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INTRAMUSCULAR AND EPIMUSCULAR MICROSTIMULATION OF SINGLE MOTOR UNITS

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SUMMARY

A new epimuscular method for stimulating single motor units (m.u.'s) in free prepared muscle is described. Unit isolation is stable and can be continued for long periods. The method is compared with an intramuscular stimulation technique and histological evidence is presented to confirm the validity of both techniques.

Conventional methods for stimulation of individual motor-units (m.u.'s), i.e. stimulation of α-motoneuron or isolated ventral roots, are time-consuming and technically demanding. Moreover such methods are not suitable for human muscles. Buchthal et al. [1] stimulated in human muscle 'bundles of muscle fibres' with intramuscular electrodes. Several investigators [7,10—12] showed that such intramuscular stimulation may excite fine motor nerve twigs, which may lead to contraction of single m.u.'s.

In this paper we will: (i) describe the difficulties involved in intramuscular stimulation of small muscles (EDL and soleus of rat); (ii) introduce 'epimuscular stimulation' to overcome these difficulties; (iii) provide histological confirmation of the validity of both intra- and epimuscular methods for single unit stimulation; (iv) discuss their limitations. The criterion we used for stimulation of a single m.u. was: perfect all-or-nothing force and EMG responses if the stimulus amplitude is adjusted close to the threshold value (I). Since our experiments demand long term stable stimulation of the same m.u. a second criterion was introduced, i.e. unvarying force and EMG responses when doubling the original threshold stimulus amplitude (II), which

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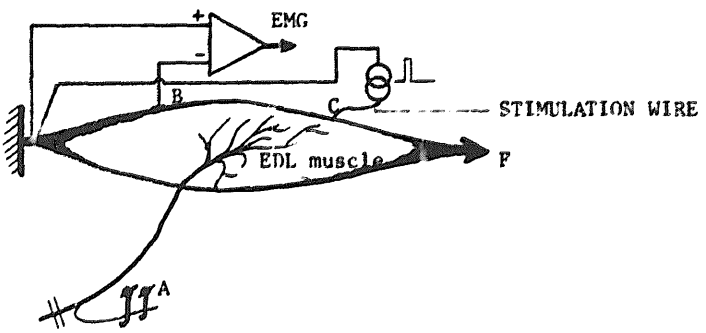
means that the stimulus amplitude was adjusted about 50% above threshold. During an experiment the absolute threshold value for a m.u. can vary within a wide range without changing the response.

When we applied the intramuscular stimulation method to dissected rat muscles, we frequently observed directly activation of muscle fibres. Moreover it took sometimes several hours to find a suitable electrode placement within the muscle, which inevitably caused considerable damage to the muscle [5]. These problems could be eliminated if the muscle was stimulated by flexible wire electrodes (diameter = 25 μm) positioned at a suitable place on the muscle surface (epimuscular microstimulation).

Figure 1 illustrates the experimental arrangements. Figure 2 shows the developed force, EMG and nerve responses under various conditions. When the stimulation wire was placed in the motor endplate region, continuously increasing stimulus amplitudes produced discrete increments of force and EMG (see Fig. 2A). After a few trials such an electrode location could always be found. It is suggested that each increment reflects recruitment of a new



SCHEMATIC DRAWING OF THE EXPERIMENTAL ARRANGEMENTS.

Fig. 1. M. Extensor digitorum longus (EDL) of rat was dissected (blood flow unimpaired, muscle in moist air at 37°C). Sciatic nerve was cut 3 cm from EDL. EDL origin tendon was rigidly fixed. Insertion tendon was cut and attached to isometric force transducer (F). EMG activity on muscle surface was recorded at electrode B (diameter = 200 μm) held in micromanipulator. A: epimuscular stimulation. Stimulus electrode (C) connected to stimulator cathode was fixed to a support. The electrode (Pt-Ir, diameter = 25 μm, length = 2 cm) was Teflon insulated except at tip and was placed under dissecting microscope in suitable position on muscle surface. The electrode, due to its mechanical characteristics, followed the movements of the muscle surface during contraction. Perfect all-or-nothing responses over a wide range of stimulus amplitudes were interpreted to indicate single m.u. activity. Anode electrode was placed on origin tendon but could be transferred to surrounding of stimulation electrode in order to reduce stimulus artefact. B: single m.u. axon stimulation. Cut end of sciatic nerve was immersed in paraffin bath and split under dissecting microscope until a few axons remained on stimulating electrode (A) containing only one functioning EDL single motor axon (criterion all-or-nothing muscle response, force and EMG, remaining unchanged with increase of stimulus amplitude to 100 μA). Same m.u.'s could be stimulated epimuscularly at C (see text) producing antidromic nerve response at A.

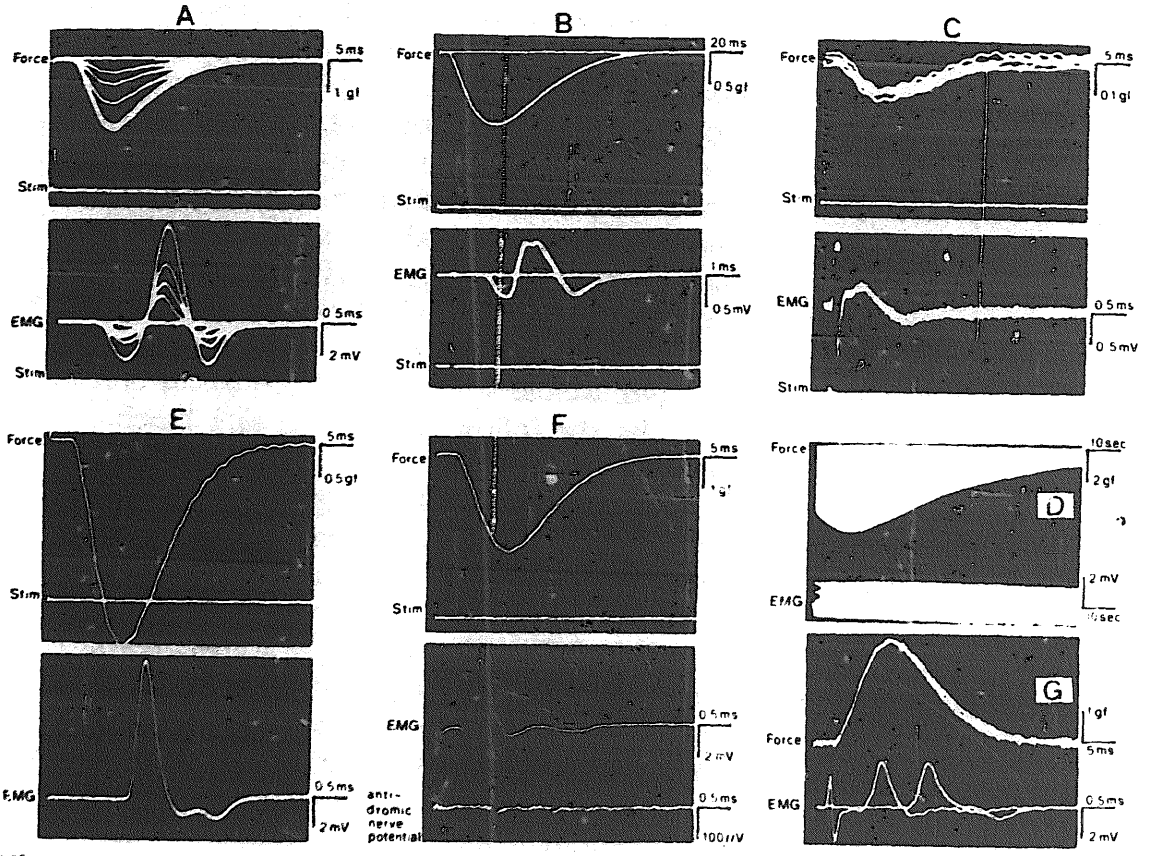


Fig. 2. A: EDL force and EMG responses with stimulus amplitude increased from 0—100 μA, stimulating electrode in endplate region on muscle surface. B: example of stable stimulation of single soleus m.u.'s. All-or-nothing force and EMG responses (I = 16 μA). Identical EMG responses (super-imposed) were obtained with I = 40 μA and I = 16 μA. C: direct stimulation of EDL muscle fibers occasionally observed. D: responses of single EDL m.u.'s to repetitive stimulation, 10 Hz. E: responses of single EDL m.u.'s after stimulation of isolated axon. F: responses of same m.u. as in 2E after epimuscular stimulation. Note antidromic nerve potential. Force calibration in F is twice that in E. G: responses of single m.u. to epimuscular and single axon stimulation. Note coinciding twitches and time delay between almost identical EMG responses. Note also inverse force scale in G (signals replayed from tape).

m.u. A single m.u. could easily be stimulated in a stable way if optimal electrode position was determined using criterion II (Fig. 2B). At the same time it can be ascertained that no muscle fibres are activated directly, on the basis of the time delay between stimulus pulse and the EMG response registered in the immediate vicinity of stimulation electrode (Fig. 2C). If the two criteria are met (Fig. 2B) the same unit can be investigated for several hours and the influence of various external conditions (i.e. muscle length, stimulus pattern) on force and EMG response of the m.u. can be studied. From the findings obtained so far it can be concluded that epimuscular stimulation is simpler, because the criteria are more easily fulfilled and it produces virtually no damage to the muscle.

A problem with all methods of microstimulation and nerve filament

stimulation is to establish their validity and reliability as methods for single unit stimulation. Only in case of intracellular recording and stimulation of motoneurons the method is entirely unequivocal. The physiological characteristics (twitch amplitude, contraction time) of the present sample of EDL m.u.'s stimulated intra- and epimuscularly are in agreement with values obtained by Close [3], using ventral rootlet stimulation. The two methods therefore appeared to be equivalent. Both resulted in a stable stimulation within the range of stimulation frequencies (up to 100 Hz). This clearly resulted from the spring-like construction of the electrode which provided a virtually free moving contact without instability due to muscle movement. The conclusion that intra- and epimuscular stimulation leads to the contraction of single m.u.'s is supported by the following anatomical, histochemical and electrophysiological evidences.

The muscle fibres activated by stimulation of a single motoneuron are spread over a large area and are histochemically uniform [2,6]. The same holds for the muscle fibres of units stimulated intra- and epimuscularly, which was demonstrated as follows. Single EDL m.u.'s were stimulated repetitively at 10 Hz, which caused exhaustion of the m.u. demonstrated by the considerable change in twitch force during the first three minutes (staircase phenomenon and exhaustion, Fig. 2D). Subsequently the muscle was excised, immediately frozen and the exhausted muscle fibres were identified in cross-sections (10 μm) on the basis of glycogen depletion [9]. In serial sections the same fibres were histochemically characterized on the basis of pH-sensitivity of myofibrillar ATPase reactions [8] and their succinate dehydrogenase activity.

Figure 3E shows a typical PAS-stained cross-section of an EDL muscle in which one m.u. was exhausted. Figures 3A—D show a typical part of an m.u. territory and the histochemical characteristics of these fibres.

In all experiments in which histochemical investigations were performed (n = 20), the spatial distribution of the muscle fibres of individual motor units agree with earlier findings [6]. The histochemical characteristics of these fibres were uniform.

If a m.u. is stimulated epi- or intramuscularly, it should be possible to stimulate the same m.u. at the axon or motoneuron level. This is illustrated in Figs. 1B and 2E. A single EDL m.u. appeared to be activated by isolated axon stimulation, since all-or-nothing response occurred during gradual increase of stimulation amplitude from 0—100 μA. The response was usually observed below 5 μA. The region where this m.u. could be stimulated epimuscularly was roughly determined on the basis of a minimal delay in the negative onset of the EMG response recorded by an exploring surface electrode (Fig. 2E). At a suitable place in this region the same m.u. could be activated by epimuscular stimulation (Fig. 2F) as by stimulation of the isolated axon as demonstrated by the antidromic response in the axon, from where the m.u. was previously stimulated. Figure 2C shows two force and two EMG responses from the same m.u. found with the two different

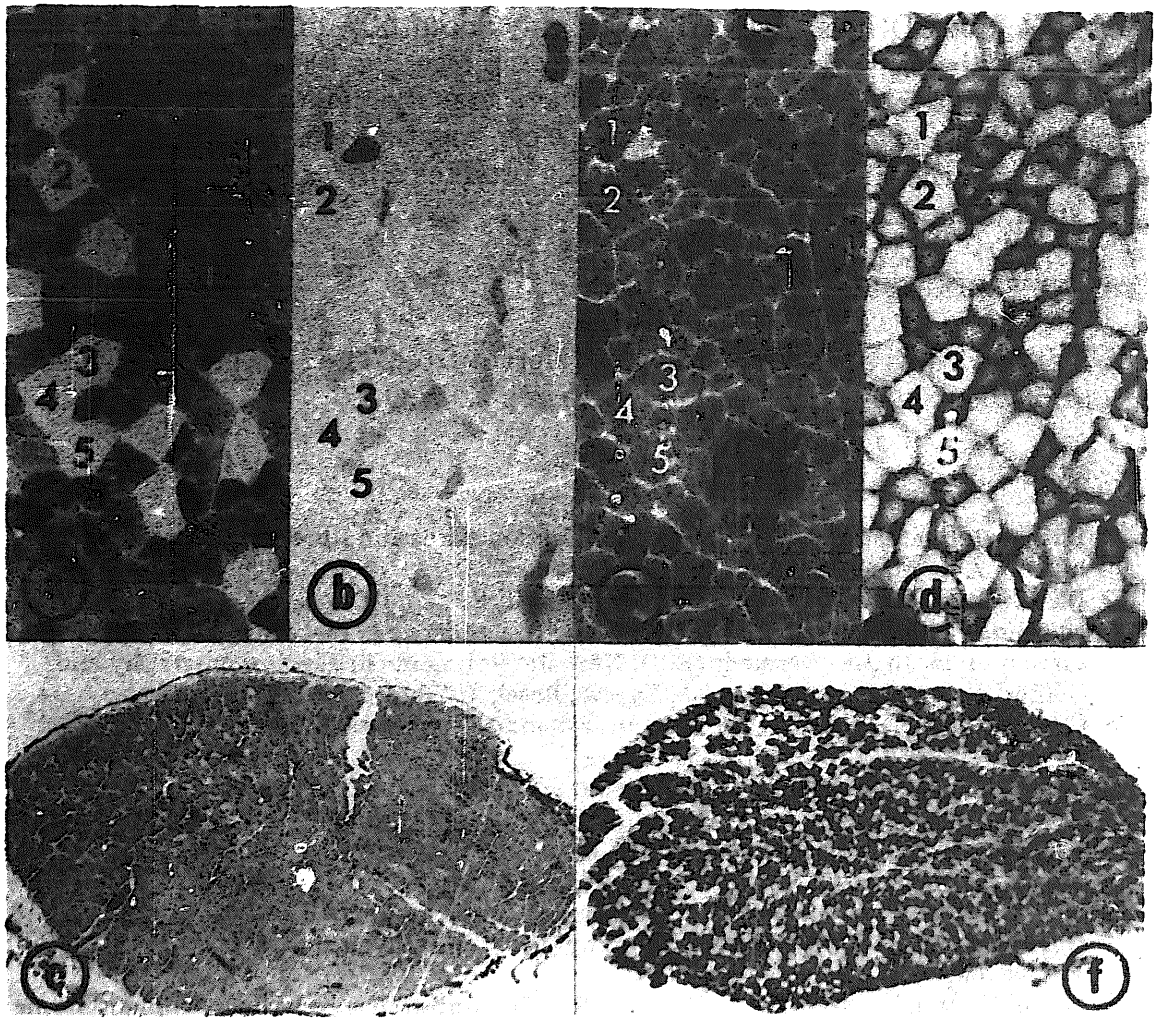


Fig. 3. Histochemical identification of fibres of EDL m.u. White fibres in (a) are glycogen depleted, (b) ATPase (acid stable), (c) ATPase (alkaline stable), (d) SDH. In four serial sections some of the fibres of the same m.u. are marked 1,2,3,4 and 5. (e) Cross-section of EDL muscle, white fibres are glycogen depleted (PAS-reaction). (f) Cross-section of EDL muscle (SDH).

stimulation methods. Note the coinciding twitches. The time delay (0.75 msec) between almost identical EMG responses is in accordance with the propagation velocity and length of the axon. (If the length of the nerve is taken to be 3 cm, the conduction velocity is 40 m/sec). The slight differences in the MUAP-shape may be caused by differences in the two methods with respect to the time synchronization of the endplate activation.

In conclusion we can state that, on the basis of the results presented here and the comparison of our m.u. characteristics with those in the literature [3], epi- and intramuscular stimulation are both valid methods for single m.u. stimulation.

Epi- and intramuscular stimulation appear to favour FF type m.u.'s. In all of the 20 experiments carried out FF m.u.'s were activated (contraction time 9 msec, low endurance, dark staining in myofibrillar ATPase after

pH 10.4, low SDH activity). However, only about 50% of the EDL muscle fibres had these histochemical characteristics (see Fig. 3F). The FF type units are relatively large and may also have large diameter terminal axon branches with low electrical threshold. This, together with the random distribution of the fibres (see Fig. 3F), may produce a low apparent threshold for such units.

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