厚生労働科学研究費補助金（こころの健康科学研究事業）

II. 分担研究報告書

筋萎縮性側索硬化症の最早期病変を求めて：運動ニューロンにおける蛋白合成系の異常と治療法開発の試み

4. ALS脊髄で増殖する細胞の有無と細胞種の同定

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研究要旨

ALS脊髄における修復機転を明らかにし、神経幹細胞による治療法の開発の可能性を探る目的から、ALS脊髄で分裂する細胞の研究を行った。17年度、私どもは本研究によって弧発型ALS患者脊髄で増殖する細胞が主としてミクログリアと血管構成細胞であることを世界でもはじめて見出した。その一方アストロサイトやシュワン細胞の増殖が顕著でないこと、増殖細胞の局在は、必ずしも病変の強さと一致しないことが分かった。18年度、ミクログリアの分裂によって、活性型ミクログリアが生じている事を見出した。またALS脊髄では、ミクログリアの増殖は前角や側索などの変性部位で強く見られるものの、変性の程度との相関は認められなかった。

A. 研究目的

ALS脊髄における増殖細胞と修復機構を明らかにし幹細胞移植治療の可能性を探る目的から、ALS脊髄における増殖細胞の存在と局在を免疫組織学的に検討落を惹起するか解析する。

本年度の研究では、私どもが見出した、ALS脊髄で分裂する細胞、特にミクログリアの分裂と、組織修復や組織障害に関与すると言われている活性型ミクログリアとの関連を調べることを目的とした。

B. 研究方法

臨床病理学的に古典型ALSと診断された日本人計8剖検例および日本人対照計5剖検例を用いた。ホルマリン固定、パラフィン包埋された頸髄および腰髄切片を、細胞増殖マーカーとされるMIB-1抗体を用いて免疫染色し陽性細胞の局在と頻度を調べた。さらにMIB-1と、各種細胞マーカー、即ち、GFAP（アストロサイト）、GST-pai（オリゴデンドログリア）、Iba1（ミクログリア）、LCA（リンパ球）、Schwann/2E（シュワン細胞）、Nestin（幹細胞）とで二重免疫染色を行って増殖してい

る細胞種を検討した。LCS・AHC の一定範囲(56.25 μm2) 中に出現する陽性細胞数を計測し、さらに LCS・AHC の変性程度を 4 段階に分類し、これらの相関を検討した。

C. 研究結果と考察・結論

(1) Iba-1 陽性細胞数は ALS が対照より多く、対照では ramified microglia が中心で、ALS では活性型ミクログリアが数多く見られた。

(2) Iba-1 陽性細胞数は症例によりばらつきがあり、変性の程度との相関は認められなかった。

(3) Iba-1・MIB-1 二重陽性細胞は ALS で多い傾向にあったが、その proliferation index は 0〜1%であった。

ヒト ALS では、ミクログリアの増殖は脊髄変性部位で強く見られるものの、変性の程度との相関は認められなかった。活性型ミクログリアの増殖は単に変性を反映するのみではなく、他因子の関与にて種々の表現型を呈すことが示唆される。また、活性型ミクログリアは、ミクログリアの分裂によって生じることが示唆された。

D. 健康危険情報

特になし。

E. 研究発表

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F. 知的財産権の出願・登録状況

1. 特許取得

なし。

2. 実用新案登録

なし。

3. その他

なし。

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III. 研究成果の刊行に関する一覧表

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

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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].

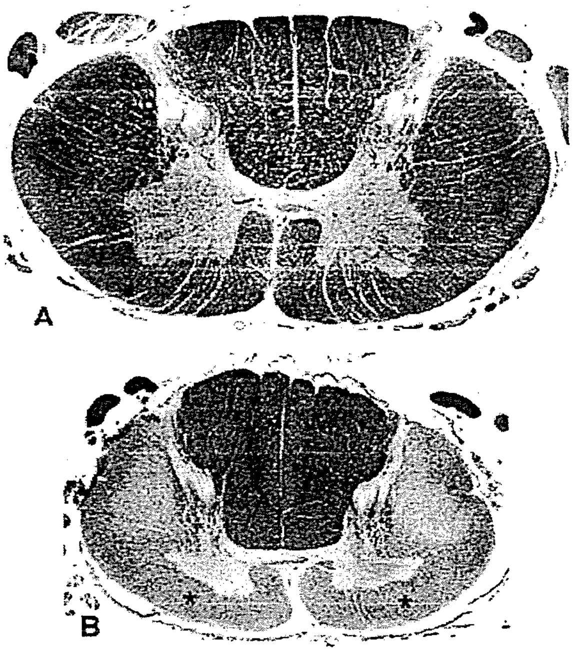

Figure 1. Cervical cord of a control subject (A) and a patient with classic ALS (B). Asterisks indicate ALF. Klüver-Barrera preparation.

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.

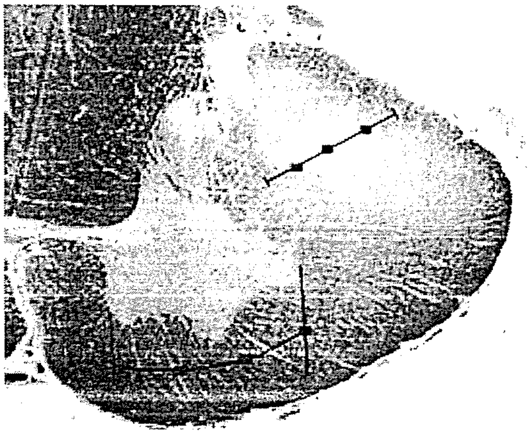

Figure 2. Photographs were taken at three points in both the anterolateral funiculusu (ALF) and lateral corticospinal tract (l-CST). For further details, see text. Cited from ref. Oyanagi et al. 1995.

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).

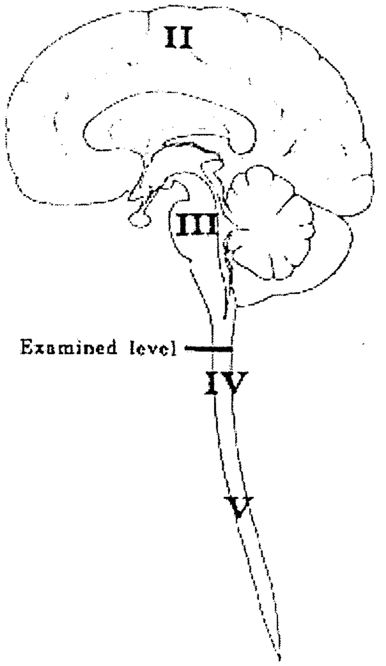

Figure 3. Myelinated fibers in the ALF of the mid-cervical spinal cord were examined quantitatively in five groups of subjects, including control subjects (Group I). The disease groups that were examined included patients with cerebral lesions showing complete degeneration of the unilateral/bilateral 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.

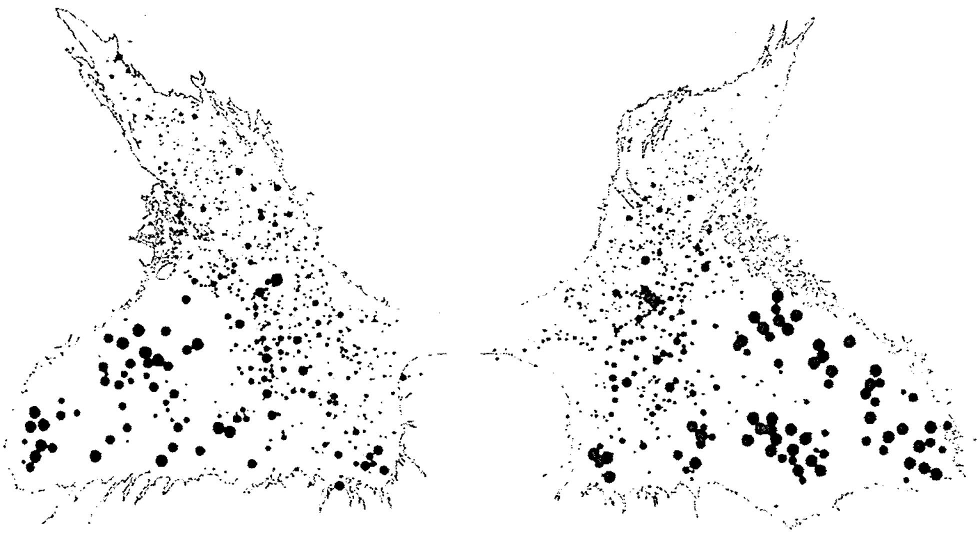

Figure 4. Size distribution of neurons in the cervical gray matter of a control subject. Large neurons, with a nuclear area greater than 151 μm^2, are located in lamina IX; middle sized neurons, 71 to 120 μm^2, in laminae IV-VIII; and numerous small neurons are distributed in laminae II, III and VII. 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.

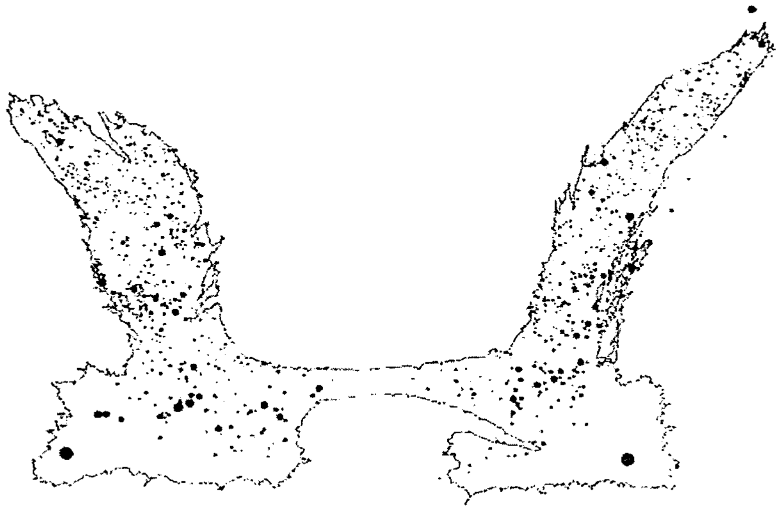

Figure 5. Size distribution of neurons in the cervical gray matter of an advanced ALS patient. The large neurons in lamina IX disappear almost completely. The middle-sized neurons located in laminae VI, VII, and VIII decrease markedly. However, the distribution of the small neurons appears the same as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

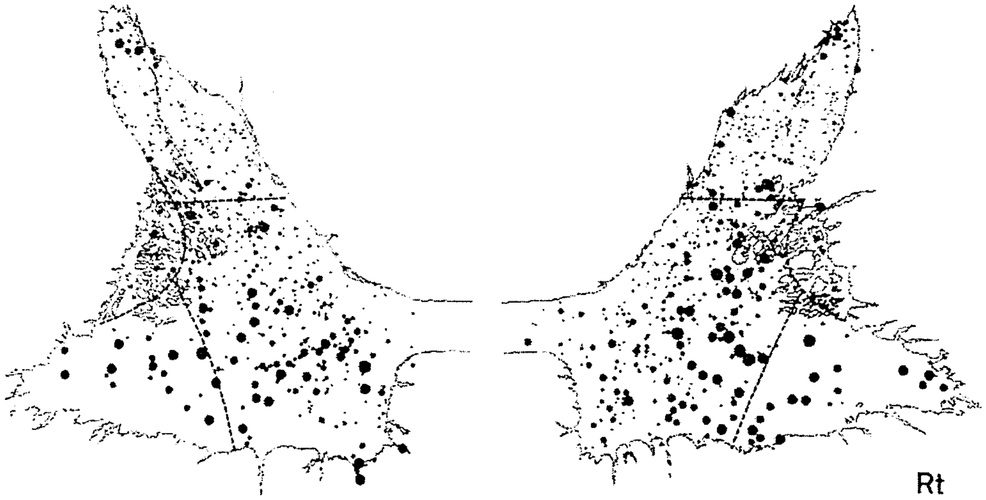

Figure 6. Size distribution of the neurons in the cervical gray matter of a ALS patient, whose muscular strength was fairly well preserved up to death. The large neurons, with a nuclear area greater than 151 μm^2, are severely decreased in Rexed's lamina IX. However, the degree of decrease of the neurons 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.

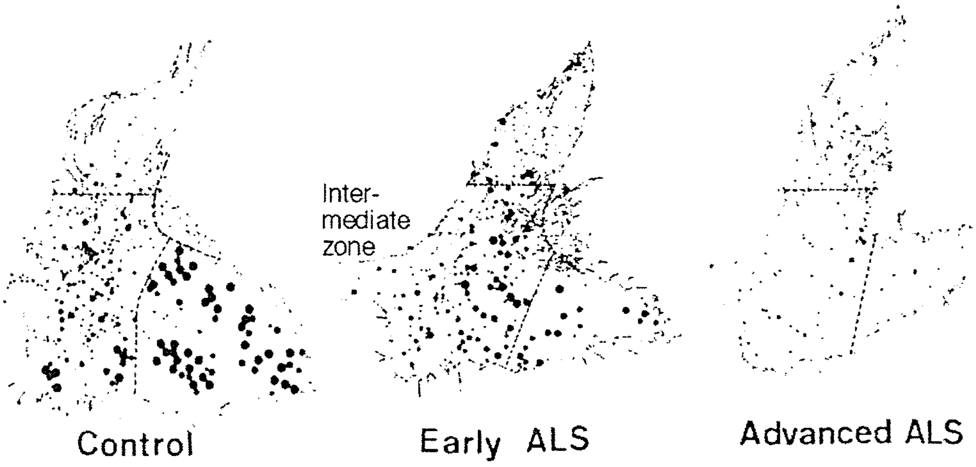

Figure 7. Progression pattern of neuronal loss in the spinal gray matter of ALS. The findings indicate that the primary degeneration may occur in the anterior horn cells and the neurons in the intermediate zone degenerate sequentially in the spinal gray matter in ALS.

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.

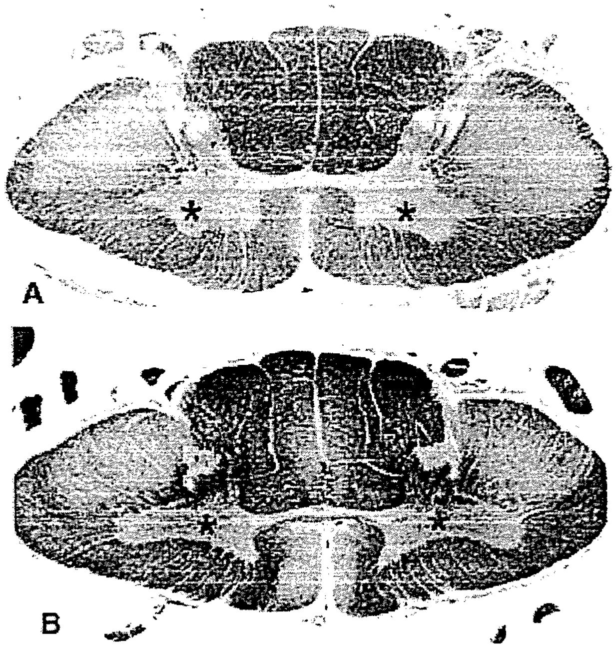

Figure 8. An ALS patient with relative preserved AHCs (asterisks) but severe degeneration of the l-CST (A). An ALS patient with marked loss of AHCs (asterisks) but relatively slight degeneration of the l-CST (B). Cervical cord. Klüver-Barrera preparation.

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).

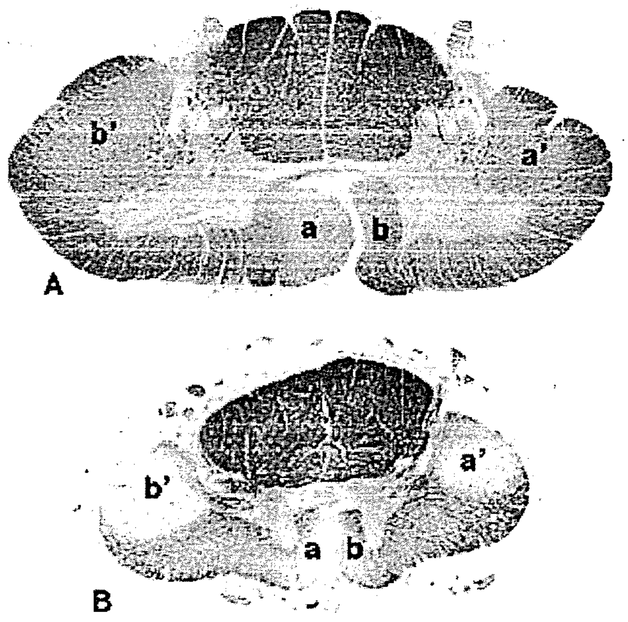

Figure 9. ALS patients showing asymmetry of the spinal white matter. Volume of the left anterior CST (a) is large, and that of right anterior CST (b) is small, but left lateral CST (b') is large, and right lateral CST (a') is small. This finding indicates a + a` = b + b'. A: cervical, B; thoracic of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

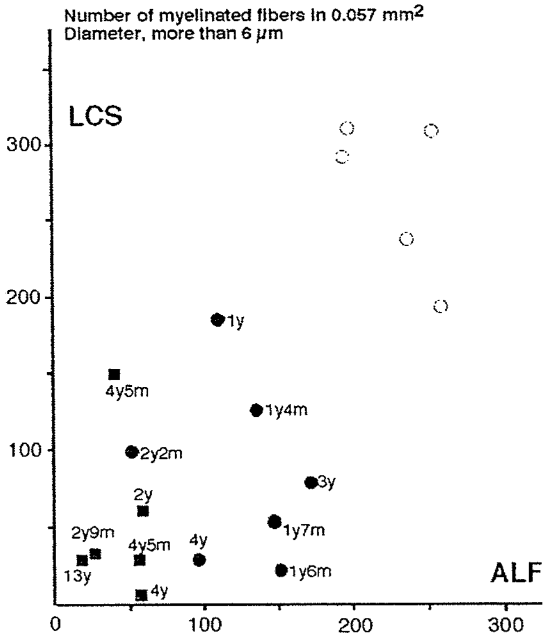

Figure 10. Evaluation of the ratio for the decrease in numbers of large myelinated fibers between the ALF and l-CST. Open circle; control subject, solid circle; ALS patient without respirator support, solid square; ALS patient with respirator support. Numbers indicate the duration of illness. Patients 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