In Vivo Studies on Fast and Slow Muscle Fibers in Cat Extraocular Muscles

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ABSTRACT In anesthetized in vivo preparations, responses of two types of extraocular muscle fibers have been studied. The small, multiply innervated slow fibers have been shown to be capable of producing propagated impulses, and thus have been labeled slow multi-innervated twitch fibers. Fast and slow multi-innervated twitch fibers are distinguished by impulse conduction velocities, by ranges of membrane potentials, by amplitudes and frequencies of the miniature end plate potentials, by responses to the intravenous administration of succinylcholine, by the frequency of stimulation required for fused tetanus, and by the velocities of conduction of the nerve fibers innervating each of the muscle fiber types.

INTRODUCTION

Two distinct types of muscle fibers, similar to the twitch and slow fibers of the frog, have recently been identified in mammalian eye muscles, both by histological studies (10, 11) and by physiological studies (11, 15, 18). Matyushkin (16) has labeled the "twitch" fibers "phasic" and the "slow" fibers "tonic." By anodal break studies he has demonstrated that slow fibers are innervated by small nerves, while twitch fibers are innervated by large fibers. The slow fibers are innervated by multiple endings of the *en grappe* type. They are smaller than the twitch fibers, and reveal differences in the disposition of the sarcoplasmic reticulum. During electrical stimulation, propagated impulse activity has been recorded from the twitch fibers, whereas slow fiber responses have been characterized by nonpropagated slow waves of depolarization, termed slow junctional potentials by Hess and Pilar (11). In the cat, slow fibers exist in the 4 recti and 2 obliques (11). Slow fibers were not found in the retractor bulbi muscle (1).

In our studies on intact cats with the eye muscle and nerve maintained under physiological conditions, both types of muscle fibers were shown to be capable of producing overshoot spike activity and propagated impulses. Thus,

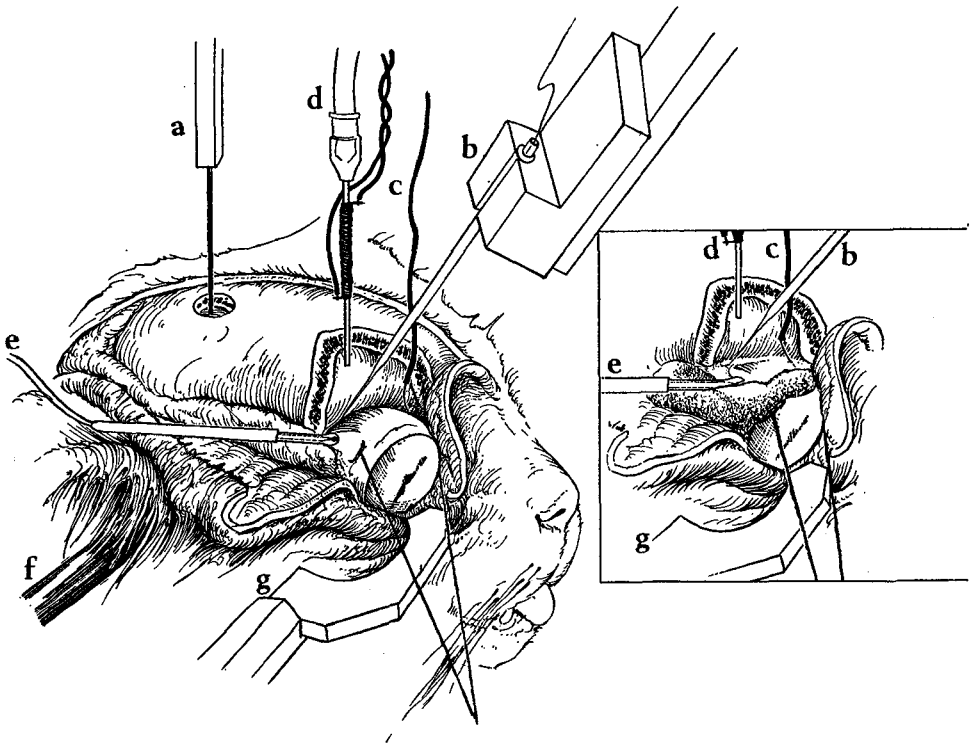

FIGURE 1. An illustration of the preparation. The head of the anesthetized cat is held in the stereotaxic instrument.

A. Superior rectus preparation.

(*a*) stimulating electrode placed stereotaxically in the third nerve nucleus.

(*b*) micropipette held in the arm of the microelectrode driver.

(*c*) indifferent electrode placed in the Ringer solution.

(*d*) Ringer's solution heater; a hypodermic needle wrapped with insulated nichrome resistance wire. The warmed Ringer solution flows drop by drop over the muscle.

(*e*) a thermistor placed in the Ringer bath to monitor the temperature.

(*f*) ear bar and

(*g*) part of the fixation plate of the stereotaxic instrument.

The insert illustrates the preparation as it appears when a wall of cotton is used to form a pool of Ringer's solution over the globe.

B. Inferior oblique preparation.

(*b*) micropipette.

(*c*) indifferent electrode.

(*d*) heater.

(*e*) thermistor.

(*g*) part of the stereotaxic instrument.

(*h*) RCA 5734 strain gauge tube and holder.

(*i*) nictitating membrane held by a suture to form an oil pool.

(*j*) platinum electrodes holding nerve to inferior oblique.

both are twitch fibers. We have labeled the two types "fast" and "slow multi-innervated" twitch fibers. Fast and slow multi-innervated twitch fibers are distinguished by their impulse conduction velocities, their ranges of membrane potentials, their amplitudes and frequencies of the miniature end plate potentials, their responses to the intravenous administration of succinylcho-

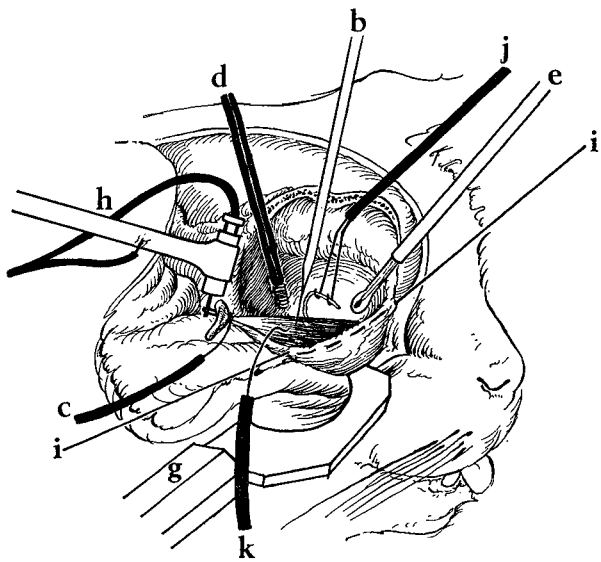

FIG. 1 B

line, by the velocities of conduction of the innervating nerve fibers, and by the frequency of stimulation required to produce fused tetanus of each fiber type.

METHODS

Preparation Eighty-eight cats (2.3 to 3.6 kg) anesthetized with Nembutal were included in the present study. The cats were mounted in a visual model stereotaxic apparatus. The cat's body temperature was maintained at 37–38°C by means of an abdominal heating pad.

Two preparations were used:

1. SUPERIOR RECTUS MUSCLE PREPARATION (Fig. 1 A) The soft tissues and bone over the orbit were removed, and the globe exposed. Needles were placed in the sclera anterior and to either side of the insertion of the superior rectus muscle to act as binding posts for silk thread sutures. Tension applied to these sutures rotated the eye downward and held it in a fixed position, so that excitation of the muscle produced an essentially isometric contraction. The muscle was bathed in Ringer's solution which flowed through a No. 20 hypodermic needle surrounded by an insulated resistance wire heating element. Temperature was maintained at 37.5°C with the aid of a thermistor.

A bipolar concentric stimulating electrode was driven into the portion of the third nerve nucleus controlling the ipsilateral superior rectus, and single square wave pulses of 0.1 msec duration and an intensity of 2 to 20 v (depending upon electrode proximity to the appropriate motoneuron) were delivered from an isolation unit of a Grass stimulator.

2. INFERIOR OBLIQUE PREPARATION (Fig. 1 B) The soft tissues and bone over the orbit were removed and the optic nerve tied and sectioned. All extraocular mus-

cles except the inferior oblique were cut away from the globe. The globe was collapsed, and the inferior oblique, together with the portion of sclera with the muscle insertion, was cut free and the globe removed. The branch of the third nerve innervating the inferior oblique muscle was dissected free and cut at the ciliary ganglion. The blood supply to the muscle remained intact. The nictitating membrane was raised by a suture to form a pool for the mineral oil. The tendon of the inferior oblique was tied with a silk suture and fixed to the stereotaxic instrument with approximately normal resting tension. The nerve was placed over a pair of platinum stimulating electrodes. The pool of oil was warmed by an insulated heating wire element, and the temperature monitored with a thermistor immersed in the oil. Temperature was maintained at 37.5°C. Usually single pulses of 0.1 msec duration were delivered from a stimulator through an isolation unit. The electrode nearest the muscle was made negative with respect to the other electrode. Twitch threshold under these conditions was usually 0.1 v or less.

Intracellular Recording Glass micropipettes filled with 3 M KCl (resistance 20 to 50 MΩ), were used to record intracellular potentials from muscle fibers. A micropipette was mounted in a micromanipulator and inserted under visual control into the muscle fibers. Intracellular potentials were led through a negative capacity dc amplifier with more than 5000 MΩ input impedance and displayed on a beam of a storage oscilloscope, while another beam was used as a zero volt reference line. Records were obtained by photographing selected stored traces with a camera. The resting potential was monitored by means of a vacuum tube voltmeter connected to the output of the direct coupled amplifier. An indifferent electrode was placed underneath the muscle near to its distal end in an inferior oblique muscle preparation, or placed in the Ringer bath in a superior rectus muscle preparation.

Miniature end plate potentials (miniature e.p.p's) were recorded in inferior oblique muscle preparations. The potentials were led through a cathode follower input dc amplifier with an input impedance of more than 500 MΩ. Records were made on a recording oscillograph with galvanometer (flat response from dc to 2.1 kc), for measuring time intervals between successive miniature e.p.p's, and simultaneously on a storage oscilloscope for measuring the amplitudes of the miniature e.p.p's.

Polarizing Current Single- and double-barreled electrodes were used in conjunction with a bridge circuit. The single electrode in a bridge circuit is illustrated in Fig. 2 A. The cat's body and the fixation apparatus were carefully insulated from ground. A constant current stimulator (output impedance up to 30,000 MΩ) was employed to pass rectangular pulses of current with a duration of 100 msec, triggered by a square wave stimulator. The bridge circuit was balanced during the passage of the direct current with the tip of the micropipette contacting the body fluid of the muscle surface or the Ringer solution. The micropipette was then driven into a muscle fiber, and pulses of current of various intensities were passed at the same setting of the potentiometer. Current through the electrode used for polarization of the muscle membrane was recorded as a potential drop across a 10 KΩ resistor inserted between the indifferent electrode and ground.

A double-barreled electrode filled with 3 M KCl, with 10 to 30 MΩ resistance, caused 100 to 200 picofarads coupling capacitance between the two electrodes. In

order to avoid a coupling resistance up to about 1 MΩ, the electrode was also constructed into a bridge circuit as shown in Fig. 2 B. The components of the circuit and the procedure for its control resembled those of the single electrode. The most success-

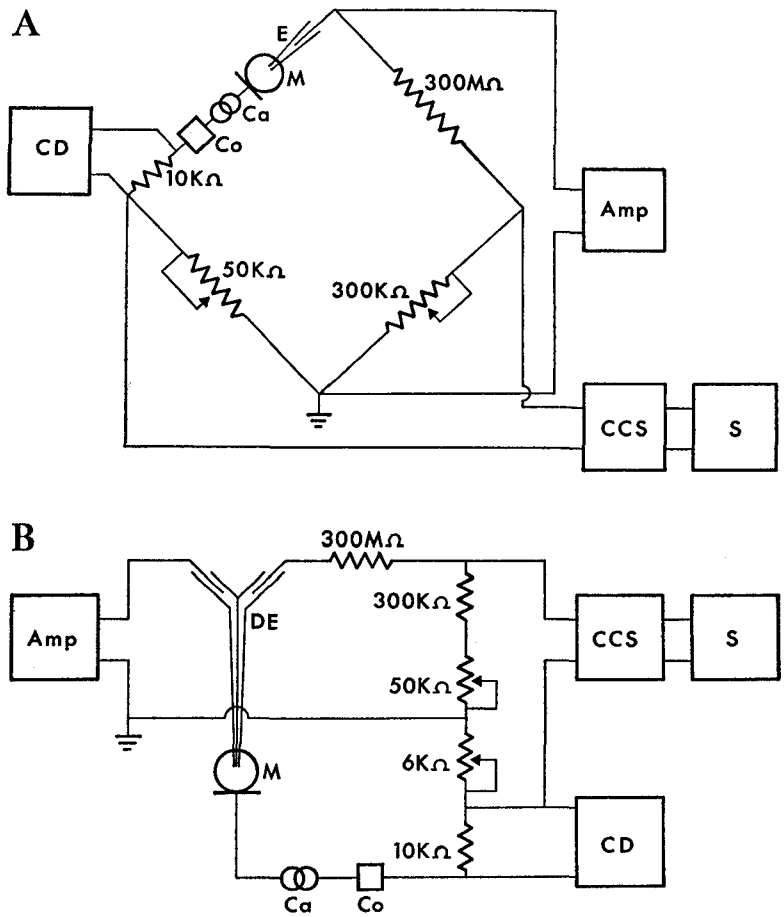

FIGURE 2. Schematic diagrams of the bridge circuits used with single (part A) and double-barreled electrodes (part B) employed for the application of polarizing currents to the muscle membrane. *Ca*, calibrator for voltage. *Co*, compensator for zero potential. *CCS*, constant current stimulator. *S*, stimulator, *Amp*, amplifier. *CD*, current detector (differential amplifier). *E*, single micropipette. *DE*, double-barreled micropipette. *M*, extraocular muscle fiber in vivo.

ful records, however, were obtained by means of an electrode without the coupling resistance.

Polarizing potentials and responses were recorded on the first beam of a storage oscilloscope through either a negative capacity dc amplifier or a cathode follower

input dc amplifier; the former was useful for recording electrical responses of the muscle, the latter for measuring time constants of the muscle membrane. The value of the polarizing current was displayed on the second beam of a storage oscilloscope through a differential input dc amplifier employed as a current detector.

Selective Stimulation of Slow Fibers The branch of the third nerve to the inferior oblique muscle usually divides into three nerve branches before entering the muscle. Thus, the surface potential recording following a nerve stimulus has a complex form. The proximal and the central branches of the nerve were cut, leaving the distal branch, which is known to include many small nerve fibers (19). In this way the surface potential could be reduced to factors involving differences in nerve conduction velocity between the large motor fibers and the small gamma range fibers, and differences in conduction velocity along the slow and fast muscle fibers. The anode

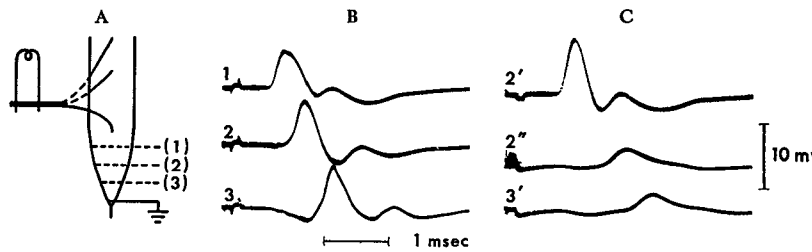

FIGURE 3. Action potentials recorded externally from an inferior oblique muscle. A. The branch of the third cranial nerve to the inferior oblique has been cut at the level of the ciliary ganglion, and placed over stimulating electrodes. At its entrance into the muscle, the nerve divides into three branches; the proximal and central branches have been cut. The recording electrode has been placed on the muscle, and moved toward the distal end in 1 mm steps. The indifferent electrode is located at the distal end. B. Responses to maximal nerve stimulation (cathode excitation); Nos. 1, 2, and 3 correspond to the numbered recording sites on the muscle in (part A). C. 2″ and 3′ are the responses to anode break stimulation, recorded from points (2) and (3), in comparison with a response (part C-2′) on maximal cathode stimulation.

break excitation method was similar to that employed by Burke and Ginsborg (6). A slowly decaying saw tooth pulse with a time constant of about 30 msec (produced from a 30 msec duration pulse), was applied to the peripheral trunk of the third nerve through a pair of platinum electrodes about 4 mm apart; the electrode nearer to the muscle was made positive with respect to the other electrode. As an indicator of this anode break excitation, extracellular responses of the muscle were recorded with a platinum electrode (0.1 mm diameter) placed on the muscle surface in an oil bath; an indifferent electrode was used in common with that of the intracellular electrode. In a range from 4 to 8 v stimulation intensities, a slow muscle component was excited selectively as shown in Fig. 3 C.

Mechanical Studies Isometric tension was measured by a mechanoelectric transducer (RCA 5734 tube) and displayed on a storage oscilloscope. The distal tendon of the muscle was tied directly to the anode pin of the tube.

RESULTS

Extracellular Recording

Fig. 3 B illustrates the response to a cathode nerve excitation of the inferior oblique muscle in which the proximal and the central nerve branches were cut, leaving only the distal nerve branch. Fast and slow propagating impulses were demonstrated.

If the indifferent electrode was kept fixed at the distal end and the other electrode moved along the surface of the muscle (Fig. 3 A), there was a variation in the latency and time course of the response. The latency was shortest in the central part of the muscle, in which the nerves were seen to enter, and increased toward each end of the muscle (Fig. 3 B). Both types of fibers in the inferior oblique muscle are able to conduct action potentials. The means of the conduction velocities were 3.00 m/sec for the faster impulse and 2.16 m/sec for the slower impulse, in eight muscles at 37.5°C muscle temperature.

When an anode break stimulation was applied to the nerve, the faster conducting impulse was eliminated, while the slower one was still present (Fig. 3 C). Even in this preparation of virtually all small nerves and slow fibers, the action potentials were able to propagate when a surface electrode was moved along the surface of the muscle as illustrated in Fig. 3 C. The average conduction velocity measured in the anode break excitation was 2.32 m/sec.

Intracellular Recording

The membrane resting potentials of muscle fibers excited by anode break stimulation were distributed in a range between 20 and 65 mv in the inferior oblique muscle preparation. In the example illustrated in Fig. 4 A, of the 153 muscle fibers which were activated only 39 were observed to respond with overshoot spikes (Fig. 5 A); the others responded with nonovershoot depolarization (Fig. 5 B). The latter appeared to be due to a reduction in resting potential caused by the insertion of the micropipette. Several factors lead to this conclusion: (*a*) Almost all the nonovershoot depolarizations were observed from the fibers having resting potentials lower than 50 mv; in contrast, the overshoot spikes were obtained from the fibers within a range between 48 and 62 mv (Fig. 4 A). (*b*) The rise time (0.77 msec) and the duration of the depolarized phase (1.86 msec) in nonovershoot depolarization were longer than those of overshoot spikes (0.33 msec rise time and 1.20 msec duration). (*c*) After insertion of a micropipette into a slow fiber, when the membrane potential decreased from 60 mv to 50 mv due to membrane injury, the overshoot spike began to be followed by a hyperpolarization. Further decrease of the membrane potential to less than 50 mv caused a change from the overshoot spike to a nonovershoot depolarization. The nonovershoot

depolarization was followed by a hyperpolarization of from 1 to 5 msec duration and up to 5 mv in amplitude. Therefore, it is unlikely that the hyperpolarization was caused by a delayed rectification property of the fiber membrane as was mentioned by Hess and Pilar (11).

The lower range of the slow fiber membrane potentials is probably due to the small size of the slow fiber: 10 to 15 μ (11). It is likely that in the depolar-

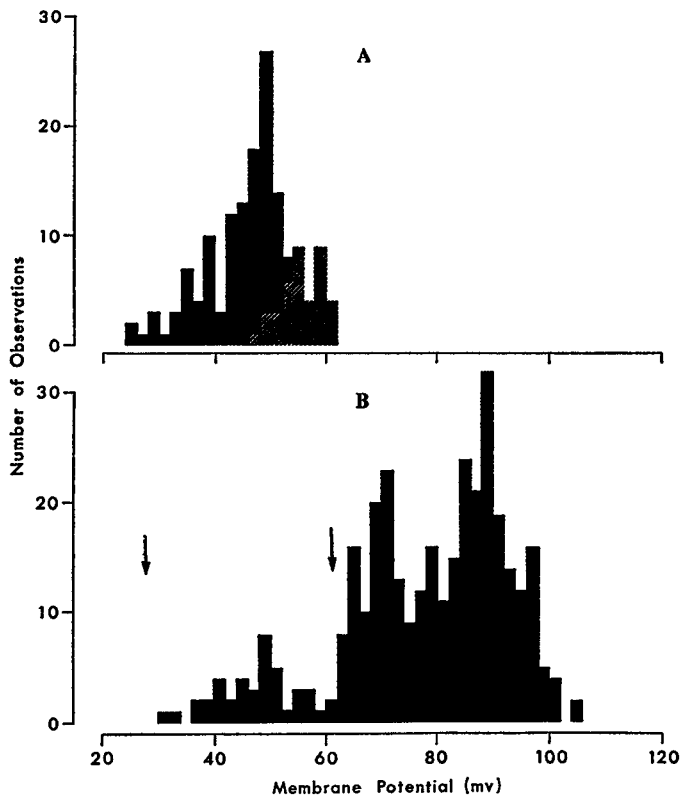

FIGURE 4. Resting potentials of muscle fibers in an inferior oblique muscle. A. The potentials of fibers that responded to anode break stimulation of the nerve; fibers that responded with overshoot spikes are represented in crosshatching. B. Potentials of fibers that did not respond to anode break excitation. The fibers in the slow fiber range of membrane potentials fall between the arrows.

ized muscle fibers the inability to produce overshoot spikes was caused by depression of the resting potential (9). In 4 preparations, the membrane potentials of 88 slow fibers with overshoot spikes to anode break excitation averaged 58.8 mv.

A large number of muscle fibers, with resting potentials from 20 to 110 mv, did not respond to anode break excitation. In each preparation there are two population peaks, similar to the example shown in Fig. 4 B. A main peak was

composed of 302 fibers distributed between 64 and 106 mv, while a small peak composed of 42 fibers (between the arrows) overlapped upon a distribution of a population of slow fiber resting potentials. The small peak may be classified as a slow fiber population, since it is likely that the absence of response in this small peak was associated with a reduction in the number of muscle fibers activated by nerve stimulus, due to the cutting of two branches of the innervating nerve.

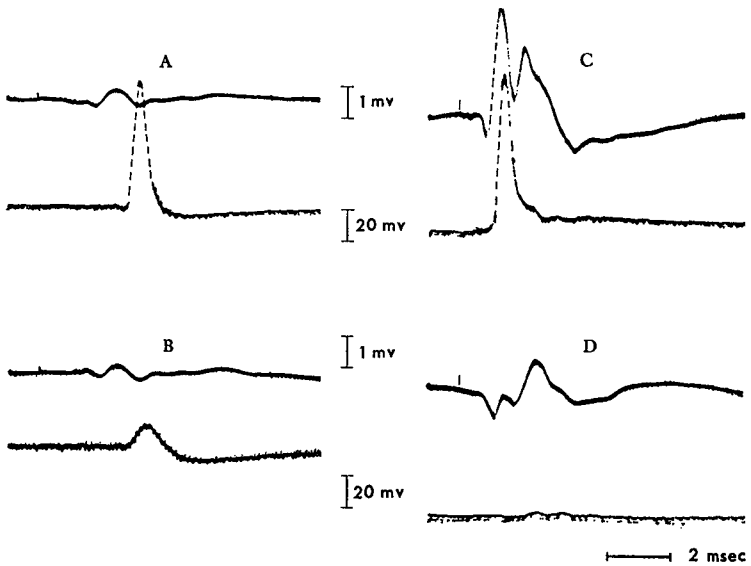

FIGURE 5. Intracellular records of responses in slow (parts A, B bottom lines) and fast (parts C, D bottom lines) muscle fibers of an inferior oblique muscle, in comparison with the extracellular responses displayed on the zero volt reference line (top lines). A. Anode break excitation followed by a long latency overshoot spike when the membrane potential was 68 mv. B. A nonovershoot response when the membrane potential (same fiber as in part A) had decreased to 45 mv. C. A short latency overshoot spike response to maximal nerve stimulation (cathode excitation) in the fast fiber. D. No response to an anode break excitation in the same fiber as part C. Parts A and C have been retouched with dashed lines.

It is not known with certainty whether the innervation of the slow multiinnervated twitch fibers is polyneuronal. However, the data presented in Fig. 4 suggest that the slow muscle fibers receive motor innervation from several nerves. Thus, although two-thirds of the nerve fibers were cut (2 of the 3 branches), more than 75% (146 out of 188) of the slow fibers were activated by stimulation of the remaining one-third (1 branch) of the innervation. In addition, nerve fibers with higher thresholds than the stimuli used may account for some of the 42 fibers that remained inactivated.

When the nerve was stimulated by a threshold strength cathode excitation,

overshoot spikes were recorded exclusively from fibers having a resting potential larger than 65 mv. In fast fibers overshoot spikes resulted from a cathode excitation, but no responses were recorded in anode break excitation, as illustrated in Fig. 5 C and D. The mean membrane potential of 875 fast fibers recorded from 4 muscles was 82.7 mv. It thus appears possible to identify slow and fast fibers by their response to anode break excitation.

Layer Organization and Population Distribution

THE INFERIOR OBLIQUE MUSCLE Fast and slow fibers were identified by their resting membrane potentials and by means of anode break excitation. When the muscle was penetrated perpendicularly by a micropipette at a point about 7 mm from the distal end of the muscle, approximately 7 layers were observed. The outer 2 or 3 were composed of slow fibers, the deeper 3 or 4 layers of fast and the deepest one again of slow fibers. In a muscle belly (15 mm from the distal end), approximately 9 layers were observed, the inner one was usually composed of slow fibers, the deeper 5 or 6 layers of fast, and the outer 2 or 3 of slow fibers. From these results it appears that the inferior oblique is composed of a population distribution with a ratio of approximately 1 slow fiber to 2 fast fibers. Such a population distribution is also suggested by Fig. 4 in which the ratio between slow and fast fibers was approximately 1 to 2. However, all layers were not equally penetrated on each electrode descent. Therefore, slow fibers located on the muscle surface may be encountered relatively more frequently than fast fibers from the deeper layers. Similar population distributions were noted at the beginning and towards the end of each experiment.

THE SUPERIOR RECTUS MUSCLE When the muscle was penetrated close to the insertion, fewer layers were observed. With the globe held in downgaze and with an electrode penetrating the muscle perpendicularly 5 mm from the insertion, approximately 5 layers were observed. In an identification of slow and fast fibers by their membrane potentials, the outer 2 or 3 were composed of slow fibers, and the deepest 2 of fast fibers. In this preparation the muscle remained in its normal position against an intact globe. Thus, the deepest layer was often not studied, since contact with the globe would break the electrode tip.

292 fibers recorded on 49 electrode penetrations in 3 preparations were distributed into 103 slow fibers and 189 fast fibers.

Spontaneous Activity

MINIATURE E. P. P'S IN FAST FIBERS Miniature e. p. p's were recorded from identified fast fibers in the inferior oblique maintained at its resting length (Fig. 6). The microelectrode was inserted in the belly of the muscle in order to place the electrode tip as close as possible to the end plate region. In

individual fibers the rise time varied between 0.3 and 0.9 msec, but there was no obvious relation between amplitude and rise time. The means of the rise time and the half-decay time were 0.61 and 1.93 msec, respectively. The mean amplitudes varied between 0.41 and 1.37 mv from one fiber to another. The frequency distribution of amplitude appears to approximate a normal

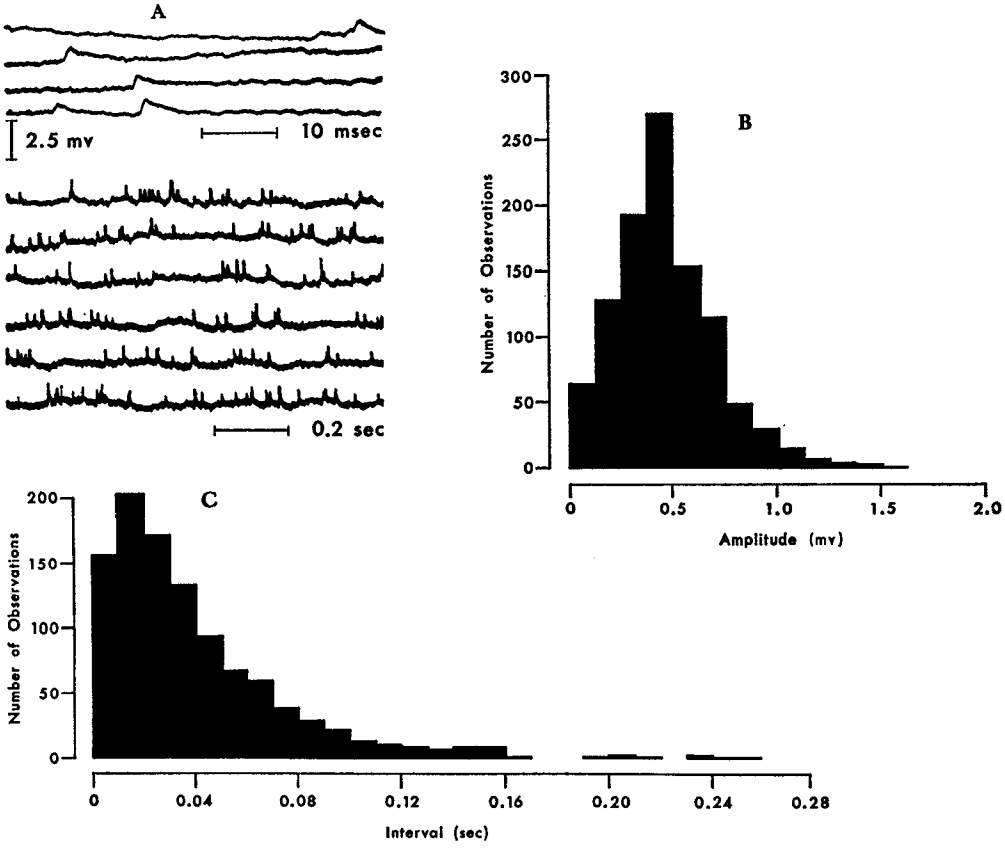

FIGURE 6. Spontaneous miniature e.p.p's recorded intracellularly from fast muscle fibers in the inferior oblique muscle. A. Oscilloscope records at fast (top) and slow (bottom) sweeps. B. Frequency distribution of the amplitudes of a series of 860 miniature e.p.p's recorded from a single fast fiber. C. Frequency distribution of the time intervals of 860 miniature e.p.p's (the same experiment as in part B).

distribution. The relative constancy of amplitude and wave form and the normal frequency distribution of amplitudes suggest that the miniature e.p.p's originate from a focal end plate near which the electrode was inserted.

The frequency of miniature e.p.p's is usually high. In 5 preparations the mean frequency varied from 5.04 to 31.0/sec. The mean frequency obtained from 13 fibers in a single muscle was 19.8/sec. It is unlikely that this high rate

was due to local excitation caused by the electrode (5), since there was no difference between the time course of the miniature e.p.p's that discharged at 5/sec and those that discharged at 31/sec. It is not probable that the rate was due to after effects of nerve stimulation applied previously for the identification of the muscle fiber type, since the records were begun at least 1 min after the nerve stimulation. The high rates observed were not due to an increase in transmitter release produced by stretching the muscle (12), since the tension applied to the muscle was no greater than the resting tension. In one fiber the frequency in the initial record was similar to that in the last record, taken about 10 min later. That the high rates result from activity in the nerve terminals is established by the fact that they are transiently abolished by the intravenous injection of 250 μg of tubocurarine.

MINIATURE E.P.P.'S IN SLOW FIBERS Hess and Pilar (11) have used the term "miniature s.j.p." for the spontaneous activity recorded from the cat's extraocular slow muscle fibers, because no propagated impulses were observed. Hyperpolarization following a response of the extraocular slow fibers, similar to the frog's slow fibers, was interpreted by them as evidence for delayed rectification of the fiber membrane. In the present experiments, however, the term "miniature e.p.p of the slow multi-innervated twitch fiber" is used, since the slow fibers were capable of producing propagated impulses, and no delayed rectification was observed.

Miniature e.p.p's from two different slow fibers are shown in Fig. 7 A. It can be seen that the size and time course vary much more than in the fast fibers. There is no strict relation between the size and the time course of the miniature e.p.p's. The means of the rise time and the half-decay time were 0.97 msec and 2.85 msec, respectively. The frequency distribution of the amplitudes is not normal, as it is approximately in the case of the fast fibers. In the slow fibers there is a relatively greater number of the larger potentials. The largest amplitudes recorded have been up to 4.3 mv. As illustrated in Fig. 7 B, two populations were distinguished, the main distribution peak was located at 0.5 mv in all cases, while the small population distribution was located between 1.0 and 3.0 mv in which no peak could be discriminated. When the rise and half-decay times of miniature e.p.p's larger than 1.5 mv were selectively analyzed, the mean values became 0.69 and 2.67 msec, respectively, which are distinctly shorter than those of the smaller miniature e.p.p's (Fig. 7 A). It is unlikely that the larger miniature e.p.p's are due to summation of two or of several individual small miniature e.p.p's. It is more likely that this variation in amplitude and time course of the miniature e.p.p's is due to the multiple innervation of the slow fibers (5, 6, 14). Potentials originating at some distance from the recording electrode will, other things being equal, be smaller and slower than those arising near the electrode. In slow fibers the miniature e.p.p's could be recorded in random microelectrode penetrations

along the length of the fibers; this was not possible in fast fibers. This characteristic is probably due to the multiplicity of slow muscle neuromuscular endings.

The frequency of miniature e.p.p's is low in comparision with that of fast fibers. In 6 preparations the mean frequencies varied between 0.8 and 5.2/sec

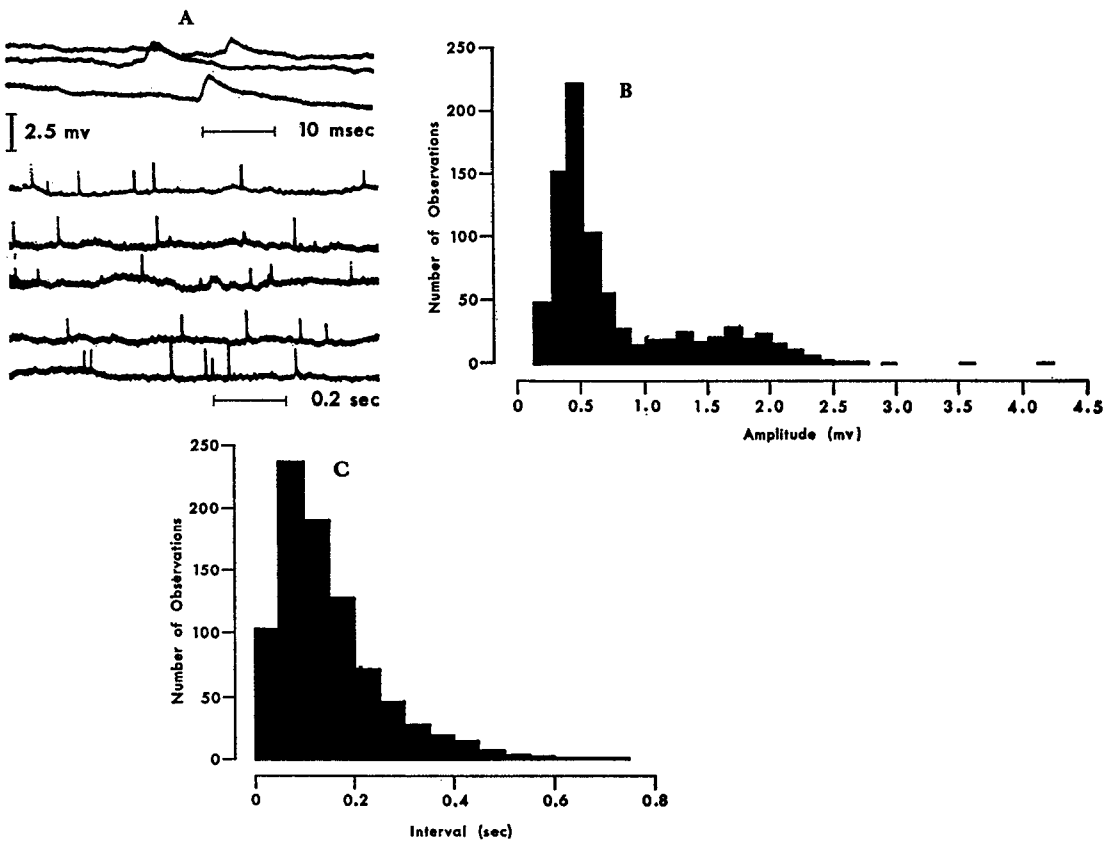

FIGURE 7. Spontaneous miniature e.p.p's recorded intracellularly from slow muscle fibers in the inferior oblique. A. Oscilloscope records at fast (top) and slow (bottom) sweeps. B. Frequency distribution of the amplitudes of a series of 1035 miniature e.p.p's recorded from a single slow fiber. C. Frequency distribution of the time intervals of 1035 miniature e.p.p's (the same experiment as in part B).

from fiber to fiber. The mean frequency obtained from 17 fibers in one muscle was 3.07/sec. The curve in Fig. 7 C shows the expected exponential distribution for a random series.

Polarizing Current

FAST FIBERS Responses of a fast fiber to direct stimulation applied through a bridge circuit, and of a fast fiber to stimulation applied through a

double-barreled electrode, are illustrated in Fig. 8 A and B. Overshoot spikes appeared when the membrane potentials were depolarized to 47 and 40 mv. These levels show a threshold for fast fibers, since the polarizing currents were applied at the site of the recording electrode in both methods. The threshold values measured from 37 fast fibers varied between 40 and 58 mv.

In all fast fibers observed, a linear relationship between the polarizing currents and the membrane potential shifts in the subthreshold range suggests the absence of a delayed rectification property of the fiber membrane. The mean of the effective membrane resistance calculated from the linear relations of 32 fibers was $11.2 \pm 2.96 \times 10^5$ Ω. If the diameter of the fiber is 30 to 45 μ (11), the specific membrane resistance is approximately 12,500 Ω cm².

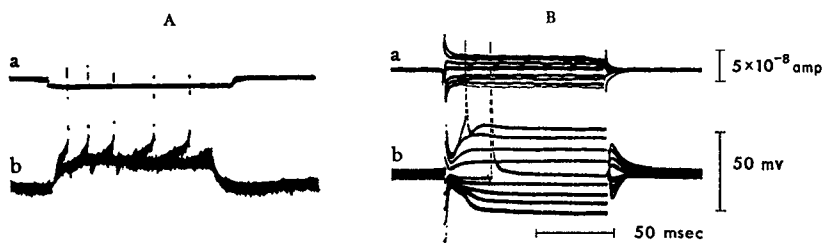

FIGURE 8. Intracellular records of action potentials from two fast muscle fibers in the inferior oblique. A. A fiber stimulated with sub- and suprathreshold depolarizing currents (2 and 4 × 10^{-8} amp) applied through a single micropipette included in a bridge circuit; the upper line represents the polarizing current, displayed on the zero volt reference line. B. *a*, The polarizing currents displayed on the zero volt reference line; depolarizing downwards and hyperpolarizing upwards. The spikes in part B have been retouched with dashed lines. B. *b*, A fiber stimulated with polarizing currents applied through a double-barreled micropipette; a reference spike was elicited by maximal nerve stimulation at the resting potential (middle line); one suprathreshold (top line, 4 × 10^{-8} amp), and three subthreshold depolarizations (next 3 lines down, 1, 2, 3 × 10^{-8} amp), and four hyperpolarizations (lowest 4 lines: 1, 2, 3, 4 × 10^{-8} amp) were superimposed.

The mean of the time constants of the fiber membranes measured from 21 records was 10.6 ± 3.62 msec, from which a specific membrane capacitance of 0.86 μ f/cm² was calculated.

SLOW FIBERS When a fiber with a membrane potential near 60 mv was observed with a single electrode included in a bridge circuit, a direct stimulation always caused an overshoot spike, as illustrated in Fig. 9 A. The threshold values of the spikes obtained from 12 fibers ranged between 40 and 55 mv, which are similar to the range of fast fiber thresholds. Although the insertion of double-barreled micropipettes into fibers of small diameter produced injury depolarization, it was possible to evoke spike potentials. If the injury depolarization did not exceed the threshold level, spikes that reached the zero volt reference

level (but did not overshoot this level) could be elicited with small applied depolarization as shown in Fig. 9 B. If the injury depolarization exceeded the threshold level, a break excitation of an electrotonic hyperpolarization was capable of producing a nonovershoot spike, while an applied depolarization caused a small response of depolarization as shown in Fig. 9 C. These results

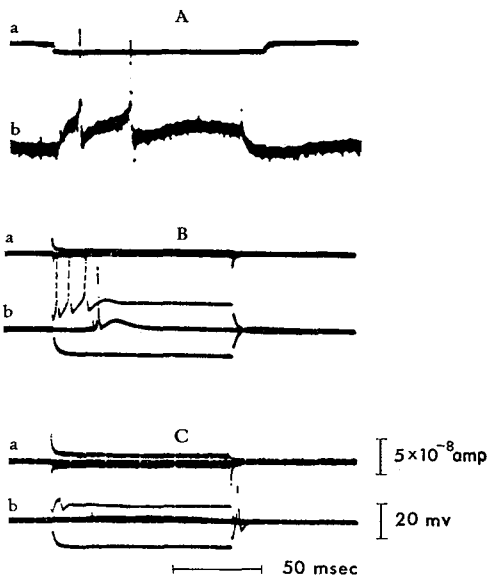

FIGURE 9. Intracellular records of action potentials from three inferior oblique slow muscle fibers (*b* in parts A, B, and C). The upper lines (*a* in parts A, B, and C) represent the polarizing currents displayed simultaneously on the zero volt reference line; depolarizing downwards and hyperpolarizing upwards. A. A fiber responded with overshoot spikes to a depolarizing current (4×10^{-8} amp) applied through a single micropipette (recording electrode). B. A fiber in which the membrane potential had decreased to 56 mv due to the insertion of a double-barreled micropipette responded with spikes to both maximal nerve stimulation (middle line of *b*) and to a depolarizing current of 1×10^{-8} amp (top line of *b*) applied through a double-barreled micropipette. No response was obtained with hyperpolarizing current of 1×10^{-8} amp (lowest line in *b*). C. A fiber in which the membrane potential had decreased to 44 mv responded with a small depolarization potential on application of a depolarizing current of 1×10^{-8} amp (top line of *b*) applied through a double-barreled micropipette; a break excitation of a hyperpolarizing current of 1×10^{-8} amp (lowest line in *b*) produced a spike. The spikes in part B have been retouched with dashed lines.

suggest that slow fibers are basically similar to fast fibers; both respond with overshoot spikes at the same threshold.

The linear relation between the applied polarizing currents and the membrane potential shifts in its subthreshold range indicated that slow fibers do not have delayed rectification properties. Some fibers, which were depolarized below the threshold for spikes, showed nonlinear relations, the resistance

being decreased by depolarization. This nonlinear relation, however, was not due to a delayed rectification property as in frog's slow fibers, but was probably due to injury of the fiber membrane caused by insertion of a double-barreled electrode into small diameter fibers.

The mean of the effective membrane resistances obtained from 45 fibers was $9.55 \pm 4.55 \times 10^5\ \Omega$. If the diameter of the fiber is 10 to 15 μ (11), the mean of the specific membrane resistance is approximately 4500 Ω cm^2. The mean time constant of the membrane, measured from 16 fibers was 10.2 ± 1.46

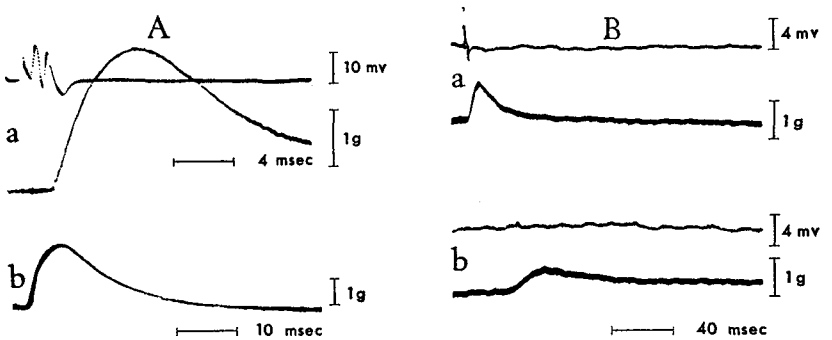

FIGURE 10. Isometric twitches in response to nerve stimulation of the inferior oblique muscle. A. Time courses of maximal twitch responses to cathode excitation of a nerve in which none of the three branches had been cut; initial tension 2.6 g (*a*) a single twitch (lower trace) and the simultaneously recorded surface potential indicating both fast and slow fibers were activated (upper trace); (*b*) the total contraction time course of a single twitch. B. Single twitch response when the proximal and central branches of the innervating nerve had been cut. Initial tension 5.5 g (*a*) a single twitch of fast fibers selectively stimulated by a threshold cathode excitation to the nerve (lower trace) and a simultaneously recorded extracellular action potential showing only the fast fiber activation (upper trace). (*b*) a single twitch of slow fibers, selectively stimulated by anode break excitation (lower trace) and the simultaneously recorded extracellular action potential showing only the slow fiber activation (upper trace).

msec, from which a specific membrane capacitance of 2.48 μ f/cm^2 was calculated.

Mechanical Studies

TWITCHES TO SINGLE NERVE STIMULATION The inferior oblique was used for all the mechanical studies. Initial tension of from 2 to 6 g was applied to the muscles. Fig. 10 A illustrates a twitch response of the inferior oblique muscle to a single maximal stimulus applied to the nerve in which none of the 3 branches to the inferior oblique had been cut. In 4 preparations the maximum twitch tension of 2.2 to 3.0 g was attained in a rise time of 5.0 to 7.2 msec. The half-decay time was 7 to 8 msec, and the total duration of the contraction was up to 200 msec. The action potential simultaneously re-

corded is composed of both fast and slow fiber components. Although some fast impulses initiated from the proximal and central end plate regions of the muscle may be included in the second peak of the potential, the twitch response appears to be constructed of two kinds of muscle contractions. In a preparation in which the proximal and central nerve branches had been cut, a single twitch response of slow fibers, produced selectively by an anode break excitation, is illustrated in comparison with a twitch response of fast fibers produced by a threshold cathode excitation in Fig. 10 B. The rise and half-decay times of the purely slow muscle twitch in 3 preparations varied between 20 and 27 msec and between 38 and 44 msec, respectively. The maximal tension measured between 0.3 and 0.7 g in the 4 muscles (in all cases, 2 of the 3 nerve branches had been cut) and the total duration of the contraction was approximately 200 msec. On the other hand, the twitch tension produced by a threshold cathode excitation appeared to be a contraction of the purely fast fibers, since the simultaneously recorded action potential included only a fast component. The rise and half-decay time observed in 3 preparations ranged from 5.7 to 6.9 msec and from 7 to 8 msec, respectively. The maximal tension was between 0.9 and 1.2 g, and the total duration of the contraction was approximately 100 msec.

TETANIC CONTRACTIONS TO REPETITIVE NERVE STIMULATION Fig. 11 A and B illustrate tetanic contractions of purely slow and fast fibers in an inferior oblique preparation in which the proximal and central nerve branches had been cut. The tetanic tensions of the slow fibers to repetitive anode break excitations appeared to fuse incompletely at 15/sec and almost completely at 25/sec, whereas those of the fast fibers to threshold cathode excitation showed individual twitches at these frequencies. When the frequency of the threshold cathode stimuli was increased, however, some small nerve fibers with a higher threshold possibly were excited, so that the tetanic contraction of the fast fibers was modulated by the additional contraction of some slow fibers.

Repetitive stimulations of maximal cathode excitation intensity were applied to the nerve trunk in which the nerve branches were intact. Under these conditions, the tetanic tension is composed of fast and slow fiber contractions. With repetitive stimulation at more than 300/sec, the maximal tension development of the tetanus attained was from 17 to 40 g, the average in 6 muscles was 27.3 g (Fig. 11 C). The rise time was 125 to 150 msec and the half-decay time varied between 13 and 20 msec from muscle to muscle. In 3 muscles, the fusion frequency, observed with high gain, was more than 450/sec (Fig. 11 D).

RESPONSES DURING APPLICATION OF SUCCINYLCHOLINE Extraocular muscles that contain multi-innervated slow muscle fibers contract on administration of succinylcholine. The extraocular muscles that do not contain slow

fibers (retractor bulbi) do not contract (1). Fast fibers in the extraocular muscles, as well as in other body musculature, are paralyzed by succinylcholine. The four recti and two obliques are composed of both multi-innervated slow fibers and fast fibers.

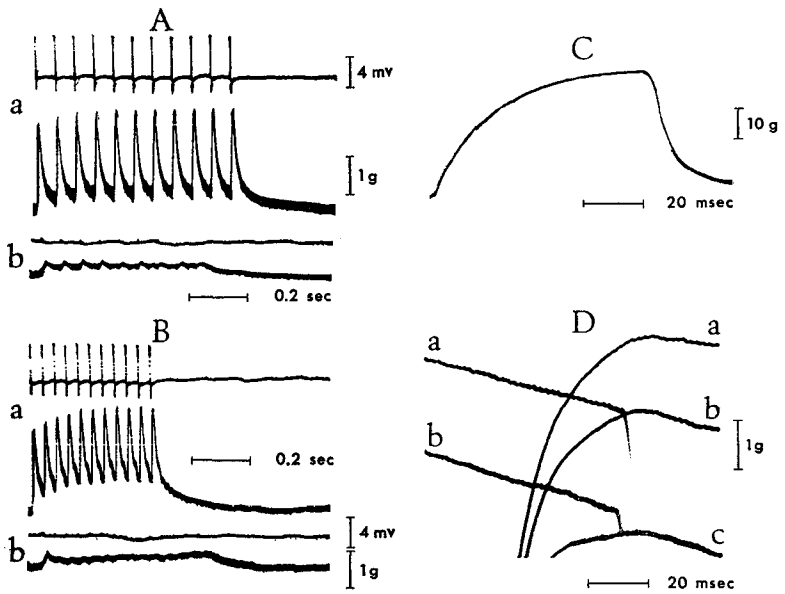

FIGURE 11. Isometric tetanic contractions of the inferior oblique muscle in response to repetitive nerve stimulation. A. Tetanic contractions when the proximal and central branches of the nerve were cut. Initial tension 5.5 g; stimulation frequency, 15/sec; (*a*) threshold cathode nerve excitation resulted in fast extracellular impulses (upper trace) and individual twitches (lower trace). (*b*) anode break excitation produced slow impulses (upper trace) and almost fused tetanus (lower trace). B. The same preparation as part A; stimulation at 25/sec (*a*) threshold cathode excitation; (*b*) anode break excitation. C. Maximal tetanic tension of an inferior oblique muscle preparation with all three branches intact; initial tension 3.8 g stimulation frequency 350/sec. D. The same preparation as in part C with high gain amplification. Maximal intensity stimulation at (*a*) 400 and (*b*) 500/sec was followed by individual peaks of contraction; at 600/sec (*c*), the tetanic contraction was apparently fused.

In order to compare the tension developed by the succinylcholine contraction to the maximal tetanic tension on nerve stimulation, 150 msec bursts of maximal cathode stimuli at 300/sec were delivered intermittently at a rate of 0.13/sec to the nerve in which the 3 branches were intact. The tetanic contraction each 7 sec showed no decay over a period of 1 min (Fig. 12 A). Eight sec after succinylcholine was injected into the femoral vein, the contracture began and reached maximum tension within 7 sec. In 6 muscles, the maximum developed with more than 100 μg/kg of succinylcholine was approxi-

mately one third of the tension developed by tetanic nerve stimulation in each case (Fig. 12 B). On the other hand, the tetanic tension decreased to one twelfth on administration of 30 μg/kg, and disappeared with 250 μg/kg of succinylcholine, which may be interpreted as being due to the paralysis of neuromuscular transmission. The concomitant decrease in the tetanic tension did not become maximal until more than 40 sec after initiation of the succinylcholine effect, and recovered within 15 min with doses of approximately 75 μg/kg.

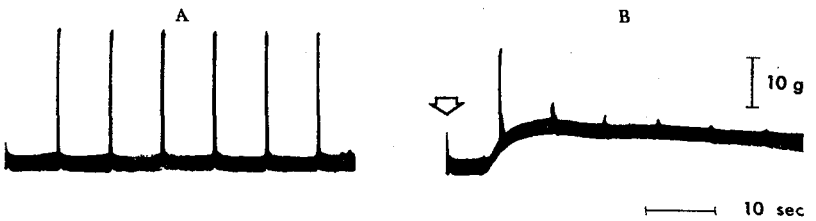

FIGURE 12. Isometric contracture produced by succinylcholine, and the simultaneous decrease in the amplitude of the tetanic contraction. Initial tension 2.6 g. A. Before the administration of succinylcholine; maximal nerve stimulation at 350/sec for 150 msec which produced maximal tetanic tension, was delivered at the rate of 0.13/sec. B. After the administration of succinylcholine; 250 μg in a 2.7 kg cat, injected in the femoral vein at the beginning of the trace (arrow).

DISCUSSION

Hess and Pilar (11) have described two morphologically distinct types of muscle fibers in the extraocular muscles of the cat: small, multi-innervated fibers, and larger, possibly singly innervated fibers. There are clear and constant differences in the size and distribution of the fibrils, and in the disposition of the sarcoplasmic reticulum between the two types of fibers. Morphologically, the two types correspond almost exactly to the twitch and slow type extrafusal muscle fibers in the frog and chicken. The two types of muscle fibers described by Hess and Pilar (11) do not correspond to the slow and fast muscle fibers studied by Buller, Eccles, and Eccles (4). These authors studied two types of fast twitch fibers, neither of which is multi-innervated.

Our experiments have shown that both types of extraocular muscle fibers are capable of producing overshoot spikes and propagated impulses. Their membrane properties were essentially similar. Avian slow muscle fibers with multiple neuromuscular junctions are also capable of producing overshoot spikes (9). However, some of the avian slow fibers that did not produce action potentials appeared to be in a state of cathodal depression due to the injury produced by the insertion of a microelectrode in a small diameter muscle fiber. In cat extraocular muscles we often encountered slow muscle fibers in-

capable of producing overshoot spikes, even with maximal nerve stimulation. In these cases we often recorded slow waves of depolarization, similar to those identified as slow muscle responses by Hess and Pilar (11), Matyushkin (15), and by Ozawa (18). However, we noted that the time course of the slow depolarization waves often coincided with the time course of the mechanical twitch response. Since the slow depolarization disappeared with anode break excitation, and since the slow fiber was able to respond with spikes to applied depolarization or break excitation of hyperpolarization (Fig. 9), the slow waves were considered to be due to mechanical artifacts.

The average speed of impulse conduction in the cat extraocular slow fibers (2.16 m/sec at 37.5°C) resembles that of the avian slow fiber (2.3 to 2.8 m/sec at 31 to 36°C). Miniature e.p.p's recorded from multiply innervated avian slow muscle fibers showed wide variations in amplitude (8). In cat extraocular slow muscle fibers, identified by anode break excitation, a similar wide distribution of miniature e.p.p amplitudes was noted, suggesting that these fibers are multiply innervated.

Boyd and Martin (3) have recorded miniature e.p.p's from cat tenuissimus muscle in vitro at 37°C. The amplitude, rise, and half-decay times in fibers of this skeletal muscle almost coincided with those observed in fast fibers of the cat extraocular muscles during in vivo recordings, but the frequency in the extraocular muscle fibers was considerably higher than that in the tenuissimus muscle.

The amplitude of the miniature e.p.p's in slow fibers was higher than in fast fibers. However, the threshold for action potentials was similar in both types of fibers, although the membrane potentials of slow fibers were lower than those of fast fibers. From these results it can be assumed that the end plate potentials in slow fibers may attain the overshoot spike threshold with the summation of less quanta than in fast fibers (7). In addition, the fusion frequency of the slow fibers is lower than for the fast fibers. It thus appears that neuromuscular transmission in slow fibers is an efficient mechanism for "tonic" muscle activity, which may include pursuit movements, vergence movements, and possibly the slow phase of vestibular nystagmus, as well as maintained contraction of the eye muscles. The tonic discharge recorded on electromyography (EMG) of extraocular muscles, even during rest, could be produced by the slow multi-innervated twitch fibers, since the present experiments show they are capable of producing spikes that, with present EMG techniques, are indistinguishable from those produced by the fast singly innervated twitch fibers.

It is possible to produce a propagated impulse from focal stimulation of a slow fiber, as our results with polarizing currents through a microelectrode have shown. This suggests that, although the slow fibers are multiply innervated, action potentials can originate from any one of many possible sites

along the muscle fiber. However, this does not exclude the possibility that these fibers may also under certain conditions show graded, nonpropagated contraction.

The microelectrode studies show that slow fibers are concentrated in the outer layers of the extraocular muscle while the fast fibers are concentrated in the inner layers, in agreement with the morphological findings of Kato (13). The microelectrode studies indicated that approximately one third of the fibers in each of the two muscles studied (superior rectus, inferior oblique) are slow. Experiments with succinylcholine, which produces selective contracture of the slow muscle fibers (1, 2), showed that the succinylcholine contracture tension is approximately one third of the maximal tetanic tension. The differential effects of succinylcholine on slow and fast fibers are shown by the differences in the time course of the action on each of the two fiber types: when approximately 100 μg/kg is administered intravenously, the slow fiber contracture reaches maximum tension 7 sec after initiation of contracture, and the tension returns to the resting level after 2 or 3 min; the fast fiber tetanic tension reduction takes 40 sec to reach maximum and lasts approximately 15 min.

Our studies with anode break excitation have indicated that the small diameter slow fibers are innervated by more slowly conducting nerve fibers than those innervating the fast fibers. O'Leary, Heinbecker, and Bishop (17) showed that approximately one third of the fibers in the third, fourth, and sixth nerves of the cat were under 8 μ in diameter. These gamma range fibers may innervate the slow fibers in the eye muscles.

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