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Morphometry of the degenerative process in the hypoglossal nerves in amyotrophic lateral sclerosis*

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Summary. The peripheral hypoglossal nerves in 13 cases of amyotrophic lateral sclerosis (ALS) and five control cases were examined using morphometrical methods to demonstrate the degenerative process of motor nerve degeneration. The total number of myelinated fibers and their histograms were analyzed according to the degree of severity of the degeneration. Reduction of the total number of myelinated fibers in ALS hypoglossal nerves were graded in three groups: mild 65%–75%, moderate 50%–65% and severe 30%–50% of the myelinated fibers in controls. Each histogram of the remaining myelinated fibers showed different patterns corresponding to the degree of the degeneration and disclosed that the progressive reduction of large myelinated fibers was the fundamental change. Small myelinated fibers were not reduced, but increased, especially in the group with a moderate grade of degeneration. In plastic section, there were clusters of regenerated myelinated fibers. The transient increase of small myelinated fibers may be a reflexion of myelinated fiber regeneration during the progressive degenerative process of the motor neurons. The correlation between the degree of severity of the hypoglossal nerve degeneration and the atrophy of the tongue muscle and the duration of bulbar symptoms was examined and discussed.

Key words: Amyotrophic lateral sclerosis – Hypoglossal nerve – Morphometrical analysis

A motor neuron consists of the anterior horn cell, anterior root and long peripheral nerve. Many pathological and morphometrical findings in the anterior horn cell or anterior roots have been described but few reports have been found on peripheral motor nerves. It has been shown by morphometrical examination that, in cases of amyotrophic lateral sclerosis (ALS), the large myelinated fibers in the anterior spinal roots, as well as the large motor neurons in the anterior horn of the spinal cord, degenerated characteristically. The small myelinated fibers, however, have been shown to remain (Wohlfart and Swank 1941; Inoue and Hirano 1979; Ohnishi et al. 1980; Sobue et al. 1981; Hanyu et al. 1982; Bradley et al. 1983). The aim of this study was to clarify the process of motor neuron degeneration in the peripheral hypoglossal nerves in cases of ALS, using morphometrical examination of the degenerated hypoglossal nerves. The correlations between hypoglossal nerve degeneration and atrophy of the tongue muscle and bulbar symptoms was examined.

Method

Hypoglossal nerves and tongue muscles of the right side were obtained from 13 autopsy cases (A1–A13) diagnosed as ALS and five control cases (C1–C5). The clinical findings are summarized in Table 1. The hypoglassal nerve was examined at the level of the posterolateral margin of the hyoglossal muscle. The specimens were fixed in 2% glutaraldehyde and 1% osmium tetroxide solutions and embedded in Araldite. Transverse sections, 1 μm thick, were stained with toluidine blue and examined. Photographs (×160) of the hypoglossal nerves of all cases containing a completely transected nerve were taken, to measure the transverse sectional area. The total number of the myelinated fibers per nerve were calculated. Photographs (×800) were taken of suitable fields of transverse sections of the nerves. The number and the diameter of all myelinated fibers in 0.24 mm^2 of the field were measured in each case and histograms of their diameters prepared. Frontal sections of the central portion of paraffin-embedded tongue muscles were stained by H.&E.

The degree of neurogenic atrophy was graded: + indicated a few atrophic fibers; + +, several atrophic fibers; + + +, many atrophic fibers in every field; and + + + +, few normal fibers

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Table 1. Clinical course, duration of bulbar symptoms and morphometrical data of hypogrossal nerves in amyotrophic lateral sclerosis (ALS) patients and controls

	Age; Sex	Clinical course	Bulbar symptoms	Hypoglossal nerve								Tongue muscle atrophy[b]
				Number of myelinated fibers					Average diameter of *MF*			
				Small *MF*	Large *MF*	(%)	Total *MF*	(%)	Small *MF*	Large *MF*	Total *MF*	
ALS												
1	28 M	4 years 1 month	1 year 10 months	1,420	5,960	72	7,380	74	2.92	7.87	6.91	++
2	57 M	2 years	2 months	1,360	5,440	66	6,800	69	2.79	7.78	6.78	+
3	60 F	1 year 3 months	3 months	1,210	5,400	65	6,610	67	3.00	8.26	7.31	+
4	68 M	1 year 6 months	1 year	2,960	3,390	41	6,350	64	2.78	6.96	5.01	+++
5	68 F	5 years	1 year	1,420	4,460	54	5,880	59	2.87	7.73	6.55	++
6	40 M	3 years	9 months	2,320	3,410	41	5,730	58	2.98	7.56	5.70	+++
7	62 F	6 months	1 month	2,010	2,980	36	4,990	50	3.04	6.97	5.82	+++
8	51 M	2 years	8 months	1,600	3,260	39	4,860	49	3.01	7.20	5.82	++
9	66 M	7 months	7 months	2,480	2,200	27	4,680	47	2.84	6.82	4.70	+++
10	63 M	1 year 3 months	9 months	1,580	2,570	31	4,150	42	2.87	7.76	5.90	++
11	63 F	2 years 7 months	3 months	1,930	2,160	26	4,090	41	2.78	7.59	5.32	++
12	33 F	2 years 7 months	2 years 1 month	920	2,480	30	3,400	34	3.08	6.64	5.67	++++
13	61 F	7 years	2 months	1,220	1,530	18	2,750	28	2.72	7.85	5.57	+++
$\bar{x}$				1,725	3,480		5,206		2.90	7.46	5.93	
SD				561	1,358		1,340		0.11	0.47	0.73	
Controls												
1	74 F			980	8,160		9,140		2.96	8.81	8.19	
2	58 M			1,830	8,480		10,310		3.16	8.04	7.18	
3	37 M			1,810	8,720		10,530		2.98	8.09	7.21	
4	58 M			1,330	8,340		9,670		3.19	7.80	7.49	
5	53 M			2,250	7,720		9,970		3.07	7.44	6.45	
$\bar{x}$				1,640	8,284		9,920		3.07	8.04	7.30	
SD				440	336		488		0.09	0.45	0.56	

MF: Myelinated fibers; SD: standard deviation

[a] Diameter of small MF <4.5 μm $<$ diameter of large MF

[b] +: Few atrophic fibers; ++: several atrophic fibers; +++: many atropic fibers; ++++: few normal fibers

in every visual field. Correlations between the types of the myelinated nerve fiber histogram and the grading of tongue muscle atrophy, and between the types of myelinated fiber histogram and the duration of bulbar symptoms were examined.

Results

The average sectional area of control hypoglossal nerves was 2.02 mm^2 and the total number of myelinated fibers per nerve was 9920 ± 490 ($m \pm SD$) (Table 1). The areas of the ALS hypoglossal nerves were from 0.78 mm^2 to 1.84 mm^2 and the average area 1.19 mm^2, which is 58.9% of the controls. The total number of myelinated fibers in each ALS hypoglossal nerve was 2750–7300, which is 28–74% of the controls. Histograms of the myelinated fibers of control hypoglossal nerves presented a large peak of large myelinated fibers at 8–9 μm and a small peak of small myelinated fibers at 3.4 μm (Fig. 1). Fibers over 4.5 μm diameter are considered large and those less than this diameter as small. The myelinated fiber histogram of the hypoglossal nerves in the ALS cases and controls are shown in Fig. 1 and arranged in order of the total number of the myelinated fibers (A1–A13). Each histogram showed different patterns according to the degree of degeneration. In mildly degenerated nerves (A1–A3, A5) the total number of the myelinated fibers decreased to 65–75% of the control; the large peak of large myelinated fibers decreased and its top moved to 7–8 μm. In moderately degenerated nerves (A4, A6, A7), the total number of the myelinated fibers decreased to 50–65% of the control, the large peak of large myelinated fibers had disappeared and the highest peak was found in the small myelinated fibers. In highly degenerated nerves (A8–A13) the total number of the myelinated fibers decreased to 30–50% of the control, both large

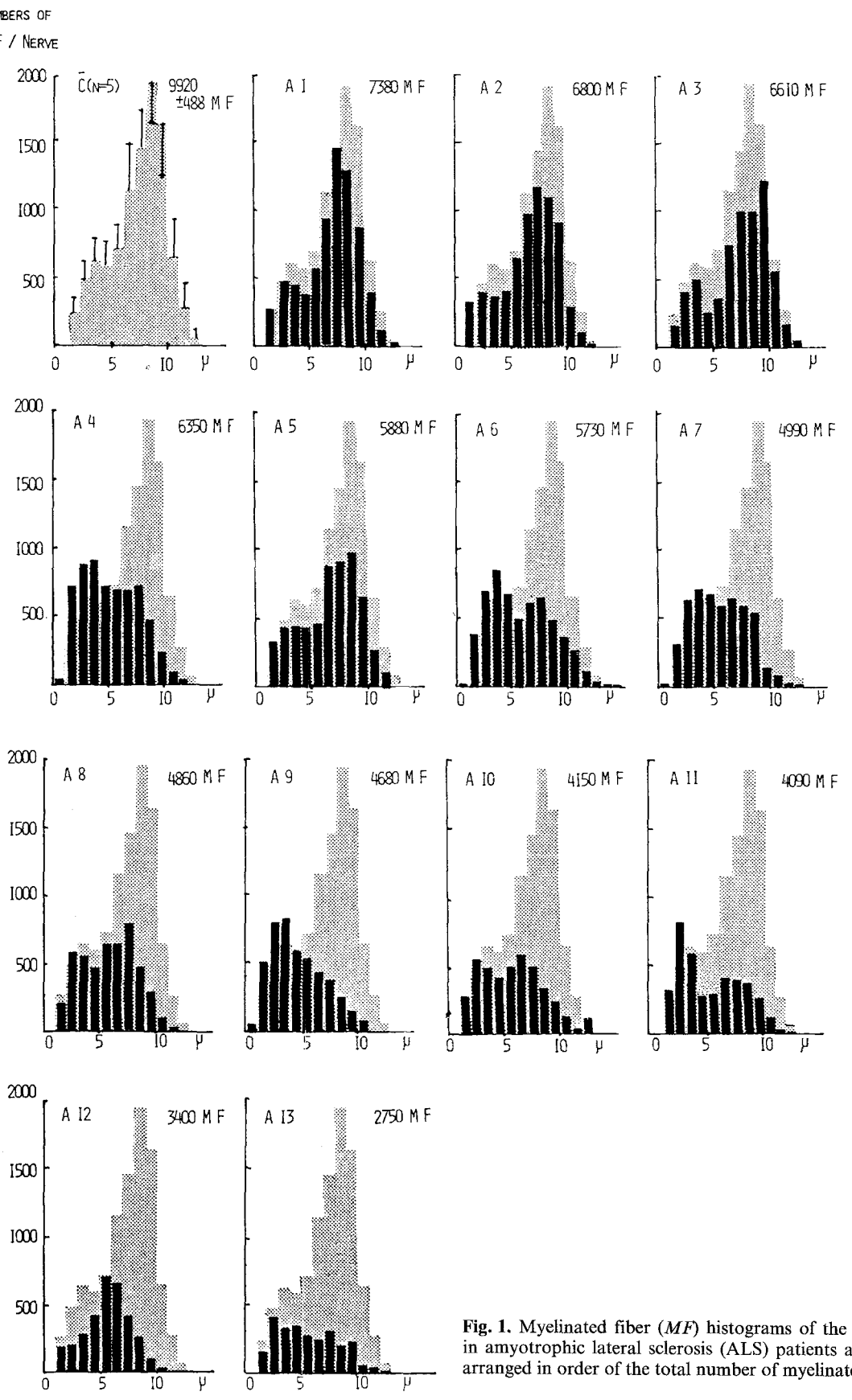

Fig. 1. Myelinated fiber (*MF*) histograms of the hypoglossal nerves in amyotrophic lateral sclerosis (ALS) patients and controls arranged in order of the total number of myelinated fibers

and small myelinated fibers were severely decreased and some specimens showed a flat pattern. To clarify the rule of the existence of two peaks in ALS hypoglossal nerves, the numbers of both large and small myelinated fibers were calculated in each nerve and are shown in Table 1. In ALS, large myelinated fibers of more than 4.5 μm were greatly reduced in number. On the other hand, the number of small myelinated fibers, less than 4.5 μm, was not reduced. There was a progressive loss of large myelinated fibers relative to the reduction of the total myelinated fibers, whereas small myelinated fibers of less than 4.5 μm diameter mostly persisted and increased transiently in number in the moderate stage of the degeneration (Fig. 2). In plastic semithin sections, there were clusters of small regenerated myelinated fibers among large myelinated fibers, some of which showed axonal degenerations (Fig. 3).

The average diameter of the myelinated fibers are shown in Table 1. The mean diameter of the total myelinated fibers in ALS cases showed a statistically significant difference compared to that of the control ($P<0.05$). Correlations between the reduced number

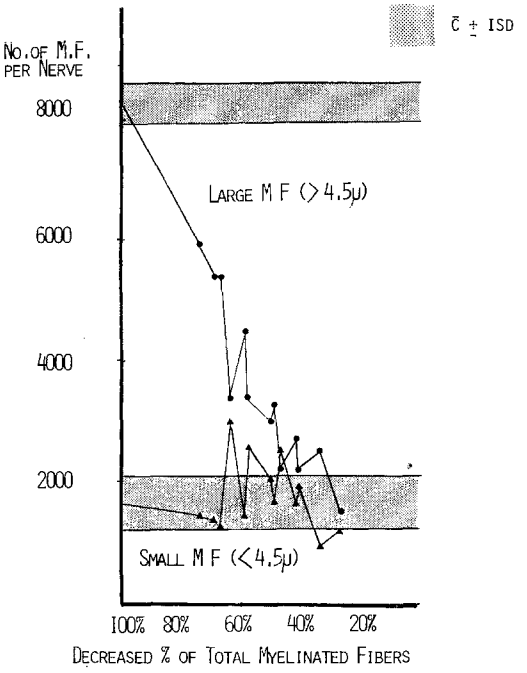

Fig. 2. Changes of numbers of large and small myelinated fibers (*MF*) according to the reduction of the total myelinated fibers

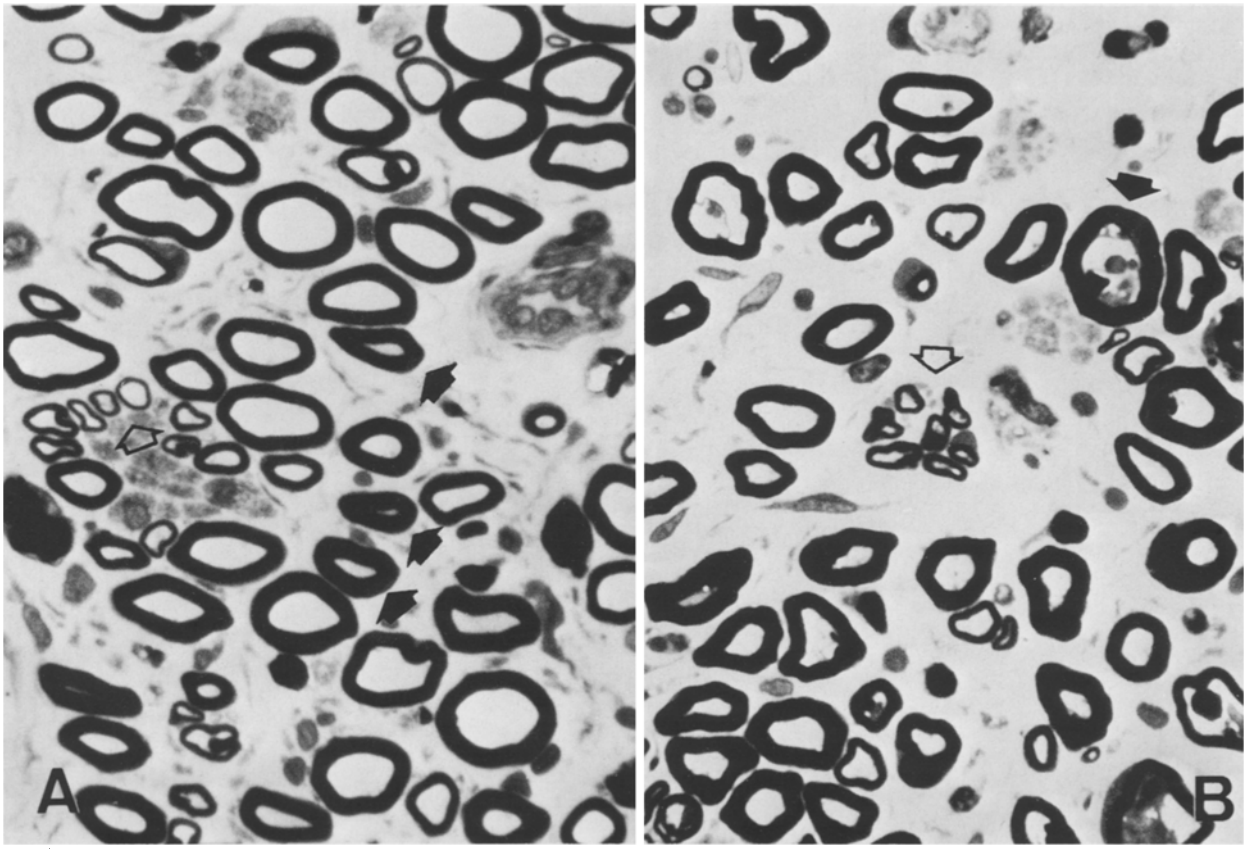

Fig. 3. A A cross section of an ALS hypoglossal nerve with relative preservation of myelinated fibers. Several fibers undergoing early axonal degeneration (*black arrow*) and a cluster of regenerating myelinated fibers (*wihite arrow*) are shown. **B** A moderately degenerated ALS hypoglossal nerve, showing loss of large myelinated fibers, a cluster of regenerating myelinated fibers (*white arrow*) and a degenerating axon. **A, B** Toluidine blue staining, ×1,000

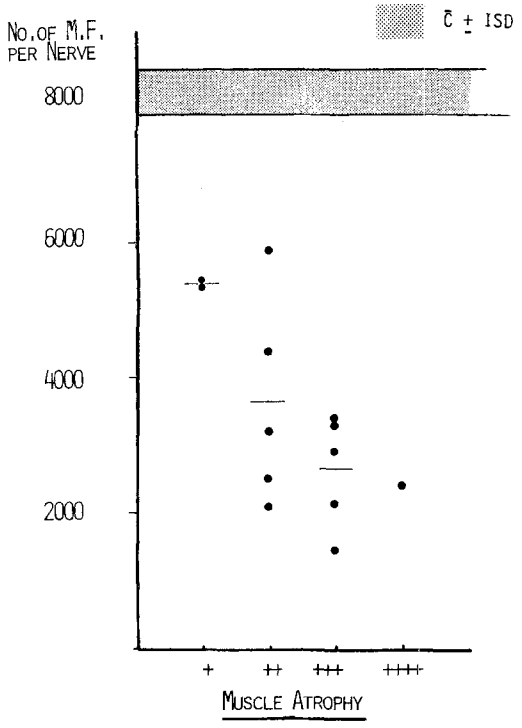

Fig. 4. Correlation between number of large myelinated fibers and grading of muscle atrophy

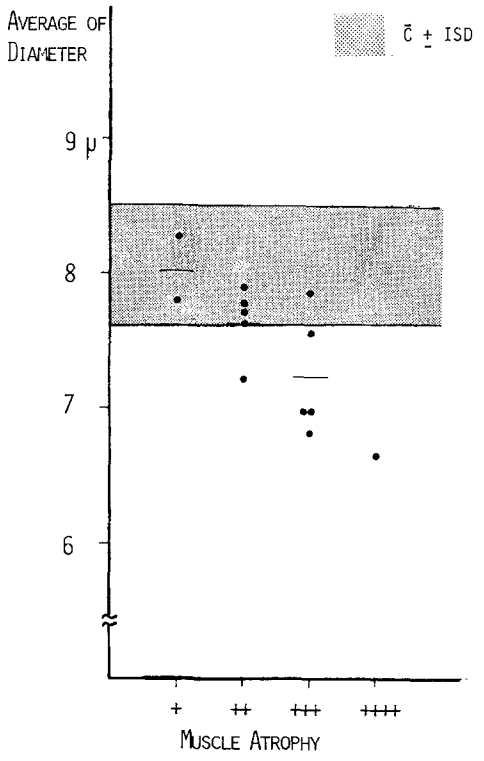

Fig. 5. Correlation between the average diameter of large myelinated fibers and grading of muscle atrophy

of the myelinated fibers, the mean diameter of the myelinated fibers and the grading of the muscular atrophy of the tongue are shown in Fig. 4 and 5. The average number and average diameter of the myelinated fibers were both reduced corresponding to the grading of the severity of the muscular atrophy of the tongue. The reduction of the mean diameter of the myelinated fibers was better correlated to the grading of the muscular atropy of the tongue than the reduction of the average number of the myelinated fibers. These findings suggest that the muscular atrophy of the tongue is more influenced by the constitution of the various kinds of the myelinated fibers than simply by the number of the myelinated fibers. The correlation between the reduction of the myelinated fibers and bulbar symptoms is shown in Fig. 6. The duration of bulbar symptoms was inversely proportional to the large myelinated fiber densities. These results suggest that the severity of change in the hypoglossal nerves strongly influences the duration of the bulbar symptoms and that the speed of the degeneration of these nerves differs in each case.

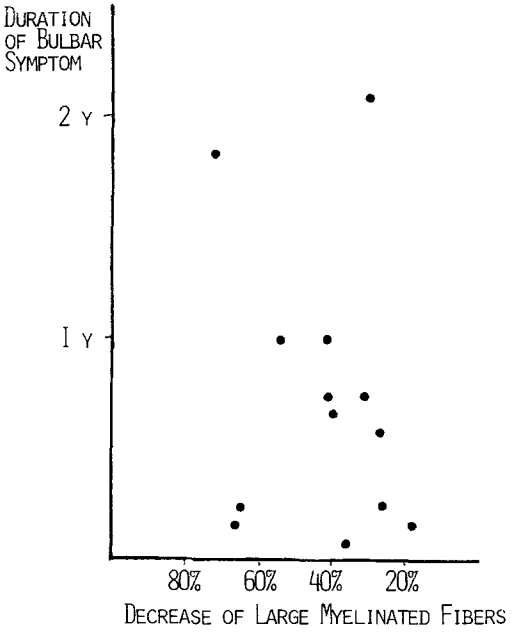

Fig. 6. Correlation between duration of bulbar symptoms and the number of large myelinated fibers

Discussion

Histograms of control hypoglossal nerves at the level of the posterolateral margin of the hyoglossal muscle showed two peaks; the average of the total number of the myelinated fibers was 9,900. These results were similar to the histogram reported by Rexed (Davis et al. 1975). Histograms of the hypoglossal nerve roots

have been reported as having a peak at 7.3 μm and a total number of the myelinated fibers of about 7,300 (Kurisaki et al. 1980). The large peak in the histogram of peripheral hypoglossal nerves in this study was similar to that of the histograms of hypoglossal nerve roots. Therefore, it is considered to consist mainly of motor nerves. The total number of the myelinated fibers in human hypoglossal nerves has been reported to be from 8,220 to 8,817 before communication, and from 9,389 to 9,876 after communication with the nodose ganglion (Wozniak and Young 1969). The site of the portions of hypoglossal nerves examined in this study was more distal than the communication point with the sympathetic nerve ganglion and also the descending branch of the cervical nerve. Therefore, autonomic fibers and afferent fibers other than motor fibers could be included in the total myelinated fibers. Muscle afferents are known to have a broad spectrum in the histogram (Rexed and Therman 1948). The small peak in this study could include the autonomic fibers.

The total number of the myelinated fibers in ALS hypoglossal nerves was always lower than that of the controls. Each hypoglossal nerve showed considerable differences in the reductions of the number of the myelinated fibers and the patterns of the histogram. In cases with a mild degree of the degeneration, which may be the initial stage of degeneration, there was a reduction of large diameter myelinated fibers of 7–8 μm. In cases with a moderate degree of the degeneration, there was a significant reduction of the large myelinated fibers and some increase of the small myelinated fibers. In cases with a severe degree of the degeneration, there was a diffuse reduction of each size of the myelinated fibers. Histograms consistently showed a reduction of large myelinated fibers, although the number of remaining myelinated fibers varied in different degrees. Reduction of large myelinated fibers in the phrenic nerve and the spinal anterior roots and also reduction of large motor neurons of the anterior horn of the spinal cord have been reported, and the significance considered to be fundamental changes in the pathology of ALS (Wohlfart and Swank 1941; Tsukagoshi et al. 1979; Kawamura et al. 1981; Bradley et al. 1983). A relative increase of small myelinated fibers in histograms of anterior spinal roots (Inoue and Hirano 1979; Ohnishi et al. 1980; Hanyu et al. 1982; Bradley et al. 1983), hypoglossal nerve roots (Kurisaki et al. 1980) and phrenic nerves (Bradley et al. 1983) have also been reported, however, the significance of this is not yet clear. There are three possibilities, namely, atrophic process, axonal regeneration, or both. In this study an increase of small myelinated fibers was found only in the group showing a moderate degeneration, not in the group showing early degeneration. In semithin sections clusters of regenerated small myelinated fibers were found, which suggest that regeneration of axons may occur. Grading of the atrophy of the tongue muscle correlated well with the reduction in the diameter of large myelinated fibers and with decrease in the average number of myelinated fibers. A good correlation between the reduction in the number of large motor neurons in the anterior horn of segment C6 of the spinal cord and the neurogenic atrophy of the biceps brachii has been reported in morphometrical analysis (Tsukagoshi et al. 1979). The correlation between the decrease of large myelinated fibers and the duration of bulbar signs showed that the degree of hypoglossal nerve change strongly influenced the duration of bulbar symptoms, and consequently the prognosis in the presence of bulbar signs. More severe changes in the hypoglossal nucleus have been reported in patients with bulbar symptoms of shorter duration (Murakami et al. 1983).

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References

Bradley WG, Good P, Rassel CG, Adelman LS (1983) Morphometric and biochemical studies of peripheral nerves in amyotrophic lateral scrolosis. Ann Neurol 14:267–277

Davis JN, Thomas PK, Spolding JMK, Harrison SP (1975) Diseases of the 9th, 10th, 11th, and 12th cranial nerves. In: Dick PJ, Thomas PK, Lambert EH, Bunge R (eds) Peripheral neuropathy. W. B. Saunders, Philadelphia, pp 614–627

Hanyu N, Oguchi K, Yanagisawa N, Tsukagoshi H (1982) Degeneration and regeneration of ventral root motorfibers in amyotrophic lateral sclerosis. J Neurol Sci 55:99–115

Inoue K, Hirano A (1979) Early pathological changes of amyotrophic lateral sclerosis. Neurol Med (Tokyo) 11:448–455

Kawamura Y, Dyck PJ, Shimono M, Okazaki H, Tateishi J, Doi H (1981) Morphometric comparison of the vulneability of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 40:667–675

Kurisaki H, Takatsu, Toyokura Y (1980) Hypoglossal nerve roots of amyotrophic lateral sclerosis, In: Research group of degenerative neurological disease. Annual report. Tokyo. Ministry of Health and Welfare, pp 65–70

Murakami N, Nakano I, Hirano A (1983) A neuropathological study of the hypoglossal nucleus in amyotrophic lateral sclerosis. Neurology (Tokyo) 23:131–139

Ohnishi Y, Makifuchi T, Ikuta F (1980) Morphometric investigation of the myelinated fibers of the anterior spinal roots in amyotrophic lateral sclerosis and Shy-Drager syndrome. Clin Neurol 20:809–815

Rexed B, Therman P (1948) Calibre spectra of motor and sensory nerve fibres to flexor and extensor muscles. J Neurophysiol 11:133–140

Sobue G, Matsuoka Y, Mukai E, Takayanagi T, Sobue I, Hashizume Y (1981) Spinal and cranial motor nerve roots in amyotrophic lateral sclerosis and X-linked nessesive bulbospinal muscular atrophy. Acta Neuropathol (Berl) 55:227–235

Tsukagoshi H, Yanagisawa N, Oguchi K, Nagashima K, Murakami T (1979) Morphometric quantification of the cervical limb moter cells in controls and in amyotrophic lateral sclerosis. J Neurol Sci 41:287–297

Wohlfart G, Swank RL (1941) Pathology of amyotrophic lateral sclerosis. Arch Neurol Psychiatry 46:783–799

Wozniak W, Young PA (1969) Further observations on human hypoglossal nerve. Anat Anz [Suppl] 125:203–205

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