Morphological evaluation of the human pyramidal tract: Gender and age differences

By

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\textbf{Summary:} Using a preparation method composed of secondary chromic acid fixation, nitrocellulose embedding and Luxol fast blue-PAS-hematoxylin stain (a discriminative staining method) for the purpose of axonal discrimination, we examined 43 human spinal cords (31 males and 12 females) at the C5 level to find the differences due to gender and aging in axons of the lateral corticospinal tract. These results can be of great help in understanding the motor functions in relation to gender differences and the aging process.

In the past, morphometric research on the nervous system had tended to focus on neuronal somata. Recently, however, research has started on the evaluation of the nervous pathways, especially of the nerve fibers\textsuperscript{1–8).} Out of the reasons for this delay may be the lack of reliable methods for the staining of axons with minimum shrinkage. For example, conventional silver impregnation methods may result in extensive axonal shrinkage. It is therefore indispensable to adopt one of the discriminative staining methods developed recently for the evaluation of nerve fibers\textsuperscript{8, 9), as it is very important to take into account the shrinkage ratio for the morphometric evaluation of human nerve tissue\textsuperscript{8, 9).} Such shrinkage should be avoided, or at least be as low as possible and have the narrowest range possible for the purpose. Although there are a few reports on the lateral corticospinal tract (LCST) at L1 level\textsuperscript{1, 2) concerning aging or gender differences, no data can be found regarding the cervical levels. In the present report, we will describe the differences due to gender and aging in nerve fiber axons in the LCST at the C5 level.

Material and methods

Spinal cords, including the dura mater coverings and the proximal parts of the spinal nerves after checking the level of spinal nerve roots were removed from the cadavers of 43 human subjects consisting of 31 males (age ranging from 41 to 97 years) and 12 females (age ranging from 59 to 92 years), after injection of a 10\% formalin (3.7–4.0\% formaldehyde) solution through the femoral artery by a pulsation pump, with the jugular and femoral veins open for drainage of the blood. The subjects showed no evidence, either clinical or pathological, of suffering from diseases affecting the nervous system. We excluded cadavers with a past history of nervous diseases or pathological findings in the nervous system such as cerebrovascular disorders, intracranial tumors, infections, neurodegenerative conditions, etc. The spinal cords prepared for the study were therefore considered to be normal. After making the transverse sections, the selected segments were confirmed to be the fifth cervical segment according to Goto’s criteria\textsuperscript{10).} Blocks of the spinal cord segments were fixed in a two-step process according to the method given in the reference section\textsuperscript{9).} For primary fixation, a 10\% solution of formalin for general purposes was used. The blocks were first immersed in the solution over a week, and were then transferred, without washing, into the secondary fixative composed of 5\% potassium dichromate and 5\% potassium chromate (1:4 in volume) and kept at room temperature for two weeks, followed by an additional week at 37°C. After washing with running water using a pipet-washer with siphon mechanism
and dehydration in graded alcohol, they were embedded in nitrocellulose\(^1\). The celloidin blocks were cut at 20 μm thickness to stain with luxol fast blue-periodic acid-Schiff-hematoxylin (LPH) triple stain\(^9\). The area occupied by the lateral corticospinal tract was defined for this study according to the Marchi findings of Wallerian degeneration of the LCST caused by the destruction of the internal capsule. The central part of the lateral pyramidal tract was selected for the evaluation under the microscope.

**Results**

The LPH stain yielded satisfactory results in the staining of axons, myelin sheaths and various kinds of cell nuclei. We found clear differences due to gender and age in the myelinated nerve axons of the LCST at C5 level under microscopic observation.

**Gender differences of the human lateral pyramidal tract:**

The large-sized myelinated axons are more dominant in number in males (Fig. 1a) than in females (Fig. 1b), while the small-sized myelinated axons are found in relatively larger numbers in females (Fig. 1b) than in males.

![Fig. 1. Gender differences in the human lateral pyramidal tract.](image)

Large-size myelinated axons are more dominant in number in males (a) than in females (b), while small-size myelinated axons are found in relatively larger numbers in females (b) than in males (a).

a: 65-year-old male, b: 71-year-old female. C5 level, LPH (luxol fast blue-PAS-hematoxylin) stain, scale bar = 10 μm.

![Fig. 2. Age differences in the human lateral pyramidal tract.](image)

Large-size myelinated axons are dominant in 41-year-old male (a) compared to 88-year-old male (b).

a: 41-year-old male, b: 88-year-old male. C5 level, LPH stain, scale bar = 10 μm.
**Age differences of the human lateral pyramidal tract:**

Large-size axons are more dominant in the younger specimens compared to the older ones, as shown in the 41-year-old male (Fig 2a) and the 88-year-old male (Fig 2b). The total number of axons is comparatively larger in the younger specimens. The amount of connective tissue increases with age, and, the shape of myelin sheaths changes in the older individuals (Fig 2b).

**Discussion**

Concerning the various staining methods, we have previously reported on the comparison of preparation methods for the evaluation of nerve axons⁸. Our conclusion was that the key point of the most suitable preparation method was to have secondary chromic acid fixation followed by nitrocellulose (or celloidine) embedding and the use of discriminative staining methods⁹ such as LPH, Masson-Goldner-Goto method⁳, modified hematoxylin-cosin stain, or selected silver impregnation methods employed after the secondary chronic fixation (Luxol fast blue-silver, Luxol fast blue-silver-periodic acid Schiff-hematoxylin). These discriminative staining methods yield good overall results concerning the lowest shrinkage ratio of tissue and the narrowest range of shrinkage ratio (10 ± 0%)⁹. The shrinkage ratios are the most important factor in the morphological study of the nervous system, central or peripheral.

There have been reported on the decrease with age of the average number and area of myelinated axons at level of L1 of the human LCST (which innervates the anterior horns supplying the lower extremities)⁹, and on the differences in axonal area between males and females: the average size is always larger in male compared to female⁹. However, no evaluation of nerve fibers in the LCST is included in the literature regarding the cervical levels, apart from decrease with age of the number of axons in the posterior funiculus at the C6 level⁷. At the C5 level, we found similar results in the LCST, which contains axons innervating the anterior horn cells supplying the upper and lower extremities, compared to the data concerning the L1 level¹⁻².

Finally, our preparation method makes it possible to easily evaluate under the microscope differences in nerve fiber axons in the LCST due to gender and/or age without the help of quantitative morphometry.

**References**