Investigating White Matter Diffusion Anisotropy Using the Dysmelinating Shiverer Mutant Mouse

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Introduction - The origin of white matter diffusion anisotropy has recently been a topic of current interest. The debate centers upon identifying the respective roles of the myelin sheath and the axoplasmic membrane, as well as other structures, such as neurofilaments, that may give rise to the observed diffusion anisotropy. In this study we describe preliminary diffusion measurements in the shiverer mutant mouse (shi) that can potentially shed light onto these questions. The shiverer has virtually no compacted myelin in the central nervous system (CNS) due to its inability to produce a major protein component of the myelin sheath, myelin basic protein (MBP). The absence of MBP is due to a deletion of the majority of the MBP gene [1]. The myelin that does exist is loose and uncompacted. In contrast, myelin in the peripheral nervous system (PNS) is nearly normal. These mice have a motor tremor, tonic seizures, and die at approximately 90 days. The shiverer is an ideal system to investigate how the absence of the myelin sheath effects both the magnitude and anisotropy of measured diffusion coefficients within CNS fiber tracts. In our initial investigation, water self-diffusion in fixed spinal cords from shi and wild-type (WT) mice was studied using microscopic diffusion tensor imaging (DTI) [2].

Methods - Wild-type (B10.PL) and shi spinal columns were prepared by perfusing anesthetized mice through the left ventricle with phosphate-buffered solution (PBS) followed by Trump's fixative (4% paraformaldehyde, 1% glutaraldehyde, in 0.1 M phosphate buffer, pH 7.4). The lumbar regions of the columns was excised, immersed in fixative, and stored at 4 °C for 36 hours before imaging. Imaging was performed on dorsal column segments that were sealed in a quartz tube surrounded by PBS. Images were acquired using a Bruker AMX500 microimaging system with a wide-bore (89 mm) 11.7 T magnet, a laboratory-built 5 mm solenoid RF probe, a laboratory-built low-noise preamplifier, and a shielded gradient coil. In each image plane, a series of (29) diffusion-weighted images (DWIs) were acquired using a 2DFT-PGSE sequence with diffusion gradients applied along seven noncollinear directions [3], b-values [2] ranging from $0 \le b \le 2000$ s/mm^2, and with diffusion gradient pulses set to a width and separation of $\delta=2$ ms and $\Delta=7.4$ ms, respectively. Transverse DWIs were acquired with 256×256 points, 20 μm in-plane resolution, 300-μm-thick slices, and TE/TR=16/2000 ms. From the DWIs, the effective diffusion tensor within each voxel was calculated using a nonlinear fitting algorithm described elsewhere [3]. The sample temperature was maintained at 8 °C.

Results - Anatomical (T_2/spin-density-weighted) and eigenvalue images (principal diffusivities), extracted from the effective diffusion tensor are displayed below. These are from the lumbar region. In shi, the anatomical images show negligible contrast between gray and white matter, which is striking in comparison to WT. Note that shi shows distinct contrast between spinal nerves and gray/white matter because the PNS is properly myelinated. The eigenvalue images ($\lambda 1, \lambda 2, \lambda 3$) are shown for each slice, where the grayscale indicates faster diffusion for higher intensity. The shi eigenvalue images look qualitatively similar to WT; white matter and spinal nerves show highly anisotropy diffusion, whereas gray matter exhibits nearly isotropic diffusion. ROI analysis within various tracts confirms this impression. Values of the anisotropy index (AI), defined as $2\lambda_1/(\lambda_2+\lambda_3)$, and the trace of the effective diffusion tensor $\mathrm{Tr(D)}=1/3(\lambda_1+\lambda_2+\lambda_3)$ ($\times 10^{-3}$ mm^2/s) for gray and white matter are shown in the table below. These values represent averages of approximately 30 voxels within each ROI and of four image slices per spinal cord. In WT, we have also averaged over 4 animals. Thus, in shi the AI and Tr(D) are comparable in magnitude to WT within uncertainties and indicates that the absence of compacted myelin does not substantially change the observed magnitude and anisotropy of diffusion. This conclusion is consistent with spectroscopic studies in other systems [4,5].

		Dorsal Column	Ventral Column	Lamina 7
WT	AI	8.5 ± 2	5.9 ± 2	1.3 ± 0.1
	Tr(D)	0.25 ± 0.02	0.23 ± 0.02	0.21 ± 0.01
shi	AI	5.5 ± 1	4.7 ± 1	1.3 ± 0.3
	Tr(D)	0.28 ± 0.03	0.29 ± 0.05	0.22 ± 0.01

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