Gray matter demyelination and remyelination detected with multimodal quantitative analysis at 11.7T in a mouse model of multiple sclerosis.

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Introduction: Myelin is a component of the nervous system that is disrupted in multiple sclerosis (MS), resulting in neuro-axonal degeneration. The development of repair therapies requires methods to monitor myelin dynamics in living individuals. Several MRI studies have shown demyelination and remyelination in the white matter (WM, e.g. corpus callosum) of mice treated with cuprizone (cpz) using different MRI techniques[1-4]. Besides WM pathology, it is now well-accepted that gray matter (GM) demyelinating lesions have a strong impact on disease evolution. We have hence investigated both GM and WM alterations during chronic intoxication and recovery in the well-characterized cpz mouse model of MS. **Methods:** All images were acquired with a 11.7-T system (Bruker Biospec 117/16 USR, 750mT/m gradients, PV5.1) and a cryoprobe. Six C57BL/6 8-week old female mice were fed with 0.2% cpz and imaged before treatment (Tx) and 12 weeks (12w) after Tx. The animals were then fed normal chow and imaged at 6w (n=5) and 12w (n=4) without Tx. Two mice were sacrificed for myelin assessment with PLP/GFP (proteolipid protein / green fluorescent protein). High-resolution anatomical T2-weighted images were acquired with a 2D RARE sequence; TR=6000ms; TE=40ms; resolution(res)=60x60μm^2; slice thickness=220μm; Nex=1; Tacq=17min. Signal ratios were calculated from coregistered images between regions-of-interest (ROIs) in the GM or WM and the signal of the ventricles (Vtr). The size of Vtr was calculated as the sum of the areas of the lateral, the third and the fourth Vtr. Parametric T2 maps were obtained from a MSME sequence (TR=5500ms; TE=15-120ms/5-ms increments; res=100x100μm^2; slice thickness=200μm). Three-dimensional diffusion echo-planar images were acquired with TR=500ms; TE=20ms; res=150x150x300μm^3; 46 directions; b=1000mm/s^2; δ=4ms; Δ=10ms; Tacq=40min. Diffusion metrics such as the fractional anisotropy (FA) and the axial and radial diffusivities (RD, as a marker of myelin damage) were measured on the same ROIs.

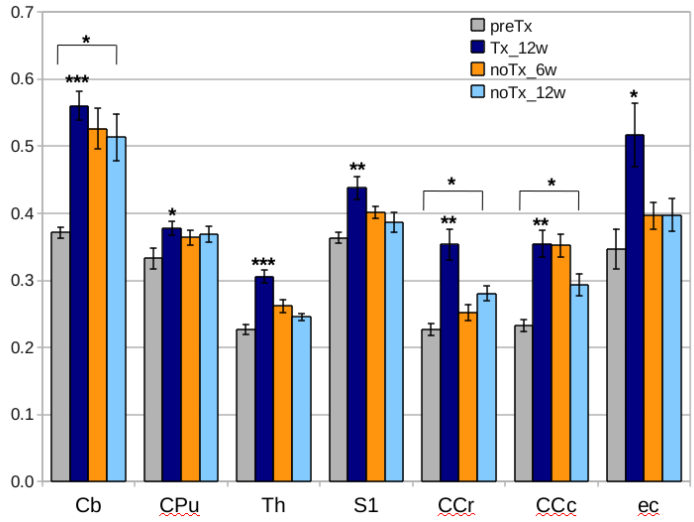

Fig.1: Mean signal ratios (error bars=mean standard error, SEM) measured before cpz Tx, 12w post Tx, then 6w and 12w without Tx. Cb: cerebellum, CPu: caudate putamen, Th: thalamus, S1: primary sensory cortex, CCr: rostral corpus callosum, CCc: caudal CC, ec: external capsule.

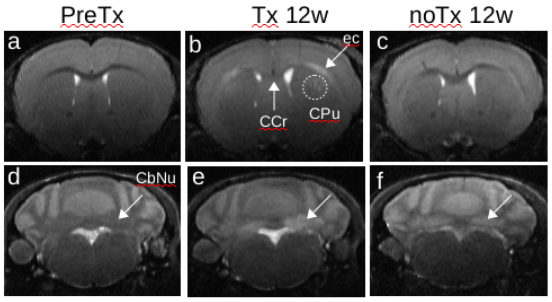

Fig.2: Representative T2-weighted images of a control mouse brain (a,d), a brain 12w after Tx (b,e) and 12w without Tx (c,f). Signal enhancement can be seen in the CCr, the ec, the CPu and in the cerebellar peduncles/nuclei (CbNu).

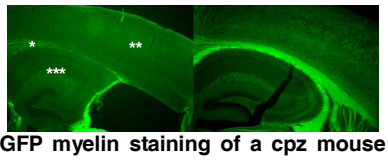

Fig.3: PLP/GFP myelin staining of a cpz mouse brain (left, 6w) and a normal brain (right) showing demyelination in the CC (*), the cortex (**) and the Hc (***).

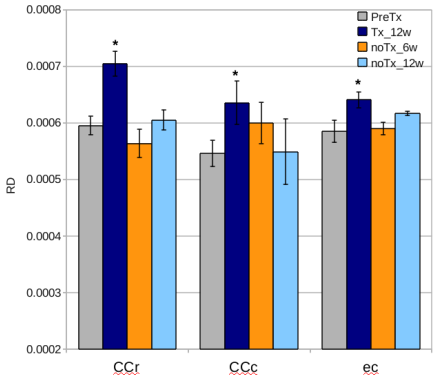

Fig.4: Mean radial diffusivity (RD) and SEM measured over the CCr, the CCc and the ec during treatment and recovery phases.

Results: Signal ratio (Sr) measurements showed significant enhancement in the the superior cerebellar peduncles and extending to the cerebellar nuclei ($p<0.0002$), the dorsal caudate putamen ($p<0.04$), the ventral posterolateral/medial thalamic nuclei ($p<0.0001$), the primary somatosensory cortex ($p<0.004$), the rostral ($p<0.002$) and caudal corpus callosum ($p<0.001$), and in the external capsule ($p<0.01$) 12w after chronic cpz Tx compared to pre-Tx (fig.1-2). After 6w and 12w of recovery (no Tx) Sr came back to its initial value in all structures and only partially in the Cb, CCc and CCr. Similarly, T2 values (data not shown) significantly increased in all structures after 12w of Tx compared to pre-Tx, then returned to initial values 6w and 12w without Tx and partially in the CCc and S1. The Vtr showed significant dilation at 12w of Tx ($p<0.001$), then returned to their initial size during the recovery phase. Histological evaluation of sectioned brains at 6w of Tx confirmed demyelination in the CC, the cortex and the Hc (fig.3). In WM regions, FA (not shown) significantly decreased and RD significantly increased after 12w of Tx (fig.4), then they recovered to initial values 6w and 12w without Tx. In GM regions, no changes in diffusion metrics were detected. **Discussion/Conclusions:** These multimodal high-resolution MRI data showed reversible alterations not only in the WM (in accordance with other published data) but also in specific GM regions. Most of these findings correspond to demyelination and remyelination processes as well as concomitant atrophy already characterized with histochemical and immunohistochemical methods in the cpz model[5]. This work will make possible MRI assessments of new treatments targeted to gray matter and more specifically to cortical demyelination and remyelination in this mouse model.

References: 1. Song et al. Neuroimage 2005;26:132-40; 2. Sun et al. MRM 2006;55:302-8; 3. Zaaraoui et al. MAGMA 2008;21(5):357-62; 4. Zhang et al. MRM 2012;67(3):750-59; 5. Skripuletz et al. Histol. Histopathol. 2011;26:1585-97.

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