Multiexponential T2, Magnetization Transfer and Quantitative Histology in White Matter Tracts of Rat Spinal Cord

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Introduction

Multi-exponential T_2 (MET_2) and quantitative magnetization transfer (qMT) have been studied as MRI-derived reporters of myelin content in white matter (WM) and nerve. With MET_2, the relative size of short-lived T_2 component (typically, T_2 = 8 - 50 ms, depending on B_0) has been defined as the myelin water fraction (MWF) and interpreted as a direct measure of myelin content. Similarly, in WM, the macromolecular protons that exchange with water are thought to be substantially constituents of myelin. That is, the qMT measure of this macromolecular pool size relative to the total water signal, sometimes called the pool-size-ratio (PSR), is believed to be largely a measure of myelin content. While both MWF and PSR have been found to correlate with myelin content when comparing normal myelinated tissue with demyelinated or dysmyelinated tissue, the exact relationship between these measures and myelin content is not well understood. In particular, the interpretation of MWF as a measure of myelin content is predicated on the assumption of slow exchange between myelin water and other water. If the rate of water exchange in and out of myelin is dependent upon the myelin thickness (which tends to correlate with axon diameter), then the slow exchange model may hold better in some myelinated tissues than others. Also, it is well known that PSR does not go to zero in the absence of myelin, and the relationship between PSR and myelin may not be constant for all myelinated tissues. In order to investigate these questions, MET_2, qMT, and quantitative histological metrics, such as axon diameter and myelin thickness, were measured in excised samples of rat spinal cord. The spinal cord provides an excellent model of variation in white matter micro-anatomy because axons with different function (and, consequently different dimensions) are grouped into various tracts.

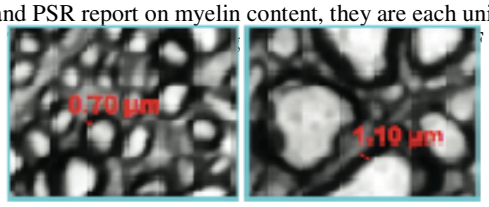
Methods

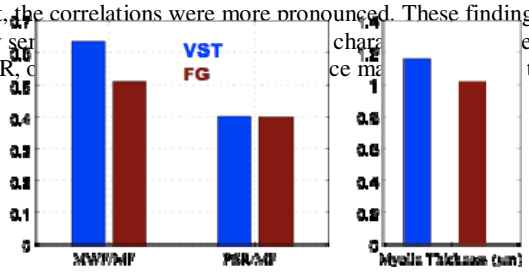
Six spinal cord samples were obtained from Sprague-Dawley rats (300 - 420 g). The animals were sacrificed with inhaled CO_2, the spinal cord was rapidly removed and cervical sections ≈ 1 cm in length were immediately placed in 0.5% paraformaldehyde/4% gluteraldehyde in phosphate buffer for 48 hrs then post-fixed in 1% osmium tetroxide in cacodylate buffer. Fixed samples were studied at 7 T using a home-built 10 mm diameter loop gap resonator. For both MET_2 and qMT imaging, 2 mm thick slices were chosen transverse to the long-axis of the spinal cord, and images were encoded using a 64 x 64 sampling over a 5 x 5 mm^2 FOV. MET_2 imaging was achieved using a single-slice 48-echo imaging sequence with appropriate spoiler gradients; first echo time at 8 ms, echo spacing of 9.2 ms up to 32 echoes, then echo spacing of 50 ms for the last 16 echoes. qMT imaging was performed using a single-slice selective inversion-inversion recovery sequence with fast spin-echo acquisition; 24 inversion times were pseudo log-spaced between 3.5 ms and 10 sec. Both sequences have been validated using phantoms as part of previous studies. After imaging, samples were dehydrated, embedded in epoxy resin, sectioned axially and stained with 1% Toluidine blue.

T_2 spectra were calculated on a voxel-by-voxel basis by fitting signal magnitudes from the 48 echo images to a broad distribution of T_2 values, log-spaced between 8 ms and 1 s using a non-negative least square method and regularized with a minimum curvature constraint. qMT analysis was also performed voxel-by-voxel by fitting the signal magnitudes of 24 images at different inversion-recovery times to a bi-exponential model. From the fitted parameters, the PSR extracted as defined in previous literature. MWF and PSR, as well as other parameters were tabulated in 5 different white matter tracts and across each of the 6 spinal cord samples. Quantitative histological evaluation included a combination of manual and automated image analysis to extract, amongst other parameter, fractional myelin area, mean myelin thickness, and mean axon diameter from each of the same 5 white matter tracts.

Results and Discussion

The figures below show example histology, MWFs and PSRs (normalized to myelin fraction, MF), and mean myelin thickness measured from two different WM tracts: VST and FG. These results summarize the broader findings, which is that MWF between different WM tracts varied not by total myelin content by rather by myelin thickness: MWFs were smaller relative to total myelin content in regions where axons were small and myelin was thin. This likely reflects the effects of inter-compartmental water exchange on the MWF measurements. In contrast, PSR estimates were relatively constant relative to total myelin content; however these values did not reduce to zero in regions of little or no myelin (grey matter). Across all WM tracts, MWF did not correlate strongly with PSR, but within each WM tract, the correlations were more pronounced. These findings indicate that while both MWF and PSR report on myelin content, they are each uniquely sen chara e tissue from which they are measured. or PSR, c ce ma than simply amount of myelin.





FG (left) and VST (right)