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Reproducibility of tract-specific magnetization transfer and diffusion tensor imaging in the cervical spinal cord at 3 tesla

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Abstract

Damage to specific white matter tracts within the spinal cord can often result in the particular neurological syndromes that characterize myelopathies such as traumatic spinal cord injury. Noninvasive visualization of these tracts with imaging techniques that are sensitive to microstructural integrity is an important clinical goal. Diffusion tensor imaging (DTI)- and magnetization transfer (MT)-derived quantities have shown promise in assessing tissue health in the central nervous system. In this paper, we demonstrate that DTI of the cervical spinal cord can reliably discriminate sensory (dorsal) and motor (lateral) columns. From data derived from nine healthy volunteers, two raters quantified column-specific parallel ($\lambda_{\parallel}$) and perpendicular ($\lambda_{\perp}$) diffusivity, fractional anisotropy (FA), mean diffusivity (MD), and MT-weighted signal intensity relative to cerebrospinal fluid (MTCSF) over two time-points separated by more than 1 week. Cross-sectional means and standard deviations of these measures in the lateral and dorsal columns were as follows: $\lambda_{\parallel}$: 2.13 ± 0.14 and $2.14 \pm 0.11 \mu\text{m}^2/\text{ms}$; $\lambda_{\perp}$: 0.67 ± 0.16 and $0.61 \pm 0.09 \mu\text{m}^2/\text{ms}$; MD: 1.15 ± 0.15 and $1.12 \pm 0.08 \mu\text{m}^2/\text{ms}$; FA: 0.68 ± 0.06 and 0.68 ± 0.05 ; MTCSF: 0.52 ± 0.05 and 0.50 ± 0.05 . We examined the variability and interrater and test-retest reliability for each metric. These column-specific MR measurements are expected to enhance understanding of the intimate structure-function relationship in the cervical spinal cord and may be useful for the assessment of disease progression.

Keywords

spinal cord; magnetic resonance imaging; diffusion tensor imaging; magnetization transfer; tractography

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INTRODUCTION

Neurologic function of the arms and legs is mediated by the spinal cord. Whereas motor information is conveyed primarily in the lateral columns, major aspects of sensory function — in particular, vibration and proprioception — are conveyed by the dorsal columns. As the integrity of the spinal cord is vital to the preservation of neurologic function, even small lesions resulting from trauma and neurodegenerative diseases such as multiple sclerosis (MS), transverse myelitis, and adrenomyeloneuropathy (AMN) can have severe ramifications. Conventional MRI (i.e. spin-density, T1- and T2-weighted) can often show the location of damage caused by inflammatory lesions in the spinal cord, but the relationship between the MRI findings (e.g. atrophy, lesion burden) and neurologic function and progression over time tends to be poor (1). Furthermore, both conventional MRI and clinical evaluation of spinal-cord health often have limited reproducibility due to the subjectivity of the assessment and to a paucity of reliable, quantitative information.

To alleviate these problems, quantitative imaging methods such as magnetization transfer (MT) (2–4) and diffusion tensor imaging (DTI) (5–8) have been applied to the cervical spinal cord. DTI has been used to investigate how diseases of the brain modify or affect white-matter fiber bundles (9–14). Recent reports have shown that diffusion-weighted imaging and DTI, including DTI-based fiber tracking, are possible in the cervical spinal cord (5–8,15–18), but the routine application of DTI in the spinal cord is still in its infancy. Moreover, a detailed study of the reproducibility of these applications to the long tracts of the spinal cord has not been reported. Additionally, MT imaging of the cervical spine can detect macromolecular tissue damage in diseases such as MS and adrenomyeloneuropathy (2,3,19–21).

There are significant technical challenges involved in the routine use of MRI, particularly for quantitative MRI methods, to assess the spinal cord. The cord is small — 1–1.5 cm in diameter at the cervical level — and its component white-matter columns are on the order of several millimeters. The high spatial resolution necessary to visualize the cord is limited by a low signal-to-noise ratio and frequent cord motion. Transverse movement of the cord resulting from cardiac and respiratory cycles and cerebrospinal-fluid pulsation, and rostrocaudal movement due to oropharyngeal motion, both cause substantial artifacts. Cardiac triggering can be used to improve stability in the face of motion (7), but the time necessary for high-resolution imaging with cardiac triggering may prohibit clinical application. Although assessment of cord area and volume can be accomplished with high fidelity, this approach ignores damage to the cord at different axial levels and in different tracts.

Previously, column-specific MT information about the cervical spinal cord was obtained in a study of patients with AMN (3,19) and more recently in a study of patients with MS (4). The importance of these studies is the high degree of correlation between the column-specific MT metrics and neurological function. However, routine application of the methods used in those studies is limited because of the need for manual placement of regions of interest (ROIs) in each column on every slice, a time-consuming and inherently variable process.

One potential solution is to incorporate the fiber-tracking capabilities of DTI to delineate and isolate the individual columns of the spinal cord. DTI-derived quantities such as FA, MD, $\lambda_{\perp}$, and $\lambda_{\parallel}$, and MT-derived quantities such as MTCSF (22), could then be analyzed in a slice-wise, column-specific manner. Compared to ROI-based assessment, tractography-based analysis might allow higher data throughput and protect, to some degree, against spurious ROI placement. However, the reliability of such an approach has not been evaluated in the spinal cord. Furthermore, profiles of DTI- and MT-derived quantities as a function of slice, as have been reported in the brain (23), have not been extensively described in the spinal cord.

We performed DTI tractography in the lateral and dorsal columns of the cervical spinal cord using coregistered, high-resolution, MT-weighted axial images for ROI placement. We report the slice-wise variation in DTI- and MT-derived quantities, referred to as spatially normalized tract profiles (24), in nine healthy volunteers. We assess the variability across volunteers and across columns, along with the test–retest reliability of these quantities. Because the tract profiles and values of the derived quantities may depend on ROI placement to some extent, we also assessed inter-rater variability.

METHODS

Data acquisition

This study was approved by the local Institutional Review Board. Signed, informed consent was obtained prior to the examination. Nine healthy volunteers (three males, six females, age range = 22–50 years, mean $\pm$ standard deviation = 36 ± 11 years) were scanned on a 3T Philips Intera MR system (Philips Medical Systems, Best, The Netherlands) with a maximum gradient strength of 60 mT/m. A second set of scans was acquired on the same volunteers at a minimum of one week following the initial session. A 16-channel neurovascular coil (eight head, two rostral neck, and six anterior-posterior caudal neck/upper thoracic elements) was used for reception, and the body coil was used for transmission. The field of view was centered at the C4 vertebral body level and spanned, at minimum, the C2 to C7 vertebral body levels in all volunteers. Parallel imaging with sensitivity encoding (SENSE) (25) and second-order shimming were used to minimize image artifacts arising from susceptibility differences between bone and tissue that occur in echo-planar-imaging (EPI) acquisitions.

Multi-slice spin-echo DTI with single-shot EPI (TR/TE = 6300/63 ms, SENSE factor = 2) was acquired with 1.5 (RL) $\times$ 1.25 (AP) mm resolution. Other parameters were: 40 slices (3 mm slice thickness, no gap), FOV = 145 (RL) $\times$ 120 (AP) mm, acquired matrix = 96 $\times$ 96, non-collinear diffusion encoding directions = 16, $b = 500$ s/mm². Scan time was 2 min per acquisition. Three acquisitions were obtained and entered into the tensor calculation as separate entities (i.e. no pre-calculation averaging was performed). The 16 non-collinear directions were derived and optimized *a priori* to sample a prolate tensor, which, to a first approximation, is more similar to the geometry of the expected fiber orientation in the spinal cord than the brain (26).

MT-weighted images were acquired using a 3D spoiled-gradient-echo sequence with multi-shot EPI readout (EPI factor = 3), TR/TE/ $\alpha = 110$ ms/13 ms/9°, and SENSE-factor = 2. Nominal resolution was 0.61 (RL) $\times$ 0.58 (AP) mm in-plane over 40 slices (3 mm slice thickness with no gap). Other parameters were: FOV = 224 (RL) $\times$ 190 (AP) mm, acquired matrix = 368 $\times$ 326. MT weighting was achieved using a 24-ms, 5-lobed, sinc-shaped saturation pulse with peak amplitude = 8.5 μ T at offset frequency = 1.5 kHz, which was empirically optimized for visual discrimination between white and gray matter. Note that the MT contrast is affected by all of these parameters and may change when other sequence parameter combinations are used. The total scan time for MT acquisition including both reference (no MT prepulse) and MT-weighted scans was 8 min. The total scan time for all sequences, including survey, SENSE reference scan, sagittal T2-weighted and STIR (short tau inversion recovery), DTI, and MT-weighted was 28 minutes. It should be noted that T2-weighted and STIR sequences were performed as part of routine MRI in all healthy volunteers to rule out disc herniation, cervical stenosis, and incidental abnormalities that could further complicate the comparison of data across healthy volunteers.

Data processing

All diffusion-weighted images were coregistered to the initial $b = 0 \text{ s/mm}^2$ (b_0) volume using a 6-degree-of-freedom, rigid-body procedure implemented in FLIRT (FMRIB's Linear Imaging Registration Tool, Oxford, UK). All diffusion-weighted images from each acquisition (including the subsequent b_0 acquisitions) were registered to the initially obtained b_0 volume. Details of the registration scheme are presented in Figure 1.

The MT dataset was coregistered to the mean b_0 in the following manner. First, the MT-weighted volume was coregistered to the mean b_0 volume using a 3D, 6-degree-of-freedom, rigid-body transformation procedure supplied in AIR (Automated Imaging Registration) (27). Thereafter, the registration was fine tuned by registering the MT-weighted volume to the mean b_0 using a 2D, slice-by-slice, 3-degree-of-freedom (two in-plane translation, and one rotation) transformation procedure (AIR) (27). Additional details are given in Figure 1.

The diffusion tensor and DTI-derived quantities were calculated using CATNAP (Coregistration, Adjustment, and Tensor-solving, a Nicely Automated Program) (28,29). Mean, parallel, and perpendicular diffusivities were calculated from the eigenvalues ($MD = (\lambda_1 + \lambda_2 + \lambda_3)/3$; $\lambda_{||} = \lambda_1$; $\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$) of the diffusion tensor and fractional anisotropy (FA) according to the following equation (10):

$$FA = \sqrt{\frac{1}{2}} \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}.$$

For fiber reconstruction, the fiber assignment by continuous tractography (FACT) method (30,31) was used with an FA threshold of 0.2 and a maximum turning angle of 60° . Tractography was performed in DtiStudio (32), available from <http://www.mristudio.org>. All other data processing was done using purpose-written software in Matlab (The Mathworks, Natick, MA).

For quantitative, inter-volunteer comparison of MT-weighted data, MTCSF (22) images were calculated using $MTCSF = S / S_{mean}^{CSF}(ROI)$ where S is the MT-weighted intensity and $S_{mean}^{CSF}(ROI)$ the mean cerebrospinal-fluid (CSF) signal intensity from the same slice of the reference (no MT preparation pulse) scan. $S_{mean}^{CSF}(ROI)$ was obtained by calculating the mean slice-by-slice signal intensity within ROIs encompassing as much CSF as possible without obvious contamination from the cord, vertebrae, or nerve roots (Fig. 1). Although MTCSF images include T_1 and T_2 effects and have an intimate relationship with the effective B_1 field, the normalization procedure makes them comparable across individuals (22). Such images have previously been used to detect changes in macromolecular (e.g. myelin) content even in the absence of inflammation (19,22).

Even after registration and downsampling, the MTCSF images show excellent contrast between gray and white matter (Fig. 2), enabling demarcation of the lateral and dorsal columns. Therefore, the coregistered and downsampled MTCSF images that now reside in DTI space were used for placement of ROIs in the left lateral (LL), right lateral (RL), and bilateral dorsal (Dor) columns (Fig. 3). The specific placement of all ROIs was performed manually in each of the columns without region-growing or semi-automatic, signal intensity thresholding being applied. These ROIs were then transferred to the DTI images and used as seed voxels for tract reconstruction. Spurious fibers, such as those that crossed from one column to the next, were manually excluded. The tractograms were overlaid on maps of FA, MD, $\lambda_{\perp}$, $\lambda_{||}$, and MTCSF.

Spatially normalized tract profiles (24) were then created to demonstrate the variation in each quantity as a function of column (lateral and dorsal) and slice. Because neck lengths vary across people, the analyzed volumes were restricted to the region between the inferior aspect of the nerve roots of C2 and the inferior aspect of the nerve roots of C6 as seen on the MTCSF images. The mean neck length over all volunteers was 75 mm (25 slices at 3 mm). To allow comparison across individuals, all tract profiles were spatially normalized, using spline interpolation, to 25 points spanning C2 to C6.

For the inter-rater comparison, two trained raters (VB and AG) independently placed ROIs on the first dataset obtained from each volunteer. For the test–retest comparison, one rater (AG) repeated the analysis on the second dataset that was obtained in each volunteer. In principle, it is straightforward to coregister data for each individual across scanning sessions. However, we chose not to perform such coregistration so as to simulate the comparison of independent examinations performed in a clinical setting.

Statistical analysis

Time-point 1, rater 1 (cross-sectional analysis)—Each MRI quantity was evaluated for differences between columns (i.e. lateral vs dorsal) and between right and left lateral columns. Two-tailed, independent *t*-tests were performed to ascertain whether or not the mean (over slices and volunteers) quantity of interest derived from the right and left lateral columns were statistically equivalent, and additionally, whether or not the mean lateral column (averaged across both right and left lateral columns) differed from the dorsal column. A significance threshold of $\alpha = 0.01$ was imposed to reduce the effects of multiple comparisons.

Time-point 1, rater 1 and rater 2 (inter-rater reliability)—For each column (lateral and dorsal), the DTI and MT indices from each of two raters were compared in two ways. Prior to statistical analysis, the mean (over slices) value for each metric was calculated for each person ($n = 9$) and rater. First, Bland-Altman (33) analysis was performed to calculate whether the differences between the two raters were significantly different from 0 (i.e. if the 95% Confidence Interval of the difference between the two raters does not encompass 0) for each MRI quantity as well as the limits of agreement (LOA), which can be used to assess whether a particular difference is outside the expected range. Second, both a Wilcoxon signed-rank (non-parametric) and a paired *t*-test (parametric) was performed to test, at the $\alpha = 0.01$ level, whether there was an observable difference in the mean values between the two raters.

Time-point 1 and 2, rater 2 (test–retest reliability)—Test–retest reliability was calculated in an analogous manner to the inter-rater reliability.

To assess the relative magnitude of variability over raters and times, and to provide data to drive future sample size calculations, we calculated the normalized Bland-Altman difference:

$$D_{BA} = \frac{D}{M} \times 100$$

where *D* is the mean difference between rater 1 and rater 2 (or scan 1 and scan 2), and *M* is the mean of the metric of interest over raters or scans.

RESULTS

Conventional sagittal STIR and T2-weighted MRI demonstrated no significant incidental abnormalities, such as cervical stenosis or large disc protrusions, in the scans of the healthy volunteers. Figure 2 shows MTCSF- and DTI-derived maps at C3 in two healthy volunteers.

In the first row, typical MTCSF images show excellent contrast between white matter (dark), the butterfly-shaped gray matter (brighter), and the surrounding CSF (brightest). Note that the MTCSF maps in Figure 2 have been reformatted to match the resolution of the DTI acquisitions.

White matter is bright on the FA maps, corresponding to high diffusion anisotropy, whereas gray matter is dark. Gray-white contrast is lower on the MD and $\lambda_{||}$ maps, although there is strong contrast between spinal cord and the surrounding cerebrospinal fluid. Finally, there is good contrast between white (dark) and gray matter (bright) on the $\lambda_{\perp}$ maps, in accord with the restriction of diffusion in this direction that is induced by tightly packed, myelinated axons within the white matter.

Figure 3 shows 3D tractograms of the right (yellow) and left (red) lateral columns and dorsal (green) column in two healthy volunteers overlaid on slices from the b_0 volume. In the small panel, the cross-sectional positions of the fiber pathways are also shown on the same mean b_0 image. Note that the orientation of the tractograms is different than the in-plane images. There is a small amount of variation in the reconstructed fiber pathways across raters (compare columns 1 and 2) and times (compare columns 2 and 3). In the bottom row, ROIs to guide tractography are shown in the two lateral columns (yellow and red) and the dorsal column (green) on these images and transferred to the DTI maps. As a visual guide to the anatomical structure, an outline of the GM as seen on the MTCSF image (orange) is propagated to each of the DTI-derived maps.

Tract profiles (mean $\pm$ standard deviation, $n = 9$) of MTCSF- and DTI-derived quantities over all volunteers for the two raters are shown in Figure 4. The profiles span the cervical spinal cord from the C2 to C6 vertebral body levels. MD, $\lambda_{||}$, and $\lambda_{\perp}$ are presented within one panel as they have the same units [$\mu\text{m}^2/\text{ms}$], which eases reading and interpreting the profiles. The mean differences between the profiles (D) for the two raters are small, and Bland-Altman analysis shows that there is no significant difference between the two raters (i.e. the 95% Confidence Interval for D overlaps zero) for any of the metrics. Additionally, neither Wilcoxon signed-rank nor paired t -tests showed significant differences ($p > 0.01$) between the raters. Detailed results are presented in Table 1.

Analysis of the differences between the left and right lateral columns and the differences between the mean lateral and dorsal column for rater 1 was performed for each of the MRI quantities. The t -tests fail to reject the null hypothesis of a difference between the right and left lateral columns (MTCSF, $p = 0.13$; FA, $p = 0.26$; MD, $p = 0.24$; $\lambda_{||}$, $p = 0.07$; and $\lambda_{\perp}$, $p = 0.40$). Similarly, although the dorsal and lateral columns have different tissue composition (e.g., axon diameter and myelin density), no difference was observed in the mean MRI quantities between dorsal and lateral columns in our images (MTCSF, $p = 0.31$; FA, $p = 0.86$; MD, $p = 0.31$; $\lambda_{||}$, $p = 0.63$; and $\lambda_{\perp}$, $p = 0.12$).

Comparison of the average ($\pm$ standard deviation) spatially normalized tract profiles across all volunteers between time-points 1 and 2 is shown in Figure 5. Again, there is little difference between the two time-points, and the Bland-Altman 95% Confidence Interval for the mean difference overlaps zero for all metrics. The Wilcoxon signed-rank and t -tests demonstrated no significant differences in the mean values for the lateral and dorsal columns across time ($p > 0.01$). Detailed results are presented in the Table.

Between raters, the average lateral-column normalized Bland-Altman difference (D_{BA}) was 2.06% (range = 0.20%–3.20%). For the dorsal column, the average D_{BA} was 1.89% (range = .08–2.81%). For the test–retest comparison, the D_{BA} was slightly larger than the D_{BA} between raters, averaging 4.54% for the lateral column (range = 0.85–8.44%) and 2.38% (range = 0.05–5.32%) for the dorsal column. Details can be found in Table 1.

DISCUSSION

The goal of this work was to demonstrate the feasibility of a column-specific analysis of the cervical spinal cord using quantitative MT- and DTI-derived quantities and to assess the associated cross-sectional, inter-rater, and test–retest variability. The assessment was performed across healthy volunteers, between the dorsal and lateral columns, and between the left and right lateral columns. The ability to reconstruct the fiber tracts with DTI in conjunction with coregistered MT data in the cervical spinal cord may increase the reliability of tract assessment in healthy and diseased spinal cords and have direct clinical relevance.

The study presented here differs from conventional ROI-based approaches in that MRI quantities are derived from tract reconstructions in a procedure known as spatially normalized tract profiling. Similar methodology has been applied to the corticospinal tracts and optic radiations in the brains of people with MS and healthy controls (23). It may seem that there is little need to apply fiber tracking to the spinal cord, where fibers are known to run generally in a rostrocaudal direction; technically, it would certainly be possible to draw ROIs on each slice on the high-spatial-resolution maps obtained with MTCSF imaging. However, the reproducibility of tractography and its relative speed compared to manual ROI placement will help to translate the methodology into more routine clinical use. Since only a few ROIs need to be drawn per subject, the tract-based approach is expected to reduce the subjectivity of manual ROI placement.

Currently, no tractography-derived, individual-column MTCSF and DTI quantities are available in the literature for comparison with our results, but whole-cord values (5,6,8,16, 18) and ROI-based examination of the MTCSF values in the columns of the spinal cord (4) have been reported. Our column-specific measurements of $\lambda_{||}$ are at the high end of the previously reported whole-cord range of 1.50–2.26 $\mu\text{m}^2/\text{ms}$, and our $\lambda_{\perp}$ measurements are in the middle of their reported range of 0.40–0.92 $\mu\text{m}^2/\text{ms}$. The column-specific MD is in the middle of the whole-cord range (0.90–1.29 $\mu\text{m}^2/\text{ms}$), whereas the measured FA values are at the higher end of the range (0.43–0.83) and are consistent with FA values in the dense white matter tracts of the brain (24). It should be noted that DTI-derived indices are sensitive to the sequence design parameters and SNR (28,29) with the former having a large impact on MD, $\lambda_{||}$, and $\lambda_{\perp}$ and the latter playing a large role in determining the bias in FA. Thus, when comparing these values to the literature, care should be taken to verify the similarity of the sequences chosen for comparison.

Mean MTCSF values for the lateral and dorsal columns cannot be compared straightforwardly with previous measures at 1.5T due to their dependence on sequence design, pulse power, and relaxation rates (which are field dependent). Despite these considerations, the delineation between white and gray matter on the MTCSF images is clearly more apparent at 3T than at 1.5T (22). To reduce cross-study variability, it would be useful to define a set of standard values for B1, irradiation offset and duration, and steady-state timing parameters. In this work, we chose to use an offset frequency = 1.5 kHz for our MTCSF calculation to visually maximize the discrimination between white and gray matter to facilitate ROI placement and tractography while still giving rise to a sufficient MT effect. While this value is suspected to be less than ideal for maximizing the MT effect, further studies and simulations may be undertaken to generate optimal scanning parameters.

A recent study explored the mean and standard deviation of MTCSF measurements derived from ROIs placed in the dorsal and lateral columns of the spinal cord in healthy volunteers and patients with multiple sclerosis (4). The normal range of the white matter MTCSF values in that paper was 0.48–0.50, slightly lower than the MTCSF values derived from the tract profiles reported here (0.50–0.53).

Reproducible measurement of column-specific, multimodality MRI quantities in the cervical spinal cord allows investigation of column-specific disease processes. Since these columns are somatotopically organized (34), disease confined to the lateral columns, for example, is more likely to impact motor function than sensory function. With the column-reconstruction method presented here, it may be possible to use profiles of each quantity to probe more specifically the structure-function relationship in health and disease. This may have applications in monitoring the effects of rehabilitative therapies or to detect column-specific damage prior to the onset of clinical symptoms.

All of the MRI quantities reported here have normalized differences <10%, however, two points of caution should be noted. First, even though the normalized difference does an adequate job of giving an impression of the percent variation in a dataset, it can be disproportionately affected by data where their means are close to zero, as is the case for test-retest $\lambda_{\perp}$ ($D_{BA} = 8.44\%$). Second, the variability presented here is sequence- and resolution-dependent. Therefore, it is conceivable that at lower resolution, where the signal-to-noise ratio is higher, the normalized differences could be smaller. However, this effect might be mitigated by worse partial-volume effects. Simulations are necessary to determine the optimal resolution to obtain reproducible data in the spinal cord.

Although parallel and perpendicular diffusivity values can be difficult to interpret in the context of the altered tissue microstructure that occurs in disease processes or in situations where multiple tracts cross (35), this situation may be less troublesome in the spinal cord, where the individual columns can be more easily separated, than in the brain. One way to increase the reproducibility of perpendicular diffusivity measurements is to study the directional diffusivity directly. Recently, our group has examined the ability of high b-value diffusion-weighted imaging with q-space analysis to detect spinal-cord damage (15). With appropriate coregistration techniques, such quantities can be examined along the column of interest in a manner similar to the one described here.

A surprising finding in our work is that both the diffusion and magnetization transfer indices that we studied do not show appreciable signal intensity differences between the lateral and dorsal columns. Histology shows that the axonal density differs by column, with the lateral column being comprised of fewer, larger, and more heavily myelinated axons than the dorsal column. With respect to MTCSF, these considerations may be offset by similar amounts of myelin in the dorsal and lateral columns (i.e. even though the individual axons of the dorsal column are surrounded by less myelin, there are more axons per voxel in the dorsal column).

Diffusion indices, on the other hand, are expected to be sensitive to the microenvironment of the tissue. However, this sensitivity is lowered by methodological limitations. The experiments presented here use a relatively low b-value ($b = 500 \text{ s/mm}^2$) for diffusion weighting compared to the generally accepted range of 700–1000 s/mm^2 for DTI in the brain. The lower b-value was chosen because it provided sufficient detectable signal in the low SNR environment, while at the same time, permitting sufficient diffusion weighting to quantify diffusion anisotropy. It is conceivable that DTI (which assumes Gaussian diffusion) at low-b-values ($<1500 \text{ s/mm}^2$) may not be capable of distinguishing the different diffusion environments since the signal attenuation in this regime is likely to be dominated by the fast diffusion which may not be sensitive to the highly restricted diffusion of water within WM fiber bundles. Recently, our group demonstrated that the signal attenuation due to water diffusion in the human spinal cord is not mono-exponential (15) when measured over a wide range of b-values (up to 5000 s/mm^2). It is hypothesized that a tract-specific study of diffusion over a wider range of b-values will demonstrate the microstructural differences that are known to exist histologically between the two columns.

We note two additional limitations of the current study. The first is the choice of two different registration schemes: FLIRT to register DTI volumes to the initially acquired b_0 and AIR to register the MT-weighted volume to the mean b_0 . In principle, either AIR or FSL could have been used to register both MT and DTI datasets. In prior work, we have optimized FLIRT-based registration of diffusion-weighted volumes (15). Although we did not perform a sophisticated analysis and comparison of registration algorithms, we found in the current work that AIR appeared to supply the most consistent registration of the MT-weighted data sets to the mean b_0 acquisition. We note that the two-step process to fine-tune the registration could potentially be simplified using other registration algorithms and cost functions.

A second limitation of our results is that, even at 3T, the resolution obtained with the DTI acquisition remains coarse relative to the sizes of the spinal-cord structures we would like to assess. The spinal cord is approximately 1.5 cm in diameter, and the individual columns are substantially smaller — on the scale of our acquired voxels. Therefore, partial volume effects heavily impact the accuracy and potentially the sensitivity of the derived quantities to differences in tissue microstructure. We expect that the higher SNR and spatial resolution that will be available at higher field strengths will enhance the ability for DTI and MT to sensitively and accurately probe microstructure in both healthy and diseased spinal cords, not only in the cervical region but also in the thoracic and lumbar regions.

CONCLUSION

This paper reports the reproducibility and variability of MRI quantities derived from DTI-reconstructed tract profiles in the lateral and dorsal columns of healthy cervical spinal cords. We hypothesize that these techniques will improve throughput in future studies because they remove much of the subjectivity of slice-by-slice ROI placement. We hypothesize that the techniques will enhance our understanding of the relationship between the structure and microstructural integrity of the spinal cord and dysfunction in neurological disease.

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Abbreviations

AMN	adrenomyeloneuropathy
AP	anterior-posterior
CSF	cerebrospinal fluid
DTI	diffusion tensor imaging

EPI	echo-planar-imaging
FA	fractional anisotropy
FOV	field of view
LL	left lateral
LOA	limit of agreement
MD	mean diffusivity
MS	multiple sclerosis
MT	magnetization transfer
MTCFSF	MT signal intensity relative to cerebrospinal fluid
RL	right lateral
ROI	regions of interest
SENSE	sensitivity encoding
STIR	short tau inversion recovery

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APPENDIX: CROSS-SECTIONAL ANALYSIS OF INDIVIDUAL TRACT PROFILES

While most of the analysis performed in this manuscript focuses on group means and differences between columns, it is important to note that the statistical significance, reproducibility, and reliability between groups is, at the core, dependent on the data from each individual. If the variability between people is large, then it is unlikely that a difference between columns, time-points, or raters could ever be appreciated.

Figure A1 shows the MT and DTI tract profiles for the first time-point and rater. The individual data is given in color and the mean ($\pm$ standard deviation, $n = 9$) is given in black. Visually, there are some features to note. For all of the DTI-derived indices, the lateral column data shows less variability over volunteers than does the dorsal column. This is most pronounced in the one volunteer where, at C5, there is a large signal elevation at C5 for MD, $\lambda_{||}$, and $\lambda_{\perp}$ which results in a signal drop in FA. Both lateral and dorsal column MTCSF values show more variability than the DTI, which we hypothesize to be a function of: (1) higher resolution and (2) registration pitfalls between MT and DTI acquisitions. Additionally, the MTCSF signal shows a deflection at each of the cervical levels. This is an interesting phenomenon and could be caused by susceptibility artifacts from intervertebral discs.

Figure 1.

Registration and MTCFS calculation scheme. The top left shows the scheme for coregistering each diffusion acquisition to the initially obtained b_0 using FLIRT. Each set of diffusion-weighted images and a second set of b_0 images are separately registered to the initial b_0 . Top right: cerebrospinal-fluid region of interest for calculation of MTCFS maps. Bottom right: MT registration followed a two-step procedure implemented in AIR: (1) 3D (6 degrees of freedom: 3 translation, 3 rotation) registration, and (2) 2D (3 degree of freedom: 2 translation, 1 rotation) registration to the mean b_0 .

Figure 2.

Representative slices of MTCSF and DTI-index maps of the at the C3 vertebral-body level in two healthy volunteers. White matter is darker than gray matter on MTCSF, mean diffusivity (MD), and perpendicular diffusivity ($\lambda_{\perp}$) maps and brighter than gray matter on fractional anisotropy (FA) and parallel diffusivity ($\lambda_{\parallel}$) maps. Cerebrospinal fluid is bright on all maps except for FA. Note that contrast between white and gray matter can best be appreciated in the MTCSF, and FA maps.

Figure 3.

Top row: Tractograms of the dorsal (green) and lateral (right = yellow, left = red) columns in one healthy volunteer. Each column of the figure shows the reconstructed fibers for a different rater or time-point. In each panel, the cross-sectional positions of the tractograms are delineated on a selected slice from the b_0 volume. Bottom row: Locations of the regions of interest drawn to seed the lateral- and dorsal-column tract reconstructions superimposed on the MTCSF map, which has been reformatted to the same resolution as the DTI. As a visual guide, an outline of the gray matter (orange) is presented and propagated to all images.

Figure 4.

Spatially normalized tract profiles (mean $\pm$ standard deviation) for each of the MTCSF- and DTI-derived quantities for the two raters. Note that MD, $\lambda_{||}$, and $\lambda_{\perp}$ are presented in the same panels for ease of viewing. Except for FA and $\lambda_{\perp}$, which are respectively lower and higher caudally than they are rostrally, there is little variation between C2 and C6. The differences between the results from the two raters are small. The right and left lateral columns are averaged together for this analysis.

Figure 5. Tract profiles (mean $\pm$ standard deviation) for the MTCSF- and DTI-derived quantities for a single rat at two time-points separated by at least 1 week. MD, $\lambda_{||}$, and $\lambda_{\perp}$ are presented in the same panel. The right and left lateral columns are averaged together for this analysis.

Figure A1.

Tract profiles for the MTCFSF- and DTI-derived quantities for each individual participant (color) as compared to the group (black) (mean $\pm$ standard deviation, $n = 9$) for a single rater. The right and left lateral columns are averaged together for this analysis.

Table 1

Mean ($\pm$ standard deviation, $n = 9$) tract-specific MRI quantities over all participants for the two raters and time-points. The table also shows the mean difference between raters and time-points (D), the Bland-Altman (BA) 95% Confidence Interval for the difference: 95% CI (D), the Bland-Altman limit-of-agreement (LOA) statistic, the paired t -test p -value for the null hypothesis that the means are equal, and the normalized difference (D_{BA}) between raters and time-points. The right and left lateral columns are averaged together for this analysis.

	Interrater					
	Rater 1	Rater 2	BA		t-test	
Lateral	mean $\pm$ std	mean $\pm$ std	difference (D)	95% CI (D)	LOA	p -value
FA	0.68 $\pm$ 0.06	0.66 $\pm$ 0.06	0.020	(-0.03, 0.07)	(-0.10, 0.14)	0.37
MD ($\mu\text{m}^2/\text{ms}$)	1.15 $\pm$ 0.15	1.15 $\pm$ 0.15	-0.012	(-0.01, 0.08)	(-0.25, 0.23)	0.78
λ_{q} ($\mu\text{m}^2/\text{ms}$)	2.13 $\pm$ 0.14	2.11 $\pm$ 0.15	0.023	(-0.06, 0.07)	(-0.17, 0.17)	0.89
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.67 $\pm$ 0.16	0.67 $\pm$ 0.16	-0.02	(-0.00, 0.09)	(-0.31, 0.27)	0.70
MTCFSF	0.52 $\pm$ 0.05	0.53 $\pm$ 0.05	-0.017	(-0.07, 0.03)	(-0.14, 0.11)	0.46
	Rater 1	Rater 2		BA		T -test
Dorsal	mean $\pm$ std	mean $\pm$ std	difference (D)	95% CI (D)	LOA	p -value
FA	0.68 $\pm$ 0.05	0.67 $\pm$ 0.05	0.010	(-0.02, 0.04)	(-0.07, 0.09)	0.48
MD ($\mu\text{m}^2/\text{ms}$)	1.12 $\pm$ 0.08	1.13 $\pm$ 0.11	-0.019	(-0.09, 0.05)	(-0.20, 0.16)	0.56
λ_{q} ($\mu\text{m}^2/\text{ms}$)	2.14 $\pm$ 0.11	2.15 $\pm$ 0.10	-0.023	(-0.1, 0.05)	(-0.20, 0.17)	0.50
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.61 $\pm$ 0.09	0.62 $\pm$ 0.12	-0.017	(-0.09, 0.06)	(-0.20, 0.18)	0.61
MTCFSF	0.50 $\pm$ 0.05	0.52 $\pm$ 0.05	-0.012	(-0.07, 0.04)	(-0.16, 0.13)	0.63
Test-Retest	Time 1	Time 2		BA		T -test
Lateral	mean $\pm$ std	mean $\pm$ std	difference (D)	95% CI (D)	LOA	p -value
FA	0.66 $\pm$ 0.06	0.69 $\pm$ 0.08	-0.03	(-0.09, 0.03)	(-0.18, 0.12)	0.26
MD ($\mu\text{m}^2/\text{ms}$)	1.15 $\pm$ 0.15	1.16 $\pm$ 0.17	-0.01	(-0.22, 0.24)	(-0.58, 0.60)	0.93
						D_{BA} (%)
						4.6
						0.9

	Interrater					
	Rater 1	Rater 2	BA		t-test	
Lateral	<i>mean ± std</i>	<i>mean ± std</i>	<i>difference (D)</i>	<i>95% CI (D)</i>	<i>LOA</i>	<i>p-value</i>
$\lambda_{ }$ ($\mu\text{m}^2/\text{ms}$)	2.11 ± 0.15	2.2 ± 0.13	-0.08	(-0.04, 0.03)	(-0.97, 0.81)	0.61
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.67 ± 0.16	0.63 ± 0.19	0.05	(-0.16, 0.27)	(-0.48, 0.59)	0.56
MTCSE	0.53 ± 0.05	0.51 ± 0.06	0.03	(0.02, 0.07)	(-0.1, 0.15)	0.24
	Time 1	Time 2		BA		T-test
Dorsal	<i>mean ± std</i>	<i>mean ± std</i>	<i>difference (D)</i>	<i>95% CI (D)</i>	<i>LOA</i>	<i>p-value</i>
FA	0.67 ± 0.05	0.66 ± 0.04	0.01	(-0.03, 0.05)	(-0.09, 0.11)	0.57
MD ($\mu\text{m}^2/\text{ms}$)	1.13 ± 0.11	1.13 ± 0.14	0.01	(-0.11, 0.14)	(-0.30, 0.32)	0.78
$\lambda_{ }$ ($\mu\text{m}^2/\text{ms}$)	2.15 ± 0.10	2.17 ± 0.15	0.01	(-0.13, 0.14)	(-0.35, 0.36)	0.99
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.62 ± 0.12	0.61 ± 0.14	0.02	(-0.09, 0.14)	(-0.28, 0.33)	0.68
MTCSE	0.52 ± 0.05	0.50 ± 0.04	0.03	(-0.02, 0.07)	(-0.09, 0.14)	0.20
						5.3