Q-space Propagator Maps of Mouse Spinal Cord Provide Insight into Regional Axonal Architecture

H. H. Ong[1], A. C. Wright[1], S. L. Wehrli[2], A. Souza[1], E. D. Schwartz[1], P. K. Saha[1], F. W. Wehrli[1]

[1]Laboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]NMR Core Facility, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

Introduction

Regional white matter (WM) spinal cord (SP) axonal architecture information could be used to assess health or recovery from injury. Axon diameters in mammalian SP are on the order of 1-2 μm and therefore not amenable to direct observation by MRI. However, q-space imaging offers potential for indirect measurement by exploiting the regularity of spin diffusion restriction in porous systems as previously applied to material science[1]. In SP, the porous system consists of water restricted by axonal membranes/myelin sheaths. The Fourier Transform of the q-space echo attenuation is known as the propagator (Pg), which is a molecular displacement probability density function. Therefore, the Pg full-width-at-half-maximum (FWHM) should correlate with the scale of restrictions, which in this case is the axon diameter. Previous work has demonstrated that spectroscopic q-space imaging could differentiate SP WM tracts[2,3]. Here we report, using a home-built 50T/m z-gradient/RF coil set[4], high-resolution Pg maps ($\Delta x=0.65\mu m$) of cervical and thoracic SP regions for three healthy adult mice. The Pg FWHM was assigned to each pixel location. Focusing on three WM tracts that showed characteristically different mean axon diameters (cuneatus, gracilis, and rubrospinal tract (RST)), the Pg maps showed significant differences in FWHM between each region that correlated with mean diameters as computed from histologic images.

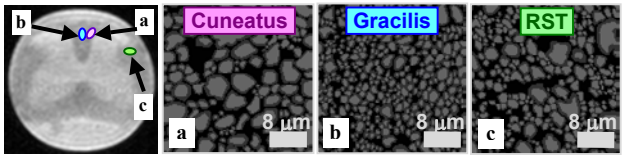

Fig. 1 Stimulated-echo image (left) of a mouse cervical SP indicating anatomic locations of cuneatus (a), gracilis (b) and RST (c) tracts along with their segmented histologic images.

Method

Three SPs were dissected from 8-10 month-old female C57 BL/6 mice that were perfusion-fixated. Optical microscopic images were obtained for one SP from the cervical and thoracic sections. Cuneatus, gracilis, and RST ROIs were selected, manually segmented (Fig.1), and mean axon diameters (with standard deviation) calculated. SP segments (~3*mm* long) were imaged with saline buffer. The experiments were performed with a custom 50 T/m z-gradient/solenoidal RF coil (3 *mm* i.d. sample bore) set designed for high-resolution q-space imaging interfaced to a 9.4 T spectrometer/micro-imaging system (Bruker DMX 400 with Micro2.5 gradients and BAFPA40 amplifiers). A diffusion-weighted stimulated-echo sequence was used: 64x64, sw=25kHz, TR=2s, TE/Δ/δ/TM=15/15/0.75/9*ms*, FOV/THK=3/1*mm*, and an ambient temperature of 19 °C. Images were zero-filled to 128x128. The diffusion gradient was applied along the z-axis (perpendicular to SP long axis) in 64 increments of q (q_{max}=1.53 μm^{-1} at 49 T/m). The q-values were numerically calculated from the actual amplifier output as monitored on an oscilloscope[5]. Q-space was zero-filled to 128 q-values. A q-space attenuation plot was obtained for each pixel and Fourier transformed to produce the Pg. FWHM, kurtosis, and zero probability of the Pg were then assigned to that pixel location. ROIs (10-30 pixels, after zero-filling) were selected within the cuneatus, gracilis, and RST tracts and the various parameters were recorded. All image processing was performed in IDL.

With such high q_{max}, SNR becomes the limiting factor. Imaging parameters were carefully chosen to avoid WM signal below the noise floor at higher q-values. To test the technique, a spectroscopic q-space experiment was performed with a packed bead (4.5 μm dia.) phantom immersed in 1mM GdDTPA doped water. The expected diffraction pattern was obtained (data not shown). Furthermore, ADC maps of water were successfully acquired that agreed with water ADC literature values (data not shown).

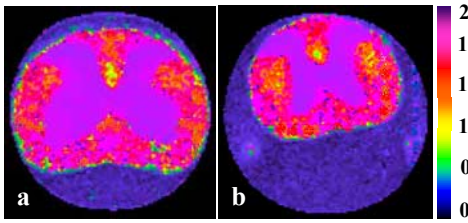

Fig. 2 Pg maps for cervical (a) and thoracic (b) regions of a mouse SP. A non-linear color scale (in μm) was chosen to highlight regional differences. Diffusion sensitization was perpendicular to SP axis (vertical image axis).

Table 1. Cervical Spinal Cord Measurements

Region	Propagator FWHM (μm)	Histologic Mean Axon diameter (μm)	Zero Probability (a.u.)	Kurtosis (a.u.)
Cuneatus	1.33 ± 0.02	1.63 ± 1.02	0.065 ± 0.003	16.6 ± 1.0
Gracilis	1.06 ± 0.05	0.90 ± 0.43	0.087 ± 0.002	25.6 ± 1.9
RST	1.20 ± 0.02	1.09 ± 0.6	0.082 ± 0.002	20.6 ± 0.7

Table 2. Thoracic Spinal Cord Measurements

Region	Propagator FWHM (μm)	Histologic Mean Axon diameter (μm)	Zero Probability (a.u.)	Kurtosis (a.u.)
Cuneatus	1.36 ± 0.05	1.33 ± 0.85	0.067 ± 0.001	16.8 ± 1.2
Gracilis	1.22 ± 0.04	1.05 ± 0.41	0.077 ± 0.006	20.9± 0.2
RST	1.24 ± 0.04	1.19 ± 0.73	0.073 ± 0.007	18.8 ± 1.8

Results and Discussion

Fig. 2 shows the displacement maps for the cervical and thoracic SP regions for one specimen. Each specimen exhibited the expected symmetry of displacements about the anterior-posterior axis. Tables 1 and 2 list the data calculated for the cervical and thoracic SP regions, respectively. With the exception of histologic mean axon diameter, listed values are an average across three specimens. Within a ROI, mean Pg FWHM, kurtosis and zero probability values correlated well with the histologic mean axon diameters. Unpaired t-tests were run between mean Pg FWHM values for various WM tracts among three specimens (cuneatus vs gracilis, RST vs cuneatus, and gracilis vs RST for both cervical and thoracic regions). With the exception of thoracic RST vs gracilis tracts, each region was significantly different from the others. Among the significant comparisons, cervical p-values ranged from 0.001 to 0.013 and thoracic p-values ranged from 0.015 to 0.029. Furthermore, unpaired t-tests between mean Pg FWHM values of the same ROI in cervical and thoracic regions showed no significant difference, except for the gracilis tract ($p = 0.012$). It should be noted that while Pg FWHM correlates with axon diameter, further investigation is needed to understand the exact relationship between FWHM and microarchitecture.

Conclusion

With increasing use of mouse models for the study of gene expression, knowledge of mouse SP micro-architecture is potentially valuable for the assessment of SP function. This work demonstrates the feasibility of generating mouse SP q-space Pg maps with sufficient resolution to distinguish WM tracts differing in mean axon diameters.

References: 1. Callaghan, PT, *Principles of NMR Microscopy*, Oxford University Press (1991). 2. Assaf Y, *et al*, *MRM*, **44**:713-722 (2000). 3. Chin CL, *et al*, *MRM*, **52**:733-740 (2004). 4. Wright AC, *et al*, Proc. ISMRM 12th Scientific Meeting, Kyoto, Japan, 2004, p. 741. 5. Ong H, *et al*, Proc. ISMRM 13th Scientific Meeting, Miami, USA 2005, p. 2. **Acknowledgements:** NIH grant R21 EB003951