Morphometric Analyses of Axons in the Lateral Corticospinal Tract with Ageing Process

By

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Summary: Morphometric analyses were carried out to reveal a relation between age and axonal changes in the lateral corticospinal tract (LCST) at the L1 level of the human spinal cord. Histological preparations of the spinal cord were made after embedding in celloidin to stain with Luxol fast blue-periodic acid-Schiff-hematoxylin or Klüver-Barrera methods. The number, average transverse area, total area and diameter of axons of the LCST were counted and measured using an image-analyser combined with a computer. The morphometric analyses revealed that (1) there was a significant decrease in the axonal number of the LCST with age (p<0.001); (2) the axons were not partially nor completely accompanied by surrounding myelin sheaths, and increased in number with age; (3) the diameter and average area of axons in the LCST became significantly reduced with age (p<0.01); (4) the total area decreased significantly with age in a unit area of 2,700 μm²; and (5) the circularity ratio showed no significant change with age.

Changes in the nervous system with age are increasingly receiving attention. Most of the neurologic signs and symptoms have been attributed to ageing (Kokmen et al., 1977; Potvin et al., 1980). A decrease in number or concentration of human myelinated axons with the ageing process has been reported regarding the spinal root (Rao and Krinke, 1983), facial nerve (Fujii and Goto, 1989), vestibulocochlear nerve (Fujii et al., 1990), sural nerve (Tohgi et al., 1987), deep peroneal nerve (Yanagisawa et al., 1994) and posterior funiculus (Zhang et al., 1995). In addition, there are reports on growth with age and body weight (Bernstein, 1966), morphometric study (Arias et al., 1987) and injury experiment research (Tator et al., 1984) of the corticospinal tract in the rat. However, there are no quantitative morphometric studies of the human being in the literature on axons in the central nervous system, especially descending tracts such as the LCST.

Fibres of the LCST are situated in the posterior part of the lateral funiculus. They descend the length of the spinal cord, give off fibres to the spinal grey matter at every level and progressively diminish in size. A lesion destroying part of this tract at any level may result in various degrees of paresis (Carpenter, 1991). We may therefore wonder if there are any relations between age and morphometric data of axons of the LCST in the spinal cord. It is important to obtain detailed data about the LCST, as it is one of the descending tracts that associate with the movements of the human body.

The purpose of this study is to reveal whether or not there are any significant relations between age and area or diameter of axons of the LCST. The morphometric method was used to evaluate quantitative analysis of myelinated axons of the spinal cord. This study may, thus, be the first to show morphometric data about the LCST of the human spinal cord and will be useful for comparison with abnormal cases in order to help produce more accurate morphometric analyses of the human pyramidal tract.

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Material and Methods

Forty human spinal cords which had been removed as a whole from cadavers for anatomical dissections were examined. The ages ranged from 41 to 97 years old (average 73.6 years). The causes of death and findings of the corpus during the anatomical dissection had no direct or indirect connection with the central or peripheral nervous systems, so that the spinal cords in the present study can be considered normal. After injection of 10% formalin (3.7–4.0% formaldehyde) through the femoral artery by a pulsation pump, with opening of the jugular and femoral veins for drainage, the selected sections were reconfirmed as being from the middle level of the first lumbar segment according to Goto's criteria (Goto, 1988). Blocks of the spinal cord segments were fixed with two-step processes according to Goto's method (Goto, 1987). For the primary fixation, a 10% solution of formalin for general purposes was used with immersion for at least one week. Then the blocks of spinal cord segments were transferred, without washing, directly into the secondary fixative composed of 5% potassium dichromate (K$_2$Cr$_2$O$_7$) and 5% potassium chromate (K$_2$CrO$_4$), 1:4 in volume and kept for two weeks at room temperature, followed by an additional week at 37°C. After washing with running water and dehydration in graded alcohol, the blocks were embedded in celloidin. Celloidin blocks were transversely cut at 20 μm thickness for staining with LPH (luxol fast blue-periodic acid-Schiff-hematoxylin) triple or K-B (Klüver-Barrera's) method.

The measured region of the LCST in this study was confirmed according to the findings of the secondary degeneration site of a lesion sample of the LCST (Fig. 1). For the measurement of axons, three sampling sites in the area of the LCST were selected. Highly enlarged images (1,600 times), as shown in Fig. 2 with a square eyepiece grid were used to count the myelinated axons in a unit area of 2,700 μm$^2$. The transverse areas of axons were measured with the help of a microscope equipped with a drawing tube, an image-analyser (Digitizer KC 3300, Graphtec Co.), a computer (PC-9801VX2, NEC) and a program written by ourselves. Data from each ten-year age group were presented as mean values ± SD (standard deviation of mean) and were statistically examined by analysis of variance (ANOVA) followed by Scheffe's multiple comparison test, where applicable. Linear regression analyses were performed to determine the relation between age and number, average area, total area, average diameter and circularity ratio (CR) of axons respectively. The significance in the present study was assumed at a level of p < 0.05 for all statistical analyses.

Results

In this study, the site of the LCST at the L1 level was examined with precision under microscope for all age groups. It was clearly shown that the connective tissue increased in volume and the myelinated axons decreased in number in the LCST of the spinal cord as age increased (Figs. 2a and 2b). Moreover, the myelin sheath became thinner with age. The morphometric values of the number, cross-sectional area and diameter of myelinated axons in all age groups are listed in Table 1. It shows that axons are thinner in the aged group than in the younger group. The change begins at least in the 80s groups.

Number of axons in the LCST

The number of axons in the LCST at the L1 level ranged from 109 to 338 (average 213) within a unit area of 2,700 μm$^2$. The axons in group D (70–79 years of age), group E (80–89 years of age) and group F (90–99 years of age) were less in number than those in group A (40–49 years of age, p < 0.05 or p < 0.001). The numbers were respectively 75.4, 67.1 and 67.6% of the numbers in group A. A linear regression analysis shows that the number of myelinated axons in the human LCST significantly decreases with age (r = -0.587, p < 0.001, Fig. 3).

Average area of axons in the LCST

The area of axons in the LCST at the L1 level
was calculated. It ranged from 1,574 to 3,863 µm² (average 2,670 µm²). The average area in groups E and F were smaller than that in group A (p < 0.01 and p < 0.001). They were respectively 85.2 and 70.9% of the value in group A. A linear regression analysis of the correlation between age and the average area of axons revealed a negative correlation in size ($r = -0.489$, p < 0.01, Fig. 4). The same
can be said about the total area of axons within a unit area of 2,700 µm², which also shows a significant reduction with age ($r = -0.714$, $p < 0.001$, Fig. 5).

**Average diameter of axons in the LCST**

The diameter of axons in the LCST at the L₁ level ranged from 1,416 to 2,218 µm (average 1,842 µm). The average diameter in groups D, E and F was smaller than in group A. A regression analysis reveals a negative correlation between age and the average diameter of axons ($r = -0.492$, $p < 0.01$, Fig. 6).

**Discussion**

Until now, no data have been available about the correlation between age and such items as the number, average area or average diameter of LCST axons. The LCST plays an major role in the movements of the human body. In the clinical field,
Potvin et al. have reported that the physical conditions and neurological functions changed with aging in 61 normal men with ages ranging from 20 to 80 years old. They found significant age-related negative linear correlations for almost all neurological functions. The decline over the age span varied from less than 10% to more than 90% for different functions (Potvin et al., 1980). The results showed that the strength of both upper and lower extremities gradually decreased with age. The most age-sensitive was the extended leg flexion strength test, and the least sensitive was the grip strength test. All hand and foot speed tests showed a significant loss in speed with age, as well as body lateralisation effects (with the exception of step tracking extension movement times).

It is obvious that paralysis and sensory loss may be attributed to the destruction of axons, but little quantitative evidence is available to support the simple relation of functional to axonal loss (Eidelberg et al., 1977). The average overall axon diameter showed a growth-related increase in adult animals, followed by a decrease reflecting senile atrophy in old rats (Rao and Krinke, 1983).

As for the peripheral nervous system, it has been reported that the numbers of axons in the facial nerve (Fuji and Goto, 1989), the transverse axonal areas of the cochlear nerve (Fujii et al., 1990), and the number and average area of the deep peroneal nerve (Yanagisawa et al., 1994) all decrease with age. Regarding the central nervous system, spinal motoneurons also decrease in number with age (Kawamura et al., 1977; Tomlinson and Irving, 1977; Tsukagoshi et al., 1979). Axonal atrophy starts appearing early in life. It is at first slight but increases with age (Fujisawa, 1988). It has already been reported that the average area of the posterior funiculus decreases with age at the levels L1 (Zhou et al., 1996) and C6 (Zhang et al., 1996) of the spinal cord. The average area of axons in the ascending tract (posterior funiculus) also decreases with age (Zhang et al., 1995). The reduction in number and size of axons in the nervous system with ageing is thus well documented. It is clear that the changes that occur in the nervous system during the ageing process appear as a reduction in the number and size of axons.

The method of LPH triple stain was employed in the present study. It has already been proved to be a reliable, consistent method in staining the axon of nerve fibres. The myelinated axons clearly appear in dark purple or black surrounded by the blue-green myelin sheath (Goto, 1987). This LPH triple staining method made it possible to conduct a quantitative study of axons in both the peripheral and central nervous systems.

In this study, the number and area of axons in the LCST were directly studied under microscope with the help of an image-analyser at the L1 level after confirming the presence of axons surrounded by myelin sheath.

The present results show that the number of myelinated axons in the LCST on one side at the L1 level ranged from 109 to 338 (average 213), the axonal area from 1,574 to 3,863 µm² (average 2,670 µm²) and the diameter of axons from 1,416 to 2,218 µm (average 1,842 µm). A significant age-related linear correlation was observed for the following parameters: number, average area, total area and average diameter of myelinated axons of the LCST within the sampling area. It can be deduced from these results that the number and area of myelinated axons in the LCST of the human spinal cord decrease with age.

Acute spinal cord injury in animal experiments (Tator et al., 1984) showed that the axonal numbers distal to the injury site diminished markedly in the pyramidal tract of the rat. In the present study, our results also show significant age-related linear decreases in the number and size of the LCST axons. It may be deduced from these results that LCST axons diminish in number and size not only in case of acute injury but also during the ageing process.

It is unclear whether age-related alterations of the nervous system are due to a unique ageing process or to a summation of the effects of known degenerative diseases, and the mechanisms underlying axonal atrophy and fibre loss with old age are unknown. The explanation could be that the rate of axonal transport in the peripheral nerves decreases with age. In particular, reduced transport of microtubule and neurofilament proteins could lead to difficulty in maintaining the cytoskeletal framework, thereby leading to axonal atrophy or fibre loss (Knox et al., 1989).

We have reported in a previous study (Zhang et al., 1995) that the number and average area of myelinated axons in the posterior funiculus decrease with age. The axonal reduction in the posterior funiculus at the C6 level was in the reverse order of age groups: 62.7% (group D), 58.3% (group E) and 40.8% (group F) in number, and 61.3% (group E) and 57.7% (group F) in the average area of the values for group A, respectively (Zhang et al., 1995). In the present study, however, it was also clearly disclosed that the number and average area of myelinated axons of LCST at the L1 level decrease with age. The axonal reduction was 75.4% (group D), 67.1% (group E) and 67.6% (group F) in number, and 85.2% (group E) and 70.9% (group F) in average area of the values for group A, respectively. It seems that, compared with
the posterior funiculus, the changes that occur in the LCST myelinated axons (descending tract) of the human spinal cord are less severe than those in the posterior funiculus (ascending tract).

One interesting result has been reported by Knox et al.: they discovered that the sural nerve did not undergo myelinated fibre-loss during ageing, while the peroneal nerve underwent a slight reduction in the number of myelinated fibres, and also showed some evidence of axonal atrophy. Motor fibres of the central and peroneal nerve in the rat were more affected than sensory fibres in the dorsal root and sural nerve (Knox et al., 1989).

Finally, we can conclude that axonal morphometry of the LCST in relation to ageing may be an indispensable procedure to understand the influences of ageing on the motor function, and to compare the standard data of ageing with other neuropathological conditions which involve the LCST such as vascular, degenerative, neoplastic and inflammatory diseases and traumas.

References