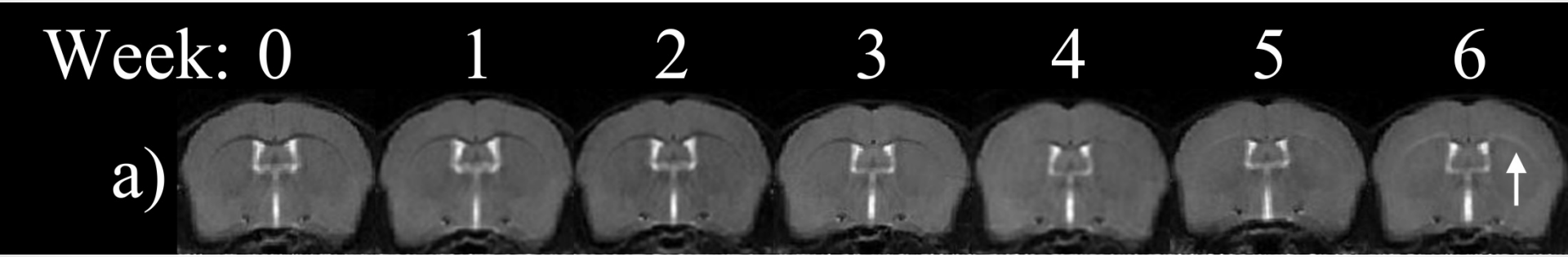
Correlating quantitative MR changes with pathological changes in the white matter of the cuprizone mouse model of demyelination



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Introduction

- Changes in the brain as seen with magnetic resonance imaging (MRI) do not correlate with signs of multiple sclerosis.
  - "Clinical Radiological Paradox"
- Many different MRI metrics are sensitive to many different types of white matter damage.
- Identifying the type of damage could aid in making the correlation

Introduction

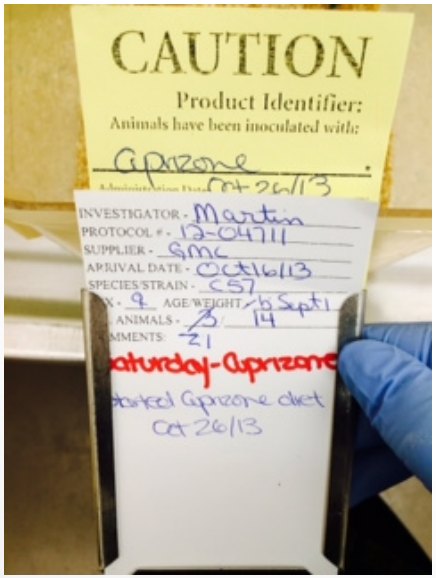
- MRI methods such as diffusion tensor imaging (DTI)[1], quantitative magnetization transfer imaging (qMTI)[2], and multicomponent T_2 relaxometry[3] might help quantify changes related to white matter damage.
- However, MRI methods are sensitive to many different pathologies making interpretation of individual results difficult.

Objective

- To understand the interplay different MRI methods have as white matter changes longitudinally in the cuprizone mouse model through:
  - Correlations between longitudinal and quantitative *in vivo* and *ex vivo* **MRI metrics** and **cellular features** found through histological examination.

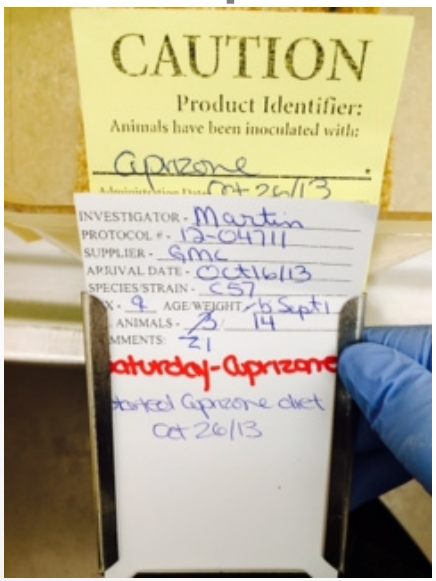
Methods

- ***Mouse Model***
  - 18 C57BL/6 mice were fed 0.3% cuprizone (CPZ) (w/w) starting at 8 weeks of age.
  - 18 C57BL/6 mice (CTL) were fed regular mouse chow.
  - Mice were imaged weekly



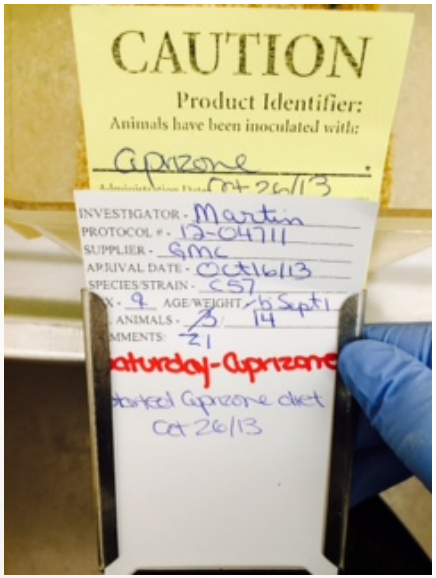
Methods

- After each week of feeding, a subset of mice was euthanatized by perfusion with phosphate buffered saline (PBS) followed by 0.5% glutaraldehyde and 2% paraformaldehyde followed by PBS as done previously[4].



Methods

- 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.

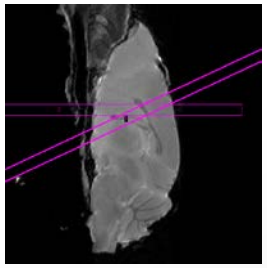


Methods

- ***MRI***
- 7T Bruker Avance III MRI system
- Mice were anesthetized using 1.5% isoflurane in O_2/N_2O.
- In order to reduce volume averaging effects, coronal slices were selected perpendicular to the rostral region of the corpus callosum (CC)[4].

Methods

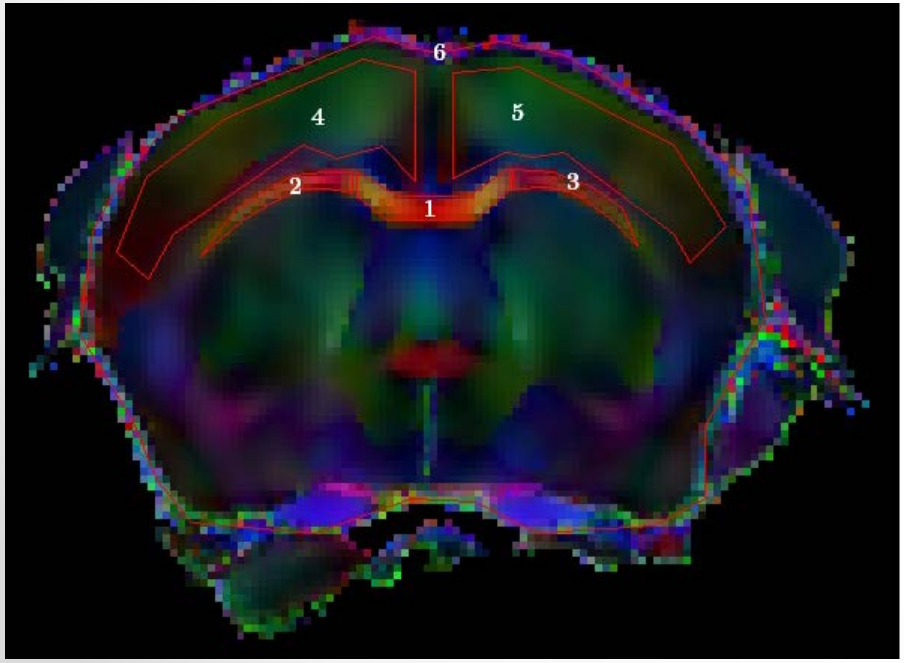
- ***MRI***
- Mice underwent *in vivo* T2w and MTI on the day the treatment began (week 0) and continued weekly for 6 weeks.
- Starting on week 1, 6 animals (3 CTL, 3 CPZ) were sacrificed each week for *ex vivo* analysis:
  - high-resolution T2w, DTI, qMTI, and T_1/T_2 relaxometry
  - Electron microscopy



Coronal CC slice selection

Methods

- ***MRI***
- Images were aligned using manual and mutual information image registration[5].
- *Ex vivo* ROIs selected in DEC map:



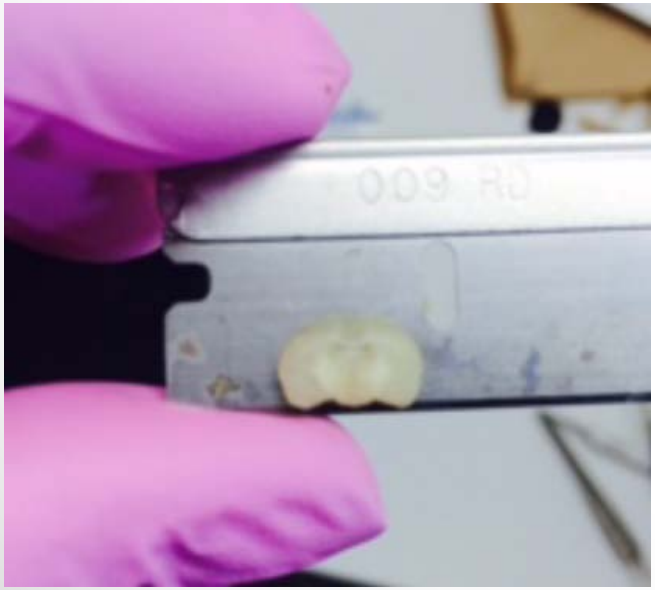
CC (1)
EC (2,3)
Cerebral Cortex (4,5)
Whole brain (6)

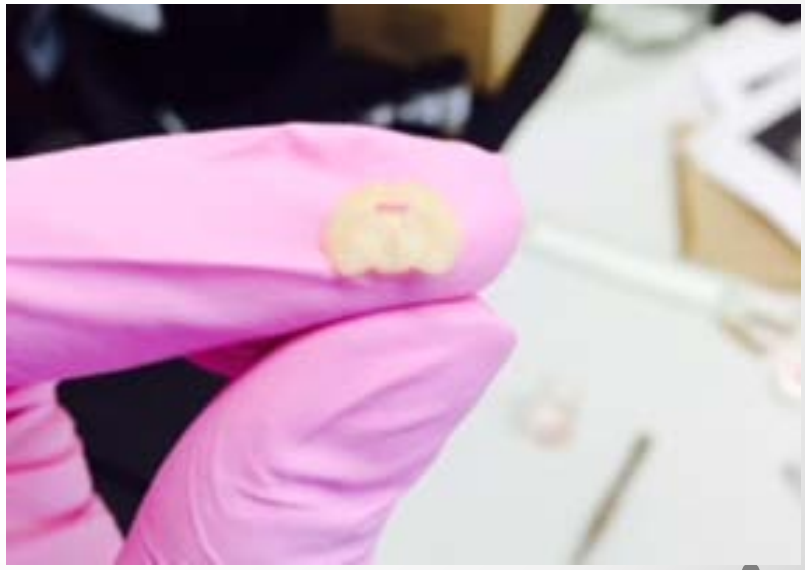
Methods

- ***MRI***
- All images were acquired on the same coronal slice with 98x98x750 μm^3 resolution.
- FOV
  - o $(2.5\ cm)^2$ *in vivo*
  - o $(1.25\ cm)^2$ *ex vivo*.
- Matrix size
  - o 256x256 *in vivo*
  - o 128x128 *ex vivo*.

Methods

- **Electron Microscopy**
- Following MRI, the brains were returned to 2% glutaraldehyde, the CC was dissected 9 months later and embedded in epoxy resin for EM.



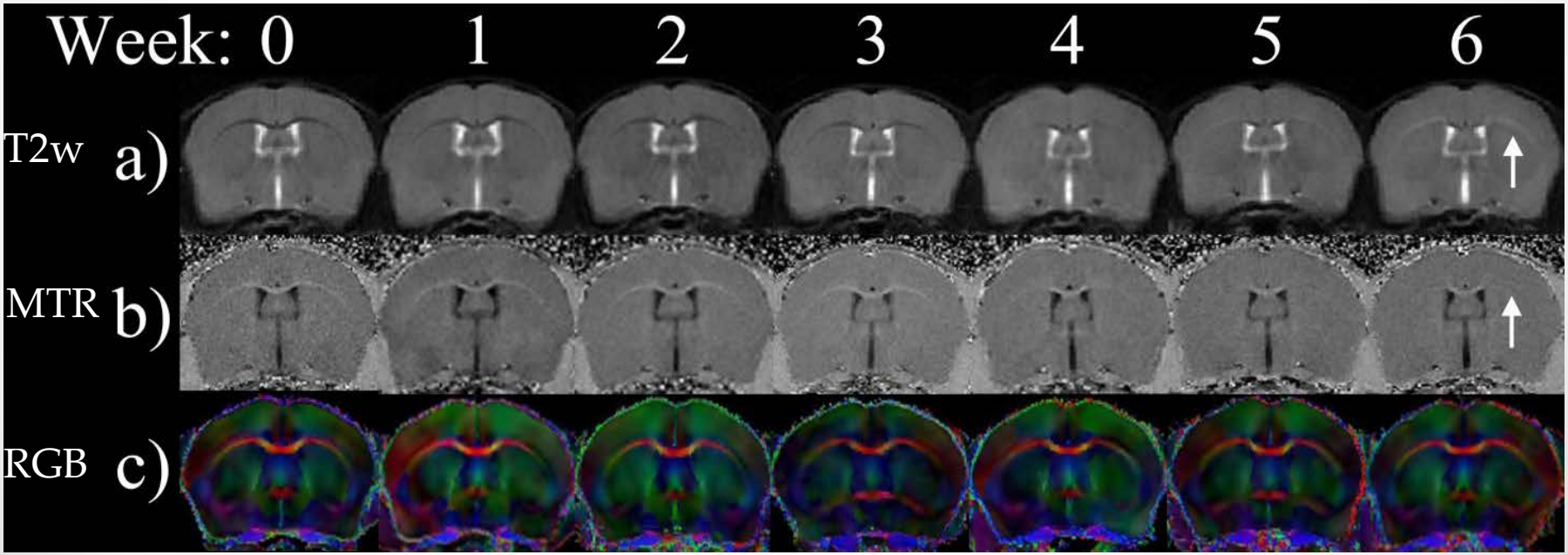


Methods

- MR metrics in each ROI were calculated
- Correlations between both longitudinal and quantitative datasets were measured in the **CC** and **EC**.

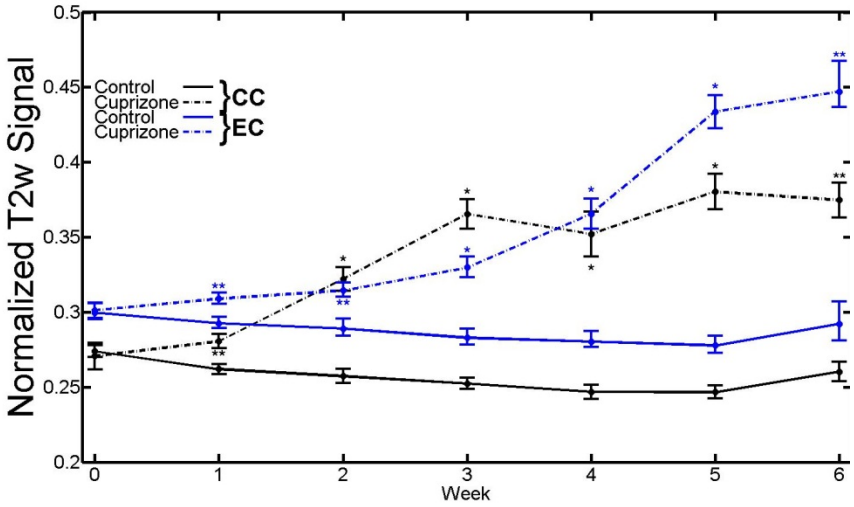
Results

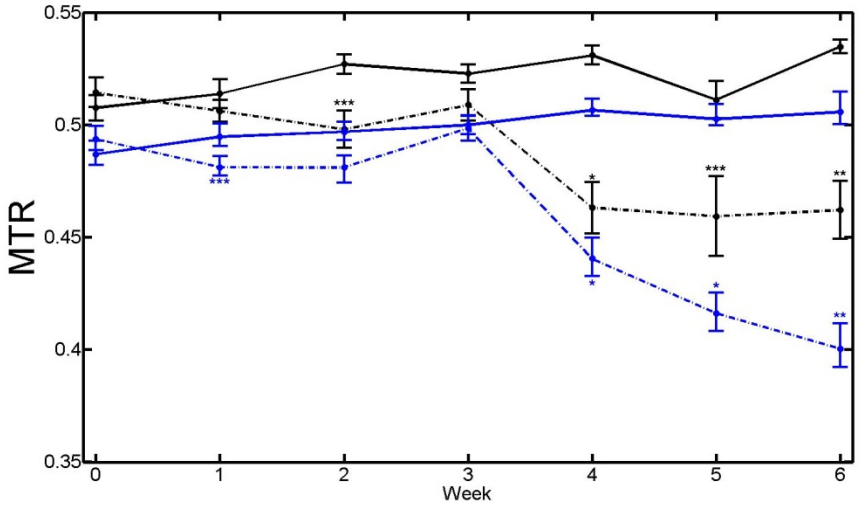
- **Representative normalized *in vivo* (a,b) and *ex vivo* (c) MR data from weeks 0-6.**



Results

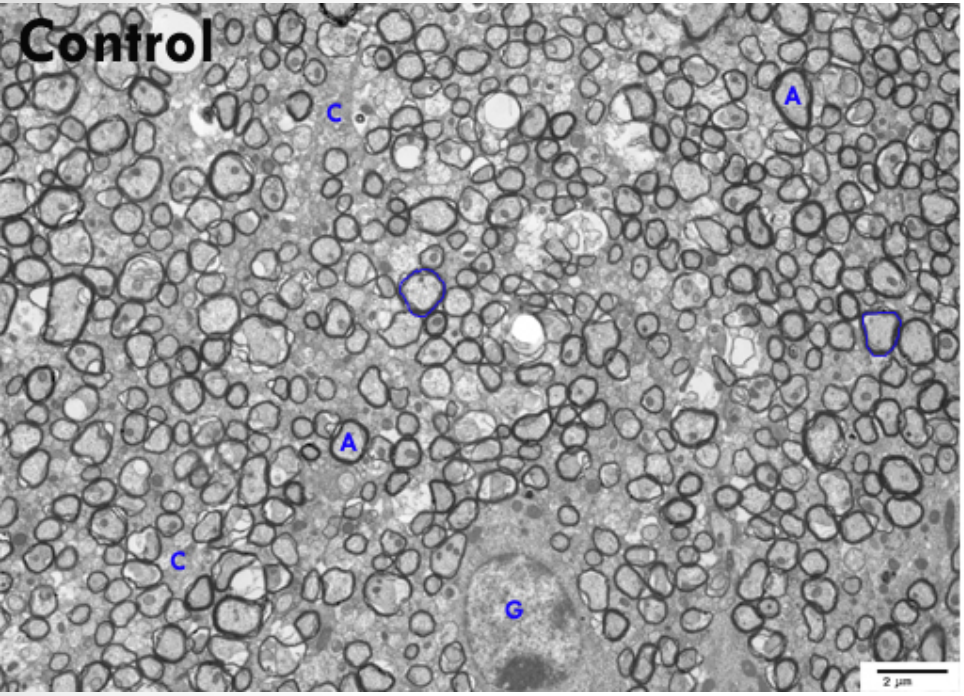
- **Weekly changes (mean ± SEM) in the CC (black) and EC (blue) in the CPZ (dotted) and CTL (solid) mice:**

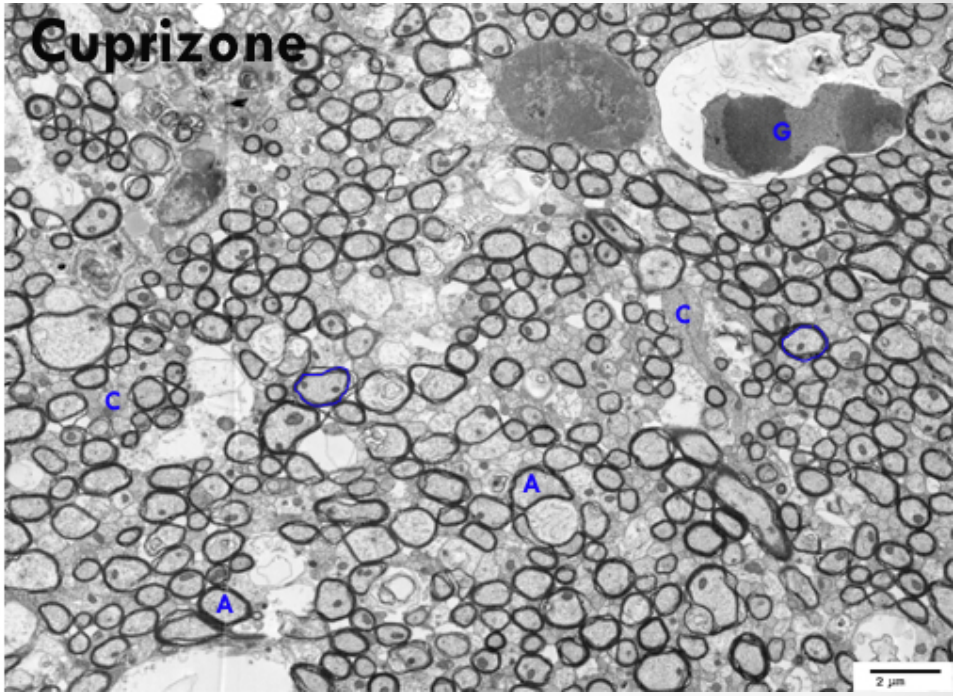




Results

- **EM of CTL and CPZ mice CC at week 3:**





- Myelinated axons (A) , Non-myelinated cells (C), Glia cell nuclei apoptosis(G), Myelin Sheath (outlines)
- Myelinated axons are apparent in both tissues.
- CPZ is associated with oligodendroglial swelling and apoptosis.

Discussion & Conclusions

- The different time courses of the MR metrics suggest that ***T_2 and MTR are sensitive to different pathological features in white matter***.
- EM analysis of the tissue is in progress for correlations with MRI metrics.
- Visually it can be seen in the EM images at week 3 that the CTL and CPZ CC show a similar amount of myelinated axons.

Discussion & Conclusions

- Our results are consistent with EM from other studies[4] suggesting ***MTR likely reflects demyelination.***
- 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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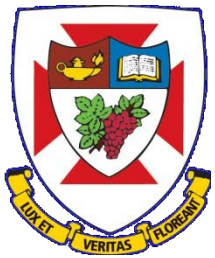

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Additional Information: Methods

- ***MRI parameters***
  - ***In vivo T2w*** RARE, NA=12, TE_{eff}/TR=80/1640 ms, RARE factor 8, 10 min.
  - ***In vivo MTI*** FLASH, NA=48, TE/TR=6/70 ms, 10° flip angle, one with an MT saturation pulse (Gaussian, 10.25 ms, 10 μT, 6 kHz off-resonance) and one without an MT saturation pulse, 2x14 min to calculate MTR maps.
  - ***Ex vivo T_1/T_2 Relaxometry*** Fit to a series of RARE images, TE_{eff}=11, 33, 55, 77, 99 ms; TR=5, 3, 1.5, 0.8, 0.4, 0.353 s; RARE factor 2; NA=8; 71 min.
  - ***Ex vivo qMTI*** 1 proton density image + 18 MTIs (5, 10, and 20μT and frequency offsets at each power of 1, 2, 4, 6, 10, and 30 kHz) NA=64 19x9.6 min;
  - ***Ex vivo DTI*** PGSE, tetraorthogonal gradient-encoding scheme, b-value=1000 s/mm^2 (δ=6 ms, Δ=14 ms), NA=6, TE/TR=26/5000 ms, 5 hr.
  - ***Ex vivo T2*** RARE, NA=36, TE_{eff}/TR=80/1640 ms, RARE factor 8, 31 min.