Age-related Reductions in Number and Size of Anterior Horn Cells at C6 Level of the Human Spinal Cord

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Summary: We examined numbers and sizes of anterior horn cells (AHCs) of the human spinal cord at C6 level with aging process. Spinal cords were obtained from twenty-four male cadavers, age ranged from 41 to 97 years without any accompanying pathological changes of the spinal cord. For quantitative investigation of the AHCs, spinal cord segments were embedded in celloidin after secondary fixation and dehydration; sections (20μm thick) were stained with luxol fast blue-periodic acid-Schiff-hematoxylin and Kluver-Barrera methods; the neuronal number, the cell body area and the perimeter of AHCs in the gray matter (equivalent to the Rexed's lamina IX) were counted and measured using a combination of a microscope with a drawing tube, a digitizer and a computer at 400 times magnification. A marked reduction in number of AHCs was found in the aged subjects. Similarly, there were significantly age-related decreases in the cross-sectional area and perimeter of AHCs at C6 segment of the spinal cord. These findings indicate that the reduction in number and size of AHCs in the human spinal cord is associated with degenerative neuronal changes and loss of motoneurons during aging process.

Various morphological and physiological changes may occur in the nervous system with increasing age. The degenerating changes of the human spinal cord with age have been recognized through many years. There have been ample data on the spinal cord with age in normal subjects, such as the length and caudal level of termination (Hara, 1987), atrophic changes (Campbell, 1894; Critchley, 1931; Duncan, 1938; Morrison, 1959; Kameyama et al., 1994; Sasaki et al., 1994; Zhang et al., 1996), the number and size of axons in the posterior funiculus (Zhang et al., 1995), loss of myelin sheaths (Duncan, 1938; Bailey, 1953; Morrison, 1959; Kameyama, 1971), etc. In addition, it has been reported that the total number of anterior horn cells (AHCs) in the spinal cord decreases in both humans (Kawamura et al., 1977; Tomlinson and Irving, 1977; Tsukagoshi et al., 1979) and animals (Wright and Spink, 1959; Kanemitsu, 1977; Ishihara et al., 1987; Hashizume et al., 1988, 1995), in which the data obtained from Wright and Spink (1959), Tomlinson and Irving (1977), Ishihara et al., (1987) and Hashizume et al., (1988) studies showed the number of motoneurons decreased with age. A decrease in the number of myelinated fibers in the ventral roots and peripheral nerves also suggests a loss of motoneurons in aging humans (Jacobs and Love, 1985; Tohgi et al., 1977; Mittal and Logmani, 1987) and animals (Samorajski, 1974; Kawamura et al., 1977; Caccia et al., 1979; Ansved and Larsson, 1990). Few reports are, however, concerned with the size of AHCs in the human spinal cord; even so, much of the available information are dispersed in the literature in the form of selected morphometric data on control subjects in papers dealing with pathological conditions of nervous system, such as, motor neuron diseases (Swash et al., 1986; Murakami, 1990; Sasaki and Iwata, 1995), cerebrovascular diseases (Qiu et al., 1991), and long-term amputation of the unilateral upper arm (Suzuki et al., 1993). Moreover, there have, as yet, been no quantitative studies on both the number and the size of AHCs or motoneurons in the gray matter of the human spinal cord with aging process.

On the other hand, it is possible to measure the areas of irregular shapes accurately, and to make the present study both accurate and easy, since the introduction of the combination of an image analyzing digitizer, a microscope with a drawing tube and a computer for structure tracing, data logging and statistical analyzing. Therefore, the present study is undertaken to make quantitative analyses of the AHCs at different ages, and to see if reduction in the...
number and size of AHCs (Rexed's lamina IX) occurs with age in the human spinal cord at C6 level.

Material and Methods

Twenty-four male spinal cords which were taken from cadavers for anatomic practice were studied. The age of cadavers were from 47 to 97 years (the average: 73.0 ± 13.9 years). The causes of death had no direct or indirect connection with the central nervous system, and microscopically, no pathological changes were found in the spinal cord. Therefore, the spinal cord in the present study was considered normal. After the injection of 10% formalin (3.7% – 4.0% formaldehyde) into the femoral artery by a pulsation pump for fixation with the opening of bilateral femoral and jugular veins for drainage of blood, the spinal cord was removed as a whole with the dural covering and placed in a 10% solution of formalin followed by the secondary fixation with a mixed solution of 5% potassium dichromate and 5% potassium chromate (1:4 in volume) for three weeks. The blocks were taken from the sixth cervical spinal cord which was reconfirmed according to the diagnostic criteria of levels of the human spinal cord by Goto (1988). They were then washed in running water, dehydrated with alcohol, embedded in celloidin, sectioned transversely into 20 μm thick slices, and stained with luxol fast blue-periodic acid-Schiff-hematoxylin (LPH) (Goto, 1987) and Klüver-Barrera methods.

For the measurement and analysis of sections, a combination of an electronic optical planimeter (Digitizer KC 3300, Graphtec Co, Japan) and a computer (PC-9801 VX2, NEC, Japan) was adopted to count the number of anterior horn cells (the number of AHCs in the present study means total number of both the left side and right side of spinal cord) and to measure the cross-sectional area and perimeter of AHCs in Rexed's lamina IX at C6 level.

Statistical analysis

The data from each decade group were presented as mean ± SEM (Standard Error of Mean) and were statistically examined by analysis of variance (ANOVA) followed by, where applicable, Fisher's multiple comparison test. The linear regression analysis was performed to determine the relation between the age and the number, average cell body area, total transverse area, and perimeter of AHCs respectively. For comparison of the number and size of AHCs between the left and right side of the same section, the two-tailed Student's t test of paired data was employed. Probability values of p < 0.05 were regarded as significant for all statistical analyses.

Results

The morphometric values of the number, cross-sectional area, and perimeter of AHCs of the spinal cord at C6 level in different decades of life were listed in Table 1.

The number of AHCs at C6 level

The number of AHCs having a nucleolus in the nucleus of the human spinal cord at C6 level was estimated from the data ranging from 50 to 127 (average 94). In Group F (age: 90–99) the number of AHCs was less than that in Group A (age: 40–49, p < 0.05). It was the 59.3% reduction comparing to Group A. The regression line showed a decrease of AHCs in number with aging (r = −0.45, p = 0.03, Fig. 1).

The transverse area of AHCs

The transverse area of AHCs in the spinal cord at C6 level ranged from 98.9 μm² to 1848.0 μm² (average

Table 1. Morphometric data of anterior horn cells (AHCs) of the human spinal cord at C6 level

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>Number of AHCs per section</th>
<th>Average area of AHCs (μm²)</th>
<th>Total areas of AHCs per section (μm²)</th>
<th>Average perimeter of AHCs (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (40–49)</td>
<td>2</td>
<td>118.0 ± 9.0</td>
<td>564.5 ± 99.7</td>
<td>65717 ± 6687</td>
<td>86.0 ± 5.8</td>
</tr>
<tr>
<td>B (50–59)</td>
<td>3</td>
<td>96.7 ± 13.9</td>
<td>585.9 ± 39.4</td>
<td>55713 ± 5853</td>
<td>86.8 ± 3.5</td>
</tr>
<tr>
<td>C (60–69)</td>
<td>3</td>
<td>104.0 ± 1.2</td>
<td>562.9 ± 62.4</td>
<td>58500 ± 6286</td>
<td>86.0 ± 2.4</td>
</tr>
<tr>
<td>D (70–79)</td>
<td>6</td>
<td>87.5 ± 7.4</td>
<td>544.9 ± 26.7</td>
<td>47284 ± 3675*</td>
<td>83.0 ± 3.7</td>
</tr>
<tr>
<td>E (80–89)</td>
<td>8</td>
<td>94.6 ± 6.6</td>
<td>503.1 ± 23.1</td>
<td>47441 ± 3729*</td>
<td>76.9 ± 2.8</td>
</tr>
<tr>
<td>F (90–99)</td>
<td>2</td>
<td>70.0 ± 20.0*</td>
<td>508.0 ± 15.3</td>
<td>35254 ± 6089**</td>
<td>77.9 ± 1.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean values ± SEM.
Significance assessed by one-way analysis of variance (ANOVA).
* p < 0.05, ** p < 0.01 vs Group A (40–49).
536.9 μm²). A scatter diagram and its regression line between the average transverse area and age were shown in Fig. 2, and revealed that the average areas of AHCs in the transverse section reduced with age advanced (r = −0.42, p < 0.05), although there were no significant differences among decade groups of life. The loss of AHCs (major in the large motoneurons) and reduction of AHCs in size were obviously found in the older subjects, comparing with the younger group (Figs. 3, 4). In addition, the total transverse area of AHCs per section decreased significantly with aging (r = −0.63, p =0.001, Fig. 5).

In Group D (age: 70−79), Group E (age: 80−89), and Group F, the total transverse areas of AHCs were less than those in Group A (p < 0.05 − p < 0.01). They were 72.0%, 72.2%, and 53.6% of Group A, respectively.

**The perimeter of AHCs**

The average perimeter of AHCs at C6 level was 81.6 μm ranging from 20.1 μm to 208.3 μm. There was a significant negative correlation between the age and average perimeter of AHCs at C6 level (r = −0.53, p < 0.01, Fig. 6).

If we compare various parameters of AHCs on both sides of the spinal cord at C6 level, we find

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**Fig. 1.** Linear regression analysis between age and the number of anterior horn cells (AHCs) in the human spinal cord at C6 level showing a decrease in number of AHCs with aging (n = 24, p = 0.03).

**Fig. 2.** Scatter diagram and regression line showing a correlation between age and the reduction of average transverse area of AHCs in the human spinal cord at C6 level (n = 24, p < 0.05).

**Fig. 3.** Microscopic photos of motoneurons in C6 spinal anterior horn (left). KB stain. Note a decrease in number (loss of large neurons) and size of neurons in a 97-year-old subject (a), comparing to a 52-year-old subject (b). Scale bar in Figs. 3a and 3b = 100 μm.
Fig. 4. Microscopic photos of motoneurons in C6 spinal anterior horn (left) under high power view. LPH triple stain. (a) indicates a smaller neuron containing lipofuscin granules in a 97-year-old subject, while (b) shows a normal neuron in a 52-year-old subject. Scale bar in Figs. 4a and 4b = 10 μm.

![Microscopic photos of motoneurons](image)

**Table 2.** Data of anterior horn cells (AHCs) at C6 level

<table>
<thead>
<tr>
<th>Side</th>
<th>N</th>
<th>Number of AHCs per section</th>
<th>Average area of AHCs (μm²)</th>
<th>Total areas of AHCs per section (μm²)</th>
<th>Average perimeter of AHCs (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>24</td>
<td>45.1 ± 2.2</td>
<td>539.4 ± 16.8</td>
<td>24059.3 ± 1179.4</td>
<td>82.5 ± 1.7</td>
</tr>
<tr>
<td>Right</td>
<td>24</td>
<td>49.1 ± 2.4</td>
<td>533.7 ± 16.3</td>
<td>26266.3 ± 1575.4</td>
<td>80.7 ± 1.9</td>
</tr>
<tr>
<td>p-value</td>
<td>0.08</td>
<td>0.67</td>
<td>0.12</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM. Two-tailed paired Student’s t test.
there are no significant differences in all parameters of AHCs (Table 2), although the number of AHCs in the right side of the spinal cord is slightly more than that in the left side.

Amyloid bodies stained in purple by LPH method were observed in transverse sections of the spinal cord at C6 level in every individual and were most commonly found in the posterior funiculus or around the entrance of the dorsal roots. A few were scattered around the glia limitans near the surface of the cord. They were also confirmed as increasing with aging process, which findings have been previously appeared elsewhere (Zhang et al., 1996).

Discussion

The butterfly-shaped gray matter of the spinal cord contains an enormous number of neurons varying in size and shape. A variety of inconsistent terminologies based on cytological features or topographical locations of cell groups within the spinal gray have been replaced by a terminology based on cytoarchitectural lamination of the spinal gray (Carpenter, 1991). Although Rexed (1952) described this neuronal lamination in thick sections (80 to 100 μm) of the cat spinal cord, it is generally accepted that a similar lamination exists in the spinal gray in all mammals, including man. Lamina IX consists of several distinct clusters of large somatic motor neurons that occupy somewhat different positions within the anterior gray horn at various spinal levels. In the cord enlargement, larger numbers of motor neurons form more numerous groups. AHCs of this lamina are large multipolar neurons (30 to 70 μm in soma diameter) with central nuclei, coarse Nissl bodies, multipolar dendrites. These large motor cells that innervate striate muscle are referred to as alpha (α) motor neurons; scattered among them are smaller gamma (γ) neurons that give rise to efferent fibers that emerge via the ventral root and innervate the contractile elements of the muscle spindle. Gamma efferent fibers play an essential role in the maintenance of muscle tone and bring the muscle spindle under control of spinal and supraspinal influences (Rexed, 1952; Carpenter, 1991).

With the combination of a microscope, a digitizer and a computer, we directly counted the number and measured the cross-sectional area of AHCs in Rexed’s lamina IX of the human spinal cord at C6 level after confirming the presence of AHCs having a nucleolus in the nucleus. A significant age-related reduction in man was found for all parameters: the number, average cell body area, total transverse area and average perimeter of AHCs (Figs. 1, 2, 5, 6). We deduced from these results that the smaller and loss of AHCs (major in large neurons) in the human spinal cord occurred with the advance of age. According to our data, the number of AHCs in the human spinal cord at C6 level ranged from 50 to 127 (average 94); the average cross-sectional area of AHCs was 536.9 μm² ranging from 98.9 μm² to 1848.0 μm²; the average perimeter of AHCs was 81.6 μm, ranging from 20.1 to 208.3 μm in 20-μm-thick sections. In addition, there were no marked differences in all parameters of AHCs at C6 level between the left and right sides of the spinal cord (Table 2).

There are many data dealing with the number of motor neurons (Morrison, 1959; Sirken and Kuhlenbeck, 1966; Irving et al., 1974; Kawamura et al., 1977; Tomlinson and Irving, 1977; Tsukagoshi et al., 1979; Murakami, 1990) and few reports on the size of motor cells (Murakami, 1990; Qiu et al., 1991; Suzuki et al., 1993) in the anterior horns of the spinal cord in normal subjects. Morrison (1959) reported that the number of AHCs at C6 level in a 24-year-old female subject was 282 (on the left side) and 249 (on the right side) in 240-μm-thick sections. Tsukagoshi et al. (1979) showed the results obtained from counting the motor cells in the limb motor nucleus of C6 segment in 10 controls aged over 38 years. The total numbers of large cells (larger than 25 μm in diameter of the soma) were 2,533 ± 454 (the mean value and standard deviation) and of small cells (smaller than 20 μm in diameter of the soma) were 1,101 ± 358 per 500-μm thickness in serial sections. Murakami (1990) reported that the cross-sectional area of cellular body in left anterior horn at C6 level in 3 controls was 346.4 ± 16.3 μm² (mean ± SEM, the numbers of the counted neurons were 298) in 20-μm-thick paraffin sections. In present study, the neuronal body area of AHCs was 536.9 ± 15.1 μm² (2,260 neurons were counted) and was larger than that of Murakami’s study (Murakami, 1990). Upon the views above-mentioned, there was a great variation in number and size of AHCs at C6 level regardless of age or sex. The reason might be explained in part by differences in scientific approaches (such as fixation, embedding, staining method, morphometry) made by independent investigators, and by populations studied, individual variations, etc. Therefore, it is desirable that many more samples be examined to cover the normal ranges about the numbers and sizes of AHCs of the human spinal cord in cadaver specimens.

It should be noted that results of this study on aging are in general agreement with the previous report made by Tomlinson et al. (1977) in spite of different segment of the spinal cord. In their study, forty-seven spinal cords at lumbar segments from subjects between 13 and 95 years of age have been
examined. No evidence exists of loss of motor neurons up to the age of 60 years, but beyond that age there is an increasing evidence of a diminishing motor neuron population, above 60 years, several cases showed motor neuron counts of only 50% of the counts in early adult life or middle age. Cell loss appeared to be uniform throughout all the segments and was not accompanied by any other striking morphological change. On the other hand, a decrease in the number of myelinated fibers in the ventral roots and peripheral nerves also suggests a loss of motoneurons in the aged humans (Jacobs and Love, 1985; Tohgi et al., 1977; Mittal and Logmani, 1987) and animals (Samorajski, 1974; Kawamura et al., 1977; Caccia et al., 1979; Ansved and Larsson, 1990). Therefore, these changes above-mentioned may be responsible for some electrophysiological alteration in amplitude and conduction of nerve, and for some clinical signs and symptoms associated with age. The electrophysiological studies have shown decline in amplitude of nerve action potentials and slowing of conduction with increasing age (Norris et al., 1953; Campbell et al., 1973; Schaumburg et al., 1983). The reduction in conduction velocities of motor fibers of the ulnar nerve has been shown in older individuals (Norris et al., 1953). Clinically, Potvin et al. (1980) reported that sixty-one normal men whose ages ranged from 20–80 years were evaluated on two occasions by means of a comprehensive series of 128 instrumental tests of neurologic function with aging process. Significant age-related linear decreases were found for almost all neurologic functions. For the upper extremities, the largest age-related declines (more than 50 percent) were in hand-force steadiness, speed of hand-arm movements and vibration sense.

In addition, a reduction in the number of motoneurons may be related to muscle atrophy, although the mechanism for these age-related changes is complex and may involve different levels of the neuromuscular system (Larsson, 1978; Ishihara et al., 1987; Kimura et al., 1995). The degenerative alterations of muscle fibers are considered to be due to central (regressive changes or loss of motoneurons) or peripheral (degeneration of muscle fibers) changes or both (Ishihara et al., 1987). Since histological samples of the upper limb muscles were not routinely taken for histology, it is not possible to state whether or not loss of motor neurons, even if severe, is always accompanied by an evidence of neurogenic atrophy in the arm muscles. However, co-workers in our laboratory have examined the human anterior tibial muscle fibers with aging process, the results suggest the number of muscle fibers declines significantly with age (Kimura et al., 1995).

Morphologic studies, such as the present one, can give only an approximate estimate of the number of alpha- and gamma-motoneuron cytons, since the physiologic identity of individual neurons cannot at this time be determined by morphologic characteristics alone (Kawamura et al., 1977). The numbers of AHCs in Rexed's lamina IX decrease with age in the present study, which means that the decline occurs in the number of alpha- or gamma-motoneurons or both. However, we can deduce that the major reduction in number of AHCs is alpha-motoneurons from our findings of the decrease of average cell body areas and loss of large cells in the older subjects (Figs. 3, 4). The similar changes have been observed in animals with age. The age-related alterations in the number and size of alpha- and gamma-motoneurons were studied in the medial gastrocnemius motor nuclei in rats at four different ages. There is a specific decrease in motoneuron number in the old and very old animals, with most of the decrease occurring among the alpha-motoneurons (Hashizume et al., 1988).

In conclusion, we have demonstrated that a marked reduction in the number of AHCs at C6 segment of the spinal cord was found in the older subjects. Similarly, there were significantly age-related decreases in the cross-sectional area and perimeter of AHCs. These findings of the present study indicate that the reduction in the number and size of AHCs in the human spinal cord is associated with degenerative neuronal changes and loss of large motoneurons during aging process.

References


