Thalamic neurodegeneration in relapsing-remitting multiple sclerosis

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Abstract—Objective: To define the extent of neuronal injury and loss in thalamic gray matter in patients with relapsing-remitting (RR) MS and to characterize how these neuronal pathologic changes are related to disease duration. Methods: The authors studied 14 patients with RRMS (Expanded Disability Status Scale score, mean 3.25, range 2.0 to 6.0) and 14 (8 men, 6 women) age-matched healthy controls. Structural MR and MRS studies were performed in a single scanning session using a 3T MR system. Results: 

Because the most prominent lesions of MS are found in the white matter (WM), until recently much less attention has been given to changes in the gray matter (GM). However, it has been long recognized that GM structures are not spared from lesions with MS. More recent work suggests that these lesions may be common and extensive. Whereas some of these GM lesions can be identified by MRI, MRI is relatively insensitive to them. An indirect approach to assessing GM involvement has been to measure progression of cortical atrophy over time. Brain GM fraction is decreased in patients with clinically definite relapsing-remitting (RR) MS, although the changes are not as great as those in WM. The precise substrate for this atrophy remains unclear.

MRS has become an important tool for assessment of axonal pathology in WM, as it allows relatively specific in vivo monitoring of neuronal integrity by measurement of the resonance signal from N-acetylaspartate (NAA), a metabolite that is almost exclusively localized to neurones and their processes in the adult brain. By allowing this pathology to be studied in life, it has improved understanding of correlations between pathology and clinical features. Cross-sectional and longitudinal MRS studies have established that WM axonal injury and loss likely are the major proximate causes of progression of disability, for example.

Recently, studies have suggested that GM involvement in MS can be detected using proton MRS. Modest but significant decreases of NAA have been reported in cortically weighted volumes. Accurate measurement of the extent of this neuronal injury or loss has been difficult, however, because it is difficult to determine precisely the volume fraction of cortical GM for these studies given the limited spatial resolution of the imaging and spectroscopy and the complex geometry of the interface of GM with CSF and WM.

We have tried to avoid these technical confounds to characterizing neuronal injury or loss in GM by study of the thalamus. The volume of this structurally well-defined GM region can be determined with precision and localized MRS can be performed without the need for partial volume corrections. In a combined MRS, MRI, and histopathologic study of patients with secondary progressive (SP) MS, we found decreases in both NAA (a measure of neuronal density) and thalamic volume to an extent suggesting that there was a 30 to 35% net decrease in neurons. Neither the MRI nor the neuropathologic study showed substantial regions of focal inflamma-

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tion. These changes imply that there is a significant neurodegenerative component in SPMS.

To test whether neurodegeneration is an integral component of MS from even earlier stages, the goal of this study was to extend our previous observations of SPMS\(^\text{13-15}\) to investigate neuronal loss in thalamic GM in patients with RRMS and to characterize how these neuronal pathologic changes are related to other parameters reflecting disease progression.

**Methods. Subjects.** We studied 14 patients with RRMS (5 men, 9 women; mean age 39.5 ± 10.6 years) with clinically definite MS. The degree of disability was assessed using the Expanded Disability Status Scale score (EDSS).\(^\text{14}\) Mean EDSS was 3.25 (range 2.0 to 6.0). Mean disease duration was 7.7 years (range 1 to 30 years). We also studied 14 (8 men, 6 women) age-matched healthy controls (mean age 45.7 ± 10.3 years). The local ethical committee approved our study and informed consent was obtained from all participants.

**MR data acquisition and analysis.** Structural MR and MRS studies were performed in a single scanning session using a 3T Varian-Inova spectrometer with a standard birdcage head coil. Structural MRI was performed using a three-dimensional magnetization-prepared fast gradient-echo sequence with inversion recovery = 500 msec, echo time (TE) = 5 msec, and repetition time (TR) = 30 msec field of view 256 mm × 256 mm, matrix 256 × 256, providing 64 3-mm-thick slices. This choice of imaging parameters was found empirically to allow easy delineation of thalamic borders.

All images were processed using Medx (Sensor Systems). Thalamic and intracranial volumes (TV and ICV) were outlined manually by an experienced operator in the coronal plane slice-by-slice (figure 1A). Ratios of TV to ICV for the whole brain volume were calculated and expressed as normalized thalamic volumes (NTV), thus correcting for the influences of global brain size differences. By using intracranial volumes, the ratio normalizes to the total brain volume before any atrophy that may be induced by the disease. Note that, as a ratio, this measure is unitless. The mean intrarater coefficient of variation for NTV was under 6%.

Third ventricle width was measured manually in an axial view at the midpoint of the dorsal-ventral extension as described previously.\(^\text{15}\)

**MRS of the thalamus and normal-appearing white matter.** Proton spectra were acquired using a PRESS sequence (TE = 26 msec, TR = 5 seconds) and WET water suppression scheme.\(^\text{16}\) These parameters (with a TR > 3 to 5 times the T1 relaxation time and a short TE) make the acquisition relatively insensitive to any changes in relaxation times between the patient and healthy control groups. For spectra from the thalami we used a specially designed 10° radiofrequency pulse in the PRESS sequence that allowed acquisition of the signal from two cubical volumes (VOI) simultaneously. These VOI (0.7 to 1 mL each) were placed on the right and left thalami using a coronal scout image (see figure 1A).

Single voxel proton spectra (TE = 26 msec, TR = 5 seconds) also were acquired (8 patients with RRMS studied and all 14 controls) from a consistently selected region of frontal normal-appearing white matter (NAWM) (figure 1, B and C). Concentrations of NAA, creatine (Cr), and choline (Cho) were estimated from the thalamic spectra using brain water as an internal concentration reference. In order to determine signal from brain water within the decay curve (TE = 0), and also to exclude any possible contribution from the CSF, the water signal was measured in 14 acquisitions with TR = 15 seconds and TE = 26, 42, 72, 92, 132, 282, 512, 612, 812, 1000, 1200, and 2000 msec. Using the biexponential fit of the obtained decay curve, the separation of CSF contribution (T2 ~1 second) from that of the thalamic water (T2 ~60 msec) was then achieved. Metabolite concentrations \(C_{\text{met}}\) were calculated using the following equation:

\[
C_{\text{met}} = \frac{S_{\text{met}}}{S_{\text{w}}} \cdot 111.1 \cdot \frac{1}{N_{\text{eq}}} \text{(mmol/kg wet tissue)}
\]

With \(S_{\text{met}}\) being the signal intensity for a given metabolite, \(S_{\text{w}}\) the signal from brain water, \(f_{\text{w}}\) the brain tissue water fraction, and \(N_{\text{eq}}\), the number of equivalent protons in a molecule of a metabolite.

Figure 1. (A) Typical PRESS volumes of interest (VOI, solid line) selected within thalamic region (dotted line) using coronal plane. Typical PRESS VOI selected within the frontal white matter, viewed in the axial plane (B) and in the coronal plane (C).

The published value of 0.798 for \(f_{\text{w}}\) of the GM\(^{\text{17}}\) was used in the calculations. The factor 111.1 corresponds to the molar concentration of protons in water.

**Measurement of water content of the thalamus.** As calculation of metabolite concentrations depends on water content, we were concerned to test whether water content was different between
the patients and healthy controls. The thalamic water content was calculated in selected patients and healthy controls using a previously described protocol.\textsuperscript{11,12} Briefly, the T2 corrected signal from brain parenchyma, $S_p$, and that from CSF, $S_{CSF}$, were expressed as fractional water signals ($S'_b$) and $S'_{CSF}$ compared to the signal from the same VOI in a pure water phantom. Assuming that MR visibility of CSF is identical to that from the pure water phantom, brain water fraction can be expressed as follows:

\[
\frac{f_b}{S'_b} = \frac{S'_b}{S'_{CSF}}
\]

In order to achieve accurate quantitation, it was necessary to correct for $B_1$ inhomogeneity and coil loading changes in the in vivo relative to the phantom experiment. This correction was based on local measurement of the $B_1$ field. The latter was achieved using a stimulated echo sequence with an additional nonselective square pulse placed between the second and third RF pulses (TM period).\textsuperscript{22}

All MRS data were processed with MRUI software (MRUI project, http://carbon.uab.es/mrui/), using frequency selective time domain fitting VARPROM\textsuperscript{23} with a Gaussian lineshape assumption after any residual water signal had been removed with use of the HSVD method.\textsuperscript{24} To reduce the contribution from broad humps, the first five points from the FID were truncated before fitting.

In order to investigate whether the data analysis technique used has a significant effect on the results obtained, all water-suppressed spectra were analyzed using the linear combination model method (LCM),\textsuperscript{25} and NAA/Cr ratios obtained with the MRUI and LCM methods then were compared. No significant differences were found (data not shown). For consistency with our previous report,\textsuperscript{26} we are reporting results from the MRUI analysis here.

Statistical analysis. Statistical analysis was performed using SPSS version 9.0 for Windows (Chicago, IL). Statistical differences between patients with MS and controls were assessed with a Mann-Whitney test. Correlations were explored with Spearman rank coefficients. Values are reported as means (with the SD in parentheses).

**Results.** NAA concentration and thalamic volume are reduced in patients with MS. Metabolite (NAA, Cr, and Cho) concentrations were measured in the thalami of healthy controls and patients with RRMS using proton MRS (figure 2, A and B). Values for the healthy controls were similar to those reported previously.\textsuperscript{24} The NAA/Cr concentration ratio in the thalami of the patients with MS was approximately 15% lower than in the healthy controls ($p < 0.02$) (table). This decrease was due to changes in NAA: absolute NAA concentrations (a measure of the apparent neuronal density) were decreased to a similar extent in the thalami of the patients with RRMS relative to controls ($p < 0.05$). There were no significant differences in Cr or in Cho concentrations. The estimated water content of the thalamus, $f_b$, for patients with RRMS ($n = 4$) (0.68 [0.09]) was not significantly different from the value obtained for controls ($n = 5$) (0.73 [0.10]).

Structural MRI was performed and the thalami were outlined manually for measurement of volumes. The patients with RRMS had an almost 25% lower normalized thalamic volume than controls ($p < 0.005$) (see the table). However, both NAA concentration and volume loss alone underestimate the true extent of injury.\textsuperscript{25} The maximum relative reduction in the product of NAA concentration and the NTV, which can be interpreted as a measure of the total loss of viable neurones, was approximately 45%.

**Thalamic NAA concentration and volume are correlated and decrease with greater disease duration.** Decreases in thalamic NAA concentration were correlated strongly with thalamic volume loss for the patients ($r = -0.85$, $p < 0.01$, figure 3), suggesting a common substrate for thalamic atrophy and NAA loss. Thalamic volume showed a significant inverse correlation with the maximum width of the third ventricle, an index of global brain atrophy ($r = 0.61$, $p = 0.01$, figure 4). These measures of thalamic pathology were related to disease progression. Both the NAA concentration ($r = -0.48$, $p < 0.04$) and NTV ($r = -0.60$, $p < 0.01$) were correlated inversely with disease duration (figure 5, A and B).

Consistent with this, the product of the NAA concentration and the NTV, which can be interpreted as a measure of the number of viable neurones, also correlated with disease duration ($r = -0.54$, $p < 0.02$) (figure 5C).

Relative NAA concentrations were also reduced in the NAWM. In all controls and in most of the patients ($n = 8$) a localized proton spectrum was acquired from a region of frontal white matter. The mean ratio of NAA/Cr for the patients (1.74 [0.21]) was lower than for the controls (1.95 [0.22]; $p = 0.03$). There was only

**Table Relative and absolute metabolite concentrations in proton MRS from volumes localized to the thalami**

<table>
<thead>
<tr>
<th>Metabolite concentrations</th>
<th>RR</th>
<th>Controls</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cr</td>
<td>1.21 (0.28)</td>
<td>1.47 (0.26)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>NAA mmol/kg wet tissue</td>
<td>9.84 (2.20)</td>
<td>11.44 (1.60)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Cr mmol/kg wet tissue</td>
<td>8.05 (0.85)</td>
<td>7.85 (1.24)</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Cho mmol/kg wet tissue</td>
<td>2.23 (0.64)</td>
<td>1.91 (0.39)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>$f_b$</td>
<td>0.68 (0.08)</td>
<td>0.73 (0.09)</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>NTV ($\times 10^6$)</td>
<td>2378 (650)</td>
<td>3067 (224)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values with one SD are given for patients with relapsing-remitting MS (RR) and healthy controls. Also given are mean (SD) values for estimates of thalamic brain water content, $f_b$, and for the normalized thalamic volume (NTV, a unit-less measure).
a trend for a correlation between thalamic NAA/Cr ratio and NAA/Cr in the frontal NAWM (\(r = 0.56, p = 0.076\)).

**Discussion.** In this study we used proton MRS and structural MRI to investigate the extent of neuronal loss in the thalamus in patients with RRMS. The main observation was of a reduction in both the NAA concentration and the thalamic volume. On the basis of our previous direct histopathologic studies demonstrating substantial neuronal density and volume loss in the later, SP stage of MS,\(^{13}\) we postulate that neuronal loss also is the substrate for these changes in RRMS. This implies that a neurodegenerative component may contribute to the pathology of MS even in the typically earlier RR stage. The maximum extent of relative neuronal loss (estimated from a reduction in the product of NAA concentration and NTV) was similar to that reported for patients with SPMS.\(^{13}\) There was a trend toward a relationship between thalamic NAA/Cr and distant NAWM changes, suggesting that there may be either a common mechanism for the WM axonal loss and thalamic neuronal injury, or that they occur by independent processes with a similar time course.

We observed an approximately 11% decrease in thalamic NAA concentration in this cohort of patients with RRMS. Previous measurements also have reported similar decreases in NAA in the neocortical GM of patients with MS\(^{11}\) and even in patients with early RRMS,\(^{12}\) but this has not been an entirely consistent finding.\(^{26}\) Accurate measurement of metabolite concentrations in cortex is particularly difficult due to possible influences of changes in the relative partial volume of WM and CSF in cortical voxels. The former is easier to correct for,\(^{7}\) but it is difficult to rule out the possibility that axonal injury in WM adjacent to cortex accounts for the apparent decreases seen with MRS of neocortically weighted volumes. In our study we chose to study the thalamus, because it is large relative to spectroscopic voxels and the borders are well defined, so that with careful

**Figure 4.** Graph demonstrates a correlation between normalized thalamic volumes (NTV) and the maximum width of the third ventricle, an index of global atrophy, for individual patients (\(r = -0.61, p < 0.01\)).

**Figure 5.** (A) Graph shows the relationship between thalamic N-acetylaspartate (NAA) concentration and disease duration for individual patients with relapsing-remitting MS. A significant inverse correlation between NAA concentration, an index of the density of viable neurons and their processes, and disease duration was observed (\(r = -0.48, p < 0.05\)). (B) A similar correlation was found between normalized thalamic volumes (NTV) and disease duration (\(r = -0.60, p < 0.02\)). (C) There also was a correlation between the product of NTV and NAA concentration, an estimate of the total number of viable neurons in the thalamus, and disease duration across the patient group (\(r = -0.53, p < 0.05\)).
placement of the VOI, contamination from CSF and WM can be prevented entirely.

We also tried to address various additional methodologic confounds that might affect the measurement of NAA changes in GM. We tested whether patients with RRMS might have increased water content in the thalamus from edema related to inflammation that could reduce the measured NAA concentration. This does not seem likely. A second potential concern is that changes in relaxation times of NAA (increased spin-lattice [T1] or decreased spin-spin [T2] relaxation times) might account for the apparent changes. Increased iron content may give rise to broader line widths in the thalamic proton spectra from the patients with MS. However, this seems unlikely to account for the apparent NAA concentration changes because the T2 is much longer than the TE used here (~250 msec27). A decrease in T2 relaxation time of at least 50% would be necessary to account for the observed changes. Also, it is unlikely that changes in T1 would be responsible for the observed decrease in NAA, because the TR (5 seconds) was much longer than the T1 of the metabolites (1 second). Further confidence in the measurements came from good agreement between the time-domain concentration measurements (VARPRO) reported in the table and frequency-domain concentration measurements (LCM) (data not shown).

The precise substrate for brain atrophy has yet to be well defined, but neuronal, axonal, and myelin loss appear likely candidates as substantial contributors.25 A potential confound to interpretation is that the measurements of volume change might reflect predominantly a decrease in thalamic myelin content rather than in neuronal volumes. Demyelination occurs in cortical GM,14 and our own postmortem studies have shown diffuse, partial myelin loss in the thalamus of patients with RRMS (A. Cifelli, M. Esiri, unpublished data). However, loss of volume with damage to myelinated tracks in thalamus should not have influenced the results greatly because the total myelin content is probably rather low in thalamus (<5% as estimated from the proportion of water with a very short T2, which is assumed to be in the myelin water pool).17

A reduction in NAA in GM could be a marker of neuronal loss, atrophy, or reduction in dendritic arborization or metabolic dysfunction. However, outside of the special contexts of recovery from an acute relapse28 or treatment with anti-inflammatory therapy,29 the generally inexorable decline in central brain NAA with increasing disease duration30,31 suggests a predominantly irreversible mechanism for NAA changes in MS generally. The correlation of NAA decreases with disease duration is consistent with the notion that the changes reported here reflect a similar largely irreversible disease process. Finally, our previous study demonstrated directly that substantial thalamic neuronal loss occurs at least in the end stage of the disease,13 although it is possible that a degree of metabolic dysfunction and contraction of dendritic arborization may accompany neuronal cell loss.

These changes could be due to local effects of thalamic lesions, either anterograde (deafferentation) or retrograde (Wallerian degeneration) changes or a diffuse process promoting neurodegeneration. The trend toward a correlation between NAWM and thalamic NAA/Cr changes is consistent with the notion that thalamic neuronal damage is related to distant lesions in WM (or cortical GM), but we cannot reject the alternative hypothesis that diffusible factors related to a common disease mechanism might be important.

In conjunction with our previous study,13 these results suggest that neuronal loss occurs in thalamic GM even in earlier stages of MS and that these changes progress with time. Our results argue for a substantial neurodegenerative component throughout the course of MS. This could help to explain the often prominent neuropsychological symptoms and signs (which are not easily explained as the consequences of disconnection32). Whereas neuronal injury may be secondary to inflammatory disease of WM, the apparent relative dissociation of effects of anti-inflammatory treatment on relapse frequency and progression of disability suggest that neurodegenerative effects may be at least partially independent. Understanding this may be a way of better unifying clinico-pathologic correlations across both primary progressive and RR disease. More practically, it suggests that treatment directed toward limiting the effects of MS on neurons either through direct neuroprotective strategies or indirectly through anti-inflammatory treatment may be an important part of care even for patients with earlier RRMS.

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