In Vivo Diffusion Tensor Imaging of the Rat Spinal Cord – Pilot Study

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Abstract. *In vivo diffusion tensor imaging (DTI) of rat thoracic spinal cord was performed on five healthy rats. Axial diffusion images were obtained using a standard spin echo diffusion weighted sequence. The fractional anisotropy (FA), longitudinal (LD) and transverse (TD) diffusivities and the mean diffusivity were estimated. Differences in these parameters between white and grey matter were in accord with theory and in the range reported in literature.*

Keywords: spinal cord, magnetic resonance imaging, diffusion tensor imaging

1. Introduction

Diffusion tensor imaging (DTI) is a magnetic resonance (MR) technique capable of measuring the magnitude and direction of diffusion of water molecules in various tissues. DTI was developed from diffusion weighted (DW) imaging, which measures the attenuation of MR signals caused by diffusion of water molecules, and was initially used for brain imaging [1].

DTI studies of spinal cord and DTI tractography can be useful in probing the integrity of white matter fiber tracts in traumatic spinal cord injuries (SCI) [2]. In the published studies both spin-echo (SE) [3,4] and echo-planar imaging (EPI) pulse sequences [5] have been presented. To minimize possible geometry distortion related to EPI, SE sequence was preferred in our study. The purpose of this pilot study is to establish and optimize DTI acquisition in rat spinal cord at our institution using our equipment. Our motivation is to step forward to in vivo rat spinal cord tractography. To the authors' knowledge so far only one in-vivo tractography of the rat spinal cord was performed [6] and the reproduction of its results is hardly possible due to the incomplete description of its acquisition and post-processing methods and parameters.

2. Methods

Animal Preparation and MRI

For this pilot study, 5 female healthy rats (250-300 g) were used. A surface coil custom made in the Institute for Clinical and Experimental Medicine (IKEM, Prague, CZ) was used for both transmitting and receiving of the radiofrequency signal. Local excitation was used to eliminate the motion artefacts due to the beating heart by not exciting it. The rats were anesthetized with isoflurane and positioned supine over the surface coil. The center of the coil was placed in the thoracic area of the spine. The rat was maintained under anesthesia (2.5% isoflurane) for the duration of imaging. A respiratory sensor (SA Instruments, Stony Brook, NY, USA) was taped over the abdomen and a rectal temperature probe (SA Instruments, Stony Brook, NY, USA) was inserted for monitoring of the animal. All experiments were in accordance with national legislature.

All imaging was performed on a 9.4T NMR system (Bruker-Biospec 94/30 USR by Bruker, Ettlingen, Germany). Fast low-angle shot (FLASH) IntraGate was used for scout imaging to locate the spinal cord. Rapid Acquisition with Refocused Echoes (RARE) sequence was used

for anatomical images of the spinal cord in the sagittal and coronal views. The parameters of the RARE sequence were as follows: TR/TE = 1300/46.6 ms, slice thickness = 0.5 mm, matrix = 256×256 and field of view = 4.98 cm × 4.98 cm, where TR and TE are the repetition and echo times, respectively. Then a standard multislice spin-echo DW sequence based on the Stejskal-Tanner diffusion preparation [7] was used. The parameters of the DTI sequence were as follows: TR/TE = 1000/28.7 ms, slice thickness = 2.0 mm, matrix = 128×128, field of view = 2.22 cm × 2.46 cm, diffusion gradient duration = 4 ms, number of b=0 images = 3, diffusion gradient separation 15 ms and b-value = 800 s/mm^2. For higher b-values, the signal to noise ratio in the acquired images was too low for a reliable DTI analysis. Images were acquired in six DW directions ([±0.33, 0.67, 0.67], [0.67, ±0.33, 0.67], [0.67, 0.67, ±0.33]). Adding directions to the DTI experiment would provide more accurate results. Nonetheless, in vivo measurements are time-limited and for the spinal cord six directions seemed to be sufficient, according to our results. The inter-slice thickness was not standardized in this pilot study. The sequence was respiration gated to avoid motion artefacts. The gating was set such that two excitations fit in one breathing cycle. The acquisition time of the DTI sequence was around 45 minutes, depending on the breathing frequency.

DTI Processing

DWI data were processed in the FSL software (Analysis group, FMRIB, Oxford, UK) to create fractional anisotropy (FA) map, eigenvalue (ε_1, ε_2, ε_3) maps and their eigenvector (v_1, v_2, v_3) maps. The longitudinal (LD) and transversal (TD) diffusivity (defined as the largest eigenvalue ε_1, and the average of the remaining two eigenvalues, $(\varepsilon_2 + \varepsilon_3)/2$, respectively) were calculated. Mean diffusivity (MD) was determined as the average of the eigenvalues, $(\varepsilon_2 + \varepsilon_3 + \varepsilon_3)/3$. [4] All maps were imported into the ImageJ software (National Institutes of Health, Bethesda, MD). For region of interest (ROI) analysis, two slices were selected for each animal. ROIs were drawn using ImageJ according to Fig. 1. An example of an eigenvector map is shown in Fig. 2.

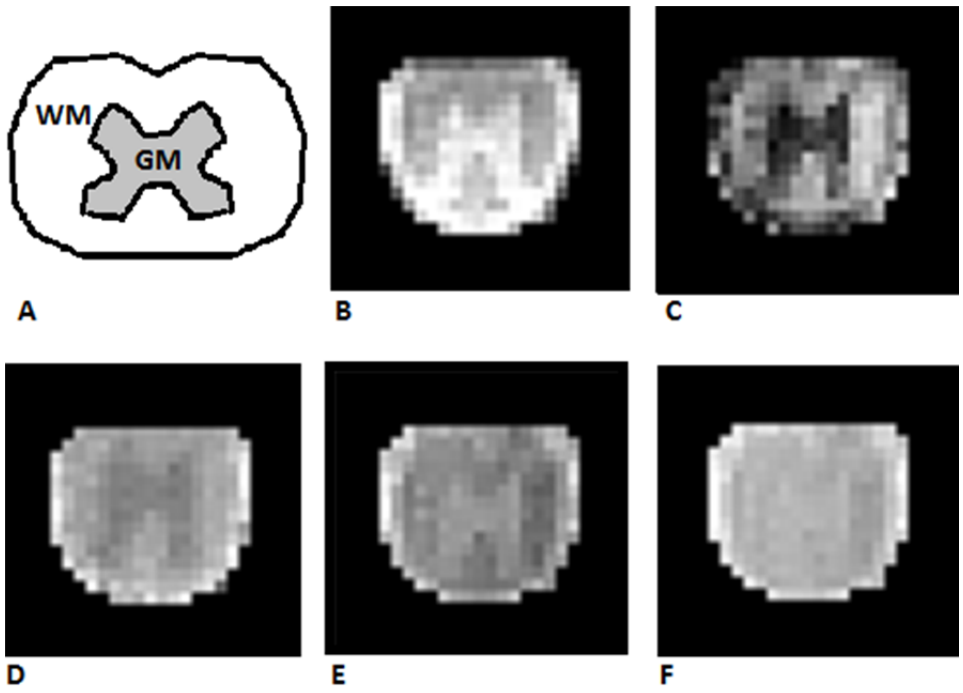

Fig 1. A: Schematic of the rat thoracic spinal cord section: WM – white matter, GM – grey matter. B: Anatomical image of the thoracic spinal cord. C-F: Maps of diffusion parameters. C: fractional anisotropy (FA) map. D: longitudinal diffusivity (LD) map. E: transverse diffusivity (TD) map. F: mean diffusivity (MD) map.

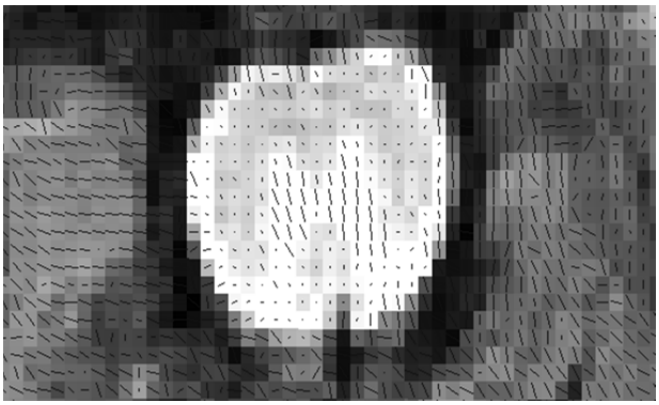

Fig 2. Map of eigenvectors of the highest eigenvalue (lines) with anatomical image of the rat thoracic spinal cord section in the background. If the vector is displayed as a dot, it indicates direction perpendicular to the imaging plane.

3. Results

The resulting diffusivity maps are shown in Fig. 1. The contrast between WM and GM was observed in the FA, LD, TD and partly in the MD maps. FA and LD are clearly higher in WM than in GM which corresponds to the rostral-caudal tracts in WM. This phenomenon can be also seen in Fig. 2 (eigenvector in WM mostly oriented along these tracts). The average DTI values for each rat are summarized in Table 1 and 2 for WM and GM, respectively.

Table 1. Values of diffusion parameters for WM. In the last four rows literature values are stated.

Rat no.	FA	LD [$\times 10^{-3}$ mm^2/sec]	TD [$\times 10^{-3}$ mm^2/sec]	MD [$\times 10^{-3}$ mm^2/sec]
1	0.73±0.11	2.31±0.58	0.65±0.23	1.48±0.31
2	0.77±0.14	2.20±0.34	0.54±0.21	1.37±0.18
3	0.88±0.16	2.46±0.60	0.30±0.30	1.37±0.33
4	0.67±0.16	1.98±0.55	0.63±0.25	1.30±0.30
5	0.68±0.17	1.84±0.38	0.65±0.18	1.20±0.19
Gullapalli[3]	0.79±0.02	1.80±0.08	0.22±0.03	1.01±0.02
Ellingson[4]	0.696±0.005	0.851±0.008	0.230±0.003	0.437±0.004
Mogatadakala[5]	0.83±0.052	1.94±0.115	0.29±0.069	0.86±0.056

Table 2. Values of diffusion parameters for GM. In the last two rows literature values are stated.

Rat no.	FA	LD [$\times 10^{-3}$ mm^2/sec]	TD [$\times 10^{-3}$ mm^2/sec]	MD [$\times 10^{-3}$ mm^2/sec]
1	0.56±0.11	1.90±0.27	0.82±0.17	1.36±0.10
2	0.48±0.13	1.63±0.20	0.80±0.14	1.21±0.08
3	0.63±0.15	1.77±0.38	0.62±0.18	1.18±0.18
4	0.40±0.12	1.36±0.16	0.77±0.10	1.06±0.08
5	0.48±0.14	1.53±0.27	0.78±0.09	1.15±0.13
Ellingson[4]	0.589±0.008	0.724±0.008	0.262±0.004	0.416±0.004
Mogatadakala[5]	0.38±0.039	1.49±0.106	0.77±0.116	1.04±0.071

4. Discussion

We have described a pilot DTI study of a rat spinal cord in the thoracic region. We have found expected differences in diffusion properties between the spinal cord WM and GM. WM showed higher FA and LD and lower TD, which corresponds to the rostral-caudal orientation of the fibre tracts in WM [2]. This was also in line with the main direction of the diffusion tensor (Fig. 2).

Our results both in WM and GM were close to literature values (Tables 1, 2). In particular, the FA values were fairly close to the listed published values. Other diffusion parameters show high variance between different authors and it can be seen that our values are consistent with the literature ranges. Overall the variability of the diffusion parameters within the ROIs and within the animal group shows fairly high reproducibility of our experiment.

In conclusion, the presented method seems to be eligible for SCI imagining. For this purpose, the measuring protocol has to be standardized, especially the positioning of the slices so that the DTI analysis is done in the same locations of the same vertebrae. For DTI tractography, more slices will be necessary. For in-vivo measurement, the acquisition time should not exceed 150 minutes. This means that with the present DTI parameters we would be able to scan at least 12 slices in one experiment. For the current slice thickness of 2.0 mm, the overall length of the imaged spinal cord would be 24 mm, which is sufficient to cover the whole lesion, which size is planned to be less than 20 mm [8]. The goal of our study is to use DTI for evaluating the integrity of white matter tracts after experimental SCI. For blunt injuries, DTI may represent a non-invasive method for longitudinal follow up after SCI.

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