Background: Diffusion tensor imaging (DTI) characterizes multiple sclerosis (MS) tissue injury, although it has remained unproven whether DTI changes in disease have functional consequences. The medial longitudinal fasciculus (MLF) is a key brainstem pathway for ocular adduction and is commonly injured in patients with MS, typically resulting in internuclear ophthalmoparesis.

Objective: To validate DTI as a physiologically relevant measure of brain tissue integrity.

Design: A correlation study of ocular dysmotility and DTI conducted between January 2004 and September 2004.

Setting: Multiple Sclerosis Center, University of Texas Southwestern Medical Center, Dallas.

Patients: Six patients with chronic, unilateral, or bilateral internuclear ophthalmoparesis and 10 healthy control subjects.

Main Outcome Measure: We used infrared oculography to correlate the velocity versional dysconjugacy index, defined as the ratio of the velocity of the abducting to adducting eye movements during horizontal saccades, and DTI measures within the MLF as measured through an anatomical overlay. Overall diffusion was measured by mean diffusivity, and anisotropy was measured by the lattice index.

Results: Within the pontine MLF, the mean diffusivity was increased compared with healthy controls (P < .005), whereas the pontine lattice index was decreased (P < .03). Correlations were observed between the velocity versional dysconjugacy index and the mean diffusivity (left: \( r = 0.65, P < .01 \); right: \( r = 0.46, P = .07 \)). Similar correlations were found between the versional dysconjugacy index and the lattice index (left: \( r = -0.43, P = .09 \); right: \( r = -0.65, P < .01 \)).

Conclusions: We identified DTI evidence of pathologic disruption of a small brainstem fiber pathway, which is crucial for accurate horizontal eye movements. In this small study, we observed correlations between the DTI changes and oculomotor dysfunction. Our preliminary observations provide criterion validity of DTI as a surrogate marker of brain tissue integrity.

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INTERNUCLEAR OPHTHALMOPARESIS (INO) is a common neurologic finding in patients with multiple sclerosis (MS). As INO manifests diplopia, oscillopsia, or difficulty with reading. Examination shows impaired eye adduction despite intact abduction of the contralateral eye and binocular convergence. An INO is commonly caused by an inflammatory demyelinating lesion that affects the medial longitudinal fasciculus (MLF), which connects the nucleus of the sixth cranial nerve to that of the contralateral medial rectus subnucleus of the oculomotor complex.1

In patients with MS with an INO, prolonged T2 relaxation (hyperintensities on T2-weighted images) is typically observed in the pons or midbrain.2 Although prolonged T2 relaxation indicates areas of tissue injury, it does not accurately characterize the degree of this injury.3,5 Similarly, gadolinium enhancement indicates areas of blood-brain barrier breakdown but not the degree of injury. Other, more advanced imaging modalities may better characterize the degree of tissue injury and may provide greater insight into brain tissue injury and its response to therapies.

Diffusion tensor imaging (DTI) is a quantitative magnetic resonance imaging (MRI) technique that measures the 3-dimensional diffusion of water molecules within tissue through the application of multiple diffusion gradients well beyond the usual resolution of MRI. In particular, DTI is useful in characterizing white matter.6,7 The power of diffusion techniques comes from their measurement of molecular probing of tissue structures by water molecules at a microscopic scale well
beyond the usual MRI resolution. By measuring the interaction of water molecules with cell membranes, myelin sheaths, and macromolecules, tissue integrity and structural injury can be inferred. Diffusion tensor imaging studies in MS have observed disrupted water diffusion. Within demyelinating lesions, mean diffusivity (MD) (a measure of overall water diffusion) is increased, and anisotropy (the degree of nonspherical diffusion of water, such as along fiber tracts) is decreased.8,9 Similar changes are seen in the normal-appearing white matter.8,10

Although a purported advantage to DTI is its quantitative nature, studies correlating DTI with functional impairment have generally used coarse measures, such as overall clinical disability.10,11 To date, no studies have correlated DTI changes with electrophysiologic measures of neurologic function, particularly within a discrete tract system. It is important to validate DTI as a functionally relevant measure of neural disruption within the central nervous system. This validation will help lay the foundation for its use to study the pathophysiology of neurologic diseases, as well as corroborate its potential as an imaging metric of neurodegeneration for use in clinical trials. In this preliminary study, we used an anatomical overlay to identify the MLF in patients with MS and correlated DTI measures with functional impairment, as measured with infrared oculographic metrics of horizontal saccadic eye movements.

METHODS

PATIENTS

Between January 2004 and September 2004, we performed DTI and quantitative ocular motility studies on 6 patients with MS (2 with relapsing-remitting disease, 3 with secondary progressive disease, and 1 with primary progressive disease; mean disease duration, 12.3 years [range, 7-18 years]; mean [SD] age, 51.8 [4.1] years) with varying severity of INO and compared them with 10 healthy controls (mean [SD] age, 36.4 [12.7] years). All patients with MS had long-standing INO documented on previous clinical examinations, often with bilateral involvement.

INFRARED OCULOGRAPHY

Eye movements were evaluated through quantitative infrared oculography. Previous discriminative analyses have identified the ratio of abduction to adduction eye peak velocity during a 20° saccade, called the velocity versional dysconjugacy index (vel-VDI), as the most accurate and reliable measure of the abnormalities of INO.12 Through use of this within-patient index, the vel-VDI minimizes interindividual and intraindividual (fellow eye) differences that occur with the quantitative analyses of monocular eye movements. The vel-VDI was converted to a z score, using a group of healthy controls as the reference population. Recently, vel-VDI z scores have been used successfully to characterize the effect of heat intolerance on ocular dysfunction.13

IMAGING

All imaging was performed on a 1.5-T GE scanner (GE NV/I; General Electric, Waukesha, Wisconsin) using a head strap to minimize motion. Pulse sequences included axial fast spin-echo T1-weighted image (echo time [TE], 8.5 milliseconds; repetition time [TR], 600 milliseconds; flip angle, 90°; bandwidth, 244 Hz per pixel; 2 averages; 128 × 128 matrix; 140 × 140-mm field of view; 4-mm section thickness), axial dual echo (first TE, 23 milliseconds; second TE, 118 milliseconds; TR, 4000 milliseconds; flip angle, 90°; bandwidth, 122 Hz per pixel; 1 average; 256 × 192 matrix; 140 × 140-mm field of view; 4-mm section thickness), axial dual echo (first TE, 23 milliseconds; second TE, 118 milliseconds; TR, 4000 milliseconds; flip angle, 90°; bandwidth, 122 Hz per pixel; 1 average; 256 × 192 matrix; 140 × 140-mm field of view; 4-mm section thickness), and 25-direction, single-shot, diffusion-
weighted, twice-refocused spin echo with echo planar imaging readout diffusion sequence with 25 diffusion-weighting gradients with \( b = 1000 \), and 3 with \( b = 0 \); TE, 89.6 milliseconds; TR, 6200 milliseconds; flip angle, 90°; bandwidth, 633 Hz per pixel; 128 × 128 matrix; 140 × 140-mm field of view; 4-mm section thickness, with 3 repeats to improve the signal to noise ratio. Singular value decomposition was used to identify its least square error inverse, which was then used to define the tensor matrix. Eigenvalues and eigenvectors were then computed from the independent elements of the tensor matrix.14,15

**IMAGE ANALYSIS**

The MLF was identified through a brainstem atlas at 7 levels that extended from the tegmentum of the pontomedullary junction to the rostral extent of the midbrain (Figure 1).16 Advanced Visualization Software (Advanced Visual Systems, Waltham, Massachusetts) was used to apply a 3 df affine transform (1 rotation, 2 scale factors) to warp a given atlas section onto the DTIs, as previously described (Figure 2).17-19

Preliminary observations found differences in DTI measures within the MLF of healthy controls along the caudo-rostral extent of the MLF. Therefore, at each brainstem level, the DTI measures of the patients with MS were subtracted from the average of the 10 healthy controls at the corresponding level. We measured the MD (a measure of overall water diffusion) and the lattice index (LI) (a measure of anisotropy that integrates the anisotropy of the surrounding voxels). The LI was used as the primary measure of anisotropy instead of the more commonly reported fractional anisotropy (FA) because our pre-
The MD and LI measures on the left were similar (data not shown). When compared with healthy controls, MD within the pontine MLF for each brainstem region. Patient DTI measures were averaged across imaging sections brain tegmentum MLF. For group comparisons, within-most rostral 3 sections, which comprised the unaffected mid-brain MLF, and the most rostral sections, which comprised the diseased pontine MLF, and the 

Therefore, the DTIs were divided into 2 groups: the caudal 4 sections for the within-patient correlation between fellow eyes, correlation analyses evaluated the right and left sides separately. Sensitivity analyses included correlations with the unaffected MLF in the midbrain.

**STATISTICAL ANALYSIS**

The differences in vel-VDI and DTI measures between patient groups were evaluated using a linear mixed model, which accounts for the within-patient correlation between fellow eyes. Pearson correlation coefficients were used to evaluate the relationship between vel-VDI and DTI measures. Because of the expected within-patient correlation between fellow eyes, correlation analyses evaluated the right and left sides separately. Sensitivity analyses included correlations with the unaffected MLF in the midbrain.

**RESULTS**

As expected from patient selection, oculography evaluations of patients with MS showed an increased vel-VDI compared with controls ($P < .001$; data not shown). Compared with healthy controls, MD within the pontine MLF of patients with MS was increased ($P < .005$, Figure 3), whereas the LI and FA were decreased (LI: $P < .03$, Figure 3; FA: $P < .03$, data not shown). The MD, LI, and FA within the unaffected midbrain MLF (ie, where no T2 lesions were seen on conventional MRI) showed less difference between patients with MS and healthy controls (MD: $P < .04$; LI: $P > .10$; FA: $P > .10$).

Significant or trend correlations were observed between the vel-VDI and both the MD and LI within each pontine MLF in the combined group of patients with MS and healthy controls (Figure 4). Weaker correlations were observed with FA (left vel-VDI and left FA: $r = -0.24$, $P > .30$; right vel-VDI and right FA: $r = -0.58$, $P = .02$). The same correlations in the midbrain showed consistently weaker correlation coefficients on each side (left vel-VDI and left MD: $r = 0.63$; right vel-VDI and right MD: $r = 0.42$; left vel-VDI and left LI: $r = 0.23$; right vel-VDI and right LI: $r = 0.52$; left vel-VDI and left FA: $r = 0.40$; right vel-VDI and right FA: $r = 0.48$).

We found altered measures of water diffusion within the pontine MLF of patients with MS with a well-characterized neuro-ophthalmologic syndrome. Specifically, we observed increased overall diffusion and decreased anisotropy, which is similar to that reported in cerebral areas of demyelination. In the midbrain tegmentum MLF, no difference was found in any DTI measure between patients with MS and healthy controls, which supports the specificity of DTI measures within the area of pontine injury for the patients we selected. Despite the small sample size, significant correlations were observed between eye movement function and MRI measures of structural injury. These observations provide direct evidence that the structural injury measured by DTI reflects a physiologically relevant disruption in neural tissues: the greater the alteration in DTI, the greater the resulting physiologic impairment. Our results are particularly conspicuous in that the MLF is an eloquent periventricular tract system at high predilection for inflammatory demyelination.

We observed a stronger correlation with the anisotropy measure LI than the more conventional anisotropy measure FA. The difference is likely due to a relatively low signal to noise ratio when measuring diffusion in a small structure such as the MLF. A low signal to noise ratio has been shown to significantly bias FA estimates more than LI. and the additional noise in the FA measure would likely weaken its correlation with validated physiologic measures. Whereas FA is calculated independently within each voxel, LI includes a spatial average over neighboring voxels. Such averaging tends to reduce the sensitivity of LI to both true physiologic variability and the effects of noise.

These studies are limited by the low signal to noise ratio of DTI performed on a 1.5-T scanner with a 25-direction pulse sequence. Errors in coregistration and warping methods used in the segmentation process may introduce variability. Higher magnetic fields, higher angular resolution of diffusion-weighted vectors, and more advanced coregistration methods should improve the ac-
curacy and reproducibility of DTI estimates, thereby improving our ability to evaluate this small fiber tract. Variability was seen in the different correlation coefficients, and this variability is likely secondary to the small sample size, limited accuracy of the pulse sequence, and errors in coregistration.

The goal of many imaging metrics is to capture the relevant characteristics of tissue integrity, although few metrics have been validated as surrogate markers of tissue integrity. Diffusion tensor imaging provides a continuous measure of tissue injury and may even differentiate axonal injury from demyelination.20,21 These characteristics make DTI an attractive marker for application to clinical trials of putative neuroprotective therapies in MS. Previous studies have demonstrated DTI’s construct validity (ie, alteration in MS lesions compared with normal-appearing white matter), but little is known about its criterion validity (ie, what does it mean functionally?).

We demonstrated DTI’s concurrent validity through its increased MD and decreased LI within the MLF of patients with MS compared with controls. The variable correlation between each side is likely secondary to the small sample size and relatively low signal to noise ratio. Convergent validity is supported by its correlation with validated functional and objective measures of ocular dysmotility.

Finally, discriminant validity was demonstrated through the weaker correlations between ocular dysmotility and DTI measures in the contralateral MLF and the unaffected midbrain in our series of patients. As expected from wallerian degeneration along the MLF, MD was modestly increased within the midbrain MLF of patients with MS compared with healthy controls, although the difference measured by the MD and LI was less than that seen in the pons. Therefore, several significant correlations were still seen between midbrain DTI measures and the vel-VDI, but in every case these correlations were weaker than their pontine equivalent.

Altogether, our observations provide preliminary validation of DTI as a functionally relevant measure of brain tissue integrity and support its implementation into MS clinical trials of neuroprotective therapies. Further studies with a larger number of patients and higher-resolution imaging (ie, using 3-T imaging systems) are planned because they will improve our understanding of the clinical relevance of DTI within this system.

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REFERENCES


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