Quantitative assessment of the white matter damage following Dorsal Column transection in rat spinal cord using frequency shift mapping

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Introduction

Gradient echo phase images provide strong gray and white matter (GM, WM) contrast in brain [1] and spinal cord that is determined by the local MR resonance frequency. The influence of fiber orientation with respect to the main magnetic field has been shown to be an important source of the contrast in phase images [1-3]. Furthermore, tissue microstructure (anisotropic WM compared to isotropic GM) was shown to play a more important role in observed GM/WM contrast [3-5]. The well-defined architecture of the rat spinal cord offers a possibility to study the influence of microstructure in different regions of the spinal cord on MR frequency shifts after injury of WM tracts. Here we investigated a dorsal column transection (DC Tx) injury model in rat spinal cords, which produces progressive levels of axonal degeneration bi-directional to the injury site, using MR frequency [6].

Methods

Rat spinal cords were excised and fixed at 3 and 8 weeks following dorsal column transection (DC Tx) injury at the C5 level [6], with 2 rats per treatment and 2 healthy controls. 3D images were acquired at 7T (Biospec 70/30, Bruker, Germany) ranging from 10 mm distal and 10mm proximal to C5 with GE sequence with in plane resolution of 33µm × 33µm × 1000µm, TE = 11.6ms, TR = 35ms, 20° flip angle, and 16 averages. Phase images were unwrapped, homodyne filtered, and converted to frequency maps [1]. Region of interest (ROI) analysis in the fasciculus gracilis (FG, ascending) and corticospinal (CST, descending) tracts in the dorsal column was performed on 1mm-thick slices at 5mm from injury on distal and proximal sides, to obtain average frequency shifts and calculate frequency shifts normalized to gray matter within each individual slice. Cords were fixed in 24% sucrose and cryoprotected, with 20um-thick cross sections corresponding to the scanned GE slices stained using eriochrome cyanide (EC) for myelin [7]. Stained sections were imaged and digitized under halogen light with a Zeiss Axioplan 2 microsope with Northern Exposure software**.** Average optical densities in each ROI, normalized to healthy ventrolateral WM, were measured with Sigma Pro 5.0 software (SPSS Inc.). Correlation between frequency shifts and eriochrome stain optical density was tested with the Pearson coefficient after the data was tested for normality with Shapiro Wilks test.

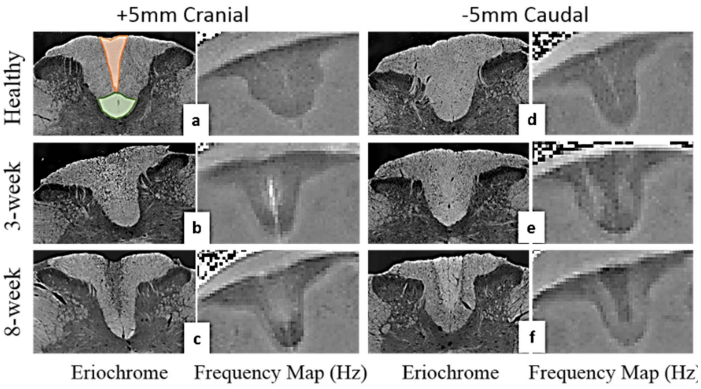

Figure 1: EC-stained sections and corresponding frequency maps for slices cranial (a-c) and caudal (d-f) to injury, with FG (ascending, orange) and CST (descending, green) tracts highlighted in (a).

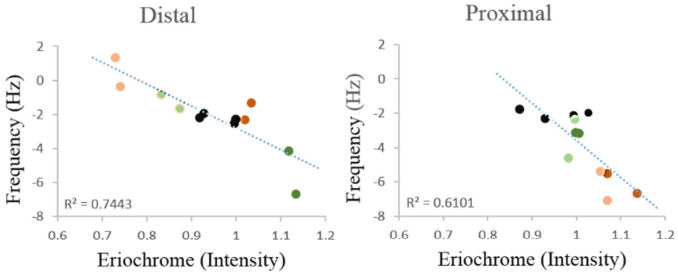

Figure 2: Frequency dependence on myelin content measured by eriochrome stain intensity. Tracts colored as in Fig. 1a, at 3-weeks (darker) and 8-weeks (lighter) post-injury. Healthy cords in black.

Results and Discussion

Distal to Injury: We observed decrease in the EC myelin stain intensity and increase in frequency shift in the FG (cranial, Fig. 1 b,c) and CST (caudal Fig. 1 e, f) WM tracts compared to healthy cords (Fig. 1 a,d).Wallerian degeneration occurs distal to the injury, causing full axon degeneration (3-4 days post-injury) and myelin sheath debris formation (several weeks post-injury). Anisotropic myelin sheaths loosen and swell, forming debris. At 3 and 8 weeks post injury the frequency shift increased distally in both tracts, and correlated strongly with the eriochrome myelin stain ($r = -0.8627$, $p < 0.0005$, Fig. 2), suggesting the main source of this increase comes from changing myelin structure caused by damage to myelin sheaths.

Proximal to Injury: Retrograde degeneration ('axonal die-back') occurs, characterized by slow gradual axonal loss leaving some myelin debris in its wake. In this case, damage to both myelin and axons contribute to frequency shift measured in FG caudally (Fig. 1 d-f) and CST cranially (Fig. 1 a-c). Though we expect some myelin debris proximal to injury, EC staining suggests the possibility that this debris formation is minimal compared to the presence of healthy intact myelin proximal to injury. However, further analysis of plastic embedded sections of myelin staining at higher magnification is required to distinguish between healthy and degenerated myelin. The observed negative frequency shift ($r = -0.7811$, $p < 0.005$, Fig. 2) could be caused by increases in the diamagnetic myelin, with additional contribution coming from the increasing proportion of the degenerated to healthy axons [8].

Conclusion

The resulting frequency shifts due to changes in myelin structure and axon integrity along the dorsal column is shown to correlate with histological stains for myelin, and provides a quantitative basis of assessing changes in local WM tissue microstructure. These preliminary results suggest that GE phase imaging of injured rat spinal cords provides a good model for assessing tissue microstructure contributions to MR frequency shifts.

References

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