ELSEVIER

Journal of Neuroscience Methods 78 (1997) 47–50

JOURNAL OF NEUROSCIENCE METHODS

Lateral rectus whole muscle and motor unit contractile measures with the extraocular muscles intact

Stephen J. Goldberg *, Mary S. Shall

Departments of Anatomy and Physical Therapy, P.O. Box 980709, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23298-0709, USA

Received 30 May 1997; accepted 20 July 1997

Abstract

Both extracellular and intracellular stimulation of single motoneurons were shown to be similarly effective and consistent in eliciting contractile responses in single lateral rectus muscle motor units. The whole muscle was activated by stimulating the sixth nerve in the brain stem. Both whole muscle and motor unit contractile characteristics, under isometric conditions, were found to remain consistent regardless of whether this extraocular muscle was detached or left attached to the globe. In addition, whole muscle twitch and maximum tetanic tension evoked by sixth nerve stimulation was significantly less than would be predicted by the linear summation of individual motor unit twitch and maximum tetanic tensions. © 1997 Elsevier Science B.V.

Keywords: Motoneuron; Abducens; Lateral rectus; Muscle; Eye movement; Oculomotor; Extraocular

1. Introduction

Previous physiological studies of the extraocular muscles and their motor units (Lennerstrand, 1974; Goldberg, 1990; Shall and Goldberg, 1992, 1995; Goldberg et al., 1997; Shall et al., 1996) have been modeled after studies of the spinal cord controlled limb musculature (Burke, 1981, 1990; Binder et al., 1996). Intracellular activation of single motoneurons was generally used to elicit muscle responses so that the contractile characteristics of extraocular single muscle units could be studied (Goldberg, 1990; Shall and Goldberg, 1992, 1995; Goldberg et al., 1997; Shall et al., 1996), but extracellular motoneuron activation has been used as well (Lennerstrand, 1974).

The spinal cord innervated muscles that are often studied with these procedures are pennate muscles (e.g. medial gastrocnemius, soleus, biceps brachii, tibialis anterior) that converge into relatively long tendons which attach to bone (Loeb and Richmond, 1994). These muscles are generally detached with their tendon intact and the tendon is then attached to a force transducer in order to measure the contractile characteristics of the muscle and its motor units. In contrast, the eye muscles are comprised of muscle fibers in series and in parallel (Mayr et al., 1975; Spencer and Porter, 1988) that insert into short, broad tendons that attach to the globe. The muscles appear, in their natural state, as flattened bands of fibers. A suture was placed through the muscle tendon and the muscle was then detached from the globe while maintaining its normal orientation (Shall and Goldberg, 1992). This procedure, however, causes the muscle tendon to converge to a point, rather than remain as a wide band.

The gross anatomical architecture of the extraocular muscles allows the possibility of examining muscle force characteristics with the muscle still attached to the globe (Miller and Robins, 1992), although this has never been done at the level of the motor unit. This would serve to leave the distal part of the muscle and its insertion in a more normal position. In the present

* Corresponding author. Tel.: +1 804 8289529; fax: +1 804 8289477; e-mail: sgoldberg@gems.vcu.edu

PII S0165-0270(97)00141-6

experiments we have examined cat lateral rectus whole muscle and muscle unit forces with the muscle tendon attached to the globe, but appropriately loaded to produce maximum isometric contractions as is done when the muscle and tendon are detached from the globe. We were then able to compare the forces produced in the same attached and then detached lateral rectus muscle.

In addition, we studied the feasibility of using extracellular stimulation of single motoneurons using glass micropipettes (1.0 μm tip diameter). We could then determine whether both extracellular and intracellular stimulation of the same motoneuron yielded identical muscle unit contractile responses. Fine tungsten wires of 3–10 μm tip diameter have been used in the past to stimulate single extraocular motoneurons (Lennerstrand, 1974). The use of reliable extracellular, rather than intracellular, stimulation could prolong the experimental life of single motoneurons and allow the examination of many more cells per experimental animal.

2. Materials and methods

Two cats weighing 2.5 and 3.1 kg were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) prior to all procedures. Supplemental doses were given intravenously throughout the experiment to maintain deep anesthesia (defined as lack of response to corneal reflex or paw pinch). Respiratory rate and expiratory CO_2 were monitored and maintained within normal ranges. The animal's body temperature was maintained between 38 and 40°C using a heating pad while the head was positioned in a stereotaxic frame throughout the experiment. The globe and extraocular muscles were exposed as previously described (Shall and Goldberg, 1992). In these experiments the superior medial and superior lateral slips as well as the inferior lateral slip (Crandall et al., 1981; Meredith et al., 1981) of the retractor bulbi muscle were disinserted from the globe. This was done so that their contractile force would not be a factor upon stimulation of the sixth nerve in the brain stem.

A length of 5-0 silk was looped beneath the lateral rectus muscle's tendon and attached to the strain gauge for the measurement of whole muscle and muscle unit forces. The loop was tied to a semiconductor strain gauge (Pixie Model 8108, Endevco) with a natural frequency of 2 KHz and a compliance of approximately 2 μm/g. The transducer was positioned to record the maximal isometric response. Muscle force was also examined with the muscle detached from the globe. The muscle tendon was sutured with a 5-0 silk loop at its distal end and detached from the globe, maintaining anatomical orientation. Similar surgical procedures and experimental setups have been previously described (Shall and Goldberg, 1992, 1995).

The sixth nerve was stimulated in the brain stem with 0.1 ms duration pulses at 0.5 Hz to evoke contractions of the lateral rectus muscle and to antidromically identify motoneurons in the abducens nucleus. These procedures have been previously described (Shall and Goldberg, 1992, 1995; Shall et al., 1996). The potassium citrate filled electrodes, used for both stimulating and recording, had impedances of 10–20 MΩ and tip diameters of $\cong$ 1.0 μm (Shall and Goldberg, 1992, 1995; Goldberg et al., 1997). The electrodes were driven using a hydraulic microdrive into the area of the abducens nucleus until a motoneuron was identified by its antidromic response to abducens nerve stimulation. We directly activated all motoneurons before intracellular penetration using 0.3 ms duration stimuli applied through the electrode to evoke muscle unit contractions (Fig. 1).

Single stimuli and stimulation trains were delivered using a programmable pulse generator (A.M.P.I. Master-8). The twitch measurements were made before the tetanic stimulation to avoid potentiation. Measures of twitch and maximum tetanic tension, twitch contraction time, fusion frequency and fatigue were all done as described previously (Shall and Goldberg, 1992, 1995; Shall et al., 1996). Muscle unit kt value (the slope of the relation between a series of single motoneuron stimulation frequencies and the resultant single muscle unit forces (Shall and Goldberg, 1992)) was also determined.

3. Results

The extracellular motoneuron stimulation protocol effectively eliminated the activation of more than one motoneuron in the following manner. When a twitch was observed upon single pulse stimulation (Fig. 1a) it immediately disappeared with a reduction of stimulus current with no gradation of twitch response (Lenner-

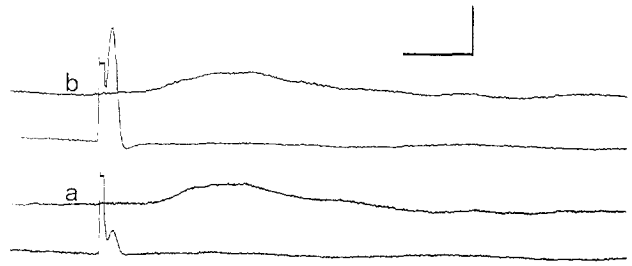

Fig. 1. Muscle unit twitch responses (average of five traces) evoked before (a) and after (b) penetration of the same motoneuron. (a) Extracellular motoneuron response to direct stimulation is 15.4 mV and twitch tension is 24.8 mg. (b) Intracellular motoneuron response to direct stimulation is 70.3 mV and twitch tension remains 24.8 mg. Horizontal bar = 5 ms for (a) and (b). Vertical bar = 30.0 mV for the bottom trace in (a) and (b); and 62.0 mg for the top trace in (a) and (b).

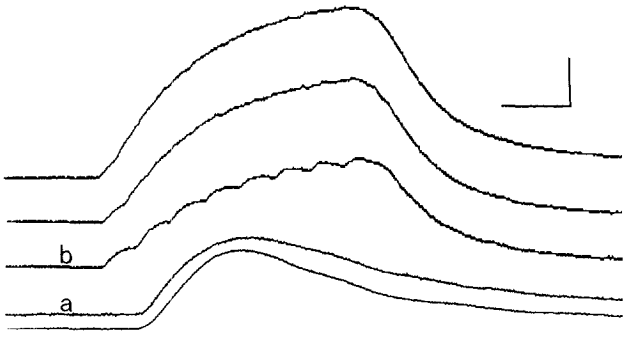

Fig. 2. Whole muscle twitch (average of five traces) and 75 ms duration tetanic responses. (a) Bottom trace is 13.8 g response with muscle attached to the globe. Top trace is 13.8 g response with muscle detached from the globe. (b) Tetanic traces from bottom to top are at 100, 150 and 190 Hz with a maximum tetanic tension of 109.7 g (at 190 Hz). Horizontal bar = 5 ms for (a) and 50 ms for (b). Vertical bar = 9.0 g for (a) and 32.0 g for (b).

strand, 1974). Increases in stimulus current rarely evoked a more powerful twitch or one of longer twitch contraction time. Such twitch force or contraction time increases would indicate that more than one motoneuron was being activated; this occurred only rarely at extremely high stimulation currents ($\cong$15–20 times threshold). We then could often penetrate the same motoneuron for intracellular activation. We noted that the antidromic latency was unchanged and that the action potential steadily increased in amplitude as the cell was approached and penetrated (Goldberg and Clamann, 1977). Intracellular stimulation evoked identical mechanical responses (Fig. 1b) and insured that our extracellular technique effectively activated one motoneuron only. This methodology effectively increases the long term viability and number of motor units which can be studied in any one animal. Fewer animals may then be necessary for future studies.

Stimulation of the sixth nerve evoked nearly identical responses from the whole lateral rectus muscle either when attached to the globe or not (Fig. 2a). The twitch ($\cong$14.0 g) and maximum tetanic tensions ($\cong$110 g, Fig. 2b) we observed were similar to those previously reported (Cooper and Eccles, 1930; Barmack et al., 1971; Goldberg, 1990). In addition, single muscle unit recordings could routinely be made with the muscle attached to the globe (Fig. 3). The range of twitch and tetanic tensions as well as twitch contraction times and fusion frequencies seen in all 12 recorded twitch units and 1 non-twitch unit were all within the ranges previously reported (Lennerstrand, 1974; Goldberg, 1990; Shall and Goldberg, 1992, 1995; Shall et al., 1996).

4. Discussion

It is evident that extracellular stimulation of motoneurons can be effective in eliciting contractile responses in single muscle units. The use of 1.0 μm glass micropipettes in the current study enabled us to stimulate the same cell both extracellularly and intracellularly. This procedure confirmed the earlier study of Lennerstrand (1974) in which fine tungsten electrodes were used for the extracellular activation of single motoneurons.

It appears that the standard surgical procedure of detaching the lateral rectus muscle from the globe is no longer necessary for the accurate recording of whole muscle and motor unit contractile characteristics. However, detaching the lateral rectus muscle from its insertion on the globe, as was routinely done in previous studies (Meredith et al., 1981; Goldberg, 1990; Shall and Goldberg, 1992, 1995; Goldberg et al., 1997; Shall et al., 1996), did not significantly alter whole muscle or muscle unit responses.

In addition, the whole lateral rectus muscle twitch force we have reported in this and many other experiments (Goldberg, 1990) is consistent with an earlier finding (Barmack et al., 1971) and lower than would be estimated based on a delineation of average twitch tension related to motor unit type (Goldberg et al., 1997). The estimate is determined as follows. First one calculates the average force produced by each motor unit type (e.g. fast fatigable units would yield 52.8 mg (Shall and Goldberg, 1992)). It has been shown that there are about 1189 lateral rectus motoneurons in the cat abducens nucleus (Steiger and Buttner-Ennever,

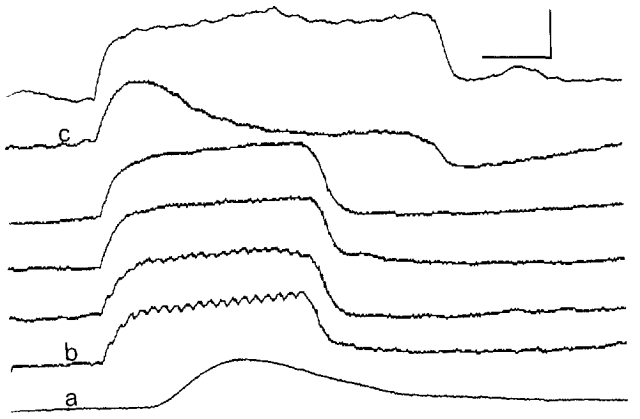

Fig. 3. Muscle unit twitch (average of five traces), 150 ms duration tetanic responses and 500 ms duration fatigue responses with whole muscle attached to the globe. (a) 114.9 mg twitch response. (b) Tetanic traces from bottom to top are at 120, 130, 140 and 150 Hz with a maximum tetanic tension of 471.4 mg (at 150 Hz). (c) Bottom trace is after 2 min of fatiguing stimulation (500 ms duration/sec at 150 Hz) and top trace is the initial response. Horizontal bar = 5 ms for (a), 50 ms for (b) and 100 ms for (c). Vertical bar = 134.0 mg for (a) and 330.0 mg for (b) and (c).

1978; Spencer et al., 1980). That force (52.8 mg in our example) is then multiplied by the number of lateral rectus motoneurons (404) which innervate fast fatigable units yielding 21.3 g of force. (We have previously shown that fast fatigable units make up 34% of the lateral rectus motoneuron pool (Shall and Goldberg, 1992)). When all the unit types are then added together one can predict what the whole muscle force should be if the units add linearly. This computation is similar to the one used by Burke et al. (1973) to predict whole muscle tension in the medial gastrocnemius muscle. The lateral rectus muscle units 'should' yield 29.0 g of twitch force (Goldberg et al., 1997) rather than the $\cong$ 15.0 g actually seen. We suggested (Goldberg et al., 1997) that the loss of predicted force may, in part, be due to the serial arrangement and branching of muscle fibers in the cat lateral rectus muscle (Katz, 1939; Mayr et al., 1975; Demieville and Partridge, 1980). We now report that the same lower than expected force is seen with tetanic tension as well. The lateral rectus muscle 'should' show 237 g at maximum tetanic tension (Shall and Goldberg, 1992) but only yields $\cong$ 110 g when the abducens nerve is stimulated (Barmack et al., 1971). Indeed, a similar maximum tetanic force (about 100 g) has been observed in the cat medial rectus muscle upon stimulation of the oculomotor nerve (Cooper and Eccles, 1930).

The ability to accurately measure lateral rectus muscle contractile force while it is still attached to the globe will now permit, in future experiments, the correlation of force measures with degree of evoked eye movement in the same animal. Such correlative measures are critical to our understanding of the eye movement control system. Furthermore, it appears clear that the observed twitch and tetanic forces of the lateral rectus muscle, upon stimulation of the sixth nerve, are much lower than would be predicted if one linearly added motor unit tensions. The manner in which this non-linear force modification is managed by the eye movement system also needs to be explored further.

Acknowledgements

This work was supported by National Eye Institute Grant EY11249.

References

Barmack NH, Bell CC, Rence BG. Tension and rate of tension development during isometric responses of extraocular muscle. J Neurophysiol 1971;34:1072–9.

Binder MD, Heckman CJ, Powers RK. The physiological control of motoneuron activity. In: Rowell LB, Shepherd JT, editors. Handbook of Physiology. Section 12, Exercise: Regulation and Integration of Multiple Systems. American Physiological Society, New York, 1996:3–53.

Burke RE. Motor units: anatomy, physiology and functional organization. In: Brooks VB, editor. Handbook of Physiology. The Nervous System. Motor Control. American Physiological Society, Bethesda, MD, 1981:345–422.

Burke RE. Motor unit types: some history and unsettled issues. In: Binder MD, Mendell LM, editors. The Segmental Motor System. Oxford University Press, New York, 1990:207–221.

Burke RE, Levine DN, Tsairis P, Zajac FE. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. J Physiol 1973;234:723–48.

Cooper S, Eccles JC. The isometric responses of mammalian muscles. J Physiol 1930;69:377–85.

Crandall WF, Goldberg SJ, Wilson JS, McClung JR. Muscle units divided among retractor bulbi muscle slips and between lateral rectus and retractor bulbi muscles in cat. Exp Neurol 1981;71:251–60.

Goldberg SJ. Mechanical properties of extraocular motor units. In: Binder MD, Mendell LM, editors. The Segmental Motor System. Oxford University Press, New York, 1990:222–238.

Goldberg SJ, Clamann HP. Effect of microelectrode impalement on the reflex discharge of motoneurons. Exp Neurol 1977;57:451–8.

Goldberg SJ, Wilson KE, Shall MS. Summation of motor unit tensions in the lateral rectus muscle of the cat. Muscle Nerve, 1997;20:1229–35.

Demieville HN, Partridge LD. Probability of peripheral interaction between motor units and implications for motor control. Am J Physiol 1980;238:R119–37.

Katz B. The relation between force and speed in muscular contraction. J Physiol 1939;96:45–64.

Lennerstrand G. Mechanical studies on the retractor bulbi muscle and its motor units in the cat. J Physiol 1974;236:43–55.

Loeb GE, Richmond FJR. Architectural features of multiarticular muscles. Hum Mov Sci 1994;13:545–56.

Mayr R, Gottschall J, Gruber H, Neuhuber W. Internal structure of cat extraocular muscle. Anat Embryol 1975;148:25–34.

Meredith MA, McClung JR, Goldberg SJ. Retractor bulbi muscle responses to oculomotor nerve and nucleus stimulation in the cat. Brain Res 1981;211:427–32.

Miller JM, Robins D. Extraocular muscle forces in alert monkey. Vis Res 1992;32:1099–113.

Shall MS, Goldberg SJ. Extraocular motor units: type classification and motoneuron stimulation frequency-muscle unit force relationships. Brain Res 1992;587:291–300.

Shall MS, Goldberg SJ. Lateral rectus EMG and contractile responses elicited by cat abducens motoneurons. Muscle Nerve 1995;18:948–55.

Shall MS, Wilson KE, Goldberg SJ. Extraocular motoneuron stimulation frequency effects on motor unit tension in cat. Acta Anat 1996;157:217–25.

Spencer RF, Porter JD. Structural organization of the extraocular muscles. In: Buttner-Ennever JA, editor. Neuroanatomy of the Oculomotor System. Elsevier, Amsterdam, 1988:33–79.

Spencer RF, Baker R, McCrea RA. Localization and morphology of cat retractor bulbi motoneurons. J Neurophysiol 1980;43:754–70.

Steiger HJ, Buttner-Ennever JA. Relationship between motoneurons and internuclear neurons in the abducens nucleus: a double retrograde tracer study in the cat. Brain Res 1978;148:181–8.