

Evidence of early cortical atrophy in MS

Relevance to white matter changes and disability

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Abstract—Objective: To assess cortical gray matter (GM) changes in MS and establish their relevance to clinical disability and to inflammatory changes of white matter (WM) in patients with the relapsing–remitting (RR) and primary progressive (PP) forms of the disease. **Methods:** Conventional MRI examinations were obtained in patients with definite MS who had either the RR or the PP form of the disease. An automated analysis tool was used with conventional T1-weighted MR images to obtain total and cortical brain volumes normalized for head size. Total brain lesion load was estimated on conventional proton density and T2-weighted MR images. The relationship between volumetric MR measures and scores of clinical disability was assessed. **Results:** Normalized cortical volumes (NCV) were lower for both RR and PP MS patients than for normal control subjects ($p < 0.001$) but were similar between the two patient groups ($p > 0.5$). NCV decreases in both patients groups were detected even in those patients with short disease duration (<5 years; $p < 0.001$ in RR MS and $p < 0.05$ in PP MS) and minimal brain lesion volume (<5 mL; $p < 0.0001$ in RR MS and $p < 0.005$ in PP MS). Measures of NCV in individual patients were negatively correlated with T2-weighted lesion volume ($r = -0.47$, $p < 0.001$) and disease duration ($r = -0.25$, $p < 0.05$) only in the patients with RR MS. NCV correlated with Expanded Disability Status Scale scores across all of the patients, but the strength of the correlation was stronger ($p < 0.05$) for PP ($r = -0.64$, $p < 0.0001$) than for RR ($r = -0.27$, $p = 0.04$) MS patients. **Conclusions:** These data confirm substantial neocortical volume loss in MS patients and suggest that neocortical GM pathology may occur early in the course of the disease in both RR and PP MS patients and contribute significantly to neurologic impairment. Although a proportion of this neocortical pathology may be secondary to WM inflammation, the extent of the changes suggests that, especially in patients with PP MS, an independent neurodegenerative process also is active.

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MS is a chronic inflammatory demyelinating disorder of the CNS. The disease involves most prominently the white matter (WM) of the brain and spinal cord with loss of myelin and axon and reactive astrogliosis.^{1,2} Over the last decade, a number of MR studies have demonstrated the relevance of this axonal loss to understanding the progression of disability^{3–8} and have shown that it occurs in the earliest stages of disease progression,^{9,10} even before the appearance of “fixed” disability.¹¹

Recently, histopathologic studies^{12,13} have demonstrated neocortical and neuronal pathology in MS. MR studies have shown lesional¹² and diffuse^{14–17} gray matter (GM) changes in brains of MS patients. Together, this work suggests that neurodegeneration is an important pathologic feature of MS. Primary neuronal loss may underlie some of the diffuse axonal destruction in the otherwise “normal-appearing” WM.¹⁸

Measurements of brain volume are sensitive to both neuronal and axonal loss. Total and regional

brain atrophy can be accurately assessed from conventional T1-weighted images by means of computational methods allowing automatic or semiautomatic measurements of cerebral volumes.^{6,19–21} With these methods, progressive decreases in total brain volumes have been consistently reported in MS patients with different forms of the disease.^{6,19–23} Cerebral GM loss seems to contribute to this overall tissue loss, at least in patients with early relapsing–remitting (RR) MS.¹⁴

Here we report measurements of neocortical volumes in patients with RR and primary progressive (PP) MS. We used an accurate, automated method for computing analysis of total and tissue class-specific brain volumes (SIENA: Structural Imaging Evaluation of Normalized Atrophy)²⁴ and performed selective measurements of the neocortical GM. The relationship of MR measurements of neocortical brain volumes with other MR and clinical measures was explored. Also, differences in neocortical GM pathology between patients with RR and PP forms of

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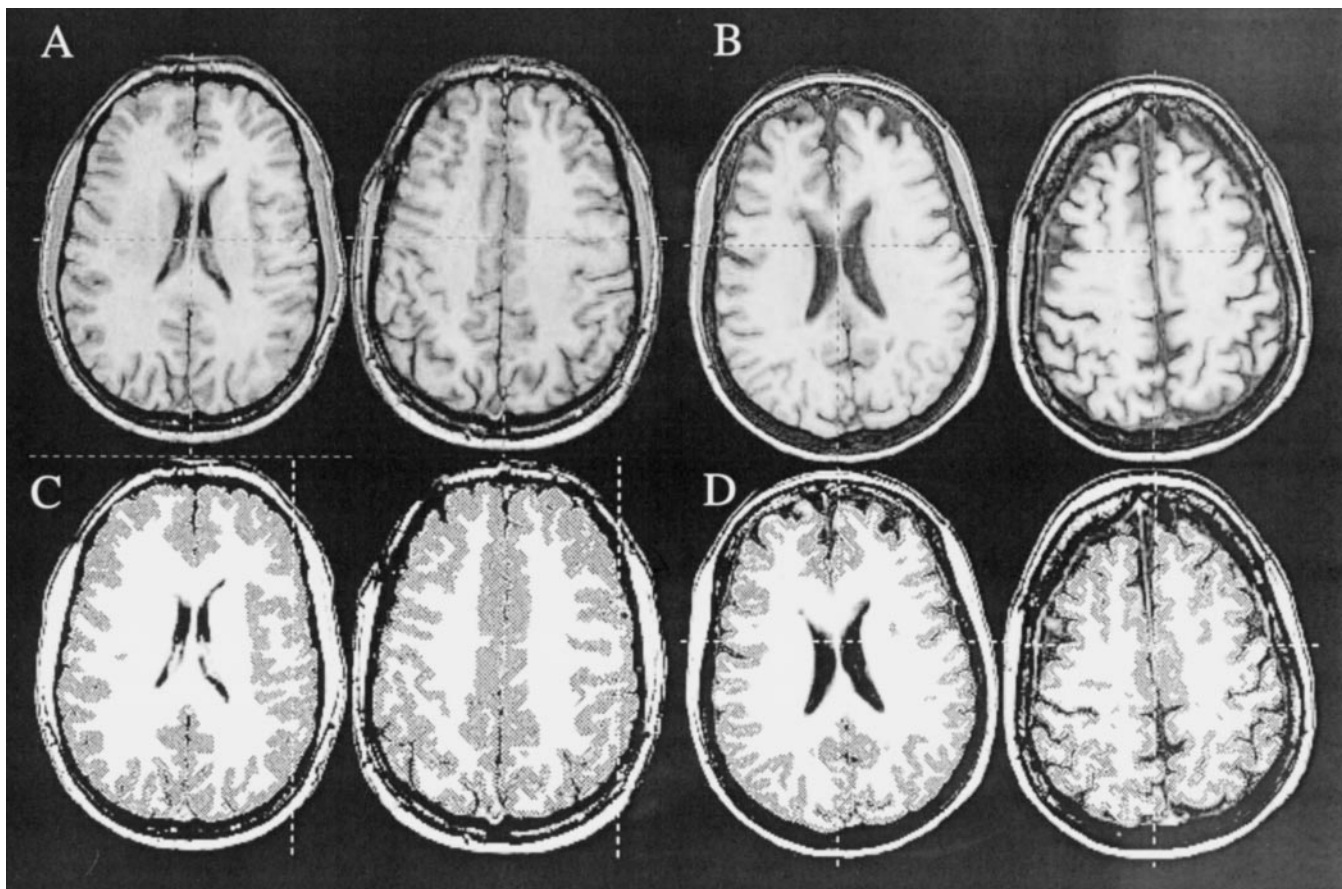


Figure 1. Typical transverse T1-weighted MR images and illustrative example of the SIENAX output for neocortex assessment of a normal control subject (A,C) and a patient of similar age with MS (B,D). Note the difference in neocortical gray matter volume between the normal control (C) and the MS patient (D).

MS at early disease duration and with minimal WM lesion accumulation were defined to test the relationship between WM inflammation and the extent of neuronal pathology.

Methods. *Patient population.* We studied 90 patients (60 women and 30 men; 18 to 67 years old, median age = 38 years) with clinically definite MS²⁵ who had either the RR (n = 65; 45 women and 20 men; 20 to 66 years old, median age = 36 years) or the PP (n = 25; 15 women and 10 men; 18 to 67 years old, median age = 50 years) form of the disease.²⁶ All RR MS patients were relapse-free for at least 1 month before study entry. When MRI scans were obtained, none of the MS patients was being treated with corticosteroids for at least 1 month, 10 of 65 RR MS patients were being treated with β -interferons, and all PP MS patients were drug treatment-free. For each patient, neurologic evaluation, which included the rating of disability using the Expanded Disability Status Scale (EDSS),²⁷ was performed within 24 hours of the performance of the MR examination by an experienced observer who was kept blinded to the MRI results. The study was approved by the Ethic Committee of the Faculty of Medicine of the University of Siena, and informed consent was obtained from all participating subjects.

MR examinations. All patients were examined using the same MR protocol. This included a transverse dual echo, turbo spin echo sequence (repetition time [TR]/first echo time [TE1]/TE2 = 2,075/30/90 milliseconds, 256 \times 256 matrix, one signal average, 250 \times 250-millimeter field of view), yielding proton density (PD) and T2-weighted images with 50 contiguous 3-mm-thick slices, acquired parallel to the line connecting the anterior and posterior commissures. Subsequently, transverse T1-weighted, gradient echo images (TR/TE = 35/10 milliseconds, 256 \times 256 matrix, one signal average, 250 \times 250-mm field of view) were acquired. This

sequence yielded image volumes of 50 slices, 3 mm thick, oriented to match exactly the PD/T2 acquisition.

MR data analysis. *Lesion volumes.* Classification of T2-weighted lesion volume (LV) was performed in each patient by a single observer unaware of the subjects' identity, employing a user-supervised thresholding technique. Lesion borders were determined primarily on PD-weighted images, but information from T2-weighted and T1-weighted images was also considered, as the software used (MEDx, Sensor Systems, Inc., Sterling, VA) offered the ability to toggle between the PD, T2-weighted, and T1-weighted images, providing the operator with convenient access to the information in both data sets while defining lesions and facilitating the discrimination of CSF from periventricular plaques. Total LV was calculated by multiplying lesion area by slice thickness and was reproducible to about 6% in serial measurements.

Total brain volumes. On T1-weighted MR images, normalized volumes of the whole of the brain parenchyma and neocortical GM were measured using a method for total and regional brain volume measurement (cross-sectional version of the SIENA software²⁴ [SIENAX]; figure 1). SIENAX uses BET (Brain Extraction Tool; part of FSL-FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl) to extract the brain and skull from the MR images, as previously described.²¹ A tissue segmentation program (FAST; another part of FSL)²⁸ is then used to segment the extracted brain image into GM and WM, CSF, and background, yielding an estimate of total brain tissue volume. In contrast to methods using binarized segmentation, FAST uses partial volume estimation for tissue segmentation. Thus, SIENAX is able to get particularly accurate volume estimates for regions that include a large number of partial volume voxels, such as neocortical GM.²⁴ The brain-extracted MR images are registered to a canonical image in a standardized space (using the skull image to provide the scaling cue), a procedure that also provides a spatial normalization (scaling) factor for each subject. For selective measurement of neocortical volumes, a

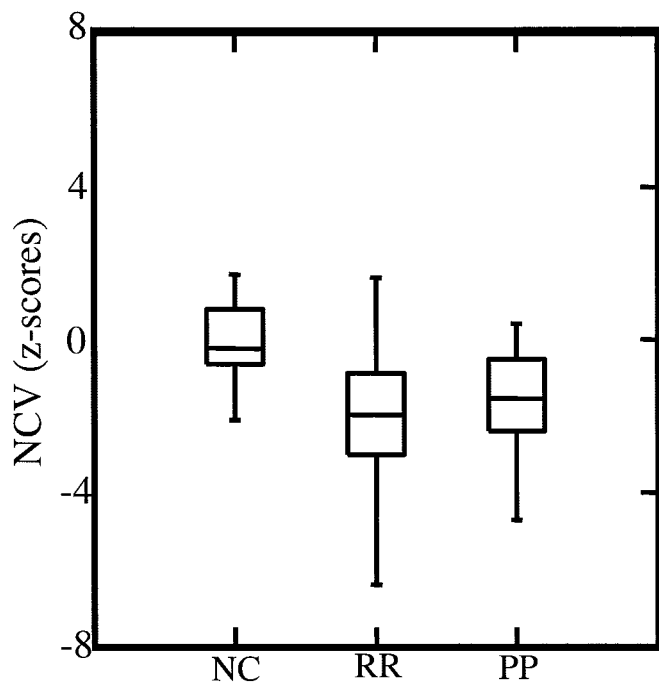


Figure 2. Box plots comparing the standardized MR measurements of normalized neocortical volume (NCV) of all patients with primary progressive (PP; $n = 25$; right) and relapsing-remitting (RR; $n = 65$, center) MS and normal control subjects (NC; left). Data are z score transformed to correct for differences in age between the two MS groups. NCV values are lower in both RR and PP MS patients than in NC ($p < 0.001$ for both), without differences between the two patient subgroups ($p > 0.5$).

standard space mask (which includes ventricles, deep GM, cerebellum, and brainstem) is used to separate segmented GM into neocortical and nonneocortical. The estimated volumes for a subject are then multiplied by the normalization factor to yield either the volume of the total brain tissue or the neocortical GM volume (NCV).

Statistical analysis. The nonparametric Mann-Whitney test was used for comparisons of the two groups of MS patients. Values of NCV for RR and PP MS patients were compared with those of a healthy normal control group. As the group of PP MS patients were significantly ($p < 0.001$) older than the RR MS patients (RR = 20 to 66 years, median age = 36 years; PP = 18 to 67 years, median age = 50 years), before statistical comparisons, MR data were corrected for by using a z-score transformation relative to an age-matched normal control group for each patient group (normal control group for comparison with RR MS patients: 18 subjects, 21 to 63 years old, median age = 38 years; normal control group for comparison with PP MS patients: 15 subjects, 30 to 68 years old, median age = 47 years). This allowed avoidance of potentially spurious results due to differences in age between patient subgroups. Normal control individuals were recruited from laboratory and hospital workers and were included in the group if they had negative history for neurologic disorders and no abnormalities on conventional brain MRI. After z-score transformation, differences between patient and normal control groups were assessed using analysis of variance followed by pairwise post hoc comparison using Tukey's highest significant difference procedure to account for multiple comparisons. Relationships between MR and clinical values of RR and PP MS patients were assessed by using the nonparametric Spearman rank order correlation. The Fisher test was used to assess significant differences between homologous correlations in the two patient subgroups. Data were considered significant at the 0.05 level. The SYSTAT software version 9 running on Windows (SPSS, Chicago, IL, 1998) was used to perform statistical calculations.

Results. The two groups of MS patients had similar disease duration (RR MS mean = 7 ± 7 years, age range = 1 to 32 years; PP MS mean = 8 ± 6.5 years, age range = 1 to 22 years; $p = 0.5$), but PP MS patients were more disabled (mean EDSS in PP MS = 4.6 ± 1.5 , range = 2 to 7.5; mean EDSS in RR MS = 1.9 ± 1 , range = 1 to 6; $p < 0.001$) and older (see Statistical analysis) than RR MS patients.

After correction for common effects of age, NCV values were lower in both RR and PP MS patients than in normal control subjects (NCV z score in RR MS = -1.96 ± 1.8 ; NCV z score in PP MS = -1.50 ± 1.3 ; $p < 0.001$ for both; figure 2), without differences between the two patient subgroups ($p > 0.5$). Decreases in NCV were different from those in normal control subjects in both patient groups, even in the subgroup with low disease duration (< 5 years; NCV z score in RR MS [$n = 31$] = -1.50 ± 1.7 , $p < 0.001$; NCV z score in PP MS [$n = 10$] = -1.3 ± 1.3 , $p < 0.05$) or minimal lesion loads (< 5 mL; NCV z score in RR MS [$n = 38$] = -1.21 ± 1.5 , $p < 0.0001$; NCV z score in PP MS [$n = 19$] = -1.25 ± 1.1 , $p < 0.005$; figure 3).

In patients with RR MS, measures of NCV showed negative correlations with measurements of both T2-weighted LV ($r = -0.47$, $p < 0.001$; figure 4) and disease duration ($r = -0.25$, $p < 0.05$). Similar relationships were not found in PP MS patients (NCV versus T2-weighted LV: $r = -0.1$, $p = 0.6$; see figure 4; NCV versus disease duration: $r = -0.08$, $p = 0.8$). In both patient subgroups, NCV correlated tightly ($p < 0.0001$) with NBV, but this correlation was much stronger ($p = 0.02$) in PP MS ($r = 0.92$) than in RR MS ($r = 0.76$) patients. Finally, NCV values correlated significantly with EDSS scores in both patient subgroups (figure 5), and this relationship also was much stronger ($p = 0.05$) in PP MS patients ($r = -0.64$, $p < 0.0001$) than in RR MS patients ($r = -0.27$, $p = 0.04$).

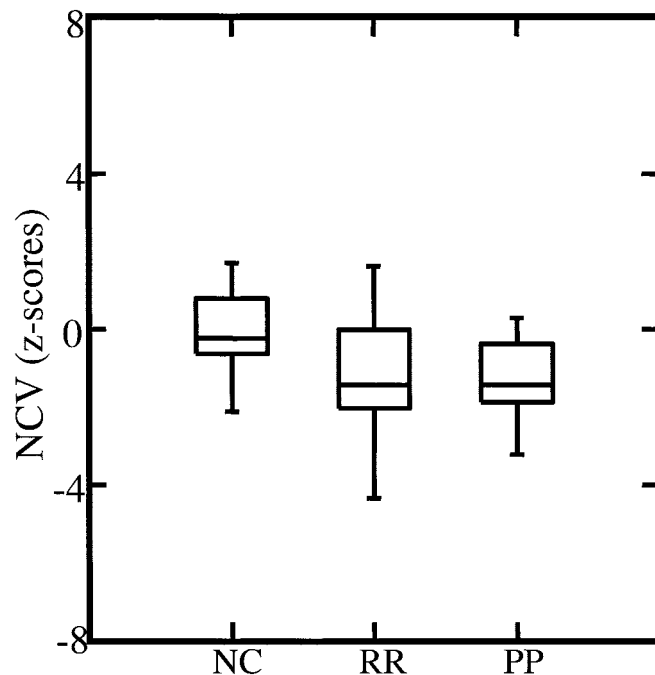


Figure 3. Box plots comparing the standardized MR measurements of normalized neocortical volume (NCV) of patients with primary progressive (PP; $n = 19$; right) and relapsing-remitting (RR; $n = 38$; center) MS with very low T2-weighted lesion volumes (< 5 mL) and normal control subjects (NC; left). Data are z score transformed to correct for differences in age between the two MS groups. NCV values are lower in both RR ($p < 0.0001$) and PP ($p < 0.005$) MS patients than in NC, without differences between the two patient subgroups ($p > 0.5$).

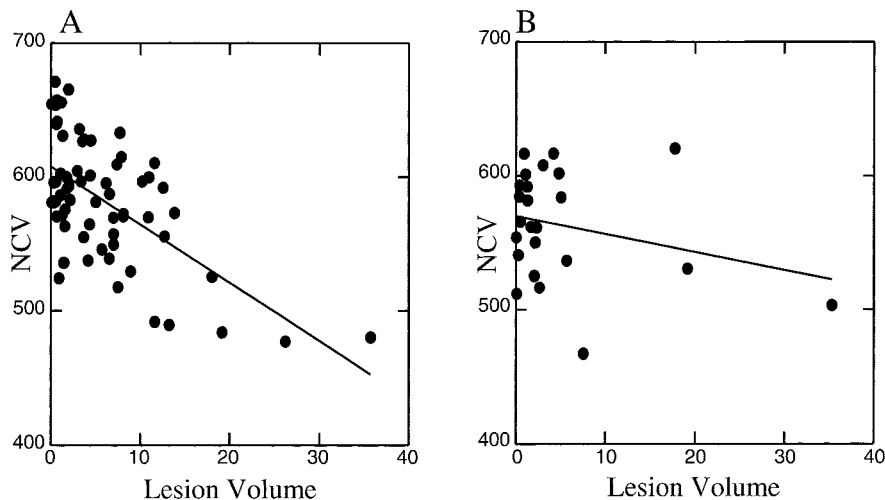


Figure 4. Data illustrating the correlation between normalized cortical volume (NCV) and T2-weighted lesion volume values in patients with relapsing–remitting (RR) MS (Spearman rank coefficient $r = -0.47$, $p < 0.001$) (A) and the absence of correlation between the two MR parameters in the primary progressive (PP) MS groups (Spearman rank coefficient $r = -0.1$, $p = 0.6$) (B).

Discussion. Pathologic and imaging studies have shown that axonal injury in WM and neocortical abnormalities occur in brains of patients with MS.^{2,12,13} Together, primary cortical pathology and cortical neuronal changes related to WM pathology could make a major contribution to the progressive brain atrophy associated with the disease.^{18–20} Axonal injury is likely associated with neuronal dystrophy through both anterograde and retrograde changes.^{29,30} However, the relative contributions of the different tissue compartments to brain atrophy and, in particular, the importance of neocortical pathology in such a process are still unclear.³¹ To investigate these aspects, we used an objective, automated method²⁴ for automatic assessments of total and regional brain volumes and selectively assessed neocortical GM volume in patients with RR and PP MS.

In contrast to the conclusion of an earlier report³² (that, however, had limitations due to the binarized segmentation method used; see Methods) and confirming the results of a recent study of RR MS patients,¹⁴ we found that neocortical atrophy is prominent in MS. Decreases in NCV can be found in both RR and PP MS patients even when patients are grouped for short disease duration (<5 years) or for

low cerebral lesion load (<5 mL of T2-weighted LV, namely, <0.5% of the total brain tissue occupied by lesions). These observations suggest that in both RR and PP MS patients, neocortical pathology not only seems to occur in the earliest stages of the disease but also appears to be significant even with minimal WM lesion accumulation.

One of the aims of the current study was to test the relationship between WM changes and the extent of neuronal pathology. In addition to the already mentioned significant NCV decreases in RR and PP MS patients with minimal WM lesion accumulation, we found a modest relationship between T2-weighted LV and NCV in RR MS patients (which explains only 25% of the variance in NCV) and the absence of this relationship in PP MS patients. These data imply that neocortical atrophy is not necessarily dependent on WM changes and suggest that neocortical pathology might be the consequence of distinct pathologic mechanisms.¹³

Our conclusions rely on accurate segmentation of GM and WM in the MR images. A methodologic concern is that apparent decreases in GM volumes might be related to the presence of occult neocortical inflammatory lesions. Such lesions can be seen con-

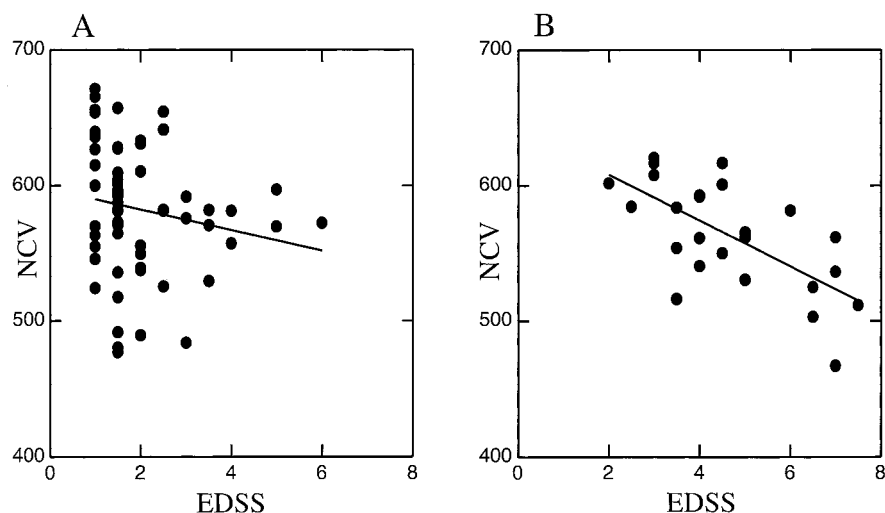


Figure 5. Data illustrating the weak although significant correlation between normalized cortical volume (NCV) and Expanded Disability Status Scale (EDSS) values in patients with relapsing–remitting (RR) MS (Spearman rank coefficient $r = -0.27$, $p = 0.04$) and the stronger ($p = 0.05$) correlation between the clinical and MR parameters in the primary progressive (PP) MS groups (Spearman rank coefficient $r = -0.64$, $p < 0.0001$).

sistently in postmortem studies^{12,13} but are rarely detected on MRI using conventional sequences.^{33,34} Thus, subtle changes in MR signal intensity due to MR-undetectable neocortical lesions could affect segmentation between the cortex and CSF such that the cortex appears to decrease in volume. On the other hand, as the T2-weighted hypointense neocortical lesions show “normal” T1 signal,³³ neocortical or juxtacortical lesions may have intensity similar to that of the GM and therefore could have been included in the volume measurement of the neocortex. However, in the current study, as we have found significant NCV decreases even in patients with minimal WM T2-weighted LV, it is very unlikely that the detected decrease in neocortical volumes could be due primarily to a neocortical lesion burden.

Clinical–pathologic differences between RR and PP patients have been stressed in several studies.^{35,36} These include a progressive myelin and axonal pathology accompanied by a mild presence of inflammatory components (and the subsequent relative paucity of MR-detectable cerebral lesions) and a more pronounced spinal cord pathology in PP MS patients than in patients with RR MS.^{37,38} In our study, differences between RR and PP MS patients were not found in terms of the extent of the NCV decrease. This finding and the associated presence of a more pronounced disability and a minor load of T2-weighted LV (data not shown) in PP with respect to RR MS patients seem to confirm the hypothesis that a pathologic involvement of the spinal cord does occur and can be clinically relevant in this complex form of MS. However, PP MS patients did not show a correlation between NCV and either T2-weighted LV or disease duration, whereas a stronger relationship was shown between NCV and EDSS than in RR MS patients. This suggests that, in patients with PP more than with RR MS, neocortical atrophy is due to a pathologic process that is dominated mostly by neurodegeneration and that, regardless of the mechanisms, seems to be very relevant to clinical disability.

Several mechanisms might contribute to neurodegenerative changes in the neocortex. We hypothesize that axonal loss, which plays a major role in the pathology of MS from the earliest disease stages,^{39,40} is an important component of this complex process. Experimental studies suggest that this may occur, at least in part, via mechanisms that are independent of those causing demyelination^{41–43} and that it is probably related to the presence of an abnormal glial–axonal interaction even with low^{13,43} or absent⁴⁴ inflammation. However, we also speculate that primary neuronal injury due to local inflammatory activity also could lead to neocortical atrophy in MS. Experimental work has shown neuronal apoptosis in experimental allergic encephalomyelitis,⁴⁵ and apoptotic neuronal death unrelated to axonal transection has been found in the cortex of MS patients¹³. Recent MR spectroscopy data, by showing decreases in the neuronal marker *N*-acetylaspartate in both

neocortical⁴⁶ and subcortical⁴⁷ GM of MS patients and, in the latter study, by correlating histopathologic work on postmortem tissue, seem to be in agreement with the opinion that primary neuronal loss could be substantial in MS brains. Thus, whereas a proportion of this neocortical pathology may be secondary to white matter inflammation, it is possible that an independent neurodegenerative process also might be active. The mechanisms of this need to be identified.

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