Monitoring Demyelination in a Cuprizone Mouse Model with Longitudinal and Quantitative MRI Measurements

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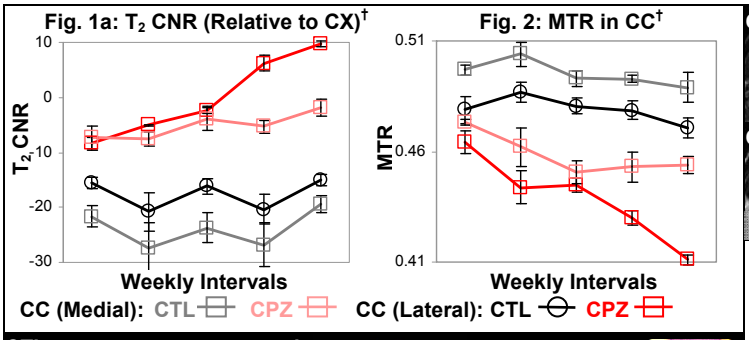
Introduction Magnetic resonance imaging (MRI) methods capable of quantifying changes due to demyelination can improve both the diagnosis and understanding of white matter diseases such as multiple sclerosis. T_2-weighted (T2w) and magnetization transfer images (MTI) were acquired weekly in control (n=4) and cuprizone-fed mice (n=4). Diffusion tensor imaging (DTI), quantitative MTI, high-resolution T2w imaging, and histopathology were used to analyze *ex vivo* tissue. All *in vivo* methods showed significant differences longitudinally in the corpus callosum (CC) of the cuprizone-fed mouse. All *in vivo* and *ex vivo* methods showed significant differences in the corpus callosum between groups.

Methods ***Mouse Model:*** Cuprizone is a toxic model of demyelination, causing cell death of oligodendrocytes followed by extensive demyelination[1]. C57BL/6 mice were fed 0.4% cuprizone (w/w) starting at 8 weeks of age. After 6 weeks of feeding, mice were perfused with PBS/saline solution followed by 4% PFA. Heads were fixed for 24 hours in 4% PFA, after which all tissue was removed external to the skull. 48 hours prior to *ex vivo* imaging, brains were transferred to a PBS solution to leach out the remaining PFA. All experiments were approved by the university's animal care committee. ***MRI:*** Experiments were performed on a 7T Bruker Avance III NMR system with Paravision 5. Mice were anesthetized using 1.5% isoflurane in O_2/N_2O. Respiration and external body temperature were monitored during imaging. 4 control (CTL) and 4 cuprizone-fed (CPZ) mice underwent *in vivo* T2w imaging and MTI at 2, 3, 4, 5 and 6 weeks after start of treatment. After sacrifice, additional high-resolution T2w, DTI and quantitative MTI datasets were acquired. Coronal slices were selected in each mouse perpendicular to a region of the CC immediately in front of the anterior commissure. *In vivo* T2w and MT images were aligned using manual and mutual information image registration. Regions of interest representing both medial and lateral regions of the CC as well as the cerebral cortex (CX) were selected in the T2w images and applied to analysis of all MR methods. All image analysis was performed using MATLAB. ***In vivo* T2w:** Rapid Acquisition with Refocused Echoes (RARE – Fast Spin Echo), 3 slices, 12 averages, (2.5 cm)^2 FOV, 98x98x750 μm^3 resolution, effective TE 80ms, TR 1640ms, RARE factor 8, 10 minutes. ***In vivo* MTI:** Fast Low Angle Shot (FLASH – Gradient Echo Sequence), 3 slices, 48 averages, (2.5 cm)^2 FOV, 98x98x750 μm^3 resolution, TE 6 ms, TR 70 ms, 10° flip angle. Two images were acquired: a magnetization transfer contrast (MTC) image with MT saturation pulse (Gaussian, 10.25ms, 10μT, 6000Hz off-resonance) and a proton density (PD) image without MT saturation pulse, 2x14 minutes. ***Ex vivo* MTI:** Same pulse sequence as *in vivo*. 1 PD image + 24 MTC images acquired with irradiation powers of 5, 10, and 20μT and frequency offsets at each power of 100, 300, 1000, 2000, 4000, 6000, 10000, and 30000 Hz. ***Ex vivo* DTI:** Pulse Gradient Spin Echo (PGSE), tetra-orthogonal gradient-encoding scheme (7-directions), b-value = 1000 s/mm^2 (δ = 6 ms, Δ = 14 ms), 1 slice, 6 averages, (1.25 cm)^2 FOV, 98x98x750 μm^3 resolution, TE 26ms, TR 5000ms, 2.5 hours. ***Ex vivo* T2w:** RARE, 1 slice, 36 averages, (1.25 cm)^2 FOV, 49x49x750 μm^3 resolution, effective TE 80ms, TR 1640ms, RARE factor 8, 31 minutes. ***Histopathology:*** 30 μm sections were stained with Luxol Fast Blue (LFB - myelin fibres appear blue, neutrophil pink, and nerve cells purple) and Periodic Acid-Schiff (PAS - glycogen appears purple-magenta).



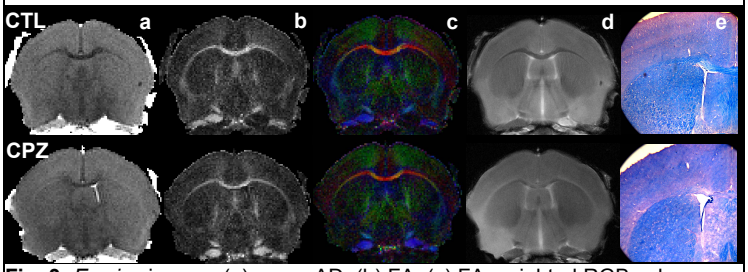


Fig. 3: *Ex vivo* images (a) mean AD, (b) FA, (c) FA-weighted RGB colormap, (d) T2w, (e) LFB-PAS pathohistology. All errors/error bars = ± SEM.

Table 1: DTI in CC	Mean AD (x 10^{-4} mm^2/s)	Axial AD (x 10^{-4} mm^2/s)	Radial AD (x 10^{-4} mm^2/s)	Fractional Anisotropy
CTL (Medial)	1.77 ± 0.05	3.57 ± 0.06	0.88 ± 0.05*	0.609 ± 0.012**
CPZ (Medial)	2.33 ± 0.16	4.26 ± 0.25	1.36 ± 0.12*	0.528 ± 0.011**
CTL (Lateral)	2.38 ± 0.07**	4.13 ± 0.20*	1.50 ± 0.09**	0.494 ± 0.033
CPZ (Lateral)	3.37 ± 0.11**	5.26 ± 0.17*	2.42 ± 0.07**	0.397 ± 0.003

†**T_2 CNR and MTR:** Two-way repeated measures ANOVA shows significant differences between measurements in the corpus callosum of CTL and CPZ mice ($p < .001$) and a paired samples t-test shows significant week-to-week changes in lateral regions of the corpus callosum of CPZ mice ($p < .05$).
DTI Metrics: Independent samples t-test shows ($p < .05$)* and ($p < .01$)**

Fig. 1b: *In Vivo* **T2w (weekly images from a CTL and a CPZ mouse)**

Results and Discussion T2w and MT images showed significant differences in the CC between control and cuprizone-fed mice (Figs. 1, 2, and 3d). Interestingly, changes in both the T_2 CNR and MTR were significant on a near weekly basis in the lateral regions of the CC, with progressive contrast inversion in the T2w images and weekly decreases in the MTR (Figs. 1 and 2). Increased T_2 relaxation times may be attributable to a loss of myelin water (T_{2m} < 30 ms) and a subsequent increase in intra- and extra-cellular fluid ($T_{2i/e}$ > 30 ms) due to demyelination and axonal degeneration. Similarly, decreased MTR suggests a smaller macromolecular pool due, in part, to demyelination. This is born out further by the significantly larger relative signal in the majority of *ex vivo* MT experiments at varying saturation frequencies and powers (data not shown). DTI metrics reveal further changes in axonal geometry (Figs. 3a, 3b, 3c and Table 1). Increased axial and radial apparent diffusivity (AD) and reduced fractional anisotropy (FA) could be due to demyelination and subsequent changes in axonal density and membrane permeability[2]. *Ex vivo* T2w and pathohistology reveal significant changes due to demyelination in the cuprizone-fed mouse (Figs. 3d and 3e). It should be noted that LFB-PAS staining may *overestimate* the degree of organized myelination since lipid debris from degraded myelin still gives a positive signal[1] (the same criticism could be leveled towards MTI). Conversely, T_2 and DTI may *underestimate* organized myelination due to the influence of varying T_2 components and axonal geometries. Ultrastructural examination with electron microscopy is still required to determine the true myelin integrity.

References [1] Matsushima, GK *et al.* Brain Pathology 11: 107-116 (2001). [2] Song, S-K *et al.* NeuroImage 26:132-140 (2005).

Conclusion All of the MR methods demonstrated significant differences between groups, however, in order to discriminate intact and degenerating myelin, a hybrid approach is necessary. This study lays the groundwork for correlating myelin-sensitive T_2-weighted and MTI methods with axonal geometry-sensitive DTI metrics. Ultimately, a hybrid MRI approach could be an important diagnostic method for measuring myelin content in the CNS.