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Changes of unmyelinated nerve fibers in sural nerve in amyotrophic lateral sclerosis, Parkinson's disease and multiple system atrophy

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Abstract Quantitative changes in unmyelinated nerve fibers (UMNFs) of sural nerves in patients of amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and multiple system atrophy (MSA) were evaluated using autopsy materials whose pathological diagnosis had been confirmed by careful postmortem examinations. Ordinary ALS cases demonstrated no involvement in cutaneous UMNFs; however, the patients with long survival due to the application of ventilatory support showed bimodality in UMNF diameter histograms, and a patient with involvement of systems other than motor pathways showed an abnormal value in two indices: a low percentage of subunits containing axon(s) and a high mean number of Schwann cell profiles per axon. A significant reduction of the mean value of UMNF density (21%) was found in PD patients. Because the density of myelinated nerve fibers did not show any significant decrease as compared with age-matched controls, the change of nerve fibers in peripheral nervous system was considered to be confined to UMNFs in PD. Elderly PD cases showed enhanced changes in the ageing process, as expressed by the two indices described above. In MSA, the mean value of UMNF density was significantly decreased (23%), and this decrease almost paralleled that of myelinated nerve fiber density. Abnormal values for the two indices described above were found and two out of four cases demonstrated bimodality in the diameter histogram of UMNFs. Unlike MSA, ALS and PD have not been included in the disorders with cutaneous UMNF involvement. Our results supply the first evidence of morphological changes in cutaneous UMNFs in PD cases. In ordinary ALS cases, the emergence of such morphological changes is suggested in cases with long survival.

Key words Amyotrophic lateral sclerosis · Morphometry · Multiple system atrophy · Parkinson's disease · Unmyelinated nerve fibres

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Introduction

Because of the postmortem artifacts and the need for electron microscopic examinations due to their small size, unmyelinated nerve fibers (UMNFs) have been virtually neglected as the object of neuropathological examination at necropsy. However, in cutaneous nerves, their density is about three to five times greater than that of myelinated nerve fibers (MNFs) and they are considered to contain not only sensory afferent fibers, subserving such modalities as pain and temperature, but also postganglionic sympathetic efferents; thus, detailed examination of cutaneous UMNFs in various neurological disorders can provide further information concerning their pathogenesis and extent of involvement.

The aim of the present article is to evaluate the changes in cutaneous UMNFs and their Schwann cells in three degenerative disorders of the central nervous system (CNS): amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and multiple system atrophy (MSA) [13]. Although the former two are not generally considered as the disorders with peripheral neuropathy or with an involvement of postganglionic sympathetic systems, some articles described the involvement of somatic afferents [5, 20] and the postganglionic autonomic nervous system [23] in these disorders; however, no precise morphological evaluation of cutaneous UMNFs has yet been provided. From the clinical point of view, recent developments in the management of these disorders, including the ventilatory support for ALS, enable some patients to live longer, although possibly accompanied by degeneration of the systems which have been believed to be "not essential" [15].

In a previous study [19], we investigated age-dependent changes in UMNFs and their Schwann cells in normal adults, and established values for nine control parameters. In this study, we compared these standard values with the values obtained for the three-degenerative disorders. The merits of using autopsy materials are also discussed.

Materials and methods

Sural nerves were obtained from 11 cases with ALS (45–84 years of age; mean ± SD, 67.4 ± 10.9 years), 9 cases with PD (60–82 years; mean 74.0 ± 6.8 years), and 4 cases with MSA (54–73 years; mean 61.5 ± 8.6 years); all samples were taken within 15 h of death (see Table 1). In all cases, the final diagnosis was established by precise postmortem examination of the CNS in addition to the records of neurological examinations and careful follow-up observations by at least three neurologists. Patients were well-nourished until death and care was taken to avoid the compression of peripheral nerve trunks of the four extremities. None of the patients had presented obvious peripheral neuropathy either clinically or electrophysiologically. Patients with diabetes mellitus, chronic renal failure, systemic vasculitis or M-proteinemia were not included in this study. Control values (normal) were obtained from 28 normal subjects (20 males and 8 females, aged 25–89 years; mean 62.1 ± 16.7 years); the details have been described previously [19]. However, since the patients with PD were significantly older than these 28 controls ($P < 0.01$), we took values for 17 of the control subjects who were over 60 of age (14 males and 3 females, aged 62–89 years; mean 73.4 ± 8.3 years) as aged controls for the comparison with PD group.

Specimens were taken just proximal to the lateral malleolous. A 3-cm length of the nerve was removed, fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer, postfixed in 1% osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and then observed under a JEOL 200CX electron microscope.

The details of the measurement procedure have also been described previously [19]. Briefly, ultrathin sections were obtained from at least three fascicles in the same plane; the total area analyzed on electron micrographs from each nerve was at least 0.03 mm^2. The final magnification used for measurement was × 10,000. Unmyelinated axons were distinguished from Schwann cells profiles by their round or oval shape with the presence of a mesaxon-like structure. The other morphological criteria for UMNFs, e.g., lighter appearance on electron micrographs and greater density of axolemma in comparison with that of Schwann cell membrane [3], were not applied in this study because they were obscured by postmortem changes. A conglomerate of Schwann cell processes with or without unmyelinated axons, enclosed by a continuous basal lamina, was designated as a "subunit" of Schwann cells [27]. The isolated projections of Schwann cells which contained a single profile and no axon on cross sections were counted separately. Collagen pockets were defined as small collections of longitudinally oriented collagen fibrils, round or oval in shape, surrounded by Schwann cell processes for at least three quarters of their outer surface. Using the criteria stated above, counts made by two observers gave identical results within 5%.

In this way, we evaluated the following nine parameters for each specimen: (1) the density and diameter histogram of unmyelinated axons, (2) the density of Schwann cell nuclei related to UMNFs, (3) the density of Schwann cell subunits with axon(s), (4) the density of Schwann cell subunits without axon, (5) the density of Schwann cell single protrusions, (6) the density of collagen pockets, (7) the percentage of subunits containing UMNFs, (8) the mean number of Schwann cell profiles per one axon, and, (9) the mean number of unmeylinated axons in one subunit [19].

Bands of Buengner and the unmyelinated axons which occurred in them were not counted. Subunits and unmyelinated axons which participated in the onion-bulb formation or in the regenerating "cluster" of MNFs were also not included.

Densities of MNFs were also estimated of 0.5-μm-thick semithin sections stained with toluidine blue and photographed at a final magnification of × 2,000 as described previously [18], to clarify whether the changes of UMNFs were independent of those of MNFs or not.

Measurements of the diameter of unmyelinated axons and statistical analysis were performed using a WT-4400 digitizer (WACOM K.K.) interfaced to a Nihon Denki PC9801VM personal computer.

In PD cases, we also evaluated the frequency of Lewy bodies in sympathetic ganglia quantitatively, using hematoxylin and eosin-stained paraffin-embedded sections of cervical sympathetic ganglia. The mean number of Lewy bodies per 100 sympathetic ganglion cell bodies in ten randomly chosen fields (each of approximately 0.07 mm^2) was counted.

Results

Unmyelinated nerve fibers

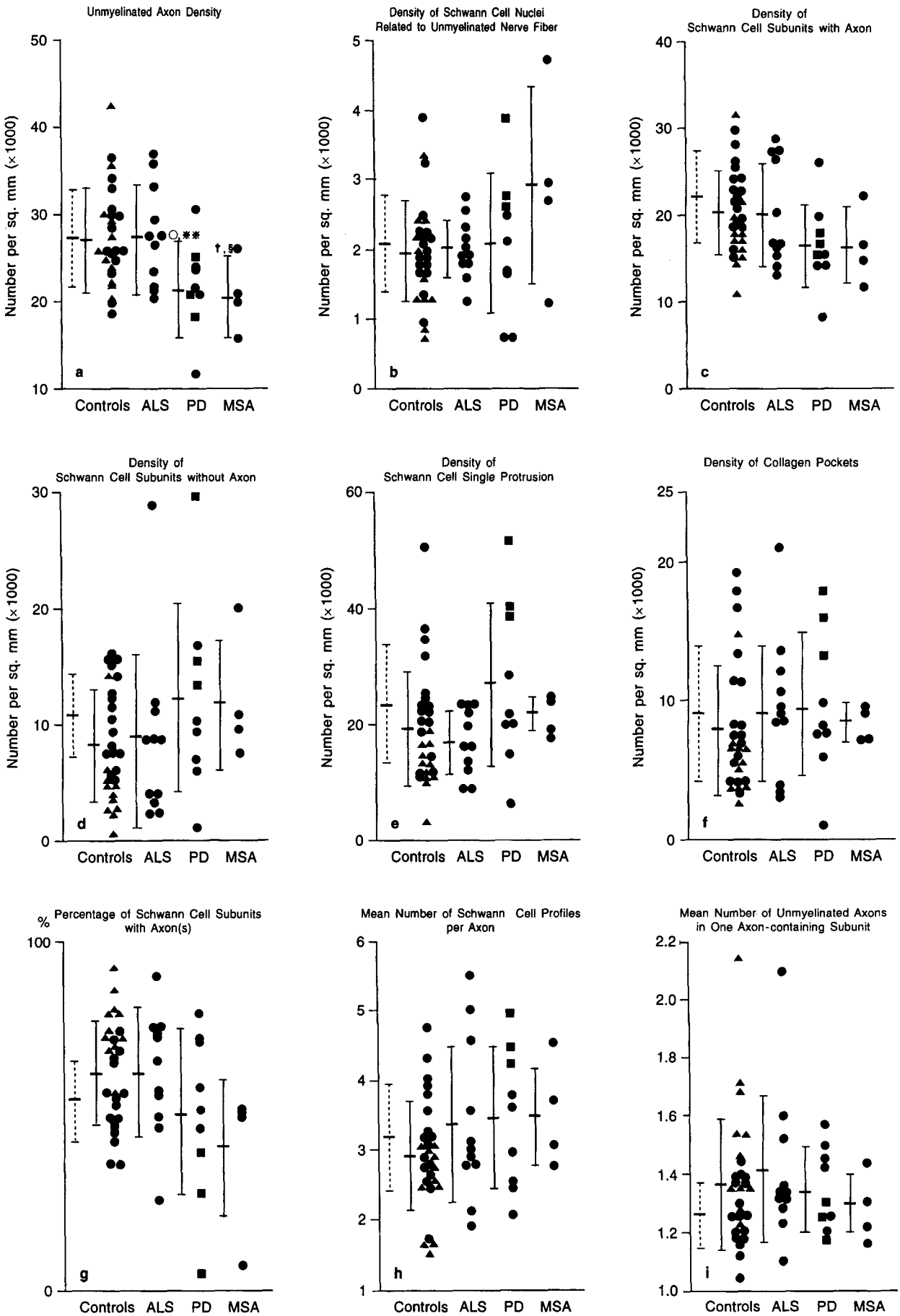
Density and diameter histogram of unmyelinated axons

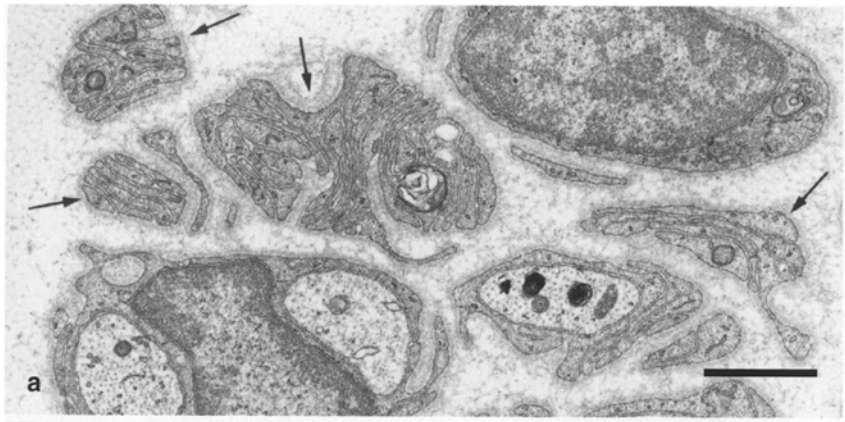
The density of unmyelinated axons in the 24 cases ranged between 20,550 and 36,100 (mean ± SD, 27,280 ± 5,620)/mm^2 in ALS, between 12,370 and 30,350 (mean 21,670 ± 4,800) in PD, and between 15,360 and 26,710 (mean 20,900 ± 4650) in MSA, as shown in Fig. 1 a. Values of normal and aged controls ranged from 19,040 to 42,520 (mean 27,260 ± 5,640) and from 19,040 to 35,970 (mean 27,500 ± 5,120), respectively. The mean value in PD was significantly smaller as compared with those in normal controls and ALS patients ($P < 0.05$) and aged controls ($P < 0.02$). The mean value in MSA patients was significantly ($P < 0.05$) smaller than ALS patients or controls.

In some of these 24 cases, the diameter histogram of unmyelinated axons showed a bimodal distribution, with the two peaks ranging from 0.2 to 0.4 μm and from 0.5 to 1.4 μm; a striking difference from that of the controls which showed unimodal distribution [19]. Bimodal patterns were observed in 2 of 11 ALS (18.2%), 2 of 9 PD (22.2%), and 2 of 4 MSA (50.0%) cases (Fig. 2).

The mean diameter of unmyelinated axons was not estimated in the present study, because this index was considered to be greatly influenced by the postmortem swelling of individual axons [19].

Fig. 1a–i Individual values of the nine parameters in controls and three disease groups. *Bars* indicate mean ± SD. In controls, values for younger (age < 60 years) subjects are marked by *solid triangles* and the mean ± SD of the aged controls (age > 60 years, $n = 17$) are expressed with *dot-dashed lines*. In Parkinson's disease (PD) values of three aged patients are marked with *solid squares*. *Open circles*, *asterisks*, *obelisks* and *section marks* indicate level of statistical significance of difference between all controls and PD patients, between aged controls and PD patients, between all controls and multiple system atrophy (MSA) patients, and between amyotrophic lateral sclerosis (ALS) patients and MSA patients, respectively: ○, *, †, §: $P < 0.05$; **: $P < 0.02$; ***: $P < 0.01$. Statistical analyses were performed using two-sided Wilcoxon rank-sum test. See also text for explanation ▶


Unmyelinated Axon Density
Number per sq. mm (×1000)
50
40
30
20
10
a
Controls ALS PD MSA
Density of Schwann Cell Nuclei Related to Unmyelinated Nerve Fiber
Number per sq. mm (×1000)
5
4
3
2
1
0
b
Controls ALS PD MSA
Density of Schwann Cell Subunits with Axon
Number per sq. mm (×1000)
40
30
20
10
0
c
Controls ALS PD MSA
Density of Schwann Cell Subunits without Axon
Number per sq. mm (×1000)
30
20
10
0
d
Controls ALS PD MSA
Density of Schwann Cell Single Protrusion
Number per sq. mm (×1000)
60
40
20
0
e
Controls ALS PD MSA
Density of Collagen Pockets
Number per sq. mm (×1000)
25
20
10
0
f
Controls ALS PD MSA
% Percentage of Schwann Cell Subunits with Axon(s)
100
0
g
Controls ALS PD MSA
Mean Number of Schwann Cell Profiles per Axon
6
5
4
3
2
1
h
Controls ALS PD MSA
Mean Number of Unmyelinated Axons in One Axon-containing Subunit
2.2
2.0
1.8
1.6
1.4
1.2
1.0
i
Controls ALS PD MSA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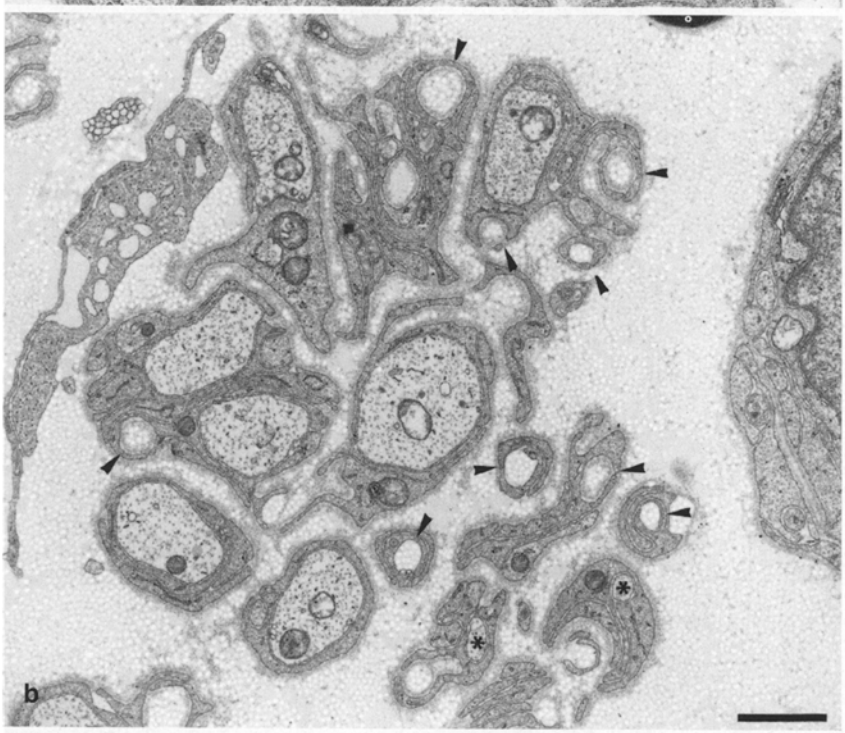
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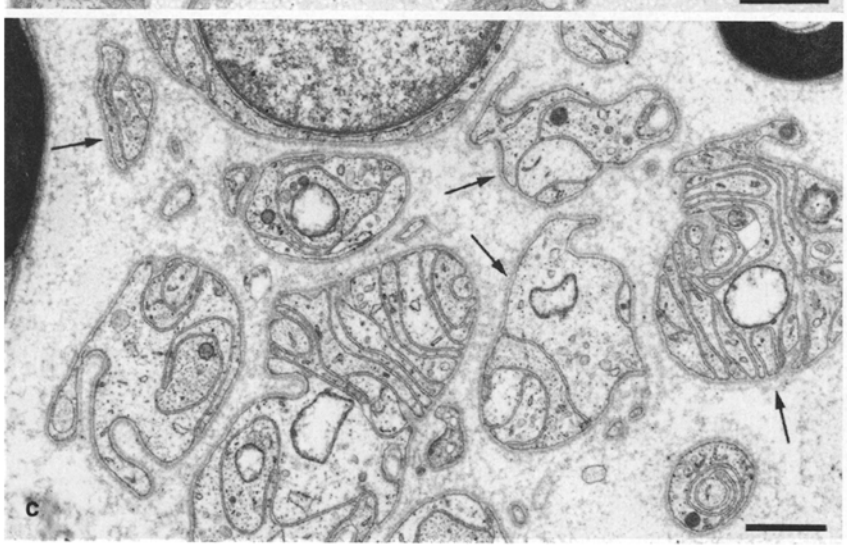
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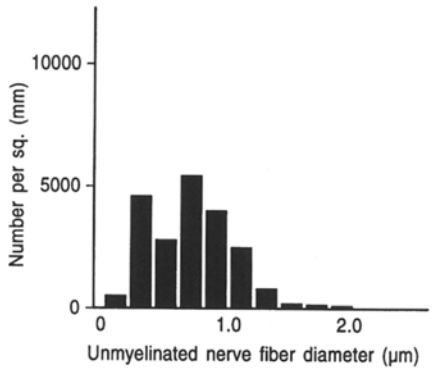
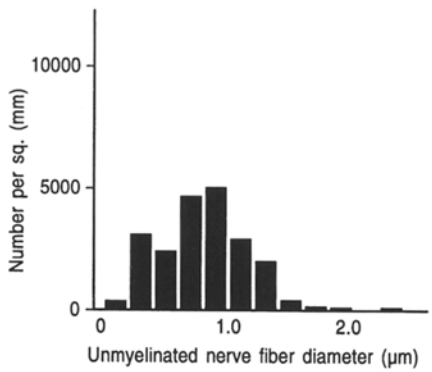
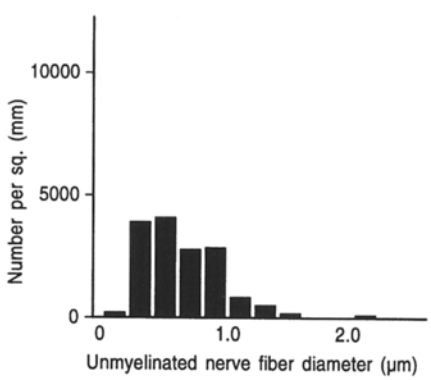

10000
5000
0
Number per sq. (mm)
0
1.0
2.0
Unmyelinated nerve fiber diameter (μm)


10000
5000
0
Number per sq. (mm)
0
1.0
2.0
Unmyelinated nerve fiber diameter (μm)


10000
5000
0
Number per sq. (mm)
0
1.0
2.0
Unmyelinated nerve fiber diameter (μm)

Density of Schwann cell nuclei related to unmyelinated nerve fibers

This ranged between 1,240 and 2,710 (mean 2,030 ± 410) in ALS, between 770 and 3,890 (mean 2,090 ± 990) in PD, and between 1,270 and 4,710 (mean 2,910 ± 1,410) in MSA (Fig. 1 b). Normal and aged control values ranged from 700 to 3,900 (mean 1,960 ± 720) and from 940 to 3,900 (mean 2,090 ± 670), respectively. Although the mean value in MSA was slightly larger than that of the others, no significant differences could be found among these four groups.

Density of Schwann cell subunits with axon(s)

This parameter ranged between 12,910 and 28,400 (mean 20,040 ± 5,820) in ALS, between 8,030 and 25,190 (mean 16,520 ± 4,560) in PD, and between 11,680 and 22,560 (mean 16,450 ± 4,570) in MSA (Fig. 1 c). Normal control values ranged from 11,670 to 31,640 (mean 20,410 ± 4,790) and aged controls from 15,120 to 29,730 (mean 22,490 ± 4,170). The mean value in PD patients was significantly smaller than that in normal controls ($P < 0.05$) and that in aged controls ($P < 0.01$).

Density of Schwann cell subunits without axon

This ranged between 2,260 and 28,130 (mean 8,570 ± 7,490) in ALS, between 1,930 and 29,430 (mean 12,210 ± 8,150) in PD, and between 7,640 and 19,760 (mean 11,890 ± 5,420) in MSA (Fig. 1 d). Normal control values ranged from 700 to 16,280 (mean 8,090 ± 4,810) and aged controls from 5,020 to 16,280 (mean 10,520 ± 3,930). The mean values in PD and MSA were larger than those in ALS and controls, but no significant difference was found.

Density of Schwann cell single protrusions

This parameter ranged between 8,770 and 23,360 (mean 16,860 ± 5,570) in ALS, between 6,010 and 51,670 (mean 26,820 ± 14,200) in PD, and between 18,400 and 24,480 (mean 21,580 ± 3,110) in MSA (Fig. 1 e). Normal control values ranged from 2,410 to 50,180 (mean 19,120 ± 9,970) and aged controls from 10,710 to 50,180 (mean 23,580 ± 10,140). Although the mean values in the latter two were larger than those in ALS and controls, the dots were greatly scattered and no significant differences could be found.

◀ **Fig. 2 a–c** Electron photomicrographs of unmyelinated nerve fibers (UMNFs) and corresponding diameter histograms of unmyelinated axons for the three representative cases, all of which show a bimodal distribution pattern in the diameter histogram. *Bars* indicate 1 μm. **a** ALS case 9, 76-year-old male. Schwann cell subunits containing a lot of Schwann cell profiles but without axon are abundant *(arrows)* **b** PD case 8, 78-year-old male. Subunits with tiny axon are occasionally observed *(asterisks)*. Collagen pockets are abundant in this area *(arrowheads)*. **c** MSA case 3, 63-year-old female. Subunits without axon containing many Schwann cell profiles are frequently seen *(arrows)*

Density of collagen pockets

This ranged between 2,860 and 20,140 (mean 8,940 ± 4,990) in ALS, between 1,120 and 17,120 (mean 9,330 ± 5,020) in PD, and between 6,730 and 9,180 (mean 7,870 ± 1,360) in MSA (Fig. 1 f). Normal control values ranged from 2,340 to 18,970 (mean 7,610 ± 4,690) and aged controls from 3,180 to 18,970 (mean 8,960 ± 5,070). The dots were scattered and no significant differences could be found among each group.

Percentage of subunits containing unmyelinated fibers

This index ranged from 47.7% to 92.6% (mean 73.7 ± 12.5%) in ALS, from 34.8% to 85.2% (mean 65.4 ± 16.4%) in PD, and from 37.2% to 67.2% (mean 58.7 ± 14.4%) in MSA (Fig. 1 g). Normal control values ranged from 55.7% to 94.3% (mean 73.9 ± 10.3%) and aged controls from 55.7% to 82.3% (68.2 ± 8.0%). The mean values in PD and MSA were smaller than those in ALS and controls, although not significantly.

Mean number of Schwann cell profiles per one axon

The mean number of Schwann cell profiles per one axon in the axon-containing Schwann cell subunit ranged between 1.87 and 5.45 (mean 3.38 ± 1.15) in ALS, between 2.08 and 4.96 (mean 3.47 ± 0.98) in PD, and between 2.77 and 4.49 (mean 3.50 ± 0.76) in MSA (Fig. 1 h). Normal control values ranged from 1.49 to 4.70 (mean 2.91 ± 0.78) and aged control from 1.73 to 4.70 (mean 3.21 ± 0.75). Although the mean values in these three disorders were larger than that of controls, no significant difference could be noticed.

Mean number of unmyelinated axons in one subunit

This parameter ranged from 1.15 to 2.09 (mean 1.41 ± 0.26) in ALS, from 1.17 to 1.57 (mean 1.34 ± 0.14) in PD, and from 1.18 to 1.43 (mean 1.29 ± 0.11) in MSA (Fig. 1 i). Normal control values ranged from 1.04 to 2.15 (mean 1.36 ± 0.22) and from 1.04 to 1.43 (mean 1.26 ± 0.11). The mean value in MSA was slightly smaller, although not significantly.

Myelinated nerve fibers

The density of MNFs in the 24 cases ranged between 4,810 and 13,010 (mean 8,440 ± 2,110) /mm^2 in ALS, between 5,620 and 9,200 (mean 7,220 ± 1,170) in PD, and between 4,250 and 7,120 (mean 6,190 ± 1,310) in MSA

Table 1 Summary of cases investigated

Amyotrophic lateral sclerosis

Case	Age/sex	Duration of		Myelinated nerve fiber density (/mm^2)	Delay in removal of sural nerves (h)
		disease (years)	respirator (years)		
1	45 M	6.2	–	9,740	4
2	57 F	6.3	–	13,010	2
3	61 F	2.4	–	6,300	6
4	63 F	1.2	–	9,380	14
5	64 F	3.1	2.2	6,770	5
6	69 M	4.8	3.8	8,390	15
7	71 F	2.5	1.1	8,240	2
8	74 M	5.0	–	10,000	3
9	76 M	8.2	5.9	4,810	11
10	77 M	2.0	–	7,700	3
11	84 F	1.6	–	8,480	2

Parkinson's disease

Case	Age/sex	Clinically overt dysautonomia	Duration of disease (years)	Myelinated nerve fiber density (/mm^2)	Lew bodies in stellate ganglia[a]	Delay in removal of sural nerves (h)
1	60 M	–	8	6,400	3.2	4
2	69 M	–	12	7,870	13.0	3
3	72 F	–	12	7,620	N/A[b]	10
4	74 M	–	15	9,200	1.0	11
5	74 F	–	11	8,580	7.8	3
6	75 M	–	22	6,510	29.5	2
7	78 M	–	7	5,620	10.6	7
8	82 M	–	13	6,340	9.3	15
9	82 F	–	4	6,830	3.0	4

Multiple system atrophy

Case	Age/sex	Clinically overt dysautonomia	Duration of		Myelinated nerve fiber density (/mm^2)	Delay in removal of sural nerves (h)
			disease (years)	dysautonomia (years)		
1	54 M	+	7	6	7,120	13
2	56 F	++	6	4	6,770	5
3	63 F	++	5.3	4.8	4,250	2
4	73 F	+	8.3	7	6,630	4

[a] The mean number of Lewy bodies per 100 sympathetic ganglion cell bodies was counted

[b] The stellate ganglia was not sampled

(Table 1). Values of normal and aged controls ranged from 6,420 to 10,860 (mean 8,270 ± 1,370 and 8,180 ± 1,370, respectively). The mean value in MSA patients was significantly ($P < 0.05$) smaller than that in ALS patients and controls (normal and aged). The mean value in ALS patients and in PD patients did not show any significant changes as compared with controls, normal and aged, respectively. Each of the 24 cases investigated and the 28 controls showed a bimodal distribution pattern for the diameter histogram of MNFs (data not shown).

The MNF density for the four groups did not show any significant relationship with patient's age or any of the nine parameters for UMNFs described above. The decrease of MNF density in MSA cases was almost in parallel with that of unmyelinated axon density ($r = 0.8783$), although not significantly.

Frequency of Lewy bodies in the sympathetic ganglion in PD cases

The mean number of Lewy bodies per 100 stellate ganglion cells in eight PD cases was 1.0 to 29.5 (mean 9.7 ± 9.0) (Table 1). This parameter showed no significant relationship with any of the nine parameters of cutaneous UMNFs described above or patient's age; however, it showed a significant correlation with the duration of disorder ($P < 0.05$; $r = 0.711$).

Unmyelinated axons and subunits associated with Buengner's band or onion bulb formation

The approximate percentage of unmyelinated axons and subunits not included in this study was less than 3% of those counted, except for PD case 7 (6.6% and 5.5%, respectively) and ALS case 9 (0.8% and 3.8%, respectively). These two cases also showed decreased MNF density (less than 6000/mm^2), suggesting MNF origin of these structures.

Discussion

Pathological changes of the UMNFs have usually been discussed for biopsied sural nerves [12, 22, 26, 30]. The merits of using biopsy materials are that the fine structures of the axonal components and Schwann cell cytoplasm are well preserved and that the postmortem swelling of the axons and/or Schwann cells can be avoided. However, an accurate clinical (premortem) diagnosis of these CNS degenerative disorders is not always easy; for example, it is often difficult to distinguish olivopontocerebellar atrophy (OPCA) from late-onset cerebello-olivary atrophy clinically, especially in the early stages of the disorder. No clear clinical distinction can be made between the two conditions with progressive autonomic failure: PD with prominent autonomic disturbances and MSA; the final diagnosis of the individual cases can often be established only after the postmortem findings are reviewed.

In this study, we used autopsy materials to evaluate the pathological changes of UMNFs in three representative CNS degenerative disorders. As regards diagnostic accuracy, we find that the study using postmortem sural nerves is superior to that on biopsied material. Furthermore, sural nerve biopsy is an invasive procedure and may leave hypesthesia or paresthesia of the areas which the excised nerve had innervated; hence, studies using biopsy materials obtained from such disorders as ALS, PD or MSA, whose sensory systems are clinically intact in many cases, can give rise to some ethical problems. Although only a few articles concerning the pathological changes of UMNFs on autopsy materials have been published so far [17, 18], we expect that the methods we have applied here will be widely used to reveal the neglected changes of UMNFs in various neurological disorders.

One of the major problems in the clinicopathological evaluation of the morphological changes of UMNFs is the proportion of the two components (sensory and sympathetic autonomic) in human sural nerve; this is still a subject of debate. Chad et al. [6] found a normal density and diameter histogram for unmyelinated axons of biopsied sural nerve from a patient after lumbar sympathectomy, and concluded that the sympathetic nervous system contributed few axons to the total population of unmyelinated axons in the sural nerve. On the other hand, analysis of the diameter spectrum of unmyelinated axons in sural nerves obtained from patients with pandysautonomia showed a shift to left, suggesting the regeneration of unmyelinated axons [2, 32]. Their results support the presence of a substantial number of sympathetic ganglia-derived unmyelinated axons in the human sural nerve. In the rat, Chad et al. [7] showed that up to a quarter of the total population of peripheral nerve unmyelinated axons are sympathetic ganglia derived.

To clarify this point, we must await future studies (for example, those using histochemical techniques which can definitely distinguish the UMNFs originated in dorsal root ganglia from those in sympathetic ganglia) to determine the exact percentage of the two components in the unmyelinated axons of human sural nerve. However, at present it is appropriate to consider the changes of UMNFs in these cases as the summation of the sensory afferent fibers and postganglionic sympathetic fibers. We discuss the morphological changes of UMNFs in the three degenerative diseases clinicopathologically from these two aspects: the involvement of primary sensory neuron and that of postganglionic sympathetic efferent.

Amyotrophic lateral sclerosis

Although ALS has long been considered to be a disorder in which lesion is confined to the primary and secondary motor neurons, the involvement of the sensory peripheral nervous system has been proved in some articles [5, 10, 20]. On the other hand, Sobue et al. [28] concluded that there was no significant reduction of preganglionic sympathetic fibers. Kennedy and Duchen [21] demonstrated a slight reduction of intermediolateral column cells in their five ALS patients but they could not show statistically significant changes. As far as the preganglionic sympathetic system is concerned, there has been no clear evidence of morphological changes in this disorder.

Morphometric data concerning the change of cutaneous UMNFs in ALS are still scanty. Bradley et al. [5] described a reduced number of small UMNFs in the four postmortem specimens of ALS. They also showed a significantly greater number of denervated Remak cell processes (i.e., Schwann cell subunit without axon in the present study) in both ALS biopsy and postmortem specimens compared with the controls. Using biopsy materials, Tohgi et al. [31] described a decrease of small and total UMNF densities in ALS patients with sensory or autonomic disturbances. They did not mention either the attitude of denervated Schwann cell subunits or the relationship between the Schwann cell profiles and unmyelinated axons. Ben Hamida et al. [4] described the reduction of small-diameter (less than 1.0 μm) UMNFs using nine samples of biopsied superficial peroneal nerve.

In our study the distribution of the values for the nine parameters in ALS patients was almost the same as that in controls (see Fig. 1) and no significant difference was observed in any of the nine parameters. Because parameters 7 and 8 are considered to be more sensitive than conventional densities of UMNFs [19], it is reasonable to conclude that cutaneous UMNFs are not involved in the cases of ordinary ALS. The inconsistency between our findings

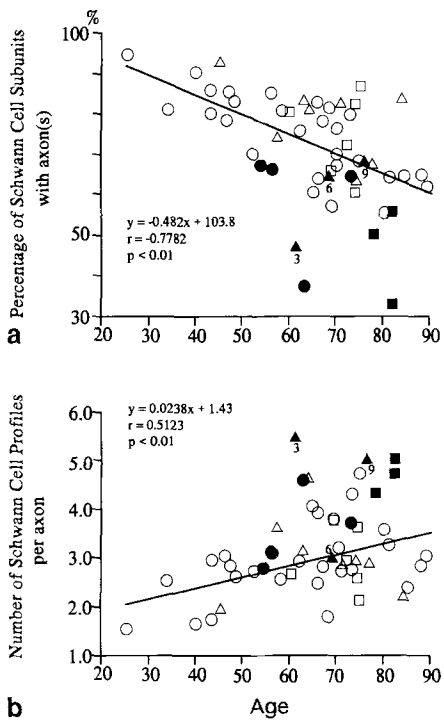


Fig. 3 The relationship between **a** percentage of Schwann cell subunits with axon(s), **b** mean number of Schwann cell profiles per one axon, and the subject's age. *Regression lines*, correlation coefficients (r) and *P* values described are for the controls. *Open circles* controls; *closed circles* MSA patients; *open triangles* ALS patients other than cases 3, 6 or 9; *closed triangles* ALS cases 3, 6 and 9. *Numerals* just *beneath the symbol* denote the number of each case; *open squares* PD cases 1–6; *closed squares* PD cases 7, 8 and 9 (elderly PD cases). The values for cases of ordinary ALS and for younger PD cases show almost the same distribution as those in controls. However, the values for elderly PD cases, MSA cases and ALS case 3 (all *closed symbols*) showed some deviation, which is more prominent in **a**

and previous reports [4, 5] might be due to the technical differences for evaluating morphometry or the diagnostic accuracy for ALS.

Two interesting results arise from this study: one, the two cases showing bimodality in the frequency distribution of diameter histogram of unmyelinated axons (ALS cases 6 and 9) are those in which the disease had progressed rapidly and a respirator was applied for ventilatory support for a long period (3.8 and 5.9 years, respectively). Both cases showed total loss of voluntary movement of the four extremities at the time of death. In addition, case 6 showed complete loss of oculomotor movement and exhibited the "total locked-in state" [15]. Pathologically, other than the lesions in the upper and lower motor neurons commonly seen in ALS, case 6 showed widespread pathological changes in various areas, including spinocerebellar tract, Clarke's column, substantia nigra, and globus pallidus (case 6 was described in detail as case 2 by Hayashi and Kato [15]). Case 9 did not demonstrate as many widespread pathological changes as case 6, but showed fibrillary gliosis in the inferior olivary nucleus and atrophy of postcentral gyrus besides the ordinary involvement in classic ALS. Case 3 showed a very low percentage of subunits containing axon(s) (47.7%) and a very high mean number of Schwann cell profiles per axon (5.45, see Fig. 3). Pathologically, involvement of the spinocerebellar tracts, Clarke's columns and posterior columns in addition to the ordinary lesion sites of classic ALS were seen. Although patient 3 did not show any family history of neurological disorders, the pathological picture resembles those of familial ALS [16].

Parkinson's disease

CNS degenerative disorders which cause peripheral neuropathy are numerous but do not include PD. Autonomic deficits, including orthostatic hypotension, are not uncommon clinical features in this disorder; however, many authors suggested that the autonomic disturbances in PD may be due to a lesion at a higher level in the nervous system above the medulla [14], or resulted from a central failure of autonomic control due to a lesion in the hypothalamus [1].

As far as the postganglionic sympathetic nervous system is concerned, only the sympathetic ganglia and adrenal medulla have been previously investigated [9, 23, 24] because these sites were frequently observed to contain Lewy bodies or adrenal bodies and were more easily accessible than the distal cutaneous nerves, including sural nerve, at autopsy. Rajput and Rozdilsky [24] commented that lesions of the sympathetic ganglia might play a major role in dysautonomia of PD because their severity approximately correlated with the degree of orthostatic hypotension in the patients. Almost all the CNS nuclei with frequent Lewy bodies in PD (e.g., substantia nigra, locus ceruleus, dorsal nucleus of nervus vagus) are accompanied by neuronal cell loss; therefore, Lewy bodies in the sympathetic ganglia are expected to be reflected in the morphology and/or number of cutaneous UMNFs.

In our study, in the majority of PD cases unmyelinated axons and their Schwann cells presented no specific qualitative changes. The mean number of Lewy bodies per 100 cell bodies in the stellate ganglia did not correlate with any of the data for the morphometric parameters for UMNFs. Positive correlation was only seen with the duration of illness. However, the mean values for the densities of unmyelinated axons and Schwann cell subunits with axon(s) were significantly decreased ($P < 0.05$ and $P < 0.01$, respectively) compared with that of controls. The mean value for the density of unmyelinated axons in the PD group was about 21% less than that of the controls. Because the densities of MNFs in PD cases did not show a significant decrease compared with those of age-matched controls, these changes are considered to be confined to UMNFs in the peripheral nervous system. These changes did not accompany the increased percentage of empty subunits or increased number of Schwann cell profiles in a subunit, which have been shown to be the two most subtle changes due to ageing [19, 26]. This suggests that the changes of cutaneous UMNFs in PD cases are different

from the processes of simple ageing. It is interesting to note that Chad et al. [7] estimated the percentage of sympathetic ganglia-derived axons as 20–25% in the rat; however, the value of unmyelinated axon density in each case was much more scattered, and it is not certain whether this quantitative change is a reflection of the reduction of postganglionic sympathetic fibers or cutaneous sensory fibers in PD.

Changes due to ageing were enhanced in the elderly PD patients: three elderly patients (two men aged 78 and 82 years and an 82-year-old woman) showed an increased percentage of empty subunits and an increased number of Schwann cell profiles in a subunit, even compared with age-matched controls (Fig. 3). Fisher [11] described the co-existence of non-parkinsonian features (e.g., pyramidal tract signs, ocular palsies, dementia or personality changes) in patients with PD of long duration and designated these cases as "Parkinson plus" or "cerebral polypathy". These additional clinical features are not considered to be essential ones; however, the classical "parkinsonian features" can be the tip of the iceberg; recent progress in the treatment and management of this disorder has enabled parkinsonian patients to live longer, and this may reveal the miscellaneous late-onset characteristics, both clinically and pathologically. Our results suggest that involvement in postganglionic sympathetic fibers or cutaneous sensory fibers might be one of them.

Multiple system atrophy

In contrast to the two disorders described above, the presence of peripheral neuropathy in MSA is widely accepted. The majority of previous articles have been concerned with changes of MNFs and histological examinations using biopsied materials have shown a preferential reduction of large myelinated fibers [12, 25, 29]. From the viewpoint of autonomic nervous system involvement, MSA has been thought to be a disorder of preganglionic fibers, but some authors have suggested that there is also an abnormality in postganglionic fibers [8]. It is reasonable to consider the abnormalities of cutaneous UMNFs in this disorder as the summation of somatic peripheral neuropathy and the derangement of autonomic postganglionic fibers.

Some authors have commented on the changes of UMNFs in MSA. Galassi et al. [12] performed a quantitative study including measurements of the densities of unmyelinated axons and associated Schwann cells, and concluded that there was no abnormality compared with the values published for controls. On the other hand, Tohgi et al. [30] described a significant reduction in the diameter of UMNFs by more than 0.5 μm and an increased number of multi-lamellated Schwann cell processes, isolated Schwann cell processes and collagen pockets in the biopsied sural nerves of cases with Shy-Drager syndrome, compared to those of OPCA which does not show autonomic dysfunction. Consequently, there is considerable disagreement among authors on this subject. Moreover, these previous studies were all on biopsied materials and had the disadvantage that a final diagnosis based on CNS pathology had not been performed.

In the present study, the values of UMNF density in MSA cases were as low as those of PD patients and the mean value was significantly smaller ($P < 0.05$) than those of ALS patients or controls. In addition, the diameter histograms of unmyelinated axons in the two older cases (MSA cases 3 and 4, aged 63 and 73 years, respectively) showed bimodal distribution. The smaller value of UMNF density, in spite of the relative increase of smaller fibers, probably means that the more marked change of UMNFs occurred in the older MSA cases. On the other hand, the two younger cases (MSA cases 1 and 2, 54 and 56 years of age, respectively) showed abnormally low values for age for the percentage of Schwann cell subunits containing axons (67.2% and 66.0%, respectively). We have demonstrated in our previous article that the value of this index in normal subjects younger than 60 years old exceeded 69% and that three-quarters of them showed the value of higher than 80% [19]. In the present study case 3 also revealed a very low value (37.2%) for this index. The values for another index recommended in our previous study, the mean number of Schwann cell profiles per axon, only showed an abnormality in case 3.

Using both conventional density and diameter histograms and the two indices of percentage of Schwann cell subunits with axon(s) (index 7) and the mean number of Schwann cell profiles per axon (index 8) we conclude that a slight but definite abnormality in the cutaneous unmyelinated axons and their Schwann cells exists in MSA. The most sensitive index for detecting the abnormality is index 7, the percentage of Schwann cell subunits with axon(s). For a routine pathological examination, the calculation of UMNF density in all cases is impractical, and a decrease of the density in each case cannot always be expected (in our series, it was apparently decreased in only one of four cases), thus, the analysis of the distribution pattern of UMNF diameter and the calculation of the two indices described above would suffice to detect abnormalities. Indeed, the first impression on observing UMNFs in MSA cases is not a decrease of UMNF density, but an increase in denervated Schwann cell bands, small unmyelinated axons and Schwann cell processes around each axon.

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