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Synapse loss in anterior horn neurons in amyotrophic lateral sclerosis

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Abstract This report deals with an ultrastructural investigation of the synapses of anterior horn neurons in the lumbar spinal cords of five patients with amyotrophic lateral sclerosis (ALS) who had mild neuronal depletion. Specimens from five age-matched, neurologically normal individuals served as controls. In each instance, the autopsy was performed within 3 h after death. A statistically significant decrease in cell body area, number of synapses and total synaptic length was found in the normal-appearing neurons of the ALS patients. The alterations were more pronounced in neurons with central chromatolysis. However, despite an approximately 20% reduction in the number of synapses, the length of the active synaptic zone of the normal-appearing neurons in the ALS patients was not diminished. This observation may be accounted for by a plasticity to the loss of synapses which maintained the active zone of the remaining synapses to increase synaptic efficiency. It is suggested that when the plasticity of the active zone reaches its limit, the continuing loss of synapses may lead to functional impairment. The capacity of the active synaptic zone to respond to progressive denervation of the anterior horn neurons may preserve motor function or slow the development of motor deficits in the early stage of degeneration of the lower motor neurons.

Key words Amyotrophic lateral sclerosis · Anterior horn neuron · Synapse · Active zone · Ultrastructure

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Introduction

Most neuropathological studies on the neurons of the spinal cord anterior horn of patients with amyotrophic lateral sclerosis (ALS) have focused mainly on cytoarchitectural alterations [3, 7, 8], but little is known about possible changes of the synaptic complex in this disease [10, 13]. As far as we are aware, there have been no reports on changes of the synaptic active zone or other synaptic alterations that occur during the process of motoneuron degeneration in ALS. Axosomatic synaptic complexes play an important role in normal neuronal function, since they are essential for the neuron to act efficiently as an integrator of electrical inputs and as an information transducer [2]. In this report we present the results of the quantitative determination of the number of synapses, the total synaptic contact length, and the length of the synaptic active zone (post-synaptic density) of anterior horn neurons of the lumbar spinal cord in ALS patients whose anterior horn neurons were relatively well preserved. The data obtained were compared with those found with control specimens from age-matched, neurologically normal individuals.

Materials and methods

This study was carried out on spinal cords of 5 patients (ages: 59, 62, 63, 67 and 81 years) with clinically and neuropathologically confirmed ALS. All 5 patients had mild depletion of the anterior horn neurons of the lumbar spinal cord and they were selected from among a total of 28 ALS patients. In 3 patients there were typical ALS symptoms, including upper and lower motor neuron signs and bulbar sign, but muscle weakness and atrophy of their legs were relatively mild when legs were compared with the upper extremities. The patients were able to walk even until the terminal stages of their disease. The other two ALS patients had a short clinical course of 10 and 16 months, respectively. Spinal cords from 5 age-matched individuals who had no neurological disease served as controls. Two of them (ages: 60 and 62 years) died of acute myocardial infarction, two others (ages: 62 and 68 years), of lung cancer and the fifth, died of rupture of an abdominal aneurysma at the age of 80. All postmortem investigations were performed within 3 h after death.

In all cases, a tissue block was obtained at autopsy from almost the same level of the lower lumbar spinal cord (L4–5), and the anterior horns of each level were fixed immediately with 2% glutaraldehyde in phosphate buffer (pH 7.4). After fixation, the anterior horns were cut transversely into pieces approximately 1 mm thick, postfixed for 2 h with 1% osmium tetroxide, dehydrated, and then embedded flat in epoxy resin. Each embedded tissue block was subsequently cut into semithin (around 1 μm thick) sections that were almost large enough to contain an entire anterior horn. Sections thus prepared were stained with toluidine blue.

After light microscopic identification of large anterior horn neurons with the nucleus, appropriate portions of the semithin sections were cut into ultrathin sections. These were stained with uranyl acetate and lead citrate for electron microscopy. Photomicrographs of normal-appearing large anterior horn cells and chromatolytic neurons were randomly taken throughout each anterior horn. In all cases, photomicrographs were taken at a magnification of 1,400 and then enlarged to a magnification of 2,660. We analyzed 184 motoneurons from the control individuals and 206 from the ALS patients. Photomicrographs taken at a magnification of 8,000 and enlarged to a magnification of 15,200 were used for examining individual synapses. Synaptic complexes consist of a presynaptic bouton and a postsynaptic membrane separated by the extracellular space (Fig. 1). The total length of each individual synapse was defined as the entire length of the synaptic contact between the presynaptic and postsynaptic sites, whereas the synaptic active zone was defined as the length of the postsynaptic density. Synapses were identified on the photographic prints by the presence of synaptic membrane thickenings that were associated with synaptic vesicles. For each motoneuron, we determined the cell body area, the number of synapses, the total synapse length, and the length of the active zone. Discontinuous synapses were counted as a single synapse. The lengths of the synaptic contact and of the active zone were not calculated in those instances in which the distance between the neuronal membrane and the presynaptic bouton was more than 1 μm.

The measurements were carried out with Kontron computerized image analyzer (Munich, Germany). The data obtained were subjected to analysis of variance (ANOVA, Scheffe's method) using a computerized statistical program. The results were expressed as mean ± standard deviation.

Results

Control subjects

A total of 184 anterior horn neurons (174 normal-appearing neurons and 10 chromatolytic neurons) were studied. The mean cross-sectional area of the normal-appearing neurons was 3,492.6 ± 723.3 μm^2 (Table 1) and that of the chromatolytic neurons, 3,002.7 ± 909.6 μm^2. This difference was not significant. A total of 2,277 synapses were counted on the cell bodies of the 174 normal-appearing anterior horn neurons (Table 2). In these neurons, the mean number of synapses was15.6 ± 6.2, the mean length of the total synaptic contact, 32.3 ± 13.3 μm and the mean length of the

Fig. 1 Synapses of an anterior horn cell of lower lumbar spinal cord, containing aggregate vesicles near the presynaptic membrane are making a synaptic contact. The total length of a synapse represents the entire length of the synaptic contact between the presynaptic and postsynaptic sites. The active zone (*arrows*) is characterized by a postsynaptic density [an amyotrophic lateral sclerosis (ALS) patient]. ×26,600

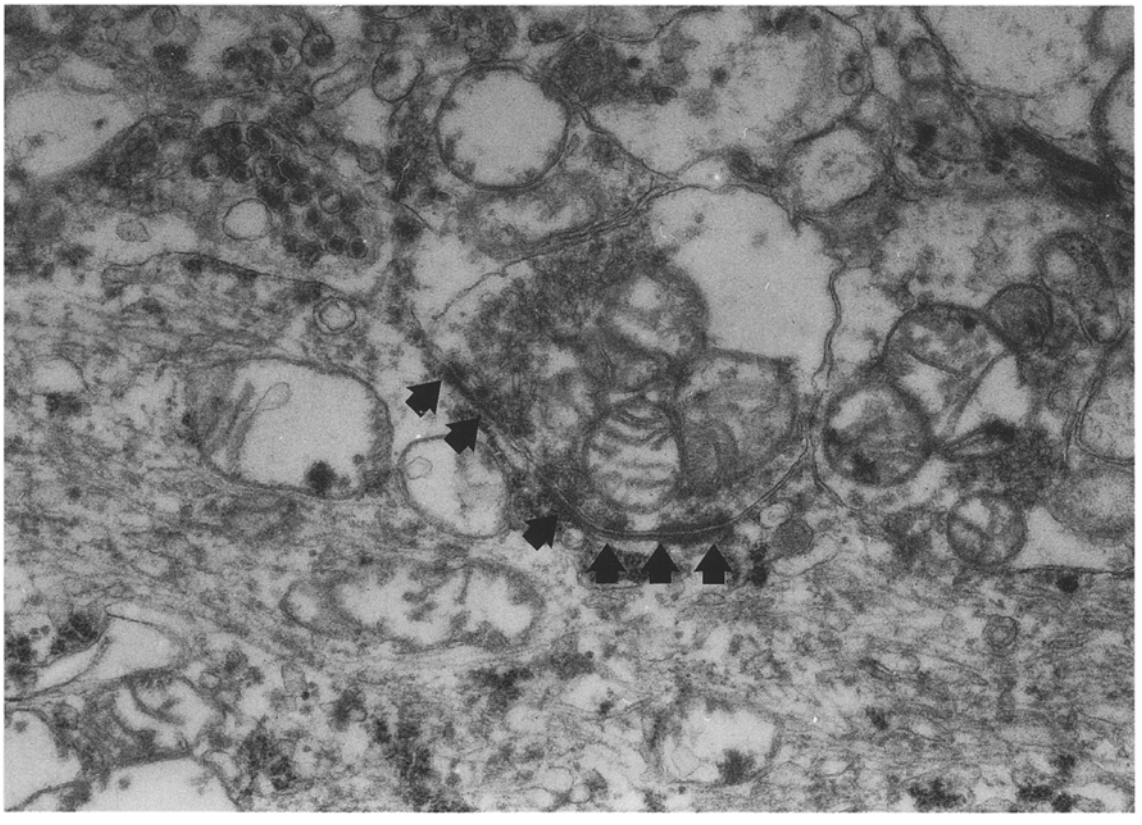

Table 1 Comparison of neuronal size between controls and ALS patients (*ALS* amyotrophic lateral sclerosis, *NS* not significant)

Cell body area (μm^2)	Control group				ALS group			
	Normal-appearing neurons (μm^2)		Chromatolytic neurons (μm^2)		Normal-appearing neurons (μm^2)		Chromatolytic neurons (μm^2)	
<2,000	1,947.9	($n=1$)	1,182.1	($n=1$)	1,691.8 ± 70.4	($n=15$)	1,457.9 ± 324.6	($n=43$)
2,000–3,000	2,686.8 ± 239.3	($n=44$)	2,435.3 ± 320.8	($n=3$)	2,634.6 ± 277.7	($n=56$)	2,319.4 ± 300.1	($n=12$)
3,000–4,000	3,446.9 ± 280.2	($n=89$)	3,437.5 ± 221.1	($n=5$)	3,455.9 ± 293.3	($n=72$)	–	
>4,000	4,519.0 ± 448.9	($n=40$)	4,351.2	($n=1$)	4,267.9 ± 179.0	($n=8$)	–	
Mean	3,492.6 ± 723.3[a]	($n=174$)	3,002.7 ± 909.6[NS]	($n=10$)	3.019.1 ± 695.3*	($n=151$)	1,645.9 ± 478.8*	($n=55$)

[a] Expressed as mean ± standard deviation
* $P<0.01$

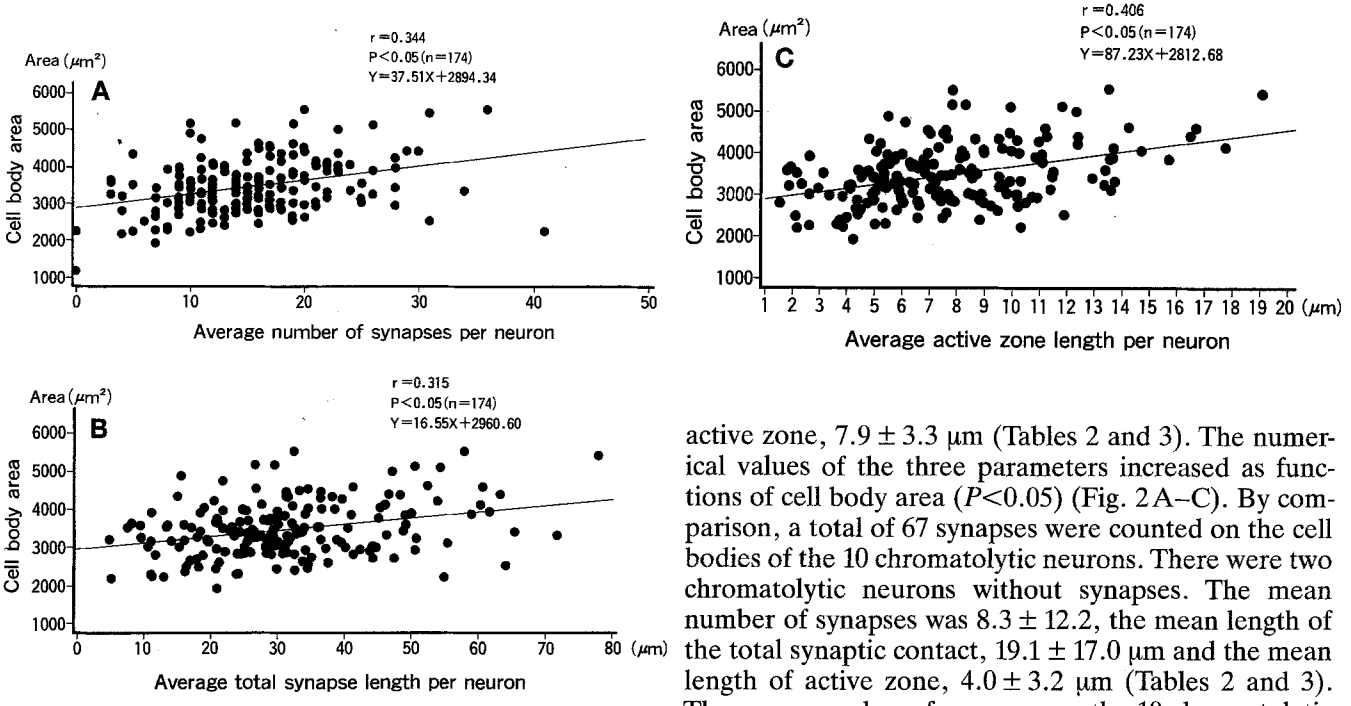

Fig. 2 **A** The average number of synapses per neuron increased as a function of cell body area ($P<0.05$). **B** Increase of average total synapse length per neuron as a function of cell body area ($P<0.05$). **C** The average active zone length per neuron increased with cell body area ($P<0.05$)

active zone, 7.9 ± 3.3 μm (Tables 2 and 3). The numerical values of the three parameters increased as functions of cell body area ($P<0.05$) (Fig. 2A–C). By comparison, a total of 67 synapses were counted on the cell bodies of the 10 chromatolytic neurons. There were two chromatolytic neurons without synapses. The mean number of synapses was 8.3 ± 12.2, the mean length of the total synaptic contact, 19.1 ± 17.0 μm and the mean length of active zone, 4.0 ± 3.2 μm (Tables 2 and 3). The mean number of synapses on the 10 chromatolytic neurons was only one half that on normal-appearing neurons ($P<0.01$) (Table 2). Similarly, the total synaptic length and the length of the active zone in the chromatolytic neurons were also significantly smaller

Table 2 Comparison of the mean number of synapses per neuron between controls and ALS patients (N total number of synapses, n number of neurons)

Cell body area (μm^2)	Number of synapses							
	Control group ($N=2,277$)				ALS group ($N = 2,157$)			
	Normal-appearing neurons		Chromatolytic neurons		Normal-appearing neurons		Chromatolytic neurons	
<2,000	7.0	($n=1$)	0	($n=1$)	7.0 ± 4.9	($n=15$)	4.3 ± 3.7	($n=43$)
2,000–3,000	13.5 ± 5.6	($n=44$)	15.0 ± 22.6	($n=3$)	12.3 ± 5.8	($n=56$)	7.6 ± 5.2	($n=12$)
3,000–4,000	15.1 ± 5.1	($n=89$)	6.6 ± 4.8	($n=5$)	13.3 ± 5.1	($n=72$)	–	
>4,000	19.4 ± 6.0	($n=40$)	5.0	($n=1$)	15.8 ± 3.7	($n=8$)	–	
Mean	15.6 ± 6.2[a]	($n=174$)	8.3 ± 12.2*	($n=10$)	12.4 ± 5.6*	($n=151$)	5.0 ± 4.2*	($n=55$)

[a] Expressed as mean ± standard deviation
* $P<0.01$

Table 3 Comparison of the total synapse length and the length of the active zone between controls and ALS patients

Cell body area (μm^2)	Total synapse length (μm)				Active zone length (μm)			
	Control group		ALS group		Control group		ALS group	
	Normal-appearing neurons	Chromatolytic neurons	Normal-appearing neurons	Chromatolytic neurons	Normal-appearing neurons	Chromatolytic neurons	Normal-appearing neurons	Chromatolytic neurons
<2,000	21.6 ($n=1$)	0 ($n=1$)	13.6 ± 9.7 ($n=15$)	8.4 ± 7.0 ($n=43$)	4.3 ($n=1$)	0 ($n=1$)	3.6 ± 2.5 ($n=15$)	2.1 ± 1.9 ($n=43$)
2,000–3,000	29.0 ± 11.6 ($n=44$)	33.5 ± 30.7 ($n=3$)	24.6 ± 11.6 ($n=56$)	14.7 ± 8.9 ($n=12$)	6.7 ± 2.6 ($n=45$)	6.0 ± 6.2 ($n=3$)	6.3 ± 2.8 ($n=56$)	3.9 ± 2.7 ($n=12$)
3,000–4,000	30.5 ± 12.7 ($n=89$)	14.1 ± 11.6 ($n=5$)	30.1 ± 11.4 ($n=72$)	–	7.4 ± 2.9 ($n=89$)	3.1 ± 2.1 ($n=5$)	7.7 ± 3.0 ($n=72$)	–
>4,000	40.1 ± 13.6 ($n=40$)	15.1 ($n=1$)	37.1 ± 9.4 ($n=8$)	–	10.3 ± 2.3 ($n=40$)	4.8 ($n=1$)	10.1 ± 2.3 ($n=8$)	–
Mean	32.3 ± 13.3[a] ($n=174$)	19.1 ± 17.0** ($n=10$)	26.8 ± 12.4** ($n=151$)	9.8 ± 7.8** ($n=55$)	7.9 ± 3.3[a] ($n=174$)	4.0 ± 3.2* ($n=10$)	6.9 ± 3.2[NS] ($n=151$)	2.5 ± 2.2** ($n=55$)

[a] Expressed as mean ± standard deviation
* $P<0.05$, ** $P<0.01$

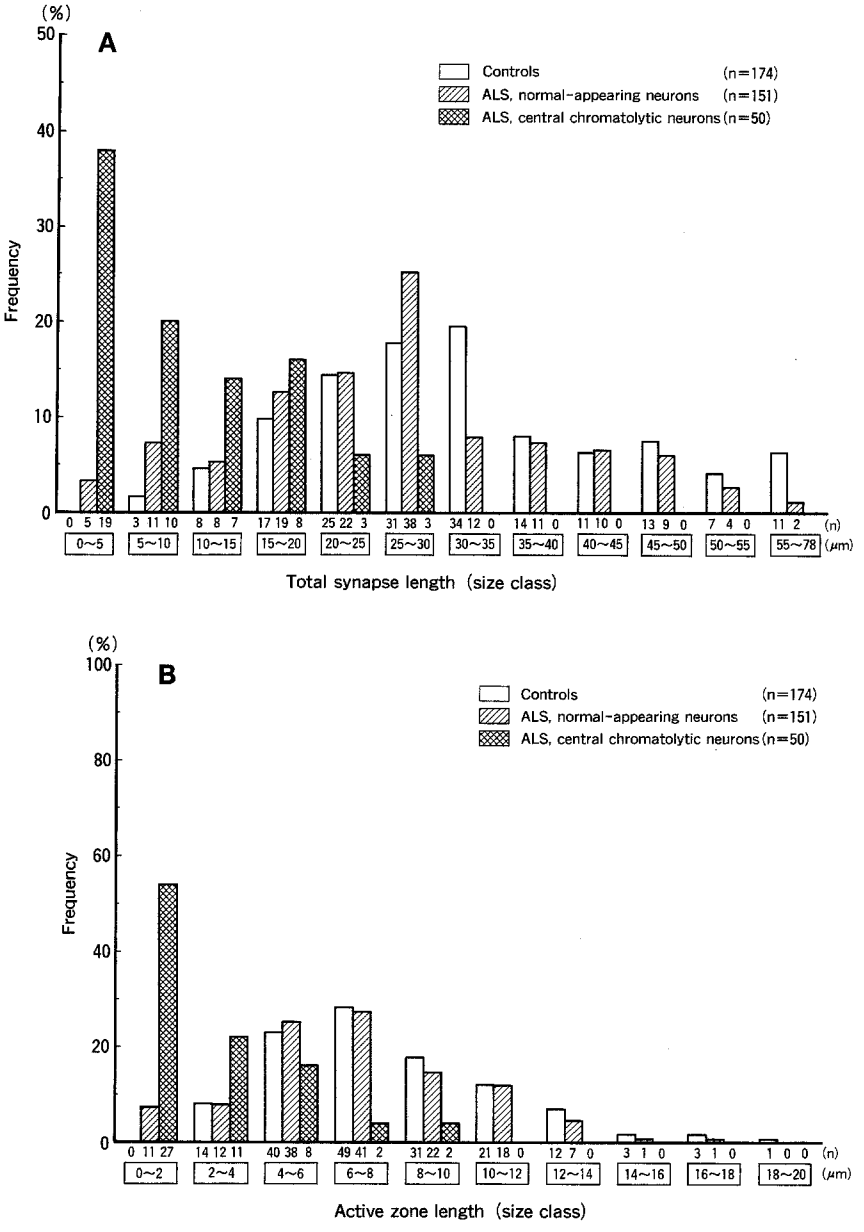

Fig. 3 **A** Frequency distribution of the total synapse length in control neurons, the normal-appearing and the chromatolytic neurons of ALS patients. **B** Frequency distribution of the length of the active zone in control neurons, the normal-appearing and the chromatolytic neurons of ALS patients

than those in the normal-appearing neurons ($P<0.01$, $P<0.05$, respectively) (Table 3). Regression analyses failed to reveal any significant correlation between the parameters measured and the age of control individuals (not shown).

ALS patients

Of a total of 206 anterior horn neurons studied in the five ALS patients, 151 appeared to be normal, with relatively abundant Nissl substance and few lipofuscin gra-

nules, while the other 55 showed central chromatolysis. The mean cell body area of the normal-appearing neurons of the ALS patients was $3,019.1 \pm 695.3$ μm^2 and that of the chromatolytic neurons was $1,645.9 \pm 478.8$ μm^2. This difference was statistically significant ($P<0.01$), as was the size difference between the neurons of the ALS patients and the controls ($P<0.01$; Table 1). A total of 2,157 synapses was counted on the neurons of the patients with ALS. There were 5 chromatolytic neurons without synapses. The mean number of synapses of the normal-appearing anterior horn neurons in the ALS group (12.4 ± 5.6) was significantly lower than that of the normal-appearing neurons in the control individuals ($P<0.01$), while the mean number of synapses of the chromatolytic neurons was further reduced (5.0 ± 4.2) as compared with the normal-appearing neurons in the controls ($P<0.01$) (Table 2). The mean total synaptic contact length of the 151 normal-appearing neurons of the ALS patients (26.8 ± 12.4 μm) was significantly shorter than that of the normal-appearing neurons in the control subjects ($P<0.01$). This length reduction was even more striking in the 55 chromatolytic neurons (9.8 ± 7.8 μm) as compared with normal-appearing neurons in the controls (Table 3 and Fig. 3A). Despite decrease in cell body area, number of synapses and synaptic contact length, the mean length of the synaptic active zone in the normal-appearing anterior horn neurons of the ALS patients did not differ from that of the normal-appearing neurons of the control individuals (Table 3 and Fig. 3B). In the chromatolytic neurons of the ALS patients, the mean length of the synaptic active zone (2.5 ± 2.2 μm) was reduced as compared with normal-appearing neurons in the controls ($P<0.01$).

The mean cell body area of the chromatolytic neurons of the ALS patients was significantly smaller than that of the chromatolytic neurons of controls ($P<0.05$), but there was no significant difference in the mean number of synapses, the mean synaptic contact length and the mean length of the synaptic active zone between them. There were no significant differences in cell body area, number of synapses, total synaptic contact lengths, and lengths of the active zone among the 5 ALS patients studied. Overall, in each stratum of cell body area, no significant difference in the number of synapses, the total synapse length and the length of active zone was seen either between normal-appearing neurons of ALS and control groups, or between chromatolytic neurons of the two groups, or between normal-appearing and chromatolytic neurons of each group, respectively (Tables 2 and 3).

Discussion

The present study shows that a spectrum of synaptic changes occurs in ALS during the process of anterior horn neuron degeneration. This investigation represents, to the best of our knowledge, the first delineation of the fate of axosomatic synapses in this disease. It has essential for carrying out computer-aided image analysis on electron microscope-generated photomicrographs that the structure of the synaptic apposition zone is highly resistant to postmortem changes as has been repeatedly documented [4, 9, 12, 14]. Thus, the use of spinal cords obtained at autopsies performed within 3 h after death, allowed us to obtain the machine-generated data presented in this report.

In the control individuals, the number of synapses, the total synaptic contact length, and the length of the active zone were directly related to cell body area. By comparison, in the normal-appearing neurons of ALS patients there were decreases in cell body area, number of synapses and synaptic contact length. These findings suggest that synaptic loss and decrease in synaptic contact length affect the anterior horn neurons even at an early stage of the disease. These alterations were even more pronounced in chromatolytic neurons. The progressive loss of synapses seemed to parallel the continuous degeneration of large anterior horn neurons. The loss of synaptic complexes terminating on neurons would give rise to dysfunction by affecting the afferent input. One of the major influences on motoneuron excitability is synaptic efficiency, which involves the interaction between presynaptic and postsynaptic elements [2]. The factors that control the synaptic efficacy of α-motoneurons include the number of synapses, the synaptic density, and the total postsynaptic membrane area on the motoneuron surface [2]. The alterations of these parameters, seen in the ALS patients studied, imply that changes of electrophysiological function may occur together with abnormalities of interneuronal communication in the motor system.

It is noteworthy that despite decreases in cell body area, synaptic numbers and synaptic contact length, the length of the active zone was not reduced in the normal-appearing neurons of the ALS patients. This finding is reminiscent of the compensation for losses, described in certain experimental animals, during the normal aging process, and in some human diseases [1, 5, 6, 15, 17, 18]. Thus, it has been speculated that the change in synaptic contact length as a function of the number of contacts may act as a compensatory mechanism for synaptic losses as a result of normal aging and certain various diseases [18]. Maintenance or enlargement of the area of existing synapses is a major form of synaptic remodeling and provides functional compensation by increasing synaptic efficacy [6]. Adams [1] has reported that the motor cortex of the human brain is capable of synaptic plasticity in response to age-induced synaptic loss. Moreover, chronic electrical stimulation of the undercut cerebral cortex increases the synaptic contact length and bouton area in the cat [15].This enlargement of synapses described in experimental animals suggests the existence of a general compensatory mechanism with some as yet undefined effect on the maintenance or restoration of neural function [18]. The preservation

of the length of the synaptic active zone seen in the ALS patients studied, probably compensates, at least in part, for the reduction in postsynaptic length as synapses are lost. The plasticity of the active zone may be triggered by the decrease in synaptic numbers, the reduction in total synapse length, or both, and it is possible that these alterations may stimulate the plasticity of adjacent synapses. Such a response seems to occur at an early stage of anterior horn neuron alterations. The suggested plasticity of the synaptic active zone may be related to its important functions in maintaining neurotransmission efficacy [19, 20]. Thus, efficient synaptic transmission would not be maintained in anterior horn cells when this compensatory mechanism is overcome by continuous synaptic loss or by the reduction in length of the active zone as in the case of chromatolytic neurons. The continuous loss of synapses implies a decrease in the global connectivity of the motor system and a decreased potential for motoneuronal interaction. The present findings also indicate that a certain capacity to respond to progressive denervation exists in the anterior horn neurons of ALS patients, and that the plasticity of the synaptic active zone may be one of the mechanisms by which motor function is preserved and/or that the development of motor dysfunction is slowed during the early stage of the disease.

Recently, we reported a decrease in synaptophysin expression in the anterior horn neuropil of lower motor neuron disease patients who had no upper motor neuron and corticospinal tract involvement [16]. On the other hand, Matsumoto et al. [11] reported no reduction of synaptophysin immunoreactivity in anterior horns of patients with descending degeneration of the corticospinal tracts secondary to cerebral and spinal cord diseases. Moreover, in the present study, most of the chromatolytic neurons in both controls and ALS patients showed a marked reduction in the number of synapses, the total synaptic length and the length of the active zone as compared to normal-appearing neurons. Thus, the substantial loss of synapses, documented in the present study, is probably caused by a primary degeneration of the anterior horn neurons associated with the plasticity of motor neuron synapse active zone. The observed loss of synapses is probably fundamental to the pathological process of ALS, in that it leads to, or reflects the damage of the anterior horn neurons.

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