Reproduciblity of *in vivo* DTI mouse spinal cord

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Introduction

Diffusion tensor imaging (DTI) has been widely used to investigate central nervous system (CNS) disorders, offering considerable insight into underlying pathology (1). Recently, *in vivo* diffusion weighted imaging (DWI) performed parallel vs. perpendicular to mouse spinal cord demonstrated enhanced signal attenuation. Considerable gray vs white matter contrast was obtained with an image resolution of $0.125 \times 0.125 \times 1$ mm^3 (2). However, DTI of mouse spinal cord *in vivo* has not been reported. Herein we describe DTI with mouse spinal cord *in vivo* and measure the axial diffusivity ($\lambda_{\parallel} = \lambda_1$), radial diffusivity [$\lambda_{\perp} = (\lambda_1 + \lambda_2)/2$], trace of diffusion tensor (Tr), and relative anisotropy (RA) of lumbar segment white matter. Intra-animal reproducibility of the *in vivo* DTI mouse spinal cord measurement is evaluated.

Methods

Animal Preparation

Ten week old female C57BL/6 mice (n=5) were employed for *in vivo* DTI spinal cord measurements. Animals were fitted with a custom nose cone to deliver inhalant anesthetics (isoflurane/oxygen mixture, 7% for induction and 0.7–1.5% for maintenance) and to monitor respiration pressure. DTI data collection was synchronized with respiration to eliminate artifacts caused by respiratory motion (3). The core temperature was maintained at 37.0 ± 0.5°C with a circulating warm water pad. An inductively coupled surface coil (20 mm × 8 mm) was used as the signal receiver. Its sensitivity profile covered lumbar cord segments 1-3. A 9 cm i.d. Helmholtz coil was utilized as the RF transmitter.

Diffusion Tensor Imaging

A spin-echo imaging sequence modified with Stesjkal-Tanner diffusion-sensitizing gradients was used to acquire the respiratory-gated diffusion-weighted images. The acquisition parameters were: repetition time (TR) 450 ms (set according to the respiratory rate), spin echo time (TE) 38 ms, time between application of gradient pulses (Δ) 20 ms, diffusion gradient on time (δ) 7 ms, 8 scans averaged per *k* space line, slice thickness 0.75 mm, field of view 1 cm, data matrix 128 × 128 (zero filled to 256 × 256). Images were obtained with diffusion sensitizing gradients applied in six directions: (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0]), (0,-1,1), and (1,0,-1). Two diffusion-sensitizing factors, or *b* values, were used: 0 and 0.785 μm^2/ms. Each multi-slice DTI data set (total of 12 slices) covering L1 - L3 of the cord was obtained with an acquisition time of *ca.* 2 hours.

Data Analysis

Lumbar cord sections were identified in reference to the location of the ilium identified on a coronal scout image (Fig. 1A). Twelve 0.75 mm thick transverse image slices were prescribed to cover vertebral segments L1 through L3. This procedure was carefully conducted on all mice examined. Values of RA, $\lambda_{\parallel}$, $\lambda_{\perp}$, and Tr were derived from each slice from regions of interest (ROIs) defined for dorsal, ventral, left lateral, and right lateral white matter.

The reproducibility of *in vivo* DTI measurements was examined using the method of Bland and Altman (4). Briefly, all parameters from each of the 12 image slices were compared between two measurements performed three days apart. For each parameter, differences between the two measurements were plotted against their mean. Results were examined to ascertain whether intra-animal measurements were within the 95% confidence limit.

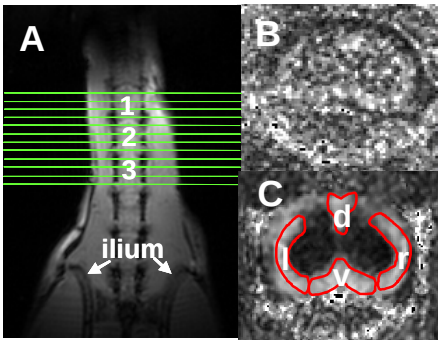

Figure 1. (A) The internal landmark, ilium, used for localization of mouse spinal cord at the lumbar level. Green lines mark the cord at lumbar 1-3 levels with a 0.75 mm slice thickness. The relative anisotropy (RA) maps of a normal mouse spinal cord without (B) and with (C) respiratory gating are shown. The ROI are defined (C): dorsal (d), left-lateral (l), right lateral (r), and ventral (v).

Results and Discussion

RA maps acquired with/without respiratory gating demonstrates the benefit from elimination of respiratory motion effects. Motion associated with respiration introduces substantial noise in the RA map obtained without gating (Fig. 1B), whereas the delineation of gray and white matter is easily achieved in the respiratory-gated acquisition (Fig. 1C). As expected, white matter has a high anisotropy indicated by its bright appearance in the RA map while the gray matter in the central region of the cord has a much lower anisotropy. Consistent with measurements of white matter in mouse brain, diffusion in the white matter of the spinal cord is highly anisotropic with the axial diffusivity being ~ 5-fold greater than radial diffusivity (Fig. 2). The intra-animal DTI parameters obtained from repeated measurements were not statistically different at the 95% confidence level (Fig. 3). The present study demonstrates that DTI of mouse spinal cord *in vivo* can be achieved with sufficiently high resolution and precision. Furthermore, the derived DTI parameters are highly reproducible and comparable to values reported for the white matter in the brains of mice.

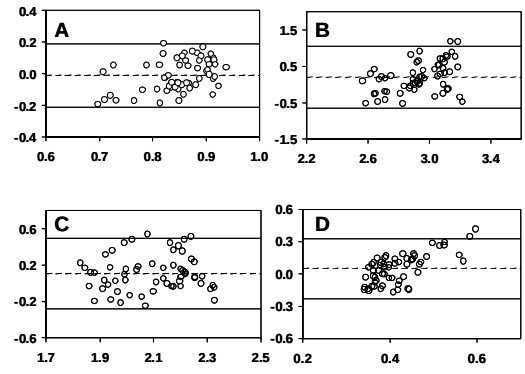

Figure 3. Bland and Altman test results: (A) relative anisotropy, (B) trace, (c) axial diffusivity, and (D) radial diffusivity. The horizontal dashed line across the middle is the mean difference of the two measurements. The horizontal axis is the mean of the two measurements. Top and bottom lines indicate 95% confidence limits.

References

1. Song *et al.*, *Neuroimage*, 20:1714-1722 (2003).
2. Bonny *et al.*, *Neurobiol. Dis.*, 15(3): 474-482 (2004).
3. Garbow *et al.*, *Concepts in Magn. Reson., Part B: Magn. Reson. Eng.* 21B:40-48 (2004).
4. Bland *et al.*, *Lancet*, 1: 307-310 (1986).

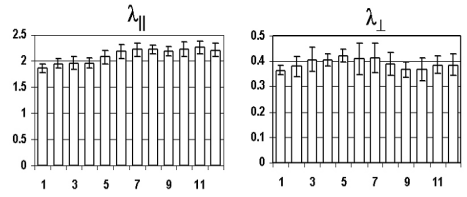

Figure 2. Bar graphs of spinal cord $\lambda_{\perp}$ and $\lambda_{\parallel}$ values from measurements of five normal mice. Bars represent mean ± standard deviation. Vertical axis: μm^2/msec. Horizontal axis: relative slice position starting with L1 = slices 1 – 4, L2 = slices 5 – 8, and L3 = slices 9 –12.