Hoshino M, Qi M-L, Yoshimura N, Miyashita T, Tagawa K, Wada Y, Enokido Y, Marubuchi S, Harjes P, Arai N, Oyanagi K Blandino G, Sudol M, Rich T, Kanazawa I, Wanker EE, Saitoe M, Okazawa H	Transcriptional repression induces a slowly progressive atypical neuronal death associated with changes of YAP isoforms and p73	J. Cell. Biol.	172	589-604	2006
Oyanagi K	The nature of the parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam and magnesium deficiency	Parkinsonism and Related Disorders	11	S17-23	2005
Yamazaki M, Hasegawa M, Mori O, Murayama S, Tsuchiya K, Ikeda K, Chen K-M, Katayama Y, Oyanagi K	Tau-positive fine granules in the cerebral white matter: a novel finding among the tauopathies exclusive to parkinsonism-dementia complex of Guam	J. Neuropathol. Exp. Neurol.	64	839-846	2005
Kadoya T, Oyanagi K, Kawakami E, Hasegawa M, Inagaki Y, Sohma Y, Horie H	Oxidized galectin-1 advances the functional recovery after peripheral nerve injury	Neurosci. Lett.	380	284-288	2005
Anamizu Y, Kawaguchi H, Seichi A, Yamaguchi S, Kawakami E, Kanda N, Matsubara S, Kuro-o M, Nabeshima Y, Nakamura K, Oyanagi K	Klotho insufficiency causes decrease of ribosomal RNA gene transcription activity, cytoplasmic RNA and rough ER in the spinal anterior horn cells	Acta Neuropathol.	109	457-466	2005
Kikuchi-Horie K, Kawakami E, Kamata M, Wada M, Hu J-G, Nakagawa H, Ohara K, Watabe K, Oyanagi K	Distinctive expression of midkine in the repair period of rat brain during neurogenesis: immunohistochemical and immunoelectron misroscopic observations.	J. Neurosci. Res.	75	678-687	2004
Mochizuki Y, Mizutani T, Isozaki E, Ohtake T, Takahashic Y	Acute limbic encephalitis: a new entity?	Neuroscience Letters	394	5-8	2006
Uchihara T, Nakamura A, Mochizuki Y, Hayashi M, Orimo S, Isozaki E, Mizutani T	Silver stainings distinguidh Lewy bodies and glial cytoplasmic inclusions: comparison between Gallysa-Braak and Campbell-Switzer methods	Acta Neuropathol.	110	255-260	2005
山岡由美子、平井　健、川田明広、磯崎英治、板東充秋、望月葉子、小森隆司、内原俊記、小柳清光、水谷俊雄、林　秀明	著しい中心前回の萎縮と注目すべき組織学的所見を伴う多系統萎縮症	脳神経	57	255-265	2005

Shiotani A, Saito K, Araki K, Moro K, Watabe K	Gene Therapy for Laryngeal Paralysis	Ann Otol Rhinol Laryngol	116	115-122	2007
Mori Y, Shiotani A, Saito K, Araki K, Ikeda K, Nakagawa M, Watabe K, Ogawa K	A novel drug therapy for recurrent laryngeal nerve injury using T-588	Laryngoscope			印刷中
Sakuraba H, Chiba Y, Kotani M, Kawashima I, Ohsawa M, Tajima Y, Takaoka Y, Jigami Y, Takahashi H, Hirai Y, Shimada T, Hashimoto Y, Ishii K, Kobayashi T, Watabe K, Fukushige T, Kanzaki T	Corrective effect on Fabry mice of yeast recombinant human alpha-galactosidase with N-linked sugar chains suitable for lysosomal delivery	J Hum Genet	51	341-352	2006
Ito Y, Wiese S, Funk N, Chittka A, Rossoll W, Boemmel H, Watabe K, Wegner M, Sendtner M	Sox10 regulates ciliary neurotrophic factor (Cntf) gene expression in Schwann cells	Proc Natl Acad Sci USA	103	7871-7876	2006
Sango K, Kato N, Hirooka H, Tanuma M, Watabe K	High glucose-induced activation of the polyol pathway and changes of gene expression profiles in immortalized adult mouse Schwann cell IMS32: a novel in vitro model for the study of diabetic neuropathy	J Neurochem	98	446-458	2006
Kobayashi H, Watabe K, Izuka S, Tani H, Matsuura Y, Barsoum J, Kaynor C, Ohashi T, Etoh Y	Successful transduction of mammalian astrocytes and oligodendrocytes by "peudotyped" baculovirus vector in vitro and in vivo	Jikeikai Med J	53	55-62	2006
Hayashi Y, Kawazoe Y, Sakamoto T, Ojima M, Wang W, Takazawa T, Miyazawa D, Ohya W, Funakoshi H, Nakamura T, Watabe K	Adenoviral gene transfer of hepatocyte growth factor prevents death of injured adult motoneurons after peripheral nerve avulsion	Brain Res	1111	Brain Res	2006
Marubuchi S, Okuda T, Tagawa K, Enokido Y, Horiuchi D, Shimokawa R, Tamura T, Qi M-L, Eishi Y, Watabe K, Shibata M, Nakagawa M, Okazawa H	Hepatoma-derived growth factor, a new trophic factor for motor neurons, is up-regulated in the spinal cord of PQBP-1 transgenic mice before onset of degeneration	J Neurochem	99	70-83	2006
Moro K, Shiotani A, Watabe K, Takeda Y, Saito K, Mori Y, Ogawa K	Adenoviral gene transfer of BDNF and GDNF synergistically prevent motoneuron loss in the nucleus ambiguus	Brain Res	1076	1-8	2006
Hozumi I, Uchida Y, Watabe K, Sakamoto T, Inuzuka T	Growth inhibitory factor (GIF) can protect brain damage after stab wounds in rat brain	Neurosci Lett	395	220-223	2006

Araki K, Shiotani A, Watabe K, Saito K, Moro K, Ogawa K	Adenoviral GDNF gene transfer enhances neurofunctional recovery after recurrent laryngeal nerve crush injury	Gene Ther	13	296-303	2006
Hayashi Y, Hashizume T, Wakida K, Satoh M, Uchida Y, Watabe K, Matsuyama Z, Kimura A, Inuzuka T, Hozumi I	Association between metallothionein genes polymorphisms and sporadic amyotrophic lateral sclerosis in a Japanese population	Amyotroph Lateral Scler Other Motor Neuron Disord	7	22-26	2006
Murata M, Kotani M, Tajima Y, Tsuji D, Ishibashi Y, Kuroki A, Itoh K, Watabe K, Sango K, Shoji Y, Sakuraba H	Establishment of immortalized Schwann cells from a Sandhoff mouse and corrective effect of recombinant human beta-hexosaminidase A on the accumulated GM2 ganglioside	J Hum Genet	50	460-467	2005
Ikeda K, Aoki M, Kawazoe Y, Sakamoto Y, Hayashi Y, Ishigaki A, Nagai M, Kamii R, Kato S, Itoyama Y, Watabe K	Motoneuron degeneration after facial nerve avulsion is exacerbated in pre-symptomatic transgenic rats expressing human mutant Cu/Zn superoxide dismutase	J Neurosci Res	82	63-70	2005
Watabe K, Hayashi Y, Kawazoe Y	Peripheral nerve avulsion injuries as experimental models for adult motoneuron degeneration	Neuropathology	25	371-380	2005
Shen J-S, Meng X-L, Yokoo T, Sakurai K, Watabe K, Ohashi T, Eto Y	Widespread and highly persistent gene transfer to the CNS by retrovirus vector *in utero*: Implication for gene therapy to Krabbe disease	J Gene Med	7	540-551	2005
Meng X-L, Shen J-S, Watabe K, Ohashi T, Eto Y	GALC transduction leads to morphological improvement of the twitcher oligodendrocytes in vivo.	Mol Genet Metab	84	332-342	2005
Sango K, Tokashiki A, Ajiki K, Horie M, Kawano H, Watabe K, Horie H, Kadoya T.	Synthesis, localization and externalization of galectin-1 in mature dorsal root ganglion neurons and Schwann cells.	Eur J Neurosci	19	55-64	2004
Shirakura M, Inoue M, Fujikawa S, Washizawa K, Komaba S, Maeda M, Watabe K, Yoshikawa Y, Hasegawa M.	Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils.	Gene Ther	11	784-790	2004

Tateno M, Sadakata H, Tanaka M, Itohara S, Shin RM, Miura M, Masuda M, Aosaki T, Urushitani M, Misawa H, Takahashi R	Calcium-permeable AMPA receptors promote misfolding of mutant SOD1 protein and development of amyotrophic lateral sclerosis in a transgenic mouse model.	Hum. Mol. Genet	13	2183-2196	2004
Oda Y, Muroishi Y, Misawa H, Suzuki S.	Comparative study of gene expression of cholinergic system-related molecules in the human spinal cord and term placenta.	Neuroscience	128	39-49	2004
Burau K, Stenull I, Huber K, Misawa H, Berse B, Unsicker K, Ernsberger U.	c-ret regulates cholinergic properties in mouse sympathetic neurons: evidence from mutant mice.	Eur. J. Neurosci.,	20	353-362	2004
Mori T, Yuxing Z, Takai H, Takeuchi M, Iseki K, Hagino S, Kitanaka J-i, Takemura M, Misawa H, Ikawa M, Okabe M, Wanaka A.	The LIM homeobox gene, L3/Lhx8, is necessary for proper development of basal forebrain cholinergic neurons.	Eur. J. Neurosci.	19	3129-3141	2004
Nakata K, Okuda T, Misawa H.	Ultrastructural localization of high-affinity choline transporter in the rat neuromuscular junction: Enrichment on synaptic vesicles.	Synapse	53	53-56	2004
Yamazaki M, Esumi E, Nakano I	Is motor neuronal cell death in amyotrophic lateral sclerosis apoptosis ?	Neuropathology	25	381-387	2005
Miyasaka T, Watanabe A, Saito Y, Murayama S, David M.A. Mann, Yamazaki M, Ravid R, Morishima-Kawashima M, Nagashima K, Ihara Y	Visualization of newly deposited tau in neurofibrillary tangles and neuropil threads	J Neuropathol Exp Neurol	64	665-674	2005
Hachiya NS, Ohkubo T, Kozuka Y, Yamazaki M, Mori O, Mizusawa H, Sakasegawa Y, Kaneko K	More than a 100-fold increase in immunoblot signals of laser-microdissected inclusion bodies with an excessive aggregation property by oligomeric actin interacting protein 2/d-lactate dehydrogenase protein 2	Anal Biochem	347	106-111	2005

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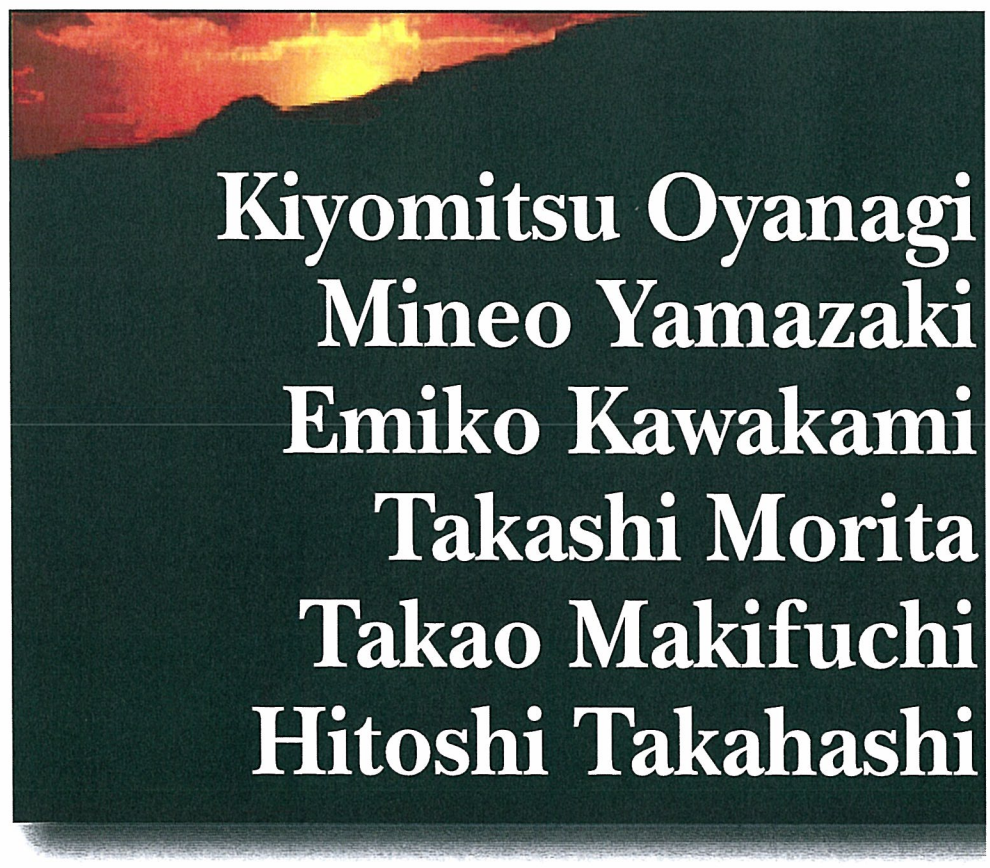

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Chapter VII

On the Origin of the Degenerated Fibers in the White Matter of the Spinal Cord in Amyotrophic Lateral Sclerosis

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ABSTRACT

The characters of the degeneration of the white matter of the spinal cord of amyotrophic lateral sclerosis (ALS) were examined, and the origins of the degenerated fibers were discussed.

1. The degree of large myelinated fiber loss in the lateral corticospinal tract (l-CST) did not correlate with either the duration of their illness or their history of respirator use. Direct correlation of disease mechanism was absent between the anterior horn cells and the l-CST in classic ALS.

2. The patients who required respirator support showed more severe degeneration of the anterolateral funiculus (ALF) in the ALS than those who required none, and the degree of myelinated fiber loss in the l-CST did not correspond with either the illness duration

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or the history of respirator use. Thus the large myelinated fibers in the l-CST and ALF degenerate independently in classic ALS.
3. The large myelinated fibers in the ALF of the mid-cervical spinal cord of humans originate from the tegmentum of the brain stem and the lower cervical spinal cord, not from the cerebrum, or the thoracic or lumbar spinal cord. The origin of the large myelinated fibers in the ALF of the spinal cord in humans, is considered to be the long-descending neurons in the brain stem tegmentum and the propriospinal neurons in the spinal cord.

Keywords: Amyotrophic lateral sclerosis, Anterolateral funiculus, Corticospinal tract, Middle root zone, Morphometry, Spinal neurons.

Introduction

Amyotrophic lateral sclerosis (ALS) is a motor neuron disease occurring in adults mainly involving the upper and lower motor neurons with relative sparing of the abducens and oculomotor nerve nuclei, and of the autonomic and sensory neurons in the brain stem and spinal cord. About 90% of ALS patients have been reported to be sporadic, and the sporadic ALS are roughly classified into classic ALS, "multi-system type ALS" [Hayashi & Kato 1989, Mizutani et al. 1992], and ALS with temporal lobe involvement [Yuasa 1970, Mitsuyama & Takamatsu 1971, Nakano et al. 1992]. Among the familial ALS patients with inheritance, superoxide dismutase (SOD)-1 gene mutation was revealed in about 20% of the patients [Shaw 2001].

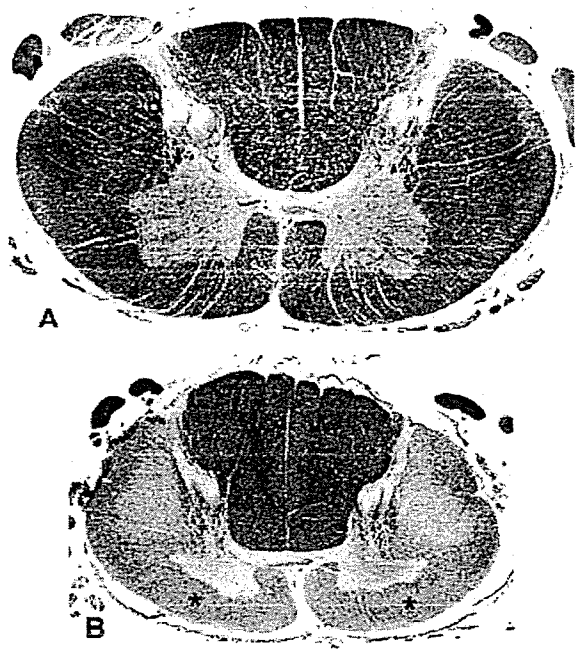

Figure 1. Cervical cord of a control subject (A) and a patient with classic ALS (B). Asterisks indicate ALF. Klüver-Barrera preparation.

In the spinal cord of ALS, degeneration of the corticospinal tract (CST), lateral and anterior, is commonly obvious. In addition to this, since being proposed by Charcot [1880], it has been reported repeatedly that degeneration of the anterolateral funiculus (ALF) occurs in the spinal cord of patients with classic ALS (Fig. 1) [Holmes 1909, Ikuta et al. 1979, 1982, Lawyer & Netsky 1953, Smith 1960]. The posterior funiculus was scarcely/occasionally involved in the classic ALS, but severely deteriorated in the "multi-system type ALS" [Hayashi & Kato 1989, Mizutani et al. 1992]. Some cases of familial ALS with or without superoxide dismutase (SOD)-1 mutation show degeneration of the posterior funiculus and spinocerebellar and spinothalamic tracts in addition to the CST degeneration.

In the present paper, the characteristic patterns of the degeneration of the white matter of the spinal cord of ALS patients were analyzed, and the origins of the degenerated fibers was discussed, based on the observation by the authors and the literature review.

Materials and Methods

1. Examined Subjects

The control subjects and patients with sporadic and familial ALS examined by the authors were Japanese, and none of them had had cardiac arrest, hypoglycemic episodes, or severe liver dysfunction. Neuropathological findings of many of the cases were previously reported by the authors [Ikuta et al. 1979, 1982, Makifuchi & Ikuta 1977, Oyanagi et al. 1983, 1989, 1995, 1999, Takahashi et al. 1992, 1993a, 1993b, 1994].

2. Light Microscopic Examination

Serial 6-μm-thick sections of the brains and spinal cords were made from 10% formalin- or 4 % paraformaldehyde in phosphate buffer-fixed-paraffin embedded blocks, and stained with hematoxylin-eosin, Klüver-Barrera, Bodian, Gallyas, phosphotungustic acid hematoxylin, periodic acid Schiff and other preparations, and examined light microscopically. Other sections (also 6 μm thick) were subjected to immunohistochemical staining using the avidin-biotin-peroxidase complex (ABC) method, using a Vectastain ABC kit (Vector, Burlingame, CA, USA). The primary antibodies used were: rabbit anti-cow ubiquitin polyclonal antibody (dilution 1:150; Dakopatts A/S, Glostrup, Denmark), a rabbit anti-cystatin C (dilution 1:1000; DAKO, Denmark), a rabbit anti-Cu/Zn superoxide dismutase (SOD; dilution 1:10000, a gift from Dr. K. Asayama, Yamanashi Medical School, Yamanashi, Japan), a mouse anti-phosphorylated neurofilament protein (SMI-31; dilution 1:10000, Sternberger Monoclonals, Baltimore, Md, USA), a rabbit anti-glial fibrillary acidic protein (GFAP) polyclonal antibody (dilution 1:500; Dakopatts A/S, Glostrup, Denmark), rabbit anti-human tau polyclonal antibody [dilution 1:1000; a gift from Prof. Y. Ihara, Tokyo University, Tokyo, Japan], anti-tau monoclonal antibody AT8 (dilution 1:1000) (Innogenetics, Belgium), and anti-α-synuclein monoclonal antibody LB509 [gift from Prof. T. Iwatsubo, Tokyo University, Tokyo, Japan]. Antigenicity was increased for ubiquitin

immunostaining by pretreating the sections with 0.025% trypsin for 15 min at room temperature, and for α-synuclein immunohistochemistry by pretreating the sections with hydrated autoclaving (121°C, 15 min). Nonspecific binding of the biotin/avidin system reagents was blocked by pretreating the sections with a blocking solution from the kit (Vector), and then incubating them with the required primary antibody overnight at 4°C. The sections were then incubated with the secondary reagent containing biotinylated anti-rabbit, or anti-mouse IgG (diluted 1:200) for 2 h, and finally with the ABC solution for 1 h. The sections were subjected to the peroxidase reaction using freshly prepared 0.02% 3,3'-diaminobenzidine-tetrahydrochloride and 0.005% hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6, for 10 min at room temperature. As antibody controls, the primary antisera were either omitted or were replaced with normal rabbit or mouse serum. Several specimens of neural and non-neural tissue from the patients served as positive or negative tissue controls.

3. Topographic and Quantitative Study of the Neurons in the Spinal Cord

Tissues were embedded in paraffin, serially sectioned at 8 μm, and stained with thionine. The thickness of the sections was verified by manipulating the fine adjustment drum of the light microscope. Five serial sections each of the 5th cervical and of the 7th thoracic segment and 4 serial sections of the second sacral segment were examined. The entire spinal gray matter of each serial section was surveyed under 1000-fold magnification. Neurons were identified by the presence of Nissl substance and prominent nucleoli. The longest diameter of the nucleus (A) and the largest dimension perpendicular to the diameter (B) were measured with an ocular micrometer (4), and the nuclear area (S) was calculated according to the formula $S=\pi AB/4$. There was a significant positive correlation coefficient ($r=0.97$) between the area obtained by calculation and that obtained by counting 1 mm squares of section paper over the enlarged photograph of the nuclei.

The frequency distribution of nuclear areas by 10 μm^2 increments was obtained for each case, and it was represented in graphs as the nuclear area x n (number of nuclei) to show that the large nuclei, though fewer in number, cover a wider area.

Spinal neurons were classified into 14 groups according to their nuclear areas. The first group was composed of neurons whose nuclear areas were smaller than 40 μm^2. Neurons with nuclear area ranging from 41 to 150 μm^2 were divided into 11 groups (2nd to 12th) in 10 μm^2 increments. The 13th group consisted of neurons with nuclear areas of 151 to 200 μm^2, and the 14th, neurons with nuclear areas greater than 201 μm^2. Each of the neurons was expressed by dots of various sizes and was plotted on the trace of the spinal gray matter magnified 100 times.

4. Quantitative Examination of the Myelinated Fibers in the Lateral Corticospinal Tract (l-CST) and Anterolateral Funiculus (ALF) of the Spinal Cord

The fourth or fifth cervical segments of the spinal cord were fixed in 20% formalin in 0.1 M phosphate buffer (PB; pH 7.3) or 3% glutaraldehyde-1% paraformaldehyde in 0.1 M PB (pH 7.3). The right half of each segment was then fixed in 1% osmium tetroxide in 0.1 M PB (pH 7.3) followed by dehydration through a graded ethanol series and embedded in Epon 812. Sections (1 μm thick) were cut, stained with toluidine blue, and then examined with the aid of a light microscope.

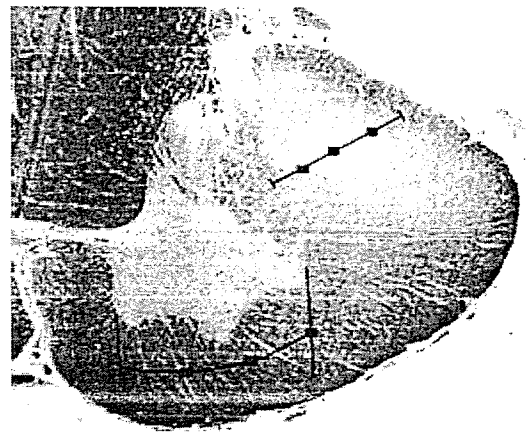

Figure 2. Photographs were taken at three points in both the anterolateral funiculusu (ALF) and lateral corticospinal tract (l-CST). For further details, see text. Cited from ref. Oyanagi et al. 1995.

Photographs (x200) were taken at three points in both the ALF and l-CST. The ALF was divided anteriorly into three equal parts from the medial to lateral margins of the anterior horn and photographs of the mid-medial, mid-lateral and lateral portions were taken (Fig. 2). Large bundles of myelinated fibers with diameters of 10-12 μm (thought to be the intramedullary portion of the anterior spinal root) crossing the ALF were avoided when taking the photographs. The l-CST was divided into four equal parts and photographs were taken at positions midway between the three dividing lines (mid-medial, medial and mid-lateral portions). Enlarged prints (x2285) were made and the mean diameter of the myelinated fibers was obtained, using a digitizer, by averaging the longest and shortest diameters (the latter was perpendicular to the former).

In the present study, in order to determine the origin of the large myelinated fibers in the ALF of the human spinal cord, the number of which is severely reduced in patients with ALS, myelinated fibers in the ALF of the mid-cervical spinal cord were examined quantitatively in five groups of subjects, including control subjects (Group I). The disease groups that were examined included patients with cerebral lesions showing complete degeneration of the unilateral/bilateral pyramis of the medulla oblongata (Group II), those with lesions of the pontine tegmentum (Group III), those with lesions of the lower cervical spinal cord (Group IV), and those with thoracic/lumbar lesions (Group V) (Fig. 3).

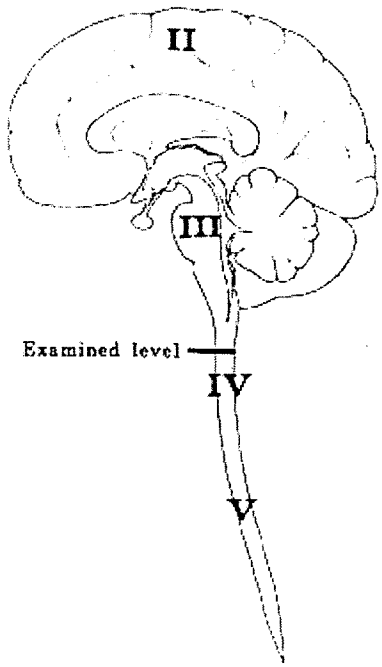

Figure 3. Myelinated fibers in the ALF of the mid-cervical spinal cord were examined quantitatively in five groups of subjects, including control subjects (Group I). The disease groups that were examined included patients with cerebral lesions showing complete degeneration of the unilateral/bilateral pyramis of the medulla oblongata (Group II), those with lesions of the pontine tegmentum (Group III), those with lesions of the lower cervical spinal cord (Group IV), and those with thoracic/lumbar lesions (Group V).

The data for the three areas in the ALF and l-CST of each patient and control subject were summed, and the frequency distribution of the myelinated fiber diameters, in 1-μm increments, was determined and represented on bar charts as the number of myelinated fibers (n) x πr^2 (r: half the mean diameter of the myelinated fibers), to show that the large myelinated fibers, although fewer in number, cover a wide area. The total area of the ALF and of the l-CST in each patient examined was 0.057 mm^2. For the control subjects, the average of each increment was expressed on the bar chars, and for the ALS cases, the data obtained for each patient and the average values for the patients with and without respirator-support were evaluated. Statistical evaluation was performed using the Mann-Whitney U test to compare the number of myelinated fibers with diameters of less than 3 μm (small), 3-6 μm (medium), and over 6 μm (large).

The myelinated fibers in the ALF and l-CST were quantitatively examined bilaterally in control subjects and in patients with cerebral lesions. Based on the obtained data that there was no difference in number between the myelinated fibers in the right and left side, those in the right side of the ALF and l-CST were examined in other groups.

Anatomic Definition of Nerve Fiber Tracts in the Spinal Cord

The white matter of the spinal cord contains bundles of various nerve fiber tracts. It has been reported that the anterolateral funiculus (ALF), which is the ventral part of the lateral funiculus [Nathan et al. 1996], contains, as long-descending tracts, the ventral pyramidal tract (human [Barnes 1901]), corticospinal tracts (human [Nathan et al. 1990]), reticulospinal tracts (cat, opossum and human [Ikuta et al, 1982, Iwamoto et al. 1990, Martin et al. 1979, Nathan et al. 1996, Nyberg-Hansen 1965, Parent 1996]), the vestibulospinal tract (cat and human [Parent 1996, Rose et al. 1996, Shinoda et al. 33]), and raphe spinal tracts (opossum [Martin et al. 1982]). In addition, the ALF contains, as long-ascending tracts, the spinothalamic tract (human [Kuru 1976, Parent 1996, Smith 1951, Smith 1957]), the spinoreticular tract (human [Parent 1996]), the spinocerebellar tract (human [Kuru 1976, Parent 1996]), and Helweg's triangular tract (human [Smith & Deacon 1981]). Propriospinal fibers (cat and human [Altermark et al. 1987, Giok 1958, Parent 1996]) have also been observed.

On the origin of these fibers, experimental studies of animals and human autopsy cases with various destructive lesions in the brain and spinal cord revealed the topographic localization of the neurons, however, the origin neurons of the above mentioned nerve tracts have not yet been completely ascertained in humans so far (Fig. 1).

Lateral Corticospinal Tract (l-CST) Degeneration and Spinal Neuron Loss

Patients with ALS of long course of the illness or on artificial respiration usually show severe loss of neurons in the spinal gray matter including anterior horn and intermediate zone (Fig. 4 & 5). On the contrary, the spinal cord of a housewife, who died at the age of 59 with 1 year-7 months-clinical course refusing the use of a respirator, and whose muscular strength was fairly well preserved up to death, shows only moderate loss of AHCs and quite well preserved neurons in the intermediate zone (Fig. 6). The findings indicate that the primary degeneration may occur in the AHCs and the neurons in the intermediate zone degenerate sequentially in the spinal gray matter in ALS (Fig. 7).

These findings indicate the occurrence of a sequential degeneration of the neurons in the intermediate zone (Rexed's [Rexed 1954] laminae V-VIII) of the spinal cord to loss of anterior horn cells (Rexed's lamina IX) in patients with ALS [Oyanagi et al. 1983, 1989]. Terao et al. [1994] also reported neuronal loss in the intermediate zone of the ALS spinal cord. Long-ascending neurons [Parent 1996], internuncial neurons [Parent 1996], and propriospinal neurons [Altermark et al. 1987, Molenaar & Kuypers 1978] have been shown to originate in the intermediate zone of the spinal cord.

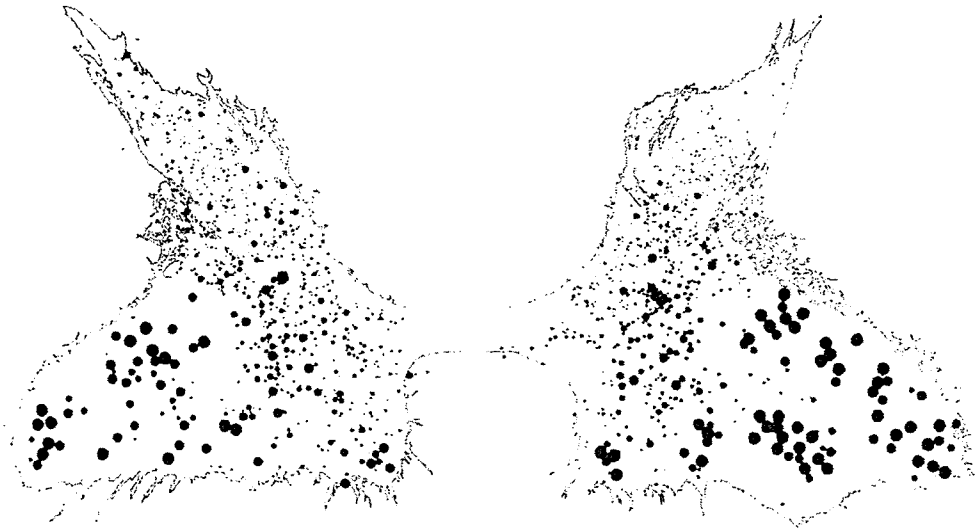

Figure 4. Size distribution of neurons in the cervical gray matter of a control subject. Large neurons, with a nuclear area greater than 151 μm^2, are located in lamina IX; middle sized neurons, 71 to 120 μm^2, in laminae IV-VIII; and numerous small neurons are distributed in laminae II, III and VII. This pattern of distribution seems to correspond to the laminar cell architecture of the cat reported by Rexed (1954). Cited from ref. Oyanagi et al. 1983.

Figure 5. Size distribution of neurons in the cervical gray matter of an advanced ALS patient. The large neurons in lamina IX disappear almost completely. The middle-sized neurons located in laminae VI, VII, and VIII decrease markedly. However, the distribution of the small neurons appears the same as in the control subjects in all parts of the spinal gray matter. Cited from ref. Oyanagi et al. 1983.

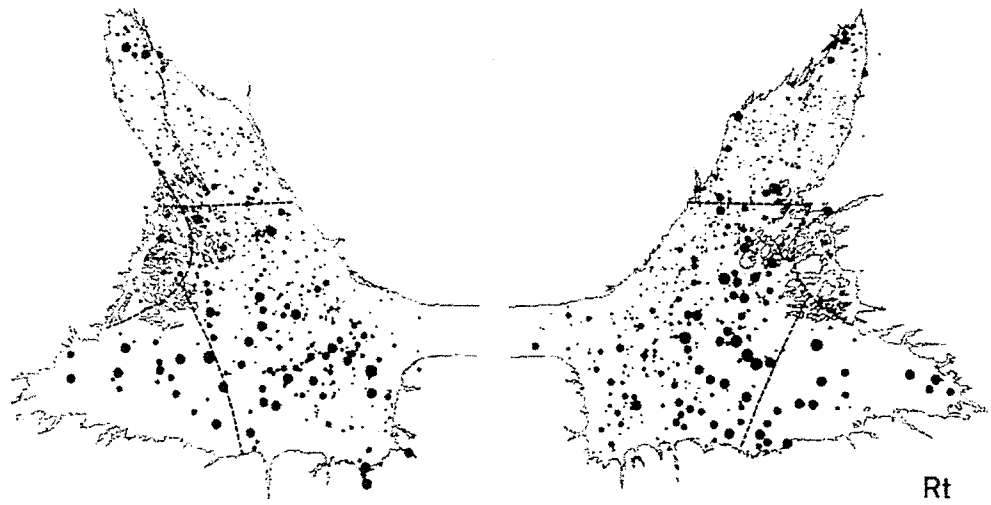

Figure 6. Size distribution of the neurons in the cervical gray matter of a ALS patient, whose muscular strength was fairly well preserved up to death. The large neurons, with a nuclear area greater than 151 μm^2, are severely decreased in Rexed's lamina IX. However, the degree of decrease of the neurons is not equal to that of advanced ALS patients. The neurons in the intermediate zone and posterior horn are quite well preserved. Cited from ref. Oyanagi et al. 1989.

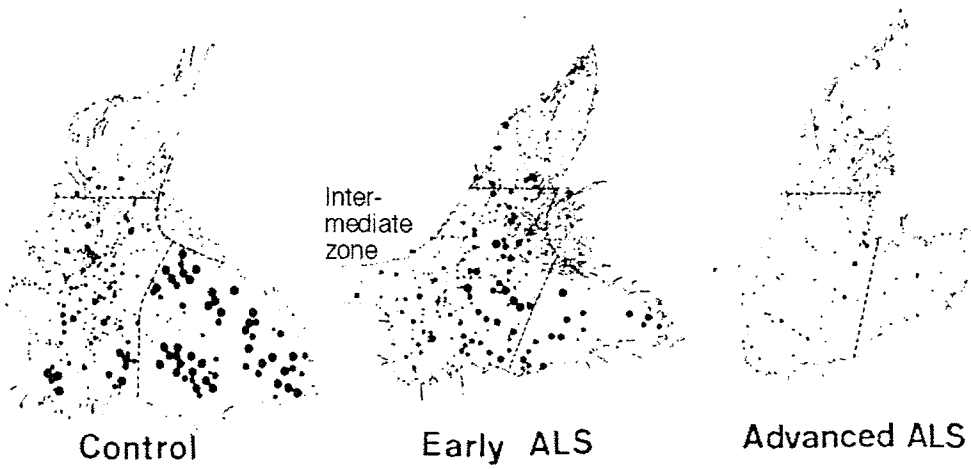

Figure 7. Progression pattern of neuronal loss in the spinal gray matter of ALS. The findings indicate that the primary degeneration may occur in the anterior horn cells and the neurons in the intermediate zone degenerate sequentially in the spinal gray matter in ALS.

It has been reported that the degeneration of the CST caused by hemispherectomy [Ikuta et al. 1982] and stroke [Terao et al. 1997] does not induce trans-synaptic degeneration in the AHCs in humans. Long duration of the illness and respirator use tend to cause severe degeneration of both the l-CST and marked loss of neurons in the spinal gray matter. However, some patients with marked loss of spinal neurons show only mild degeneration of the l-CST (Fig.8). Based on these findings, the degree of myelinated fiber loss in the l-CST did not completely correlate with either the duration of their illness or their history of

respirator use [Oyanagi et al. 1995] These findings mean absence of direct correlation of disease mechanism between the AHCs and the l-CST in ALS.

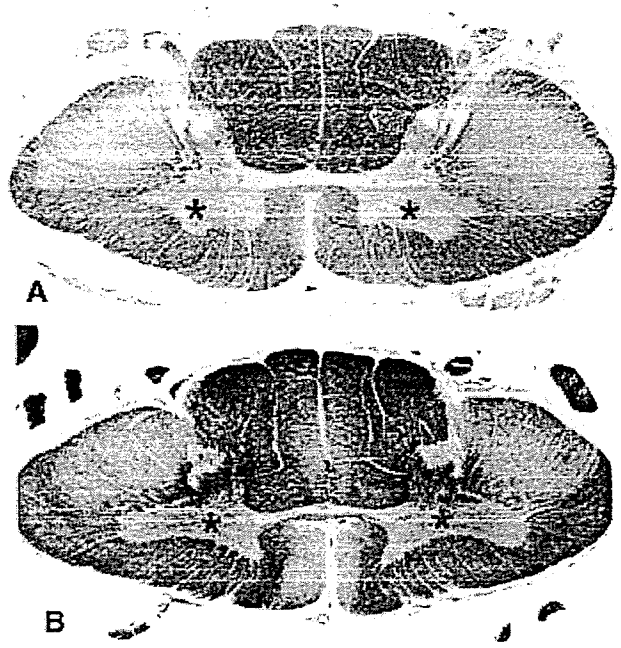

Figure 8. An ALS patient with relative preserved AHCs (asterisks) but severe degeneration of the l-CST (A). An ALS patient with marked loss of AHCs (asterisks) but relatively slight degeneration of the l-CST (B). Cervical cord. Klüver-Barrera preparation.

Laterality of Corticospinal Tract (CST) Degenerration in ALS: Proportion of Crossed and Uncrossed Fibers

Symptoms of most of the ALS patients occurs in unilateral extremity at the initiation. According to the course of the disease, muscle weakness usually progressed to the other extremity in the patients, and the neuropathological examination reveals symmetric or almost symmetric degeneration of the CST and loss of AHCs. However, occasionally, there have been reported asymmetric degeneration of the l-CST [Reuter 1931, Swash et al. 1988] and loss of AHCs [Mochizuki et al. 1995].

The proportion of the crossed (lateral) and uncrossed (anterior) CST should be considered, when asymmetric degeneration of the CST is present in the spinal cord, since dysproportion, probably developmental, of the crossed and uncrossed CST is observed occasionally in Japanese subjects in routine neuropathological examination. It has been observed that the apparent asymmetry of the CST degeneration was caused not by the severity of the degeneration, but by the dyspropotion of the nerve fibers of the crossed and uncrossed CST (Fig. 9).

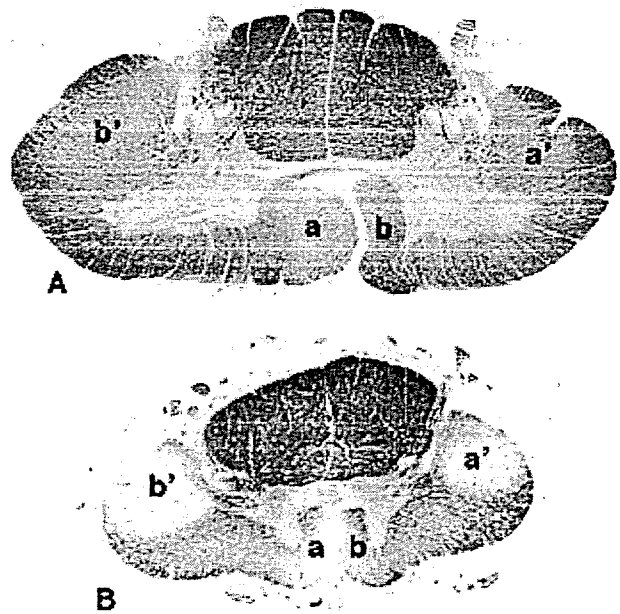

Figure 9. ALS patients showing asymmetry of the spinal white matter. Volume of the left anterior CST (a) is large, and that of right anterior CST (b) is small, but left lateral CST (b') is large, and right lateral CST (a') is small. This finding indicates a + a` = b + b'. A: cervical, B; thoracic of a different patient. Klüver-Barrera preparation.

Loss of Myelinated Fibers in the Anterolateral Funiculus (ALF) in Classic ALS

In the white matter of most the patients with sporadic ALS, the ALF degenerates along with the lateral and anterior CST [Charcot 1880, Holmes 1909, Ikuta et al. 1979, 1982, Lawyer & Netsky 1953, Oyanagi et al. 1995, Smith 1960]. In the present morphometric study on the myelinated fibers in the ALF and l-CST of the cervical segment revealed that: (1) marked and significant loss of large myelinated fibers in the ALF of ALS patients, (2) the patients who required respirator support showed more severe degeneration of the ALF in the ALS than those who required none, and (3) the degree of myelinated fiber loss in the l-CST did not correspond with either the illness duration or the history of respirator use, (4) large myelinated fibers in the l-CST and ALF degenerate independently in classic ALS [Oyanagi et al. 1995] (Fig. 10).

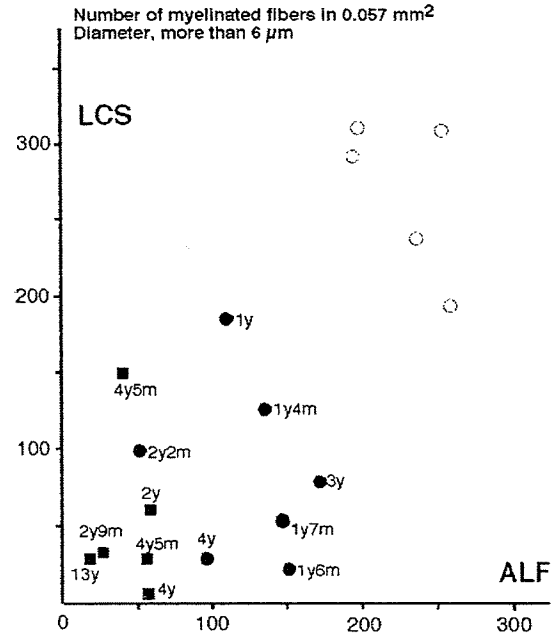

Figure 10. Evaluation of the ratio for the decrease in numbers of large myelinated fibers between the ALF and l-CST. Open circle; control subject, solid circle; ALS patient without respirator support, solid square; ALS patient with respirator support. Numbers indicate the duration of illness. Patients who required respirator support showed more severe degeneration of the ALF in the ALS than those who required none, and the degree of myelinated fiber loss in the l-CST did not correspond with either the illness duration or the history of respirator use. Cited from ref. Oyanagi et al. 1995.

Origin of the Degenerated Fibers of the ALF in Classic ALS

The neurons originating the reticulospinal tract, the neurons in the spinal cord [Ikuta et al. 1982], and the propriospinal neurons [Oyanagi et al. 1983, 1989] have been proposed as the origins of the degenerated fibers observed in the ALF of patients with ALS.

In the present study, in order to determine the origin of the large myelinated fibers in the ALF of the human spinal cord, the number of which is severely reduced in patients with ALS, myelinated fibers in the ALF of the mid-cervical spinal cord were examined quantitatively in five groups of subjects, including control subjects (Group I). The disease groups that were examined included patients with cerebral lesions showing complete degeneration of the unilateral/bilateral pyramis of the medulla oblongata (Group II), those with lesions of the pontine tegmentum (Group III), those with lesions of the lower cervical spinal cord (Group IV), and those with thoracic/lumbar lesions (Group V) (Fig. 3).

The results of the present study have revealed that: (1) large myelinated fibers in the ALF of the mid-cervical spinal cord originate from the tegmentum of the brain stem and from the lower cervical spinal cord. (2) large and medium-sized myelinated fibers in the ALF of the mid-cervical spinal cord are not corticospinal tracts, (3) nor are these fibers long-ascending tracts from the thoracic and lumbar spinal cord (Table 1).

A histological and quantitative study has revealed neither degenerative fibers, loss of myelinated fibers, nor atrophy of the ALF in patients with hemispherectomy [Ueki 1966]. The finding concurs with the results of the present study.

The number of large and medium-sized myelinated fibers in the ALF of the mid-cervical spinal cord in patients with complete transverse myelopathy at the thoracic level was not reduced. This indicates that the long-ascending tracts, such as the spinothalamic [Kuru 1976, Parent 1996, Smith 1951, Smith 1957], spinoreticular [Parent 1996], spinocerebellar [Kuru 1976, Parent 1996], and Helweg's triangular [Smith & Deacon 1981] tracts are either not composed of large or medium-sized myelinated fibers, or else do not pass through the areas investigated in the present study.

Table 1. The number of myelinated fibers in the ALF. The mean ± SD of the values are indicated. The examined area is 0.057 mm^2 in each subject or patient. Statistical evaluation was performed using the Mann-Whitney U test to compare the numbers of myelinated fibers with diameters of less than 3 μm, 3-6 μm and over 6 μm. In the patients with lesions of the pontine tegmentum (group III) and with lesions of the lower cervical cord (group IV), the number of myelinated fibers with a diameter of over 6 μm had decreased significantly. Cited from ref. Oyanagi et al. 1999

		Diameter		
		< 3.0 μm	3-6 μm	> 6.0 μm
I. Control	(n=5)	2645.2 +/- 247.6	605.2 +/- 62.8	158.4 +/- 11.6
II. Cerebral lesions	(n=4)	2335.5 +/- 400.9	627.0 +/- 83.6	183.0 +/- 91.6 *
III. Pontine tegmentum lesions	(n=5)	2745.4 +/- 607.6	620.2 +/- 54.5	56.4 +/- 19.3 *
IV. Lower cervical lesions	(n=5)	3472.4 +/- 802.8	519.2 +/- 117.9	88.6 +/- 23.6
V. Thoracic/lumbar lesions	(n=4)	2403.0 +/- 83.2	607.0 +/- 91.6	148.0 +/- 25.6

* P< 0.01

The result of the present study shows that a proportion of the large myelinated fibers in the ALF of the mid-cervical spinal cord originate from the lower cervical cord, and that the large myelinated fibers in the ALF are not long-ascending fibers from the thoracic and lumbar spinal cord. This finding suggests that the large myelinated fibers reduced in number in patients with lower cervical involvement are not long-ascending fibers, but propriospinal fibers connecting neighboring segments. The present study has also revealed that the large myelinated fibers in the ALF of the mid-cervical segment originate from the tegmentum of the brain stem and the lower cervical spinal cord, and their origins are considered to be reticulo-, vestibulo- and/or raphe-spinal neurons, and propriospinal neurons.

In advanced ALS patients who require the long-term use of a respirator, an extensive reduction in the number of neurons other than motor neurons has been observed in addition to complete loss of the anterior horn cells [Hayashi & Kato 1989, Mizutani et al. 1992]. It has also been noted that the tegmentum of the brain stem and the intermediate zone of the spinal cord exhibit extremely severe atrophy and neuronal loss, and that the ALF degenerates

markedly in these ALS patients [Hayashi & Kato 1989, Mizutani et al. 1992]. Bunina bodies and ubiqutin-immunopositive inclusions have been observed in the neurons of the reticular formation of the medulla oblongata in patients with ALS [Bergmann 1993, Nakano et al. 1993]. This indicates that an ALS specific disease process exists in the neurons in the reticular formation.

It has been shown that propriospinal neurons at the cervical segment are located in laminae V-VII (cat and monkey [Molenaar and Kuypers 1978]) of Rexed [Rexed 1954], and that these neurons play roles in movement control and sensorimotor integration of the extremities [Martin 1996, Pierrot-Deseilligny 1996]. The reticulospinal and propriospinal tracts terminate in laminae V-VIII in the cervical cord (cat [Barilari & Kuypers 1969]). Thus, the reticulospinal neurons in the brain stem and propriospinal neurons in the spinal cord are considered to be closely linked to the degeneration of the ALF in classic ALS.

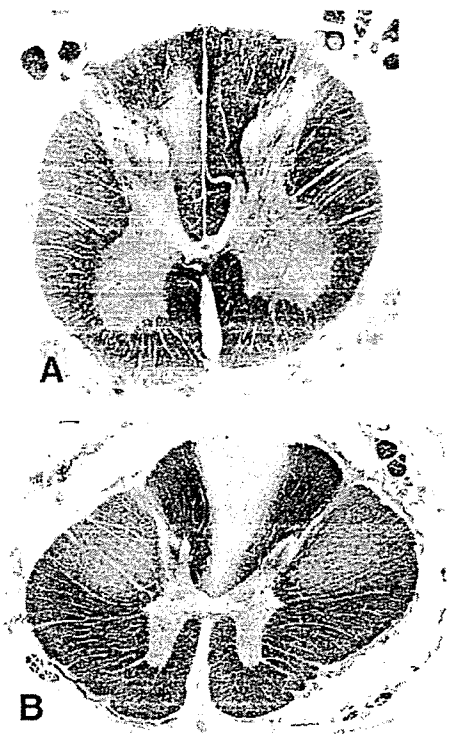

Figure 11. A familial ALS patient with SOD-1 gene mutation showing a degeneration of the left middle root zone (A) of the 12th thoracic segment. Mid-thoracic cord of a patient with SMON (B). Klüver-Barrera preparation.

Mechanism of the Middle Root Zone Degeneration in Familial ALS

Some patients of familial ALS with or without SOD-1 gene mutation show degeneration of the posterior funiculus, that is "middle root zone degeneration" (Fig. 11A) [Makifuchi and Ikuta 1977, Takahashi et al. 1994]. The pattern is quite different from that of the Wallerian