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ELECTRICAL PROPERTIES OF FIBRES FROM STRIDULATORY AND FLIGHT MUSCLES OF A TETTIGONIID

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SUMMARY

1. The mesothoracic dorsal longitudinal muscle (DLM) of the katydid *Neoconocephalus robustus* is used in stridulation and flight; the metathoracic DLM is used in flight only. The DLM's in the two segments have radically different maximum operating frequencies, 200 Hz for the mesothoracic muscle during stridulation and 20 Hz for the metathoracic muscle during flight.

2. Cable analysis was used to determine the passive electrical properties of mesothoracic and metathoracic DLM fibres. Fibres in the two segments are of similar diameter and have similar sarcoplasmic resistivity. The apparent membrane resistance is lower, the apparent membrane capacitance higher, and the time constant shorter in mesothoracic fibres than in the metathoracic homologues.

3. The depolarization evoked by neural stimulation in both mesothoracic and metathoracic fibres is principally an excitatory junctional potential (e.j.p.) with little or no contribution from voltage-dependent, inward current channels. At short interstimulus intervals the second e.j.p. of a pair is reduced in amplitude relative to the first e.j.p. The period of e.j.p. depression is shorter in mesothoracic than in metathoracic fibres. It is suggested that the faster recovery of e.j.p.'s in mesothoracic fibres is due to more rapid recovery of the transmitter release mechanism in their motorneurones.

4. In mesothoracic but not metathoracic fibres the voltage response to large depolarizing currents is usually oscillatory, and the recovery of e.j.p. amplitude as a function of time in paired shock experiments is sometimes oscillatory. The oscillation frequency is 250–300 Hz (35 °C) which is higher than the natural operating frequency of the muscle.

INTRODUCTION

Adult, male katydids and crickets (Families Tettigoniidae and Gryllidae) stridulate by rubbing their forewings together. The muscles used in stridulation are the wing muscles of the mesothoracic segment, and are the same muscles as those used to power the forewings during flight. The metathoracic wing muscles, the muscles

which move the hind wings, are used in flight but not stridulation. The wing stroke frequency during flight can be nearly an order of magnitude less than the stridulation frequency. In *N. robustus*, in which the stridulatory frequency may exceed 200 Hz (Josephson & Halverson, 1971; Walker, Whitesell & Alexander, 1973), the wing stroke frequency during flight is 20 Hz (N. Ready, unpublished observations). In the related tettigoniid *Euconocephalus nasutus* the stridulation frequency is 160 Hz and the flight frequency 19 Hz (Josephson, 1973). Thus homologous wing muscles in the two adjacent alate segments may have radically different maximum operating frequencies. The wing muscles in katydids and crickets, and as far as is known in all Orthoptera, are synchronous muscles with muscle fibre depolarization for each contraction.

The difference in maximum operating frequencies of mesothoracic and metathoracic wing muscles in tettigoniids with high stridulation frequencies is reflected in the ultrastructure of these muscles. In both *N. robustus* and *E. nasutus*, the sarcoplasmic reticulum is considerably more abundant in mesothoracic than in metathoracic wing muscles, and the relative muscle volume occupied by mitochondria is greater in mesothoracic muscles than in their metathoracic homologues (Elder, 1971, and unpublished observations).

A previous study (Josephson, Stokes & Chen, 1975) has indicated that the membrane properties of the forewing muscles of *N. robustus* are modified for high frequency performance, specifically in having a very low apparent membrane resistance and therefore a short electrical time constant. The method used to determine the membrane resistance was rather indirect (see Discussion). The following study extends the observations on the electrical properties of mesothoracic wing muscles in *N. robustus*, and compares these with the electrical properties of metathoracic wing muscles in order to better identify those properties of mesothoracic muscle which are modifications for high frequency performance.

METHODS

Adult, male *N. robustus* from the vicinity of Woods Hole, Massachusetts, were kept in the laboratory on a diet of fresh grass. Animals were used within a few days of capture.

The muscle examined was the dorsal longitudinal muscle (DLM) which is the thinnest of the major flight muscles. The wings and legs of an animal were removed and the ventral nerve cord was exposed and transected in the neck. The oesophagus was ligated in the neck and transected anterior to the ligation. The gut was then removed through a longitudinal slit in the abdomen. The animal was mounted on its side in a dish lined with Sylgard resin (Dow Corning Co., Midland, Michigan) using insect pins bent over the body. The uppermost pleuron and the dorso-ventral muscles were removed to expose the upper DLM of either the mesothoracic or the metathoracic segment. Care was taken to leave the tracheation of the DLM as intact as possible. The ganglion in the segment of interest was removed after transection of its nerves and connectives. It was often found useful to reduce spontaneous movement in the preparation by removing the other thoracic ganglia and those of the anterior abdominal

segments. Fine insect pins inserted between fibres of the muscle into the Sylgard and up against the anterior and posterior phragma stabilized the muscle for electrical recording. For mechanical recordings, only the posterior phragma was fixed, and the transducer was hooked behind the anterior phragma. The preparation was perfused with saline (Usherwood, 1968) adjusted to pH 7 with NaOH. In later experiments 10 mM sucrose was added to the saline which seemed to improve the longevity of the preparation.

The electrical and mechanical recording techniques used are described in detail elsewhere (Josephson *et al.* 1975). Intracellular recordings were made with capillary microelectrodes filled with 3 M-KCl and with resistances of 10–30 MΩ. The nerve to the DLM was stimulated with a glass suction electrode. Floating microelectrodes were used to record from fibres of stimulated and contracting muscles. Muscle tension was recorded using a transducer constructed from a pair of semiconductor elements (Pixie 8101, Endevco Co., San Juan Capistrano, California; see Fig. 4–4 in Miller, 1979, for details on construction of the transducer). Tension recordings were essentially isometric. Muscle temperature was monitored with a thermocouple probe (0·3 mm o.d.) placed adjacent to the muscle in the haemolymph. The muscle temperature was controlled by changing the intensity of a microscope lamp directed at the muscle. Most measurements were made at both 25 °C and 35 °C. The thoracic temperature during singing is approximately 35 °C (Heath & Josephson, 1970). Additional techniques are described in appropriate sections of the text.

RESULTS

Contraction kinetics

Stimulating the motor nerve to the mesothoracic DLM with shocks of gradually increasing intensity initiated muscle twitches in four discrete tension increments. This indicates that the mesothoracic DLM is innervated by four excitatory axons which each evoke significant twitch tension (Josephson *et al.* 1975). Similarly, activating the nerve to the metathoracic DLM evoked twitches with five amplitude increments (Fig. 1); the metathoracic DLM thus receives five excitatory axons which each produce a measureable twitch. There may be a slow excitatory axons to the muscles as well which do not evoke measurable twitches, but we have seen no evidence for their existence in responses to single shocks or multiple stimuli. In the mesothoracic DLM, one of the tension increments, that produced by the dorsal band of the muscle, is distinctly slower than the other three (Stokes, Josephson & Price, 1975). In the metathoracic DLM each of the twitch increments had approximately the same time course and therefore this muscle is composed of temporally homogeneous motor units. No evidence for inhibitory innervation to either the mesothoracic or the metathoracic DLM was obtained in mechanical or electrical recordings from these muscles. The twitches of the mesothoracic muscle were considerably shorter than those of the metathoracic muscle (Table 1). The shorter twitch duration is clearly related to the higher repetition frequency at which the mesothoracic muscle may operate.

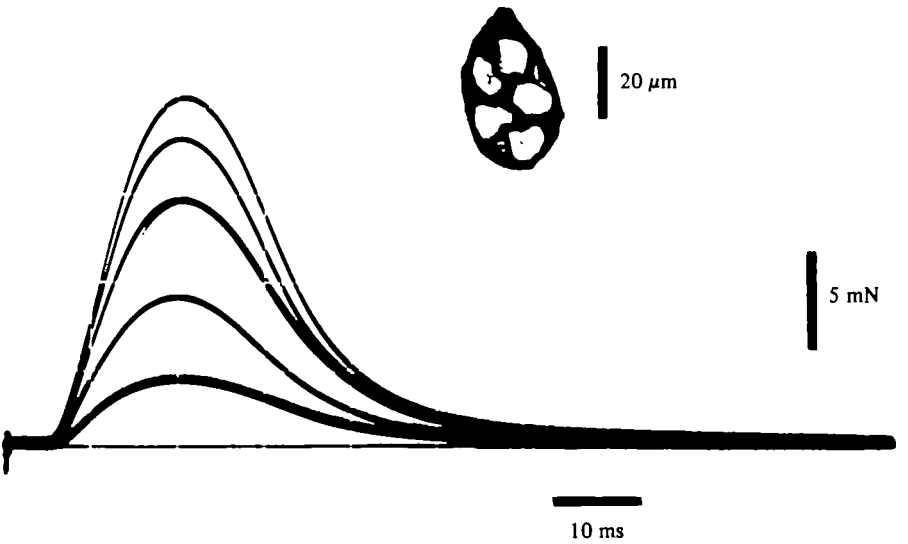

Fig. 1. Mechanical responses of the metathoracic DLM to single shocks of gradually increasing intensity, 25 °C. Each trace is several superimposed sweeps representing a range of stimulus intensities. The inset is a cross-section of the motor nerve to the DLM. Note the five tension increments and, correspondingly, the five large axon profiles in the nerve.

Table 1. *Twitch time course in mesothoracic and metathoracic DLM muscles*

(Mean ± S.E.)

	Mesothoracic* ($n = 6$)		Metathoracic† ($n = 9$)	
	25°	35°	25°	35°
Twitch rise time (ms)	4·2 ± 0·2	3·4 ± 0·4	11·8 ± 0·9	8·6 ± 0·5
Twitch duration, onset to 50% return (ms)	12·8 ± 1·7	7·6 ± 0·6	20·7 ± 1·4	15·2 ± 0·8

* The data for the mesothoracic muscles is from Josephson *et al.* (1975). The three faster motor units of this muscle together produce considerably more tension than does the single slower unit, so the values given apply essentially to the faster portion of the muscle, the medial and ventral bands.

† The data for the metathoracic muscle is from Stokes *et al.* (1975) supplemented by more recent measurements. The value of 18·8 ms given in Stokes *et al.* (1975) for the twitch duration of the metathoracic DLM at 35 °C is a typographical error; the correct average for that data set was 13·8 ms.

Passive electrical properties of muscle fibres

Cable analysis was used to determine the membrane properties of mesothoracic and metathoracic DLM fibres. A muscle fibre was injected with hyperpolarizing current pulses (100 nA, 50 ms) through a microelectrode. The low frequency current–voltage relationship was linear for hyperpolarizing current pulses up to at least 50 nA in both mesothoracic and metathoracic fibres. The change in membrane potential due to injected current was measured with a second microelectrode inserted sequentially at several distances from the current injection site. The distance between the current electrode and the recording electrode at each site was measured to the nearest 10 μm with an ocular micrometer. The mesothoracic DLM fibres examined were all from the ventral and medial bands. The current electrode in these experiments was

filled with a 3% Lucifer Yellow CH, a fluorescent dye (Stewart, 1978). Some Lucifer Yellow was ionophoretically injected into the muscle cell with each of the current pulses used in determining membrane impedance. At the end of the experiment, additional dye was injected into the cell using 50 ms, 100 nA, negative current pulses at 2–5 Hz for several minutes. The dye-filled fibres were then viewed at 200× under epi-illumination with UV light. There was no spread of dye to other muscle fibres, nor was electrical coupling ever detected between fibres. The dye-filled cells were always on the exposed lateral surface of the DLM so there was no light-scattering from overlying tissues which might obscure the cell boundaries. The widths of the marked fibres were measured to the nearest 2 μm.

In the analysis, the muscle fibres were treated as simple cylinders of diameter equal to the width measured with the fluorescence microscope. The assumption that fibres can be treated as cylinders is in two ways an inaccurate simplification. First, wing muscle fibres in *N. robustus* are ovoid or polygonal in cross-section (Elder, 1971; Stokes *et al.* 1975). The measured width of a fibre was the horizontal projection of that fibre, and this could be greater than or less than the average width of the fibre depending on how the fibre lay in the field of view. Treating a fibre as a cylinder of diameter equal to the measured width may either overestimate or underestimate the fibre's cross-sectional area and surface area depending on the shape and orientation of the fibre. Second, wing muscles of *N. robustus* have well-developed transverse tubules which open to the surface of the muscle fibres. Some part of the current flow between the cytoplasm and the external fluid is presumably through the transverse tubules (cf. Falk & Fatt, 1964). Treating a muscle fibre as a cylinder and considering only the area of the outer surface underestimates the membrane area available as a current pathway and therefore underestimates the specific membrane resistivity and overestimates the specific membrane capacitance. The calculated values for specific membrane resistance and capacitance will be termed the apparent membrane resistivity and the apparent membrane capacitance to emphasize that they apply only with the simplifying assumption of cylindrical geometry.

The fibres of the mesothoracic DLM are 3·5–5 mm long, those of the metathoracic DLM 3–3·5 mm long. The space constants of fibres in the two muscles are around 200 μm and 400 μm respectively (see below), so a current electrode near the middle of a fibre is several space constants from either end. The fibres can therefore be treated as infinitely-long cables and no corrections are needed for cable termination effects.

The natural logarithm of the potential change due to injected current ($\ln \Delta V$) was plotted as a function of inter-electrode distance (Fig. 2). The ordinal intercept of the resulting straight line is the logarithm of the potential change at the current injection site; the slope of the line is the reciprocal of the space constant (i.e. λ^{-1}). Fibres that did not meet all of the following criteria were rejected from further analysis: (1) measurements of ΔV were obtained at 4 or more distances from the current electrode; (2) the resting potential recorded with the current electrode did not vary by more than 10 mV during the course of the experiments, and the resting potential at each voltage recording site was approximately the same as that recorded from the current electrode; and (3) the relation between $\ln \Delta V$ and inter-electrode distance was approximately linear and the absolute value of the linear correlation coefficient was greater than

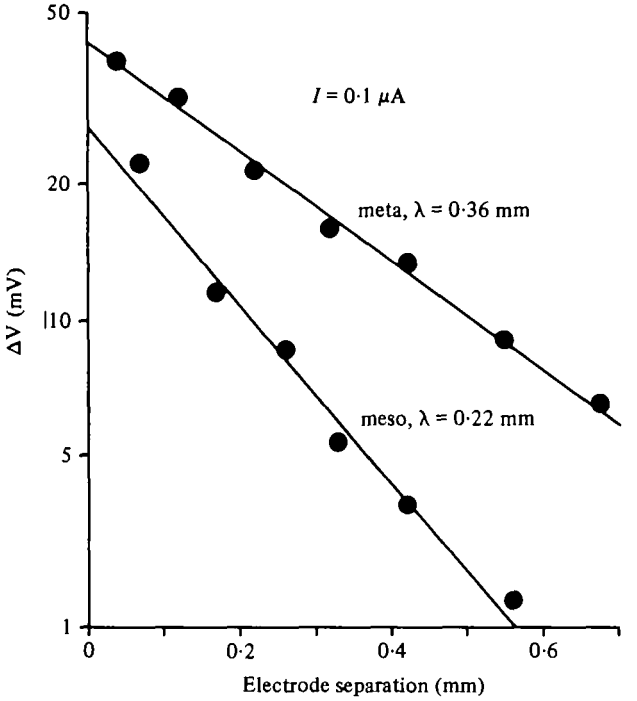

Fig. 2. Decline in the measured potential change to a hyperpolarizing current pulse with increasing distance between the current and voltage electrodes. The data shown are from a single mesothoracic fibre and a single metathoracic fibre of the same measured diameter (20 μm).

0·94. The apparent membrane resistance (R_m) and cytoplasmic resistivity (R_i) were calculated for fibres meeting these criteria using standard cable equations (e.g. Jack, Noble & Tsien, 1975).

The membrane time constant of a cable can be determined from the time course of the voltage change resulting from an imposed current pulse. It is convenient to discuss this in terms of the ratio between the voltage change at an elapsed time equal to one time constant and the asymptotic value of the voltage change, i.e. $\Delta V_{t=\tau}/\Delta V_{t=\infty}$. In a cable of infinite length the ratio is 0·84 at the current source and becomes smaller with increasing distance from the point of current injection; for example at a distance of one space constant the ratio is 0·63 (Hodgkin & Rushton, 1946). The minimum distance between current and voltage electrodes in the experiments being considered ranged from 60 to 140 μm, which is 26–62% of the average space constant for mesothoracic fibres and 15–34% of the average space constant for metathoracic fibres. To take this into account, the following procedure was adopted to determine the time constant of the DLM fibres. Values tabulated in Hodgkin & Rushton (1946) were used to plot a graph of $\Delta V_{t=\tau}/\Delta V_{t=\infty}$ against X/λ, where X is the inter-electrode distance and λ the space constant. For each fibre the minimal inter-electrode distance was expressed as a fraction of the space constant of that fibre. Using the constructed graph, the value of $\Delta V_{t=\tau}/\Delta V_{t=\infty}$ was determined for the minimal electrode distance. The time taken to reach this fraction of the total voltage

Table 2. *Passive electrical properties of DLM fibres from mesothoracic and metathoracic segments*

(Mean ± S.E. The abbreviations are: λ, space constant; R_{in}, input resistance; R_i, cytoplasmic resistivity; R_m, apparent specific membrane resistivity; τ, membrane time constant; C_m, apparent membrane capacitance.)

	Mesothoracic (n = 11)	Metathoracic (n = 12)
Fibre width (μm)	22·1 ± 1·4	23·6 ± 1·2
λ (μm)	227 ± 9	412 ± 27
R_{in} (kΩ)	256 ± 11	367 ± 35
R_i (Ω cm)	92 ± 10	90 ± 22
R_m (Ω cm^2)	84 ± 8	210 ± 13
τ (ms)	1·4 ± 0·1	2·1 ± 0·1
C_m (μF/cm^2)	16·5 ± 1·8	10·6 ± 1·2

Table 3. *E.J.P. duration in mesothoracic and metathoracic fibres, onset to 50% return*

(Mean ± S.E., sample size in parentheses. Each measurement was from a separate fibre. The difference between e.j.p. duration in mesothoracic and metathoracic fibres is statistically significant at each temperature ($P < 0{\cdot}01$, 2-tailed t-test).)

	Mesothoracic	Metathoracic
25°	2·35 ± 0·04 (152)	2·91 ± 0·05 (149)
35°	1·37 ± 0·02 (169)	1·65 ± 0·03 (61)

change, i.e. the time constant, was measured from oscilloscope photographs of the voltage response to an injected current pulse. For example, in one mesothoracic muscle fibre the minimal inter-electrode distance was 100 μm and the space constant 240 μm, thus the ratio of X/λ was 0·42. The value of $\Delta V_{t=\tau}/\Delta V_{t=\infty}$ corresponding to X/λ of 0·42 is 0·77. In this fibre the voltage change to an injected current pulse reached 0·77 of its asymptotic value in 1·3 ms; this then is the time constant of the fibre. Apparent membrane capacitance was calculated as the ratio of the time constant to the apparent membrane resistance. Values for the cable properties of mesothoracic and metathoracic fibres are summarized in Table 2.

Neurally evoked potentials

Stimulating the motor nerve to a DLM evoked a brief depolarization of the fibres of the muscle which approached and sometimes even overshot the zero potential level. The depolarization was frequently followed by a hyperpolarizing after potential (Fig. 4) which we attribute to the activation of voltage sensitive, outward current channels, probably potassium channels. The responses were slightly shorter in mesothoracic than in metathoracic fibres (Table 3). The neurally-evoked responses had a time course and shape rather like that of typical action potentials in nerve and muscle fibres. Nevertheless the responses are not action potentials but rather are almost entirely synaptic potentials, the muscle fibres lack significant voltage-dependent, inward current channels. This was shown earlier for mesothoracic fibres (Josephson *et al.* 1975) and was verified here for both mesothoracic and metathoracic fibres as follows. The current–voltage relation for both mesothoracic and metathoracic DLM fibres was linear for hyperpolarizing current pulses of all amplitudes tested (up to

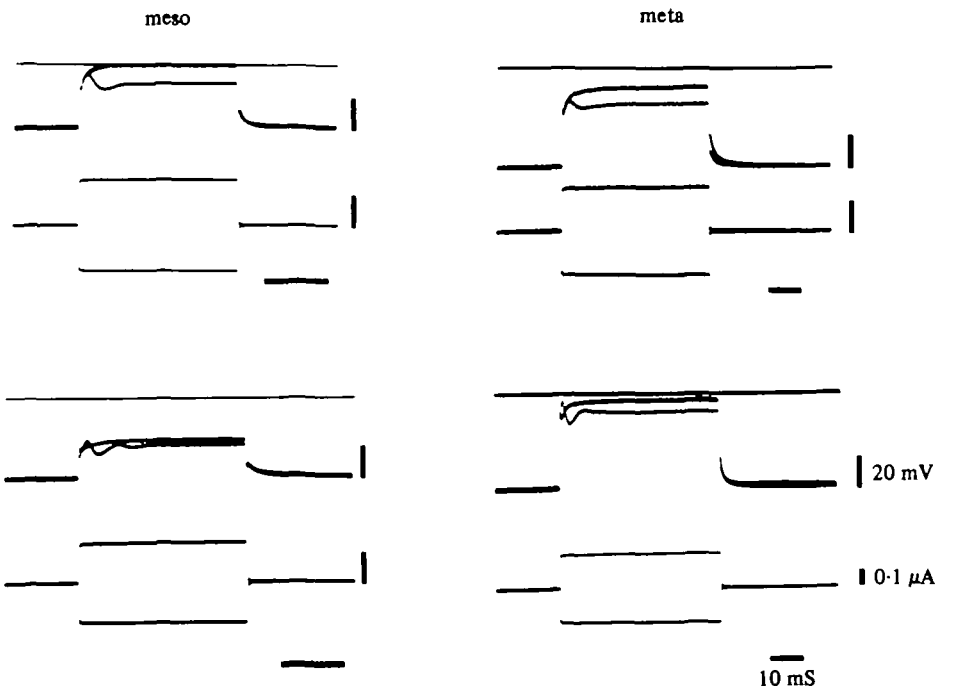

Fig. 3. Potential changes to hyperpolarizing and depolarizing current pulses in mesothoracic and metathoracic fibres, T = 25 °C. The current and voltage electrodes were separated by less than 0·1 mm. In each set the response to a hyperpolarizing current pulse was superimposed on that to a depolarizing pulse by inverting the voltage display before the hyperpolarizing pulse and repositioning the display so that the traces were aligned when no current was injected. The upper sets were selected as examples in which the absolute magnitude of the depolarizing response was never greater than that of the hyperpolarizing response. The lower sets show instances in which the initial peak of the depolarizing response was greater than the hyperpolarizing response at an equivalent time during the current pulse.

0·5 μA) and for small depolarizing current pulses (see Fig. 8 in Josephson *et al.* 1975). In some fibres the response to large depolarizing current pulses was also linear and essentially the mirror image of responses to hyperpolarizing currents of the same amplitude. In most fibres, however, the voltage change to a large depolarizing current pulse had an initial peak followed by a partial return toward the resting level (Fig. 3). In mesothoracic but not metathoracic fibres the initial peak was often part of a series of damped oscillations. In many mesothoracic and metathoracic fibres the potential at the initial peak was no greater than would be predicted from the response at the same time to a hyperpolarizing current pulse of the same amplitude (Fig. 3, upper). In such fibres the initial voltage peak seemed to be due entirely to a delayed conductance increase whose onset terminated the voltage rise. In other fibres the initial peak was somewhat greater than would be predicted from the passive electrical properties of the fibres (Fig. 3, lower). Here there may be contributions from voltage-dependent, inward current channels. But the increase in potential at the peak over that expected from the passive electrical properties of the fibres was never very great; electrically excitable, depolarizing electrogenesis is not a significant membrane

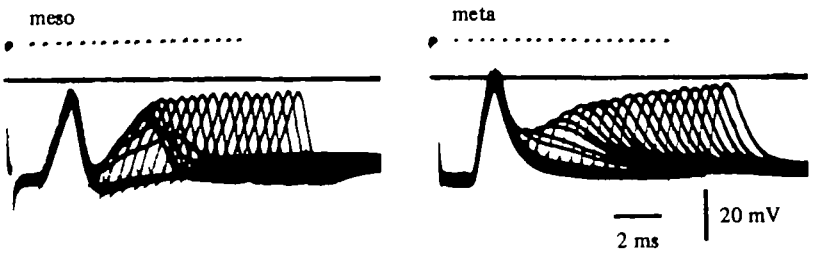

Fig. 4. Excitatory junction potentials to paired stimuli at varied interstimulus intervals, $T = 35$ °C. The upper dots mark the times at which the nerve was stimulated. The straight line is the zero potential level for the e.j.p.'s below. In the mesothoracic fibre the e.j.p. at the shortest effective interstimulus interval is the smallest of the series and is delayed relative to e.j.p.'s at somewhat longer intervals.

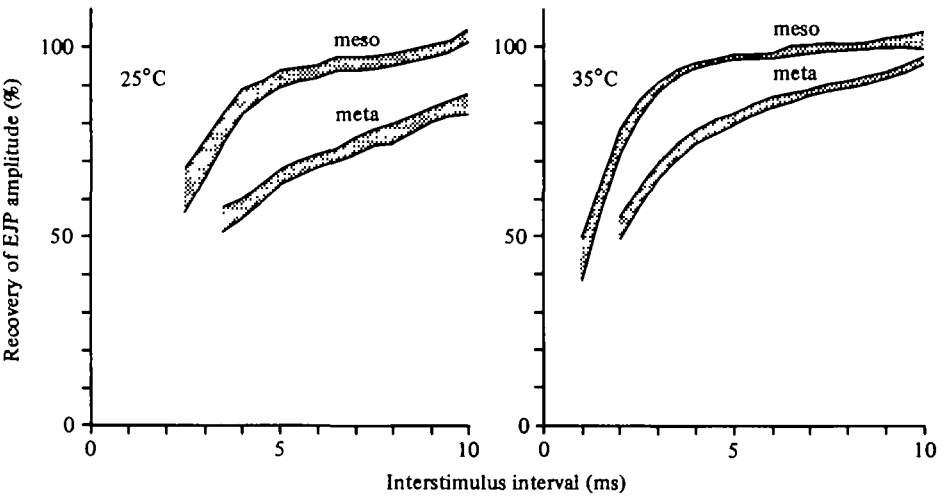

Fig. 5. Amplitude of the second e.j.p. of a pair relative to that of the first e.j.p. at differing interstimulus intervals. The shaded area is the mean ± S.E., $n = 10$.

property in these muscles. Therefore the large responses to nerve stimulation must be excitatory junctional potentials (e.j.p.'s) and not electrically-excited action potentials.

Refractoriness of neuromuscular transmission

If the motor nerve to the DLM was stimulated twice within a short interval, the amplitude of the second e.j.p. was depressed relative to that of the first response, and the depression was greater the shorter the interstimulus interval (Fig. 4). The reduction in the second e.j.p. was smoothly graded with decreasing interstimulus interval and there was no hint of an abrupt loss of an all-or-nothing component. This is further evidence that the neurally-evoked responses are solely e.j.p.'s. Certainly there was no evidence for regenerative action potentials as have been found in some insect muscle fibres (e.g. Washio, 1972; Deitmer, 1977).

The time course of e.j.p. depression was investigated with paired shocks at interstimulus intervals which were varied in 0·5 ms increments. E.j.p.'s were recorded with

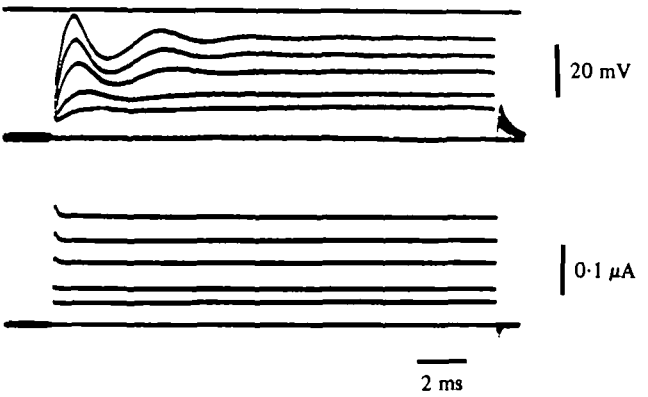

Fig. 6. Oscillatory voltage changes in a mesothoracic DLM fibre to long, depolarizing current pulses, $T = 35$ °C. The upper line is the zero potential level for the voltage traces below.

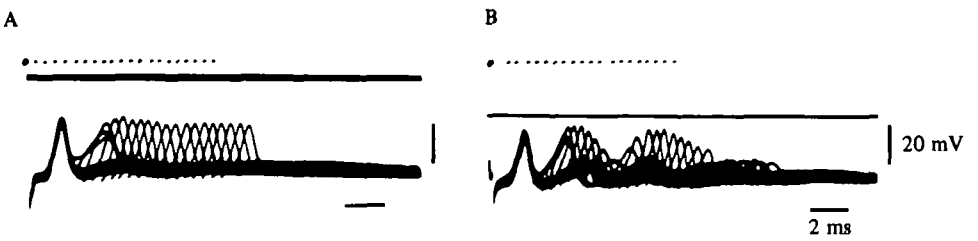

Fig. 7. E.j.p.'s in mesothoracic DLM fibres to paired stimuli at varied interstimulus intervals, $T = 35$ °C. The upper dots mark the stimuli, the straight line is the zero potential level for the e.j.p's below.

a floating microelectrode. The following criteria were used in selecting records for analysis: (1) The resting potential remained stable and the e.j.p. to the first stimulus remained of consistent size and shape through the series. (2) The records did not contain obvious movement artifact. Often recordings made with a floating microelectrode include a slower potential change following the e.j.p. This second potential is thought to be a movement artifact because it is coincident with muscle contraction and has about the same time course as does a twitch. (3) The increase in e.j.p. amplitude with increasing interstimulus interval was monotonic. In some mesothoracic fibres the peak amplitude of the second e.j.p. of a pair waxed and waned in a regular manner as the interstimulus interval was progressively lengthened (Fig. 7). This oscillation in amplitude is considered further below. (4) E.j.p.'s were obtainable with stimuli separated by 3·5 ms or less at 25 °C and 2 ms or less at 35 °C. In some preparations e.j.p.'s ceased abruptly at interstimulus intervals of 4–5 ms even though the e.j.p. at the shortest effective interval was still relatively large. Such abrupt failure is probably due to axonal refractoriness rather than depressed neuromuscular transmission.

Data from the fibres meeting the selection criteria are summarized in Fig. 5. The time axis in Fig. 5 is based on interstimulus interval rather than inter-e.j.p. interval as measured, for example, by the interval between e.j.p. peaks, because in meso-

thoracic fibres e.j.p.'s to the second stimulus may be inordinately delayed at short interstimulus intervals (Fig. 4). Thus the peak of the reduced e.j.p. to the second of two closely-spaced stimuli may actually occur later than the peaks of larger e.j.p.'s to more delayed stimuli. Several features emerge from Fig. 5. First, the recovery of e.j.p. amplitude following an initial response was more rapid at 35 °C than at 25 °C. Second, at either temperature recovery was significantly faster in mesothoracic than in metathoracic fibres. Finally, the recovery was remarkably fast in mesothoracic fibres; at 35 °C responses initiated at an interstimulus interval of 3 ms were already 90% as large as those to single stimuli.

Responses of mesothoracic fibres to paired current pulses

The reduced size of the second e.j.p. of a pair at short inter-stimulus intervals could be due to an increase in membrane conductance resulting from the opening of voltage sensitive, ionic channels during the first e.j.p. This possibility was examined by comparing the voltage responses of mesothoracic fibres at 35 °C to the first and second pulses of a pair of 2 ms, depolarizing current pulses separated by 0·5 ms. Current amplitude was sufficient to drive the membrane potential nearly to or slightly beyond zero during the first current pulse. The voltage response to the first current pulse was a shortened version of the response to long current pulses (e.g. Fig. 3); the potential reached a peak and began to fall during the current pulse. The voltage change to the second current pulse had a very rapid rise which was obscured by a capacitive coupling artifact between current and voltage electrodes. Subsequently the potential either fell slightly before rising or rose monotonically throughout the current pulse. In the seven fibres examined, in which the average depolarization to the first current pulse was 46 mV to an average peak potential of +6 mV, the average ratio of the peak depolarization during the second current pulse to that during the first was 0·90 (S.D. = 0·05). The minimum potential change during the second pulse after the coupling artifact averaged 0·73 (S.D. = 0·14) of the peak depolarization during the first pulse. Thus, the entire range of the amplitude of the second voltage response, excluding the very onset which was lost in the capacitive transient, lay between 70 and 90% of the peak response to the first pulse. This compares with a reduction of e.j.p. amplitude of more than 50% at short interstimulus intervals in mesothoracic fibres at the same temperature (Fig. 5). The conclusion is that most of the e.j.p. depression at short interstimulus intervals cannot be accounted for on the basis of a persistent, depolarization-induced increase in membrane conductance. The depression could be due to receptor desensitization or to reduction in transmitter release.

Oscillatory responses of mesothoracic fibres

(1) *Oscillations to long current pulses.* The potential change in a metathoracic DLM fibre to a depolarizing current pulse of 100–300 nA was either a monotonic depolarization or, as in the responses shown in Fig. 3, an initial depolarizing peak followed by a partial return to the resting potential. Similar responses could be recorded from some mesothoracic fibres in response to large depolarizing current pulses, but in over half the mesothoracic fibres examined the response was a damped potential oscillation

(Fig. 6). The amplitude of the oscillations, and to a lesser extent their frequency, increased with increasing current amplitude.

The oscillatory responses of mesothoracic fibres suggested that the membranes have a resonant frequency, and should be most responsive to inputs at that frequency. The oscillation frequency for mesothoracic fibres was determined for current pulses producing an initial depolarization close to the zero potential level because the peak of an e.j.p. is typically close to zero potential. Only fibres giving oscillations with a pronounced second peak were considered. In 19 fibres examined at 35 °C and in which the potential at the initial depolarizing peak averaged −4·4 mV (S.D. = 7·8 mV, range = +2 to −22 mV), the average interval between the first and second peaks of the voltage response averaged 3·5 ms (S.D. = 0·6 ms) which is equivalent to an oscillation frequency of 286 Hz. The equivalent oscillation frequency calculated separately for each fibre averaged 294 Hz. For 18 fibres at 25 °C with an average potential at the depolarized peak of −7·1 mV (S.D. = 6·7 mV, range = +2 to −19 mV), the average interval between the first and second voltage peaks was 5·4 ms (S.D. = 0·5 ms), equivalent to a frequency of 185 Hz.

(2) *Oscillation of e.j.p. amplitude.* Mesothoracic fibres in which paired e.j.p.'s were examined were classifiable into three response types on the basis of the recovery of e.j.p. amplitude with time following an initial e.j.p.: type 1, in which there was a continual increase to a plateau in e.j.p. amplitude with increasing inter-stimulus interval (Fig. 4); type 2, in which there was a small but discernible peak in e.j.p. amplitude with increasing inter-stimulus interval (Fig. 7*A*); and type 3, in which there was a pronounced early peak often followed by smaller peaks at still longer inter-stimulus intervals (Fig. 7*B*). Metathoracic fibres were all type 1. Forty-four mesothoracic fibres from 14 animals were tested with paired stimuli at 25 °C. Of these, 82% were of type 1, 9% were of type 2, and four fibres could not be classified because their resting potentials were unstable and the e.j.p. amplitudes, including those of the priming e.j.p.'s, varied erratically, presumably because of incomplete sealing about the recording electrode. The responses of 110 fibres from 17 animals were examined at 35 °C. Forty-three per cent were type 1, 29% were type 2, 12% were type 3, and 26 fibres could not be classified because of the variability in priming and test e.j.p.'s. The fibres giving oscillatory responses tended to have somewhat lower resting potentials than those that did not. In the fibres examined at 35°, the resting potential averaged 51·9 mV (S.E. = 1·0 mV) in type 1 fibres, 47·7 mV (S.E. = 1·6 mV) in type 2 fibres, and 42·1 mV (S.E. = 1·5 mV) in type 3 fibres. These differences in mean resting potential between fibre types are statistically significant ($P < 0{\cdot}05$).

In those type 2 and type 3 fibres for which the envelope formed by the peaks of the second responses had a sufficiently pronounced maximum that its time of occurrence could be measured with some accuracy, the interval between the peak of the first e.j.p. and the early maximum in the amplitude of following e.j.p.'s averaged 5·4 ms (S.D. = 0·8 ms, $n = 4$) at 25 °C and 3·9 ms (S.D. = 1·1 ms, $n = 36$) at 35 °C. These values correspond to frequencies of 185 Hz and 256 Hz respectively. In five type 3 fibres at 35 °C the interval between the first and second maxima in e.j.p. amplitude was measurable, and averaged 4·6 ms (S.D. = 0·4 ms) which corresponds to a frequency of 217 Hz.

DISCUSSION

Passive electrical properties

There are striking differences between the passive electrical properties of fibres from the mesothoracic DLM, which are used in stridulation and flight, and those of the metathoracic DLM which are used only in flight (Table 2). Although the fibres of both muscles are of similar diameter and have essentially identical sarcoplasmic resistivity, mesothoracic fibres have a significantly shorter space constant and a lower input resistance than do metathoracic fibres. This is a consequence of the much lower apparent membrane resistivity in mesothoracic fibres. The time constant of mesothoracic fibres is shorter than that of metathoracic fibres even though the apparent membrane capacitance of mesothoracic fibres is greater than that of metathoracic ones. The short time constant is also a consequence of the low membrane resistivity of the mesothoracic fibres. Of the differences in passive electrical properties between the two types of fibres, it is probably the short time constant of the mesothoracic fibres which is of greatest functional significance. The short resting time constant of stridulatory muscle fibres, augmented by a delayed conductance increase with depolarization (cf. Fig. 3), facilitates rapid charging and discharging of the membrane capacitance and contributes to the brevity of the synaptic potentials (Table 3). It is, of course, necessary that individual membrane depolarizations be brief for the stridulatory muscle to operate at the high frequencies which it achieves.

The values reported here for the passive electrical properties of mesothoracic DLM fibres are somewhat different from those given in an earlier study (Josephson *et al.* 1975). In the earlier study the passive electrical properties were calculated from: (1) an estimate of cytoplasmic resistivity based on the increase in resistive coupling between the two channels of a double-barrel electrode upon insertion of the electrode into a muscle fibre; (2) the input resistance, determined as the ratio of change in membrane potential to the amplitude of injected current pulses using intracellular current and voltage electrodes separated by 20–200 μm; (3) the voltage rise time during injected current pulses; and (4) the average fibre circumference and cross-sectional area determined from fixed and sectioned muscles. The average values in the earlier study, followed in parentheses by those from the present study for comparison, were: fibre circumference, 167 μm (69 μm assuming cylindrical geometry); input resistance, R_{in}, 191 kΩ (256 kΩ); sarcoplasmic resistivity, R_i, 248 Ω cm (92 Ω cm); membrane resistance, R_m, 162 Ω cm^2 (84 Ω cm^2); time constant, τ, 1·5 ms (1·4 ms). The difference between the sarcoplasmic resistivity obtained earlier and the newer value is unexplained; the earlier method should have given reasonable values. The fibre widths, and therefore fibre circumferences, determined from dye-filled, living mesothoracic fibres, are smaller than those reported earlier. The dye-filled fibres were all on the lateral edge of the muscle; the earlier measurements were made from fibres selected randomly from throughout the muscle. The difference between the new and old values may indicate that there is a lateral to medial gradient in fibre size across the DLM. The earlier value for input resistance was an underestimate because it was based on measurements made at electrode separations which were significant fractions of a space constant. The differences in measured values for

input resistance, fibre diameter, and sarcoplasmic resistivity together contribute to the difference in apparent membrane resistance between this and the earlier study. It should be emphasized that the newer, and presumably better, values emphasize rather than change the principal conclusion in the earlier study about the passive electrical properties; namely that in fibres of this unusually fast muscle the apparent membrane resistivity is unusually low and, as a consequence, the membrane time constant has an unusually short value for a muscle fibre.

The apparent membrane capacitance of fibres from either the mesothoracic or metathoracic DLM of *N. robustus* lies within the range of values previously reported for the capacitance of insect skeletal muscle fibres, which extends from about 5 to over 20 μF/cm^2 (Malpus, 1968; Deitmer, 1977). However, the apparent membrane resistances of both mesothoracic and metathoracic DLM fibres of *N. robustus* are distinctly lower than any yet reported for insect skeletal muscle (range about 500 Ω cm^2 to several kΩ cm^2; Yamaguchi, Lockshin & Woodward, 1972; Patlak, 1976; Ashcroft, 1980). The wing muscles of *N. robustus* are the first synchronous flight muscles of an insect for which data is available on passive electrical properties. As synchronous wing muscles, both the mesothoracic and metathoracic DLM's would be expected to produce faster twitches than the other insect muscles which have been examined which are body wall muscles, leg muscles, or asynchronous flight muscles whose neurally-evoked, non-oscillatory contractions are presumably quite slow (cf. Josephson & Young, 1981). As indicated above, a low effective membrane resistivity, and therefore a short time constant, would be advantageous for a muscle producing brief twitches. A direct relation between twitch duration, apparent membrane resistance, and membrane time constant has also been found in comparisons of fast and slow muscle fibres of crabs (Atwood, 1963), frogs (Adrian & Peachey, 1965), and mice (Luff & Atwood, 1972) so this may be a general phenomenon in striated muscles.

Twitches of the mesothoracic DLM are much shorter than those of the metathoracic muscle (Table 1) and, associated with this, mesothoracic wing muscle fibres in *N. robustus* have more abundant sarcoplasmic reticulum than do metathoracic wing muscle fibres (Elder, 1971). It has not yet been examined directly but it is likely that the T-tubular system is better developed in mesothoracic than in metathoracic fibres. A more elaborate T-system could account, at least in part, for the differences in electrical properties between mesothoracic and metathoracic fibres since an increase in the area of T-tubule membrane should increase apparent membrane capacitance and decrease the apparent membrane resistance (e.g. Jack *et al.* 1975). If 1 μF/cm^2 is allowed for the capacitance of the surface membrane (cf. Schneider, 1970; Valdiosera, Clausen & Eisenberg, 1974), the portion of the total capacitance ascribable to the T-system is 15·5 μF per cm^2 of fibre surface in mesothoracic fibres and 9·6 μF per cm^2 for metathoracic fibres. Assuming that the T-tubule capacitance is proportional to the surface area of the T-tubules, the total area of T-tubule membrane in a given volume of cytoplasm should be 1·6 times greater in mesothoracic fibres than in metathoracic ones. The ratio of apparent membrane conductance ($= 1/R_m$) for mesothoracic and metathoracic fibres is 2·5 which is larger than the predicted ratio for T-tubule area or total membrane area. This suggests that the specific membrane resistivity is lower in mesothoracic fibres than in metathoracic ones, in addition to there being greater membrane area in mesothoracic fibres.

Other features of mesothoracic muscles related to high-frequency performance

In addition to having different ultrastructure, contraction kinetics, and passive electrical properties, mesothoracic and metathoracic DLM muscle fibres differ in the rate of recovery of the second e.j.p. of a pair as a function of interstimulus interval (Fig. 5) and in that responses of mesothoracic fibres can be oscillatory, both to imposed current and to neural inputs (Figs. 6, 7). Part, but only part, of the depression of the second e.j.p. of a pair can be accounted for on the basis of a slowly-recovering, increased membrane conductance initiated by the preceding depolarization. Much of the e.j.p. depression must be due to desensitization of the post-synaptic receptors for the neuromuscular transmitter or to decreased transmitter release from the presynaptic terminals. Depressed transmitter release has been observed in mammalian muscles, where the depression of the second e.j.p. of a pair is due to reduced quantal content of the e.j.p. to the second stimulus (Thies, 1965). The amount of transmitter released per impulse depends on conditions in the presynaptic terminal and the extent to which the terminals are invaded by action potentials. If, as seems likely, the reduction in e.j.p. amplitude at very short inter-stimulus intervals in tettigoniid muscles is of pre-synaptic origin, the more rapid recovery in mesothoracic fibres indicates that the mechanisms controlling transmitter release recover more quickly in mesothoracic nerve terminals than in metathoracic ones. This would mean that the motoneurones to the mesothoracic DLM as well as the fibres of the DLM are modified for high-frequency performance. It should be noted that in both mesothoracic and metathoracic muscles the recovery of e.j.p. amplitude following a conditioning response is essentially complete in 10 ms or less at 35 °C (Fig. 5), much less than depression lasting more than a second in a similar two-shock experiment with guinea pig muscles (Thies, 1965). E.j.p. recovery is also rapid in leg muscles of the grasshopper *Romalea microptera*, where the response to the second shock of a pair is essentially the same size as that to the conditioning shock at interstimulus intervals greater than about 20 ms at 22–26 °C (Cerf *et al.* 1959).

Damped, oscillatory potential changes during imposed current pulses, like those in mesothoracic DLM fibres of *N. robustus*, have been found in fibres of a number of arthropod limb, wing and body wall muscles (e.g. Fatt & Katz, 1953; Washio, 1972; Patlak, 1976; Deitmer, 1977). These damped oscillations presumably arise from cyclic conductance changes which result in the membrane acting like a partially resonant circuit (for analysis of a similar phenomenon in squid axons see Mauro *et al.* 1970). Periodic inputs should be most effective in driving such a system when the input frequency matches the resonant frequency. Oscillatory responses to depolarizing current pulses occur in mesothoracic but not metathoracic DLM fibres of *N. robustus*, suggesting that the oscillatory behaviour is related to stridulation. Surprisingly, the frequency of electrical oscillations (about 290 Hz, 35°) is distinctly higher than the stridulatory frequency (about 200 Hz). The voltage change to injected current is spatially non-uniform, being greatest at the current injection site. It might be argued that the high oscillation frequency is somehow a consequence of the spatial gradient in electrical potential change. The oscillatory responses seen with paired e.j.p.'s suggest that this is not the case. E.j.p.'s probably depolarize the muscle uniformly. The enve-

lope formed by the second e.j.p. of a pair at varied interstimulus intervals is oscillatory in some mesothoracic fibres (Fig. 7), probably for the same reasons that voltage responses to injected current are oscillatory. However, it is possible that transmitter release following a priming stimulus is itself oscillatory, as has been suggested for neuromuscular transmission at a locust leg muscle (Usherwood, 1972), in which case the oscillation in e.j.p. amplitude would be at least partially of presynaptic origin. The oscillatory frequency for e.j.p. amplitude (256 Hz, at 35 °C), like the oscillation frequency to imposed current, is higher than the stridulation frequency. Thus, for some reason, neuromuscular transmission to the stridulatory muscle, like the muscle fibres themselves, is tuned to a higher frequency than the normal contraction frequency.

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