

Sensorimotor dysfunction in multiple sclerosis and column-specific magnetization transfer-imaging abnormalities in the spinal cord

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The human spinal cord contains segregated sensory and motor pathways that have been difficult to quantify using conventional magnetic resonance imaging (MRI) techniques. Multiple sclerosis is characterized by both focal and spatially diffuse spinal cord lesions with heterogeneous pathologies that have limited attempts at linking MRI and behaviour. We used a novel magnetization-transfer-weighted imaging approach to quantify damage to spinal white matter columns and tested its association with sensorimotor impairment. We studied 42 participants with multiple sclerosis who each underwent MRI at 3 Tesla and quantitative tests of sensorimotor function. We measured cerebrospinal-fluid-normalized magnetization-transfer signals in the dorsal and lateral columns and grey matter of the cervical cord. We also measured brain lesion volume, cervical spinal cord lesion number and cross-sectional area, vibration sensation, strength, walking velocity and standing balance. We used linear regression to assess the relationship between sensorimotor impairment and MRI abnormalities. We found that the dorsal column cerebrospinal-fluid-normalized magnetization-transfer signal specifically correlated with vibration sensation ($R=0.58$, $P<0.001$) and the lateral column signal with strength ($R=-0.45$, $P=0.003$). Spinal cord signal measures also correlated with walking and balance dysfunction. A stepwise multiple regression showed that the dorsal column signal and diagnosis subtype alone explained a significant portion of the variance in sensation ($R^2=0.54$, $P<0.001$), whereas the lateral column signal and diagnosis subtype explained a significant portion of the variance in strength ($R^2=0.30$, $P<0.001$). These results help to understand the anatomic basis of sensorimotor disability in multiple sclerosis and have implications for testing the effects of neuroprotective and reparative interventions.

Keywords: strength; sensation; corticospinal tract; dorsal column medial lemniscal tract; magnetic resonance imaging

Abbreviations: EDSS = expanded disability status scale; FLAIR = fluid attenuated inversion recovery; MRI = magnetic resonance imaging; MT = magnetization transfer; MTCSF = cerebrospinal-fluid-normalized magnetization-transfer

Introduction

The accumulation of sensorimotor disability in multiple sclerosis is largely dependent on spinal cord disease (Ikuta and Zimmerman, 1976), and prior studies have shown a relationship between spinal cord disease and functional abnormality in multiple sclerosis (Brex *et al.*, 2002; Rocca *et al.*, 2005; Agosta *et al.*, 2007a, b). Several studies have evaluated abnormalities of the spinal cord using conventional magnetic resonance imaging (MRI), including T₁- and T₂-weighted sequences (Schreiber *et al.*, 2001; Brex *et al.*, 2002; Rovaris *et al.*, 2003) and gadolinium enhancement (Kappos *et al.*, 1999; Bot *et al.*, 2004a), and advanced techniques such as magnetization transfer (MT) imaging (Agosta *et al.*, 2007b). These MRI properties were studied in white and grey matter and related to global disability measures such as the multiple sclerosis functional composite.

The spinal cord is somatotopically organized. We hypothesized that those MRI techniques of sufficient resolution to distinguish individual white matter columns would detect abnormalities related to the specific function of that tract. MT imaging is a technique that indirectly assesses the status of water protons associated with macromolecular structures in tissues such as myelin. It is especially useful for studying white matter integrity because of their high myelin content (van Buchem *et al.*, 1999; Sled and Pike, 2001). MT imaging therefore provides a sensitive means to quantify white matter abnormalities in both brain and spinal cord (Agosta *et al.*, 2007a, b; Wu *et al.*, 2007). However, conventional spinal cord MRI has been limited by a low signal-to-noise ratio and high sensitivity to motion, which is exacerbated for MT MRI due to the need for a reference scan to determine MT ratio values. Smith *et al.* (2005) developed a novel approach to quantify MT effects in the spine using cerebrospinal fluid as an internal intensity standard, allowing inter-individual comparison of MT-weighted data without the need for a reference scan. This so-called cerebrospinal-fluid-normalized magnetization-transfer (MTCSF) approach allows quantitative evaluation of the integrity of specific spinal columns or grey matter, as was first demonstrated in a study of demyelination in adrenomyeloneuropathy (Fatemi *et al.*, 2005; Smith *et al.*, 2005, 2009).

Sensorimotor measures of impairment, such as sensation and strength, are specific to particular white matter pathways and report objectively on neurologic dysfunction. However, quantifying the magnitude of specific neurologic disability is difficult, because the rating scales used to evaluate physical function do not distinguish between the effects of sensory versus motor abnormalities and often utilize categorical and nonlinear measures. Clinical studies typically evaluate a patient's physical disability using timed tests or rating scales such as the 25-foot walk test (Gardner, 1993; Ciccarelli *et al.*, 2007), maximum distance walked (Schwid *et al.*, 1997) or the multiple sclerosis functional composite (Rudick *et al.*, 1997; Hobart *et al.*, 2004). These scales measure physical disability in a broad, general manner with the goal of determining overall disease severity, but blur the effects of clinically important focal deficits such as single-extremity weakness or sensory impairment. When considering diseases in which disability is time varying and heterogeneous, such as multiple sclerosis, the effects of the underlying pathology can be missed. Here, to

systematically evaluate sensory and motor function, we quantify lower extremity strength using dynamometry and vibration sensation thresholds using a Vibratron device. We also use a motion measurement system to quantify walking speed and a force plate to quantify features of balance.

In this study, we use MTCSF MRI to visualize and quantify the integrity of specific white matter columns in the spinal cord that carry motor and sensory information to and from the extremities. We further study the relationship between spinal cord structure and function by quantifying the relationship between this integrity and the degree of overt sensorimotor impairment. In addition, we quantify brain lesion volume, spinal cord lesion number and cross-sectional area to characterize our multiple sclerosis cohort. Last, we directly examine a portion of the grey matter in the cervical spinal cord. Our data in multiple sclerosis show that column-specific, spinal cord MTCSF can explain a significant portion of the variance in specific impairments of vibration sensation and strength as well as the higher-level functions of walking speed and balance. The information gained from this study can be used for defining the anatomic substrates of disability in this disease. The results are expected to provide a foundation for designing specific rehabilitation programs that are based on structure–function relationships and for quantifying the effects of future neuroprotective and reparative interventions.

Methods

Participants

We examined 42 individuals with multiple sclerosis (25 women; mean age: 45 ± 10 years; median disease duration: 6.5 years) using both spinal cord MRI and measures of sensorimotor impairment (measures of strength, sensation, walking and balance). Twenty-three participants had relapsing remitting multiple sclerosis (median disease duration: 7 years), 11 secondary progressive multiple sclerosis (16 years) and 8 primary progressive multiple sclerosis (4 years). Four of these participants had one, and one participant had two, sensorimotor impairment(s) that could not be quantified, because the deficits were beyond the dynamic range of the instrument; we used all the data that could be quantified. We obtained MRI data from 18 healthy controls (mean age: 35 ± 9 years). For strength and walking speed measures, we used published controls (Bohannon, 1997a, b). For vibration measures, we used information from Vibratron packaging insert. For sway amplitude, we used a collection of 52 healthy controls in our laboratory (unpublished data). All participants provided signed, informed consent in accordance with Internal Review Board regulations at Johns Hopkins University and Kennedy Krieger Institute.

Movement and MRI studies were done within 2 weeks of one another. Disease subtype and duration was obtained by retrospective chart review and interviews with the participants.

Image acquisition

MRI scans of the cervical spinal cord and the brain were performed with the same 3-Tesla Intera scanner (Philips Medical Systems, Best, The Netherlands) using body coil excitation and two-element phased array surface coil reception. Forty contiguous 2.25 mm slices were acquired from the C2–C6 vertebral body levels, with nominal in-plane

resolution of 0.6×0.6 mm. MT-weighted images were obtained using an MT prepulse applied 1.5 kHz off resonance (24 ms, five-lobed sinc-gauss pulse with maximum amplitude $9.5 \mu\text{T}$). Other parameters: repetition time 110 ms, echo time 13 ms, flip angle 9° , echo planar imaging factor 3 and SENSE acceleration factor 2. Prior to image assessment, the scans were resliced to 60 1.5 mm slices.

Cerebrospinal fluid-normalized MT signal intensity

In each slice, cerebrospinal fluid-normalized MT (MTCSF) was calculated for three manually drawn regions of interest (ROIs, Fig. 1): right lateral column, left lateral column and combined right and left dorsal columns of the white matter. Five slices centered on the C2–C3 disc were further examined for grey matter abnormalities using the method described in Smith *et al.* (2005). ROIs were placed in the dorsolateral horn grey matter of each slice then averaged for each person. The MTCSF in each ROI was defined by dividing the MT-weighted signal by the mean signal intensity of the surrounding cerebrospinal fluid in the reference scan at the same level (Smith *et al.*, 2005).

Since neck lengths differ among participants, MT signal intensities were interpolated to fit 40 slices to the average distance from the nerve roots of C2–C6. Slice 1 was fixed to the inferior aspect of the exiting C6 nerve roots and slice 40 to the inferior aspect of the C2 nerve roots. The column-specific MTCSF measurements that

were compared to sensorimotor functions were the averages over all 40 normalized slices.

Spinal cord lesion count

Cervical spinal cord lesions were identified and counted by a neuro-radiologist (DSR) on the axial MT scans.

Spinal cord cross-sectional area

For each of three axial slices centered around the C2–C3 disc, we drew ROIs around the spinal cord using DtiStudio (Jiang *et al.*, 2006). Care was taken to minimize partial volume averaging with the surrounding cerebrospinal fluid. The average area across the three slices was recorded for each case.

Brain lesion segmentation

We obtained and coregistered FLAIR sequences covering the brain from the medulla to the vertex, or just caudal, to it for each participant. Lesions were identified on the each slice of the coregistered FLAIR images by a neuroradiologist who was blinded to the participant's identity and level of disability. They were then outlined with a region growing technique (implemented in DtiStudio) that extended a contiguous area around hand-selected seed points until a threshold intensity was reached (van Walderveen *et al.*, 1995). The threshold

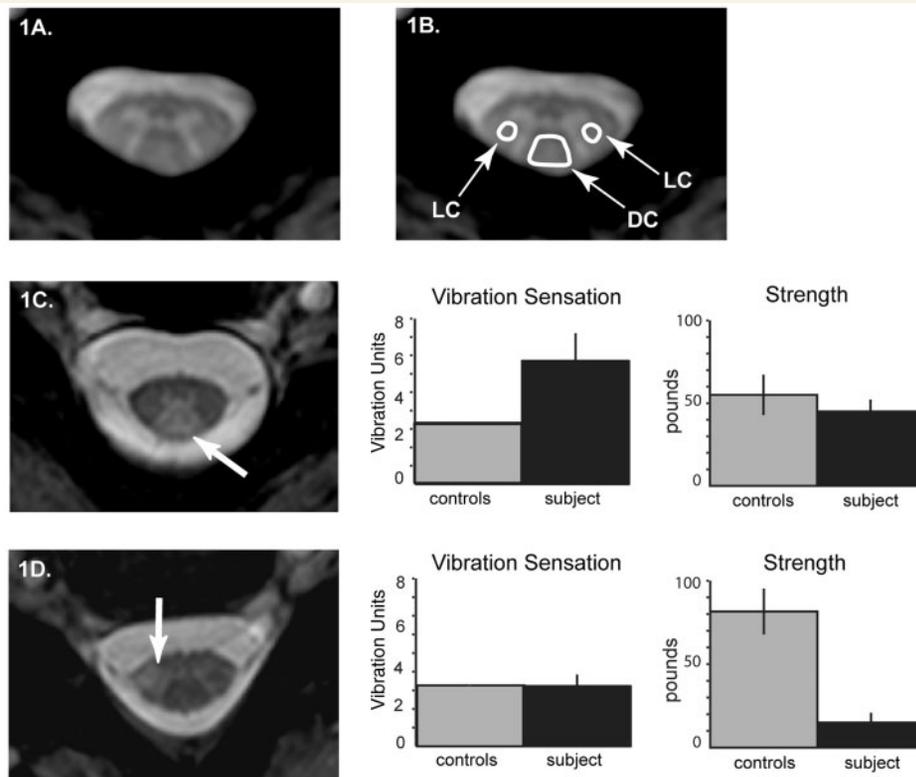


Figure 1 Representative MTCSF axial spinal cord slices for one healthy participant and two individuals with multiple sclerosis with a lesion in either the dorsal column or lateral column. (A) A 50-year-old individual showing clear delineation of grey and white matter with no detectable lesion. (B) Same individual as in (A), with white outlines to show examples of regions of interest drawn for dorsal and lateral columns, (C) A 43-year-old individual with multiple sclerosis with a lesion in the dorsal column showing significantly decreased vibration sensation (6.5 vibration units), but normal ankle strength, (D) A 47-year-old individual with multiple sclerosis and a lesion in the right lateral column in the expected location of the corticospinal tract. This person had significantly decreased strength in both ankles (right = 15.0 lbs, left = 37.5 lbs) and decreased walking speed (0.57 m/s), but vibration sensation was normal.

was adjusted for optimal qualitative capture of the lesions with minimal inclusion of adjacent NAWM.

Impairment measures

Dorsal column involvement was quantified using a series of tests assessing vibratory sense and degree of postural sway (i.e. static standing balance). Vibration sensation thresholds for the right and left great toes were noninvasively quantified for 86 of 88 toes using the Vibratron II (Physitemp, Huron, NJ) (Arezzo *et al.*, 1985). Postural sway data (1 kHz sampling rate) were collected on 42 of 44 participants using a Kistler 9281 force plate (Kistler Instrument Corp., Switzerland). Participants stood on the force plate with feet apart (25–35 cm) and eyes open for 20 s. The centre-of-pressure *x*- and *y*-coordinates were determined, and postural sway amplitude was calculated as the mean vector distance between the origin of pressure to each new centre of pressure point (Prieto *et al.*, 1996). The mean of the two independent trials was the sway amplitude score.

Lateral column involvement was assessed using ankle dorsiflexion strength (84 of 84 ankles) and walking velocity (42 of 44 participants). For strength, we collected two maximal ankle dorsiflexion efforts at each ankle using a Microfet2 handheld dynamometer (Bohannon, 1997b). We chose ankle strength, because it could be reliably quantified, and clinically the ankle is one of the most common sites of weakness and less likely to be confounded by disuse or steroid-induced atrophy than the hip flexors. Walking velocity data were collected at 100 Hz using a 3D Optotrak Motion Measurement System (Northern Digital Inc., Waterloo, Ontario). Participants walked across a 20-foot walkway at their fastest walking speed. The mean walking velocity score was the average of three to five trials.

Statistics

To compare sensorimotor impairment scores between healthy controls and multiple sclerosis patients, we converted raw values to *z*-scores based on published norms for ankle strength (age- and gender-matched) and walking speed (age-matched) and on a collection of 52 healthy controls collected in our laboratory for sway amplitude (unpublished values). Two-tailed *t*-tests were then used to compare the resulting *z*-scores to 0. For toe vibration, for which age-specific means and standard deviations were not available, we used the 2.5 SD thresholds listed in the Vibratron package insert. We then applied the cumulative binomial distribution to determine the probability that the

number of observed abnormal cases could be expected by chance alone. To determine abnormalities in column-specific MTCSF, grey matter MTCSF and spinal cord cross-sectional area, we conducted partial correlation analysis adjusting for age and sex. To compare the different multiple sclerosis subtypes, we used one-way ANOVA for each of the following MRI variables: lateral column MTCSF, dorsal column MTCSF, cross-sectional area, spinal cord lesion number, brain lesion volume and grey matter MTCSF.

Pearson product moment correlations were used to evaluate the relationships between sensorimotor impairments, MTCSF for lateral columns, dorsal columns and grey matter and the Expanded Disability Status Scale (EDSS). Strength measurements from the weaker of the two ankles were related to MTCSF from the ipsilateral lateral column; similarly, toe vibration sensation from the toe with the poorer sensation was related to dorsal column MTCSF. To assess the extent to which the MRI variables can explain the variance in sensation or strength, we performed a stepwise regression analysis using Statistica software (StatSoft, Tulsa, OK). Eight regressor variables were included: multiple sclerosis subtype, age, disease duration, brain lesion volume, spinal cord cross-sectional area, spinal cord lesion number, ipsilateral lateral column MTCSF and ipsilateral dorsal column MTCSF. To enter the stepwise model, each variable was required to have a significant correlation of $P < 0.05$.

In general, we report *P*-values directly, without adjustment for multiple comparisons, and limited discussion of statistical significance to the extent possible. As many of the variables tested here are correlated with one another, applying a simple Bonferroni correction would not be appropriate.

Results

Sensorimotor impairments

Compared to mean controls, multiple sclerosis participants showed considerable significant abnormalities in the four sensorimotor impairment measures ($P < 0.001$). The greatest abnormalities were seen in secondary progressive multiple sclerosis and the least in relapsing remitting multiple sclerosis (Table 1). A one-way ANOVA showed a significant effect of multiple sclerosis subtype on each of the sensorimotor impairments. Sixty-one of 78 toes (78%), had abnormal vibration sensation, defined as at

Table 1 Sensorimotor impairments and clinical disability

	All MS	RRMS	SPMS	PPMS	ANOVA
Number of participants	42	22	11	9	
Age	44.7 ± 10.0	39.2 ± 9.7	49.5 ± 5.8	52.3 ± 7.2	0.0002
EDSS	3.7 ± 2.0	2.3 ± 1.3	5.7 ± 1.1	4.5 ± 1.6	<0.0001
Sensory					
Vibration	4.53 ± 3.74 (<0.0001)	2.78 ± 1.77 (<0.0001)	6.36 ± 2.94 (<0.0001)	6.60 ± 5.92 (<0.0001)	0.08
Sway (mm)	6.02 ± 2.89 (0.0002)	4.77 ± 2.15 (0.18)	6.74 ± 1.71 (0.002)	8.11 ± 4.03 (0.019)	0.11
Motor					
Ankle dorsiflexion (lbs)	35.8 ± 18.9 (<0.0001)	46.1 ± 12.8 (0.0001)	18.5 ± 18.8 (<0.0001)	31.8 ± 15.7 (0.0004)	0.0002
Walking velocity (m/s)	1.34 ± 0.61 (<0.0001)	1.73 ± 0.34 (<0.0001)	0.67 ± 0.48 (<0.0001)	1.17 ± 0.55 (0.0007)	0.0001

Data are reported as mean ± SD. For impairment scores, *P*-values assessing age- and sex-adjusted differences from healthy controls are listed in parentheses; see 'Methods' section for details on statistical tests. Vibration units are amplitudes, proportional to the square of the applied voltage. Data in the ANOVA column are *P*-values assessing a main effect of MS subtype on each variable, adjusting for age and sex. EDSS = expanded disability status score; RRMS = relapsing-remitting MS; SPMS = secondary-progressive MS; PPMS = primary progressive MS.

least 2 SD above the control mean (Arezzo *et al.*, 1985). Over one-third of toes were severely impaired (at least 6.5 SD above control mean), largely in progressive multiple sclerosis (Table 1). Of 40 participants, 27 (67%) had abnormal balance as measured by a sway amplitude more than 1 SD above mean controls.

For ankle dorsiflexion strength, 57 of 84 ankles (68%) were more than 1 SD weaker than controls. All patient values were significantly below ($P < 0.05$) the mean dominant ankle strength in healthy controls, age and gender matched, between 20 and 70 years old (Bohannon, 1997b). Of 40 participants, 34 (85%) had walking velocities more than 1 SD below control. These were significantly different from mean walking velocity ($P < 0.001$) for healthy controls, age- and gender-matched, between 20 and 70 years (Bohannon, 1997a).

MRI abnormalities in the spinal cord and brain

Table 2 shows MRI results for the study population, including spinal cord lesion number, spinal cord cross-sectional area, brain lesion volume and MTCSF in the dorsal columns, lateral columns and grey matter. One-way ANOVA conducted for each variable showed weak effects of multiple sclerosis subtype on lateral column lesion number and on lateral column and dorsal column MTCSF, but not on any of the other variables tested. Cross-sectional area was slightly lower in the secondary progressive multiple sclerosis group than in controls ($P = 0.023$). In our multiple sclerosis cohort, dorsal column MTCSF was significantly different from controls in the secondary progressive multiple sclerosis group. For the lateral column, MTCSF for the entire multiple sclerosis group and for both progressive subgroups was also significantly higher than controls. No significant abnormalities were found in the relapsing remitting subgroup.

White and grey matter MTCSF-disability correlations

The EDSS was significantly correlated with column-specific MTCSF, sensorimotor impairments and function ($P < 0.05$). Not surprisingly, given its emphasis on walking, EDSS correlated most highly with walking velocity ($R = -0.83$, $P < 0.001$) and least with standing

balance ($R = 0.35$, $P = 0.02$). The EDSS correlated significantly and similarly with dorsal column and lateral column MTCSF ($R = 0.41$ and $R = 0.59$, respectively) as well as with sensation and strength ($R = 0.59$ and $R = -0.66$, respectively).

Figure 1C and D show examples of a single participant's imaging and corresponding strength and sensation data. Group results (Fig. 2) show that column-specific MTCSF correlates with sensorimotor measures. Great toe vibration sensation correlated with dorsal column MTCSF (Fig. 2A, $R = 0.58$, $P < 0.001$); a correlation with lateral column MTCSF was weaker ($R = 0.50$, $P < 0.001$). The functional measure of standing balance (sway amplitude) was more weakly associated with dorsal column MTCSF (Fig. 2B, $R = 0.32$, $P = 0.02$). Ankle strength was associated with lateral column MTCSF (Fig. 2C, $R = -0.45$, $P = 0.003$) and less strongly with dorsal column MTCSF ($R = -0.39$, $P = 0.01$). Walking velocity, a functional measure that may be influenced by strength, was significantly associated with lateral column MTCSF (Fig. 2D, $R = -0.51$, $P < 0.001$).

We also measured correlations of sensorimotor impairments and disability with grey matter MTCSF and spinal cord atrophy. A one-way ANOVA showed no effect of multiple sclerosis subtype on grey matter abnormalities, and grey matter involvement in multiple sclerosis was not significantly different from controls ($P < 0.1$). Pearson product moment correlations showed that grey matter MTCSF was not significantly correlated with cross-sectional area of the spinal cord (our measures of cord atrophy). In contrast, grey matter MTCSF was significantly correlated with lateral column and dorsal column MTCSF ($R = 0.51$ and $R = 0.55$, $P < 0.01$, respectively) and to EDSS ($R = 0.39$, $P = 0.02$), but not to our global measures of sensorimotor function, walking velocity and balance ($P > 0.05$).

Stepwise multiple regression and partial correlations

We used stepwise multiple regression modelling to determine whether MTCSF abnormalities can explain a significant portion of the variance in toe vibration sensation and ankle dorsiflexion strength, because sensation and strength have important functional significance, are quantifiable and may relate to specific white matter fibre tracts (e.g. strength to the CST). For the

Table 2 MRI results

	Controls	All MS	RRMS	SPMS	PPMS	ANOVA
Brain lesion volume (cm ³)	0	13.8 ± 13.0	12.0 ± 13.4	14.7 ± 12.0	17.4 ± 14.0	0.71
Spinal cord area (mm ²)	144 ± 12	139 ± 16 (0.73)	142 ± 16 (0.90)	137 ± 15 (0.023)	136 ± 18 (0.10)	0.68
Lesion number						
Lateral column	0	2.0 ± 1.5	2.1 ± 1.8	2.3 ± 1.0	1.2 ± 1.1	0.032
Dorsal column	0	1.9 ± 1.6	2.3 ± 1.7	1.7 ± 1.2	1.1 ± 1.5	0.60
MTCSF (normalized units)						
Lateral column	0.50 ± 0.05	0.55 ± 0.07 (0.008)	0.52 ± 0.06 (0.11)	0.60 ± 0.05 (<0.001)	0.56 ± 0.08 (0.016)	0.038
Dorsal column	0.48 ± 0.05	0.51 ± 0.06 (0.14)	0.49 ± 0.05 (0.44)	0.56 ± 0.06 (0.009)	0.50 ± 0.05 (0.73)	0.016
Grey matter	0.55 ± 0.07	0.56 ± 0.08 (0.98)	0.54 ± 0.07 (0.40)	0.61 ± 0.04 (0.005)	0.55 ± 0.11 (0.40)	0.12

Data are reported as mean ± SD. Partial correlation analysis, adjusting for age and sex, was used to determine differences from healthy controls for spinal cord area and MTCSF values; P -values are listed in parentheses. Data in the ANOVA column are P -values assessing a main effect of MS subtype on each variable, adjusting for age and sex. Abbreviations as in Table 1.

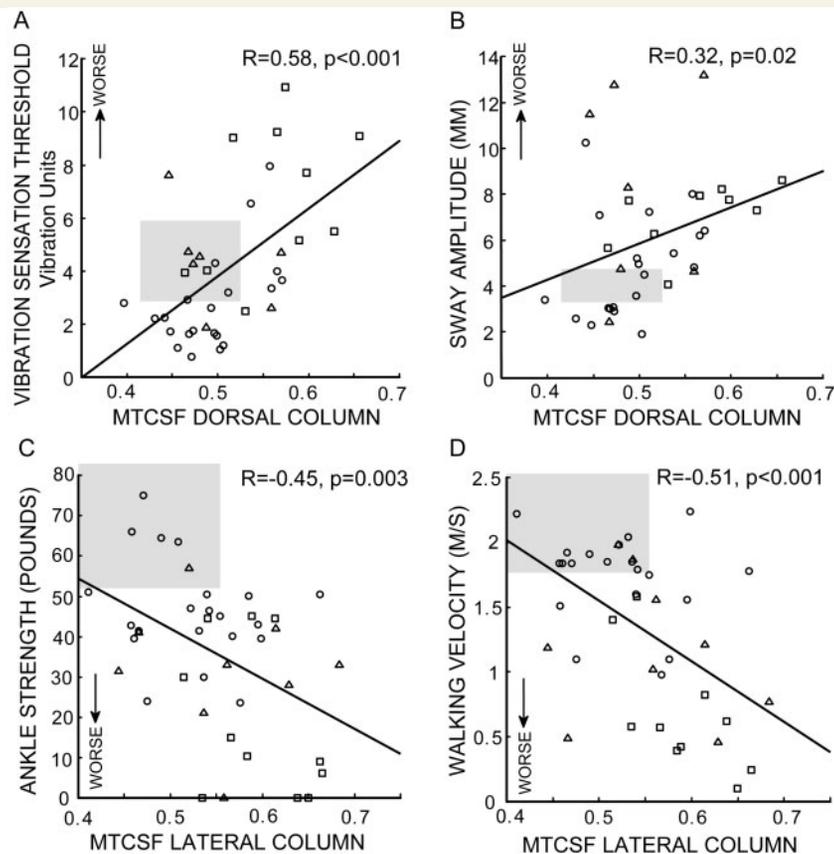


Figure 2 Correlation between column-specific MTCSF and sensorimotor impairment and function. (A) Great toe vibration sensation threshold from the more impaired toe (right or left) versus MTCSF of the dorsal column. Vibration units are the amplitude of vibration (proportional to the square of the applied voltage). (B) Sway amplitude, a measure of static standing balance versus MTCSF of the dorsal column. (C) Ankle strength from the weaker side (right or left) versus MTCSF of the ipsilateral lateral column. (D) Walking velocity versus MTCSF of ipsilateral lateral column from (C). Circles: relapsing remitting multiple sclerosis, triangles: secondary progressive multiple sclerosis, squares: primary progressive multiple sclerosis. Grey-shaded boxes indicate control ranges.

Table 3 Partial correlations from multiple linear regression model

	Ankle strength	Toe vibration sensation
Diagnosis subtype	-0.40 ($P=0.02$)	0.46 ($P<0.01$)
Age	-0.12 ($P=0.51$)	0.13 ($P=0.46$)
Disease duration	-0.22 ($P=0.23$)	-0.03 ($P=0.85$)
Brain lesion vol (cm^3)	0.32 ($P=0.07$)	0.31 ($P=0.08$)
Spinal cord lesion number	-0.24 ($P=0.17$)	0.14 ($P=0.45$)
Spinal cord cross-sectional area (mm^2)	0.26 ($P=0.14$)	-0.05 ($P=0.77$)
MTCSF of LC	-0.35 ($P=0.04$)	0.03 ($P=0.88$)
MTCSF of DC	-0.13 ($P=0.49$)	0.42 ($P=0.01$)

Each subject contributes only the weaker ankle (right or left) and the great toe with the worse sensation. Spinal cord lesion no. indicates spinal cord lesion number. Partial correlation coefficients are used. All listed variables are included in the multiple regression analysis.

regression model, we included several clinical multiple sclerosis variables (diagnosis subtype, age and disease duration) as well as MRI variables including spinal cord lesion number, cross-sectional area and brain lesion volume, in addition to lateral

column and dorsal column MTCSF. To represent the unique contribution of each variable to strength or sensation, we report partial correlation coefficients in Table 3.

Figure 3A shows the scatterplot of observed versus predicted values with ankle strength as the dependent variable. Partial correlation analysis (Table 3) indicates that the strongest and only significant correlates of ankle strength were lateral column MTCSF and disease subtype, together explaining 36% of the overall variance in strength ($P<0.001$). Figure 3B shows the corresponding scatterplot for toe vibration sensation. The strongest and only significant correlations of toe vibration sensation were dorsal column MTCSF and disease subtype, together explaining 48% of the overall variance in sensation ($P<0.0001$).

Discussion

Our results show that abnormalities in column-specific cervical spinal cord MTCSF explains a portion of the disability that is related to its relevant column. Thus, the mean MTCSF signal change reflects real-world impairments of individuals with multiple sclerosis. Since MT imaging is thought to have increased

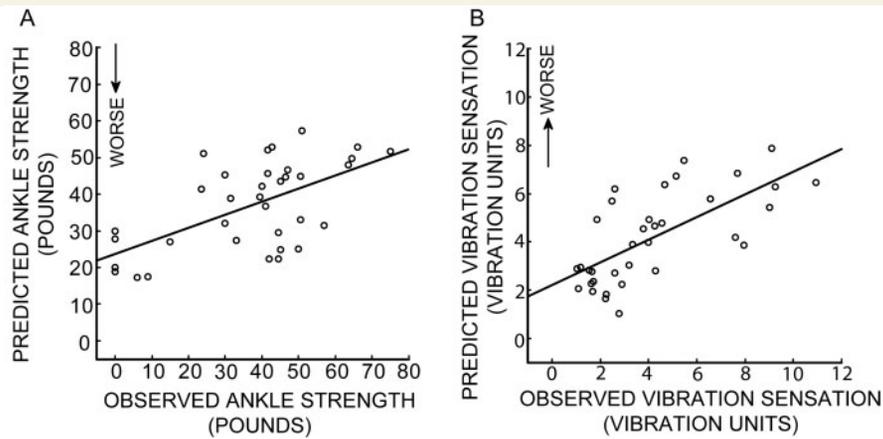


Figure 3 Results from the stepwise multiple linear regression analyses, indicating the predictive value of the MTCFS measures. (A) Ankle strength. (B) Toe vibration sensation.

specificity for demyelination (Schmierer *et al.*, 2004; Smith *et al.*, 2005), our results may provide greater insight into the pathologic substrate underlying sensorimotor dysfunction.

Relating MT imaging to sensorimotor impairment

The increased use of MRI for diagnosis and classification of patients with multiple sclerosis has been a driving force for understanding how lesions affect clinical manifestations and progression of the disease. Unfortunately, the role of lesions seen on spinal cord MRI remains poorly understood and has been limited to the detection of large inflammatory lesions (Bakshi *et al.*, 2008). Recently however, Ciccarelli *et al.* used diffusion tractography and MR spectroscopy to evaluate the spinal cord in 14 patients with relapsing remitting multiple sclerosis who were experiencing an acute relapse (Ciccarelli, 2007). By using these more advanced MRI techniques, they were able to show, for the first time, stronger and more significant correlations with motor function than what had been noted previously. Our results extend their findings in that we show—in a larger, more diverse sample of individuals with multiple sclerosis (and using 3T MRI)—that column-specific MTCFS not only correlates with motor and sensory disability but also explains a significant portion of their variance. We take these results one step further by correlating column-specific MTCFS data with functional measures of walking velocity and standing balance.

Our data also show that both lateral column and dorsal column MTCFS are significantly correlated with the EDSS. Even though the EDSS is a categorical, nonlinear, clinical rating scale that lacks specific information about impairment (i.e. strength or sensation), it correlated almost as well as the lateral column MTCFS with ankle dorsiflexion strength. This is probably due to the fact that patients with higher levels of disability tend to have weakness at the ankle dorsiflexors as well as at other joints, resulting in high-EDSS scores. Past studies have hypothesized, but not tested, that spinal cord abnormalities would relate strongly to measures of physical disability (Zivadinov *et al.*, 2007) and weakness in multiple sclerosis (Reich *et al.*, 2007). These hypotheses

have been supported by pathological findings of spinal cord atrophy (DeLuca *et al.*, 2004; Evangelou *et al.*, 2005) and spectroscopic findings of reduced levels of *N*-acetyl aspartate, a marker of overall axonal integrity, in the spinal cord (Blamire *et al.*, 2007). These are thought to represent primary axonal damage in the spinal cord and/or secondary damage such as Wallerian degeneration.

In validating a new biomarker (column-specific MTCFS), it is useful to have it relate to the gold standard biomarker (EDSS), but offer some advantages. The correlations between dorsal column MTCFS and sensation are better than the EDSS. Lateral column correlations are only slightly better than EDSS. One possible reason for the moderately strong correlation of lateral column MTCFS and strength is that our measure of strength is not comprehensive enough—fibres controlling the ankle dorsiflexors only account for a small fraction of the lateral column. In addition, weakness may result from lesions below the level at which we are assessing the lateral column.

Nonetheless, our regression results show that lateral column MTCFS explains a significant portion of the variance in strength. It is known that MT provides information about demyelination (Schmierer *et al.*, 2004, 2007) thus, using this tool, we gain greater insight into the neuropathology that may be contributing to the sensorimotor dysfunction.

We used great toe vibration perception in multiple sclerosis participants as an index of sensation, which is critical for control of static standing balance. Vibration sensation impairment is important for at least two reasons: (i) it is specific to the dorsal column, which also carries proprioceptive information, and therefore it has the specificity needed to be quantified using MRI; and (ii) it has functional relevance. It is known that poor sensory function contributes to poor balance and a higher incidence of falls and that balance problems are common in people with multiple sclerosis (Horak, 2006; Cameron *et al.*, 2008). Our findings show that both vibration sensation and sway amplitude (i.e. standing balance) are highly correlated with dorsal column MTCFS. A previous study on participants with adrenomyeloneuropathy showed a similar relationship between decreased vibration sensation and MTCFS abnormalities of the dorsal column

(Fatemi *et al.*, 2005). We hypothesize that our measure of vibration sensation could be used as a surrogate marker of dorsal column abnormalities in the spinal cord. A surrogate marker of vibration sensation would have important clinical implications for testing therapeutic interventions aimed at improving poor vibration sensation and balance.

Anatomic specificity of the link between MTCSF and sensorimotor impairment

Since the spinal cord is so small, and since tracts are positioned in close proximity to one another, we were interested in whether we could detect imaging abnormalities that correlate with tract-specific functions. To this end, we used multiple regression analyses to determine the variables that best explain the variance in vibration sensation or strength. We chose a number of dependent variables for our regression, because past studies have suggested that these are important variables to consider in multiple sclerosis (Gauthier *et al.*, 2007; Miller *et al.*, 2007; Reich *et al.*, 2007). Disease subtype (relapsing remitting, secondary progressive or primary progressive) was found to be an important independent variable for explaining the variance in vibration sensation and strength. This is not surprising given that clinically progressive patients generally have more significant disability, as was true in our sample: only two people with relapsing remitting multiple sclerosis had an EDSS of five or greater, indicating impairment of full daily activities. By contrast, 12 of 19 individuals with progressive multiple sclerosis had an EDSS of five or greater. However, although multiple sclerosis subtype alone was not sensitive enough to differentiate between weakness and vibratory loss, such differentiation could be achieved with column-specific MTCSF. In particular, dorsal column MTCSF was most strongly correlated with great toe vibration sensation and lateral column MTCSF with ankle weakness (Table 3). Other spinal cord imaging abnormalities and clinical variables were not independently associated with disability.

Grey matter involvement

Grey matter atrophy in the brain contributes to disability in individuals with multiple sclerosis (Fisniku *et al.*, 2008). A previous study has shown that cervical cord grey matter is also abnormal and is an additional factor contributing to the disability of multiple sclerosis patients (Agosta *et al.*, 2007a). That study, however, used a small and specific sample of patients with no visible MRI lesions and used the MT ratio to quantify grey matter. Measurement of the MT ratio requires accurate co-registration of two sets of images, which can lead to image degradation—a particular problem in the spinal cord (Smith *et al.*, 2005); we therefore focused on the MTCSF rather than the ratio. Our results support the findings of Agosta *et al.* (2007a) in that grey matter MTCSF was not significantly correlated with spinal cord atrophy but was correlated with clinical disability. Since we examined grey matter at the C2–C3 level, where there are no neurons that control ankle dorsiflexion and vibration sensation in the toes, this

result is somewhat surprising. Possible explanations include partial volume averaging with adjacent white matter, since the spinal cord is so small, and other technical factors that limit evaluation (Geurts and Barkhof, 2008). Further work will determine whether grey matter MTCSF, even in areas remote from those that relate to specific functions, is a good marker for diffuse spinal cord abnormality. Nevertheless, grey matter abnormalities were less strongly and specifically associated with tract-specific clinical dysfunction than dorsal column and lateral column abnormalities.

Histopathologic correlation

It is important to consider our results in light of histopathologic results. Because of the sensitivity of MT imaging to demyelination (Bot *et al.*, 2004a, b), our results are consistent with evidence that demyelination plays an important role in generating clinical disability in multiple sclerosis. Histopathologic data also suggest that axon loss occurs independently of demyelination in areas of both focal and diffuse signal abnormality and is often present in normal appearing white matter (Bot and Barkhof, 2009). It is possible that developing a column-specific imaging method, such as diffusion tensor imaging (DeBoy *et al.*, 2007; Naismith *et al.*, 2008), that is more sensitive to axon loss will improve the association with disability.

Limitations

Our measure of strength is objective and reliable (Bohannon, 1997b); however, it only incorporates one muscle, the tibialis anterior. We chose this muscle, because weakness of the tibialis anterior muscle typically results in foot drop, a common clinical walking problem in people with multiple sclerosis (Byrne *et al.*, 2007). Use of a combination of muscle strengths, such as the tibialis anterior and hip flexors, might strengthen the correlations and represent leg weakness more completely but could also introduce confounding variables due to proximal weakness from disuse, steroid use and vitamin D deficiency. By the same token, imaging the cord with higher resolution might allow more specific assessment of the portions of the lateral column that control tibialis anterior function. Similarly, our use of the Vibratron to measure vibration sensation is reliable and more objective than the commonly used tuning fork. However, the device is limited to testing at the great toe or the index finger; ideally, we would evaluate vibration sensation at multiple points on the body.

Similar to MT ratio measurements, MTCSF values depend on many technical variables such as the properties (length, shape and power) of the radiofrequency pulse in the MT pulse sequence (Smith *et al.*, 2005). Thus, MT ratio and MTCSF values may differ across equipment and sequence parameters, limiting the effectiveness of MT imaging in multi-site clinical trials. We chose to use MTCSF, rather than MT ratio, to limit the effects of patient motion and to accentuate contributions from T_1 , T_2^* and water density that can make abnormalities more conspicuous (though less specifically related to MT effects). Combining MTCSF with other quantitative MRI metrics such as diffusion tensor imaging and MR spectroscopy should add important information about the

relationships between tract specific spinal cord abnormalities and clinical dysfunction.

There are obvious limitations of MTCSF grey matter measurements that need discussion. Previous work using MT in evaluating grey matter (Agosta *et al.*, 2007a) acknowledge, as do we, that the small size of the spinal cord make it difficult to avoid partial volume effects. In addition, we know that MTCSF is sensitive to inflammation therefore the signal associated with a lesion can look similar to grey matter in the spinal cord. This made it difficult, in some cases, to visualize all borders of the grey matter and draw an accurate region of interest. We evaluated each case as carefully as possible to steer clear of such effects, but this remains an unavoidable technical limitation. Finally, interpretation of the MT signal in grey matter is challenging. Previous MT experiments often report being sensitive to myelin changes. In grey matter, the pathological substrate responsible for changes in the spinal cord is unclear. This makes it difficult to draw conclusions between grey matter changes and MTCSF.

An additional limitation of our study is the primary focus on spinal cord abnormalities. Our group has previously studied a combination of diffusion tensor imaging and MT imaging to evaluate the corticospinal tracts in multiple sclerosis (Reich *et al.*, 2007). This multiparametric approach allowed more sensitive detection, localization and characterization of tract-specific abnormalities in the brain. In addition, results showed that this type of approach correlated with clinical disability, including ankle dorsiflexion strength—although the correlation was relatively weak compared to the results presented here (Reich *et al.*, 2008). Ohgiya *et al.* (2007) applied diffusion tensor imaging to the cervical spinal cord of multiple sclerosis patients and found that it is more sensitive than T₂-weighted imaging in assessing disease burden. We suspect that use of a multiparametric imaging approach in combination with specific and quantifiable clinical measures will be the most effective way to gain information about the pathology of the disease, its relationship to clinical disability and ultimately better treatments for multiple sclerosis.

Conclusions

We have shown that spinal cord MTCSF imaging, a technique sensitive to myelin damage, can explain a significant amount of the variance associated with sensory and motor dysfunction in individuals with multiple sclerosis. Since myelin damage and physical disability are both hallmarks of multiple sclerosis, the techniques presented here provide a new tool for evaluating spinal cord pathology in multiple sclerosis and have the potential to improve patient care. We are currently using these methods to investigate, in a larger multiple sclerosis cohort, how MT and sensorimotor dysfunction evolve over time.

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