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A multiple-contact EMG recording array for mapping single muscle unit territories

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The glycogen-depletion technique has become a well-established method for determining histologically the cross-sectional distribution of a single muscle unit. A major drawback of this method is its low yield of one depleted unit per experiment. Furthermore, this technique is particularly unsuited for determining the longitudinal distribution of single muscle units in long, broad muscles because of the formidable serial sectioning job that would be required. Our alternative, electrophysiological method utilizes a multiple-contact, two-dimensional EMG recording array to map efficiently the cross-sectional and longitudinal distributions of numerous single muscle units in anatomically diverse muscles. Additionally, architectural information on muscle fiber lengths, end-plate locations, motor subunit (MSU) arrangements and muscle conduction velocities can be determined.

Introduction

The muscle territory occupied by the fibers of a single muscle unit varies widely from muscle to muscle. For example, in the unipinnate medial gastrocnemius and soleus muscles, single unit distribution extends over approximately 25–50% of the muscle cross-section (Burke and Tsairis, 1973; Burke et al., 1974). In contrast, electrical stimulation of either a single motor unit or a discrete muscular region of the long, parallel-fibered sartorius muscle causes contraction of a narrow column (6 mm) of fibers spanning the length of this broad, sheet-like muscle (Cooper, 1929; Loeb et al., 1986; Pratt and Chanaud, 1986). Such restricted muscle unit territories may provide the anatomical/architectural basis for the selective neural activation of functionally homogeneous regions of kinematically complex muscles (Loeb et al., 1983; Chanaud et al., 1986; Loeb et al., 1986). In order to facilitate mapping the distribution of single muscle unit territories, we have developed a novel electrophys-

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iological technique, utilizing a two-dimensional EMG recording array. This technique offers several distinctive benefits over the tedious and low-yielding glycogen-depletion method: (1) architectural and physiological data can be generated on muscle fiber lengths, fiber and motor end-plate distributions, muscle activation latency and conduction velocity (CV); (2) the relationship between fiber length and muscle length can be examined while varying muscle length; (3) numerous units within one muscle can be analyzed in a single preparation; and (4) the technique can be adapted readily to muscles of diverse morphometries.

Materials and Methods

Surgery and stimulation paradigm

Using acute surgical techniques under pentobarbitol anesthesia, the cat hindlimb was dissected to expose the target muscle and free it from surrounding connective tissue, while leaving its innervation and blood supply intact. To reduce movement artifact and possible sources of EMG cross-talk, the innervation to nearby muscles was severed. The EMG recording array was carefully inserted underneath the muscle and bathed in a radiantly heated mineral oil pool (35–37 °C). Appropriate lumbar and/or sacral ventral roots, accessed via laminectomy, were cut proximally and divided into filaments. The distal ends of the fine filaments, placed on hook electrodes within a heated mineral oil pool, were stimulated with constant current (2 × threshold), low frequency (1–10 pps, to prevent fatigue) and biphasic pulses (0.1 ms phase duration).

Two criteria were used to verify that the filament contained a single axon innervating the muscle of interest: (1) the presence of an all-or-none EMG as stimulus strength was varied widely and (2) the constancy of the EMG waveform when the stimulus was set to a threshold strength at which the unit fired approximately 50% of the time. If more than one unit was present, the waveform changed shape from stimulus to stimulus as one of the units fired intermittently.

EMG recording technique

EMGs are recorded using custom-built, flexible printed circuits (Flex-Link Products, San Fernando, CA). Each circuit (Fig. 1) contains a row of 40 electrode contacts, photolithographically defined and etched on a flexible circuit substrate (Kapton polyimide). The copper conductors are insulated with polyimide, except for the exposed gold-plated electrode contacts (3 mm in length, 0.5 mm in width and spaced 3 mm apart; Fig. 1, inset). Thirty-nine serial pairs of bipolar EMG recording sites are available for recording longitudinally from the muscle (electrode array should be aligned parallel to fiber direction). The physical flexibility of this 0.5 mm thick circuit allows it to conform to the shape of the muscle, facilitating uniform electrical contact and minimizing the possibility of damaging muscle or nerve. Several such circuits can be placed side-by-side to form a two-dimensional array (minimal transverse spacing is 6–7 mm center-to-center), for recording EMG along both the longitudinal and transverse axes of long, broad muscles.

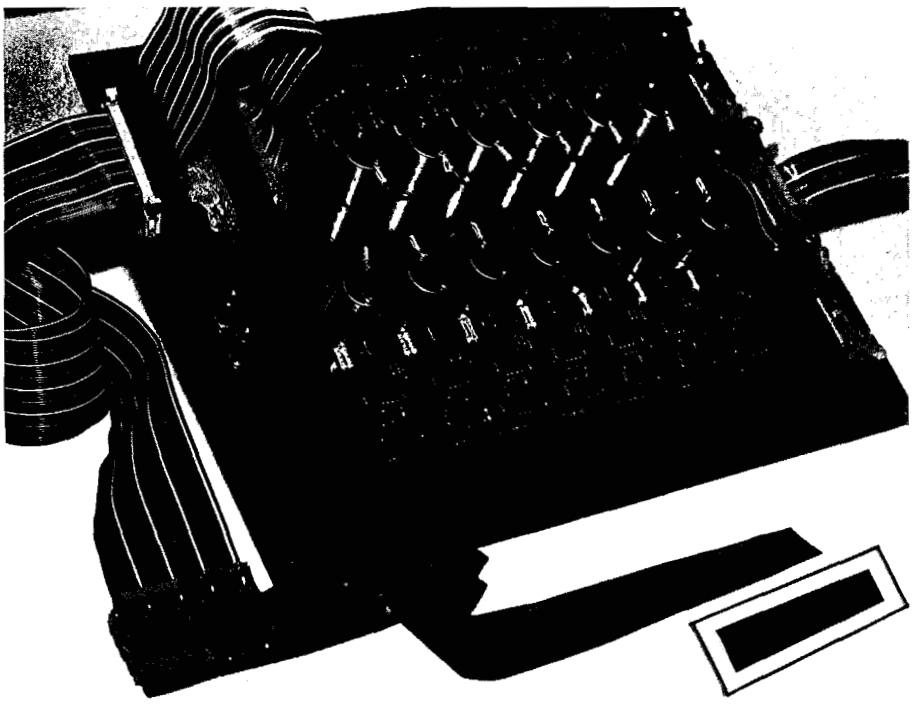


Fig. 1. Two-dimensional EMG recording array and 13-channel amplification circuit board. Several arrays (bottom), placed side-by-side, form the recording array (note curl to demonstrate flexibility). Inset (lower right) shows gold-plated electrode contacts and 4-mil copper conducting lines. Single arrays are connected to amplification board via a flexible ribbon cable and 40-pin dual connector (upper left). The adjacent jumper uses a 40-pin strip connector that can be shifted successively by one pin position to select a particular 13 pair subset of the 39 possible inputs to the differential amplifiers. Three sets of amplifier outputs are available (connectors on the right edge) providing fixed gains of 500, 2000 and 8000, respectively.

A 13-channel amplifier (Fig. 1), custom-built on a printed circuit board, is equipped with a special connector arrangement that allows for rapid selection of 13 of the total 39 pairs of electrodes to be sampled simultaneously. Each amplifier channel incorporates an impedance-matching transformer input (Stein et al., 1977) to a high common-mode rejection differential circuit with 50–5000 Hz bandwidth and total gain of 500, 2000 or 8000. Each channel is digitized sequentially at 10 kilosamples/s using an on-line PDP-11/23 computer and a data acquisition program written in PASCAL. All 39 recording sites for each array can be displayed simultaneously for quick visual inspection.

Data analysis

Fig. 2A shows the polyphasic EMG traces produced by stimulation of a single motor unit and recorded at 3 mm intervals along a 3.3 cm length of the tenuissimus

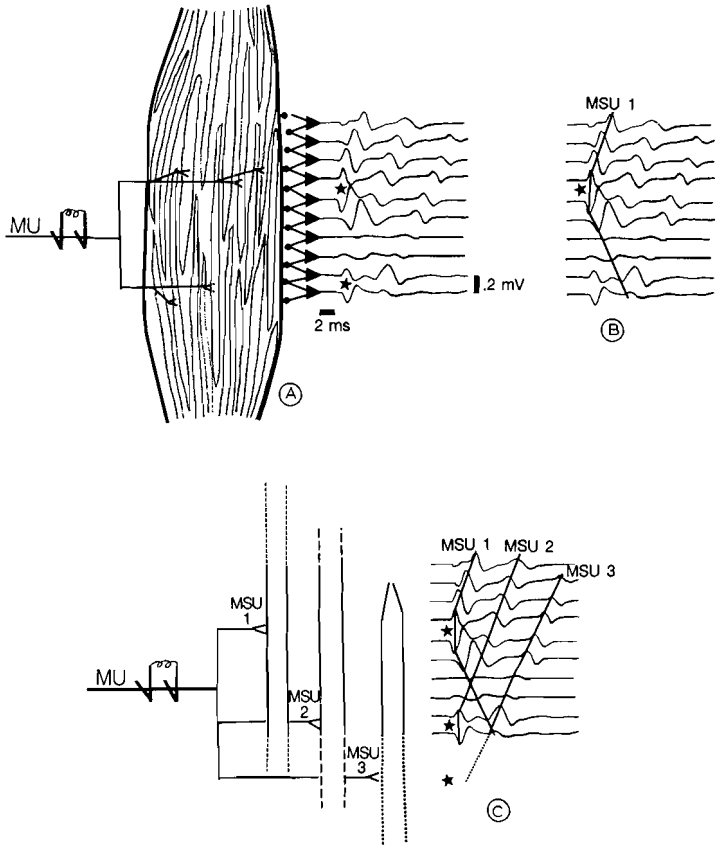


Fig. 2. A: on the right, EMG recordings from tenuissimus following stimulation of a single motor unit. End-plates (stars) are indicated by short latency waveforms and polarity reversals. Each end-plate region actually consists of a cluster of end-plates innervating a group of fibers. (The muscle apparently was making poor contact with two electrodes — note smaller amplitude in 3rd and 4th traces from bottom.) B: the biphasic potentials travelling away from an end-plate, and forming a motor subunit (MSU), are connected by a line. C: three end-plates and their MSUs are labelled on the right. The schematic map on the left shows relative end-plate placements and MSU lengths (each fiber represents a group of fibers).

muscle. The regular spacing between traces is proportional to the spacing between recording electrodes, providing a temporospatial map of the spread of activity along the muscle.

Each complete set of EMG records can be analyzed for the following information:

(1) Topographic distribution of the muscle unit within the muscle.

(2) Location and number of end-plates and inter-end-plate distances. Motor end-plates (stars in Fig. 2A) can be inferred from the sites with the shortest latency waveforms and a polarity reversal in the two waveforms recorded from adjacent recording sites. The polarity reversal occurs because, within individual fibers, the postsynaptic action potential propagates in opposite directions.

(3) Number of motor subunits (MSU), MSU arrangements within the overall muscle unit, and MSU lengths and overlap. The biphasic potential produced by a group of fibers innervated by an end-plate cluster (as defined by a single innervation band shown by acetylcholinesterase staining) can be followed from trace to trace with the latency increasing as the distance from the end-plate increases (Fig. 2B). Note that the biphasic potential decreases in amplitude and increases in duration as the potential travels away from the end-plate. In tenuissimus, two likely explanations for this change in shape and duration of the waveform are: (1) that the range of CV in the muscle fibers increases as some fibers taper to an end-point (Loeb et al., 1986) increasing the temporal dispersion of summed action potentials and (2) as fibers terminate, the summed potential distally decreases in amplitude.

An end-plate cluster and the fibers producing the associated set of peaks is referred to as a motor subunit (MSU), not to be confused with the concept of motor subunit used by Rosenfalck and Buchthal (1970) to interpret microelectrode recordings. (For a discussion of the muscle unit action potential as the sum of individual fiber action potentials, see Wani and Guha, 1980.) MSU length is determined by measuring the distance between the longest latency positive-leading peak and the longest latency negative-leading peak. This length represents the upper limit for lengths of individual fibers within the motor subunit. Presumably the motor subunits are composed of multiple fibers innervated by the same end-plate cluster, thus actual single fiber lengths cannot be determined by this method.

(4) Conduction velocities of muscle fiber action potentials. The slope of the line connecting the waveform peaks equals conduction velocity (Fig. 2B).

(5) Measurements from (2) and (3) are used to draw schematic muscle unit architectural maps, as shown in Fig. 2C. The three end-plate clusters and their motor subunits are labelled on the right of Fig. 2C. The schematic on the left shows the inferred relationship between the muscle unit, end-plates and fibers (each fiber in the diagram actually represents a group of fibers).

Results

Fig. 3 shows a set of EMG traces recorded from a tenuissimus muscle unit. All 27 single unit recordings demonstrated that tenuissimus muscle units extended the entire 12–14 cm length of the muscle. End-plates within a single unit were spaced at regular intervals, 0.6–1.2 cm, in agreement with acetylcholinesterase staining patterns (Loeb et al., 1986). Motor subunit lengths were short, 2.4–7.4 cm, relative to muscle length, 12–14 cm. These motor subunit lengths are consistent with the reported values of tenuissimus single fiber lengths (Adrian, 1925; Loeb et al., 1986). Tenuissimus motor subunits appeared to be highly overlapping, suggesting that individual fibers are not situated end-to-end, but are interdigitated along the length of the muscle. Muscle fiber conduction velocity, 2.5–3.5 m/s, was slow relative to alpha motor axons, 60–90 m/s, in agreement with other reports (Buchtal et al., 1955; Lennerstrand, 1974; Emerson and Zahalak, 1981; Sadoyama et al., 1985; Jones et al., 1985). Similar EMG records (Fig. 4) produced by stimulation of a

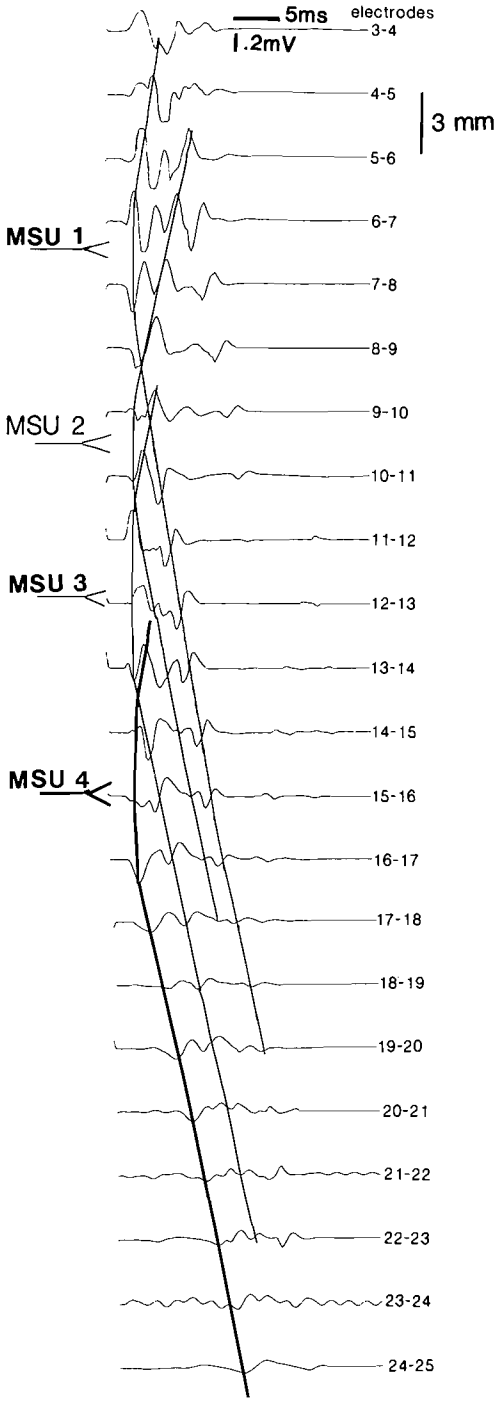
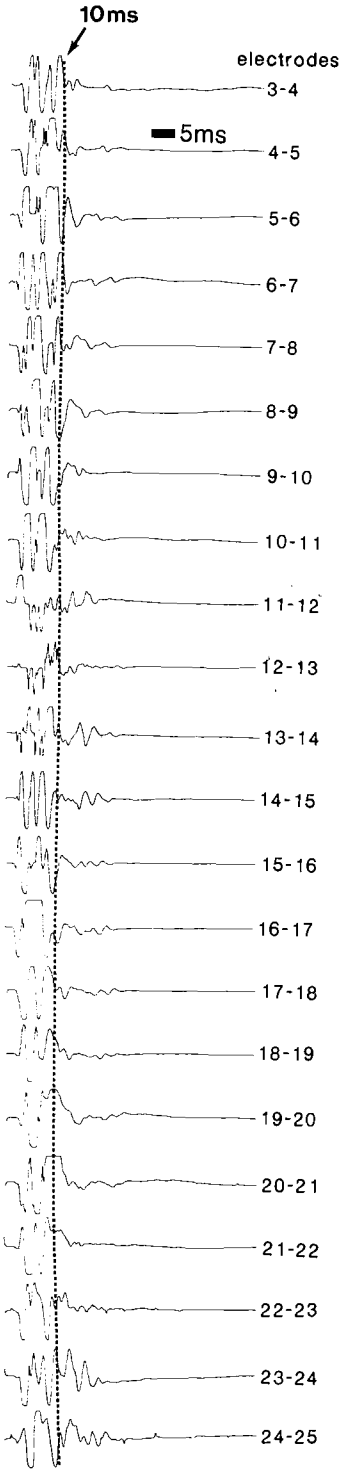

5ms
1.2mV
electrodes
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
17-18
18-19
19-20
20-21
21-22
22-23
23-24
24-25
3 mm
MSU 1
MSU 2
MSU 3
MSU 4

Fig. 3


10ms
electrodes
3-4
5ms
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
17-18
18-19
19-20
20-21
21-22
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23-24
24-25

Fig. 4

ventral root containing approximately half of the 20 alpha motor axons innervating tenuissimus (Boyd and Davey, 1960), suggested that 80–90% of the muscle can be activated at a short latency (< 10 ms) following motoneuron firing.

Discussion

The short fibers of the long, parallel-fibered tenuissimus muscle are arranged from origin to insertion in a highly regular, interdigitated fashion. This pattern of organization may serve to solve the problem of activating this very long muscle in a relatively brief time. Muscle fiber activation time equals conduction velocity (CV) of the fiber action potential multiplied by fiber length. Assuming CV = 3 m/s and innervation at the fiber midpoint, a fiber spanning the 14 cm length of the muscle would require 24 ms to complete activation, which would probably result in the central muscle belly contracting against the still-inactive ends. However, the shorter fiber lengths found in tenuissimus require just 8–12 ms for electrical activation. These values are less than the latencies to peak tension development measured in tenuissimus (Lev-Tov et al., 1984). The interdigitated arrangement of short fibers found in this muscle provides a mechanism for both synchronous electrical activation of the whole muscle and uniform contraction and force transmission from the tendon of origin to the tendon of insertion.

Several studies have mapped single or multiple unit distributions by recording EMG with a roving ball electrode from the exposed surface of the muscle (Lennerstrand, 1974; Brink et al., 1981; Loeb et al., 1986). Both the surface ball electrode method and the method described in this paper lose some resolution for units located deep within a thick muscle. However, the multiple-contact EMG recording array provides a unique and efficient electrophysiological method for mapping the distribution of large numbers of muscle units in a single experiment. Experimental results produced by this method are readily compared to findings of traditional techniques such as glycogen-depletion, motor end-plate staining and single fiber dissection. Linear electrode arrays developed by other investigators (Lynn, 1979; Emerson and Zahalak, 1981; Sadoyama et al., 1985) have been used solely for the purpose of recording surface EMG in humans to measure muscle conduction velocity. An added feature of our method is the detailed architectural and functional anatomical information supplied regarding the temporospatial development of activation in the whole muscle unit.

Fig. 3. EMG recordings from a 7.5 cm length of tenuissimus following stimulation of a single motor unit in a L_7 ventral root filament, showing that the muscle unit extends the full recorded length. Individual MSUs are labelled. Note the regular spacing between end-plates and the overlap of MSUs, indicating a high degree of fiber interdigitation.

Fig. 4. EMG recordings from tenuissimus following stimulation of the whole S_1 ventral root (square peaks indicate saturation of the amplifier). Note that most electrical activity occurs in <10 ms (dotted line).

In future experiments, two-dimensional EMG recording arrays will be applied to the more complex, transversely compartmentalized muscles of the cat hindlimb. We expect these studies to further our understanding of the relationship between fiber architecture and muscle mechanics, and to elucidate the muscle unit organization which parallels the capacity of the CNS for selective recruitment of restricted regions within a single muscle.

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