Morphometric Analysis of the Human Femoral Nerve and its Ageing Process

By

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Summary: We analysed the sizes of nerve fibres in the human femoral nerve which innervates the quadriceps femoris muscles. The material was taken from 14 cadavers aged from 61 to 97 years. A linear regression analysis disclosed a significant age-related decrease of the mean transverse area of axons. Such decrease with age may be an indication of motoneuron atrophy. Our results could help in the understanding of the correlation between morphology and function during the ageing process.

Postural instability has been identified as a major factor in the sudden loss of balance. Postural adjustment depends on antigravity muscles³, among which the quadriceps femoris is the most important for keeping the upright standing position. Therefore, a deterioration in their function may give rise to postural disturbance.

Since the motoneurons innervating the lower limb muscles begin to disappear from the age of 60², ⁷, it would seem that the deterioration of muscular function with age may in part be due to age-related changes in the motoneurons. Although past studies have analysed the physiological functions of the femoral nerve by measuring H-reflex activities⁸, ¹⁰, there has been no quantitative research on the transverse areas of anterior horn cells, as far as we know.

In the present study, we conducted a morphometric analysis of the myelinated axons of the human femoral nerve innervating the quadriceps femoris muscle to study age-related changes.

Material and Methods

Small sections of the femoral nerve beneath the inguinal ligament innervating the quadriceps femoris muscle were removed from 14 human cadavers (7 males and 7 females) for anatomic dissection. The age of cadavers ranged from 61 to 97 years (average and SD: 80.1 ± 11.1 years). The causes of death indicated no direct or indirect influence on the nervous system. The femoral nerve was therefore considered to be normal. We employed the same methods as in our previous reports regarding fixation, washing, dehydration, embedding, staining and morphometry. We adopted the Luxol fast blue-periodic acid-Schiff-hematoxylin stain (LPH) for 6 cadavers and Goto's modification of Masson-Goldner's method (MGG) for 8 cadavers⁵, ⁶ (Table 1). Regarding the shrinkage of sections, both staining methods showed practically the same ratio: 10 ± 0% in length.

The systemic sampling method was employed for the measurement of axons. Five sampling sites were selected: in the centre of the nerve, and in four marginal sites. Highly enlarged images (3,500 times with oil immersion) of areas in a square eyepiece grid were selected to measure the transverse area of axons.

For the measurement and analysis of sections, we employed (a) a microscope equipped with a

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drawing tube (BH2 with BH2-DA, Olympus, Japan), (b) a digitiser (Digitizer KC 3300, Graphtec Co., Japan), (c) a computer (PC-9821V13, NEC, Japan) with original analysing software for storing data and calculation. The data were presented as mean ± S.D. A linear regression analysis was performed to determine the correlation between age and the mean area of the axons.

Fig. 1. High-power transverse view microphotograph of the human femoral nerve of a 79-year-old female. Masson-Goldner-Goto’s stain, scale bar = 10 μm.

Fig. 2. High-power transverse view microphotograph of the human femoral nerve of a 92-year-old female. Masson-Goldner-Goto’s stain, scale bar = 10 μm.
Results

Microscopic findings
A microscopic study of the femoral nerve in all 14 subjects revealed a marked difference in the number and size of axons depending on age. This is evidenced in the high-power-view photograph shown in Fig. 1 (79-year-old female) and Fig. 2 (92-year-old female). Most of the fibres are myelinated. In both figures, the axons appear dark-blue in colour surrounded by a pink-coloured myelin sheath. The axons in Fig. 1 are definitely larger and more numerous than in Fig. 2.

Morphometric data
The mean transverse area of axons and mean perimeter of axons in all subjects are listed in Table 1.

The mean transverse area of axons ranged from 3.252 to 8.620 μm² (mean ± SD: 5.858 ± 1.799 μm², Table 1). Fig. 3 shows that the mean transverse area of axons decreased with age. A regression analysis reveals a significant negative correlation between age and the mean transverse area of axons (r = -0.734, p < 0.01).

Discussion
There have been several studies on the changes in the musculoskeletal system during ageing process. Aniansson et al.1) reported lower extremity muscle strength shows a reduction by as much as 40% between 30 and 80 years of age. This condition is more severe in older persons with a history of falls. In such subjects, the mean knee muscle strength was half that of nonfallers13). In recent physiological studies, Morita et al.10) indicate that the facilitation of the monosynaptic Ia fibres in the femoral nerve decreased linearly with age. A similar finding has also been reported by Koceja et al.8).

In the present study, we found a significant age-related reduction of the mean transverse area of axons in the femoral nerve. Yanagisawa et al.17) found similar results in the deep peroneal nerve innervating the tibialis anterior muscle.

As a long-term denervation process can be observed only in the largest and fastest-conducting motoneurons7,9), it is important to measure accurately the size of axons in nerve fibres. This we could achieve thanks to the use of the LPH and MGG5,6) which both enable clear discrimination of structures (axons, myelin sheaths, nuclei, amyloid bodies, vessels, connective tissue, etc.) with minimal shrinkage (only about 10% in length). Several studies using these methods4,11-17) found age-related changes in various other nerves.

Although many other factors affect the human postural control systems, our findings of age-related changes in the femoral nerve may provide critical information concerning the mechanism of postural instability in older people.

References
1) Aniansson A, Hedberg M and Henning G. Muscle mor-

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Mean 80.1  5.858  8.526  SD 11.1  1.799  1.338

MTA: mean transverse area of axons (μm²), MPA: mean perimeter of axons (μm), LPH: Luxol fast blue-periodic acid-Schiff-hematoxylin stain, MGG: Masson-Goldner-Goto’s stain.

Fig. 3. Linear regression analysis between the mean area of axons and age (n = 14, p < 0.01).
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