Morphometric Analysis of the Human Trigeminal Nerve

By

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Summary: We evaluated the components of nerve fibers of the motor and sensory roots of the trigeminal nerve by morphometric analysis. Trigeminal nerves were obtained from 5 cadavers (males, aged 67–95) were stained by Masson-Goldner-Goto method and examined under the microscope using a morphometric image-analyzer. The area and perimeter of axons were larger in the motor root than in the sensory root. The size distribution of axons was wider in the motor root than in the sensory root and the distribution pattern was unimodal type. These findings suggested that nerve fibers of the human trigeminal nerve gave similar findings to those of other peripheral nerves, regarding axonal size distribution and relative size of motor and sensory nerve axons.

The trigeminal nerve has two roots (motor and sensory) and supplies to most of the oromaxillo-facial region. Its anatomical organization is very important for the analysis of such mechanisms as mastication and pain in peripheral organs (facial skin, masticatory muscles, teeth, gums). There have been many previous studies of nerves of peripheral region of the trigeminal nerves such as the ophthalmic¹⁾ and maxillary⁶⁾ and infra-alveolar nerves⁵⁾, and a few report of motor^{9,12)} and sensory roots^{7,9,13)} but they have not desultorily a comparison with motor and sensory root.

The aim of this study is, therefore to clarify the fiber component of the motor and sensory root of the trigeminal nerve and the comparison of the trigeminal nerve by morphometrically the difference between the motor and sensory root, in few of fact that the roots are thought not to contain the autonomic nerve fibers originated from other nerve.

Material and Methods

Trigeminal nerves were obtained from 5 cadavers (males, aged from 67 to 95 years) without any disease of the central and peripheral nervous

systems. The trigeminal nerve was cut near the surface of the pons and after cutting, removing the brain from the cranial cavity and we obtained the trigeminal roots with ganglion (Fig. 1). After fixation in a solution of 10% neutral formalin for 2 weeks, the specimens were transferred into a secondary fixative solution composed of 5% potassium dichromate and 5% potassium chromate (1:4, in volume) and were left there for 2 weeks at room temperature and for 1 week at 37°C. After the secondary fixation, the specimens were washed in running water overnight and dehydrated in graded series of ethanol, before embedding nitrocellulose (Shiojirin, Showa Ether Co., Tokyo, Japan). The tissue blocks were then transversely sectioned at 30 µm thickness. The sections were stained by Masson-Goldner-Goto method.

For morphometry, we used an original morphometric image-analyzer comprising a light microscope was equipped with a drawing tube (BH2 with BH2-DA, Olympus, Tokyo, Japan), a digitizer (Crystizer α KC3300, Yokohama, Japan) and a computer (PC-9821 V13, NEC, Tokyo, Japan) running original programming software for data storing and analysis. We measured analyses of each transverse sectional area of motor and sensory

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roots as well as the area and number of nerve fiber axons of the trigeminal nerve. Enlarged images (70 times) were used to measure the transverse sectional area of nerve bundles excluding connective tissue. As for the analysis of nerve fibers, the systemic sampling method was employed. Five sampling sites (each $22.5 \times 22.5 \mu\text{m}$ in size) were selected in each transverse section of the motor and sensory roots (Fig. 2). Highly enlarged images (3500 times with oil immersion) were used to measure the area of axons and count their number.

Results

Histological Observations

With the Masson-Goldner-Goto staining method, the axons are stained in blue and the myelin sheaths in red (Fig. 3). The trigeminal nerve is composed of several uniform fiber bundles. We counted 4–5 in the motor root, and about 70 in the sensory root. Most of the fibers were myelinated,

with only a few unmyelinated both in the motor and sensory roots.

Morphometric Analysis

The transverse sectional area of the nerves ranged from 0.176 to 0.385 mm^2 (average: 0.295 mm^2) in the motor root, and from 1.810 to 2.525 mm^2 (average: 2.147 mm^2) in the sensory root (Tables 1 and 2).

The area of axons in the motor root was $1.612 \mu\text{m}^2$ to $3.040 \mu\text{m}^2$ (average $\pm$ SD: $2.526 \pm 0.565 \mu\text{m}^2$), while it was $0.679 \mu\text{m}^2$ to $1.295 \mu\text{m}^2$ (average $\pm$ SD: $0.896 \pm 0.250 \mu\text{m}^2$) in the sensory root (Tables 1 and 2).

The perimeter of axons ranged from $4.215 \mu\text{m}$ to $6.103 \mu\text{m}$ (average $\pm$ SD: $5.400 \pm 0.734 \mu\text{m}$) in the motor root, and from $2.594 \mu\text{m}$ to $3.943 \mu\text{m}$ (average $\pm$ SD: $3.078 \pm 0.533 \mu\text{m}$) in the sensory root (Tables 1 and 2).

The total number of axons ranged from 3,486 to 6,857 (average: 5,268) in the motor root and from 38,042 to 69,833 (average: 51,862) in the sensory

Fig. 2. Schematic drawing of systemic sampling method.

Five sampling sites were selected from each transverse section of motor and sensory roots. Highly enlarged images (3500 times) of each sampling site were used to measure axonal area and count the number of axons. Axons inside the square and on the lower and right lines (thick lines) included in the measurement; those on the upper and left lines (thin lines) were excluded.

Table 1. Morphometric data of the motor root of the trigeminal nerve

Specimen number	Sex	Age	Transverse sectional area of nerve (mm ²)	Average number of axons in analysis unit	Total number of axons	Area of axons (μm ²)		Perimeter of axons (μm)	
						average	SD	average	SD
1541	Male	67	0.385	9	6857	2.381	1.038	5.215	1.228
1537	Male	72	0.302	9	5366	2.745	1.275	5.733	1.408
1533	Male	89	0.375	8	5931	2.854	1.424	5.736	1.488
1540	Male	90	0.176	10	3486	3.040	1.234	6.103	1.373
1515	Male	95	0.238	10	4702	1.612	0.838	4.215	1.148
average		83	0.295	9	5268	2.526	0.565	5.400	0.734

Table 2. Morphometric data of the sensory root of the trigeminal nerve

Specimen number	Sex	Age	Transverse sectional area of nerve (mm ²)	Average number of axons in analysis unit	Total number of axons	Area of axons (μm ²)		Perimeter of axons (μm)	
						average	SD	average	SD
1541	Male	67	2.525	14	69833	0.982	0.509	3.204	0.900
1537	Male	72	1.810	13	46494	0.785	0.359	2.887	0.708
1533	Male	89	2.140	9	38042	0.739	0.294	2.761	0.578
1540	Male	90	1.968	13	50534	1.295	0.649	3.943	1.069
1515	Male	95	2.295	12	54406	0.679	0.497	2.594	0.817
average		83	2.147	12	51862	0.896	0.250	3.078	0.533

root (Tables 1 and 2).

The distribution of axonal area was unimodal in both motor and sensory roots and, while their range was wider in the motor root than in the sensory root (Fig. 4).

Discussion

Various factors may affect the data derived from morphometric analysis, and should be taken into account. For instance, tissues may undergo deformation or shrinkage during fixation and embedding. Schwarzacher¹⁰⁾ reported that the human peripheral nerve might shrink by up to 40% with paraffin embedding and silver impregnation and Nakamura⁸⁾ reported similar shrinkage ratio in the case of brains with 10% formalin fixation and celloidin embedding. However, the fixation and embedding method we used⁴⁾ causes only 10%. As for staining method employed, Masson-Goldner-Goto staining can distinguish the axon and the myelin sheath more easily than other methods (e.g. silver impregnation). Our choice of magnification (3500 times, instead of 1,000 times in previous studies) also helped reduce the error range, as it is generally

agreed that the error range decrease in reverse ratio to the magnification. In the fact, we believe that our method may be the most suitable for morphometric analysis.

According to the traditional morphology and physiology, and previous studies^{2,3)}, the motor nerve fiber of peripheral nerve are larger than the sensory nerve. Furthermore, Fujii and Goto²⁾ and Fujii *et al.*³⁾ reported that the size distribution of axons was wide in the facial nerve (only motor root), which is a "pure motor" nerve, and narrow in the cochlear nerve, which is a "pure sensory" nerve, while a unimodal size distribution of axon was reported both for motor (motor root of the facial nerve in Fujii and Goto²⁾, and the oculomotor nerve in Takeshita *et al.*¹¹⁾) and sensory (the cochlear nerve in Fujii *et al.*³⁾) nerve fiber.

Our precise finding of the human trigeminal nerve gave similar findings to there of other peripheral nerves, regarding axonal size distribution, as well as the relative size of motor nerve axons and sensory nerve axons.

Fig. 4. Axon size distribution in the motor and sensory roots of the trigeminal nerve.

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Explanation of Figures

Plate I

Fig. 1. Macrophotograph of motor and sensory roots of the trigeminal nerve with trigeminal ganglion. M, Motor root; S, Sensory root; G, Trigeminal ganglion; Scale Bar = 10 mm.

Fig. 3. Microphotograph of transverse sections of the trigeminal nerve. (a), Motor root; (b), Sensory root. Scale Bar = 10 μm.