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Detection of demyelination of the sensory and motor cortex in a mild EAE mouse model using diffusion weighted MRI

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Introduction: Multiple sclerosis (MS) is a central nervous system disease characterized by neuroinflammation and demyelination that mainly affects the white matter (WM) structures such as the corpus callosum and the optic nerve. Recently, diffuse pathological changes have been documented in the gray matter (GM) and cortical areas in patients with MS as well as in animal models of this disease [1, 2]. MS is one of the major causes of neurological disability worldwide; patient symptoms vary according to the disease stage and treatment outcomes. The major hallmarks of MS include neurodegeneration, demyelination, axonal damage and nerve injury [3, 4]. Various rodent models of MS have been developed to investigate specific aspects of this disease and explore underlying mechanisms. Experimental autoimmune encephalomyelitis (EAE) in the mouse is a classical MS model, which is characterized by development of CNS neuroinflammation that leads to demyelination and neurodegeneration. EAE disease severity in mice varies according to the mouse strain and the immunization protocols utilized [5]. In order to assess sensory deficits in EAE-mice, the immunization protocol was optimised to produce mild relapsing remitting MS clinical symptoms with partial recovery between relapses. Using this model, we assessed possible correlations between clinical scores and pain behaviour with changes in the motor and sensory areas of the brain by MRI [6]. Here we describe the use of high spatial and angular resolution *ex vivo* diffusion-weighted imaging (HARDI) to investigate neuropathological changes in the brains of our EAE-mouse model of remitting-relapsing MS.

Methods: Animal model: EAE was induced in 4-6 week old C57BL/6 male mice by subcutaneous injection of MOG_{35-55} (200µg) emulsified in saponin adjuvant (Quil-A) (45µg) and an intraperitoneal injection of pertussis toxin (PT) (200-250ng) as adjuvant and to temporarily open the blood brain barrier. A second, identical injection of pertussis toxin was administered after 48 h. Sham mice received Quil-A and pertussis toxin only. Mice were monitored once daily over the 60-day experimental period for clinical scoring using a 0-5 point scale with half-point gradations. Sample preparation: *Ex-vivo* MRI samples were prepared using C57 BL6 mice in the following groups: (i) non-disease control animals (n=8); (ii) acute (day 13-16 post injection): 7 sham and 7 EAE, (iii) chronic (day 54-57 post injection): 7 sham and 9 EAE. Mice were anaesthetized and transcardially perfused with 0.1 M phosphate-buffered solution (PBS) followed by immersion of mouse heads in 4% PFA for 24 h. Following extraction, brain tissues were washed with PBS for 48h prior to MR imaging [7]. HARDI acquisition: MRI data were acquired at 16.4 Tesla using a Bruker 89mm MR micro-MR imaging system equipped with a 15 mm i.d. linearly polarized birdcage RF coil. 3D HARDI data were acquired using the Stejskal-Tanner pulse-field gradient spin echo sequence [7], with 30 diffusion gradients directions at b = 3000 s/mm^2, δ/Δ = 2.4/6.4 ms, TR/TE = 400/14.5 ms, two b_0, and at 100 micron isotropic resolution. The total acquisition time was 14 h 44 mins. FID was zero-filled by a factor of 1.5 prior to a Fourier transform, resulting in 67micron 3D isotropic resolution. Image processing: Diffusion tensors imaging (DTI) parametric maps were calculated using the MRtrix program [8]. A fractional anisotropy (FA) map template was created from all datasets using the build template script of the program ANTS. All brain samples were registered to the template using the FLIRT/FNIRT of the program FSL. Statistical data analysis: (i) Twelve regions of interest (ROIs) were drawn manually on the FA template using ITK-snap at various white matter (WM) structures, and used to read out the values from each brain sample [9]. (ii) Voxel based morphometric (VBM) analysis was performed on all registered brain samples using the Statistical Parametric Mapping program (SPM 5), with Gaussian smoothing factor = 5x voxel size (0.33 mm) and False Discovery Rate correction with $p<0.05$ [10]. Histology: Black Gold II (BGII) myelin staining was used to visualize myelinated axons and to confirm the MRI observations. BGII stain highlighted large myelinated fibers such as corpus callosum in red-brown color and small individual fibers in dark-brown color [11].

Results: EAE-mice exhibited a remitting-relapsing disease course with a mean score of 1.5 (limp tail and distinct hind limb weakness characterized by poor grip and unsteady gait) while sham and control-mice showed no evidence of clinical disease**.** Comparison of DTI parametric maps (FA, axial, radial and mean diffusivity) using manually drawn ROIs in the major WM structures did not reveal significant changes between EAE, sham and normal controls in acute and chronic stages of the disease. Data analysis using VBM, however, revealed significant reductions of FA in chronic EAE-mice in several GM brain areas including the primary and secondary motor areas, primary somatosensory area, anterior cingulate and rostral CA1 hippocampal regions, and a small portion of WM external capsule (Figure1). The FA of affected primary and secondary motor region in chronic EAE is 0.17±0.01 compared to 0.19±0.01 in the control animals. BGII histology confirmed the presence of extensive demyelination in these gray matter areas. These FA changes were not detected during the acute phase. Our findings suggest a correlation between functional impairment in motor and sensory areas with a reduction in FA.

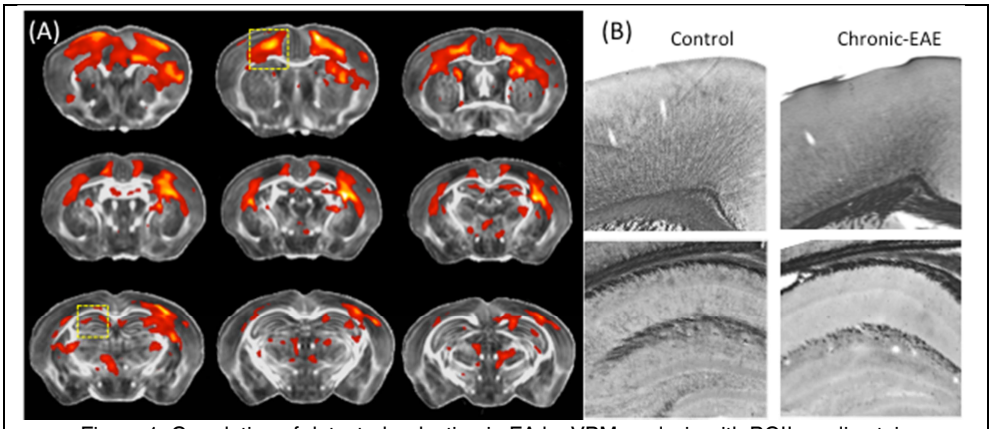

Figure 1. Correlation of detected reduction in FA by VBM analysis with BGII myelin stain

Discussion: ROI analysis of change due to disease can be problematic if target anatomical areas for suspected pathological change are unknown. In this study, ROI analysis produced negative results because initial analyses were directed towards detecting classical MS demyelination in the major WM structures. In addition, even at high spatial-resolution (67 micron), the mouse brain WM structures are relatively small, being only 2-3 voxels in width such that ROI-based analysis may be prone to error due to partial volume effects and in areas containing crossing fibers. VBM analysis proved more sensitive in detecting changes in the whole brain, including areas exhibiting lower anisotropy such as the cortex and hippocampus. The axial, radial and mean diffusivity may be less sensitive to detect these changes *ex-vivo* as they are more affected by the variability of fixation compared to FA (12).

Conclusion: This remitting-relapsing EAE-mouse model of MS is mild in terms of clinical scores enabling pain behaviours to be assessed without confounding motor deficits. The model provides an opportunity to gain better insight into neuropathological changes during the remitting-relapsing disease course. *Ex vivo* MRI at 16.4T provided the sensitivity to study this mild EAE model by providing data acquisition using high-spatial resolution, high-angular diffusion encoding resolution and large b-value. Finally, this study shows that FA is sensitive for detecting MS demyelination in GM structures.

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