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Diffusion Tensor Imaging of Mouse Brain Stem and Cervical Spinal Cord

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Abstract

In vivo diffusion tensor imaging measurements of the mouse brain stem and cervical spinal cord are presented. Utilizing actively decoupled transmit/receive coils, high resolution diffusion images ($117 \times 59 \times 500 \mu\text{m}^3$) were acquired at 4.7 T within an hour. Both brain stem and cervical spine displayed clear gray-white matter contrast. The cervical spinal cord white matter showed similar tissue characteristics as seen in the thoracic cord. The coherent fiber orientation in the white matter was observed in both the brain stem and the cervical spinal cord. The results may serve as a reference for future inter-lab comparison in mouse brain stem and cervical spine diffusion measurements.

Keywords

Diffusion tensor imaging; Mice; Brain stem; Cervical spinal cord; Axial and radial diffusivity; Diffusion anisotropy

1. Introduction

Diffusion tensor imaging (DTI) is one of the most versatile magnetic resonance imaging (MRI) modalities for longitudinal evaluations of central nervous system (CNS) disorders in rodent models of human diseases and in patients (Dong et al. 2004; Le Bihan et al. 2001). Recently, *in vivo* DTI derived $\lambda_{\parallel}$ and $\lambda_{\perp}$ have been shown to correlate well with axon and myelin integrity respectively (Kim et al. 2006). Findings from animal models suggest that *in vivo* DTI acquisition is well suited to detect CNS white matter injury. Most preclinical rodent DTI studies have been conducted on brains since respiratory and cardiac motions are less of a concern. Efforts to overcome motion artifacts and characterize diffusion properties in the spinal cord using DTI have been reported in rats (Ellingson et al. 2008; Franconi et al. 2000; Gullapalli et al. 2006; Madi et al. 2005).

In the use of rodents for preclinical studies, mouse models of human diseases have been widely employed because of its low cost, easy handling, and the readily achieved genetic manipulation. Numerous experimental mouse models have been developed for CNS disorders including

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multiple sclerosis (Cross et al. 1993), amyotrophic lateral sclerosis (ALS) (Bruijn et al. 2004), traumatic spinal cord injury (Kim et al. 2007a), etc. Studies have been performed with transgenic, and wild type mice to investigate the underlying mechanism responsible for the neurological deficit and to pursue the effective therapy (Halder et al. 2007). Damages to the cervical spine are commonly seen in multiple sclerosis, traumatic spinal cord injury, and neurodegenerative diseases. For example, motor neuron degeneration from cortex to brain stem with reported spinal cord lesions has been reported in ALS (Bruijn et al. 2004). As mouse models of human CNS lesions are disseminated temporally and spatially mimicking human clinical cases, a noninvasive assessment of the axonal and neuronal change in cervical cord and the upper and lower brain is critical to understand the fundamental morphological and pathophysiological changes.

Compared to rats (Ellingson et al. 2008; Franconi et al. 2000; Gullapalli et al. 2006; Madi et al. 2005), in vivo DTI observations of mouse spinal cord have been less common with only ex vivo (Niessen et al. 2006; Ong et al. 2008) and in vivo observations in mid thoracic spinal cords (Bilgen et al. 2005; Bonny et al. 2004; Kim et al. 2007a; Kim et al. 2007b). In this study, we present in vivo DTI maps of the mouse brain stem and cervical spinal cord at 4.7 T using the actively decoupled RF transmitter and receiver. The pixel based in vivo DTI parameters including diffusion anisotropy, directional diffusivities, principal eigenvector, and diffusion ellipsoid are reported to reflect tissue structures.

2. Materials and Method

2.1. In Vivo DTI

Five 10-week-old female C57BL/6 mice were anesthetized using isoflurane/oxygen mixture (7% for induction and 0.7 – 1.5% for maintenance) with warm water circulating in a pad for maintaining body temperature at 37 °C. A customized stereotaxic brain holder with ear bar and nose cone was employed to hold the mouse in a prone position allowing the spine holder, similar to that described previously (Kim et al. 2007a), to immobilize the cervical spine as well as position the surface receive coil. The nose cone was used to deliver isoflurane/oxygen mixture and to detect the change of inline pressure during the respiratory cycle. The detected inline pressure change was detected using a pressure transducer and converted to a TTL signal for synchronization of MR data acquisition with animal's respiratory motion. The gated acquisition device as employed has been reported previously (Kim et al. 2007a).

Actively detuned two-coil system was employed for DTI data acquisition. Improved coil performance through active detuning of MR coils with pin diode has been reported previously (Barberi et al. 2000; Mellor and Checkley 1995; Vaughan et al. 2002). The simplified circuit design of coils used in this study is shown in Fig. 1a. The volume RF transmit coil is 6 cm in diameter and 10-cm long. Two 1 - 30 pF tunable capacitors (Voltronics, New Jersey) for matching and tuning along with a 4.7 pF chip capacitors (American Technical Ceramics, New York) were used in the RF transmitter. Two 0.5 - 10 pF ceramic tunable capacitors (Voltronics, New Jersey) were used to control matching and tuning of the surface receive coil. The shape of the surface coil was fit to the posterior quarter of the mouse brain and the cervical spine combining circular shape with 1.5 cm diameter covering the brain stem with the saddle shape legs of 1 cm in length to cover the cervical spine (Fig. 1 a).

The entire preparation was placed in an Oxford Instruments magnet (4.7 T, 40-cm clear bore) equipped with a 10-cm inner diameter, actively shielded Magnex gradient coil (up to 60 G/cm, 200 μ s rise time). The magnet, gradient coil, and gradient power supply were interfaced with a Varian NMR systems (Palo Alto, CA) UNITY INOVA console controlled by a Sun Blade 1500 workstation (Sun Microsystems, Santa Clara, CA).

The anatomical image (Fig. 1b) was acquired using a respiratory gated conventional spin-echo imaging sequence: repetition time (TR) 1.2 s (modulated by the respiratory rate), spin echo time (TE) 10 ms, slice thickness 0.5 mm, field of view $1.5 \times 1.5 \text{ cm}^2$, data matrix 128×256 (phase encoding $\times$ readout), zero filled to 256×256 .

Diffusion weighted images were acquired using a conventional spin-echo imaging sequence modified by adding Stejskal-Tanner diffusion weighting gradients (Stejskal 1965) without motion compensation. The acquisition parameters were: repetition time (TR) 1.2 s (slightly varied with the respiratory rate), spin echo time (TE) 36 ms, time between application of gradient pulses (Δ) 21 ms, diffusion gradient duration (δ) 6 ms, slice thickness 0.5 mm, diffusion sensitizing gradients orientations: $(G_x, G_y, G_z) = (1, 1, 0), (1, 0, 1), (0, 1, 1), (-1, 1, 0), (0, -1, 1), \text{ and } (1, 0, -1)$. Based on our previous experience on in vivo DTI of mouse spine, the b value of $1.414 \text{ ms}/\mu\text{m}^2$ resulted in the best diffusion results in mouse spine. Ten transverse images of brain stem and cervical spinal cord were planned in horizontal scout image centered at C1 spinal cord (Fig. 1b) with 1.0 mm gap covering from brain stem to C5 cervical spinal cord with the following parameters: field of view $1.5 \times 1.5 \text{ cm}^2$, mediolateral phase encoding and dorsoventral read out gradients (data matrix of 128×256 zero-filled to 256×256), four scans averaged per k space line, and the total acquisition of one hour.

2.2. Data analyses

Diffusion tensors were estimated independently for each pixel from the diffusion-weighted images using a weighted linear least-squares method (Koay et al. 2006). The eigenvalue decomposition was then applied to the tensor, yielding a set of eigenvalues ($\lambda_1 \geq \lambda_2 \geq \lambda_3$) and eigenvectors for each pixel. Maps of diffusion indices including the axial diffusivity ($\lambda_{\parallel}$), radial diffusivity ($\lambda_{\perp}$), fractional anisotropy (FA), and mean diffusivity ($\langle D \rangle$) were generated by applying the following equations for each pixel:

$$\langle D \rangle = (\lambda_1 + \lambda_2 + \lambda_3) / 3 \quad [1]$$

$$\lambda_{\parallel} = \lambda_1 \quad [2]$$

$$\lambda_{\perp} = (\lambda_2 + \lambda_3) / 2 \quad [3]$$

$$\text{FA} = \frac{\sqrt{3} \sqrt{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}}{\sqrt{2} \sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad [4]$$

The eigenvalue decomposition of the diffusion tensor was also used to generate diffusion ellipsoids, constructed for each pixel by aligning the axes of the ellipsoid with the eigenvectors of the diffusion tensor setting the principal radii proportional to the corresponding eigenvalues (Pierpaoli and Bassar 1996).

The FA map was additionally color coded to include information regarding the primary direction of diffusion (i.e., the direction of the largest eigenvalue). Specifically, the colors blue, red, and green correspond to anteroposterior, mediolateral, and superoinferior directions,

respectively, with the brightness of the color maps weighted by the FA (Pajevic and Pierpaoli 1999).

Region of interest (ROI) analysis was performed for brain stem and cervical spinal cord using Image J v1.37. The diffusion parameters of brain stem, gray matter, and dorsal and ventrolateral white matter were derived using a ROI analysis on DTI index maps and T2 weighted images ($b = 0$). From $b = 0$ image, signal to noise ratio (SNR) was calculated as signal intensity of ROIs divided by standard deviation of back ground noise. Student t-test was performed to compare DTI parameters of different ROI at the same cord segment. One way ANOVA was performed to examine the craniocaudal dependence of DTI parameters. Statistical significance was accepted as $p < 0.05$.

3. Results

The in vivo DTI maps, displayed in scales of $0 - 1$ (FA), $0 - 1 \mu\text{m}^2/\text{ms}$ ($\lambda_{\perp}$), $0 - 3 \mu\text{m}^2/\text{ms}$ ($\lambda_{\parallel}$), and $0 - 2 \mu\text{m}^2/\text{ms}$ ($\langle D \rangle$), demonstrate a better gray-white matter contrast (Fig. 2) than those of the conventional images (Fig. 1b), comparable with those previously reported on the thoracic cord (Kim et al. 2007a). The coherent axonal fiber direction, mostly parallel to the axis of the spine (blue), is seen in the color coded principal eigenvector map (Fig. 2).

The diffusion ellipsoids reflecting detailed diffusion characteristics of the cervical spinal cord (Fig. 3) are axially elongated in blue for white matter and are rounded for gray matter with no coherent color (Figs. 3b and c). In the magnified view, a more detailed structural difference is seen between dorsal (DGM) and ventral gray matter (VGM) (Fig. 3c) where DGM exhibited more directional coherence than that in the VGM as evidenced by the more coherent color. The fiber coherence in DGM was also seen in the FA map with relatively higher anisotropy than that in the VGM. The anisotropy of DGM is much lower than that in dorsal white matter (DWM) and ventrolateral white matter (VLWM) (Fig. 3c).

The quantitative ROI analysis of DTI parameters was performed in brain stem (Fig. 2) and cervical spinal cord (Fig. 3a). The mean diffusivity ($\langle D \rangle$) exhibited no significant regional or gray-white matter difference in the brain stem or cervical spinal cord. The $\langle D \rangle$ of both nuclei VII and XII, known to be affected in motor neuron degenerative diseases, is not different from the rest of the brain stem (Table 1). In cervical cord, there was no significant $\langle D \rangle$ difference between gray (VGM and DGM) and white (VLWM and DWM) matter (Table 2). In general, the cervical spine gray matter (VGM and DGM) anisotropy is about 40% of that in the white matter (DWM and VLWM). Both DWM and VLWM exhibit high anisotropy similar to that previously observed in the thoracic and lumbar spine (Kim et al. 2006; Kim et al. 2007a) with $\lambda_{\parallel} \sim 6 \times \lambda_{\perp}$. Quantitatively, DGM anisotropy is higher (about 10 %) than VGM while still much lower than both DWM and VLWM.

The diffusion tensor parameters are different from rostral to caudal segments (Fig. 4). Specifically, FA, $\lambda_{\perp}$, and $\lambda_{\parallel}$ of DGM and DWM show more spatial dependence than those of VGM and VLWM. However, no significant spatial $\lambda_{\perp}$ difference was seen in either DWM or VLWM.

4. Discussion

The current results demonstrate that high quality in vivo diffusion MR measurements of mouse brain stem and cervical spinal cord in a single setting could be achieved at 4.7 T. The produced high quality in vivo images are comparable to those reported ex vivo (Niessen et al. 2006; Ong et al. 2008) and in vivo at 9.4 T (Bilgen 2007; Huang et al. 2007) measurements from mouse spinal cords. The diffusion parameters of brain stem and cervical spinal cord obtained are

consistent among animals examined and could be representative of the normative values in mice allowing inter-lab comparisons.

The derived diffusion parameters reflect the structural characteristics where white matter exhibits higher anisotropy than gray matter in both brain stem and cervical cord due to the more coherently orientated myelinated axons. In the brain stem, both trigeminal and pyramidal tracts show typical high anisotropy of white matter (Fig. 2). Both nuclei VII and XII are isotropic as expected for the typical gray matter with FA ~ 0.3 (Table 1). The cervical spine exhibits similar diffusion characteristics as seen in the thoracic and lumbar segments (Kim et al. 2006; Kim et al. 2007a) with white to gray matter FA ratio of ~ 2.5 , ventrolateral white matter $\lambda_{\parallel} \sim 6 \times \lambda_{\perp}$, and white matter $\lambda_{\perp} \sim 50\%$ the of gray matter value (Table 2). White matter shows almost two times higher water diffusion in axial direction than gray matter, which shows faster radial diffusion than white matter with isotropic diffusion. The $\langle D \rangle$ of cervical spinal cord white matter is $\sim 10\%$ smaller than that of mid thoracic cord. The packing density and axonal caliber may be different in the cervical spine from that in thoracic and lumbar segments resulting in the different apparent water diffusion (Schwartz et al. 2005).

As shown in Fig. 4, DTI parameters vary from C1 through C5 cervical spinal cord as has been reported in the rat (Ellingson et al. 2008). There is no gray-white matter tissue contrast change throughout the length of the cervical cord. Regional differences in addition to the lengthwise change are also present for cervical spinal cord white matter. Statistically significant differences in DTI parameters observed between DWM and VLWM are consistent with the recent finding that DWM axons pack more densely and are smaller in diameter compared with those in VLWM (Ong et al. 2008).

Similarly, the distinct structural difference between DGM and VGM could also readily be recognized in gray scale FA and diffusion ellipsoids (Figs. 2 and 3). DGM has higher FA (0.44 ± 0.07) than VGM (0.32 ± 0.05) (Table 2). DGM exhibits a higher $\lambda_{\parallel}$ but lower $\lambda_{\perp}$ than that of VGM, probably reflects the more numerous axons of the existing nerve roots of the peripheral nerve. Indeed, it has also been reported that dorsal (or posterior) horn neurons pack more densely and are smaller in size than those at ventral horn (Rottkamp et al. 2008; Stephens et al. 2006). These findings suggest that DTI parameters may be used to examine morphological or pathophysiological changes in mouse cervical spinal cords.

The accuracy of in vivo diffusion measurements is known to be SNR dependent. A SNR threshold for obtaining λ_1 , λ_2 and λ_3 without noise effect has been suggested previously (Anderson 2001; Bastin et al. 1998). Low SNR results in higher λ_1 , and lower λ_2 and λ_3 compared to those obtained with sufficiently higher SNR. Previously, we have established that the SNR threshold of ~ 40 , derived from the image of $b = 0$, is necessary for in vivo DTI of mouse spinal cord. This SNR was achieved previously with 2.5-hr acquisition using passively decoupled two-coil system on the thoracic and lumbar segments of the mouse spinal cord (Kim et al. 2007a; Kim et al. 2007b). In the present study, this SNR threshold was achieved in an hour. The actively decoupled two-coil system is necessary to eliminate the coupling between the transmit and receive coils since the orthogonality between the two coils is no longer possible for the different portion of the non-coplanar receiver coil (Mellor and Checkley 1995). The significantly improved sensitivity seen in the present study may be due to the improved excitation profile resulting in the better refocused echoes, thus the detected signal. The use of an actively detuned two-coil system was demonstrated to generate a better quality image than that obtained using a birdcage coil from the rat brain (Ludwig et al. 2004). Thus, the sensitivity gain seen in the present study is not likely to be achieved using a volume coil.

In general, rodent spinal cord diffusion imaging requires a thicker slice (from 1 to 2 mm) than the typical brain image (0.5 to 1 mm), and less than 128 phase encoding step with larger field

of view (from 2×2 to 3×3 cm²) to improve SNR (Bilgen et al. 2005; Bonny et al. 2004; Gullapalli et al. 2006). The current study is unique in that both high SNR and spatial resolution (0.5 mm slice thickness, and 128 phase encoding steps with 1.5×1.5 mm² field of view) were achieved. To improve both sensitivity and resolution of the acquired image, a well designed implantable coil has been employed for rodent thoracic spine resulting in improved image resolution and SNR at 9.4 T (Bilgen 2007). However, the use of such coil has not resulted in a sufficient SNR gain to generate the high quality DTI maps shown in the present report. The sensitivity to motion artifact and the more significant tissue loading effect may be the primary cause of the worse result in using the implanted coil at 9.4T. It would be necessary to evaluate the implanted vs. the anatomy fitted surface coils more systemically to determine the best set up for rodent spinal cord imaging. However, based on the current finding and the reported implanted coil measurements, the implanted coil may not be necessary for the mouse cervical spinal cord.

Echo-planar imaging (EPI) is one of the most commonly employed sequences to reduce the acquisition time. It has recently been applied to acquire *in vivo* DTI on rodent spinal cords (Madi et al. 2005). Ultra fast data acquisition using EPI diffusion sequences offers the advantage of reducing motional artifacts and physiological stress of the animal. Unfortunately, the required image resolution to delineate tissue structures in rodent spinal cord results in lengthened echo time for the measurement leading to significant susceptibility effect. The current report demonstrates that the long data acquisition using the conventional spin echo diffusion sequence may be significantly reduced while retaining the high image quality. The data acquisition time of this report is comparable with the previously reported EPI based diffusion measurements (Madi et al. 2005) while producing the higher quality diffusion tensor images. Recently, the segmented EPI measurements on brain (Guilfoyle and Hrabec 2006) and spinal cord (Callot et al. 2007) were reported using high magnet strength like 7 or 12 T. The image quality has been significantly improved in these reports. However, the choice between segmented EPI and spin echo diffusion measurements has to be determined locally by the individual laboratory since hardware requirements are not trivial for EPI diffusion measurements.

In conclusion, the *in vivo* DTI of the brain stem and cervical spine may be acquired in a single setting, reflecting the underlying structural characteristics. The use of actively decoupled two-coil system produced diffusion parameter maps with high spatial resolution and SNR. The results demonstrate that DTI parameters not only can detect the general difference between gray and white matter, but also reveals the spatial differences among spinal cord tissues. The results suggest that the current set up is ideal to study rodent models of CNS disorders involving brain stem and cervical spine.

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Figure 1.

The circuit diagram of actively decoupled RF transmit (left) and receive (right) coils are demonstrated in panel (a). The isolation between the two coils is achieved by the application of +5 V DC to the receive coil during RF excitation to the PIN diode (Voltronics, New Jersey). The RF choke (Coilcraft, Illinois) is used to prevent noise breakthrough. The coil loop reflects the actual shape of the receive coil conforming to the anatomy of brain stem and cervical spine (b). The transverse images are centered at C1 segment of the spine. Each image is 0.5-mm thick with 1.0-mm gap between images. The typical gray (bright, the arrows) and white (dark, the arrow heads) matter contrast is seen.

Figure 2.

The transverse *in vivo* diffusion parameter maps of brainstem and cervical spine. Fractional anisotropy (FA; color-coded: red – x, green – y, and blue – z), radial ($\lambda_{\perp}$), axial ($\lambda_{\parallel}$), and mean ($\langle D \rangle$) diffusivities are displayed at the scale of 0 – 1.0, 0 – 1.0 $\mu\text{m}^2/\text{ms}$, 0 – 3.0 $\mu\text{m}^2/\text{ms}$, and 0 – 2.0 $\mu\text{m}^2/\text{ms}$ respectively. Trigeminal (t) and pyramidal (p) tracts in brain stem and dorsal (d) and ventrolateral (v) white matter of cervical spinal cord are brighter in $\lambda_{\parallel}$ but darker in $\lambda_{\perp}$ map than gray matter. The coherent fiber orientation of the high anisotropic white matter tracts can be seen as the consistent color (blue) in RGB-coded FA map. There is no visible tissue contrast in $\langle D \rangle$ maps. The isotropic cerebral spinal fluid (CSF), indicated with arrow head, surrounding central nervous system (CNS) is bright in $\lambda_{\parallel}$ and $\lambda_{\perp}$ maps, and dark in FA map. The diffusion parameters obtained from brain stem and cervical cord are reported in Tables 1 and 2.

Figure 3.

The representative transverse (in x-y plane) FA map is shown with ROI definitions for ventral gray matter (VGM), dorsal gray matter (DGM), ventrolateral white matter (VLWM), and dorsal white matter (DWM) (a). The color coded diffusion ellipsoids of the cervical spine (b) shows axially (in z-direction) elongated ellipsoids of pure blue in white matter tracts and arbitrarily oriented round ellipsoids of various shades of colors in the gray matter. The ROI of the four major tissue types, VGM, DGM, VLWM, and DWM, are selected (b) and expanded for a closer examination (c), slightly rotated in x and y axis for a better visualization of the diffusion ellipsoid. The elongated and pure blue color ellipsoids of the DWM and VLWM reflect the highly anisotropic and coherent fiber tracts in the spinal cord white matter. The coherent fiber orientation of the DGM is readily seen as the round, i.e., less anisotropic, ellipsoids of pure color. In contrast, the rounded ellipsoids of mixed colors from the VGM suggest incoherent fibers in this region. Brain stem white matter is also seen as the axially elongated pure blue diffusion ellipsoid (outer rim), while the gray matter is seen as the rounded ellipsoid with mixed colors (d).

Figure 4.

The DTI parameters of cervical cord, i.e., FA (a), $\langle D \rangle$ (b), $\lambda_{\perp}$ (c), and $\lambda_{\parallel}$ (d), expressed as mean $\pm$ standard deviation ($n = 5$) are shown at each transverse image location (0.5-mm slice thickness with 1.0-mm gap) covering from C1 to C5. As shown in Table 2, gray matter (solid line, VGM (Δ) and DGM ($\square$)) exhibits statistically significantly lower FA and $\lambda_{\parallel}$, but higher $\lambda_{\perp}$ comparing with the white matter (dotted line, VLWM ($\blacktriangle$) and DWM ($\blacksquare$)). No statistically significant differences in $\langle D \rangle$ were observed in gray and white matter throughout the examined cord. Despite the statistically significant difference between DWM and VLWM, only less than 5 % difference was observed in FA and $\lambda_{\parallel}$ at C4 and C5. At C3 to C5, DGM shows significantly higher FA than VGM resulting from higher $\lambda_{\parallel}$ and lower $\lambda_{\perp}$. * p (DWM vs. VLWM) < 0.05 , and # p (DGM vs. VGM) < 0.05 . The significance of longitudinal difference is noted with arrows ($p < 0.05$).

Table 1

In vivo brain stem DTI parameters.

Mean $\pm$ SD (n = 5)	NuVII ^a	NuXII ^b	Brain Stem
FA	0.32 $\pm$ 0.06	0.35 $\pm$ 0.06	0.39 $\pm$ 0.05
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.44 $\pm$ 0.06	0.49 $\pm$ 0.04	0.41 $\pm$ 0.05
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	0.73 $\pm$ 0.06	0.82 $\pm$ 0.06	0.78 $\pm$ 0.06
$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	0.55 $\pm$ 0.08	0.58 $\pm$ 0.06	0.55 $\pm$ 0.08

^aNucleus VII^bNucleus XII

The average pixel numbers in the ROI: 250 (NuVII), 100 (NuXII), and 3000 (brain stem).

The signal to noise ratio (SNR) is about 40 for each ROI.

Table 2

In vivo DTI parameters of the cervical spinal cord.

Mean ± SD (n = 5)	Gray Matter (G)		White Matter (W)			$P_{(G\ vs\ W)}$
	VGM ^a	DGM ^b	$P_{(a\ vs\ b)}$	VLWM ^c	DWM ^d	$P_{(c\ vs\ d)}$
FA	0.32 ± 0.07	0.44 ± 0.05	**	0.78 ± 0.05	0.81 ± 0.03	***
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.51 ± 0.03	0.41 ± 0.02	**	0.23 ± 0.02	0.21 ± 0.02	***
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	0.79 ± 0.05	0.88 ± 0.03	*	1.38 ± 0.03	1.41 ± 0.03	***
<D> ($\mu\text{m}^2/\text{ms}$)	0.60 ± 0.02	0.59 ± 0.03	#	0.62 ± 0.02	0.61 ± 0.02	#

^aVentral gray matter,
^bDorsal gray matter,
^cVentrolateral white matter,
^dDorsal white matter.

$p > 0.2$, * $p < 0.01$, ** $p < 0.001$, and *** $p < 0.0001$.

The average pixel numbers are ~350 for both ventral gray and white matter and ~70 for both dorsal gray and white matter. The signal to noise ratio is about 40 for each ROI. The longitudinal spatial dependence of DTI parameters are shown in Fig. 4.