Motor Unit Size of M. Stylohyoideus and M. Stapedius in the Mouse

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Summary: To elucidate the relation between the caliber of myelinated nerve fibers (MNFs) and motor unit size (i.e., number of muscle fibers per motor unit), motor unit size was measured for the mouse m. stylohyoideus, innervated predominantly by extra large MNFs, and compared with that of the mouse m. stapedius, innervated predominantly by large MNFs. All muscle fibers and MNFs were counted morphologically, and mean motor unit size was calculated for each muscle. The results showed that the average of 12 mean motor unit sizes for the m. stylohyoideus (35.8 ± 8.2), innervated predominantly by extra large MNFs, was greater than that for the m. stapedius (6.5 ± 1.3), innervated predominantly by large MNFs.

Edgeworth (1899) found on the dog nervus facialis that nerve branches to the venter caudalis of the m. digastricus and m. stylohyoideus consisted of larger myelinated nerve fibers (MNFs) than nerve branches to the other facial muscles. In a study on the mouse n. facialis, Shimozawa and Nakamura (1985) also found that MNFs of the r. digastricus and r. stylohyoideus consisted of considerably larger MNFs than the truncus facialis distal to the foramen stylomastoideum innervating the skeletal muscles in the face (Shimozawa and Furuta, 1984).

It is generally considered that, in vertebrates, the large motor MNFs innervate the white (fast twitch) muscle fibers and the small motor MNFs innervate the red (slow twitch) muscle fibers (Tasaki and Mizutani, 1944; Kuffler and Williams, 1953). Moreover, electrophysiological studies by Henneman and Olson (1965) in the cat m. soleus and m. gastrocnemius indicated that the diameter of the axon may be related to the number, type and size of the muscle fibers innervated by the axon. To examine the relationship between the caliber of MNFs and the types of muscle fibers, Shimozawa and Ishizuya-Oka (1987) performed a quantitative analysis of the muscle fiber types in the mouse m. digastricus and m. stylohyoideus, innervated by larger MNFs, and the m. zygomaticus and m. buccinator, innervated by smaller MNFs. However, the results showed that the m. digastricus and m. stylohyoideus do not have a higher ratio of white muscle fibers than the m. zygomaticus and m. buccinator.

Shimozawa and Nakamura (1993) roughly classified the caliber of MNFs of the mouse into 4 classes: small (myelin sheath; MS 1–2 μm, axon; A 0.5–1 μm), medium (MS 2–3.5 μm, A 1–2 μm), large (MS 3.5–6 μm, A 2–4 μm) and extra-large (MS 6–12 μm, A 4–9 μm), according to electron microscopic measurements. The r. stylohyoideus consists of predominantly extra large MNFs (Shimozawa and Nakamura, 1985) and the r. stapedius consists of predominantly large MNFs (Shimozawa, 1978) in the mouse. To elucidate the relation between the caliber of MNFs and motor unit size, we performed a comparison between the motor unit size of the mouse m. stylohyoideus and that of the m. stapedius.

Materials and Methods

Twelve m. stapedius from 7 adult mice (ddy, 8 weeks old, 30.9 ± 0.9 g body weight) and 12 m. stylohyoideus from 6 adult mice (ddy, 8 weeks old, 29.1 ± 0.4 g body weight) were used in this study. The muscle fibers and MNFs of each muscle were counted from the same side of each mouse.

1) Muscle Fiber Enumeration

All muscle fibers in each m. stapedius were teased out and directly counted following modified nitric acid digestion of the connective tissue (Aikawa et al., 1993). Mice were anesthetized with ether, weighed, and killed by decapitation. The heads were cut in half sagittally and immersed in 15% nitric acid solution in containers at 30°C for approximately 4 hr to digest the connective tissue, and then the solution
was gradually replaced by distilled water. The m.
stapedius was removed and placed in distilled water.
All muscle fibers were carefully teased free from
the muscle and counted with the aid of fine-tipped
forceps under a dissecting microscope.

In the same manner mentioned above, we have
confirmed that almost all muscle fibers of the m.
stylohyoideus run the full distance from the origin
to the insertion without myo-myonal junctions (Aikawa
and Shimozawa, in press). Hence, we regarded the
muscle fiber number at one transverse section as the
total muscle fiber number of the m. stylohyoideus,
and counted the muscle fibers histologically in the
same manner used for nerve fiber enumeration.

2) Nerve Fiber Enumeration
The nerve was fixed for 4—5 hr in Dalton’s fixative
(Dalton, 1955) at room temperature, dehydrated
with ethanol and embedded in methacrylate
resin from the Polysciences JB-4 embedding kit
(Polysciences, Inc., U.S.A.). The nerve was then
sectioned transversely (2—5 μm thick) using a glass
knife on a Sorval MT-1 Ultratome. The section was
stained with cresyl fast violet (Nguyen and Pender,
1989). The total MNF count of each nerve was done
on a magnified photomicrograph (×3, 180) of the
section by checking the original slide under the
microscope.

3) Motor Unit Size Calculation
The mean size of the motor unit (total muscle
fiber number/total motoneuron number) for each
muscle was calculated by dividing the total muscle
fiber number by 84% of the total MNF number in
the r. stapedius or by the total MNF number in the r.
stylohyoideus under the following assumptions:
(1) the motoneuron number is about the same as the
α-motor MNF number, (2) 16% of the total MNFs in
the r. stapedius are afferent (Lyon, 1979), (3) the r.
stylohyoideus contains very few (less than 0.3%)
afferent MNFs (Semba and Egger, 1986), and (4)
since we could not find typical muscle spindles in
the two muscles examined in this study, the γ-motor
MNFs to the intrafusal muscle fibers are negligible;
thus the α-motor MNF number is about the same as
the efferent MNF number.

Results
1) Motor Unit Size in M. Stapedius (Table 1)
The total muscle fiber number in the m. stapedius
was 489.6 ± 59.7 (average ± S.D., n = 12), and the
total MNF number in the r. stapedius was 92.2 ± 16.7
(n = 12), resulting in an innervation ratio (total
muscle fiber number/total MNF number) in the
mouse m. stapedius of 5.4 ± 1.0 (n = 12). The
α-MNF number was estimated as 77.4 ± 14.0
(n = 12), and the average of 12 mean motor unit
sizes as 6.5 ± 1.3 in the mouse m. stapedius.

2) Motor Unit Size in M. Stylohyoideus (Table 2)
The total muscle fiber number in the m. stylo-
hyoideus was 542.5 ± 59.6 (average ± S.D., n = 12),

Table 1. Innervation ratio and mean motor unit size in the mouse m. stapedius.

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Body weight (g)</th>
<th>MNF number</th>
<th>α-MNF number*</th>
<th>MF number</th>
<th>Innervation ratio**</th>
<th>Mean motor unit size***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Right</td>
<td>29.6</td>
<td>110</td>
<td>92.4</td>
<td>503</td>
<td>4.6</td>
<td>5.4</td>
</tr>
<tr>
<td>1Left</td>
<td>32.0</td>
<td>68</td>
<td>63.0</td>
<td>465</td>
<td>6.2</td>
<td>7.4</td>
</tr>
<tr>
<td>2R</td>
<td>31.5</td>
<td>78</td>
<td>73.9</td>
<td>443</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>2L</td>
<td>31.5</td>
<td>120</td>
<td>100.8</td>
<td>514</td>
<td>4.3</td>
<td>5.1</td>
</tr>
<tr>
<td>3R</td>
<td>30.2</td>
<td>119</td>
<td>100.0</td>
<td>533</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td>3L</td>
<td>30.2</td>
<td>95</td>
<td>79.8</td>
<td>603</td>
<td>6.3</td>
<td>7.6</td>
</tr>
<tr>
<td>4R</td>
<td>36.0</td>
<td>78</td>
<td>65.5</td>
<td>583</td>
<td>7.5</td>
<td>8.9</td>
</tr>
<tr>
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<td>36.0</td>
<td>97</td>
<td>81.5</td>
<td>459</td>
<td>4.7</td>
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<tr>
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<td>88</td>
<td>73.9</td>
<td>442</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
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<td>73</td>
<td>61.3</td>
<td>507</td>
<td>6.9</td>
<td>8.3</td>
</tr>
<tr>
<td>6R</td>
<td>36.0</td>
<td>95</td>
<td>79.8</td>
<td>412</td>
<td>4.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Average | 30.9 ± 0.9 | 92.2 ± 16.7 | 77.4 ± 14.0 | 489.6 ± 59.7 | 5.4 ± 1.0 | 6.5 ± 1.3 |

*: 84% of the MNF number
**: muscle fiber (MF) number/myelinated nerve fiber (MNF) number
***: MF number/α-motor MNF number
Table 2. Innervation ratio and mean motor unit size in the mouse m. stylohyoideus.

<table>
<thead>
<tr>
<th>Mouse No., side</th>
<th>Body weight (g)</th>
<th>MNF number*</th>
<th>MF number</th>
<th>Mean motor unit size**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Right</td>
<td>29.7</td>
<td>21</td>
<td>623</td>
<td>29.7</td>
</tr>
<tr>
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<td>29.2</td>
<td>16</td>
<td>450</td>
<td>36.4</td>
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<tr>
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<td>28.7</td>
<td>18</td>
<td>441</td>
<td>24.5</td>
</tr>
<tr>
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<td>28.7</td>
<td>11</td>
<td>647</td>
<td>58.8</td>
</tr>
<tr>
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<td>28.7</td>
<td>15</td>
<td>534</td>
<td>35.6</td>
</tr>
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<td>15</td>
<td>486</td>
<td>32.4</td>
</tr>
<tr>
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<td>14</td>
<td>534</td>
<td>38.1</td>
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<tr>
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<td>16</td>
<td>565</td>
<td>35.3</td>
</tr>
<tr>
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<td>28.5</td>
<td>16</td>
<td>531</td>
<td>33.2</td>
</tr>
<tr>
<td>6R</td>
<td>28.5</td>
<td>16</td>
<td>579</td>
<td>36.2</td>
</tr>
<tr>
<td>6L</td>
<td>28.5</td>
<td>14</td>
<td>574</td>
<td>41.0</td>
</tr>
</tbody>
</table>

*: MNF number = α-motor MNF number
**: Motor unit size = MF number/MNF number = innervation ratio

and the total MNF number in the r. stylohyoideus was 15.6 ± 2.3 (n = 12), resulting in an innervation ratio in the m. stylohyoideus of 35.8 ± 8.2 (n = 12) in the mouse. The α-MNF number was estimated as 15.6 ± 2.3 (n = 12), and the average of 12 mean motor unit sizes as 35.8 ± 8.2 in the mouse m. stylohyoideus.

Discussion

To elucidate the relation between the caliber of the MNF and motor unit size, individual motor unit size must be examined. But it is very difficult to investigate single motor unit size by tracing a single α-motor MNF in serial histological sections. Therefore, in the present study, we examined mean motor unit size in the individual muscle. A motor unit consists of a motoneuron and the muscle fibers innervated by the motoneuron. In general it is believed that branching of the axon rarely occurs except in the region near the terminus. Therefore, in the present study, we calculated mean motor unit size (total muscle fiber number/α-motor MNF number) on the basis of the assumption that the α-motor MNF number is about the same as the motor neuron number. But the axon may divide into some branches on the way to the muscle (Eccles and Sherrington, 1930), so the axon number counted in the nerve may be greater than the actual neuron number. Therefore, it is postulated that the mean motor unit size estimated in our present study (total muscle fiber number/α-motor MNF number) is somewhat smaller than the actual size (total muscle fiber number/total motoneuron number).

Motor Unit Size in M. Stapedius

The average sizes of the motor unit of the m. stapedius of the cat (Blevins, 1964), rabbit (Berlendis and De Caro, 1955; Malmfors and Wersäll, 1960), and human (Blevins, 1967, 1968) have been reported. In determining the number of α-motor MNFs, one must consider that there are not only α-motor MNFs but also γ-motor and afferent MNFs in the nerve. All these previous studies applied the Sherrington values of afferent MNFs for the nervi spinales (1/3—1/2 of the total MNF number) (Sherrington, 1894/1895) to the r. stapedius. However, the afferent MNF population of the r. stapedius may differ somewhat among species. Neither muscle spindles nor free sensory endings were detected in the cat m. stapedius (Blevins, 1964); a few atypical spindles were found in the m. stapedius of the cat (Ohta, 1958) and human (Nojiri, 1963); a few muscle spindles and intratendinous sensory endings were found in the human m. stapedius (Blevins, 1967); and typical muscle spindles were not found in the mouse m. stapedius in the present study. Thus the γ-MNFs to the intrafusal muscle fibers in the r. stapedius may be negligible. Therefore, 84% of the total MNF number in the mouse r. stapedius was used as the population of the α-MNFs in the present study.

The m. stapedius shows a bipennate muscle fiber arrangement in which the muscle fibers pass obliquely between the superficial and internal tendons, and not all of the muscle fibers of the muscle appear in any one cross-section (Aikawa et al., 1993). Thus it had been difficult to determine the total muscle fiber number for the m. stapedius by the histological method. All the previous studies (Blevins, 1964; Berlendis and De Caro, 1955; Malmfors and Wersäll, 1960; Blevins, 1967, 1968) estimated the total muscle fiber number for the m. stapedius from a histological cross section through the widest part of the muscle. Gollnik et al. (1981) first directly counted the total muscle fiber number of rat leg muscles by the nitric acid digestion and tearing method. In the present study, we directly counted the total muscle fiber number of the mouse m. stapedius by the modified nitric acid digestion and tearing method (Aikawa et al., 1993).
Motor Unit Size in M. Stylohyoideus

When Semba and Egger (1986) applied horse-radish peroxidase to the r. stylohyoideus and r. digastricus of the rat n. facialis, less than 0.3% of the geniculate ganglion cells were labelled. Thus, they reported that those branches do not contain a significant number of afferent MNFs. From their results, we consider afferent MNFs in the mouse r. stylohyoideus to be negligible. Since typical muscle spindles were not found in the mouse m. stylohyoideus in the present study, we consider γ-MNFs in the r. stylohyoideus also to be negligible. If the populations of afferent and γ-MNFs are negligible in the mouse r. stylohyoideus, the α-MNF number may be about the same as the total MNF number.

The present study showed that the average of 12 mean motor unit sizes for the mouse m. stylohyoideus (35.8 ± 8.2), innervated predominantly by extra large MNFs, was greater than that for the m. stapedius (6.5 ± 1.3), innervated predominantly by large MNFs. Therefore, it is suggested that MNF caliber is correlated with motor unit size. The present result is generally in agreement with that of Clark (1931) on the m. soleus and m. extensor digitorum longus of the cat, but is not in agreement with that of Fernand and Young (1951) on the m. depressor labii inferioris, m. sternothyroideus, m. tensor fasciae cruris and m. interosseous pedis of the rabbit. Our present result also may correspond to the electrophysiological studies of Eccles and Sherrington (1930) on the m. gastrocnemius, m. soleus, m. semitendinosus and m. extensor digitorum longus of the cat, McPhedran et al. (1965) on the m. soleus of the cat, Wuerker et al. (1965) on the m. gastrocnemius of the cat, and Proske and Waite (1974) on the m. gastrocnemius of the cat. Henneman and his coworkers showed electrophysiologically in the m. triceps surae of the cat that the size of the motoneuron relates to the diameter of the axon (Henneman et al., 1965; Cullheim, 1978), and the conduction velocity of the axon (i.e. the axon diameter; Hursh, 1939; Cullheim, 1978) to the contraction tension of the motor unit (i.e. motor unit size; Clark, 1931) (Henneman and Olson, 1965; McPhedran et al., 1965; Wuerker et al., 1965). Henneman et al. stated that both the neural and contractile properties of a motor unit are generally correlated with the size of its motoneuron, and they advocated this concept as the “size principle” (Henneman and Olson, 1965; Henneman et al., 1965, 1974). Our present result may support Henneman’s “size principle.” Since the motor unit is the basic functional unit of muscular contraction, it is important to study motor unit size to obtain an understanding of muscle movement control. The above-mentioned reports indicate that MNF caliber might be correlated with axonal conduction velocity, contraction tension and motor unit size. We wanted to determine why the r. stylohyoideus and r. digastricus of the mouse n. facialis consist of predominately extra large MNFs, in contrast with the nerves which innervate the other muscles and consist of predominantly large MNFs. Our present study on the mouse m. stylohyoideus and m. stapedius clarified that MNF caliber is correlated with motor unit size. The m. digastricus and m. stylohyoideus are concerned with mastication and deglutition, so it is assumed that those muscles might have to contract more rapidly for survival than the other muscles, particularly in a carnivore. Thus the caliber of MNFs innervating the m. digastricus and m. stylohyoideus might have had to evolve phylogenetically to become larger, for faster conduction velocity, and the larger MNFs might come to innervate more numerous muscle fibers than the smaller MNFs, resulting in the motor unit size of the former becoming larger than that of the latter. Further morphological and functional investigations on motor unit size will be needed to substantiate this assumption.

References

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Motor Unit Sizes in Mouse Head Muscles


