Contribution of White Matter Lesions to Gray Matter Atrophy in Multiple Sclerosis

Evidence From Voxel-Based Analysis of T1 Lesions in the Visual Pathway

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Background: The biological basis of gray matter (GM) atrophy in multiple sclerosis is not well understood, but GM damage seems to be the most critical factor leading to permanent disability.

Objective: To assess to what extent white matter (WM) lesions contribute to regional GM atrophy in multiple sclerosis.

Design: Because optic pathway GM atrophy and optic radiation lesions, rather than being related to each other, could be independent results of the disease, we applied a nonaprioristic WM method to analyze the interrelationships of both phenomena. On a voxel-by-voxel basis, we correlated T1 magnetic resonance imaging–derived lesion probability maps of the entire brain with atrophy of the lateral geniculate nuclei and calcarine/pericalcarine cortices.

Setting: Multiple sclerosis center, University of Navarra, Pamplona, Spain.

PATIENTS: Sixty-one patients with multiple sclerosis.

Main Outcome Measure: Mapping of WM regions contributing to GM atrophy in the optic pathway.

Results: Patients with multiple sclerosis had lateral geniculate nucleus atrophy, which correlated with the presence of lesions specifically in the optic radiations but not in the rest of the brain. Optic pathway lesions explained up to 28% of the change of variance in lateral geniculate nucleus atrophy. Patients also had occipital cortex atrophy, which did not correlate with lesions in the optic radiations or any other WM region.

Conclusions: Focal WM damage is associated with upstream GM atrophy, suggesting that retrograde damage of the perikarya from axonal injury in multiple sclerosis plaques is one of the significant factors in the genesis of GM atrophy, although other neurodegenerative processes are probably at work as well.


MULTIPLE SCLEROSIS (MS) is a chronic multifocal inflammatory and degenerative disease of the central nervous system. Although it affects predominantly the white matter (WM), it is now known that the gray matter (GM) is also involved, even at early stages. Different mechanisms underlying GM atrophy in MS have been postulated. Some of them involve primary damage of the GM, such as GM plaques that induce demyelination, neuronal loss, and pruning of dendrites and synapses, or even long-distance effects such as transsynaptic degeneration in areas of normal-appearing GM. However, GM atrophy could also be secondary to axonal transection in the WM plaques, causing wallerian degeneration with attendant transsynaptic degeneration or a dying-back process affecting the neuronal perikarya, giving rise to the transected axon. Many experimental studies have shown that neuronal degeneration often occurs when its axon is transected. That WM lesions may induce GM atrophy is supported by a positive correlation between WM lesion load and whole GM atrophy. However, it could be countered that the same mechanisms that cause greater WM damage may also cause greater GM damage, independent of axonal transection in the WM. Thus, the real contribution of WM lesions to regional GM atrophy remains unclear.

This issue may be addressed by determining whether there is a specific correlation between volume loss in a given GM structure and the presence of lesions in the WM pathways originating from or ending in the same GM structure. In this sense, the visual pathway could be an excellent model for studying axonal degeneration.
Figure 1. Experimental approach. The optic pathway is well described anatomically, including the gray matter regions (lateral geniculate nucleus and occipital cortex) and white matter tracts (pregeniculate pathway and optic radiations) (top). Lesions in multiple sclerosis (in red) are distributed all through the brain, including the white matter of the optic pathway. By correlating the volume of the lateral geniculate nucleus and occipital cortex with all voxels containing multiple sclerosis lesions, only lesions transecting axons that originate in or target such gray matter areas will be identified, as is the case with the optic radiations in our study. Either anterograde or retrograde axonal degeneration can contribute to gray matter atrophy (bottom).
in MS. We hypothesized that lesions of the optic radiations, but not anywhere else in the brain, would correlate with atrophy in the lateral geniculate nucleus (LGN) and visual cortex in the calcarine sulcus of the occipital lobe. To test this hypothesis, a method should be used that avoids an aprioristic knowledge about the relationship among these anatomic structures and queries the entire brain, asking the following question: lesions of what regions (if any) of the WM of the entire brain correlate with LGN or calcarine cortex atrophy? To answer this question while avoiding WM aprioristic biases, we used lesion probability maps (LPMs) of the entire brain, obtained from high-resolution structural magnetic resonance (MR) images and an optimized voxel-based morphometry method (Figure 1).

METHODS

SUBJECTS

We analyzed a sample of 61 patients who fulfilled the revised McDonald criteria for the diagnosis of MS. They were originally recruited to assess disease activity and retinal changes in MS. All subjects were recruited at the MS Center of the University of Navarra after approval by the ethical review committee of this institution and were studied after having provided informed consent. Because our study was designed to identify the relationship between visible WM damage and GM volume independent of the subtype of the disease, we included patients with all evolution subtypes of MS. Because of the reduced number of patients with the progressive subtype of MS, we did not compare disease subtypes. The use of immunomodulatory drugs was allowed. Patients were excluded if they were taking corticosteroids or had had a clinical relapse within the previous 3 months. A control sample consisted of 20 sex- and age-matched healthy controls. Individuals were excluded from both groups if they had ophthalmologic diseases that might bias the study (eg, diabetes mellitus or glaucoma). Subject demographics are listed in Table 1.

MR IMAGE ACQUISITION

Magnetic resonance imaging was performed with a 1.5-T imager (Symphony; Siemens, Erlangen, Germany). A 3-dimensional (3D) T1-weighted MR image of the whole head (2-mm axial sections; repetition time, 2140 milliseconds; echo time, 5.04 milliseconds; 256 × 256 matrix size; field of view, 25 cm; flip angle, 30°; in-plane resolution, 1 × 1 mm) was acquired for all subjects. We used T1- rather than T2-weighted images because they more accurately identify the existence of axonal damage in patients with MS. In addition, a 3D T1-weighted MR image with the same acquisition settings and a single dose of gadolinium (0.1 mmol/kg) (Magnevist; Bayer Schering Pharma AG, Berlin, Germany) was obtained to identify acute lesions in the study sample.

MR IMAGING DATA ANALYSIS

Voxel-Based Morphometry Protocol to Obtain GM Segmented Images

We used a modified version of the optimized voxel-based morphometry protocol to obtain the normalized and segmented GM images from each subject’s T1 MR image, while avoiding the bias introduced by WM lesions in the normalization and segmentation procedures. For this purpose we used statistical parametric mapping (SPM2) software (Wellcome Department of Cognitive Neurology, University College of London, London, England; http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB version 6.5 (MathWorks Inc, Natick, Massachusetts). To preserve the total within-voxel volume, all voxel signal intensities in the final GM segmented images were multiplied by the Jacobian determinants (Jacobian modulation) derived from the spatial normalization. Finally, images were smoothed with a 12-mm full-width at half-maximum isotropic gaussian filter to increase the signal-to-noise ratio and to account for variations in normal gyral anatomy.

White Matter LPMs

To obtain the WM LPMs, 2 independent observers outlined at voxel level all of the WM lesions on each of the transverse sections of the 3D T1-weighted MR image of each patient by means of MRicro software (Chris Rorden, PhD, University of Nottingham, Nottingham, England; http://www.sph.sc.edu/comd /rorden/mricro.html). Interrater reliability was excellent (intraclass correlation coefficient=0.89; P < .001). In this first step, we obtained a 3D binary mask for each patient. We then normalized each lesion mask from a native space to a stereotactic space by using the SPM2 software and the settings obtained from the previous voxel-based morphometry procedure (see the preceding section). Finally, to approximate the neuropathological characteristics of MS with greater tissue damage in the center of the lesions, lesion masks were smoothed and converted to LPMs by applying an isotropic gaussian kernel (12-mm full-width at half-maximum). Therefore, we obtained 3D LPMs where each voxel had a probability value of being classified as lesion (ranging from 0 to 1), with a higher.

Table 1. Characteristics of the Healthy Control and MS Groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n=20)</th>
<th>MS (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>37.4 (8.7)</td>
<td>36.4 (9.0)</td>
</tr>
<tr>
<td>Sex, No. M:F</td>
<td>8:12</td>
<td>22:39</td>
</tr>
<tr>
<td>Duration of MS, mean (SD), y</td>
<td>NA (7.0)</td>
<td>5.7 (7.0)</td>
</tr>
<tr>
<td>Disease subtype.</td>
<td>NA</td>
<td>22/28/5/6</td>
</tr>
<tr>
<td>No. CIS/RR/SP/PP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS, median (range)</td>
<td>NA (0-7)</td>
<td>2 (0-7)</td>
</tr>
<tr>
<td>MSFC, mean (SD)</td>
<td>0.64 (0.76)</td>
<td></td>
</tr>
<tr>
<td>History of optic neuritis, No.</td>
<td>21 (13 R/5 L /3 bilateral)</td>
<td></td>
</tr>
</tbody>
</table>

Signal intensity, mean (SD) of L lateral geniculate nucleus 0.175 (0.016) 0.141 (0.017)
R lateral geniculate nucleus 0.161 (0.019) 0.132 (0.018)
L calcarine cortex 0.459 (0.056) 0.404 (0.052)
R calcarine cortex 0.453 (0.064) 0.395 (0.047)
L superior occipital cortex 0.310 (0.037) 0.259 (0.035)
R superior occipital cortex 0.286 (0.030) 0.224 (0.029)
L middle occipital cortex 0.320 (0.042) 0.271 (0.031)
R middle occipital cortex 0.300 (0.038) 0.233 (0.030)
L inferior occipital cortex 0.390 (0.055) 0.317 (0.043)
R inferior occipital cortex 0.365 (0.057) 0.249 (0.038)

Abbreviations: CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; L, left; MS, multiple sclerosis; MSFC, MS Functional Composite; NA, not applicable; R, right; RR, relapsing-remitting; PP, primary progressive; SP, secondary progressive.

a No significant differences between groups.
b Signal intensity values (adimensional eigenvalues) extracted from each gray matter region of interest.
c P < .001.

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value at the center of the lesion than in the periphery (Figure 2A and B).

STATISTICAL ANALYSIS

As we focused our study on the visual system, a set of brain structures with well-defined anatomy, we used a region of interest (ROI)–level analysis for the assessment of GM atrophy by means of the Marsbar automated parcellation method.28 Specifically, we studied the LGNs, calcarine cortices (Brodmann area 17/18), and pericalcarine cortices (Brodmann area 18/19) of the occipital lobe, divided into superior, inferior, and middle occipital cortex ROIs (Figure 2C–G). All normalized ROIs were obtained from Marsbar (occipital cortex ROI) and Voitool (LGN ROI) (http://www.ihb.spb.ru/~pet_lab/VTO/VTOMain.html) SPM toolboxes. The GM volumes in patients with MS and in healthy controls were compared by means of the SPSS 13.0 statistical package (SPSS Inc, Chicago, Illinois). The normal distribution of the variables was assessed with the Kolmogorov-Smirnov test. To search for correlations between GM atrophy and WM lesions, we used the framework of the general linear model implemented in the SPM2 software. We applied voxel-by-voxel multiple linear regressions to correlate the WM LPMs (of the entire brain; nonaprioristic WM analysis) and the GM ROI extracted data. This analysis strategy allowed us to look into the WM of the entire brain for lesions associated with GM atrophy, while avoiding the introduction of any a priori knowledge of possible WM anatomic target locations (such as the optic radiations). All correlations were adjusted by (1) sex and age and (2) the regional predilection of MS lesions, which, for instance, tend to cluster in the periventricular regions. The last adjustment was made by using a WM regional lesion frequency mask, created with the average of the individual 3D binary masks. The use of this mask ensured that only voxels of WM were included in the analysis, excluding from the analysis any GM voxels highlighted by the smoothing protocol used to create the LPM. The mean signal intensity values (adimensional eigenvalues) of each significant cluster were extracted with the VOI (volume of interest) toolbox of SPM2 to show the global $R^2$ and $P$ values of every correlation. The level of significance for the results was set at $P<.05$ after correction for multiple comparisons to minimize type I error (false discovery rate method).29 To achieve consistent results, only clusters with 100 or more voxels were retained.30

RESULTS

To evaluate the contribution of WM lesions to GM atrophy in MS, we first calculated the GM volume reduction in the optic pathway (LGN and occipital cortex) in patients compared with controls. After that, locations of WM lesions associated with GM reduction were identified by a voxel-based lesional approach. This method allowed us to evaluate the correlation between MS lesion location and GM reduction (Figure 1) while avoiding any a priori knowledge about anatomic WM target locations.22,31 Finally, we calculated the percentage of the variance in the GM atrophy explained by the presence of pre-

Figure 2. Lesion probability map for a single patient, shown in color (A) and in gray scale overlapped with a T1 magnetic resonance imaging template (B). Also shown are gray matter regions of interest used in the analysis of the visual system: lateral geniculate nucleus (C), calcarine cortex (D), inferior occipital cortex (E), middle occipital cortex (F), and superior occipital cortex (G).
previously identified WM lesions. Compared with healthy controls, patients with MS had smaller right and left LGN ($P < .001$ in both cases; Table 1).

In the WM lesions analysis, we found that LGN volume loss correlated with the presence of lesions in the optic radiations in both the right (Figure 3A) and left (Figure 3B) hemispheres. No other WM lesions, either within or outside of the optic pathway, correlated with LGN atrophy. The presence of WM lesions in the right optic radiations explained 28% of variance changes for right LGN atrophy, and the presence of WM lesions in the left optic radiations explained 13% of the variance change for left LGN atrophy (Table 2). Despite the anatomic specificity of the results in the left hemisphere, this cluster did not remain significant after multiple-comparison correction. No significant difference was found in LGN volume between patients with and without a history of optic neuritis. Twelve subjects had gadolinium-enhancing lesions, but none of them involved the optic radiations.

Finally, we also found that patients with MS had significant volume decrease in both right and left occipital cortices ($P < .001$ in all cortical ROIs: calcarine, superior occipital, inferior occipital, middle occipital; Table 1). However, we did not find any correlation between occipital cortex volume and the presence of WM plaques anywhere in the brain.

![Figure 3. Axial projections showing white matter lesion locations correlated with lateral geniculate nuclei volumes (color bar indicates $F$ statistic values). A, Right lateral geniculate nucleus volume decrease was specifically correlated with white matter lesions in the right optic radiations. B, Left lateral geniculate nucleus volume decrease was specifically correlated with white matter lesions in the left optic radiations, although this cluster was not retained after multiple-test correction.](image)

### Table 2. Correlation Between Lateral Geniculate Nucleus Volume and White Matter Lesions

<table>
<thead>
<tr>
<th>White Matter Lesion Location$^a$</th>
<th>Right Optic Radiations</th>
<th>Left Optic Radiations</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of voxels</td>
<td>2815</td>
<td>1977</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$&lt;.001$</td>
<td>.002</td>
</tr>
<tr>
<td>Uncorrected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With FDR correction</td>
<td>.02</td>
<td>.06</td>
</tr>
<tr>
<td>$F$ statistic value</td>
<td>26.23</td>
<td>10.04</td>
</tr>
<tr>
<td>Maximum coordinates x, y, z</td>
<td>36, −36, 15</td>
<td>−27, −66, 9</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.280 (28)</td>
<td>0.136 (13)</td>
</tr>
</tbody>
</table>

Abbreviation: FDR, false discovery rate.

$^a$Locations are correlated with right and left lateral geniculate nucleus, respectively.

$^b$Derived from the mean signal intensity values (eigenvalues) of each significant cluster.

### COMMENT

Different mechanisms have been proposed to explain GM damage in MS. Transsynaptic degeneration has received particular attention in the visual system. Lesions in the optic nerve are associated with transsynaptic changes in the LGN, particularly in the parvocellular layer, and in the visual cortex. Ciccarelli et al have shown reduced connectivity in the optic radiations, measured by tractography-based mapping, 1 year after an episode of optic neuritis, suggesting not only transsynaptic effects but also axonal loss in the optic radiations corresponding to neuronal loss in the LGN. However, the effect of optic radiation lesions on the volume of the LGN and occipital cortex has not been previously studied, to our knowledge.

Our results indicate that the presence of WM lesions in the optic radiations, originating in the LGN, explains up to 28% of the change of variance for the LGN total volume, reinforcing the concept that retrograde damage of the neurons (perikarya) due to injury of their axons in the MS plaques contributes significantly to GM atrophy. It is important that the WM of the entire brain was tested against LGN atrophy, by means of a WM non-aprioristic approach. Only lesions in either of the optic radiations, and not anywhere else in the brain, were associated with LGN atrophy. However, the lesions explained more volume variance change on the right side.
than the left side probably because our combined sample had a greater lesion load in the right hemisphere.

In summary, because our method prevented any bias introduced by the prior knowledge of anatomic correlations among WM structures, the finding of an association between LGN atrophy and lesions in the optic radiations of both hemispheres is robust and could confirm our hypothesis that lesions in the optic radiations cause atrophy in the LGN. However, because more than 70% of LGN volume variance remains unexplained, the effect of optic radiation lesions on the development of GM atrophy is limited and other factors clearly could play a role in the genesis of LGN atrophy. It is possible that lesions of the WM not visible on MR images (normal-appearing WM changes), pregeniculate lesions,32-34 (although we did not find any differences in LGN volume between patients with and without previous optic neuritis), or GM in situ processes may contribute to the rest of the variance.

Finally, patients with MS had highly significant atrophy of the occipital cortex that was not explained by the presence of WM lesions in its related pathways. This result is in concordance with previously published data using magnetic transfer ratio in the visual cortex of patients with and without lesions in the optic radiations.35 To explain this apparent paradox, it could be argued that the occipital cortex is a highly connected area and therefore the impact of axonal damage in the optic radiations was not enough to cause atrophy. Loss in connectivity, however, may not be the only cause of the occipital cortex atrophy observed in MS. The convergence of the results from different MR imaging techniques, showing the lack of correlation with lesions in the optic radiations, suggests the existence of other neurodegenerative or GM in situ processes.

In conclusion, our results suggest that neuronal loss from retrograde degeneration due to axonal damage within MS plaques could be one of the significant factors in the genesis of GM atrophy. Because this mechanism explains less than half of the variance, other neurodegenerative processes are probably at work as well.

Accepted for Publication: May 4, 2008.

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Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Sepulcre, Masdeu, and Villoslada. Acquisition of data: Villoslada. Analysis and interpretation of data: Sepulcre, Góthi, Masdeu, Bejarano, Vélez de Mendizábal, Toledo, and Villoslada. Drafting of the manuscript: Sepulcre, Masdeu, Toledo, and Villoslada. Critical revision of the manuscript for important intellectual content: Góthi, Masdeu, Bejarano, Vélez de Mendizábal, and Villoslada. Statistical analysis: Sepulcre, Góthi, and Toledo. Obtained funding: Villoslada. Administrative, technical, and material support: Toledo. Study supervision: Góthi, Masdeu, Bejarano, Vélez de Mendizábal, and Villoslada. Financial Disclosure: None reported.

Funding/Support: This study was supported by the European Union (grant 512146/LSH-2003-1.2.2.-2 to Dr Masdeu), the Spanish Ministry of Health (grant CM05/00222 to Dr Sepulcre; FIS#PI052520 to Dr Masdeu; and FIS#PI051201 to Dr Villoslada), the Navarra government (Mr Goni and Dr Masdeu), the Basque Country government (Ms Vélez de Mendizábal), the foundation “UTE project CIMA” (Dr Masdeu), and the Fundación Uriach (Dr Villoslada).

Additional Contributions: We thank the MS Society of Navarra and the subjects who kindly agreed to take part in this study.

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