Axonal Injury or Loss in the Internal Capsule and Motor Impairment in Multiple Sclerosis

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Objective: To test the hypothesis that axonal damage extending into primarily normal-appearing white matter is clinically important by comparing the concentrations of N-acetylaspartate (NAA) bilaterally within the internal capsule with laterization of motor impairment in patients with multiple sclerosis (MS) and persistent asymmetrical motor deficit.

Design: We performed magnetic resonance spectroscopy and T2-weighted imaging of the internal capsule, calculated central motor conduction times, and related these results to measures of motor function asymmetry in 12 patients with MS.

Results: Levels of NAA from normal-appearing white matter of the internal capsule in patients with MS were significantly lower than those in control subjects (P = .05). Side-to-side differences in NAA levels were also significantly greater in patients with MS than in controls (P = .01). There was a correlation between asymmetry in motor function for the left and right limbs and asymmetry of internal capsule NAA concentrations (r = 0.60; P = .04). This correlation seemed slightly stronger when tests specifically of arm and hand motor asymmetry were considered alone. Central motor conduction times were abnormal in most patients with MS and showed a side-to-side difference that also correlated with asymmetry in motor function.

Conclusion: Our demonstration of a graded association between NAA concentrations within primarily normal-appearing white matter of a specific tract and functional impairments referable to that tract suggests that axonal pathology distant from macroscopic lesions might be an important determinant of disability in MS.

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T HAS BECOME clear from results of animal1 and human2 studies that neurologic function can fully recover after acute inflammation, despite persistent demyelination. The mechanisms of recovery might include axonal adaptations such as the redistribution of sodium channels.3,4 Thus, the focus of interest has recently been directed toward testing the hypothesis that axonal loss and damage are responsible for the persistent functional deficits found in multiple sclerosis (MS) (the axonal hypothesis).5

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Results of postmortem studies demonstrated that substantial axonal loss and injury occurs in MS in acute6,7 and chronic8,9 lesions. Magnetic resonance spectroscopy (MRS) has allowed the investigation of axonal loss and damage in vivo by allowing the quantification of N-acetylaspartate (NAA), an amino acid localized almost exclusively to neurons within the adult central nervous system. Results of in vivo MRS studies demonstrated reduced NAA levels in chronic10,11 and acute12,13 lesions. Moreover, results of MRS studies12-15 showed a significant reduction in NAA concentration in normal-appearing white matter (NAWM), suggesting damage to axons distant from areas of focal inflammation. Although the conventionally obvious pathological features in MS lie in macroscopic lesions, significant and functionally important axonal damage might also occur within the more extensive NAWM.

An axonal hypothesis of disability can be tested by defining the relationship between axonal pathology and persistent neurologic dysfunction. Indirect magnetic resonance markers of axonal loss and damage, such as cerebral16 and spinal cord17 atrophy and T1-weighted “black holes,”18 have been shown to correlate with disability, as measured by the Extended Disability Status Scale (EDSS). Recently, Fu et al19 showed in a cohort of patients with MS followed up longitudinally that decreases in brain NAA concentrations correlate with the progression of disability. A more demanding test of the axonal hypothesis would be a demonstration of a graded relationship between specific markers of axonal loss (eg, brain NAA concentration) and impairment within a single functional system. The only study known...
PATIENTS AND METHODS

Twelve patients with MS and a wide range of disabilities (EDSS score, 2.5-8.0) and persistent asymmetrical motor impairment were recruited from neurology outpatient clinics at the Radcliffe Infirmary, Oxford, England. Patients with a history of relapse within the last 3 months were excluded. Twelve control subjects with a similar mean age were recruited for MRS. The study was approved by the Central Oxford Regional Ethics Committee, and informed consent was obtained before the study.

CLINICAL ASSESSMENT

Clinical assessment was carried out by a single observer (M.A.L.) before MRS and magnetic resonance imaging examination. Overall disability was assessed using the EDSS. Specific clinical measures of motor function obtained from patients were the Motricity Index score,$^{21,22}$ 9-hole peg test time,$^{23}$ grip strength (measured using a modified strain gauge),$^{24}$ and leg extension power (measured using a leg extensor rig dynamometer). Hand preference was assessed using the Salmaso hand preference index.$^{24}$ Primary comparison was made between differences in NAA levels within the internal capsule and percentage of motor asymmetry within patients using a composite asymmetry score. Within-patient motor asymmetry comparisons were used to avoid difficulties inherent in establishing control values for motor tasks across participants. Composite score was generated by calculating the mean of the percentage differences between the worse and less affect sides, as a percentage of the least affected side, for the Motricity Index score (arm and leg), grip strength, 9-hole peg test time, and leg extensor measurements. Mean composite asymmetry index was assigned a positive value if the weaker side was on the right, and a negative value if on the left. Hence, for each patient, a single signed score was generated reflecting the mean of 5 predefined clinical tests of motor asymmetry and the lateralization of the deficit. Results of previous studies$^{25}$ demonstrate the importance of the corticospinal tract in the control of fine upper-limb movements. It was also likely that a proportion of patients would have significant spinal cord disease that might be poorly reflected by axonal changes within the internal capsule. In an attempt to compensate for these factors, differences in NAA levels were also compared with motor asymmetry measured using tests of upper limb function alone.

MAGNETIC RESONANCE IMAGING AND MRS

Imaging and spectroscopy were performed in a 2-T whole-body magnet using a spectrometer (Avance; Bruker Medical, Ettlingen, Germany). A single spectroscopic voxel of interest (VOI) was used to isolate the internal capsule as fully as possible. Phantom experiments demonstrated voxel localization accuracy to within ±1 mm in all 3 axes. Spectroscopic volume selection was performed using a point-resolved spectroscopy sequence.$^{26}$ To avoid significant chemical shift displacement of the VOI for the signal of interest (NAA), an offset frequency of −228 Hz relative to the water frequency was applied to all 3 pulses of the point resolved spectroscopy sequence. This ensured that the NAA signal was collected from precisely that volume of tissue prescribed by the visually defined VOI.

Care was taken to standardize head positioning across study participants. All images and spectra were obtained using a birdcage quadrature coil tuned to 83.2 MHz. Voxel localization accuracy was validated using the 3D T2 sequence. Middle cerebellar peduncle voxel was used to assess the mean voxel intensity of the middle cerebellar peduncle. The mean voxel intensity of the middle cerebellar peduncle was compared between groups. The percentage difference in NAA concentration between the left and right middle cerebellar peduncle voxels was calculated. The mean voxel intensity of the middle cerebellar peduncle was significantly reduced in patients with MS compared with controls (P < .05).

RESULTS

Clinical details for the patients with MS are shown in Table 1. There was no significant difference in ages of patients with MS vs controls (49.0 ± 8.5 vs 40.6 ± 15.9 years; P = .20).

Spectroscopic, imaging, and electrophysiologic results for patients with MS are presented in Table 2. Spectroscopic control data are presented in Table 3. The internal capsule NAA concentration in patients with MS (0.167 ± 0.021 U) was significantly less than that in controls (0.176 ± 0.011 U) (P = .04) (Figure 2). Even NAA levels from voxels containing entirely NAWM (16 of 24 voxels; 0.166 ± 0.018 U) were significantly reduced compared with those in the control group (P = .05).

The percentage difference in NAA concentration between the right and left internal capsule in patients with MS (15.9% ± 11.2%) was significantly greater than that in controls (3.5% ± 3.1%) (P = .01). There was a significant correlation between motor asymmetry within patients (as measured by the composite motor score) and differences in relative NAA concentrations within the internal capsule (r = 0.60; P = .04) (Figure 3). The gradient of this regression was 0.42, ie, a difference in motor function score of 50% (on average) between sides was associated with a difference of approximately 20% in NAA concentrations between the left and right internal capsule VOIs. The strength of the correlation between NAA, as a measure of axonal damage, and motor impairment might be increased when more specific tests of arm and hand function are considered alone (for the correlation of peg-time asymmetry and NAA asymmetry, r = 0.70; P = .03, and for the correlation between first dorsal interossei myometry asymmetry and NAA asymmetry, r = 0.89; P < .01). A more modest relationship was found for absolute measures of up-
MHz. Axial fast spin echo T2-weighted (T2) images were collected with the following variables: repetition time, 3100 milliseconds; echo time, 82 milliseconds; 3-mm-thick contiguous slices with nominal in-plane resolution of 1 mm; 256 × 190 matrix; 25.6-cm field of view; 2 averages; and zero filling to 256. Spectroscopic VOI was prescribed using the T2 images, and was centered on the left and right posterior limb of the internal capsule in turn (Figure 1). Placement of the left and right voxels was performed on the same slice within each patient at the level of the third ventricle, and symmetry of placement was confirmed by 2 observers (M.A.L. and A.M.B.). Care was taken to ensure that the voxel did not contain ventricle. The VOI had nominal dimensions of 2.0 × 1.5 × 2.0 cm (volume, 6 cm³). Spectroscopic acquisition variables were as follows: repetition time, 1500 milliseconds; echo time, 90 milliseconds; and 2048 data points collected with a spectral width of 2500 Hz. Each spectrum was the average of 256 acquisitions. Water suppression was produced using a chemical shift selective sequence. Non–water-suppressed spectra were also collected, with 16 averages and no offset frequency. This generated a spectrum dominated by the water peak, which was used to normalize the NAA signal to correct for differing experimental conditions across patients. Spectral analysis was performed with the operator (M.A.L.) masked to the lateralization of motor deficit. Four hertz of exponential line broadening was applied before Fourier transformation. Automatic line fitting and integration was done with a software package (1D WIN-NMR; Bruker Franzen Analytik GmbH, Bremen, Germany). Hyperintense T2 lesion area was measured in each patient using a manually defined thresholding technique by a masked operator (M.A.L.).

**NEUROPHYSIOLOGICAL VARIABLES**

Cortical magnetic stimulation was delivered with a stimulator (MagStim 200; The MagStim Co Ltd, Dyfed, Wales). A figure eight coil with a loop diameter of 8 cm was placed 5 cm lateral to the vertex in the interaural line. Compound muscle action potentials (CMAPs) were recorded from the contralateral relaxed first dorsal interosseus. The coil was angled 45° to the parasagittal plane. Stimuli were delivered at 110% of the upper motor threshold, defined as the stimulator output necessary to elicit CMAPs at a gain of 100 µV per division in 10 of 10 consecutive stimulations. Each participant was seated in a chair and electromyographic activity was recorded over the relaxed first dorsal interosseus. Signals were converted from analog to digital format before being analyzed with a computer software package (CED SIGAVG; Cambridge Electronic Design, Cambridge, England). Central motor conduction times were calculated from cortex to C8 using the following equation: CMCT = CMAP latency − [(F + M − 1)/2], in which F is the F wave latency and M is the distal motor latency. The shortest latency obtained from 10 stimuli was used, with the reference CMCT range in our laboratory being 3.5 to 7.9 milliseconds. The side of cortical stimulation for CMCTs is the side referred to in the “Results” section. If significantly prolonged, CMCTs are considered an electrophysiologic marker of demyelination. Responses typically show delay and dispersion in patients with MS. However, small delays in conduction may occur in conditions characterized by axonal degeneration.

**STATISTICS**

Null hypotheses were tested using a Mann-Whitney U test and correlations were performed using the Spearman rank correlation test. Values are given as mean ± SD.

per-limb function and NAA levels across patients (for the correlation of peg test times and NAA levels, \( r = -0.35; P < .1 \)). No significant correlation was found between mean NAA levels within the small internal capsule volumes and EDSS scores across the MS group (\( r = -0.26; P = .4 \)).

Hyperintense T2 lesions were found in 6 of the 24 VOIs from which the NAA signal was measured. However, the mean volume of T2 lesions (0.06 ± 0.03 cm³) identified occupied only about 1% of the VOI (6 cm³), and there was no significant difference between NAA levels from voxels with T2 lesions (0.170 ± 0.021 U) and those without (0.166 ± 0.018 U) (\( P = .08 \)). T2 lesion volume within the VOI did not correlate with asymmetry of motor function or NAA levels.

There was no significant correlation between cerebral T2 lesion volume asymmetry and composite motor score asymmetry (\( r = 0.31; P = .32 \)) or the more specific tests of upper limb asymmetry.

Bilateral motor conduction times were obtained in all patients (range, 5.1-22.0 milliseconds). Fifteen of 24 conduction times (62%) were abnormal, as defined by an upper reference limit of 7.9 milliseconds (defined as 2 SDs above the mean normal latency of 5.7 milliseconds). Latencies were prolonged bilaterally in 5 patients and unilaterally in 5 patients. In all but 1 patient, latencies were longer with stimulation contralateral to the most severely affected limb. There was a correlation between CMCT asymmetry and composite motor score asymmetry (\( r = -0.73; P = .006 \)) for individual patients (Figure 4). In contrast to the MRS results, this correlation was less strong for measures of upper limb motor asymmetry alone (peg asymmetry: \( r = -0.42; P = .23 \), and first dorsal interosseus myometry asymmetry: \( r = -0.6; P = .04 \)). Mean CMCT across patient groups was correlated with the EDSS score (\( r = 0.75; P = .02 \)). There was a trend for asymmetry in CMCT to be negatively correlated with asymmetry in NAA level within the internal capsule in patients with MS (\( r = -0.5; P = .10 \)).

Stimulation threshold levels for cortical motor stimulation were significantly higher in the MS group (73.1% ± 13.7%) than were previously reported healthy control values from our laboratory (\( P < .001 \)). A significant correlation was found between threshold asymmetry and composite motor score asymmetry (\( r = -0.84; P = .001 \)).

**COMMENT**

Using in vivo MRS, we demonstrated a significant correlation between decreases in NAA levels (a measure of axonal damage and dysfunction) in primarily NAWM confined to a specific functional pathway and persistent functional impairments referable to that pathway. This rela-
The relationship was somewhat strengthened when more specific tests of upper limb function were used. These results extend those of previous studies suggesting that axonal damage and loss is the major cause of chronic disability in MS.

The fact that our observations were conducted on volumes, including the corticospinal tract, that were largely free of T2-hyperintense signal is particularly important to the interpretation of our results. Even in the 6 of 24 VOIs that included some T2 lesions, the mean volume of lesion was only 1% of the VOI and would not have accounted for a mean reduction in NAA concentration compared with the contralateral side of 16% in patients with MS. Prolonged T1 and T2 relaxation times, altered saturation transfer, diffusion-weighted abnormalities, and more recently, results of spectroscopic studies suggest that significant biochemical changes occur within the NAWM. Our results support the hypothesis that this axonal damage within the NAWM is clinically significant. The pathological mechanisms underlying NAWM axonal damage and loss are not defined but might include wallerian degenera

Figure 1. Axial T2-weighted image (radiological in convention) showing placement of spectroscopic voxels of interest to include the right and left posterior limb of the internal capsule with associated spectra. Spectra are from a patient (patient 12) with greater motor dysfunction on the right side compared with the left side. Chol indicates signal from choline-containing compounds; Cr, signal from creatine and phosphocreatine; NAA, signal from N-acetylaspartate.

Table 1. Clinical Data for the Patients With Multiple Sclerosis

<table>
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<tr>
<th>Patient No./Sex/Age, y</th>
<th>EDSS Score</th>
<th>Disease Type</th>
<th>Disease Duration, y</th>
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<td>RR</td>
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*EDSS indicates Expanded Disability Severity Scale; RR, relapsing-remitting; and SP, secondary-progressive.

Table 2. MRS, MRI, and CMCT Results in Patients With Multiple Sclerosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hand Preference</th>
<th>Motor Asymmetry, % (R Weakness + ve)†</th>
<th>IC NAA, U</th>
<th>T2 Lesion Size (Cerebral), cm³</th>
<th>CMCT (Central), ms</th>
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<td>0.16</td>
<td>0.21</td>
<td>5.61</td>
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</table>

*MRS indicates magnetic resonance spectrometry; MRI, magnetic resonance imaging; CMCT, central motor conduction time; IC NAA, internal capsule N-acetylaspartate level.
†Motor asymmetry is measured using the composite of 5 predefined clinical motor scores (motricity index for arm and leg, grip strength, 9-hole peg test time, and leg extensor power). A positive value indicates greater weakness on the right side.
Mean internal capsule NAA levels did not correlate significantly with disability, as measured by the EDSS across the patient population. This might reflect experimental variation inherent in quantifying NAA concentration within a small volume of white matter across patients, the relatively small number of patients studied, or the functional nonspecificity of the EDSS.

A good correlation was found between CMCT asymmetry and the composite motor asymmetry score. A moderate correlation was demonstrated between CMCT asymmetry and NAA asymmetry, although this did not reach statistical significance \( (r = 0.60; P = .04) \). Positive values on the x-axis reflect increasing right-sided weakness relative to the left side; negative values reflect increasing left-sided weakness relative to the right side.

Several factors might have reduced the sensitivity of the acquired NAA signal to reflect axonal damage within the descending motor tracts of the internal capsule. First, the effects of demyelination and axonal loss. Second, the relationship between CMCTs and the lateralization of motor deficits might also reflect the effects of demyelination on the axon. Chronic demyelination might result in distant axonal changes by interruption of cytoskeletal axoplasmic transport. A relationship between CMCTs and functional impairment or axonal damage might therefore be expected. Further evidence that the association of CMCTs with chronic neurologic dysfunction should not be interpreted as implying that demyelination causes chronic neurologic dysfunction comes from recent work demonstrating significant progression of disability in patients with MS and stable, but abnormal, CMCTs. The more modest relationship between NAA asymmetry and CMCT asymmetry suggests that demyelination can also occur independently of axonal damage in MS.

Several factors might have reduced the sensitivity of the acquired NAA signal to reflect axonal damage within the descending motor tracts of the internal capsule. First,
as a consequence of the desire to include all of the posterior limb of the internal capsule within the VOI, a significant proportion of nuclear gray matter from the thalamus and striatum was inevitably included. Because gray matter is relatively unaffected in MS, this would have reduced the power to detect NAA concentration changes within the tract of interest due to partial volume effects. Also, despite careful positioning, there might have been small side-to-side differences in the amount of nuclear gray matter included within the VOI. However, differences in NAA levels between normal gray and white matter are not well defined, with greater,6,7 similar,41 and reduced44 gray matter compared with white matter NAA levels having been described.40-42 We believe the effect of different gray matter volume inclusion within the voxel between sides to be modest and unbiased because side-to-side variations in controls were significantly less than those in patients with MS (mean difference in controls vs patients with MS, 3.5% vs 15.9%) and equally distributed (Table 3).

In summary, our results and those of previous studies suggest that axonal damage and loss is strongly associated with the development of persistent, progressive functional impairment in MS. Such findings might be more significant in light of recent study results showing that axonal damage in acute multiple sclerosis lesions can be quantified using MRS.47-51 We believe the effect of different gray matter volume inclusion within the voxel between sides to be modest and unbiased because side-to-side variations in controls were significantly less than those in patients with MS (mean difference in controls vs patients with MS, 3.5% vs 15.9%) and equally distributed (Table 3).

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