Understanding white matter pathology through correlating longitudinal and quantitative MRI metrics weekly in the cuprizone mouse model of demyelination

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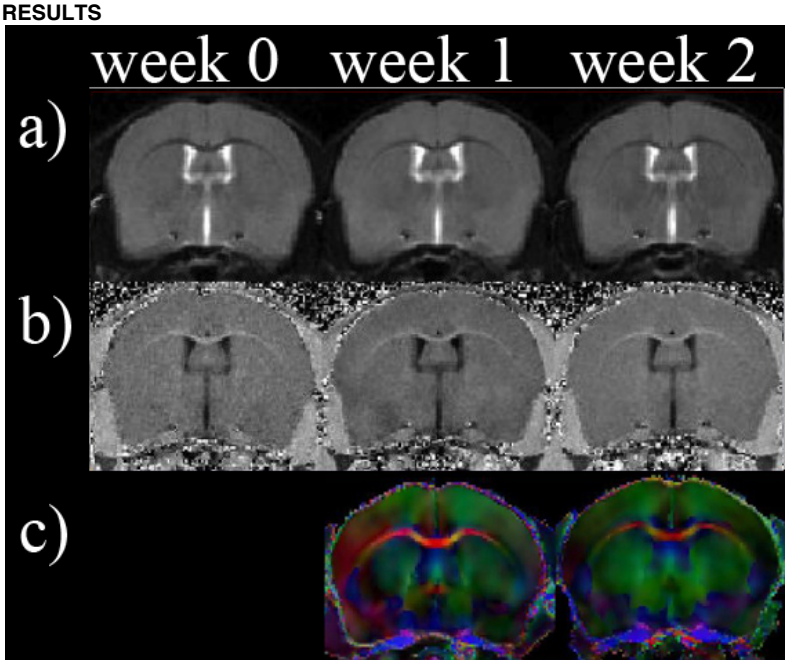
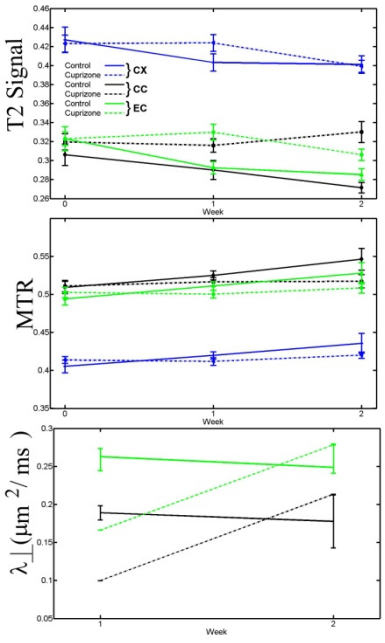
INTRODUCTION: MRI methods such as diffusion tensor imaging (DTI)[1], quantitative magnetization transfer imaging (qMTI)[2], and multicomponent T_2 relaxometry[3] might help quantify changes related to demyelination. To understand the interplay different MRI methods have as white matter changes longitudinally in the cuprizone mouse model, *in vivo* T_2-weighted (T2w) and magnetization transfer images (MTI) were acquired weekly in control (CTL) (n=18) and cuprizone-fed (CPZ) (n=18) mice. As well, weekly DTI, qMTI, T_1/T_2 relaxometry, T2w imaging, and electron microscopy (EM) were used to analyze *ex vivo* tissue after each week of cuprizone delivery (n=3 per group each week). Correlation between both longitudinal and quantitative datasets was measured with a focus on the corpus callosum (CC) and external capsule (EC). A previous study examined correlations between MR metrics and EM measures after 6 weeks of feeding[4]. The addition of weekly *ex vivo* tissue analysis allows for a more complete understanding of the correlations between MR metrics and EM measures of tissue pathology.

METHODS: ***Mouse Model*** C57BL/6 mice were fed 0.3% cuprizone (w/w) starting at 8 weeks of age. After each week of feeding, a subset of mice was perfused with 10 ml of 0.1M phosphate buffered saline (PBS) for ~2 min followed by 0.5% glutaraldehyde and 2% paraformaldehyde (PFA) for ~10 min. This was followed by another 10 ml of 0.1M PBS to flush out any remaining fixative. All tissue external to the skull was removed and the mouse head was stored in PBS prior to overnight imaging. All experiments were approved by the university's animal care committee. ***MRI*** Experiments were performed on a 7T Bruker Avance III NMR system. Mice were anesthetized using 1.5% isoflurane in O_2/N_2O. Respiration and external body temperature were monitored during imaging. In order to reduce volume averaging effects, coronal slices were selected in each mouse perpendicular to the rostral region of the CC. Initially, 18 CTL and 18 CPZ mice underwent *in vivo* T2w imaging and MTI on the day the treatment began (week 0) and one week later (week 1). Starting on week 1, 6 animals (3 CTL, 3 CUP) were sacrificed each week for *ex vivo* analysis. After sacrifice, additional high-resolution T2w, DTI, qMTI, and T_1/T_2 relaxometry datasets were acquired. *In vivo* T2w and MT images were aligned using manual and mutual information image registration[5]. Regions of interest representing both the CC and the EC as well as the cerebral cortex were selected in the *in vivo* MT contrast images and *ex vivo* DTI directionality encoded color maps and applied to analysis of all MR methods. All images were acquired on the same 3 coronal slices with 1.25 mm inter-slice spacing and 98x98x750 μm^3 resolution. FOV/matrix size was $(2.5\ cm)^2$/256x256 *in vivo* and $(1.25\ cm)^2$/128x128 *ex vivo*. ***In vivo T2w*** RARE, 12 averages, effective TE/TR = 80/1640 ms, RARE factor 8, 10 minutes. ***In vivo MTI*** FLASH, 48 averages, TE/TR = 6/70 ms, 10° flip angle. In order to calculate the magnetization transfer ratio (MTR), images were acquired with an MT saturation pulse (Gaussian, 10.25 ms, 10 μT, 6000 Hz off-resonance) and without an MT saturation pulse, 2x14 minutes. ***Ex vivo T_1/T_2 Relaxometry*** Fit to a series of RARE images, effective TE = 11, 33, 55, 77, 99 ms; TR = 5000, 3000, 1500, 800, 400, 353 ms; RARE factor 2; 8 averages; 71 minutes. ***Ex vivo qMTI*** 1 proton density image + 18 MT images acquired with irradiation powers of 5, 10, and 20μT and frequency offsets at each power of 1000, 2000, 4000, 6000, 10000, and 30000 Hz, 64 averages 9.6 min/image x 19images; ***Ex vivo DTI*** PGSE, tetraorthogonal gradient-encoding scheme (7-directions), b-value = 1000 s/mm2 (δ = 6 ms, Δ = 14 ms), 1 slice, 6 averages, TE/TR = 26/5000 ms, 5 hours. ***Ex vivo T2w*** RARE, 1 slice, 36 averages, effective TE/TR = 80/1640 ms, RARE factor 8, 31 minutes. Presented here are *in vivo* and *ex vivo* MR data from weeks 0-2.

RESULTS





As expected, weekly *in vivo* (a,b) and *ex vivo* (c) imaging shows no change in the normalized T_2 signal intensity (a) or MTR (b) or RGB map, $\lambda_\perp$ (c) or other parameters (data not shown) in the cuprizone mouse. Changes are expected to start in week 3. **Figure** Weekly changes in the cortex (blue), CC (black), EC (green) in the Cuprizone (dotted) Control (solid) mice are shown on the right. Data for *in vivo* images (top and middle) are shown for weeks 0, 1 and 2. Data for *ex vivo* images (bottom) are shown for weeks 1 and 2.

DISCUSSION AND CONCLUSION Changes in white matter pathology are expected to begin at week 3. EM analysis of the tissue still needs to be done for correlations with white matter pathology. Weekly imaging out to week 6 is currently underway. The addition of the weekly *ex vivo* tissue analysis allows for a more complete understanding of the correlations between MR metrics and EM measures of tissue pathology.

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