Whole-Muscle and Motor-Unit Contractile Properties of the Styloglossus Muscle in Rat

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Sutlive, Thomas G., J. Ross McClung, and Stephen J. Goldberg. Whole-muscle and motor-unit contractile properties of the styloglossus muscle in rat. J. Neurophysiol. 82: 584–592, 1999. Investigations of whole muscle and motor-unit contractile properties have provided valuable information for our understanding of the spinal cord and extraocular motor systems. However, no previous investigation has examined these properties in an isolated tongue muscle. The purpose of this study was to determine the contractile properties and muscle fiber types of the rat styloglossus muscle. The styloglossus is one of three extrinsic tongue muscles and serves to retract the tongue within the oral cavity. Adult male Sprague-Dawley rats (n = 19) were used in these experiments. The contractile characteristics of the whole styloglossus muscle (n = 9) were measured in response to stimulation of the hypoglossal nerve branch to the muscle. The average twitch tension produced was 3.30 g with a mean twitch contraction time of 13.81 ms. The mean maximum tetanic tension was 19.66 g and occurred at or near the fusion frequency, which averaged 109 Hz. The styloglossus muscle was resistant to fatigue [fatigue index (F. I.) = 0.76]. In separate experiments (n = 7), the contractile characteristics of 37 single motor units were measured in response to extracellular stimulation of hypoglossal motoneurons. The twitch tension generated by styloglossus motor units averaged 35.7 mg, and the mean twitch contraction time was 12.46 ms. The mean fusion frequency was 92 Hz. Maximum tetanic tension averaged 177.8 mg. Styloglossus single motor units were resistant to fatigue (F. I. = 0.74). The sites of stimulation that yielded a contractile response in the styloglossus muscle were consistent with the location of the styloglossus motoneuron pool reported in earlier anatomy studies. Muscle fiber typing was determined in three animals based on the myofibrillar ATPase reaction at pH 9.8, 4.6, and 4.3. The styloglossus muscle was composed of ~99% type IIA fibers with a few scattered type I fibers present in the study sample. On the basis of the combined findings of the physiology and histochemistry experiments, the styloglossus muscle appeared to be a homogeneous muscle composed almost exclusively of fast, fatigue-resistant motor units. These properties of the styloglossus muscle and its motor units were compared with findings in other rat skeletal muscles.

INTRODUCTION

Studies of motor-unit contractile properties have provided valuable information about the structure and function of skeletal muscles. The properties of motor units in the spinal cord and extraocular motor systems have been the most frequently studied (see reviews by Binder et al. 1996; Burke 1981; Goldberg 1990). Like the extremity and extraocular muscles, the tongue is composed of skeletal muscle tissue. There are very few studies, however, describing the physiological features of the tongue muscles or the hypoglossal (XIIth) motor system, which controls tongue muscle function.

Tongue muscles

Normal function of the tongue is required for a number of vital processes, including chewing, respiration, suckling, swallowing, and speech (Lowe 1981). To perform these functions, the tongue must be able to assume a variety of shapes and positions within the oral cavity. Its complex muscular structure enables it to do so. The tongue is composed of both extrinsic and intrinsic muscles. The extrinsic muscles originate from bony attachments to insert into the body of the tongue, and their primary function is to position the base of the tongue. The extrinsic muscles include the styloglossus and hyoglossus, both tongue retractors, and the genioglossus, the primary tongue protrusor. The intrinsic muscles have no bony attachments; they are contained within the body of the tongue. These muscles are oriented along the tongue’s transverse, vertical, and longitudinal axes. The intrinsic muscles shape the body and tip of the tongue. Coordination of extrinsic and intrinsic muscle activity is required for the execution of precise tongue movements.

ANATOMIC STUDIES. Retrograde labeling studies in the rat (Aldes 1995; Altschuler et al. 1994; Dobbins and Feldman 1995; Krammer et al. 1979; Lewis et al. 1971; McClung and Goldberg 1999; O’Reilly and Fitzgerald 1990; Sokoloff 1993; Uemura-Sumi et al. 1988) have demonstrated two subdivisions in the hypoglossal motor system. Motoneurons within the ventral subcompartment of the hypoglossal nucleus form the medial branch of the XIIth nerve. These motoneurons innervate the genioglossus and some intrinsic muscles (protrusor subdivision). Motoneurons in the dorsal sub compartment form the lateral branch of the XIIth nerve and innervate the retractor muscles, the styloglossus and hyoglossus. Studies in the cat (Uemura et al. 1979), dog (Uemura-Sumi et al. 1988), monkey (Sokoloff and Deacon 1992; Uemura-Sumi et al. 1981), and rabbit (Uemura-Sumi et al. 1988) have reported similar findings.

PHYSIOLOGICAL STUDIES. There are few studies describing the physiological properties of the hypoglossal motor system. Hayashimoto (1960) (reported by Lowe 1981) and Morimoto et al. (1966) mapped, in a general way, the cat hypoglossal nucleus. Their results basically agreed with the anatomic studies regarding the dorsal and ventral arrangement of the nucleus for retraction and protrusion.

Hellstrand (1981) studied the contraction speeds of the tongue’s intrinsic and extrinsic muscles in cat using a light
reflection transducer. An average contraction time (CT) of 33 ms for the extrinsic muscles and 22 ms for the intrinsic muscles was reported. Van Lunteren and Manubay (1992) examined the mechanical properties of excised strips of cat genioglossus muscle in vitro. They reported a CT of 38 ms, twitch tension of 3.1 g, and fatigue index (F.I.) of 0.36.

Gilliam and Goldberg (1995) studied the tongue’s contractile properties with the tip of the tongue attached to a force transducer. Their results generally confirmed the earlier anatomic divisions of the hypoglossal nucleus and delineated the force characteristics for the extrinsic and intrinsic muscles. They did, however, point out the need for studies of the tongue muscles in isolation so that more precise measures of their contractile characteristics could be made.

Histochemical studies. Hellstrand (1980) found that the cat styloglossus muscle was composed predominantly of fast (type II), fatigue-resistant or -intermediate fibers. Smith (1989) reported that the extrinsic tongue muscles of hooded rats consisted almost exclusively of fast, fatigue-resistant fibers. And Sato et al. (1990) found that the rat genioglossus muscle contained 37% red (slow), 30% white (fast), and 33% intermediate fibers. The intrinsic muscles were found to be composed generally of fast, fatigue-resistant fibers (DePaul and Abbs 1996; Hellstrand 1980; Sato et al. 1990; Smith 1989).

The purpose of this study was to describe the physiological properties of the styloglossus muscle, one of the three extrinsic tongue muscles. The styloglossus muscle is active during most functional tongue movements. Electromyographic (EMG) and tongue movement studies have demonstrated styloglossus muscle activity during chewing (Liu et al. 1998), respiration (Yasui et al. 1993), licking (Dinardo and Travers 1994; Liu et al. 1998), sucking (Liu et al. 1998), swallowing (Amri et al. 1989; Tomonume and Takata 1988; Travers and Jackson 1992), and speech (Stone and Lundberg 1996). The delineation of the contractile characteristics of tongue muscle motor units, then, is a basic step toward understanding tongue function. Specifically, the aims of this study were to measure the contractile properties of the whole styloglossus muscle in response to stimulation of the isolated XIIth nerve branch to the muscle, to measure the contractile properties of single styloglossus motor units in response to extracellular stimulation of motoneurons within the XIIth nucleus, and to determine the muscle fiber types of the styloglossus using the myofibrillar ATPase reaction. To our knowledge, no previous investigation has examined the contractile properties of an individually isolated tongue muscle as has routinely been done for other skeletal muscles. Such studies (Binder et al. 1996; Burke 1981; Goldberg 1990) are clearly important to our understanding of the movement dynamics of motor control systems.

Methods

Animals and surgical preparation

Nineteen adult male Sprague-Dawley rats weighing 250–400 g were anesthetized with an intraperitoneal injection of urethane (1.3 g/kg) before surgery and given supplemental doses (300 mg/kg) as needed to maintain deep anesthesia (no withdrawal to paw pinch). The trachea was cannulated to maintain a patent airway. The respiratory rate was continuously monitored, and a heating pad was used to maintain body temperature between 38 and 40°C.

Using a ventral approach, the hypoglossal nerve and its medial and lateral branches were exposed. The nerve to the geniohyoid muscle was excised, the medial branch was severed, and its proximal stump was cauterized to prevent current spread. The styloglossus muscle and lateral nerve branch were separated from the hyoglossus muscle along the fascial plane between those two muscles. Special care was taken to preserve the blood supply to the styloglossus muscle as it was separated from the hyoglossus muscle. The tapered, distal end of the styloglossus muscle was cut at its point of insertion (the muscle does not have a tendon on this end) into the body of the tongue. This preserved the entire styloglossus muscle belly, and no bleeding was noted during the procedure. A silk loop was sutured into the distal end of the muscle and later attached to a strain gauge.

After the initial surgery, the animal was secured in the prone position in a Kopf stereotaxic frame. In the case of single-unit experiments, a craniotomy was performed at this point to expose the cerebellum and brain stem. A small portion of the midline, caudal cerebellum was aspirated to fully expose the obex and floor of the fourth ventricle. This exposure allowed clear access to the XIIth nucleus, which is located near the midline, both rostral and caudal to the obex. Mineral oil was placed on the brain stem to keep it moist and warm.

Stimulating electrode, strain gauge, and EMG electrode placement

Next the animal was rotated in the stereotaxic frame to the supine position. For whole-muscle experiments, a bipolar silver-silver chloride hook electrode was placed around the hypoglossal nerve, proximal to its bifurcation into medial and lateral branches (recall that the medial nerve branch had been cut and cauterized).

While still supine, the styloglossus muscle was attached to a semiconductor strain gauge (Pixie Model 8101, ENDEVCO) via the silk suture loop. The strain gauge has a compliance of 2 μm/g and a resonant frequency of 2 kHz. The transducer is capable of linearly resolving forces from <1 mg to 40 g. To attach the styloglossus to the strain gauge, the distal end of the muscle had to be positioned just below the mandible so that the angle of pull deviated slightly from its natural position. Every effort was made to minimize deviation from the natural line of pull.

A bipolar, fine-wire EMG electrode (25 μm diam) with a separation of ~4.0 mm between the poles was inserted (using a 28-gauge needle) into the muscle belly, perpendicular to the long axis of the muscle. The electrode was insulated except for the final 3 mm (the width of the muscle belly), which terminated in a small hook to anchor into the muscle tissue. Mineral oil and petroleum jelly were applied to the muscle and nerve preparation to keep it moist and to prevent current spread during stimulation.

Whole-muscle experiments

Whole-muscle experiments were conducted in nine animals. The styloglossus muscle was aligned with the strain gauge, and the maximal isometric tension was determined in response to supramaximal single pulse stimulation. Twitch characteristics were measured in response to 0.1-ms duration pulses (300–500 μA) delivered to the whole nerve at a rate of one per second. Twitch tension, twitch contraction time and half-decay time were averaged over 10 trials. The twitch measurements were made before tetanic stimulation and therefore were unpotentiated responses. Tetanic forces and fusion frequency were assessed using 200-ms trains with stimulation rates ranging from 30 to 170 Hz. Fatigability of the muscle was measured in response to tetanic stimulation of the nerve at 90 Hz at a rate of one 500-ms train delivered per second. This stimulus paradigm was delivered for a period of 2 min. Muscle fatigability was expressed as the F.I., which is the ratio of tension remaining after 2 min of stimulation to the tension generated by the initial train (Burke et al. 1973).
Single-unit experiments

In seven separate animals, data were collected on single motor units. The experimental set up was similar to that described in the preceding section for the whole-muscle experiments, with the following differences. First, the craniotomy was performed to allow clear access to the XIIth nucleus. Second, to ensure that there were no conduction blocks along the length of the XIIth nerve, the hook electrode was not used. Therefore a modified technique was needed to set the isometric tension of the muscle. This was accomplished by stimulating in the rostral XIIth nucleus with single pulses using a large-diameter (30–40 μm tip) glass electrode, to activate a number of motoneurons and thereby set muscle isometric tension. Glass electrodes filled with 1.6 M potassium citrate (tip diameters ≤2 μm and >5.0 MΩ resistance) then were used for extracellular stimulation of individual motoneurons within the nucleus. Single units were identified (Fig. 1) by concurrent “all-or-none” responses of the force and EMG signals (Goldberg et al. 1998; Maciefield et al. 1996; Powers and Binder 1991). To ensure that single-unit isolation was maintained, the EMG potential was monitored continuously at high gain (Fig. 1, inset). This allowed for the detection of changes in EMG signal shape or amplitude, which would indicate the recruitment of another motor unit. The mechanical properties of each unit were measured and the site of stimulation within the nucleus was noted.

Equipment and data analysis

An A.M.P.I. digital, programmable stimulator and stimulus isolation units were used to provide electrical stimulation. Data were visualized during the experiment on a Tektronix 2221 digital storage oscilloscope and simultaneously recorded on a Vetter 420-H FM magnetic tape recorder for subsequent analysis. Data were analyzed using Microsoft Excel spreadsheet software. Descriptive statistics were calculated for the whole-muscle and motor-unit contractile properties.

![FIG. 1. Unitary force and electromyographic (EMG) responses of a single styloglossus motor unit to extracellular stimulation within the XIIth nucleus. A: at threshold level of stimulation. Top: twitch tension = 31.3 mg and twitch contraction time = 11.4 ms. Bottom: unitary EMG response in styloglossus muscle to stimulation of the same cell. B: at stimulation intensity below threshold, there is a concurrent drop-out of both the force and EMG signals. C: at suprathreshold level of stimulation, there is no change in the twitch or EMG characteristics when compared with A. Inset: high-gain and fast-speed EMG monitoring ensured that the EMG signal was consistent. Calibration bars in C apply to A and B. Horizontal bar is 5.0 ms in all figures. Vertical bar is 52 mg for the top traces and 285 μV for the bottom traces. Vertical line to left of EMG potential in bottom traces is stimulus artifact.](http://jn.physiology.org/lookup/doi/10.2203/335.5.19)

Histochemical analysis

In three animals, the muscle fiber types of the styloglossus muscle were determined using the myofibrillar ATPase reaction (Dubowitz 1985). After surgically isolating the muscle as described in the preceding text, it was excised and placed in tissue embedding medium. The muscle immediately was frozen in liquid nitrogen-cooled isopentane. The frozen muscle blocks were stored in a freezer maintained at −70°C.

The frozen blocks were trimmed and serial sections of 10-μm thickness were cut from the muscle with a cryostat kept at −20°C. The unfixed frozen section were thaw-mounted on glass slides. The serial sections then were stained for the demonstration of myofibrillar ATPase at pH 9.8 after alkaline (pH 9.8) and acidic (pH 4.6 and 4.3) preincubations. The sections were mounted in artificial resin.

Muscle fiber types were determined based on their staining affinity for myofibrillar ATPase at each of the pH levels. Muscle fibers that stained lightly at pH 9.8, demonstrating low ATPase activity, were classified as type I fibers. Type II fibers stained darkly at pH 9.8. After acidic preincubation, type II fibers could be subdivided further into type IIA (inhibited at both acidic levels) and type IIB (inhibited at pH 4.3 only). Type I fibers stained darkly at both acidic levels. The Neuromolucida (MicroBrightField, Colchester, VT) imaging analysis system was used to determine the total muscle fiber count and percentage of muscle fiber type for each muscle, as well as the mean least diameter (Dubowitz 1985) and mean cross-sectional area (CSA) of the muscle fibers. A one-way ANOVA with post hoc analysis (Scheffe test) was used to test for differences in mean CSA and mean least diameter among the three muscles.

RESULTS

Whole-muscle contractile properties

The contractile properties of the whole styloglossus muscle were measured in nine experiments. The whole muscles produced an average twitch tension of 3.30 ± 0.72 (SD) g (range: 1.95–4.25 g). The mean contraction time was 13.81 ± 1.11 ms (range: 12.0–15.8 ms), and half-decay time averaged 10.82 ± 2.43 ms (range: 7.2–13.7 ms) (Fig. 2A).

The mean fusion frequency was 109 ± 15.29 Hz (range: 90–140 Hz). Tetanic tension at fusion averaged 19.14 ± 5.97 g (range: 9.91–27.32 g). Maximum tetanic tension averaged 19.66 ± 5.68 g (range: 10.44–27.32 g) and usually occurred at or near fusion (Fig. 2B). Tetanic-to-twitch tension ratios were calculated for each muscle and ranged from 3.7 to 7.1 with a mean of 5.9 ± 0.97.

A 2-min fatigue test was performed in three whole-muscle experiments. The F.I. describes the amount of tetanic tension remaining after 2 min of repetitive stimulation compared with the initial tension generated at the same stimulation frequency. The F.I. averaged 0.76 ± 0.09 (range: 0.67–0.85; Fig. 2C).

Single-motor-unit contractile properties

The twitch, tetanic, and fatigue properties of styloglossus muscle single motor units were evaluated (Fig. 3). The units were identified on the basis of concurrent all-or-none force and EMG responses to single pulse stimulation (Fig. 3A). The average twitch tension (Fig. 4A; n = 37) produced was 35.7 ± 14.98 mg (range: 17.4–80.0 mg). The mean contraction time (Fig. 4B; n = 37) was 12.46 ± 1.46 ms (range: 10.6–17.0 ms), and half-decay time (n = 37) averaged 13.17 ± 2.96 ms (range: 8.4–19.4 ms).

Tetanic responses were measured in 27 of the single units.
scattered type I fibers were found and accounted for <1.0% of any of the three muscles.

Mean muscle-fiber size was calculated using both the CSA and least-diameter measurement techniques. The mean CSA for all muscle fibers averaged $828 \pm 513 \mu m^2$. The mean type I CSA was $869 \pm 413 \mu m^2$ and the type IIA CSA averaged $827 \pm 513 \mu m^2$. The average least diameter for all muscle fibers, as well as for the type IIA fibers, was $25.9 \pm 8.2 \mu m$. The mean diameter for the type I fibers was $26.9 \pm 8.6 \mu m$.

Although the CSAs of all three of the whole muscles were similar, the mean muscle-fiber CSAs, mean muscle-fiber diameters and total fiber counts were varied (Table 1). The mean fiber CSAs were significantly different for each muscle ($P < 0.001$) as were the mean fiber diameters ($P < 0.002$). These variations in muscle-fiber size appeared to correlate with differences in total fiber count for each muscle.

**DISCUSSION**

The major findings of this study were that the twitch contraction time, fusion frequency, twitch tension, maximum tetanic tension, and fatigue properties of the rat whole styloglossus muscle and its motor units were delineated, the muscle appeared to be homogeneous and composed almost exclusively of FR motor units, and the sites of extracellular stimulation that yielded a contractile response in the styloglossus muscle were consistent with the findings of earlier anatomy and physiology studies that showed the styloglossus muscle motoneuron pool to be located in the dorsorstral XIIth nucleus.

**Contractile properties of the whole styloglossus muscle**

The forces and speeds generated by the whole styloglossus muscle are compared with those produced by other rat muscles in Table 2. The twitch and tetanic tensions appear to scale...
appropriately, according to whole-muscle size, with the tensions generated by the rat extraocular and limb muscles. Also, the styloglossus muscle contractile properties appear to correlate with the force and speed measures of whole tongue retraction seen in response to stimulation of the lateral branch of the XIIth nerve (Gilliam and Goldberg 1995).

The rat styloglossus muscle was extremely resistant to fatigue. Table 3 compares the fatigue resistance of the styloglossus with the fatigability of some rat extremity muscles. The fatigue test was based on similar tests for extremity muscle (Burke et al. 1973), extraocular muscle (Nelson et al. 1986; Shall and Goldberg 1992), and tongue muscle (Gilliam and Goldberg 1995). A stimulation frequency of 90 Hz was selected because it approximated the fusion frequency for the styloglossus muscle, and a train duration of 500 ms was selected because the styloglossus muscle has been shown to be active for ~0.5 s for every second during a typical lick/swallow cycle in the rat (Travers and Jackson 1992). We observed an average F.I. (0.76) for the whole styloglossus muscle that was higher than that observed for the whole rat soleus muscle (0.58) (Eken and Gundersen 1988). However, Eken and Gundersen (1988) used a stimulation rate of 77 Hz, which is much higher than the 18-Hz fusion frequency observed previously (Lieber et al. 1986). In addition, Leterme and Falempin (1996) recently have shown rat soleus motor unit F.I.s >0.9 using the more common 40-Hz stimulation rate (Burke et al. 1974) for that muscle.

Classification of motor units: contractile properties and histochemistry

Measures of contraction speed (twitch contraction time and fusion frequency) have been used to classify motor units into fast and slow groups in other motor systems (Burke 1967; Shall and Goldberg 1992). In the present study, as in cat and primate extraocular muscle (Goldberg et al. 1998; Shall and Goldberg 1992), the distribution for fusion frequencies was continuous.

![Histograms showing frequency distributions of single unit twitch tensions (A), twitch contraction times (B), fusion frequencies (C), maximum tetanic tensions (D), and fatigue indices (E), and a 3-dimensional graph displaying the multivariate physiological profiles of 27 styloglossus motor units (F).](http://jn.physiology.org/)

**FIG. 4.** Histograms showing frequency distributions of single unit twitch tensions (A), twitch contraction times (B), fusion frequencies (C), maximum tetanic tensions (D), and fatigue indices (E), and a 3-dimensional graph displaying the multivariate physiological profiles of 27 styloglossus motor units (F).
However, the contraction times of the motor units showed a possible separation between 14.3 and 16.0 ms (Fig. 4, B and F). This observation, coupled with the muscle histochemical findings of a small but consistent percentage of type I fibers (Fig. 6), caused us to classify single units with a contraction time >16.0 ms as slow (S) units.

Another criterion frequently used to distinguish fast and slow motor unit types is the “sag” phenomenon, originally described in the fast motor units of the cat medial gastrocnemius (Burke et al. 1973). Fast units exhibit a sag in their tension output during unfused tetani, slow units do not. The sag phenomenon was not seen during tetanic stimulation in any of the rat styloglossus units. This is consistent with findings reported in studies of cat and primate extraocular motor units (Goldberg et al. 1998; Nelson et al. 1986; Shall and Goldberg 1992). Because none of the styloglossus single units demonstrated a sag, we could not use this criterion to divide the units into fast and slow types.

The fatigability of single units is frequently used to subdivide fast motor unit populations. The F.I. for styloglossus motor units showed an apparent bimodal distribution with a separation occurring at an F.I. of 0.50 (Fig. 4E). This small separation equivocally divided the fast styloglossus units into “fatigable” (F.I. ≤ 0.50) and “fatigue resistant” (F.I. > 0.50) categories.

On the basis of the physiology alone, one might say that there were three motor unit types present in the styloglossus muscle: slow (S), fast fatigue resistant (FR), and fast fatigable (FF). The histochemical analysis, however, identified only two muscle fiber types. The styloglossus was composed of ≈99% type IIA and 1% type I muscle fibers. The results of glycogen-depletion studies in other muscles have shown that S motor units are composed of type I muscle fibers, whereas FR units are composed of type IIA fibers (Chamberlain and Lewis 1989). Thus on the basis of the combined findings of the physiology experiments and the histochemical analysis of the whole muscle, the styloglossus motor units were divided into two populations. The styloglossus muscle is composed almost exclusively of FR motor units. Two S units were seen in the present study sample of 27 units with complete physiological data. The two (FR and S) motor unit populations are shown in Fig. 4F.

That styloglossus muscle units are predominantly fast and fatigue resistant, with a relatively narrow range of contractile properties, appears to be consonant with the known involvement of the muscle in fast, repetitive retractions of the tongue during chewing, licking, swallowing, and respiration. Coupled to this, we have noted previously that styloglossus motoneurons have a somewhat restricted range of cell body sizes (McClung and Goldberg 1999). Although these findings of relative similarity in styloglossus muscle motor-unit characteristics may have implications regarding the manner in which the units are recruited during particular movements, we did not study recruitment directly. We also need to know more about the characteristics of the other extrinsic muscle motor units, hyoglossus and genioglossus, to see how they compare with the styloglossus motor units. For instance, we might predict that hyoglossus motor units would be similar to those in the styloglossus since the two muscles appear to act in synergy (Amri et al. 1989).

Sites of extracellular stimulation

All styloglossus single units were identified by stimulating in or near the ipsilateral XIIth nucleus. Contractile responses in the styloglossus muscle were elicited when stimulating between 200 and 1,000 μm rostral to the level of the obex, and from 100 to 400 μm lateral to the midline (Fig. 5B). These physiologically defined boundaries are generally in agreement with the range of extracellular stimulation sites that yielded tongue retraction with the lateral branch intact (Gilliam and Goldberg 1995). The rostrocaudal extent of the stimulation sites is consistent with the location of the styloglossus muscle.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Weight of Animal, g</th>
<th>Whole-muscle CSA, mm²</th>
<th>Total Muscle-Fiber Count</th>
<th>Mean Fiber CSA, μm²</th>
<th>Mean Fiber Diameter, μm</th>
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<tbody>
<tr>
<td>1</td>
<td>299</td>
<td>1.60</td>
<td>1361</td>
<td>1181</td>
<td>30.9</td>
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<tr>
<td>2</td>
<td>321</td>
<td>1.33</td>
<td>1753</td>
<td>757</td>
<td>24.7</td>
</tr>
<tr>
<td>3</td>
<td>337</td>
<td>1.48</td>
<td>2220</td>
<td>666</td>
<td>23.9</td>
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</table>
toneuron pool described in recent anatomic studies (Guo et al. 1996; McClung and Goldberg 1999).

Contractile responses were evoked in the styloglossus muscle during extracellular stimulation between 0.5 and 2.5 mm deep to the brain stem surface (Fig. 5A). Single-motor-unit responses were elicited consistently within a 1.0-mm region, between depths of 0.5 and 1.5 mm. Because no responses were elicited before the electrode tip had reached a depth of 0.5 mm, it is reasonable to assume that a number of the contractile responses were elicited by stimulation of single axons deep to the styloglossus cell bodies (Altschuler et al. 1994).

Quantification of motoneurons and muscle fibers

Previous anatomic investigations in our laboratory have indicated that the motoneuron pool for the rat styloglossus muscle contains \approx 50 neurons with cell diameters averaging 30.6 \mu m (McClung and Goldberg 1999). The belly of the styloglossus muscle was found to have 2,200 muscle fibers (McClung and Goldberg 1999). The average innervation ratio, based on the numbers in the preceding text, would then be \approx 44 muscle fibers per motoneuron. It is interesting to note, in this regard, that we never found \geq 22 type I muscle fibers in any of the three muscles. We suspect that these fibers represent no more than two (possibly one) muscle units per muscle. Also, great care was taken in those previous anatomic studies to inject small quantities (2–5 \mu l) of low-concentration (0.1\%) cholera toxin conjugate of horseradish peroxidase (CTHRP) into the principal innervation zone of the muscle (McClung and Goldberg 1999). Although this procedure ensured that unwanted muscles were not inadvertently labeled, it likely resulted in a somewhat low number of labeled styloglossus motoneurons (McClung and Goldberg 1999) that could increase the innervation ratio.

Additionally, this probable low motoneuron number may help explain why our average motor-unit twitch (35.7 mg) and tetanic (177.8 mg) tensions, when multiplied by 50 motoneurons, do not add up to the average whole-muscle twitch (3.3 g) and tetanic (19.7 g) tensions observed in response to stimulation of the whole-muscle nerve. If these numbers are accurate, it would appear that \approx 100, rather than 50, styloglossus muscle motoneurons would be expected in the nucleus. Indeed, high-

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Twitch Contraction Time, ms</th>
<th>Twitch Tension, g</th>
<th>Maximum Tetanic Tension, g</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior rectus</td>
<td>4.4</td>
<td>0.66</td>
<td>5.1</td>
<td>Close and Luff (1974)</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>13.1</td>
<td>50.5</td>
<td>290.1</td>
<td>Eken and Gundersen (1988)</td>
</tr>
<tr>
<td>Styloglossus</td>
<td>13.8</td>
<td>3.3</td>
<td>19.7</td>
<td>Present study</td>
</tr>
<tr>
<td>Tongue:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral branch (styloglossus, hyoglossus, intrinsics)</td>
<td>13.4</td>
<td>12.0</td>
<td>36.8</td>
<td>Gilliam and Goldberg (1995)</td>
</tr>
<tr>
<td>Fourth lumbrical</td>
<td>22.7</td>
<td>3.4</td>
<td>12.0</td>
<td>Gates et al. (1991)</td>
</tr>
<tr>
<td>Soleus</td>
<td>36.7</td>
<td>39.5</td>
<td>188.0</td>
<td>Eken and Gundersen (1988)</td>
</tr>
</tbody>
</table>

TABLE 3. Fatigue index of selected muscles in the rat

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Fatigue Index</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantaris</td>
<td>0.22</td>
<td>Gardiner and Olha (1987)</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>0.23</td>
<td>Eken and Gundersen (1988)</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.58</td>
<td>Eken and Gundersen (1988)</td>
</tr>
<tr>
<td>Styloglossus</td>
<td>0.76</td>
<td>Present study</td>
</tr>
</tbody>
</table>

FIG. 6. Photomicrographs of serial sections of a styloglossus muscle. Sections were stained for the myofibrillar ATPase reaction after preincubation at pH 9.8 (A), 4.6 (B), and 4.3 (C). \( \uparrow \) 2 type I muscle fibers. All other fibers are type IIA. Calibration bar in C = 50 \mu m and applies to A and B.
volume (50–60 μl) CTHRP injections into the tongue muscles resulted in labeling ~800 motoneurons in the dorsal subdivision of the hypoglossal nucleus, compared with a cell count of ~400 motoneurons seen with low-volume (2–5 μl) labeling of individual muscle innervation zones (McClung and Goldberg 1999).

Although the various muscles of the tongue must coordinate their contractions in a complex manner during normal movements (Lowe 1981), we have shown that it is still important to study the individual muscles in isolation (Giilliam and Goldberg 1995). Using this experimental design, one can begin to evaluate the force, contraction speed, and fatigue properties that each muscle contributes to the tongue’s participation in numerous vital functions. Current investigations in this laboratory are directed at documenting the innervation ratios of identified single motor units in the styloglossus muscle as well as delineating the motor-unit and muscle-fiber types of the isolated genioglossus muscle of the rat.

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