The contribution of demyelination to axonal loss in multiple sclerosis

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The traditional notion that multiple sclerosis is a primary demyelinating disease has led to a plaque-centred view of both aetiology and the pathogenesis of disease progression. The presence of axonal loss has received increasing recognition. However, the relative roles of demyelination and axonal loss have not been fully clarified in multiple sclerosis nor have their possible interrelationships been elucidated. Post-mortem material from the cerebrum, brainstem and spinal cord of 55 multiple sclerosis patients (29 males) with an age range of 25–83 years (mean = 57.5 years) and length of disease history ranging from 2 to 43 years (mean = 17.1 years) was stained for myelin. Plaque load was calculated by summing the relative proportion of plaque area compared with total white matter area of the corticospinal and sensory tracts at each level. This was related to estimates of axonal density and of total axon number in these tracts in the spinal cord. Our results indicate that plaque load did not correlate with brain weight. Unexpectedly, after adjusting for sex, age and duration of disease, correlations between total plaque load and axonal loss in both the corticospinal tract and sensory tracts were weak or absent at each level investigated. Since there was little correlation between plaque load and axonal loss, the possibility that demyelination is not the primary determinant of spinal cord axonal loss warrants consideration.

Keywords: demyelination; axon; axonal degeneration; multiple sclerosis; neuropathology

Abbreviation: NAWM = normal appearing white matter

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Introduction

Multiple sclerosis has long been classified as an inflammatory demyelinating disease, associated with the formation of discrete or confluent foci of myelin loss and relative preservation of axons. It is unambiguous that plaque accumulation and progression of disability are temporally linked. However, the belief that the acquisition of progressive disability in multiple sclerosis results from the cumulative effect of plaques is an assumption for which there has been little direct evidence. The rediscovery of axonal damage and loss in plaques has sharpened the focus on these demyelinated regions (Ferguson et al., 1997; Trapp et al., 1998; Lovas et al., 2000). The plaque-centred view of disease progression has come under scrutiny, as the traditional demyelinating paradigm in which plaques determine outcome is challenged by a substantial number of clinical observations.

A number of the clinical features of multiple sclerosis may be considered to challenge the concept that myelin-related pathology accounts for progressive disability. These include the following: (i) individual plaques as measured by relapses are not crucial to long-term clinical outcome (Kremenchutzky et al., 2006; Confavreux et al., 2000); (ii) the location of the first plaque and the extent to which a patient recovers from that first plaque have no influence on long-term outcome (Confavreux et al., 2003; Kremenchutzky et al., 2006); (iii) only early relapse frequency has been shown to associate with outcome (Weinshenker et al., 1989b), and although relapses impact time to onset of progression, they do not have an appreciable effect on the rate of progression once a progressive phase begins (Kremenchutzky et al., 1999) or a certain level of disability is reached (Confavreux et al., 2000; Pittock et al., 2004); (iv) treatment with interferon suppresses plaque load (T2 lesion burden) without affecting long-term MRI measured atrophy (Gasperini et al., 2002; Horsfield and Filippi, 2003; Lin et al., 2003) and impact on disability remains uncertain.

From a clinical perspective, it could be argued that the role of demyelination in axonal loss is most relevantly examined in the corticospinal tracts. These are typically the first to
manifest the onset of the chronic progressive phase and they play the largest role in disability as measured by the ambulation-dependent Extended Disability Status Scale (EDSS). Furthermore, they are discrete at several levels of the neuraxis, allowing serial anatomical measurement in a centripetal fashion. It is possible that the long processes are selectively vulnerable by virtue of being more likely to be affected by plaques than are shorter ones. The corticospinal axons, owing to their extraordinary length, are potentially vulnerable to degenerative processes. However, there are undoubtedly many other influences on the topography of axonal degeneration. It is not known why the combined degeneration of both the corticospinal and sensory tracts is a common pattern in several metabolic and degenerative disorders such as sub-acute combined degeneration of the spinal cord, Friedreich’s ataxia and hereditary spastic paraplegia (HSP) (Behan and Maia, 1974; Said et al., 1986; Jitpimolmard et al., 1993; Hemmer et al., 1998). Even when a gene mutation is known and its expression is characterized, the site-specific axonal degeneration observed in these conditions cannot be easily explained. Nevertheless, selective tract vulnerability appears to be a common feature of processes that might otherwise be considered to be generalized or diffuse.

We have previously shown that there is a symmetric, size-dependent selective loss of axons in the corticospinal and posterior column tracts in multiple sclerosis, a pattern implying differential sensitivity of the axons within these tracts. Accordingly, it was anticipated that axonal loss might not be easily attributable to the anatomical location of plaques alone (Ganter et al., 1999). In the case of the corticospinal tracts in multiple sclerosis, axonal loss is actually more selective than in HSP (DeLuca et al., 2004b).

The present study utilizes what may be one of the largest reported pathological cohorts of multiple sclerosis cases to examine relationships among atrophy, plaque load and axonal density/loss using quantitative techniques. By examining the population of axons in the corticospinal and sensory tracts throughout the length of the spinal cord (as described in our previous study) (DeLuca et al., 2004a) and plaque load impinging on the respective tracts, the following questions were addressed: (i) what is the nature and extent of the axonal loss? and (ii) what is the relationship between total plaque and axonal loss?

**Material and methods**

**Clinical material**

Human autopsy material of 55 pathologically confirmed cases of multiple sclerosis (29 males and 26 females) with an age range of 25–83 years (mean = 57.5 years) was studied. The length of disease history ranged from 2 to 43 years (mean = 17.1 years). The post-mortem material was derived from the autopsy brain and spinal cord archive from the Neuropathology Department, Oxford Radcliffe NHS Trust, and was obtained with consent from the next-of-kin for use of tissue from research. The study was approved by the local research ethics committee.

**Pathological preparation**

For each of the multiple sclerosis cases, formalin-fixed paraffin-embedded sections were taken from the cerebrum (coronal section at internal capsule including the posterior limb), brainstem (transverse sections from each of the mid-brain, mid-pons and mid-medulla) and spinal cord (transverse sections at the high and low cervical, high and low thoracic, and lumbar levels). Adjacent myelin-stained and axon-stained sections from each level were obtained using the Luxol fast blue cresyl violet and Palmgren silver stains, respectively. Plaque load measures were related to estimates of axonal density and total axon numbers reported in our previous study, reproduced with permission in Fig. 1 (DeLuca et al., 2004a).

To assess more thoroughly the extent of plaque load impinging on the corticospinal and sensory tracts throughout the length of the spinal cord, spinal cords from a subset of 10 cases were sectioned at 5 mm intervals from cervical to lumbar cord levels. The subset of spinal cords comprised five cases, each lying at the opposite ends of initial total plaque load estimates obtained from the nine levels as previously outlined. A total of 1355 discrete spinal cord sections from these cases were stained for myelin so that total plaque load measures could be obtained and subsequently related to estimates of axonal density and total axon numbers as described above.

**Quantification of axonal loss**

Sections stained with Palmgren silver were used to demonstrate axons. The axons in the corticospinal tract and sensory tract of each side were examined via microscopy (×400). Five fields from each side of the corticospinal tracts and six fields from the sensory tracts on each side were digitized and then transferred to the NIH Image software program, where the fibre counts were performed automatically after setting of a threshold. To ensure that the oedema and high cellular infiltration of active lesions would not confound axonal counts, measures of axonal density were obtained in normal appearing white matter (NAWM).

To validate our automated image analysis procedure, several different automated settings (i.e. filters, thresholds, etc.) were applied to the images previously counted manually by two observers so that the axon numbers for each method could be compared. A threshold was established that gave a strong correlation between the final automated counts and manual counts (r = 0.956, P < 0.001).

To determine the distribution of axonal sizes observed in each of the tracts, histograms were constructed. The distribution of axonal sizes was used to categorize axonal fibres as either large or small using a size range of >3 μm² for the large fibres and ≤3 μm² for the small ones.

In order to obtain an estimate of the total number of axons in the corticospinal and sensory tracts, we took the product of the axonal density measures (axons/mm²) and cross-sectional areas (mm²) of each respective tract.

**Quantification of plaque load**

To quantify the extent of demyelination affecting the corticospinal and sensory tracts in the multiple sclerosis cases, the cross-sectional area of the multiple sclerosis lesions impinging on each tract were identified and then measured. For the corticospinal tracts, plaque load was quantified for cerebral and brainstem sections by a point-counting method (Fig. 2). Plaques affecting the corticospinal and sensory tracts in the spinal cord were quantified using a computerized image analysis system running NIH image software. Measures of relative plaque load were obtained by estimating the area of...
demyelination in relation to the total white matter area at each level investigated. Lesions that were demyelinated (both active and inactive) were included in the calculation of plaque load measures, with the majority of cases (52 out of 55) containing only chronic lesions. Of the three active cases, only a small proportion of lesions (7 out of 161) could be classified as active as defined by macrophages labelled with PG-M1 Luxol fast blue/cresyl violet inclusions. Fewer than 5% of the lesions showed evidence of remyelination in all of the cases studied. When present, remyelination was often confined to a narrow ribbon bordering the edges of chronic plaques. As areas of remyelination contributed marginally to global measures of plaque load, they were not included in global measures of plaque load.

Measures of plaque load were estimated by summing the relative plaque load measures of the cerebrum, brainstem and spinal cord for the corticospinal tracts and by summing relative plaque load measures of the spinal cord for the sensory tracts. To account for the variation in the number of levels available in each case, total plaque load measures were divided by the number of levels sampled. One-tailed Pearson correlations between total plaque load and each of axonal density, cross-sectional area and total axonal number were calculated for each tract, respectively (under the assumption that

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Fig. 1 Bar graphs of axonal density and total axon number of the corticospinal tract (A, C) and the posterior sensory tract (B, D) in controls and multiple sclerosis cases [modified from DeLuca et al. (2004a)].

Fig. 2 Coronal section of the cerebrum at the level of the internal capsule stained for myelin with Luxol fast blue cresyl violet (×10). The area of white matter (outlined in red) and the area of demyelination (yellow shading) were quantified by a point-counting method. Measures of relative plaque load were obtained by estimating the area of demyelination in relation to the total white matter area.
increases in area and total number of axons were not possible in the patient group). As the total number of axons in the sensory tract was significantly reduced only in the cervical and upper thoracic spinal cord, total measures of plaque load were correlated with axonal loss only at these levels. The relationship between relative changes in lesion areas and regional axonal counts was tested by multiple regression analysis. For the subset of cases ($n = 10$) in which the spinal cords were sampled exhaustively, stepwise regression analysis was performed using total plaque load as the first variable. Other variables utilized in the regression analyses include sex, age and duration of disease. All statistical calculations were performed with SPSS for Windows version 11.3. $P$-values $< 0.05$ were deemed statistically significant.

To evaluate axonal loss in plaques both within and between cases, measures of axonal density within chronic plaques were compared with measures of axonal density in NAWM. To control for variations of axonal number at different levels of the neuraxis, comparisons of axonal density inside and outside of plaques were restricted to levels wherein axonal counts within a chronic plaque involving the entire area of the lateral column on one side could be compared with the NAWM of the other lateral column with no evidence of plaque pathology. A total of 20 plaques from 16 cases met the specified criteria.

Cerebral hemispheres

To evaluate more thoroughly the extent of cerebral plaque load in the cerebrum, the cerebral hemispheres from six of the cases studied were sectioned coronally every 1 cm, as reported in a previous study (Evangelou et al., 2000b). These cases were selected because they showed a clear correlation between global measures of cerebral plaque load and axonal loss in the corpus callosum. All multiple sclerosis lesions were identified macroscopically and the cross-sectional area of each lesion was measured using the stereological technique of point counting (Howard et al., 1998). The volume of the lesions was calculated by multiplying the surface area by the depth of the section (1 cm). Lesion volumes for each case were normalized by dividing the total volumes by the brain weight reported at autopsy. Normalized cerebral lesion volumes were related to estimates of axonal density and total axon numbers in the corticospinal tracts. Cerebral lesions were not restricted to the corticospinal tracts, in order to provide an estimate of the effect of global cerebral plaque load on axonal loss.

Results

Corticospinal tracts

Axonal loss

In the corticospinal tract, it was found that there was a significant reduction in axonal density and total axonal loss throughout all levels of the neuraxis in multiple sclerosis cases when compared with controls. The results are summarized in Fig. 1.

In the crossed corticospinal tracts in multiple sclerosis, fibres of smaller diameter ($\leq 3 \mu m^2$) were preferentially lost in contrast to the larger diameter fibres ($>3 \mu m^2$), which were relatively preserved (data not shown). These findings are described in more detail in DeLuca et al. (2004a).

Plaque load

Plaque load impinging on the corticospinal tracts was variable among cases. The mean of the proportion of total white matter in the lateral column affected by demyelination of the five levels initially studied was 0.1616 (range = 0.0197–0.5362). Relative plaque load measures from the cerebrum (mean = 0.1101, range = 0.001–0.340), brainstem (mean = 0.1236, range = 0.0–0.4848) and spinal cord (mean = 0.1949, range = 0–0.80) contributed to total plaque load estimates. Plaque load measures were similar for both sexes. Measures of total plaque load did not correlate well with brain weight ($r = 0.069, P = 0.323$) nor with duration of disease ($r = -0.145, P = 0.186$).

Comprehensive assessment of total spinal cord plaque load of the subset of 10 cases revealed that the mean of the proportion of total white matter in the lateral column affected by demyelination was 0.0148 (range = 0–0.0534). The lower cervical cord was the level of the neuraxis most significantly burdened with plaque pathology affecting the corticospinal tracts. Measures of total spinal cord plaque load did not correlate well with brain weight ($r = -0.138, P = 0.373$) nor with length of disease history ($r = 0.306, P = 0.230$).

For the subset of cases selected for detailed examination of cerebral plaque load, the average lesion volume was 13.92 cm$^3$ (range = 0.24–30.76 cm$^3$). Measures of total cerebral plaque load did not correlate well with brain weight ($r = -0.567, P = 0.319$) nor with duration of disease ($r = 0.021, P = 0.968$).

Sensory tracts

Axonal loss

In the posterior white matter columns, there was a reduction in axonal density and total axonal number in multiple sclerosis cases compared with controls throughout the length of the spinal cord (Fig. 1). However, it was only at the cervical and high thoracic cord levels that the reduction in total axonal number reached statistical significance. There was a statistically significant reduction in axonal density in the smaller diameter fibres but the larger diameter fibres showed no significant reduction. These findings are described in more detail in DeLuca et al. (2004a).
For the subset of cases surveyed for total spinal cord plaque load in detail, the average lesion load was 0.0282 (range = 0–0.1269). Measures of total spinal cord plaque load affecting the sensory tracts did not correlate well with brain weight ($r = 0.193, P = 0.323$). No significant relationship between total spinal cord plaque load and duration of disease was noted ($r = 0.327, P = 0.214$).

**Correlations of plaque load with axonal loss**

**Corticospinal tracts**

Estimates of plaque load of the nine levels of the neuraxis (from cerebrum to lumbar spinal cord) did not correlate well with axonal density measures or with estimates of total axon number at each level of the spinal cord surveyed. The results are summarized in Table 1. The only level of the cord at which the correlation between plaque load and total axonal loss was significant was the upper thoracic.

Detailed measurement of total spinal cord plaque load from the subset of cases sampled every 5 mm did not correlate well with axonal density measures or with estimates of total axon number at each level of the spinal cord surveyed. The results are summarized in Table 2. Upon consideration of the plaque subtype, chronic and active plaques each yielded similarly poor correlations with axonal counts at each level investigated (data not shown).

Upon more thorough evaluation of the plaque load in the cerebral hemispheres in the subset of cases examined, we found that cerebral lesion volume did not correlate with axonal density measures nor with estimates of total axonal number at each level of the cord investigated (data not shown).

**Sensory tracts**

Measures of plaque load did not correlate well with axonal density in the upper cervical cord. In contrast, plaque load correlated well with total axon number in the upper cervical cord. These results are summarized in Table 4.

Upon comprehensive examination of total spinal cord plaque load from the subset of 10 cases, measures of plaque load did not correlate well with axonal density nor with total axon number at the levels investigated. The results are presented in Table 5. Upon consideration of the plaque subtype, chronic and active plaques each yielded similarly poor correlations with axonal counts at each level investigated (data not shown).

**Axonal counts within chronic plaques compared with NAWM**

Measures of axonal density within chronic plaques did not differ significantly from those obtained in NAWM both within cases and between cases [axonal density (axons/mm$^2$) (i) within

### Table 1: Corticospinal tract: simple and *partial correlations of total plaque load and axonal loss in multiple sclerosis*

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>Correlations</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple ($r$)</td>
<td>$P$-value</td>
<td>Partial ($r$) $P$-value</td>
</tr>
<tr>
<td>Upper cervical</td>
<td>0.417</td>
<td>0.03</td>
<td>0.299</td>
</tr>
<tr>
<td>Density</td>
<td>0.364</td>
<td>0.063</td>
<td>0.247</td>
</tr>
<tr>
<td>Total</td>
<td>0.17</td>
<td>0.237</td>
<td>0.062</td>
</tr>
<tr>
<td>Density</td>
<td>0.262</td>
<td>0.147</td>
<td>0.281</td>
</tr>
<tr>
<td>Total</td>
<td>0.288</td>
<td>0.065</td>
<td>0.299</td>
</tr>
<tr>
<td>Density</td>
<td>0.464</td>
<td>0.007</td>
<td>0.519</td>
</tr>
<tr>
<td>Total</td>
<td>0.262</td>
<td>0.067</td>
<td>0.275</td>
</tr>
<tr>
<td>Density</td>
<td>0.224</td>
<td>0.113</td>
<td>0.249</td>
</tr>
<tr>
<td>Lumbar</td>
<td>0.135</td>
<td>0.239</td>
<td>0.022</td>
</tr>
<tr>
<td>Density</td>
<td>0.307</td>
<td>0.053</td>
<td>0.233</td>
</tr>
</tbody>
</table>

Density = axonal density (axons/mm$^2$); total = total number of axons. *Adjusted to account for sex, age and duration of disease.

### Table 2: Corticospinal tract: correlations of total spinal cord plaque load and axonal loss in multiple sclerosis*

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>Total Plaque load</th>
<th>Correlations</th>
<th>$R$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper cervical</td>
<td>0.001</td>
<td>-0.491</td>
<td>0.201</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>-0.761</td>
<td>0.067</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.069</td>
<td>-0.413</td>
<td>0.245</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>-0.518</td>
<td>0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.013</td>
<td>0.037</td>
<td>0.472</td>
<td></td>
</tr>
<tr>
<td>Upper thoracic</td>
<td>0.002</td>
<td>0.045</td>
<td>0.462</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>-0.586</td>
<td>0.083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-0.435</td>
<td>0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>0.005</td>
<td>-0.318</td>
<td>0.269</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>-0.514</td>
<td>0.149</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Density = axonal density (axons/mm$^2$); total = total number of axons.

plagues = 14 050 (9150–24 625); and (ii) NAWM = 15 450 (5775–31 925), $P = 0.614$[).

### Regression analyses

Upon consideration of the covariates sex, age and duration of disease when comparing the effect of plaque load on axonal density and total axonal number by multiple regression models, the partial correlations for each tract were calculated. The partial correlations for the corticospinal tracts and for the
sensory tracts are presented in Tables 1 and 4, respectively. They showed no significant correlations between plaque load and axonal number. For the subset of cases selected for detailed quantification of total spinal cord load, stepwise regression was carried out, with total spinal cord plaque load being the first variable. No significant correlations between total spinal cord plaque load and axonal density and total axon number were found. Sex, age and duration of disease had no appreciable effect on the correlations between total spinal cord plaque load and axonal number.

### Discussion

Traditionally, multiple sclerosis has been viewed as a primary demyelinating disease (Lumsden, 1971; McAlpine et al., 1972; Poser, 1986). Accordingly, much attention has focused on the role episodic inflammatory insults play in the formation of plaques throughout the CNS. The observations that axonal injury is associated with acute plaques and that extensive axonal loss can occur in gliotic lesions in chronic multiple sclerosis have engendered support for the concept that axonal pathology is the consequence of inflammation-induced demyelination (Trapp et al., 1998). Detailed neuropathological reports in combination with neuroimaging and magnetic resonance spectroscopy studies in life validate the existence of such axonal damage and loss, quantify the extent to which it occurs and postulate how it may contribute to clinical disability (Davie et al., 1995; Filippi et al., 1995; Losseff et al., 1996; Ferguson et al., 1997; Ganter et al., 1999; Trapp et al., 1999; Lovas et al., 2000; DeLuca et al., 2004a). However, recent evidence that substantial loss of axons occurs in areas apparently not affected by demyelination in the disease (Bjartmar et al., 2001), and that correlations between MRI measures of plaque load and axonal damage are poor, challenge the plaque-centred view of axonal degeneration (Iannucci et al., 1999; Mainiero et al., 2001; Bergers et al., 2002). The relative importance of axonal loss attributable to acute plaques, the axonal loss occurring in tracts without plaques and the Wallerian (or ‘dying back’) nature of this axonal loss and its widely believed relationship to plaque presence all remain unclear. As a result, the purpose of the present study was to examine the distribution and extent of axonal loss in the corticospinal and sensory tracts and its relationship to plaque load throughout the length of the neuraxis to gain better insight into the nature of axonal damage and loss in multiple sclerosis.

The examination of such a large cohort of multiple sclerosis cases provides valuable insight into the nature of demyelination in the disease. Measures of plaque load impinging on the corticospinal and sensory tracts varied significantly between cases. The wide range of plaque load measures between cases in these tracts reflects the variability of the disease process and, more important to this study, enables...
the thorough examination of the extent to which total measures of plaque load influence axonal loss in these tracts. As supported by epidemiological data, no significant differences were found in plaque load measures between the sexes (Weinshenker et al., 1989a, 1991). Plaque load did not correlate well with brain weight, suggesting that pathological mechanisms of tissue loss independent of demyelination may be important in determining the extent of atrophy in the brain, including axonal loss (McGavern et al., 2000). Upon consideration of the relationship between plaque load and duration of disease, it was observed that the extent of demyelination in the corticospinal and sensory tracts did not correlate with the length of disease history. However, it must be considered that a cohort of multiple sclerosis cases selected at autopsy is likely to have more aggressive disease and/or increased disability than the average. Therefore, the poor relationship between plaque load and disease duration in the present study may reflect (i) acute multiple sclerosis cases wherein death occurs early in the course of their disease; or (ii) multiple sclerosis cases with long-standing disease with chronic, ‘burnt out’ plaques that do not contribute to an increased number of plaques observed despite longer durations of disease. Fewer than 5% of plaques in the cohort examined showed evidence of remyelination, which confirms the documented observation that the extent to which remyelination occurs is highly dependent on duration of disease, with cases with increasing lengths of disease history showing increasingly less evidence of remyelination (Ozawa et al., 1994). The relative paucity of remyelination in the spinal cord lesions in our cohort contrasts the degree of remyelination reported to occur in the cerebral hemispheres (Prineas et al., 1993). Although it must be considered that the identification of remyelination in the spinal cord may be more difficult than in brain lesions and that some areas classified as NAWM may represent lesions with extensive remyelination, it is more likely that the question of remyelination is a possible confound for some lesions but seems unlikely to account for the overall observations presented in this study.

It has been proposed that the pathogenesis of axonal loss in NAWM may occur via two distinct mechanisms. On the one hand, axonal loss may result from a Wallerian degenerative process in plaques, where inflammation and demyelination are periodically exacerbated. In this circumstance, it would be predicted that the distribution and extent of axonal damage is determined by the distribution and extent of demyelinated plaques and may help explain relapse-related disability. On the other hand, the nature of the process may be one of neurodegeneration wherein an underlying diffuse axonopathy, independent of inflammatory demyelination, contributes significantly to axonal loss and results in the unrelenting accumulation of disability often seen in the progressive phase of the disease. In the latter scenario, it would be predicted that the relationship between plaque load and axonal loss would not be strong. It remains plausible that both processes may operate to varying extents in different patients and in different tracts, reflecting the marked pathological heterogeneity encountered in the disease. This is also the view supported by Kutzelnigg et al. (2005) in a study in which cortical pathology was thought to contribute to diffuse white matter injury.

We have found that axonal loss in the corticospinal tract is symmetric and size-selective (small fibres ≤3 μm² are preferentially lost), and occurs throughout the length of the neuraxis. In the corticospinal tract, plaque load did not correlate well with decreases in axonal density or with total number of axons at any of the levels investigated. Consideration of the covariates sex, age and length of disease duration resulted in similarly poor correlations between plaque load and axonal loss. The comprehensive survey of total plaque load in a subset of spinal cords also did not reveal any significant correlation between plaque load and axonal density nor total axon number. Both plaque subtypes (active and inactive) each yielded similarly poor correlations with axonal loss at each level investigated. To ensure that the effect of cerebral plaque load on axonal loss was not underestimated, the cerebral plaque load from a subset of cases was evaluated in detail. The cerebral plaque load in these selected cases did not correlate with decreases in axonal density or with total number of axons at any of the levels investigated. These findings suggest that inflammation-induced demyelination does not determine fully the extent to which axons are lost in this tract. Even though it has been demonstrated that axonal injury is associated with acute plaques, it cannot be assumed that the formation of lesions is necessarily causative of such axonal pathology and that all axonal injury in these lesions is irreversible. The observations that axonal loss in this tract is symmetric and size-selective despite the fact that lesions involve the central white matter in a disseminated fashion (Fog, 1950; Ikuta and Zimmerman, 1976; Oppenheimer, 1978), and that multiple sclerosis patients rarely develop isolated progressive hemiparesis, lend support to an underlying neurodegenerative process (Coles et al., 1999). It is feasible that discrete changes in the axonal microenvironment secondary to an underlying axonopathy may elicit an immune response targeted at the axon itself and/or surrounding myelin sheath that lead to the formation of the demyelinating lesion (Silber and Sharief, 1999). It is also possible that lesions impinging on this tract in sites not investigated could play a role in the axonal pathology observed. As a result, the data cannot exclude the possibility that inflammation and demyelination may contribute to the axonal loss observed in the corticospinal tracts in multiple sclerosis. However, plaques affecting the corticospinal tracts were sampled at more levels than in any other neuropathological study. Nevertheless, it demonstrates that the widely accepted plaque-centred view of the disease may not be sufficient to explain the axonal loss in this tract and that alternative explanations for axonal loss need to be considered.

In contrast to the corticospinal tracts, the sensory tracts showed a significant, symmetric and size-selective reduction
in axonal density and total axonal number only in the upper part of the spinal cord. Upon analysis of the relationship between plaque load and axonal loss, there was a significant, although modest, correlation between plaque load and total axonal loss in the sensory tract in the upper cervical cord, the region of most marked axonal loss. When the covariates sex, age and duration of disease are taken into account, any significant relationship between plaque load and axonal loss in the sensory tract disappeared. Further supporting the dissociation of the relationship between plaque load and axonal loss, the extensive sampling of the subset of spinal cords at 5 mm intervals revealed surprisingly weak correlations between total plaque load and total axon number. When considered separately, active and inactive plaques each showed similarly weak correlations between measures of plaque load and axonal loss. As the sensory tract is largely contained within the spinal cord with its axons terminating at the gracile and cuneatus nuclei of the medulla, this finding is particularly interesting for two reasons: first, plaque load was surveyed meticulously throughout the length of the spinal cord, thereby providing ample opportunity to discover a possible relationship between plaque load and axonal loss in this tract; and second, plaque load from the cerebrum, a common site of plaque pathology, does not directly influence the axonal viability of these sensory axons. These observations in the sensory tract mirror the poor correlations between plaque load and axonal loss found in the corticospinal tract. In short, the lack of impressive correlations between plaque load and axonal loss in the long nerve tracts in multiple sclerosis implies that mechanisms other than inflammation-induced demyelination may play an important role in axonal loss in multiple sclerosis, particularly in the spinal cord.

Pathologically, axonal loss in multiple sclerosis has been observed in a number of tracts such as the anterior optic pathways, corpus callosum, corticospinal tracts and sensory tracts (Ganter et al., 1999; Evangelou et al., 2000a, 2001; Lovas et al., 2000; DeLuca et al., 2004a). Correlations between plaque load and axonal loss in these tracts are variable. As axonal loss correlated well with the plaque load in the cerebral hemispheres in the corpus callosum, it was expected that a similar relationship between plaque load and axonal loss would be seen in the long tracts in multiple sclerosis. However, the results of the present study were very different in that they highlighted the limited role plaque load appears to play in axonal loss in the corticospinal and sensory tracts. The observation that axonal counts did not differ significantly between chronic plaques and NAWM has been reported elsewhere (Lovas et al., 2000), and further underlines the poor relationship between plaque pathology and axonal loss. Even though good correlations between plaque load and axonal loss do not signify causality, it remains plausible that plaque pathology may affect axonal viability to varying extents in different nerve fibre tracts perhaps reflecting differential genetic influences at different sites. The unifying feature of size-selective axonal loss in these anatomically and functionally distinct tracts lends support to the idea that a neurodegenerative process is operative.

There are some limitations in this study. All of the ascertainment biases common to autopsy studies surely will apply. Although plaque load was examined in an exhaustive fashion throughout the neuraxis, especially within the spinal cord, the assessment of plaque load may not have determined the true extent of demyelination in the multiple sclerosis cases. Cerebral, brainstem and spinal cord plaque load may be weak reciprocal surrogates. Unfortunately, imaging information was not available on the subjects studied to supplement the pathological observations. However, the number of levels investigated is substantial and widespread, extending from the cerebrum to the lumbar spinal cord for the corticospinal tracts. The comprehensive assessment of total spinal cord plaque load in a subset of cases failed to reveal any significant correlation between plaque load and axonal loss in either the corticospinal or sensory tracts. To study the contribution of cerebral plaque load to axonal loss in more detail, we comprehensively examined the plaque load of the cerebral hemispheres from a subset of cases. These cases had previously shown a strong correlation between plaque load and corpus callosum axonal loss (Evangelou et al., 2000b). However, in contrast to the corpus callosum findings, no such relationship could be demonstrated in the corticospinal tract. While it should be acknowledged that the extent of axonal loss may vary between different plaques of the same patient as well as between lesions of different patients, it could be argued that if a clear relationship between plaque load and axonal loss in the corticospinal and sensory tracts were to exist, then it should have been more readily apparent in the current study. Despite such considerations, the fact remains that a good correlation was found between cerebral plaque load and corpus callosum axon loss in the study by Evangelou et al. (2000b). As neuropathological studies can only provide a static picture of a dynamic process, it is not possible to prove definitively that axonal loss in the long tracts of the multiple sclerosis spinal cord occurs independently of demyelination affecting these tracts. The present study, however, provides several lines of evidence to suggest that axonal loss in the multiple sclerosis spinal cord may occur, in part, independently of inflammation-induced demyelination, providing support to the claim that an underlying neurodegenerative process may be operative in the disease. Aside from the difficulties with acquiring pathological material, the lack of retrospective clinical information made it difficult to relate the observed pathological findings to established clinical scales, such as the EDSS.

Nevertheless, the current study may be the largest pathological cohort of multiple sclerosis sampled for plaque load and tract-specific axonal loss in a comprehensive fashion. The sample size has allowed the documentation of a number of findings not described elsewhere. It has been observed that (i) no close relationship between plaque load and axonal loss exists; and (ii) axonal loss appears to be symmetric, size-selective and tract-specific. These findings suggest that...
the complex pathogenesis of multiple sclerosis may encompass parallel neurodegenerative and Wallerian mechanisms of axonal loss that affect different tracts, and even fibres within these tracts, to varying extents. Recognition of such pathological heterogeneity within axonal bundles may not only help explain the marked clinical variability seen in patients but also may help elucidate key aetiological factors that drive the course in multiple sclerosis.

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