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## Extraocular motor unit and whole-muscle contractile properties in the squirrel monkey

### Summation of forces and fiber morphology

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**Abstract** In order to understand the neural control of movement, many investigations have examined the contractile properties of single motor units contracting in isolation, and a great majority of those studies have been done in the cat. Fewer studies, again primarily in the cat, have examined motor units acting in concert in both hind-limb and extraocular muscles. It has been shown, in general, that when individual motor unit forces are added together they do not always add linearly, which makes our understanding of motor control somewhat more complicated. In addition, complex neuronal firing patterns can yield unexpected force outputs or muscle positions whether those patterns occur naturally or are induced through motoneuron stimulation. The current investigation extends these findings of nonlinearity to the primate extraocular system. In studies of the squirrel monkey lateral rectus muscle and its motor units, we show that individual units lose an average of 45% of their force output when they fire in concert with a small number of other motor units. Also, when individual motor units are stimulated at a constant rate of 100 Hz, the force output is most often dramatically different if that constant 100-Hz stimulation is preceded by brief (25 ms), high-frequency stimulation burst or pulse, as occurs during saccades. The force at 100 Hz is usually significantly higher than when no pulse is delivered. However, we now show that an identical stimulation

pattern applied to a number of motor units simultaneously does not always yield these force differences. These “nonlinearities” are addressed in terms of the complex muscle architecture that we show in the squirrel monkey lateral rectus muscle. Muscle fibers do not always run in parallel from tendon to tendon. Instead, they may branch or attach to each other laterally or end to end, serially.

**Keywords** Oculomotor system · Eye movements · Abducens · Skeletal muscle · Motoneuron

### Introduction

That the transformation of motoneuron action potentials into the contractions of muscle-unit populations resulting in movements is an important mechanism is evident. It is germane to our basic understanding of motor control as well as in the treatment of motor disorders. The extraocular motor control system is a good model to use in studying this transformation (Dean 1996), because much is known about “normal” motoneuronal firing patterns as alert animals execute precise and repeatable movements in order to acquire and fixate visual targets.

While many investigators have examined the contractile properties of single motor units contracting in isolation, we and others have begun studying motor units acting in concert. Basically, it has been found that when individual motor unit forces are added together they do not always add linearly (Clamann and Schelhorn 1988; Emonet-Denand et al. 1990; Powers and Binder 1991). In regard to the cat extraocular system, we have previously reported an apparent loss of twitch motor-unit force in the lateral rectus muscle when single motor units were stimulated simultaneously (Goldberg et al. 1997). This loss of force was attributed, in part, to the complex arrangement of muscle fibers within the studied muscles (Mayr et al. 1975; Alvarado-Mallart and Pinçon-Raymond 1976). It has been shown that extraocular muscle fibers (EOMs) do not always run in parallel from tendon to tendon but show branching as well as end-to-end and

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side-to-side attachments (Mayr et al. 1975; Alvarado-Mallart and Pinçon-Raymond 1976). Trotter et al. (1995) and Monti et al. (2001) have recently reviewed this literature as it pertains to skeletal muscle, with much of the work having been done in the rat and cat.

The present study was undertaken to see whether similar muscle physiological and anatomical findings were to be found in the primate extraocular motor control system. While the cat extraocular motor system is certainly similar to that in man, most studies of extraocular motoneuron firing during eye movements have been done in the primate and many of the models of the eye movement control system are based on those studies. Also, recent anatomical and physiological studies of squirrel monkey abducens nucleus motoneurons appeared to indicate that motor units firing in unison would not add linearly (Goldberg et al. 1998; McClung et al. 2001). Some of these results have been presented in preliminary form (Goldberg and Shall 2000).

## Materials and methods

All procedures for the care and use of monkeys complied with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Two adult male squirrel monkeys (*Saimiri sciureus*, 0.84 and 0.88 kg) were used for the muscle anatomical studies. Monkeys were premedicated with 15 mg ketamine/kg, 0.1 mg acepromazine/kg, and 10 mg glycopyrrolate/kg, all administered intramuscularly. The animals were given pentobarbital sodium (15 mg/kg i.p.). Under anesthesia, left and right lateral rectus muscles were excised. The muscles were frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

A modified nitric acid fiber digestion technique (Tamaki et al. 1989, 1992) was used to remove the connective tissue from around and between the muscle fibers while protecting the inner morphology of the muscle cells as much as possible. First, each muscle was thawed in distilled water before treatment with 15% nitric acid for 2 h at room temperature. The vial containing the solution and muscle was placed on a platform shaker to facilitate constant circulation and loosening of the intramuscular connective tissue. After nitric acid treatment, the muscles were washed in distilled water and cooled for 1 h at  $4^{\circ}\text{C}$ . The muscle was then placed in 0.01 M phosphate-buffered saline (pH 7.4) and placed in a warm ultrasonic bath for 3 min. Small groups of muscle fibers could then be dissected apart with minimal trauma to the fibers. Some single branched fibers were bluntly dissected free using 28-gauge needles.

Small groups of muscle fibers were fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer (pH 7.4) for 1 h at room temperature and postfixed with 1% osmium tetroxide/0.1 M cacodylate buffer (pH 7.4) for 1 h. After fixation, the muscle fibers were dehydrated with a graded ethanol series and propylene oxide and embedded in Poly/Bed 812 epoxy (Luft formulation with DMP-30 as hardener; Polysciences, Warrington, Pa.). Ultra-thin sections of the fibers were obtained with an ultramicrotome and stained with 5% uranyl acetate and Reynolds lead citrate. Stained sections were observed by electron microscopy (Zeiss EM10CA; LEO Electron Microscopy).

### Muscle fiber type analysis

Muscle histology was completed on normal monkey EOMs using an ATPase reaction. The EOMs were placed in an embedding

medium in heavy aluminum foil and quickly frozen in liquid nitrogen-cooled isopentane and stored at  $-70^{\circ}\text{C}$  until processing.

Cross-sections (10  $\mu\text{m}$ ) were cut from the mid-belly portion of the muscle, using a cryostat at  $20^{\circ}\text{C}$ , and mounted on gelatin-coated slides. Sections were stained for myofibrillar adenosine triphosphatase (myofibrillar ATPase) at an acidic (pH 4.3) preincubation according to Guth and Samaha (1970). All sections were dehydrated in ascending alcohols, cleared in xylene, and mounted in Permount. Sections were analyzed for total number of muscle fibers and fiber diameter in each layer (global and orbital) using the NeuroLucida MicroBrightField image-analysis system (Colchester, Vt.) with a Nikon Optiphot-2 light microscope. The means and standard deviations are reported in the results.

Lateral rectus muscles were removed from three cats (3–3.5 kg) that had been anesthetized in the same manner as described above. The muscles were frozen in liquid nitrogen, sectioned, stained for ATPase, and analyzed in the same way as described for the monkey.

### Muscle physiology

Two adult male squirrel monkeys (0.87 and 0.91 kg) were used for the physiological studies. They were premedicated and anesthetized as delineated above. Supplemental doses of pentobarbital were administered intravenously to insure complete anesthesia throughout the experiment. Respiratory rate, expiratory  $\text{CO}_2$ , and EKG were continuously monitored and maintained within normal ranges. A detailed description of the physiological methodology has been presented in a recent publication (Goldberg et al. 1998), so only a brief recapitulation is given below.

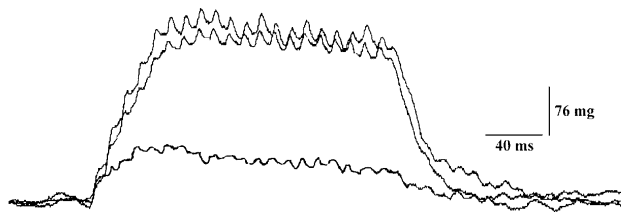
The abducens nerve was stimulated in the brainstem with a bipolar electrode in order to evoke antidromic responses in abducens nucleus motoneurons recorded extracellularly with glass micropipettes (2–3- $\mu\text{m}$  tip diameter). The same neurons could then be extracellularly stimulated with the same electrode to evoke contractions of single motor units in the ipsilateral lateral rectus muscle. Contractile responses of the whole muscle, or a number of motor units, could also be elicited by stimulation of the nerve in the brainstem. Supramaximal stimulation was used to elicit whole-muscle responses, while lower stimulus intensities were used to evoke a partial response of the muscle. All contractile responses at the muscle were recorded with a sensitive strain gauge attached to the muscle's distal tendon, but with the tendon still attached to the globe (Goldberg and Shall 1997). The muscle's tension was set for a maximal isometric twitch response to single-pulse stimulation of the VI<sup>th</sup> nerve.

### Stimulation paradigms

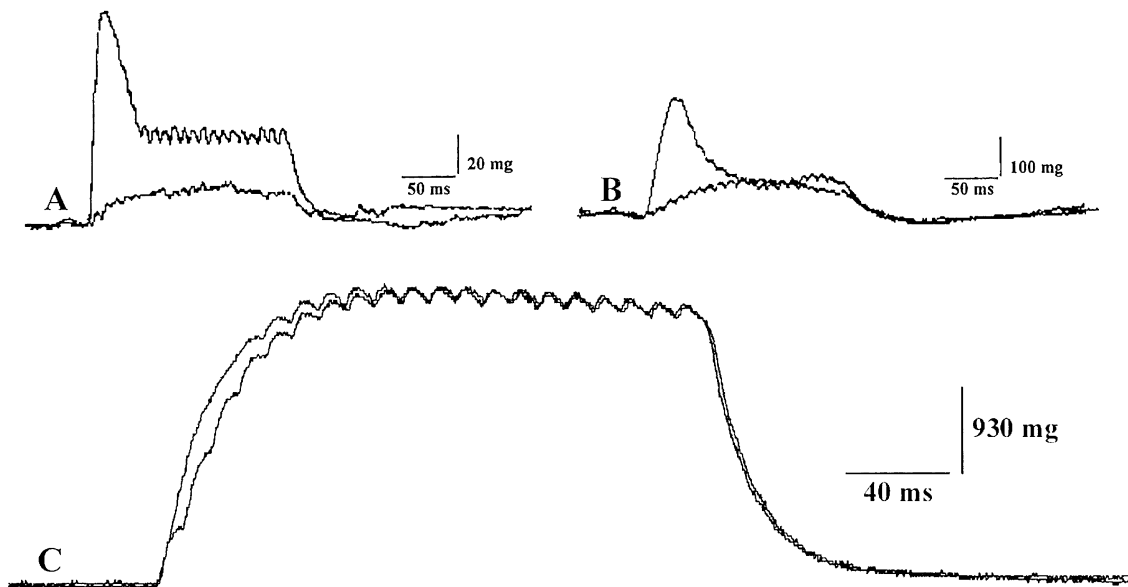
Whole-muscle and single motor-unit mechanical properties were measured in response to 0.1- to 0.2-ms duration stimuli applied through the nerve-stimulating electrodes or glass micropipette electrodes at various frequencies (Shall et al. 1996). All stimulation trains were delivered using a programmable pulse generator (A.M.P.I. Master-8).

Whole-muscle and motor-unit tetanic tension were assessed as follows. Tetanic tension measurements were taken in response to a constant frequency, 200-ms duration train at a stimulation rate of 100 Hz to determine motor-unit summation. We chose a tetanic stimulation frequency of 100 Hz for this summation study, since it has been shown that primate lateral rectus motoneurons show an average firing frequency of 97 Hz during fixation at primary position (Goldstein and Robinson 1986).

Tension assessments were also made in response to a 200-ms, pulse/slide/step tetanic train. Each stimulation train included a 25-ms, high-frequency pulse/slide phase (500 Hz for 10 ms and 250 Hz for 15 ms) followed by a 175-ms step phase at 100 Hz. Tensions reached during the step phase were compared with those recorded in response to constant-frequency stimulation in order to determine if there was a tension "hysteresis" (Shall et al. 1996). That is, single



**Fig. 1** Superimposed traces showing lack of motor-unit summation at a stimulation rate of 100 Hz. *Bottom trace:* Single motor-unit shows a tension of 81.6 mg. *Middle trace:* VIth nerve activated units show a tension of 287.0 mg. *Top trace:* Simultaneous activation of motor unit and nerve-activated units show a tension of 319.0 mg; 49.6 mg of force is “lost”



**Fig. 2A-C** Hysteresis of single motor-units and among motor units activated simultaneously. **A** Single motor-unit activation. *Bottom trace* shows tetanic response to constant-frequency stimulation at 100 Hz (20 mg). *Top trace* shows tetanic response to a pulse/slide/step stimulation paradigm: 500 Hz for 15 ms, 250 Hz for 10 ms, followed by 100 Hz (52 mg) for 175 ms. Note the pulse/slide/step response and the difference in muscle force at 100 Hz. **B** Another single motor-unit activated with the same stimulation pattern as in **A**. Note pulse/slide/step response, but lack of hysteresis at 100 Hz

(100 mg). **C** Superimposed traces of a number of units activated simultaneously through stimulation of the VIth nerve. *Bottom trace* is a response to constant-frequency stimulation and *top trace* shows a response to pulse/slide/step stimulation (as in **A** and **B**). Note the lack of the pulse/slide/step response and the lack of hysteresis. However, the rise time is significantly faster with the pulse/slide. (Panels **A** and **B** of this figure are reprinted with permission from the *Journal of Neuroscience*. They appeared in Goldberg et al. 1998)

muscle-unit force in various skeletal muscles has been shown to vary in response to identical motoneuron stimulation rates depending on the immediately previous frequency of stimulation.

See the Results section and legends for Figs. 1 and 2 for further explanation of the stimulation sequences.

## Results

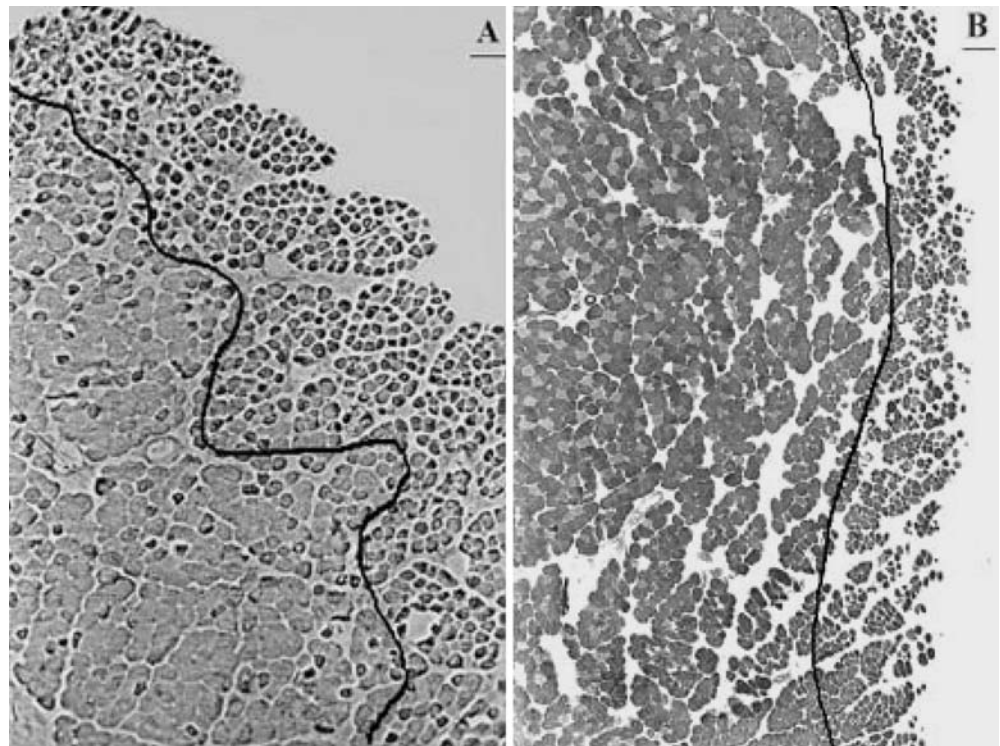
### Motor-unit contractile characteristics

Eight single lateral rectus motor-units were examined in one squirrel monkey. The lateral rectus motor-unit contractile characteristics found in this experiment (Table 1) were generally similar to those found in a previous study (Goldberg et al. 1998). Submaximal stimulation of abducens nerve axons with the bipolar electrode at 100 Hz

**Table 1** Single muscle units adding to nerve-activated muscle units

	Proportion of single-unit force that added to nerve-activated force	Twitch tension (mg)	Fusion frequency	Fatigue (% units that are fatigue resistant)	Tetanic tension generated at 100 Hz	Maximum tetanic tension
All units ( $n=8$ )		$16.58 \pm 3.83$	$180 \pm 20$	14.3	$76.92 \pm 42.7$	$132.38 \pm 61.23$
Units adding <i>less</i> than expected force ( $n=7$ )	Mean $0.55 \pm 0.13$ Range $0.35-0.72$	$15.54 \pm 2.68$	$185 \pm 16$	16.6	$68.49 \pm 38.24$	$122.64 \pm 59.06$
Units adding <i>more</i> than expected force ( $n=1$ )	1.08	23.8	150.0	0.0	136.0	200.6

**Fig. 3** **A** Micrograph ( $\times 600$ ) of a cross section of the superior edge and orbital fiber side of the squirrel monkey lateral rectus muscle using an ATPase reaction. The orbital layer makes up about one-half ( $\approx 300 \mu\text{m}$ ) of the ( $\approx 700 \mu\text{m}$ ) muscle. *Scale bar*  $50 \mu\text{m}$ . **B** Micrograph ( $\times 420$ ) of a cross section of the orbital side of the cat lateral rectus muscle. The orbital layer makes up a smaller percentage ( $\approx 400 \mu\text{m}$ ) of this larger ( $\approx 2.5 \text{ mm}$ ) muscle. *Scale bar*  $125 \mu\text{m}$ . *Curved lines in A and B* approximate the border between the orbital layer (to the right) and global layer (to the left)



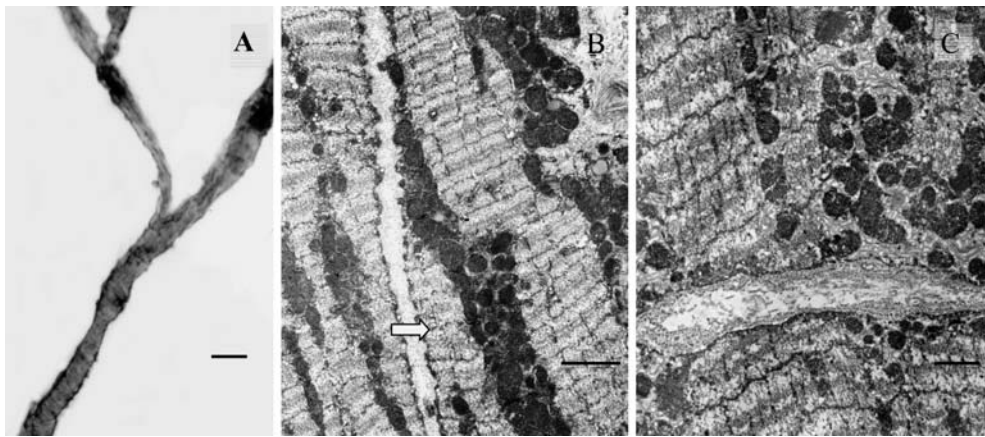
was used to activate a small group of motor units each time a new single motoneuron was isolated in the brain stem. This submaximal stimulation was adjusted each time to produce about 300 mg of force at 100 Hz. Only one of the eight single motor units studied was seen to add its full twitch force to the nerve-activated units. The other seven units added only a percentage of their full force (Table 1 and Fig. 1). This force addition was evaluated using constant-frequency stimulus trains at 100 Hz delivered simultaneously through the nerve-stimulating electrode and the extracellular glass micropipette within the abducens nucleus. The seven motor units added only 55.2% of their force, indicating a “loss” of 44.8% of the force generated when stimulated in isolation. Figure 1 illustrates an example of the force generated by a group of motor units via 100-Hz stimulation of the abducens nerve compared with the force produced by a single motor unit when its motoneuron is also stimulated at 100 Hz. When the single motor unit is stimulated at the same time as the group, only 39.2% of the force of the single unit is added to the force generated by the original group.

Hysteresis was examined in the whole muscle of two squirrel monkeys. We have previously shown a pronounced hysteresis effect in single units at the mid-range firing frequency of 100 Hz in squirrel monkeys (Goldberg et al. 1998) and cats (Shall and Goldberg 1992; Shall et al. 1996). Figure 2A is an example of a motor unit showing a sharp rise in tension caused by a 25-ms pulse of high-frequency stimulation followed by the response to the 175-ms step stimulation at 100 Hz). The 100-Hz step tension remains significantly greater than the tetanic response to constant-frequency stimulation at 100 Hz

(lower trace). This unit, therefore, shows a force hysteresis at 100 Hz. Seventy-five percent of squirrel monkey motor units showed this hysteresis effect (Goldberg et al. 1998). Figure 2B is an example of a motor unit that shows similar tensions at 100 Hz either preceded or not by a high-frequency stimulation pulse. Five percent of the motor units studied showed this type of response without hysteresis (Goldberg et al. 1998). In contrast to the usual motor-unit response, neither a pulse response nor a hysteresis effect was evident when a number of motor units were stimulated using the same paradigms as in Fig. 2A and B. Figure 2C displays the trace of the force produced when a group of motor units were stimulated with a constant frequency of 100 Hz or with the pulse/slide/step paradigm. Both paradigms generated 3.41 g of tension, though the rise in tension was more rapid with the pulse/step paradigm.

#### Muscle fiber analysis

The whole lateral rectus muscle of the squirrel monkey was sectioned in order to count the number of muscle fibers. The range of muscle fiber diameters was also observed. These findings were compared with that in the cat in order to partially replicate a previous report (Peachey 1971) in which a number of extraocular muscles were anatomically studied. The thinner fibers of the squirrel monkey orbital layer constitute about half (0.3 mm) of the 0.7-mm-thick squirrel monkey muscle (Fig. 3A). The average diameter of squirrel monkey orbital fibers is  $14.62 \mu\text{m} \pm 2.57 \mu\text{m}$  compared with global



**Fig. 4** **A** Micrograph ( $\times 1,200$ ) of a single branched muscle fiber teased from a squirrel monkey lateral rectus. *Scale bar* 100  $\mu\text{m}$ . **B** Electron micrograph ( $\times 6,000$ ) of a squirrel monkey lateral rectus muscle fiber. Note the smaller muscle fiber (on the *right* with *arrow*), which is attached to the side of the larger primary muscle fiber (on the *left*). The two fibers are separated by the basement

membrane and connective tissue. *Scale bar* 3  $\mu\text{m}$ . **C** Electron micrograph ( $\times 7,500$ ) of an end to end muscle fiber attachment. Note the sarcomeres, mitochondria, and basement membrane of two muscle fibers terminating at a space filled with connective tissue fibers. *Scale bar* 1  $\mu\text{m}$

fibers that average  $21.70 \mu\text{m} \pm 4.24 \mu\text{m}$  in diameter. The number of muscle fibers at the muscle's mid-belly is approximately 5,000. Since approximately 10,000 fibers were reported previously for the Rhesus and 15,000 were reported for the cat (Peachey 1971), we rechecked the cat (Fig. 3B) and confirmed that approximately 15,000 fibers were present at the mid-belly of the muscle. The orbital layer of the cat appears to comprise about one-fifth of the muscle (mean fiber diameter,  $22.43 \mu\text{m} \pm 5.58 \mu\text{m}$ ), and the global- and intermediate-layer muscle fibers make up a larger portion of the muscle (mean fiber diameter,  $38.26 \mu\text{m} \pm 7.31 \mu\text{m}$ ).

It has been suggested that connections between muscle fibers may distort the production of muscle force so that there is not a simple linear summation of single muscle-unit forces (Monti et al. 2001). Teasing muscle fibers from the lateral rectus muscle under the light microscope showed a number of branching fibers (Fig. 4A). This technique allowed us to see the lateral branching but not end-to-end junctions. Analysis of longitudinal sections under the electron microscope revealed many lateral connections of muscle fibers (Fig. 4B) and blunt junctions between the ends of muscle fibers (Fig. 4C). Each of these connections is separated by a space ( $\approx 1 \mu\text{m}$ ) containing loose connective tissue.

## Discussion

An observation by Martin J. Pinter (personal communication) triggered a series of experiments regarding extraocular motor units firing in unison. Pinter observed that supramaximal stimulation of the whole superior oblique muscle nerve (Waldeck et al. 1995) yielded whole-muscle contractile forces well below those that would be expected if the average twitch tension of individual superior oblique motor units was multiplied by

the number of motoneurons in the trochlear nucleus. The mean twitch tension for motor units of the cat superior oblique muscle was 27.5 mg (Waldeck et al. 1995), with about 1,000 neurons in the trochlear nucleus (Murphy et al. 1990). Therefore, the expected whole-muscle twitch tension would be about 27.5 g in contrast to the observed value of 4 to 5 g (Waldeck et al. 1995). This represents about an 85% "loss" of twitch force. This was contrary to the finding of Burke et al. (1973) in the cat medial gastrocnemius muscle, wherein single muscle-unit tension, when multiplied by motoneuron number, yielded a total whole-muscle tension equivalent to that seen upon supramaximal stimulation of the muscle nerve. We confirmed Pinter's "loss of force" observation in studies of the cat lateral rectus muscle for both twitch (Goldberg et al. 1997) and tetanic tensions (Goldberg and Shall 1997). These studies revealed a "loss" of cat lateral rectus muscle force of about 50% for both twitch and tetanic stimulation. We speculated (Goldberg et al. 1997, 1998; Goldberg and Shall 1999; McClung et al. 2001) that the complex arrangement of muscle fibers in the cat lateral rectus muscle (Mayr et al. 1975; Alvarado-Mallart and Pinçon-Raymond 1976) contributed to the loss of force seen when motor units contracted in unison and that innervation of single muscle fibers by more than one motoneuron (polyinnervation) might also play a role (Goldberg and Shall 1997; McClung et al. 2001). Recent anatomical studies have also indicated that extraocular pulleys coupled to the orbit may also redirect some force in intact animals (Demer et al. 2000). It has also been suggested that the smoothness of eye movements and force gradations may be implemented by the buffering of forces that occurs due to complex muscle fiber arrangements and the interaction of active and inactive fibers (Scudder et al. 2002).

In light of these findings regarding the cat superior oblique and lateral rectus muscles, we revisited data

presented in previous reports of the cat inferior oblique and medial rectus muscles. The inferior oblique whole muscle showed a mean twitch tension of 4.5 g and a maximum tetanic tension of 35.7 g in response to whole nerve stimulation (Shall et al. 1995). We used the graphs presented by Lennerstrand (1974) to approximate the average twitch and tetanic tensions of single inferior oblique motor units. We calculate a  $\approx 14$  mg twitch force and a  $\approx 179$  mg tetanic force from the figures presented. If we multiply these figures by a very conservative estimate for the number of inferior oblique motoneurons, 1,000 (Miyazaki 1985), we would expect a 14-g twitch and a 179-g tetanic response. This represents an approximate 70% loss of twitch and an 80% loss of tetanic force. For medial rectus, an average whole-muscle twitch force of 13.7 g (Meredith and Goldberg 1986) and a 100-g tetanic force (Cooper and Eccles 1930) have been observed. These compare to an average motor-unit twitch force of 43 mg and a tetanic force of 262 mg (Meredith and Goldberg 1986). Again, multiplying by a very conservative motoneuron number of 1,000 (Miyazaki 1985), we would expect a whole-muscle twitch force of 43 g and a tetanic force of 262 g. This represents an approximate 68% loss of twitch force and a 62% loss of tetanic force for the cat medial rectus muscle. Furthermore, if we compare the observed whole-muscle twitch and tetanic responses of 1.1 g and 13.7 g with the expected values of 21.4 g and 372.4 g (McClung et al. 2001) there is an approximate 95% loss of the twitch force and a 96% loss of tetanic force in the squirrel monkey lateral rectus muscle.

Monti et al. (2001) have suggested that the interactive architecture of muscle fibers and connective tissue are the main contributing factors to the nonaddition of motor-unit force to the whole-muscle force. Complex fiber arrangements have been seen in a variety of skeletal muscles and species (Trotter 1993), with tapering muscle fibers transmitting their force laterally and blunt endings terminating on tendons or in-series to other muscle fibers. Anatomical studies of the mouse superior rectus muscle have found that the orbital layer does not extend the full length of the muscle, terminating approximately at the middle of the distal third (Davidowitz et al. 1996). Multiple connections may account for the maintenance of near-normal forces in spite of injury to the muscle (Street 1983; Goldberg et al. 1997).

We now can add that the lateral rectus muscle in the primate (squirrel monkey) is at least as complex as that previously shown in the cat (Mayr et al. 1975; Alvarado-Mallart and Pinçon-Raymond 1976). There is also a clear lack of linear summation of motor-unit force as has also been reported in the cat (Goldberg et al. 1997; Goldberg and Shall 1997). However, the force lost when studying small numbers of motor units does not appear directly comparable with that lost when comparing whole-muscle actual and predicted force. Some 25% of cat lateral rectus motor units showed an average 50% loss of their twitch force, while most of the other units showed linear force addition (Goldberg et al. 1997). [We focused on twitch

force in the cat study (Goldberg et al. 1997), since the average firing frequency of lateral rectus motoneurons is about 57 Hz during fixation at primary position and single motor-unit force summation is generally not seen at that frequency. Results from 9 units studied with a tetanic frequency of 150 Hz were inconsistent with regard to summation (Goldberg et al. 1997.)] In this study, 7 of 8 squirrel monkey lateral rectus motor units lost about 45% of their tetanic force at 100 Hz (the average firing frequency of primate lateral rectus motoneurons during fixation at primary position). It is of interest to note here that a very recent paper using the cat soleus muscle (Perreault et al. 2003) has shown superadditive (higher than expected force) summation at low force levels involving only a few motor units and subadditive (loss of force) summation at higher force levels involving a greater number of motor units. Other factors which may play a role in revealing nonlinear force summation are stimulation frequency and motor-unit type (Troiani et al. 1999).

But there does appear to be some consistency in the extraocular muscles. The muscles studied to date (lateral and medial recti plus superior and inferior obliques) all show an apparent loss of whole-muscle force when comparing actual force values to expected or predicted force values. And the motor units also appear to reflect, at least to some degree, that loss of force when they are added together. Spinal cord innervated motor units also show nonlinearities in force addition, but their whole muscle actual and expected force values would appear to be, to the best of our knowledge, consistent (Burke et al. 1973; Kernell et al. 1985).

In addition, there may be some tendency indicating a generally greater loss of extraocular muscle force as the innervation ratio decreases. Using the graphs of muscle fiber counts at the mid-belly of the cat extraocular muscles provided by Peachey (1971), we estimate 17,000 medial rectus, 15,000 lateral rectus, 8,000 inferior oblique, and 7,000 superior oblique muscle fibers. These numbers divided by approximately 1,000 motoneurons for each nucleus yield innervation ratios of 17 ( $\approx 65\%$  loss), 15 ( $\approx 50\%$  loss; see also Guéritaud et al. 1985 for innervation ratio of cat lateral rectus), 8 ( $\approx 75\%$  loss), and 7 ( $\approx 85\%$  loss), respectively. The squirrel monkey lateral rectus muscle has about 5,000 muscle fibers divided by approximately 2,000 lateral rectus muscle motoneurons (McClung et al. 2001) for an innervation ratio of 2.5 ( $\approx 95\%$  loss).

Another line of inquiry pertaining to the predictability and repeatability of muscle force output involves the notion of "hysteresis." That is, extraocular muscle motoneurons, including those in the primate, have been shown to fire at different steady rates during eye fixation at identical positions depending on the direction of movement prior to that (identical) fixation position (Eckmiller 1974; Delgado-Garcia et al. 1986; Goldstein and Robinson 1986; Stahl and Simpson 1995). This was termed a motoneuron firing hysteresis by Eckmiller (1974). Similarly, single muscle-unit force in various

skeletal muscles has been shown to vary in response to identical motoneuron stimulation rates depending on the immediately previous frequency of stimulation (Burke et al. 1976; Binder-Macleod and Clamann 1989; Kernell 1995; Shall et al. 1996; Goldberg et al. 1998). Most of these muscle-unit studies were done in the cat. We have previously shown a muscle-unit force hysteresis in a large percentage (95%) of squirrel monkey lateral rectus muscle motor units (Goldberg et al. 1998). It is interesting, however, that when several motor units were stimulated simultaneously there was an apparent lack of hysteresis as well as an absence of the high-force response to the high-frequency pulse of stimulation. Perhaps the complex arrangement of muscle fibers plays a role here in “damping” out this response. But we feel that further study is needed to look at a range of stimulation frequencies, muscle fiber types and possibly the territory of the whole muscle occupied by the particular muscle units activated.

These findings, taken together, clearly demonstrate that our ability to predict muscle force based on motor-unit number and force output as well as the complex firing patterns of the active motoneurons can be a complicated matter.

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