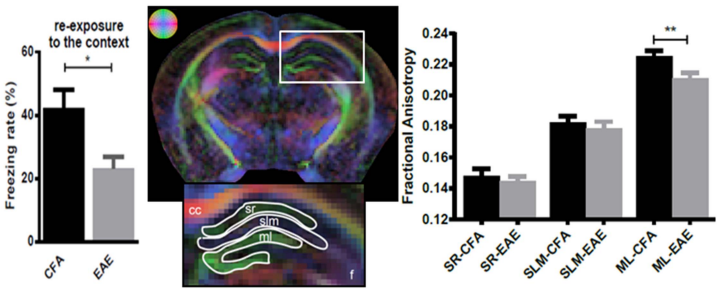

Figure 1: EAE-mice showed early memory impairment as measured by a significant decrease of the freezing rate when re-exposed to a conditioning context (*p<0.05). High resolution DTI allowed the identification of 3 hippocampal layers: stratum radiatium (SR), stratum lacunosum molecular (SLM) and the molecular layer (ML) of the dentate gyrus. "CC" states for the corpus callosum and "f" for the fimbria. A significant decrease of FA (driven by axial diffusivity) was found selectively within the ML of EAE-mice compared to CFA-mice (**p<0.01) while FA was not modified in the other layers

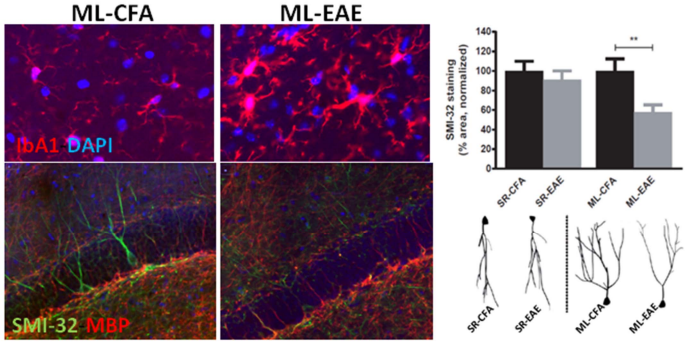

Figure 2: Significant microglia activation was found within the whole hippocampus of EAE-mice (IbA1 staining) but dendrites (SMI-32 staining) were selectively lost within the molecular layer (ML) of the dentate gyrus of EAE-mice compared to CFA while dendrites of the stratum radiatum (SR) were spared (**p<0.01). Illustrative example of neurons traced with neurolucida software show the decreased complexity of neurons of the ML in EAE compared to CFA while the neurons of the SR are not affected.

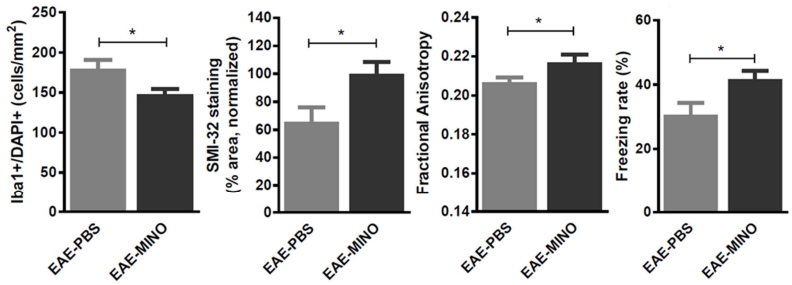

Figure 3: Microglia proliferation (Iba1+/DAPI+ cells) was partially suppressed in EAE-mice treated daily with minocycline compared to EAE-mice treated with PBS. Such microglia inhibition protected against the dendritic loss (SMI-32 staining) in the ML of the dentate gyrus, and FA went back up in minocycline-treated mice compared to PBS-treated mice. Altogether minocycline preserved the memory performances of EAE-mice that showed a higher freezing rate after re-exposure to the conditioning context (*p<0.05).

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