We describe a 52-year-old man with body weight loss and bulbar palsy, who exhibited muscle atrophy and weakness with fasciculation especially in the respiratory muscles 4 years prior death, necessitating respiratory support for 4 years, but who was able to walk until the end stage. He had no significant family history. Neuropathological examination revealed severe loss of motor neurons in the spinal cord and brainstem, and ubiquitin-positive skein-like inclusions and Bunina bodies in the remaining neurons. In addition, prominent degeneration of the anterolateral funiculus and severe loss of neurons in the intermediate zone of the spinal cord were evident, without marked alteration of the corticospinal tracts. Degeneration of the subthalamic nucleus, increased iron deposition in the substantia nigra, and axonal swelling, residual nodules and acidophilic granules in the spinal ganglia were found. The patient's condition was considered to have been a forme fruste or incipient form of widespread-type amyotrophic lateral sclerosis (ALS) or motor neuron disease (MND) with pallido-nigro-luysian atrophy (PNLA). The neuropathological features of the present case appear to be important for understanding the nature of widespread-type ALS and MND with PNLA.

Key words: amyotrophic lateral sclerosis, anterolateral funiculus, pallido-nigro-luysian atrophy, subthalamic nucleus, spinal ganglia.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder in the elderly, clinically manifested by weakness and wasting of the affected muscles with pyramidal signs. The pathological hallmarks of sporadic ALS are severe degeneration of the spinal anterior horn cells and corticospinal tracts (CST) of the spinal cord and characteristic Bunina bodies and ubiquitinated inclusions.[1,2]

Some patients with sporadic ALS who survive for a long time with respiratory support develop a totally locked-in state, or widespread-type ALS.[3-8] Such patients show extensive pathological involvement far beyond the motor neuron system, and usually show impairment of voluntary ocular movements.[3-5,7,8] In widespread-type ALS, the pallidoluysian system is one of the most vulnerable regions, and the spinal ganglia show frequent degeneration.[3] The pallidoluysian system has been reported to be involved also in motor neuron disease (MND) combined with pallido-nigro-luysian atrophy (PNLA).[9-14]

We report here the neuropathological findings in a 52-year-old male patient with lower-motor-neuron-predominant ALS, who showed unusual pathological features such as prominent degeneration of the anterolateral funiculus (ALF) in the spinal cord without marked alteration of the corticospinal tract (CST), degeneration of the subthalamic nucleus, increased iron deposition in the substantia nigra, and axonal swelling, residual nodules and acidophilic granules in the spinal ganglia. The significance of these findings is discussed.

Clinical summary

A Japanese man with no family history of neurological or psychiatric disease or relevant medical history suffered body weight loss of about 11 kg (from 67 to 56 kg) during a one-year period at the age of 48. One year later, mild to moderate muscle atrophy and weakness with fasciculation became evident in the tongue, sternocleidomastoid, upper limb, and intercostal muscles. The deep tendon reflexes of the bilateral lower limbs were mildly exaggerated, but no pathological reflexes were seen. Dysarthria and dysphagia were not observed, but arterial blood gas examination revealed moderate hypercapnia and hypoxia. His pulmonary vital capacity was only 59%. Electromyography (EMG) showed systemic neurogenic changes in the tongue, truncal muscles, arms and legs. He was diagnosed clinically as having ALS. Thirteen months after onset, respiratory support (non-invasive positive pressure ventilation) was initiated for night apnea. These neurological symptoms gradually progressed, and bulbar palsy and weakness of the facial muscles were evident at the age of 51. Arterial oxygen partial pressure decreased progressively, and the respiratory support time was extended. However, the patient remained capable of swallowing food and walking without support until just before his death. He was found dead in his home at the age of 52 years, 42 months after disease onset. Throughout the clinical course of the disease, the patient's mental status had remained unimpaired and extrapyramidal symptoms such as resting tremor, akinesia and rigidity were not observed.

The serum creatine kinase level was slightly elevated (455 IU/L), but HbA1c was normal. Results of other investigations, such as cerebrospinal fluid analysis and a nerve conduction study, were normal.

Pathological findings

A general autopsy was performed 5 h after the patient's death. Pulmonary emphysema was observed, but no other visceral organs exhibited significant pathological abnormality.

The brain with the dura mater weighed 1517g before fixation. The brain and spinal cord were fixed with 20% buffered formalin, and some parts of the cervical and lumbar enlargements were fixed in 2.5% glutaraldehyde-1% paraformaldehyde in 0.1 M cacodylate buffer solution (CB) at autopsy. Coronally cut surfaces of the brain showed that the subthalamic nucleus was small and brownish in color, and the pigmentation of the substantia nigra and locus ceruleus was mildly decreased. In terms of size, the pyramis of the medulla oblongata and the cerebral peduncle looked well preserved, but the volume of the spinal cord and the anterior roots at the cervical and lumbar enlargements appeared moderately decreased.

Histological examinations were performed using 10-μm-thick sections stained with hematoxylin and eosin (HE), Klüver-Barrera (KB), Bodian, Holzer, Gallyas-Braak, Berlin blue, periodic-acid Schiff (PAS), Luxol fast blue (LFB) and cresyl violet (CV).

Selected sections were immunostained using the labeled streptavidin-biotinylated antibody (LSAB) method (Dako, Kyoto, Japan) or avidin-biotin-peroxidase (ABC) method (Vector, Berlingame, CA, USA) with diaminobenzidine as the chromogen. The primary antibodies used were rabbit

polyclonal antibody against ubiquitin (Dako, Glostrup, Denmark; 1:600), goat polyclonal antibody against α-synuclein (N-19, SantaCruz, CA; 1:200), and mouse monoclonal antibodies against phosphorylation-dependent tau (AT8; Innogenetics, Ghent, Belgium; 1:500), neurofilament (Dako, Glostrup, Denmark; 1:100), glial fibrillary acidic protein (GFAP) (Novocastra, Newcastle-upon-Tyne, UK; 1:100) and expanded polyglutamine stretches (1C2; Chemicon, Temecula, CA; 1:8000, stained by Dr. M. Yamada, Department of Pathology, Brain Research Institute, Niigata University, Japan).

For the ultrastructural study, the cervical and lumbar segments of the spinal cord and dorsal root ganglia were post-fixed with 4% osmium tetroxide in 0.2 M CB, followed by dehydration through a graded ethanol series and embedding in Epon 812. Toluidine blue-stained semithin sections were observed by light microscopy, and ultrathin sections of the selected areas were stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope (H-9000, Hitachi, Tokyo, Japan).

Histologically, severe volume loss and degeneration of the ALF was noted in the spinal cord, and atrophy of the anterior horn (AH) and intermediate zone (IMZ) was observed especially in the cervical enlargement. The ALF showed severe loss of myelinated fibers. The lateral corticospinal tract (LCS) appeared to be preserved in K-B-stained sections, but showed slight loss of myelinated fibers in Epon-embedded toluidine blue-stained sections. Severe loss of neurons and myelinated fibers was observed in the spinal AH and IMZ (Fig. 1A-C, 2A, B), and remaining neurons in the

IMZ were severely shrunken (Fig. 2C). The neurons of Clark's column and the intermediolateral nucleus were relatively well preserved. Bunina bodies (Fig.1D) and ubiquitin-immunopositive skein-like inclusions (Fig.1E) were detected in the lumbar and sacral anterior horn cells. Several spheroid bodies and globules were also observed in the anterior horn, especially in the lumbar segments (Fig.1F). Severe loss of large myelinated fibers and GFAP-immunopositive glial bundles were noted in the anterior spinal nerve roots. Mild degeneration was also observed in the fasciculus cuneatus (Fig. 1A).

In the brainstem, severe loss of neurons and gliosis were observed in the hypoglossal nucleus, and moderate loss in the trigeminal motor, facial and ambiguous nuclei, with relative sparing of the oculomotor and trochlear nuclei. There was moderate loss of myelinated fibers in the reticular formation, and mild loss in the pyramid of the medulla oblongata (Fig. 2D). In the motor cortex, Betz cells appeared atrophic, but their number was relatively well preserved.

In the muscles, grouped atrophy was confirmed, being severe in the sternocleidomastoideus and basophilic fibers, moderate in the 4th intercostal muscles, diaphragm and tongue, and mild in the illiopsoas.

The subthalamic nucleus showed marked gliosis with moderate neuronal loss (Fig. 3A, B). In the medial part of the substantia nigra, mild neuronal loss, several foamy spheroid bodies, and an increased number of Berlin blue-positive granules were observed (Fig.3C, D). Some iron granules were found in the astrocytes, neurons (Fig. 3D) and foamy spheroid bodies in the substantia nigra. A few iron granules were also

detected in the globus pallidus, putamen and dentate nucleus. No neurofibrillary tangles, as shown by Gallyas and Bodian staining, were present in the putamen, globus pallidus, substantia nigra, or subthalamic and dentate nuclei. Purkinje cells were well preserved and the dentate and inferior olivary nuclei appeared intact.

In the spinal ganglia, several residual nodules were found in the cervical segment (Fig. 4A). Axonal swelling was evident around the neurons (Fig. 4A, B), but onion bulbs were not detected. Many acidophilic granules, varying from 1 to 6 μm in diameter, appeared either singly or in groups in the neurons of the spinal ganglia (Fig. 4C). Histochemically, positive staining with PAS, LFB and CV suggested that these granules contained glycoprotein and phospholipids (Fig. 4D, E). Electron microscopy revealed that the acidophilic granules were electron-dense, homogeneous and amorphous round bodies, which showed fusion with each other. All of the fusing round bodies were surrounded by a double-layered limiting membrane, suggesting that they were mitochondria (Fig.4F). The surface of the round bodies was thorny (Fig. 4G).

There was no evidence of degeneration in the CA1 to subiculum transitional areas or motor neuron disease inclusions. There were no 1C2-immunopositive cytoplasmic and intranuclear inclusions or glial cytoplasmic inclusions in the brain and spinal cord.

Discussion

Severe involvement of the lower motor neurons in the brainstem and spinal cord with relative sparing of the oculomotor, trochlear and abducens nuclei and presence of

ubiquitin-immunopositive skein-like inclusions and Bunina bodies indicated that the present patient had sporadic ALS. The most noteworthy feature was prominent degeneration of the ALF, whereas only slight alteration of the LCS was evident. It has been reported that degeneration of the ALF is roughly correlated with the severity of degeneration of the LCS in patients with sporadic ALS.[15,16] However, it seems that the LCS and ALF of the spinal cord degenerate independently in some cases of sporadic ALS,[15,16] and only one case showing severe degeneration of the ALF despite mild degeneration of the CST has been reported previously.[17] In the experience of the authors during laboratory service, the proportion of patients showing prominent ALF degeneration with faint alteration of the LCS is approximately 2% among patients with sporadic ALS. In previous studies, the propriospinal neurons of the IMZ and neurons in the medullary reticular formation have been proposed to be the origin of the degenerated fibers in the ALF of ALS patients.[15,16,18,19] The present authors consider that the marked degeneration of the ALF in the present patient was due mainly to severe neuronal loss in the IMZ.

Some patients with ALS who survive for a long period with respirator support show widespread involvement beyond the motor neurons system. In these patients, Betz cells, the globus pallidus, subthalamic nucleus, red nucleus, substantia nigra, dentate nucleus, locus ceruleus, oculomotor, trochlear and abducens nuclei, reticular formation, medial longitudinal fasciculus (MLF), Clark's column, the intermediolateral nucleus, Onufrowicz nucleus, spinocerebellar tract and middle root zone of the posterior column[3-7] have been reported to be severely affected. In the present patient,

however, degeneration of the Betz cells was mild. Accordingly, the present case appears to have been a forme fruste or incipient form of the widespread type of sporadic ALS (Table 1).

MND patients with degeneration of the striatonigral-pallidoluysian systems have been reported previously.[9-13] Even in patients with a relatively short disease duration,[8-11. 14] all parts of the striatonigral-pallidoluysian systems were evidently involved, and the initial site of the degeneration has not yet been reported. Furthermore, iron deposition was evidently increased in the substantia nigra, putamen, globus pallidus and subthalamic nucleus in the affected patients. It has been claimed that these pathological findings are compatible with PNLA.[20] As the present patient showed iron deposition in the putamen, globus pallidus and substantia nigra, a forme fruste or an incipient form of MND may also have been combined with PNLA (Table 1). Iron deposition may play a role in generating free radicals, thus inducing neuronal degeneration in the PNLA lesions.[21]

There have been previous reports of onion bulbs being detected in the spinal ganglia in ALS,[22] and neuronal loss and residual nodules being evident in the spinal ganglia in widespread-type ALS.[3] However, axonal swelling in the spinal ganglia has not been reported in ALS. A recent study demonstrated that a neuronal intermediate filament protein "peripherin" was associated with axonal spheroids in ALS, and that its overexpression caused the death of not only motor neurons, but also spinal ganglia neurons in vitro.[23]

Many intracytoplasmic acidophilic granules were observed in the spinal ganglia

of the present patient. Similar granules have been reported in the locus ceruleus, substantia nigra and spinal ganglia in humans, and appear to be mitochondrial in origin.[24,25] The relationship between degeneration of the spinal ganglia and these acidophilic granules is still unclear.[26]

In conclusion, the present patient showing unusual degeneration of the spinal white matter with degeneration of the subthalamic nucleus and iron deposition in the putamen, globus pallidus and substantia nigra was considered to have had a forme fruste or incipient form of widespread-type ALS or MND with PNLA. The neuropathological findings in this case seem to be important for understanding the nature of widespread-type ALS and MND with PNLA.

Acknowledgements

We are deeply indebted to Ms. J. Motoki and Mr. A. Ishihara, Department of Pathology, Tokyo Metropolitan Neurological Hospital, Fuchu, Tokyo, Japan, and Ms. E. Kawakami, Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo, Japan, for their technical assistance. We are also grateful to Dr. M. Shibuya, Department of Diagnostic Pathology, Tokyo Medical University Hachioji Medical Center, Hachioji, Tokyo, Japan, for comments about visceral organs, and Dr. M. Yamada, Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan, for 1C2 immunostaining. This work was supported in part by a grant from the Japanese Ministry of Health, Labor and Welfare (Research on Psychiatric and Neurological Diseases and Mental Health; H16-kokoro-017 to KO).

References

1. Sasaki S. Bunina body (in Japanese): *Neurological Medicine* 1986;24:463-470

2. Leigh PN, Anderton BH, Dodson A et al. Ubiquitin deposits in anterior horn cells in motor neuron disease: *Neurosci Lett* 1988;93:197-203

3. Akiyama K, Tsutsumi Y, Onoda N et al. An autopsy case of amyotrophic lateral sclerosis associated with sensory disturbance and eye movement disorder: Pathological consideration on development of multisystem degeneration of the nervous system in a patient with prolonged survial (in Japanese) : *Pathology and Clinical Medicine* 1987;5:921-927

4. Oda M, Kato S, Hayashi H et al. Pathological study of spino-cerebellar, dentate-rubro-pallido-luysian and thalamic degenerations in ALS (in Japanese with English abstract) : *Annual report of the Research Committee of Ataxic Disease, the Ministry of Health and Welfare of Japan* 1989;63:74-78

5. Hayashi H, Kato S.Total manifestations of amyotrophic lateral sclerosis. ALS in the totally locked-in state : *J Neurol Sci* 1989;93:19-35

6. Sasaki S, Tsutsumi Y, Yamane K et al. Sporadic amyotrophic lateral sclerosis with

extensive neurological involvement : *Acta Neuropathol* 1992;84:211-215

7. Mizutani T, Sakamaki S, Tsuchiya N et al. Amyotrophic lateral sclerosis with ophthalmoplegia and multisystem degeneration in patients on long-term use of respirators : *Acta Neuropathol* 1992;84:372-377

8. Takeda S, Yamada M, Kawasaki K et al. Motor neuron disease with multi-system involvement presenting as tetraparesis, ophthalmoplegia and sensori-autonomic dysfunction: *Acta Neuropathol* 1994;88:193-200

9. Gray F, De Baecque C, Serdaru M et al. Pallido-luyso-nigral atrophy and amyotrophic lateral sclerosis : *Acta Neuropathol* 1981;suppl.VII:348-351

10. Gray F, Eizenbaum J F, Gherardi R et al. Luyso-pallido-nigral atrophy and amyotrophic lateral sclerosis : *Acta Neuropathol* 1985;66:78-82

11. Bergmann M, Kuchelmeister K, Migheli A et al. Motor neuron disease with pallido-luysio-nigral atrophy : *Acta Neuropathol* 1993;86:105-108

12. Kato S, Oda M, Murahashi M et al. Motor neuron disease with involvement of the pallido-luysio-nigral system and mesencephalic tegmentum : *Clin Neuropathol* 1995;14:241-244

13. Hasegawa K, Kowa H, Yagishita S. Extrapyramidal system involvement in motor

neuron disease : *J Neurol Sci* 1992;108:137-148

14. Sudo S, Fukutani Y, Matsubara R et al. Motor neuron disease with dementia combined with degeneration of striatonigral and pallidoluysian systems : *Acta Neuropathol* 2002;103:521-525

15. Ikuta F, Makifuchi T, Ohama T et al. Tract degeneration of the human spinal cord: Some observations on ALS and hemispherectomized human (in Japanese) : *Shinkei Kenkyu No Shimpo* 1982;26:710-736

16. Oyanagi K, Makifuchi T, Ikuta F. The anterolateral funiculus in the spinal cord in amyotrophic lateral sclerosis : *Acta Neuropathol* 1995;90:221-227

17. Watabe K, Tanaka J et al. An autopsy case of amyotrophic lateral sclerosis with lower motor neuron symptoms and suggestive of spinal muscular atrophy (in Japanese): *Jikeikai Med J* 1995;110:147-152

18. Oyanagi K, Makifuchi T, Ikuta F. The anterolateral funiculus in the spinal cord in amyotrophic lateral sclerosis : *Biomed Res* 1983;4:211-224

19. Oyanagi K, Ikuta F, Horikawa Y. Evidence for sequential degeneration of the neurons in the intermediate zone of the spinal cord in amyotrophic lateral sclerosis: a topographic and quantitative investigation : *Acta Neuropathol*

1989;77:343-349

20. Kawai J, Sasahara M, Hazama F et al. Pallidonigroluysian degeneration with iron deposition: a study of three autopsy cases : *Acta Neuropathol* 1993;86:609-616

21. Ben-Shachar D, Riederer P, Youdim MBH. Iron-melanin interaction and lipid peroxidation: implications for Parkinson's disease : *J Neurochem* 1991;57:1607-1614

22. Murayama S, Bouldin TW, Suzuki K. Onion bulb formation in the initial complex of neurons in human dorsal root ganglion: their significance and alterations in amyotrophic lateral sclerosis : *Acta Neuropathol* 1991;82:462-470

23. Robertson J, Beaulieu JM, Doroudchi MM et al. Apoptotic death of neurons exhibiting peripherion aggregates is mediated by the proinflammatory cytokine tumor necrosis facter-α : *J Cell Biol* 2001; 155:217-226

24. Sasaki S, Hirano A. Study of intracytoplasmic acidophilic granules in the human dorsal root ganglia (in Japanese) : *Neurological Medicine* 1983;19:263-268

25. Sekiya S, Tanaka M, Hayashi S et al. Light- and electron-microscopic studies of intracytoplasmic acidophilic granules in the human locus ceruleus and substantia nigra: *Acta Neuropathol* 1982; 56:78-80

Legends

Fig. 1

A: Spinal cord at the 6th cervical (C6), 3rd thoracic (T3) and 5th lumbar (L5) segments; moderate atrophy of the spinal cord and anterior horns, and severe volume loss of the anterolateral region. Relatively well preserved anterior and lateral corticospinal tracts (CST), but severely degenerated anterolateral funiculus (ALF). Degeneration of fasciculus cuneatus in the cervical segment. (Klüver-Barrera (KB)). Bar; 1mm.

B: Severely decreased number of large myelinated fibers, in addition to moderate decrease of medium-sized and small myelinated fibers in the ALF (Toluidine blue preparation). Bar; 50 μm.

C: Decreased number of large myelinated fibers with relatively increased number of small myelinated fibers in the lateral corticospinal tract (LCS) (Toluidine blue preparation). Bar; 50 μm.

D: Bunina bodies in a lumbar anterior horn cell (Hematoxylin and eosin (HE)). Bar; 30 μm.

E: Ubiquitin-positive filamentous structures (skein-like inclusion) in a lumbar anterior horn cell (Immunohistochemistry for ubiquitin). Bar; 30 μm.

F: Spheroid in the lumbar anterior horn (HE). Bar; 30 μm.

Fig. 2

A, B: Moderate loss of neurons in the intermediate zone (IMZ) of the spinal cord at the 6th cervical (C6) (A) and 5th lumbar (L5) (B) segments ; (KB). Bar; 400 μm, (dotted line; boundaries between Rexed's lamina VI and VII, VIII and IX)

C: High-power micrograph of the IMZ of the spinal cord at the C6 segment; shrinkage of remaining neurons (KB). Bar; 50 μm.

D: Myelin pallor of longitudinal fibers in the reticular formation (asterisk) and that of the pyramis at the medulla oblongata (KB). Bar; 5 mm.

Fig. 3

A: Fibrillary gliosis is the subthalamic nucleus (arrowhead) (Holzer). Bar; 1mm.

B: Moderate neuronal loss with marked gliosis in the subthalamic nucleus (HE). Bar; 250 μm.

C: Mild neuronal loss in the medial part of the substantia nigra (double asterisk). Cerebral peduncle (asterisk) (KB). Bar; 400 μm.

D: Iron deposition in the neurons and astrocytes of the substantia nigra (Berlin blue). Bar; 30 μm.

Fig. 4

A: Degeneration of the cervical spinal ganglion. Scattered Nageott's residual nodules with loss of neurons. Arrow indicates swollen axon (HE). Bar; 100 μm.

B: A neuron (asterisk) in a dorsal root ganglion at the 7th cervical segment with axonal swelling (double asterisk) (immunohistochemistry for neurofilament). Bar; 50 μm.

C: Acidophilic granules in a neuron of the dorsal root ganglion (arrows) (HE). Bar; 50 μm.

D, E: Acidophilic granules in the spinal ganglion positive for periodic-acid Schiff (D), and luxol fast blue (E) staining. Bar; 30 μm.

F: Ultrastructure of acidophilic granules in the lumbar spinal ganglia. Amorphous and globular bodies of homogeneous material, and surrounded by a double-layered limiting membrane. Mitochondrial cristae (arrows) are evident. Bar; 1μm.

G: Surface of the bodies is thorny (F-G, Uranyl acetate and lead citrate). Bar; 1μm.

Table1 Neuropathological comparison between MND with iron deposited PNLA ,[14] ALS with widespread degeneration,[4] and present patient.

Report	Present patient	Sudo et al[14]	Oda et al[4]	
Age at death, gender	52 M	60 M	69 M	69 M
Duration	3y8m	5y	4y9m	9y9m
Respirator	2y7m	-	3y9m	8y5m
Brain weight (g)	1,517	1,040	1,190	1,050
Cerebral cortex	+/-	++/+	+++/+++	+++/+++
Putamen	+/+(Fe+)	++/++(Fe++)	-/-	-/-
GPi	+/+(Fe+)	+++/+++(Fe++)	++/++	++/++
GPe	+/+	++/+(Fe++)	+++/+++	+++/+++
Subthalamic n.	++/+++	+++/++(Fe++)	+/++	+/+++
Substantia nigra	+/+(Fe++)	+++/+++(Fe++)	++/++	++/++
Inferior olivary n.	-/-	-/-	-/-	-/-
Dentate n.	-/-	-/-	++/++	+++/+++
Hypoglossal n.	+++/+++	+/-	+++/+++	+++/+++
Pyramidal tract	+/+	++/+++	++/n.a.	++/n.a.
Spinal AHC	+++/+++	+/-	+++/+++	+++/+++
Bunina body	+	+	-	-
Skein-like inclusion	+	-	-	-

Neuronal or myelin loss /gliosis; - absent, + mild, ++ moderate, +++ severe,

Fe; iron deposition; – absent, + several, ++ many, n.a. not available

(MND: motor neuron disease, PNLA: pallido-nigro-luysian atrophy, M: male, y: year, m: month,

GPi: internal segment of globus pallidus, GPe: external segment of globus pallidus,

n.a.: not available, n.: nucleus, AHC: anterior horn cell)

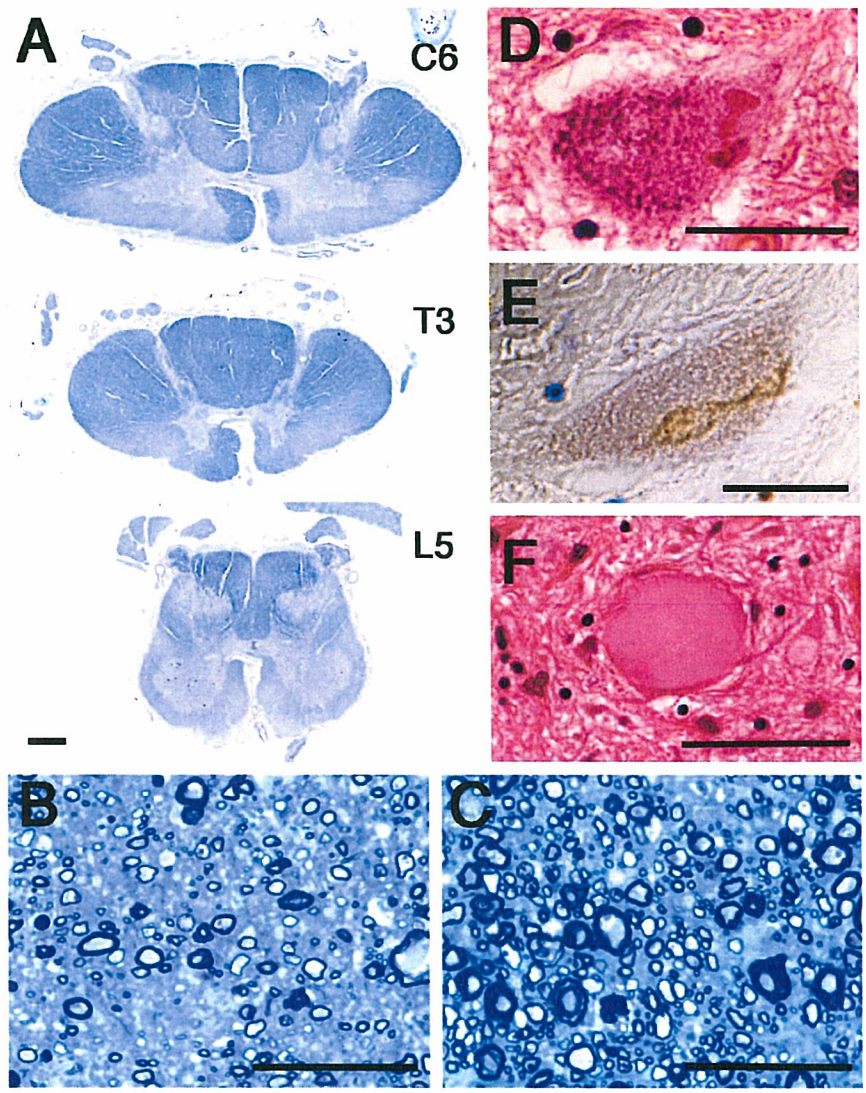

Fig. 1 A: Spinal cord at the 6th cervical (C6), 3rd thoracic (T3) and 5th lumbar (L5) segments; moderate atrophy of the spinal cord and anterior horns, and severe volume loss of the anterolateral region. Relatively well preserved anterior and lateral corticospinal tracts (CST), but severely degenerated anterolateral funiculus (ALF). Degeneration of fasciculus cuneatus in the cervical segment. (Klüver-Barrera (KB)). Bar; 1mm. B: Severely decreased number of large myelinated fibers, in addition to moderate decrease of medium-sized and small myelinated fibers in the ALF (Toluidine blue preparation). Bar; 50 µm. C: Decreased number of large myelinated fibers with relatively increased number of small myelinated fibers in the lateral corticospinal tract (LCS) (Toluidine blue preparation). Bar; 50 µm. D: Bunina bodies in a lumbar anterior horn cell (Hematoxylin and eosin (HE)). Bar; 30 µm. E: Ubiquitin-positive filamentous structures (skein-like inclusion) in a lumbar anterior horn cell (Immunohistochemistry for ubiquitin). Bar; 30 µm. F: Spheroid in the lumbar anterior horn (HE). Bar; 30 µm.

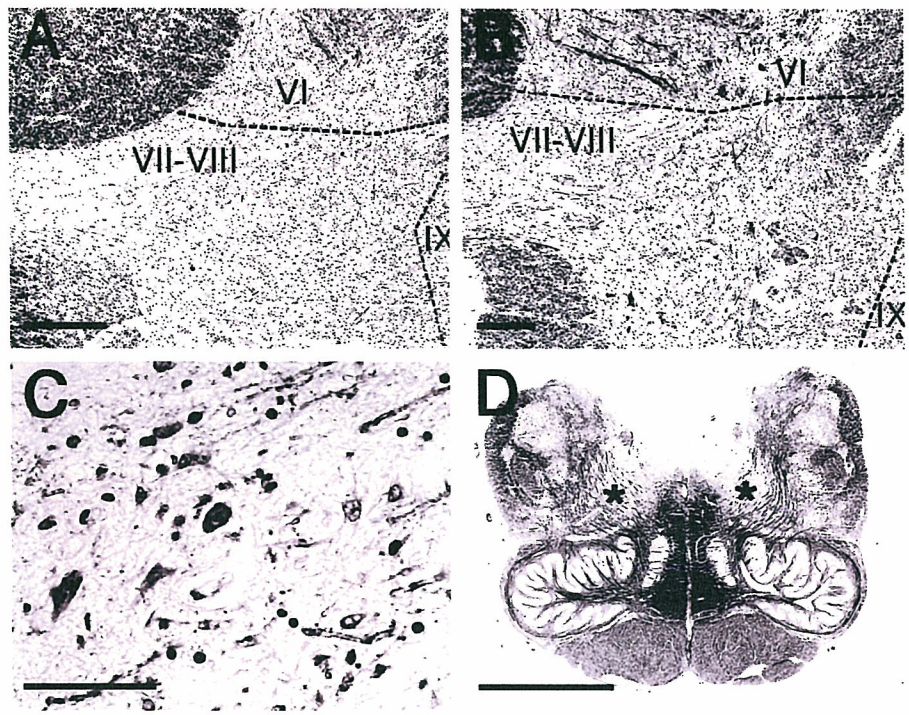

Fig. 2 A, B: Moderate loss of neurons in the intermediate zone (IMZ) of the spinal cord at the 6th cervical (C6) (A) and 5th lumbar (L5) (B) segments ; (KB). Bar; 400 µm, (dotted line; boundaries between Rexed□fs lamina VI and VII, VIII and IX) C: High-power micrograph of the IMZ of the spinal cord at the C6 segment; shrinkage of remaining neurons (KB). Bar; 50 µm. D: Myelin pallor of longitudinal fibers in the reticular formation (asterisk) and that of the pyramis at the medulla oblongata (KB). Bar; 5 mm.