Localization and Contractile Properties of Intrinsic Longitudinal Motor Units of the Rat Tongue

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Sokoloff, Alan J. Localization and contractile properties of intrinsic longitudinal motor units of the rat tongue. J Neurophysiol 84: 827–835, 2000. Tongue dysfunction is a hallmark of many human clinical disorders, yet we lack even a rudimentary understanding of tongue neural control. Here, the location and contractile properties of intrinsic longitudinal motor units (MUs) of the rat tongue body are described to provide a foundation for developing and testing theories of tongue motor control. One hundred and sixty-five MUs were studied by microelectrode penetration and stimulation of individual motor axons coursing in the terminal portion of the lateral (retrusor) branch of the hypoglossal nerve in the rat. Uniaxial MU force was recorded by a transducer attached to the protruded tongue tip, and MU location was estimated by electromyographic (EMG) electrodes implanted into the anterior, middle, and posterior portions of the tongue body. All MUs produced retrusive force. MU twitch force ranged from 2–129 mg (mean = 35 mg) and tetanic force ranged from 9–394 mg (mean = 95 mg). MUs reached maximal twitch force in 8–33 ms (mean = 15 ms) and were resistant to fatigue; following 2 min of stimulation, MUs (n = 11) produced 78–131% of initial force. EMG data were collected for 105 MUs. For 65 of these MUs, the EMG response was confined to a single electrode location: for 26 MUs to the anterior, 21 MUs to the middle, and 18 MUs to the posterior portion of the tongue. Of the remaining MUs, EMG responses were observed in two (38/40) or all three (2/40) tongue regions. These data provide the first contractile measures of identified intrinsic tongue body MUs and the first evidence that intrinsic longitudinal MUs are restricted to a portion of tongue length. Localization of MU territory suggests a role for intrinsic MU in the regional control of the mammalian tongue observed during feeding and speech.

INTRODUCTION

Despite the importance of the mammalian tongue in normal oro-motor behavior and the association of tongue dysfunction with many human clinical syndromes (e.g., cerebral palsy, Down’s syndrome, obstructive sleep apnea, tongue thrusting; Adachi et al. 1993; Dent 1995; Guilleminault et al. 1995; Lowe 1981; Murdoch et al. 1995; Yarom et al. 1986), we lack even a basic understanding of the physiological organization of the fundamental output elements of the tongue motor system, i.e., the hypoglossal (tongue) motor units (MUs). Study of hypoglossal MU organization is hampered by the complexity of tongue muscular architecture. Eight muscles are present in the tongue of many mammals: four originating outside the tongue body [the “extrinsic” muscles genioglossus (GG), hyoglossus (HG), styloglossus (SG), and palatoglossus] and four having both origin and insertion within the tongue body [the “intrinsic” muscles inferior longitudinalis (IL), superior longitudinalis (SL), transversus (T) and verticalis (V)]. Extrinsic and intrinsic muscles interdigitate extensively within the tongue, making functional isolation of tongue body musculature, and thus of identified MUs, difficult.

Due in large part to the complexity of tongue anatomy, studies of MU organization have focused either on MUs of extrinsic muscles (e.g., Fuller et al. 1998; Yokota et al. 1974; see references in Lowe 1981) or on MUs commonly classified as “protrusor” (motor axons coursing through the medial branch of the hypoglossal nerve) or “retrusor” (motor axons coursing through the lateral branch) (DiNardo and Travers 1994; Gilliam and Goldberg 1995; Kaku 1984; Sumi 1970; Travers and Jackson 1992). Few of these studies have included contractile characterization of MUs, and thus the relationship between MU activity and unit contractile properties is unknown.

Further, none of these studies have considered the organization of MUs of identified intrinsic muscles. Yet information on the organization of MUs of intrinsic muscles is likely important for developing and testing models of tongue motor control. For example, one current theory of tongue control, the muscular-hydrostat theory, posits a central role for intrinsic muscles in both tongue movements and posture (Kier and Smith 1985). Additionally, by virtue of their anatomical distribution, intrinsic muscles may be involved in the independent control of different tongue regions observed during feeding in mammals (Hiitemae et al. 1995) and in the complex changes in tongue shape observed during speech in humans (Stone 1990).

The neural mechanisms responsible for the control of regional tongue movements are not known, but presumably involve selective activation of MUs of localized territory within the tongue body. As part of an ongoing investigation of the physiology and activity of hypoglossal MUs, here I describe the contractile properties and location of intrinsic longitudinal MUs, i.e., MUs of intrinsic muscles with axons coursing through the terminal branch of lateral hypoglossal nerve. Two questions necessary to define the role of these MUs in regional tongue control are addressed. First, are intrinsic longitudinal MUs localized within the tongue body? And, second, if localized, do MU properties vary in a systematic way with location? Findings indicate that intrinsic longitudinal
MUs produce small forces, are nonfatiguing, and are localized within the tongue body. These results are discussed in light of current theories of tongue control.

Methods

Nine adult male rats (Sprague-Dawley, 300–350 g) were used in terminal experiments. All surgeries were done in accordance with the NIH Guidelines on the Use of Animals for Research and were approved by the Institutional Animal Care and Use Committee at Emory University. Sodium pentobarbital (40 mg/kg) was administered intraperitoneally, and surgery proceeded when withdrawal and eye-blink reflexes were completely suppressed. Supplemental doses (0.4 mg/kg, ip) were given as needed to maintain a surgical plane of anesthesia. At the end of the experiment, rats were euthanized by barbiturate overdose (sodium pentobarbital, iv).

Surgical procedure

Cannulae were secured in the right femoral artery to measure blood pressure and in the right femoral vein for infusion of dextrose (5%) in lactated Ringer solution to support mean blood pressure above 80 mmHg. Body temperature was continuously monitored and maintained at 36–38°C by radiant heat. A cannula was inserted into the trachea, and during the experiment, the rate and volume of respiration were adjusted, when necessary, to maintain end-tidal CO2 between 3–5%. Rats were placed supine in a rigid frame and secured with ear bars and maxilla clamp. The suprahypoid region was dissected to expose the branches of the left hypoglossal nerve (see Data collection). Throughout, care was taken to preserve the blood supply to muscles and nerves. The mylohyoid muscles and anterior digastric muscles were removed bilaterally, the left geniohyoid muscle was separated from the hyoid bone, reflected medially, and its nerve was cut. The main trunk of the hypoglossal nerve was carefully exposed at its emergence deep to the digastric tendon and the proximal portions of the medial and lateral hypoglossal nerve branches were exposed, as were the nerve branches to the GG muscle, the HG muscle, and the SG muscle. Following nerve dissection, the hyoid bone, origin of muscle and connective structures of the tongue, was clamped to ensure that subsequent force measurements would not be attenuated by hyoid movement.

A piece of 2-0 silk suture was secured into the tongue tip and attached to a strain gauge (Kulite) with an estimated minimal sensitivity of −0.2 mg resolution for measurement of contractile properties (see Gilliam and Goldberg 1995; Hellstrand 1981). Previous study has demonstrated that similar contractile measures are obtained whether the tongue is attached to a transducer or is free to move (Hellstrand 1981). In five experiments, three indwelling bipolar electrodes (50-µm wire diameter, 0.5-mm exposed tips) were placed in the center of anterior, middle, and posterior sections of the left tongue-body to record MU electromyographic (EMG) signals. In these experiments, MUs were assigned to one of six categories based on the location of EMG response: anterior, middle, posterior, antero-middle, postero-middle, or whole tongue. The entire exposure (i.e., supra and infraphyoid dissections, the oral cavity, and the tongue) was submerged in mineral oil maintained at 36–38°C. Following data collection, the tongue was removed and dissected to confirm nerve pattern and EMG electrode placement.

Data collection

ANATOMICAL ISOLATION OF INTRINSIC LONGITUDINAL MUS. The population of MUs that project to intrinsic longitudinal muscles of the left side of the tongue body was isolated by transection of the hypoglossal nerve branches that project to all other muscles. First, nerve branches to the left GG, HG, and SG were cut, leaving intact the motor axons that project to intrinsic tongue muscles. Second, the medial hypoglossal nerve was cut, disrupting the motor axons that project to transverse and vertical intrinsic muscles (Hellstrand 1981; O’Reilly and Fitzgerald 1990); this eliminated the potential study of transversus and verticalis muscles which, although oriented perpendicular to the long axis of the tongue, may produce retrusive forces on the transducer when the tongue is protruded (see Gilliam and Goldberg 1995). Following these sections, only the axons coursing in the terminal portion of the lateral hypoglossal nerve branch were left intact. In many mammals, these axons are thought to project to both superior and inferior intrinsic longitudinal muscles; in some mammals, however, superior longitudinal muscles are innervated by branches of the medial hypoglossal nerve (Hellstrand 1981; O’Reilly and Fitzgerald 1990; for recent discussion see Mu and Sanders 1999). A glycogen depletion experiment was performed to definitively identify the MU population examined in the present study. In one rat, left hypoglossal nerve branches were cut as described above; the left hypoglossal nerve trunk was placed on a bipolar electrode and stimulated for 2 h [330-ms burst, 100 parts per second (pps), 1 burst/s], and the tongue was removed and processed for glycogen (following Edström and Kugelberg 1968). In this experiment, depletion of glycogen was observed in all ipsilateral intrinsic longitudinal muscles, i.e., including the superior longitudinal muscles (Fig. 1). Thus, axons left in continuity in the present study are classified as “intrinsic retrusor tongue body MUs,” i.e., lateral nerve branch motor axons that innervate inferior, lateral, and superior longitudinal muscle fibers.

ACTIVE TENSION OF INTRINSIC RETRUSOR MU POPULATION. MU contractile properties were measured in eight animals. Prior to isolation of individual MUs, the effect of tongue length on forces generated by the entire population of intrinsic longitudinal MUs was measured to establish an optimal tongue length for MU characterization. In each experiment, the left hypoglossal nerve trunk was isolated to the midsagittal plane of tongue. Magnification, X70.
was placed on a bipolar electrode and stimulated at 2 times twitch threshold. Tongue length was increased in 10 or more 1-mm increment steps, corresponding to a range of passive tensions 0 and 10 g, and twitch force was determined at each length. The tongue length at which the largest force was produced was considered optimal for single unit studies.

PHYSIOLOGICAL ISOLATION AND STIMULATION OF INDIVIDUAL MUS. Conventional glass microelectrodes (15–25 mΩ, 2 M K-ace-tate) were driven into single axons in the left hypoglossal nerve trunk to measure contractile properties of individual MUs (following techniques of Cope and Clark 1991). Single motor axons were isolated by intra-axonal injection of depolarizing current (0.5–3.0 nA) in bursts delivered every 4 s (100 pps, 40-μs duration, 330-ms burst) as the electrode was lowered in 2-μm intervals (Transvertex Microdrive, Harvard Apparatus). Penetration of an isolated motor axon was determined by a change in force profile coincident with stimulation, and when possible (see RESULTS), the presence of EMG signal coincident with stimulation. In some penetrations, current strength was graded (to 3 times threshold) to verify that multiple MUs were not activated during stimulation. Microelectrode penetration of isolated motor axons has been successfully employed to study contractile properties of single MUs in hindlimb muscles (see Cope and Clark 1991; Tansey and Botterman 1996). Advantages of the intra-axonal technique include isolation of single motor axons with certainty and the ability to regularly record from many MUs in single experiments (up to 44 in the present study) allowing for within-animal analysis of MU measures.

Isometric contractile properties of isolated MUs were studied by the following intra-axonal stimulation protocols. Maximal MU force was determined during a train of stimuli (40-μs duration, 200 pps, 600-ms burst) delivered every 4 s, and twitch force and twitch contraction time were determined during a single stimulus delivered 2.0 s following each tetanic stimulation (following Burke et al. 1973; Cope and Clark 1991; Gilliam and Goldberg 1995). Multiple stimulation records (2–20 per unit) were collected for later off-line averaging to eliminate any artifact in background force produced by respiratory movements. MUs were tested for fatigue with 330 ms bursts of 40 pps delivered each second for 60 or 120 s (following Burke et al. 1973). Fusion frequency was determined by activation of MUs at a range of stimulation frequencies between 10 and 200 pps. All force and EMG (low-pass filter 6 kHz) waveforms were sampled at 3 kHz (CED, 1401 plus; Pentium, Gateway).

Data analysis

Contractile data were analyzed in SigAvg software (CED). MU twitch contraction time was measured from the beginning of the force deflection to peak force and MU fatigue was determined by comparing initial MU force to force produced following 120-s stimulation or, in less stable penetrations, following 60-s stimulation. To allow extensive comparisons to studies of other muscles, nonparametric tests of correlation (Spearman R) were used to identify relationships between MU properties. Whole nerve stimulation indicated that, for the entire population of MUs, force varied substantially with tongue length (see RESULTS). To investigate whether there was an effect of tongue length on the properties of individual MUs, a Mann-Whitney U Test (Statistica Software) was performed for groups of MUs sampled at different tongue lengths. A one-way analysis of variance (ANOVA) with passive tongue tension (an indirect measure of tongue length) as a covariate was also computed to compare contractile measures for MUs located in different tongue regions; comparisons between treatment means were made using the Tukey honest significant difference test (Statistica Software).

TABLE 1. Contractile properties of intrinsic longitudinal motor units

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Motor Unit Number (Ptet/Ptw)</th>
<th>Ptet Range (mg) (Ave, SD)</th>
<th>Ptw Range (mg) (Ave, SD)</th>
<th>CT Range (ms) (Ave)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/3</td>
<td>42–133 (80, 47)</td>
<td>11–21 (17, 5)</td>
<td>9–11</td>
</tr>
<tr>
<td>2</td>
<td>14/10</td>
<td>32–200 (125, 56)</td>
<td>16–95 (51, 28)</td>
<td>10–16 (12.5)</td>
</tr>
<tr>
<td>3</td>
<td>18/14</td>
<td>9–281 (76, 64)</td>
<td>4–129 (33, 31)</td>
<td>8–15 (11.6)</td>
</tr>
<tr>
<td>4</td>
<td>16/11</td>
<td>12–114 (51, 30)</td>
<td>7–40 (21, 11)</td>
<td>10–15 (11.8)</td>
</tr>
<tr>
<td>5</td>
<td>34/24</td>
<td>15–247 (83, 58)</td>
<td>2–90 (32, 21)</td>
<td>9–27 (16.7)</td>
</tr>
<tr>
<td>6</td>
<td>27/21</td>
<td>9–323 (118, 80)</td>
<td>6–83 (42, 24)</td>
<td>11–20 (16.6)</td>
</tr>
<tr>
<td>7</td>
<td>44/32</td>
<td>15–394 (99, 78)</td>
<td>5–115 (34, 24)</td>
<td>10–33 (16.0)</td>
</tr>
<tr>
<td>8</td>
<td>9/9</td>
<td>16–340 (123, 104)</td>
<td>7–100 (42, 35)</td>
<td>11–17 (15.1)</td>
</tr>
<tr>
<td>Total</td>
<td>165/124</td>
<td>9–394 (95, 71)</td>
<td>2–129 (35, 25)</td>
<td>8–33 (14.8)</td>
</tr>
</tbody>
</table>

Ptet, tetanic force; Ptw, twitch force; Ave, average; SD, standard deviation; CT, contraction time.
responses of a single unit are shown in Fig. 3. It was not possible to obtain all measures for each MU, nor was it possible to attain a passive tension of \( \pm 2 \) g for tests of all MUs. For 165 MUs, tetanic force ranged from 9 to 394 mg (95 \( \pm 71 \) mg, mean \( \pm \) SD); for 124 MUs, twitch force ranged from 2–129 mg (35 \( \pm 25 \) mg), and contraction times ranged from 8 to 33 ms (15 \( \pm 4 \) ms; see Table 1). Twitch/tetanic ratios ranged from 0.08 to 0.77 (0.36 \( \pm 0.11 \)).

MU FATIGUE AND FUSION FREQUENCY. Following 120 s of repetitive stimulation (see METHODS), 11 MUs produced from 78 to 131% of initial force [i.e., fatigue index (FI) of 0.78–1.31; Fig. 4A]. An additional six units produced from 74 to 117% of initial force after 60 s of stimulation. By these measures, 16/17 tested MUs are nonfatiguing, and 1/17 is fatigue-intermediate, following the classification of Burke et al. (1973). Tests of fusion frequency were completed in five units (Fig. 4B). Summation of twitch force was evident in all units at 50-Hz stimulation and maximum tetanic force was obtained between 100 and 125 Hz stimulation.

Relationships between MU contractile properties

Significant correlations between MU contractile properties were present in the MU sample (Fig. 5; Table 2). MU twitch
force; CT, contraction time.

animals. Significance indicated by parentheses. Ptet, tetanic force; Ptw, twitch

force was strongly and positively correlated with tetanic force

R

0.01), twitch force (P

0.26, P

0.05; Table 2). MU fatigue (n = 17, see Data collection) was not significantly correlated with any contractile measure. Significant differences in tetanic and twitch force were not present in comparisons between MUs assigned to one of two groups based on contraction time (Mann-Whitney U test) for groups demarcated at 14.5, 19.0, and 20.0 ms in contrast to findings in other rat muscles (e.g., lateral gastrocnemius and soleus, Gillespie et al. 1987; medial gastrocnemius, Kanda and Hashizume 1992; Gardiner 1993; plantaris, Gardiner and Olha 1987).

Relationships between passive tongue tension and MU properties

The demonstration that increases in passive tongue tension from 0 to 2 g were associated with increases in the active twitch tension of the total MU population (see Fig. 2) suggested an effect of passive tongue tension on individual MU measures. Indeed, for the entire MU sample, weak but significant correlations were observed between passive tongue tension and MU tetanic force (R = 0.21, P < 0.01; Table 2) and between passive tongue tension and twitch force (R = 0.26, P < 0.05; Table 2). MU fatigue (n = 17, see Data collection) was not significantly correlated with any contractile measure. Significant differences in tetanic and twitch force were not present in comparisons between MUs assigned to one of two groups based on contraction time (Mann-Whitney U test) for groups demarcated at 14.5, 19.0, and 20.0 ms in contrast to findings in other rat muscles (e.g., lateral gastrocnemius and soleus, Gillespie et al. 1987; medial gastrocnemius, Kanda and Hashizume 1992; Gardiner 1993; plantaris, Gardiner and Olha 1987).

![FIG. 6. Scatter plots showing the relationships between MU contractile properties and the passive tongue tensions at which contractile measurements were made. A: weak, significant correlation between passive tongue tension and MU tetanic force (R = 0.21, P < 0.01). B: weak, significant correlation between passive tongue tension and MU twitch force (R = 0.26, P < 0.005). C: strong, significant correlation between passive tongue tension and MU contraction time (R = 0.62, P < 0.0001). Differences in contractile measures are also observed when the population of MUs studied at passive tongue tensions of <2 g (open circles) is compared with the population of MUs studied at passive tongue tensions of ≥2 g (closed circles). Although there is substantial overlap in the contractile measures of these two groups, positive, significant differences are present for tetanic force, P < 0.01, twitch force, P < 0.05, and contraction time, P < 0.001 (Mann-Whitney U test).]

Analysis of individual experiments

One strength of the intra-axonal technique is the ability to collect large samples in single experiments allowing within-

animal analysis. In all seven experiments for which nine or more MUs were studied, tetanic and twitch force measures were highly significantly correlated (Spearman R values 0.78–0.97; P < 0.005). In contrast, MU contraction time measures were significantly correlated with tetanic force measures in only 2/7 experiments, contraction time increasing with tetanic force in one (R = 0.59, P < 0.01) and decreasing in another (R = –0.42, P < 0.05). MU contraction time measures were significantly correlated with twitch force measures in only 1/8 experiments (R = 0.58, P < 0.01). The effect of passive tongue tension on contractile measures was also minimal, with signif-

### TABLE 2. Spearman rank order correlations between contractile properties of total motor unit sample

<table>
<thead>
<tr>
<th>Passive tongue tension</th>
<th>Ptet</th>
<th>Ptw</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21 (0.01)</td>
<td>0.26 (0.005)</td>
<td>0.62 (0.0001)</td>
<td></td>
</tr>
<tr>
<td>0.89 (0.0001)</td>
<td>0.19 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.23 (0.05)</td>
<td></td>
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</tr>
</tbody>
</table>

Spearman R values for combined sample of 165 motor units (Ptet(passive) and 124 motor units (all other correlations) for motor units sampled from nine animals. Significance indicated by parentheses. Ptet, tetanic force; Ptw, twitch force; CT, contraction time.
incidence attained in only 2 of 21 possible correlations across the seven experiments, with twitch force in experiment 8 (R = 0.68, P < 0.05) and twitch contraction time in experiment 5 (R = 0.41, P < 0.05). Thus, many relationships observed for the entire MU sample are not observed within individual animals.

**MU location**

EMG signatures were recorded for 105 MUs. For many of these MUs, the EMG response was confined to a single electrode location: for 26 MUs to the anterior tongue, 21 MUs to the middle tongue, and 18 MUs to the posterior tongue. An example of a MU with EMG signature confined to the middle tongue is shown in Fig. 3. Of the remaining MUs, EMG responses were observed in anterior-middle (14/40), middle-posterior (24/40), or all three (2/40) tongue regions. A one-way ANOVA, with passive tongue tension as a covariate (because of its effect on MU properties, see Relationships between passive tongue tension and MU properties), was computed to compare the tetanic force, twitch force, and contraction times of MUs with EMG responses in anterior, middle, and posterior regions. For the combined sample, the only significant effect of location was found for tetanic force (P < 0.05). Tukey posthoc analysis indicated significant difference (P < 0.05) in the tetanic force of posterior (mean = 0.121 g) versus anterior (mean = 0.061 g) MUs. Significant differences were not found for contraction time or for twitch force measures. Additional analysis including MUs localized to anterior-middle and middle-posterior tongue regions revealed a similar pattern: in all significant comparisons, MU force was larger in the most posterior of the groups compared.

Samples were sufficiently large to allow tests of location effects on MU properties in three individual experiments. Significant differences were only observed for twitch and tetanic forces in one experiment (P < 0.05); for each measure, larger values were observed for the most posterior of the tongue regions compared (Tukey posthoc analysis). There was no effect of location on contraction time.

**Estimation of MU number**

Studies in the cat hindlimb have shown that MU forces do not sum linearly and that single MU force measures may be influenced by common compliance and by the internal friction produced by adjacent passive muscle fibers (see Clamann and Schelhorn 1988; Powers and Binder 1991). Mechanical interactions among MUs have not been studied in the tongue, but may be particularly complex due both to the organization of tongue muscle and connective tissue structures and to the localization of individual MU territories. Nevertheless, an estimate of the number of MUs that comprise the intrinsic longitudinal muscle was made in the present study. In seven experiments, the following measures were obtained: 1) average MU twitch force, 2) average passive tongue tension at which MU measures were made, and 3) maximal twitch force produced by stimulation of hypoglossal nerve (following nerve transections described in Methods) with the tongue length near average passive tension (i.e., within 0.050–0.550 g average passive tension). Maximal twitch force was divided by average MU twitch force (see Fig. 2). By these estimates, the number of MUs in the seven experiments ranged from the 131 to 511 (average = 345 ± 119).

**DISCUSSION**

This study provides the first detailed description of the contractile properties of identified intrinsic tongue MUs. The major findings of this study are that intrinsic longitudinal MUs of the rat tongue body 1) produce small twitch and tetanic forces compared with other rat MUs, 2) have twitch contraction times similar to other rat MUs, 3) are nonfatiguing, and 4) are localized to a portion of anterior-to-posterior tongue length.

**Contractile properties: relation to other studies**

In previous studies of rat hypoglossal nucleus organization, Goldberg and colleagues used extracellular stimulation to activate motoneurons innervating tongue muscle generally (Gilliam and Goldberg 1995) and innervating the styloglossus muscle specifically (Sutlive et al. 1999). Similar to the present study, the activation of motoneurons whose axons course in the lateral branch of the hypoglossal nerve [i.e., supplying HG, SG, IL, and SL (see Methods)] produces contractions that reach maximal twitch force in 13.5 ms, have a high resistance to fatigue, and reach tetanic fusion at an average of 92.5 Hz (Gilliam and Goldberg 1995; see also Sutlive et al. 1999). The average twitch force of styloglossus MUs (36 mg) is also similar to measures reported here for intrinsic longitudinal MUs.

Differences between the contractile properties of intrinsic longitudinal MUs and the properties of other rat hypoglossal MUs, however, are also apparent. MUs of the styloglossus express a narrower range of twitch force measures (17.4–80.0 mg; Sutlive et al. 1999) than do MUs of the longitudinal intrinsic tongue muscles (2–129 mg). Intrinsic longitudinal MUs express a narrower range of fatigue measure [all FI > 0.70] than do styloglossus MUs (30% with FI < 0.70). Additionally, the average force values produced by extracellular stimulation of unidentified motoneurons in the lateral nerve (86 mg average MU twitch force and 755 mg average MU tetanic force, Gilliam and Goldberg 1995) are much higher than average values for either styloglossus (Sutlive et al. 1999) or intrinsic longitudinal MUs (present study). The >2–8 fold difference in these measures compared with the present study may be due to the inclusion of HG in the study of Gilliam and Goldberg, a possibility that would suggest that HG MUs produce on average more force than other retractor MUs. Differences in force measures may also be related to the passive tongue tension at which MUs were studied (5 g in Gilliam and Goldberg 1995, versus an average of 3.3 g in the present study), although the effect of passive tongue tension on MU force is weak (see Results). It is also possible that extracellular stimulation of the hypoglossal nucleus activated multiple MUs (Gilliam and Goldberg 1995).

In measures of contraction times, intrinsic longitudinal tongue MUs are similar to MUs of other rat muscles. Twitch contraction times of rat tibialis anterior MUs range from 11 to 18 ms (Bakels and Kernell 1993a), with an average of 15.5 ms (Totosy de Zepetnek et al. 1992), and twitch contraction times of medial gastrocnemius MUs range from 12.9 to 36.2 ms (Kanda and Hashizume 1992; see also Gardiner and Olha
1987). Tongue MUs differ from hind limb MUs, however, in measures of force and fatigability. Tibialis anterior MUs produce 5–441 mN tetanic force (Totosy de Zepetnek et al. 1992; see also Bakels and Kernell 1993a) and 1.5–34.5 mN twitch force (Bakels and Kernell 1993a), and gastrocnemius MUs have similar values for these contractile measures (Bakels and Kernell 1993b; Kanda and Hashizume 1989). Thus despite similar ranges in contraction time, single intrinsic longitudinal tongue body MUs produce some 100–1000 times less force than rat hind limb MUs. Intrinsic longitudinal tongue MUs have low measures of fatigability when compared with other rat muscles (FI of 0.05–1.35 measured in tibialis anterior and medial gastrocnemius MUs; Kanda and Hashizume 1992; Totosy de Zepetnek et al. 1992). Twitch/tetanus ratios of tongue MUs are similar to ratios of rat lumbrical and medial gastrocnemius MUs (Gates et al. 1991; Kanda and Hashizume 1989), but greater than twitch/tetanus ratios of rat tibialis anterior, plantaris, and lateral gastrocnemius MUs (Bakels and Kernell 1993a; Gardiner and Olha 1987; Seburn and Gardiner 1995).

The forces produced by rat intrinsic longitudinal tongue MUs are similar to those of oculomotor MUs in the cat and monkey. In the cat superior oblique muscle, for example, MU twitch forces range from 3 to 237 mg (mean = 27.5) and tetanic forces from 11 to 1327 mg (mean = 141) (Waldeck et al. 1995). MUs of similar force are found in the primate lateral rectus (means of 10.7 mg twitch force and 186.2 mg tetanus force; Goldberg et al. 1998). Unlike rat tongue MUs, however, oculomotor MUs contract very rapidly (<15 ms, Goldberg et al. 1998; Waldeck et al. 1995), have a high fusion frequency (e.g., 150–260 Hz, Goldberg et al. 1998), and express a wide range of fatigabilities (FI of 0.01–1.15; Shal and Goldberg 1995).

There are few studies of the muscle fiber histochemistry of the rat tongue. Myofibrillar ATPase staining revealed that virtually all muscle fibers of the rat styloglossus are type Ila (Sutlive et al. 1999). Sato et al. (1989) reported that 86% of longitudinal muscle fibers in the rat tongue are “red” or “intermediate” (i.e., strongly or moderately oxidative). These findings, in concert with the large percentage of fatigue-resistant MUs in the styloglossus and intrinsic longitudinal muscles (Sutlive et al. 1999), suggest that the contractile/histochemical relationships of tongue MUs are similar to those of other muscle systems (see Burke 1981; Sutlive et al. 1999). In the present study, however, fatigable units were not encountered as would be expected of “white” fibers (14% of longitudinal muscle fibers in Sato et al. 1989); this may reflect the relatively small number of MUs for which fatigue measures were recorded in the present study, and/or differences in population of the MUs investigated.

**Contractile properties: correlations**

Among intrinsic longitudinal tongue MUs, twitch force is strongly and positively correlated with tetanic force ($R = 0.89$). A strong positive correlation between force measures is also present in all seven individual animal analyses ($R > 0.77$). In contrast, correlations between contraction time and force measures for the total sample are weak ($R < 0.24$) and are significant in only 2 of 7 animals. In this respect, intrinsic longitudinal tongue MUs are similar to MUs of muscles which have either very weak and negative or nonsignificant relationship between MU contraction time and force measures, e.g., the rat tibialis anterior (Bakels and Kernell 1993a), cat soleus, tibialis anterior, and extensor digitorum longus (Goslow et al. 1977; Mosher et al. 1972), rabbit masseter (Kwa et al. 1995), and human masseter and nasal dilator (Goldberg and Derfler 1977; Mateika et al. 1998).

MUs are commonly categorized into fast or slow “types” based, respectively, on the presence or absence of a decline in force during unfused tetanus (i.e., the “sag” property, see Burke et al. 1973). “Fast” and “slow” MUs often differ in other contractile properties, with fast units usually producing more force and contracting more rapidly than slow units. In the present study, sag was not tested, and MUs were not classified according to type. However, when MUs were segregated into groups based on contraction times that distinguish fast from slow units in other rat muscles (e.g., 14.5 ms in the tibialis anterior, 19 ms in the plantaris; Bakels and Kernell 1993a; Gardiner and Olha 1987), no significant differences between the force measures of the groups were observed.

The independence of force and contraction time measures in intrinsic longitudinal tongue MUs raises the possibility that MUs may be selected into activity for either property, i.e., speed or force, in different behaviors. This contrasts with the organization of many muscles in which MU force and speed measures are interrelated such that recruitment of MUs of increasing force results in the obligate recruitment of MUs of increasingly fast contraction speeds (Burke 1981; Cope and Clark 1991; for discussion see Bigland-Ritchie et al. 1998).

**Contractile properties: effect of passive tongue tension**

During oro-motor behaviors, the mammal tongue may extend 50–100% of its resting length (Kier and Smith 1985). Here, weak correlations were found between passive tongue tension and two measures of MU force ($R < 0.27$); a stronger correlation was found between passive tongue tension and contraction time ($R = 0.62$). Differences in MU force and speed of contraction were also evident when the sample of MUs measured at <2 g passive tension was compared with the sample of MUs measured at ≥2 g. This effect of tongue tension on MU properties was observed over a range of tongue tensions that corresponded to tongue positions in normal rat oromotor behaviors (i.e., tongue tip within the oral cavity to 13 mm anterior to the incisors, see Whishaw and Tompkins 1988), suggesting that the contractile effects of individual MUs may change during tongue movement. Direct studies of the length-tension properties of individual MUs are necessary to fully explore these relationships.

**MU localization and functional morphology of the tongue**

Independent kinematic control of different tongue regions has been documented during mammal feeding (Hiiemae et al. 1995) and human speech (Stone 1990). The neural bases of regional tongue control have not been described, but likely involve the differential activation of MUs located in different tongue body regions. Due to their circumscribed location within regions of the rat tongue body, intrinsic longitudinal tongue MUs are candidates for coordinating regional control of tongue movements. The large number of intrinsic longitudinal MUs, estimated at 345 in the present study, suggests that...
REFERENCES


