J. Physiol. (1965), **178**, *pp.* 359–367
With 4 text-figures
Printed in Great Britain

SINGLE MOTOR UNITS OF MAMMALIAN MUSCLE

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(*Received* 13 *October* 1964)

The motor unit ('a single functional entity'; Eccles & Sherrington, 1930) was defined by Sherrington (1925) as 'together with the muscle fibres innervated by the unit, the whole axon of the motoneurone from its hillock in the perikaryon down to its terminals in the muscle'. In this paper the term 'motor unit' will be used in a slightly different context as applying only to the group of muscle fibres innervated by a single motor axon. The size of contraction of such a bundle of muscle fibres has been estimated by several methods. Single axons may be isolated by dissection or by threshold stimulation either of the appropriate ventral root or of the muscle nerve (Krnjević & Miledi, 1958). Electrical stimulation of such a single axon would lead to the production of a muscular contraction limited to the muscle fibres innervated by that particular axon (Denslow & Gutensohn, 1950; Krnjević & Miledi, 1958; Eccles & Iggo, 1961; Norris & Irwin, 1961). Single motor units may also be obtained by weak voluntary movement (Buchthal, Guld & Rosenfalck, 1957) or by reflex stimulation (Porter, 1929; Gordon & Holbourn, 1949; Gordon & Phillips, 1953).

Mean values for the contraction of motor units in a particular muscle have been obtained by dividing the maximum twitch by the number of motor nerve fibres (Eccles & Sherrington, 1930). However, as pointed out by O'Leary, Heinbecker & Bishop (1935), the maximum twitch should be divided by the number of alpha motor fibres rather than all the motor fibres as was originally done.

In the present investigation single motor units were activated by stimulating motoneurones by means of current pulses passed between an intracellular micro-electrode and an indifferent electrode. The impulse so generated in a particular motoneurone propagated down the axon and activated the muscle fibres of the motor unit. In this way it was possible

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to record the mechanical responses of the motor unit and to correlate them with the properties of the motoneurone and its axon.

METHODS

Young cats (3·0–3·2 g) were anaesthetized with sodium pentobarbital (40 mg/kg). In the left leg the following nerves were completely freed from the surrounding connective tissue for about 2 cm but not severed; medial gastrocnemius (MG); plantaris (PL); flexor digitorum longus (FDL) and flexor hallucis longus (FHL) together; the soleus nerve (SOL) was dissected apart from the lateral gastrocnemius nerve branches which were cut leaving only SOL in continuity. Both the posterior biceps and semitendinosus (PBST) and peronei and deep peroneal (PDP) nerves were cut peripherally and prepared for electrical stimulation. Hooks were tied to the tendons of the following muscles (MG, PL, FDL, FHL, SOL) for mechanical recording of muscle twitches (Buller, Eccles & Eccles, 1960). The muscles were freed from the surrounding connective tissue, care being taken that there was minimal interference with the blood supply. A laminectomy was made from S_1 to L_2 and the cord cut in L_2. On the left side the dorsal roots which supply the lumbar 5, 6, 7 and sacral 1 and 2 segments were transected. Micro-electrodes (filled with 3 M-KCl; resistance 5–7 MΩ) were inserted into the motoneurone in the usual manner, i.e. from the dorso-lateral surface of the lumbar sacral cord.

The leg was held rigid by the attachment of clamps to the drills inserted into the distal end of the femur and the distal ends of the tibia and fibula (Buller *et al.* 1960). The clamps and strain gauges were securely bolted on to the animal frame. Full details of the mechanical arrangement have been given already (Buller *et al.* 1960). Unfortunately the temperature range tended to be slightly lower (35–36° C) than that maintained by Buller *et al.* (1960) and by Gordon & Phillips (1953) and hence the twitches were somewhat slower.

Measurements of the contractions of single units were easily achieved with the more sensitive strain gauge (Statham Gl-8-350) whilst the less sensitive gauge (Statham Gl-80-35) was required to record the twitch of the whole muscle. Contractions of single units were recorded at several resting tensions. The criterion for the optimum resting tension was that at which the twitch contraction for the motor unit was maximum (fig. 1 in Buller *et al.* 1960). There is a technical difficulty in that it is difficult to maintain a micro-electrode intracellularly when muscle twitches are recorded. No matter how much care is taken with fixation of the cat spinal cord, a small pressure wave sometimes travels throughout the preparation, dislodging micro-electrodes from cells. It is not a problem easily disposed of since it is essential in locating motor nuclei to apply electrical shocks to the nerves in continuity with the muscles they innervate and thereby to initiate powerful muscle contractions.

The impaled motoneurone was identified either by antidromic invasion on stimulating the nerve or by passing a square pulse through the micro-electrode and observing the motor unit contracting. If the unit belonged to one of the muscles prepared, the conduction velocity of the nerve impulse, the antidromic spike potential and the after-hyperpolarization of the motoneurone were recorded before the muscle was attached to the 8 oz. Statham gauge (Gl–8–350). The motoneurone was then stimulated with square pulses (1 msec in duration) produced by a Tektronix stimulator (161) which was connected to the micro-electrode or, in later experiments, by a Grass stimulator (S 4) with an isolation unit (SIU–4 B). In this way it was possible to correlate some of the properties of the motoneurone itself with the single or tetanic contractions of the unit it innervated.

In the table are assembled the results of all the motor units for which most of the information was obtained both on properties of motoneurones themselves as well as the units they innervated. Passage of pulses of current through an intracellular micro-electrode could prevent entry of the antidromic impulse into a motoneurone so that, in a few instances,

values for the duration of the after-hyperpolarization (AHP) and the conduction velocity (C.V.) were not available. Values for the spike potentials (60–80 mV) are not given as they were often not recorded.

RESULTS

In Fig. 1 the responses are assembled from a typical motor unit belonging to MG. The stimulus artifact in *E* is indicated by an arrow so that the antidromic conduction time from the electrode on the muscle nerve to this particular MG motoneurone was less than 2 msec. The duration of the after-hyperpolarization was about 85 msec and is seen to be superimposed on a small recurrent inhibition when the stimulus strength just straddled the threshold for the axon (Fig. 1*F*).

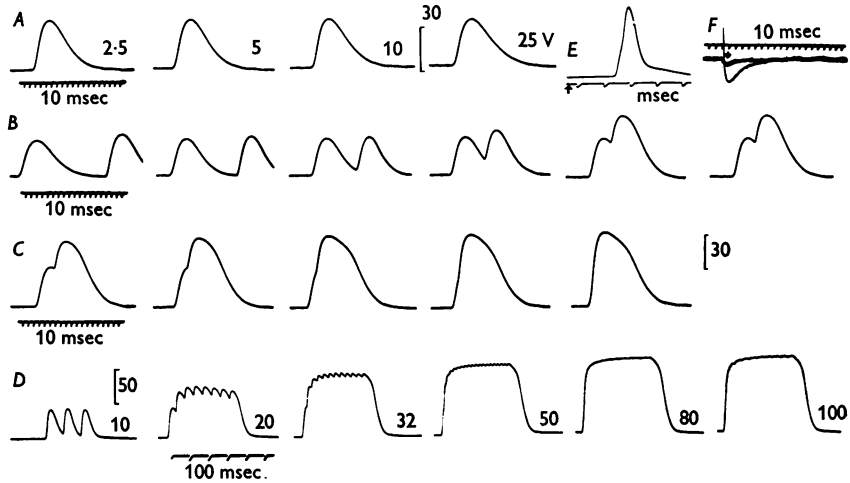

Fig. 1. Records *A–D* give the isometric twitch contraction of a MG motor unit innervated by a motoneurone whose resting potential was −65 mV. The spike potential (no potential scale) is shown in *E*, and after-hyperpolarization (no potential scale) is shown in *F* at the appropriate time scales. Vertical scales are for tension, 30 g for row *A*; another 30 g for *B* and *C*; and 50 g for *D*. In *A* the responses were recorded to single square pulses of different voltages; figures adjacent to records refer to stimulus strengths in volts. In *B* and *C* the responses to double stimuli are shown. In *D* are the contractions of the unit to repetitive stimulation (from 10 to 100 c/s). Unless otherwise indicated, each row has its own time scale.

Row *A* illustrates that an increase in the voltage driving the square pulse applied through the micro-electrode from 2·5 to 25 V does not alter the twitch height, about 30 g, evoked by the square pulse. Below 2·5 V no muscular response could be recorded. Therefore it may be said that the motoneurone behaves in an all-or-nothing manner. It is either stimulated or not and a tenfold increase in the stimulus does not lead to the recruitment of other units owing to a spread of current to nearby motoneurones.

Similar square current pulses applied through micro-electrodes lying extracellularly to motoneurones or even in motoneurones of low resting potentials (20 mV or less) produced no muscular contraction and hence must have failed to stimulate motoneurones. Occasionally, if very strong current pulses were employed, a sudden increase in twitch height was recorded. This seemed to be due to two units but the strength of current required was many times that required for the unit under observation. Therefore it can be concluded that, when stimulating with intracellular electrodes at a stimulus strength just above threshold, there is little chance that adjacent motoneurones will be excited. In Fig. 1*B* and *C* two square pulses were applied at various intervals to the intracellular electrode. It will be noted that, when the stimuli were close together, the motor unit responded with

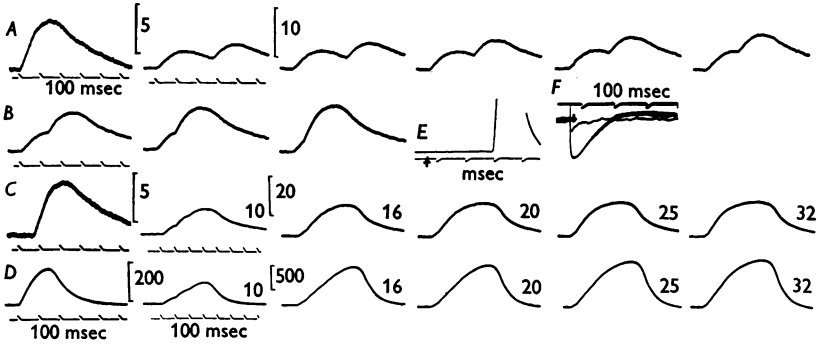

Fig. 2. Records of a motor unit innervated by a soleus motoneurone (R.P. = −60 mV) whose conduction velocity and after-hyperpolarization are shown in *E* and *F* respectively. The stimulus artifact is indicated by an arrow in *E*. The first record in *A* is the response to a single square pulse (5 g scale); in the other records in *A* and *B* two square pulses (at different intervals) were applied to the soleus motoneurone (note the different gain, 10 g). In *C*, at a slightly higher resting tension, the twitch was repeated at 5 g gain and then repetitive stimuli were applied at the frequencies indicated (10–32 c/s) and recorded on the 20 g scale. In *D*, the twitch (200 g scale) and the repetitive responses (500 g scale) were recorded for the whole soleus muscle about 1 hr later.

a twitch twice the height of a single one. Finally, in Fig. 1*D* the responses of the motor unit are shown to the application of repetitive pulses to the motoneurone. The maximum tension was reached by the third or fourth impulse, and at higher frequencies (32/sec to 100/sec) this plateau was maintained throughout the tetanus. The responses closely resemble those obtained from the whole muscle (Buller *et al.* 1960).

A soleus motor unit illustrates a typical response from this muscle. In Fig. 2*E* the antidromic impulse in the axon required about half a msec longer to reach the motoneurone than that in the MG axon illustrated in Fig. 1*E*. The after-hyperpolarization was 180 msec in duration (Fig. 2*F*).

The maximum twitch tension of the motor unit itself was smaller, being only about 5 g. In the remainder of row *A* and *B* are a series of the muscular responses following the application of double volleys to the motoneurone. It is obvious that these twitches become fused even when an interval of 50 msec separates the two pulses, whereas an interval of 10 msec or less is required for fusion of the faster motor unit (Fig. 1). In Fig. 2*C* at a slightly higher initial resting tension the twitch was again recorded and was followed by a series of responses to repetitive stimulation that were recorded at lower amplification. In *D* the results of stimulating the whole of the soleus nerve and recording the muscular contraction of the soleus muscle are shown to a single volley on the 200 g scale, and repetitively on the 500 g scale. Comparison between *C* and *D* shows that the characteristics of the soleus motor unit resemble those of the soleus

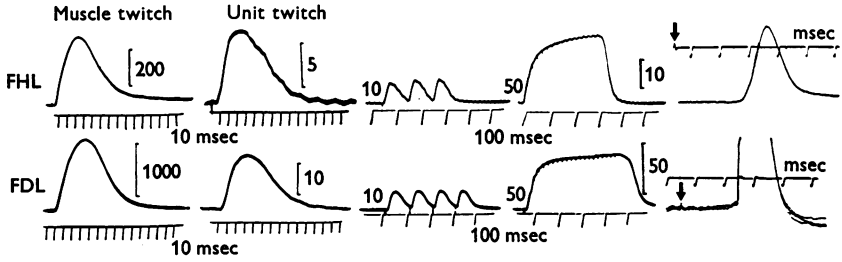

Fig. 3. The responses of FHL muscle, the FHL unit (upper row) are compared with the responses of FDL muscle and FDL unit. The muscle twitches are shown with their appropriate scales 200 g and 1 kg. The twitch response of a single FHL unit at 5 g; an FDL unit at 10 g. The repetitive responses of the unit at 10 c/s and 50 c/s were at lower gains, 10 and 50 g respectively. The final records indicate the latency of the axon supplying the motoneurone. Appropriate time scales accompany all responses.

muscle. The contraction times and half relaxation times for the muscle are of the same magnitude, approximately 150 msec (Fig. 2*C*, *D*), compared to the 30 msec required for the fast muscle motor unit (Fig. 1) or the fast muscles themselves (Buller *et al.* 1960; Buller, 1963). A ripple was still discernible on the traces at a frequency of 16 c/s, whereas at 20 c/s fusion had occurred (Fig. 2*C*, *D*). In the MG motor unit, on the other hand, fusion of the individual responses required a frequency as high as 80 c/s.

In Fig. 3 the responses of a typical unit of flexor hallucis longus (FHL) are compared with the responses of a typical flexor digitorum longus unit (FDL). Both muscles are toe extensors and belong to the category of fast muscles with contraction times about 30 msec and relaxation times of about 20 msec (Buller *et al.* 1960). However, the FHL muscle is only about

a quarter the size in weight of the FDL muscle and the muscle twitches show this marked difference (e.g. the twitches given in Fig. 3). Similarly, the twitches of the single motor unit reflect these differences, the twitch of this FHL unit being 8·35 g, whereas the twitch response of the FDL unit was 18·0 g. There is little difference in the time to peak, i.e. contraction time is 29·0 msec for FHL, 30 msec for FDL; and the half relaxation times are 30·4 msec FHL and 31·2 msec FDL. The repetitive responses show similar rates required for fusion (50 c/s). The time of conduction for both units (taken in two animals of the same weight and length) are approximately the same. Here then are two muscles, both fast, lying side by side and with the same function yet the twitches of the FHL units were always small (average 6·59 g from the table) and the twitches of FDL always large (17·35 g from the table).

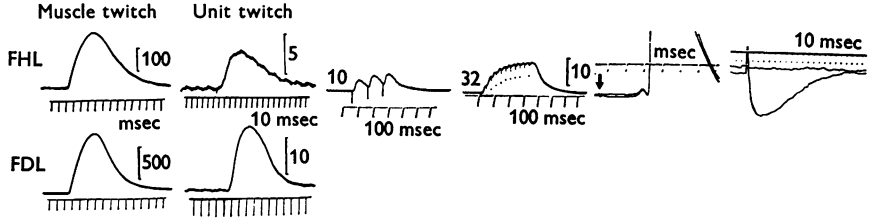

Fig. 4. The responses of FHL muscle and an FHL unit of an experiment other than Fig. 3 are recorded in the upper row. The muscle twitch at 100 g is compared to the twitch response of a unit at 5 g, and the repetitive responses of the unit at 10 c/s and 32 c/s on the 10 g scale. The final records are of latency and after-hyperpolarization (no potential scales being given for these). All records have their own time scales. Arrows on both Figs. 3 and Fig. 4 mark the stimulus artifact. Below are the twitch response for the FDL muscle and a typical FDL motor unit in the same animal. Vertical scale indicates tension in grams.

In Fig. 4 the responses of a slight variant to the rule that FHL is fast is illustrated. The whole muscle in this particular cat was slower than normal and this is reflected in the contraction time, relaxation time and lower frequency for fusion of an FHL unit. When the properties of this motoneurone were examined it was noticed that the spike required nearly 1 msec more than usual for conduction. The small potential before the spike seemed to be due to the fields generated by the nearby faster-conducting FDL motor axons in the ventral horn. The after-hyperpolarization of 190 msec was well outside the range for all other FHL units and as long as the average soleus motoneurone. Unfortunately only one unit was successfully recorded from in spite of a diligent search with the micro-electrode in the FHL–FDL motor nucleus.

DISCUSSION

This present study has been limited to muscles which are relatively pure, i.e. either fast like MG or slow like soleus; there seems to have been no admixture as reported for the cat tibialis anterior muscle (Gordon & Holbourn, 1949) with fast units superficial (mean contraction time of 28·9 msec) and slow units deeper (mean contraction time of 62·6 msec).

In Table 1 the responses from the successfully impaled motoneurones are grouped according to the muscle innervated. The eight soleus motor units have smaller twitch and tetanic tensions and longer contraction times (CT) and half-relaxation times ($\frac{1}{2}$RT) than any of the units of the fast muscles, MG, PL, FDL and FHL. The CT's and $\frac{1}{2}$RT's of soleus are linked with the lower fusion frequency, 20 c/s, compared to the 64–80 c/s required for faster muscles. Similarly, the soleus motoneurones have longer after-hyperpolarizations (mean = 167·7 msec) and slower conducting axons (mean = 73 m/sec) than the other motoneurones (Eccles, Eccles & Lundberg, 1958).

TABLE 1. Summary of the results on motor units

Motor units	RP	AHP	C.V.	Twitch tension	CT	$\frac{1}{2}$RT	Tetanic tension	T_e/T_w	Frequency of fusion
MG (11)	57	97	96	40·1 (13·6–59)	37·0	38·0	79·6 (57–103)	2·41	64
SOL (8)	53	167·5	73·0	3·26 (0·8–5·8)	96·9	107·1	14·3 (9–24·4)	4·97	16
PL (15)	64·7	96	98	21·8 (6·7–39)	26·3	24·3	79·3 (20–110)	4·00	64
FHL (9)	—	112	—	6·59 (3·4–9)	29·6	32·4	23·8 (12–36)	4·14	64
FDL (29)	63	93	—	17·35 (11–26)	29·7	23·7	54·85 (27–85)	3·27	64

Each column gives the means of different properties of the motoneurones (first three columns) or the motor units they innervate (last six columns). The columns show the following measurements: the resting potential of the motoneurones in mV (RP); the duration of the after-hyperpolarization (AHP) in msec; the conduction velocity (CV) of the axons in m/sec; the maximum isometric twitch tension in g; the contraction time (CT) in msec; the half-relaxation time ($\frac{1}{2}$RT) in msec; the maximum isometric tetanic tension in g; the ratio of tetanus to twitch; the approximate frequency of repetitive stimulation required for fusion in c/s. The numbers of units investigated for twitches are in parentheses; some of these did not have records of the motoneuronal properties taken. The range of values for both twitch and tetanic tensions are given in parentheses below the mean value.

Few values exist in the literature for the comparison of twitch tension of single units. The twitch tensions for the mean motor unit for medial gastrocnemius and soleus were given by Eccles & Sherrington (1930). These were derived by dividing the maximum twitch tension developed by the whole muscle by the number of motor fibres both alpha and gamma. The soleus value (3·26 g) in Table 1 is about the same size as the earlier

mean value (2·48) when allowance has been made for the gamma motor fibres. However, soleus units were rarely held for more than a few minutes and it is likely that these twitch tensions were not always taken under the optimum conditions. Therefore the mean soleus motor unit contraction may have been slightly higher than the value in Table 1. For MG motor units Eccles & Sherrington (1930) recorded a mean of 6·4 g which is closer to 10 g, after rejecting the gamma fibres from the motor fibre count. This is considerably smaller than 40·1 g given for MG units in Table 1. Twitch tensions from 0·5 to 15·4 g and tetanic tensions from 0·7 to 55·4 g were given for MG units by Wuerker & Henneman (1963). These are small and more variable than those used in the construction of Table 1 where the mean twitch for MG was 40·1 g (range 13·6–59); and the mean tetanic contraction was 79·6 g (range 57·3–103).

The differences between FDL and FHL units were interesting since both act as physiological toe extensors. However the mean twitch and tetanus from FHL were only 6·59 and 23·8 g respectively compared to the mean twitch of 17·35 g and the mean tetanus of 54·85 g for FDL. The values for FDL are not unlike those reported by Hunt & Kuffler (1951) for an FDL motor unit, a twitch of 15 g and a tetanus of 42·5 g. The smallness of the FHL motor unit may be related to control of movement, since a finer control is provided by a large group of small units than by a small group of large units.

This method of investigating motor units by stimulating the motoneurone is laborious but there is little likelihood that two motor units can be stimulated together, which is a slight possibility when dissecting single motor axons in ventral roots (Eccles & Iggo, 1961; Norris & Irwin, 1961) or in the motor nerve (Krnjević & Miledi, 1958). A further advantage is that the properties of the motoneurone and its axon can be correlated with the muscle fibres it innervates. However, this procedure has the disadvantage that it is easier to impale successfully the larger cells, so that the mean value so derived could be weighted in favour of the larger motoneurones, which probably innervate larger motor units. Similarly, isolation of single motor axons would tend to lead to selection of the larger motor axons, which probably innervate the larger motor units (Norris & Irwin, 1961). Mean values for motor units for any muscle must be based on the maximum twitch height of the muscle divided by the number of alpha motor fibres even though this is 'but a rough estimate of value of the prevalent unit' (Eccles & Sherrington, 1930).

SUMMARY

1. Under optimal conditions the isometric twitch and tetanic tensions have been measured for individual motor units of some of the cat's hind

limb muscles. Each motor unit was activated by a stimulus applied through a microelectrode situated intracellularly in its motoneurone.

2. A correlation was obtained showing that units innervated by motoneurones whose spike potentials were followed by after hyperpolarizations less than 100 msec, always had times to peak and times to half-relaxations for the isometric twitch tensions of less than 40 msec.

3. Similarly, motoneurones whose spike potentials were followed by after-hyperpolarizations of 150 msec or more always innervated motor units with times to peak for the twitch tension of more than 80 msec.

4. It is suggested that the motor units of the fast muscle, flexor digitorum hallucis, which have a mean twitch tension of 6·59 g, compared to 17·35 g for flexor digitorum longus, have an important role in the fine control of movement of the hind limb digits.

We wish to thank Professor Sir John Eccles for his encouragement and Mr G. J. Winsbury for help with some of the experiments. The authors also acknowledge Dr R. I. Close's criticisms of the manuscript.

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