Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts

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Summary
Clinical, imaging, and pathological studies in multiple sclerosis have generally emphasized the relative preservation of axons in comparison with myelin. Recent evidence, however, demonstrates that axonal loss is also significant, affects long tracts such as the corticospinal and sensory tracts and relates closely to functional disability. Accordingly, the distribution and extent of this axonal loss is the focus of the current investigation.

Post-mortem material of 55 multiple sclerosis patients and 32 matched controls was used to examine quantitatively the population of axons in the corticospinal tracts from the medulla to the lumbar spinal cord and the sensory tracts from the lumbar to the upper cervical spinal cord. Myelin- and axon-stained sections have been prepared to estimate the notional area and axon density, respectively of both tracts. Our results indicate that in the corticospinal tracts there is a significant reduction of the area and axon density at all levels investigated in multiple sclerosis cases when compared with controls. In contrast, the sensory tracts in multiple sclerosis cases showed a significant reduction in area and axon density only in the upper regions of the spinal cord. As has been found with MRI plaque load and T2 burden, correlations of axonal loss with duration of disease were not strong. Of the fibres lost in multiple sclerosis, we have found that small fibres (<3 μm²) seem to be particularly affected, with large fibres remaining relatively preserved in both the corticospinal and sensory tracts. In multiple sclerosis, axonal loss is widespread, and its extent is tract specific and size selective.

Keywords: axonal loss, multiple sclerosis, neuropathology

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Introduction
Clinical, imaging, and pathological studies in multiple sclerosis have supported the general view that this disorder is primarily demyelinating in nature (Lumsden, 1971; McAlpine et al., 1972; Poser, 1986; Filippi et al., 1995). It has been widely believed that episodic inflammation results in the formation of plaques throughout the CNS and the cumulative damage in these plaques overcomes both plasticity and capacity for repair to produce unremitting injury. Emphasis on the relative preservation of axons dates to the time of Charcot (1868). Clinical descriptions have often failed to point out the fact that the predilection for myelin is only relative or have left unmentioned the development of axonal loss even though this has been a consistent theme in most careful pathological descriptions (Charcot, 1868; Dawson, 1916; Ganter et al., 1999; Bjartmar et al., 2000; Evangelou et al., 2000a, b; Lovas et al., 2000; Lassmann, 2003). More recently, the recognition that axonal loss occurs in acute plaques, combined with neuroimaging and magnetic resonance spectroscopy studies in life, have aided in the rediscovery of the concept that axonal damage occurs in multiple sclerosis and that this may relate to an extent to functional disability (Davie et al., 1995; Filippi et al., 1995; Losseff et al., 1996; De Stefano et al., 1998; Wujek et al., 2002). Pathological studies validate such axonal damage and build on the significance of axonal pathology in multiple sclerosis by revealing that substantial loss of axons occurs in major white matter tracts such as the corpus callosum, the corticospinal tracts and sensory tracts (Ferguson et al., 1997; Ganter et al., 1999; Lovas et al., 2000). Cumulative loss of axons, therefore, is an important element of multiple sclerosis pathology and seems likely to play a significant role in the progressive neurological disability observed in patients afflicted with the disease.

Clues to the understanding of the cause of axonal pathology may come from a systematic assessment of the...
distribution and extent of axonal loss throughout the length of the neuraxis. To date, previous studies investigating axonal loss in multiple sclerosis have focused on particular sites such as the corpus callosum, optic nerves and tracts, or the cervical and upper thoracic regions of the spinal cord (Ganter et al., 1999; Evangelou et al., 2000a, b; Lovas et al., 2000). However, the relative roles of axonal loss and demyelination have not been clarified in multiple sclerosis, nor have their possible interrelationships been elucidated.

From a clinical perspective, it might be expected that the roles of axonal loss would be most relevantly examined in the corticospinal tracts. These are usually 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. Furthermore, they are discrete at several levels of the neuraxis, allowing serial anatomical measurement in a centripetal fashion. The extraordinary neurobiology of the long corticospinal axons, able to maintain cell processes a metre or more from their cell body, makes them a potential bellwether for degenerative processes which impair the health of the cell body or more proximal parts of the axon itself. We have shown previously that there is size-dependent selective loss of axons in the corticospinal tract in multiple sclerosis, a pattern implying differential sensitivity of the axons within this tract. Accordingly, it was anticipated that axonal loss might not be easily attributable to the anatomical location of plaques alone (Ganter et al., 1999).

The present study may be the largest pathological cohort of multiple sclerosis cases studied to date using a quantitative technique. We have examined the population of axons in the corticospinal tracts at six levels from medulla to lumbar spinal cord and the sensory tracts in the spinal cord from
lumbar to upper cervical levels. We report our findings on the distribution and extent of axonal loss throughout the length of the neuraxis in multiple sclerosis cases compared with controls. Our aim was to see if such a systematic approach could provide better insight into the nature of the axonal damage and loss in multiple sclerosis.

Materials and methods
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). Most multiple sclerosis cases had developed spastic weakness of the lower limbs by the time of death. As control material, we had 32 cases (14 males and 18 females) with an age range of 31–81 years (mean 57.9 years) without evidence of spinal cord disease. 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 next-of-kin for use of tissue for research. The study was approved by the Oxfordshire research ethics committee. Some of these cases have been the subject of an earlier report on upper levels of the spinal cord, and the same cohort has been used in a recent study of cord atrophy in multiple sclerosis (Ganter, 1999).

For each of the multiple sclerosis and control cases, formalin-fixed paraffin-embedded transverse sections were taken at several levels of the brainstem and spinal cord for microscopy. Specifically, adjacent myelin- and axon-stained sections of the spinal cord at five levels (lumbar, low and high thoracic, low and high cervical) and similar sections of the brainstem at mid-medulla were prepared. The aim of the spinal cord sections was to determine (i) the area of the lateral and posterior white matter columns in which run the crossed corticospinal tracts and major sensory tracts, respectively; and (ii) the axonal density in the same regions. Axonal density measurements in the multiple sclerosis cases were made at spinal cord levels at which no plaques occurred in adjacent myelin-stained sections. Thus, the axonal density measurements obtained represented those of normal-appearing white matter.

Brainstem myelin-stained sections of the medullary pyramids were used to obtain area measures of the corticospinal tract, while axon-stained brainstem sections from levels that lacked plaques were used to obtain axonal density measurements of the medullary pyramids.

All area and axonal density measurements were made using a semi-automated computerized image analysis system running NIH Image software. Cases were coded so that these measurements could be made with the observer blind to clinical disease category.

Area measurements
Sections, 15 μm thick, were stained for myelin with luxol fast blue/cresyl violet and were examined at low power (12.5×) (Olympus SZH10) and digitized (Sony DKC5000 photocamera). The areas containing the lateral corticospinal and sensory tracts on each side of the spinal cord were outlined by hand. To standardize the area assessed in the lateral column, a horizontal line extended laterally from the most posterior part of the grey matter commissure out to the lateral border of the cord was used to define the anterior border, with the circumferential edge of the spinal cord and the dorsal horn representing the lateral and medial borders, respectively (Fig. 1). To measure the area of the posterior column, a line drawn from the most posterior part of the grey matter commissure was used to define the anterior border; the lateral border was demarcated by the dorsal horn, the posterior border by the circumferential edge of the spinal cord and the medial border by the central median fissure (Fig. 1). These measurements were repeated three times on each side of each level and averaged; the results were found to be robust and reproducible (coefficient of error <5%).

A shrinkage factor of 0.71 was applied to the measured areas to correct for changes in tissue block size observed before and after the fixation and embedding process.

Fibre counts
Sections, 10 μm thick, were stained with Palmgren silver to demonstrate axons. The axons in the corticospinal 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. The selection of the fields in each tract was systematic and is outlined in Fig. 1.

Validation of Palmgren stain to demonstrate axons
To determine whether to use the Palmgren silver stain or a neurofilament immunostain for staining of axons, we compared the two stains on 10 cases which showed variable quality of Palmgren staining from six controls and four multiple sclerosis cases (Fig. 2). Each spinal cord level to be included in the study was represented. One image from the centre of the lateral corticospinal tract from each side of the spinal cords was acquired via the Sony-DKC5000 digital photocamera for sections stained with Palmgren and with anti-neurofilament antibody. Manual counts of the axons stained by Palmgren and anti-neurofilament antibody were made three times for each image by two independent observers. With no significant difference in axonal counts between observers, we found that there was no significant difference in axonal counts between sections stained by Palmgren and anti-neurofilament antibody. As the Palmgren method more consistently demonstrated axons throughout the length of the cord (especially at lumbar regions) compared with the anti-neurofilament antibody, we decided to use the Palmgren silver stain for the study.

Validation of semi-automated axon fibre counting method
All axon density measurements were made using an automated computerized image analysis system running NIH Image software. 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 (Fig. 3).

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.
Estimation of total axon numbers

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.

Statistics

Statistical methods used to analyse the data were parametric where data are normally distributed and non-parametric otherwise, to compare findings in multiple sclerosis and controls. Multiple regression analyses were undertaken of factors determining axonal density and estimates of total axons and small and large axons, based on area as well as axonal density. Factors considered in these analyses include age, sex, disease category and length of disease history at each level sampled.

Results

Changes in axonal density in multiple sclerosis

Axonal density, expressed as axons/mm², was determined at all levels of the neuraxis investigated (Fig. 5).

Corticospinal tract

In the corticospinal tract, it was found that there was a significant reduction in axonal density throughout all levels of the neuraxis in multiple sclerosis cases when compared with controls. The results are summarized in Table 1 and Fig. 5A.

In the crossed corticospinal tracts in multiple sclerosis, fibres of smaller diameter (≤3 μm²) were lost preferentially in contrast to the larger diameter fibres (>3 μm²) which were relatively preserved (Table 3, Fig. 4). In control and multiple sclerosis cases, there were no significant correlations of reduction in axonal density with age and sex at any of the levels surveyed. The length of disease history in multiple sclerosis cases did not correlate with the reduction in axonal density at any of the levels of the neuraxis investigated (medulla, r = −0.032, P = 0.855; upper cervical, r = −0.013, P = 0.956; lower cervical, r = −0.192, P = 0.418; upper thoracic, r = −0.131, P = 0.499; lower thoracic, r = −0.030, P = 0.838; lumbar, r = −0.202, P = 0.275).

Sensory tract

In the posterior white matter columns, there was a reduction in axonal density in multiple sclerosis cases compared with controls throughout the length of the spinal cord (Fig. 5B). However, it was only at the upper cervical level that the reduction in axonal density reached statistical significance. A summary of the extent to which the axonal density was reduced throughout the spinal cord is found in Table 2. At the upper cervical level, there was a statistically significant
reduction in axonal density in the smaller diameter fibres, but the larger diameter fibres showed no significant reduction. The axonal densities in the posterior sensory tracts in control and multiple sclerosis cases are presented for small and large fibres separately in Table 4.

Due to the distinct anatomy of the fasciculi at the level of the upper and lower cervical cord, the axonal densities of the gracilis and cuneatus fasciculi of the sensory tracts were analysed separately in these regions. At the upper cervical region, both the gracilis and cuneatus fasciculi demonstrated a statistically significant reduction in axonal density in multiple sclerosis cases versus controls [18% reduction ($P = 0.023$) and 17% reduction ($P = 0.017$), respectively]. In the lower cervical spinal cord, the reduction of axonal density in the gracilis fasciculus (13%, $P = 0.098$) was greater than in the cuneatus fasciculus (6%, $P = 0.321$), although the reduction of axonal densities in both fasciculi did not reach statistical significance at this level.

In controls, age and sex showed no significant correlation with axonal density at any of the levels of the neuraxis studied. In multiple sclerosis cases, age and sex did not correlate with reduced axonal density measures in the sensory tracts at any of the levels investigated except at the upper thoracic cord where age was negatively correlated with axonal density ($r = -0.399$, $P = 0.018$). Similarly, there were no significant correlations of length of disease with axonal density: upper cervical, $r = 0.198$, $P = 0.354$; lower cervical, $r = 0.105$, $P = 0.580$; upper thoracic, $r = 0.007$, $P = 0.972$; lower thoracic, $r = 0.072$, $P = 0.712$; lumbar, $r = 0.208$, $P = 0.238$.

Changes in area
The area (in mm$^2$) of the lateral and posterior white matter columns was determined for each side of the spinal cord. As there were no significant differences between the area measures obtained from the left and right sides of the spinal cord, the mean of the area measures of both sides was used to compare changes in area in multiple sclerosis cases and controls (Fig. 5).

Corticospinal white matter columns
The area of the corticospinal white matter columns was reduced at all levels of the neuraxis investigated in multiple sclerosis cases compared with controls. The results are summarized in Table 1 and Fig. 5C.

Of the levels examined, it was only in the upper regions of the spinal cord (i.e. upper cervical, lower cervical and upper thoracic levels) that the reduction in cross-sectional area of the corticospinal white column in multiple sclerosis cases in comparison with controls was statistically significant. In both multiple sclerosis cases and control subjects, age and sex parameters did not influence significantly the areas of the corticospinal white matter columns at any of the levels of the neuraxis studied. However, in multiple sclerosis cases, the length of disease history correlated significantly with reduction in area measures in the white matter columns at all of the levels except the lumbar cord (upper cervical, $r = -0.700$, $P < 0.001$; lower cervical, $r = -0.500$, $P = 0.003$; upper thoracic, $r = -0.521$, $P = 0.001$; lower thoracic, $r = -0.357$, $P = 0.038$; lumbar, $r = -0.168$, $P = 0.342$).

Posterior white matter columns
Appreciable reduction in the area of the posterior white matter columns in multiple sclerosis cases was only demonstrated in the lower cervical and upper thoracic regions of the spinal cord, with only the upper thoracic level reaching statistical significance (Table 2). The area measures in

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**Fig. 4** Palmgren-stained transverse section of corticospinal tract axons in the spinal cord in a control case and in a case of multiple sclerosis ($\times 400$). There is a selective reduction in the density of small fibres compared with large fibres, which are relatively preserved.
controls compared with multiple sclerosis cases are displayed in Fig. 5D.

In controls and multiple sclerosis cases, age and sex showed no relationship with area of the posterior white matter columns at all spinal cord levels, except at the lower thoracic level where increased age correlated negatively with area of the sensory columns ($r = -0.366$, $P = 0.031$). In multiple sclerosis cases, however, there did exist strong correlations between length of disease history and reduction in posterior column area at the rostral end of the spinal cord: upper cervical, $r = -0.561$, $P = 0.019$; lower cervical, $r = -0.419$, $P = 0.033$; upper thoracic, $r = -0.347$, $P = 0.061$; lower thoracic, $r = -0.279$, $P = 0.143$; lumbar, $r = -0.184$, $P = 0.290$.

**Correlations between axonal density and area measures**

In the corticospinal tracts of multiple sclerosis cases, there was no relationship between the reduction in axonal density and the reduction in notional cross-sectional area of the corticospinal tracts throughout the length of the neuraxis.

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**Fig. 5** Bar graphs of axonal density, cross-sectional area and total axon number of (A, C and E) the corticospinal tract and (B, D and F) the posterior sensory tract in controls and multiple sclerosis cases.
(medulla, \( r = 0.027, P = 0.879 \); upper cervical, \( r = 0.193, P = 0.334 \); lower cervical, \( r = -0.280, P = 0.166 \); upper thoracic, \( r = -0.157, P = 0.347 \); lower thoracic, \( r = -0.099, P = 0.537 \); lumbar, \( r = 0.022, P = 0.896 \)). However, in the sensory tracts in multiple sclerosis, there was a significant correlation between axonal density and cross-sectional area of the posterior white matter columns at all levels studied (upper cervical, \( r = -0.544, P = 0.05 \); lower cervical, \( r = -0.619, P < 0.001 \); upper thoracic, \( r = -0.565, P = 0.001 \); lower thoracic, \( r = -0.663, P < 0.001 \); lumbar, \( r = -0.417, P = 0.004 \)).

### Table 1 Corticospinal tract: reduction in density, area and axon number in multiple sclerosis compared with controls

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>% Reduction</th>
<th>Density</th>
<th>Area</th>
<th>Total axon number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulla</td>
<td></td>
<td>15*</td>
<td>7</td>
<td>19*</td>
</tr>
<tr>
<td>UpC</td>
<td></td>
<td>33**</td>
<td>17**</td>
<td>41**</td>
</tr>
<tr>
<td>LowC</td>
<td></td>
<td>33**</td>
<td>22**</td>
<td>44**</td>
</tr>
<tr>
<td>UpT</td>
<td></td>
<td>27**</td>
<td>20**</td>
<td>40**</td>
</tr>
<tr>
<td>LowT</td>
<td></td>
<td>32**</td>
<td>10</td>
<td>33**</td>
</tr>
<tr>
<td>Lumbar</td>
<td></td>
<td>30**</td>
<td>11</td>
<td>26*</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.

UpC = upper cervical; LowC = lower cervical; UpT = upper thoracic; LowT = lower thoracic.

### Table 2 Sensory tract: reduction in density, area and axon number in multiple sclerosis compared with controls

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>% Reduction</th>
<th>Density</th>
<th>Area</th>
<th>Total axon number</th>
</tr>
</thead>
<tbody>
<tr>
<td>UpC</td>
<td></td>
<td>17*</td>
<td>N/A</td>
<td>24**</td>
</tr>
<tr>
<td>LowC</td>
<td></td>
<td>10</td>
<td>9</td>
<td>19**</td>
</tr>
<tr>
<td>UpT</td>
<td></td>
<td>9</td>
<td>8*</td>
<td>12*</td>
</tr>
<tr>
<td>LowT</td>
<td></td>
<td>14</td>
<td>N/A</td>
<td>5</td>
</tr>
<tr>
<td>Lumbar</td>
<td></td>
<td>14</td>
<td>N/A</td>
<td>10</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.

N/A = not applicable (as at those levels there was no reduction observed); UpC = upper cervical; LowC = lower cervical; UpT = upper thoracic; LowT = lower thoracic.

### Changes in total axon number

Through calculating the product of the axonal density (axons/mm\(^2\)) and area (mm\(^2\)), it was possible to estimate the total number of axons in normal-appearing white matter of both the corticospinal and posterior sensory tracts for each level of the neuraxis investigated.

### Corticospinal tract

The estimated total number of axons in the corticospinal tract was markedly reduced in multiple sclerosis cases in comparison with controls at all levels examined (Table 1 and Fig. 5E).

In multiple sclerosis cases, length of disease history did not correlate with reduction in total axon numbers at any of the spinal cord levels (medulla, \( r = -0.178, P = 0.385 \); upper cervical, \( r = -0.255, P = 0.293 \); lower cervical, \( r = -0.021, P = 0.934 \); upper thoracic, \( r = -0.227, P = 0.255 \); lower thoracic, \( r = -0.191, P = 0.304 \); lumbar, \( r = 0.244, P = 0.202 \)).

### Sensory tract

The total number of fibres in the sensory tract was reduced at all levels of the cord, however, only to a significant extent in the upper region of the cord (Table 2 and Fig. 5F).

No significant relationships were found between length of disease history and reduction in total number of axons throughout the length of the spinal cord (upper cervical, \( r = -0.081, P = 0.719 \); lower cervical, \( r = -0.255, P = 0.240 \); upper thoracic, \( r = -0.402, P = 0.051 \); lower thoracic, \( r = -0.209, P = 0.307 \); lumbar, \( r = 0.013, P = 0.943 \)).

### Discussion

Even though pathological axonal changes in multiple sclerosis have been documented for more than a century, the substantial extent of axonal damage and its contribution to functional disability have been investigated only recently (Davie et al., 1995; Losseff et al., 1996; Kornek et al., 2000; Wujek et al., 2002). Despite correlations of surrogates of axonal loss with progressive neurological disability in recent imaging studies of multiple sclerosis, descriptions of the

### Table 3 Corticospinal tract loss (small versus large fibre density per mm\(^2\) ± SE) in multiple sclerosis compared with controls

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>Small fibres</th>
<th></th>
<th>Large fibres</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Multiple sclerosis</td>
<td>Control</td>
<td>Multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>16 652 ± 1168</td>
<td>13 675 ± 2579*</td>
<td>2473 ± 230</td>
<td>2579 ± 167</td>
<td>19 125 ± 1144</td>
</tr>
<tr>
<td>UpC</td>
<td>17 380 ± 1099</td>
<td>11 073 ± 907**</td>
<td>1801 ± 289</td>
<td>1801 ± 200</td>
<td>19 181 ± 1209</td>
</tr>
<tr>
<td>LowC</td>
<td>16 341 ± 1071</td>
<td>10 640 ± 687**</td>
<td>2161 ± 207</td>
<td>2018 ± 202</td>
<td>18 502 ± 1057</td>
</tr>
<tr>
<td>UpT</td>
<td>15 392 ± 1136</td>
<td>10 783 ± 563**</td>
<td>2142 ± 381</td>
<td>2091 ± 190</td>
<td>17 654 ± 1074</td>
</tr>
<tr>
<td>LowT</td>
<td>14 048 ± 1402</td>
<td>9 559 ± 563**</td>
<td>2619 ± 381</td>
<td>1742 ± 190</td>
<td>16 667 ± 1303</td>
</tr>
<tr>
<td>Lumbar</td>
<td>15 503 ± 1333</td>
<td>10 496 ± 741**</td>
<td>1750 ± 486</td>
<td>1545 ± 195</td>
<td>17 253 ± 1473</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01. UpC = upper cervical; LowC = lower cervical; UpT = upper thoracic; LowT = lower thoracic.
distribution and extent of axonal changes that occur in normal-appearing white matter in functionally important white matter tracts, such as the corticospinal and sensory tracts, at autopsy have been incomplete. Accordingly, the purpose of the present study was to reveal the distribution and extent of axonal loss in the corticospinal and sensory tracts throughout the length of the lower brainstem and spinal cord in human post-mortem tissue.

In concordance with previous reports, we have found marked, symmetrical reductions in both the nerve fibre density and total number of fibres in the corticospinal tracts in multiple sclerosis cases compared with controls at all levels investigated (Ganter et al., 1999; Lovas et al., 2000). The notional cross-sectional area of the lateral white matter, in which these crossed corticospinal tract fibres lie, was also reduced in multiple sclerosis cases, but only in the upper regions of the cord and not to the same degree as the nerve fibre loss. This finding suggests that MRI studies correlating measures of atrophy to clinical outcome may appreciably underestimate the true extent of axonal loss. The unexpected lack of a substantive correlation between the decrease in the white matter area and axonal density in the corticospinal tract in the multiple sclerosis cohort authenticates the notion that the extent to which cord atrophy accompanies nerve fibre loss or atrophy is variable. Reported changes to the extracellular environment in the vicinity of axonal pathology, such as influx of inflammatory infiltrates, astrocytes and oedema, may explain, in part, the absence of a significant relationship between atrophy of the cord and reduction in nerve fibre density (Shi and Blight, 1996; Trapp et al., 1998; Kornek et al., 2000).

The contrasting fates of axons in the posterior columns and corticospinal tracts described here are interesting. Unlike the corticospinal tracts, the dorsal sensory tracts showed a significant reduction in axonal density, total axon number and cross-sectional area only in the upper regions of the spinal cord. Thus, they appeared generally to survive better than the corticospinal tracts. However, as in the corticospinal tracts, the percentage decrease in area of the posterior columns was considerably smaller than that observed for both axonal density and total axon number in multiple sclerosis cases when compared with controls. Once again, this illustrates that measures of atrophy at both sites poorly reflect the magnitude of axonal loss. Contrary to the poor relationship between area of the lateral white matter column and nerve fibre density of the corticospinal tract, there did exist a clear correlation between posterior column white matter area and nerve fibre density in multiple sclerosis cases. The reduction of nerve fibre density in the sensory tract was not reflected in the degree of posterior column white matter atrophy evidenced by the inverse relationship between reduction of nerve fibre density and reduction in area in this part of the spinal cord. These differences in the correlations between atrophy and axonal density in the corticospinal and sensory tracts suggest that there may be different factors that determine the extent of axonal loss in a given tract and/or the extent to which extracellular changes occur as a result of such axonal loss in different tracts. It also needs to be acknowledged that the posterior columns exclusively carry sensory fibres within them and are, therefore, more homogeneous than the lateral columns which convey some other upward- and downward-directed fibres in addition to those of the crossed corticospinal tracts.

In the evaluation of the potential sources of cord atrophy in multiple sclerosis, it is important to consider the roles of axonal loss and axonal atrophy. If axonal atrophy were to play a notable role in spinal cord atrophy, then one would expect a reduction in the density of large diameter nerve fibres. However, of the nerve fibres lost in multiple sclerosis, we observed selective loss of the small diameter fibres (<3 μm²), with the large nerve fibres (>3 μm²) being relatively preserved, which is in accordance with other studies examining axonal loss in the cord. The size-selective reduction of nerve fibre density in multiple sclerosis raises several interesting questions. (i) What is the cortical origin of the small and large fibres? (ii) What are the specific roles of small and large fibres in the corticospinal and sensory tracts? (iii) Why are small fibres particularly vulnerable to degeneration compared with large fibres? The answers to these questions are essential to advance the understanding of the pathogenic mechanism(s) of size-selective axonal loss in multiple sclerosis and its associated functional consequences.

The reported distribution and extent of axonal loss in the corticospinal tracts and posterior columns result in unique functional consequences. In the most rostral aspect of the cord, the descending corticospinal tract axons in a given cross-sectional plane will terminate at a multitude of cord levels. As a result, the observed mean 41% reduction in total axon number in the upper cervical region of the cord could potentially impact the voluntary movements of muscles in all limbs. In contrast, the reported mean 26% axonal loss in the most caudal regions of the cord where corticospinal tract

### Table 4

<table>
<thead>
<tr>
<th>Level of neuraxis</th>
<th>Small fibres</th>
<th>Large fibres</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Multiple sclerosis</td>
<td>Control</td>
</tr>
<tr>
<td>UpC</td>
<td>15 223 ± 865</td>
<td>13 293 ± 669*</td>
<td>2183 ± 375</td>
</tr>
</tbody>
</table>

*P < 0.05.

UpC = upper cervical; other levels showed no significant difference in reduction in axonal density between control and multiple sclerosis.
nerve fibres primarily innervate the lower limbs, though less than in the cervical region, may have a much more significant impact on lower limb motor function. The loss of corticospinal tract axons in the lumbar region, therefore, may help to explain why spasticity and paralysis of the lower limbs are unifying features of disease progression. For the sensory nerve fibres, on the other hand, we have found that the reduction in axonal density was most pronounced at the level of the cervical cord. This finding is consistent with the clinical observation that multiple sclerosis patients most commonly experience sensory disturbances in their upper extremities. Unfortunately, the lack of retrospective clinical information made it difficult to relate the observed pathological findings in our study to established clinical scales, such as the Extended Disability Status Scale.

As axonal pathology may play a role in determining functional disability in multiple sclerosis, it is important to evaluate the time course of axonal loss. Previous studies have shown that axonal injury is an early feature of the disease (Ferguson et al., 1997; Trapp et al., 1998). In both the corticospinal and sensory tracts, neither the reduction of axonal density nor the reduction of total axon numbers correlated with length of disease history at any of the levels examined, validating the idea that axonal loss begins at an early stage of the disease, at least in some patients. In this respect, it is similar to the weak correlations of MRI plaque load and T2 burden with duration of disease. With regard to area measures, however, the extent of tissue atrophy correlated significantly with length of disease history in both the corticospinal and sensory tracts throughout the length of the neuraxis, perhaps reflecting loss of oedema and inflammation and shrinkage of fibrous astrocytes in chronic plaques (Ganter et al., 1999). This result has implications for the interpretation of MRI measures of spinal cord atrophy as an indicator of clinical outcome because it suggests that changes in spinal cord area are only realized some time after axonal loss has begun.

Autopsy material is necessarily going to be biased towards including patients with increased disability and longer duration of disease than might be ideal in understanding the dynamics of axonal loss. We have examined this question as closely as possible by comparing axonal loss with duration of disease, and have found that even patients with short duration of disease have extensive axonal loss. We acknowledge, nevertheless, that even patients with short disease duration coming to autopsy will have been selected for more aggressive disease.

The regional distribution of axonal loss in the corticospinal and sensory tracts may provide valuable insight into the mechanism by which axons are lost in multiple sclerosis. It is interesting that the most severe reduction in axons of both tracts occurred in the cervical region despite the fact that the tracts run in different directions and terminate at different points. Given that the cervical region is most susceptible to plaque formation in the spinal cord, it is feasible that plaques may play an important role in the promotion of axonal loss in this region of the cord. The medullary pyramids, containing corticospinal tract fibres rostral to the cervical cord, have a notably smaller reduction in axonal density and total axonal loss compared with more distal regions of the neuraxis containing corticospinal tract fibres. Based on our observation that axonal loss is more significant distally in both tracts at the cervical level, it is possible that Wallerian degeneration secondary to axonal damage in plaques may be operative in the corticospinal and sensory tracts (Bjartmar et al., 2001). However, it is also possible that tract-specific mechanisms other than plaque-induced Wallerian degeneration may influence axon viability (Simon et al., 2000). Several myelin-related proteins such as myelin-associated glycoprotein, peripheral myelin protein 22 and proteolipid protein-2 have been shown to play a role in long-term axonal survival (Griffiths et al., 1998; Yin et al., 1998; Bjartmar et al., 1999; Scherer et al., 1999). Interestingly, it has been demonstrated that proteolipid protein-2-deficient mice undergo length-dependent axonal degeneration, with the longest axons (i.e. axons in the gracile column of the sensory tract, and axons in the lumbar cord of the corticospinal tract) being most severely affected (Garbern et al., 2002). It is most likely that the axonal loss observed in the corticospinal and sensory tracts is due to several concerted pathological mechanisms that operate to varying degrees during the course of the disease. Future studies investigating the relationship between plaque load and axonal loss along the neuraxis of these tracts will be critical to advance our knowledge of the mechanisms by which axons are lost in multiple sclerosis.

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