Evidence for the Tapering of Nerve Axons

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Abstract: Recently, the transverse areas of axons have been used as an index for pathological study based on the supposition that the transverse area of the axon is normally maintained constant. It is generally accepted that nerve conduction velocity is directly proportional to the fiber diameter, under the condition mentioned in the proceeding sentence. To verify this supposition, we measured the transverse areas of the facial nerve axons at three positions: proximal, middle and distal in three subjects. We found that the average cross-section area of the axons decreased moderately in proportion to the distance from the soma of each nerve. The diminution ratio averaged about 2.6% per millimeter.

Key words: facial nerve, axon, morphometry, nerve fiber analysis, nerve conduction velocity

Introduction

There are two main methods of neuromorphological assessment of the human peripheral nervous system. One is by electron-microscopic observation of the nerve tissue, and the other is a quantitative assessment of the total number of nerve fibers obtained by their counting and of the transverse area or diameter of each nerve fiber obtained by actual measurement. Nerve fiber analysis has a long history1-4) but has provided few results in humans. The number and the size of nerve fibers in the human facial nerve have been measured4-7): the average number is 6,000 and the average cross-section area of the axons is $6 \mu m^2$5) and their changes with age have been reported5). The next step is to clarify whether the size of nerve fibers (the transverse area of axons) is constant or whether the axons taper.

The principle that the conduction velocity in myelinated fibers is directly proportional to the fiber diameter was proposed by Hursh8) and Tasaki et al.9); the relationships between the conduction velocity and fiber diameter have been investigated in several primates10,11). This principle was based on the supposition that the cross-section area of an axon is constant. However, this supposition has never been re-evaluated: the cross-section of an axon as a function of distance from the soma has never been experimentally determined.

Here, nerve fiber analyses were conducted on the human facial nerve, using a new staining method, luxol fast blue-PAS-hematoxylin (LHP) stain12), which is one of the discriminative staining methods for the nervous system that permits simultaneous observation of the axon.
and the surrounding myelin sheath. The following combination of equipment was used in the study: a microscope with a drawing tube and an image analyzing computer system for structure tracing, data logging, and statistical analysis.

**Material and Methods**

Three human facial nerve trunks were cut off inside the brain stems and near the fundus of the internal auditory meatus, of three cadavers. The body was placed in a coffin after death with enough dry ice to minimize the postmortem changes until it was put into a cold chamber at 4 degrees C. Twenty-four hours after death, the cadaver was injected with formaldehyde into the arterial system for fixation. The causes of death, past history, and dissection findings indicated no direct or indirect connection with the facial nerve, so the nerves studied were considered normal. Each nerve was collected from three individuals: subjects 1–3, aged 55, 61, and 73, respectively. The methodology followed the same procedure used in our previous reports. After fixation of the nerves in 10% formalin and a secondary fixative with a mixed solution of 5% potassium dichromate and 5% potassium chromate (1:4, volume/volume), the nerves were embedded in celloidin after washing in running water and dehydration with alcohol. The celloidin blocks were sectioned transversely into 10 μm thick slices and 20 μm thick slices at regular intervals and stained with LPH. The shrinkage of the sections during their preparation amounted to about 20% in length. After each section was observed, the transverse areas of the axons from each subject were measured at three (proximal, middle and distal) positions, respectively. For measurement and analysis of the facial nerve, a microscope with a drawing tube (or camera lucida) and an image analysing computer system (MOP-100, Carl Zeiss Vision Co., Tokyo) were used. Highly enlarged images (1,340 times) were used with oil immersion to measure the transverse areas of axons. For the measurement, a sampling site with an area of 6,184 μm² was selected in the center of each section, because no clear difference of cross-section area was evident among the various sites of a single facial nerve section.

**Results**

The cross-section areas of the facial nerve axon decreased with distance from the brain stem toward the fundus of the internal auditory meatus, in all three subjects (Fig. 1). The mean transverse areas of axons at the proximal, middle and distal positions of Subject 1 were 5.1, 4.5 and 3.5 μm², respectively; those of Subject 2 were 4.1, 3.3 and 2.6 μm², respectively; and those of Subject 3 were 7.5, 6.3 and 4.9 μm², respectively. Thus, the average cross-section area of the axons at the proximal position was greater than that at the middle position (p<0.01), and the area at the middle position was greater than that at the distal position (p<0.01) of each nerve (Table 1). The average rate of decrease of the cross-section area was 2.6% per millimeter (Table 1). These results indicate that the facial nerve axon tapers gradually from the proximal to the distal position in each subject, although these three subjects had differing average cross-section areas values of the axons (Fig. 2).

**Discussion**

Books of neurology, especially neuromorphology, seldom discuss whether or not an axon maintains a constant cross-section area. The reasons for this may be the same as the reasons for the relatively few reports of nerve fiber analysis, as mentioned in a previous article: it
has only recently become possible to stain the axon and myelin sheath, such that they can be discriminated by light microscopy. The development of electron microscopic procedures suitable for observation of nerve fibers has not contributed much to quantitative nerve fiber analysis in humans. We have been studying the human nerves morphologically and quantitatively, and our research was aided by several unique contributions: 1) we directly mea-

Fig. 1. High-power view of facial nerve (LPH stain). a: proximal position of Subject 3; b: distal position of Subject 3; bar = 20 μm, Ax: axon; Ms: myelin sheath.
Table 1. Transverse area of facial nerve axons.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Sampling position</th>
<th>Sampling number</th>
<th>Transverse area of facial nerve (mm²)</th>
<th>Mean transverse area of axons with S.D. (μm²)</th>
<th>Diminution ratio*** (%/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>M</td>
<td>proximal</td>
<td>93</td>
<td>0.990</td>
<td>5.1±2.1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>middle</td>
<td>73</td>
<td>0.994</td>
<td>4.5±1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>distal</td>
<td>75</td>
<td>1.032</td>
<td>3.5±1.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>M</td>
<td>proximal</td>
<td>88</td>
<td>0.784</td>
<td>4.1±1.9</td>
<td>2.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>middle</td>
<td>116</td>
<td>0.577</td>
<td>3.3±1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>distal</td>
<td>60</td>
<td>0.798</td>
<td>2.6±1.1</td>
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</tr>
<tr>
<td>3</td>
<td>73</td>
<td>M</td>
<td>proximal</td>
<td>93</td>
<td>0.880</td>
<td>7.5±2.5</td>
<td>2.7</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>middle</td>
<td>124</td>
<td>0.770</td>
<td>6.3±2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>distal</td>
<td>74</td>
<td>0.980</td>
<td>4.9±1.6</td>
<td></td>
</tr>
</tbody>
</table>


* The distance from the brain stem to the proximal position is 6 mm, 3 mm and 7 mm each in the order of subject number; to the middle position 15, 13 and 13 mm; to the distal position 17, 20 and 20 mm.

** Number of axons in a sampling area of 6,184 μm². The number of axons was large for the middle sampling position of subjects 2 and 3. They were tightly packed because of slight intervening and surrounding connective tissue, blood vessels and fat tissue (transverse areas of the facial nerve were small in such position).

*** The diminution ratio was calculated as follows: \( \frac{(pta-dta)/pta}{(pD-dD)} \)

pta: transverse area of axons at the proximal position; dta: transverse area of axons at the distal position; pD: distance from the brain stem to the proximal position; dD: distance from the brain stem to the distal position. Significant differences of mean transverse area of axons were observed among the proximal, middle and distal positions of the three subjects (p<0.01).

It is highly probable that facial nerve axons are tapered and do not have a constant cross-section area. It is widely accepted that the conduction velocity in myelinated fibers is directly proportional to the fiber diameter. Hursh proposed this principle in the cat, and reported no evidence of tapering in any of the preparations upon which velocity measurement had been made, since the largest fiber in the peripheral segment of the preparations was not smaller than the largest fiber in the middle and proximal segments. However, he did not show the distributions of the diameter measurements of the peripheral, middle and proximal segments, and the problem is that he made the sections after fixation in either 1% osmic acid or 10% formalin and embedding in paraffin which distorted the specimens by shrinkage. It is necessary to embed the specimens in celloidin after the formaldehyde fixation of the nerves followed by secondary fixation with chromic acid, which makes tissue hard and minimizes tissue shrinkage. This fixation method is suitable for the morphological measurements.
Fig. 2. Scatter diagrams and regression lines between average transverse area of facial nerve axons and distance from the brain stem (r = -0.88, -1.00 & -1.00 in the order of subject number). A schema in the bottom of the figure shows tapering of the facial nerve axon. A: proximal position; B: middle position; C: distal position.

When human autopsy material is used, various sources of error must be considered in performing histologic morphometry: 1) time and care before autopsy, 2) fixation technique and fixative, 3) embedding, 4) section thickness, and 5) measurement. Time and care before autopsy (see Material and Methods) is very important for minimizing postmortem changes. Postmortem tissue anoxia for extended periods of time may cause shrinkage of plasmalemma. The phenomenon of autolysis may also be accelerated when a cadaver is kept at high temperature. Fixation is one of the most important operations in morphometric analysis. Formaldehyde fixation, for example, may cause extreme shrinkage of tissue during histologic processing. When secondary fixation with chromic acid is employed after formaldehyde fixation, tissue shrinkage is reduced (about 10% in the length of each of the three dimensions). In the present study, the celloidin blocks were hardened more than usual because we needed thinner sections (10 and 20 µm thick slices), and linear shrinkage of the sections amounted to approximately 20%.

The only literature we could find that discussed the tapering of nerve fibers was that of Hursh. This suggests the necessity for re-evaluation of nerve fiber tapering, and the principle of nerve conduction velocity. It is necessary to confirm that the conduction velocity in myelinated fibers is directly proportional to the fiber diameter.

Before we evaluate the transverse area of axons pathologically and study the transverse area of the axons of one nerve with regard to aging, it must be determined as to whether
the axon maintains a constant transverse area. We suggest that other nerve axons be further examined morphometrically under light and electron microscopes.

Acknowledgements

We thank Dr. T. Sato, Department of Physiology, Showa University School of Medicine, for his advice on electrophysiology and Mr. S. Endo, Nippon Steel Corporation, for reading the manuscript.

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[Received April 27, 1994: Accepted May 19, 1994]