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NEUROMUSCULAR PHYSIOLOGY OF THE CLOSER MUSCLES IN THE PEDIPALP OF THE SCORPION *LEIURUS QUINQUESTRIATUS*

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INTRODUCTION

Most of the studies on neuromuscular systems in arthropods have been made on crustaceans (for review cf. Atwood, 1967*a*) and insects (for review cf. Usherwood, 1967). Only a few studies have been made on spiders (Rathmayer, 1965) and *Limulus* (Hoyle, 1958; Parnas *et al.* 1968). Very little knowledge is available on the properties of neuromuscular systems of lower arachnids, and to the best of our knowledge only one electrophysiological study has been made on the neuromuscular physiology of scorpions (Rao, 1964).

The claw of the scorpion is similar to the claw of crustaceans and *Limulus*. In both the latter the movement of the claw is controlled by a pair of antagonistic muscles (closer and opener). But the scorpion has only a set of agonistic closer muscles (Snodgrass, 1952) and the opening of the claw is passive (Mathew, 1965). It was suggested by Hoyle & Smyth (1963) and Rathmayer (1965) that peripheral inhibition in arthropods is found only in systems composed of antagonistic muscles. Indeed, no peripheral inhibition was found in the *flexor metatarsi bilobatus* muscle of *Eurypelma* (Rathmayer, 1965). In this respect, the closer muscle of the *Limulus* claw was considered to be an exception because Hoyle (1958) was unable to demonstrate inhibition. Recently, it was found that the closer muscle in the *Limulus* claw receives inhibitory input (Parnas *et al.* 1968). The scorpion claw thus represents a special anatomical situation in which the hypothesis of Hoyle & Smyth (1963) and Rathmayer (1965) could be tested.

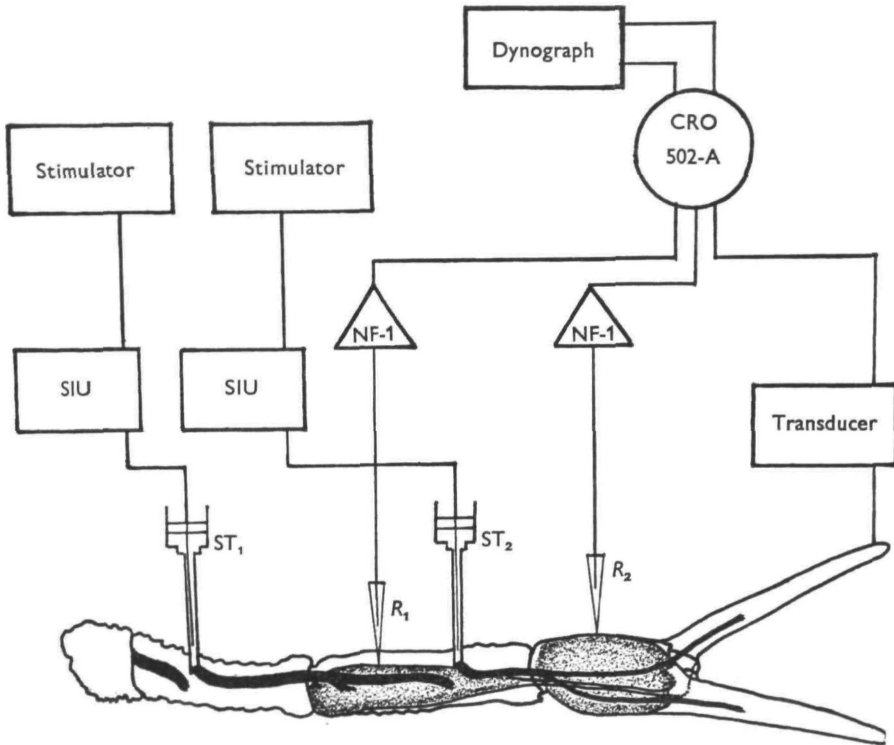
In the present study we have examined the neuromuscular physiology of the closer muscles in the pedipalp of the scorpion *Leiurus quinquestriatus* H. et E. (Buthidae; Scorpiones). The pattern and properties of excitatory inputs are described. It was not possible to demonstrate either the presence of inhibitory inputs or any effect of possible transmitters such as glutamate or GABA.

MATERIALS AND METHODS

Animals. The scorpions were collected in the southern part of the Judean desert. Each scorpion was kept separately at a temperature of 20–28° C and was fed once a week with maggots and water.

Preparation. A schematic representation of the preparation and the general assembly of the apparatus is given in Text-fig. 1. The closer muscles of the isolated pedipalp

claw were used. The closer muscles and the pedipalp nerve were exposed (Text-figs. 1, 2) and the pedipalp nerve was cut at the level of the patella in order to stimulate each closer muscle separately (Text-fig. 1). The nerve was stimulated with a pulse of 0·1 msec. duration through a suction electrode. Intracellular recording was accomplished with glass microelectrodes connected to a negative capacity amplifier (NF-1, Bioelectric Instruments); the microelectrodes were filled with 3 M-KCl and had a resistance of 10–20 MΩ. To reduce movement artifacts intracellular recordings were made with floating electrodes (Woodbury & Brady, 1956).



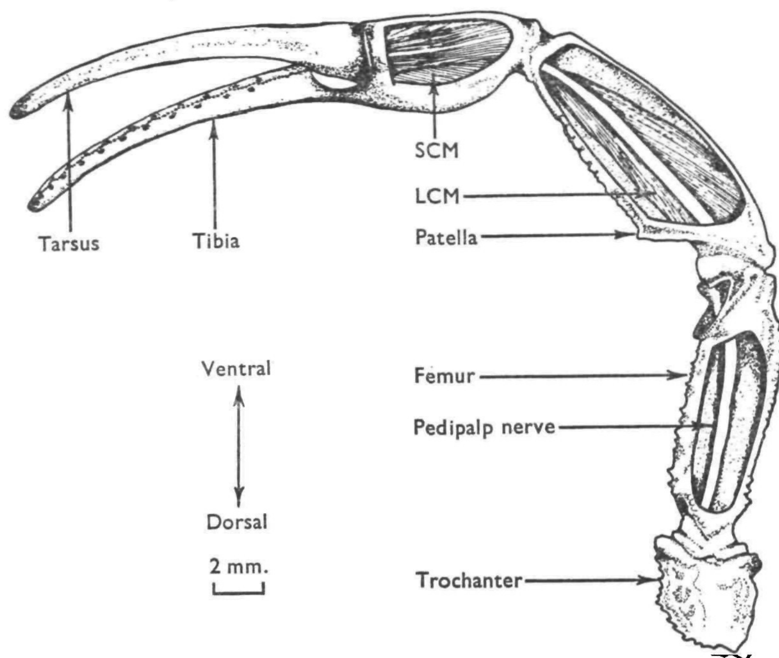
Text-fig. 1. Schematic representation of the preparation and apparatus. R_1, R_2, Floating microelectrodes for intracellular recording. ST_1, ST_2, Suction electrodes for nerve stimulation. NF-1, Amplifier. SIU, Stimulus isolation unit.

Intracellular currents were applied with microelectrodes filled with 1 M Na-citrate. Current–voltage curves were obtained directly with an oscilloscope used as an X–Y recorder. Tension was recorded by connecting the tarsus to an RCA 5734 mechano-electrical transducer.

Solutions. Known saline solutions for other scorpions (Kanungo, 1955; Zwicky, 1967; Padmanabhanaida, 1967) were tried. These solutions were found to be unsatisfactory as the preparation lasted less than half an hour. The ionic composition of the hemolymph of *Leiurus* was determined by flame photometry and the osmotic pressure was measured cryoscopically. A saline solution for *Leiurus* was composed of the following chloride salts: Na^+ 250 mM/l., K^+ 7·7 mM/l. Ca^{2+} 5·0 mM/l., Mg^{2+} 1·0 mM/l. The

pH was adjusted to 7·0 with 0·1 M-HCl or NaOH. In this saline solution the muscle membrane potential was found to be 62 ± 5 mV. and the preparation lasted for several hours.

Histological preparations: The muscles were fixed in Bouin's or Gilson's fluid while held at rest length, and subsequently embedded in paraffin, sectioned (8 μ) and stained with hematoxylin-eosin.



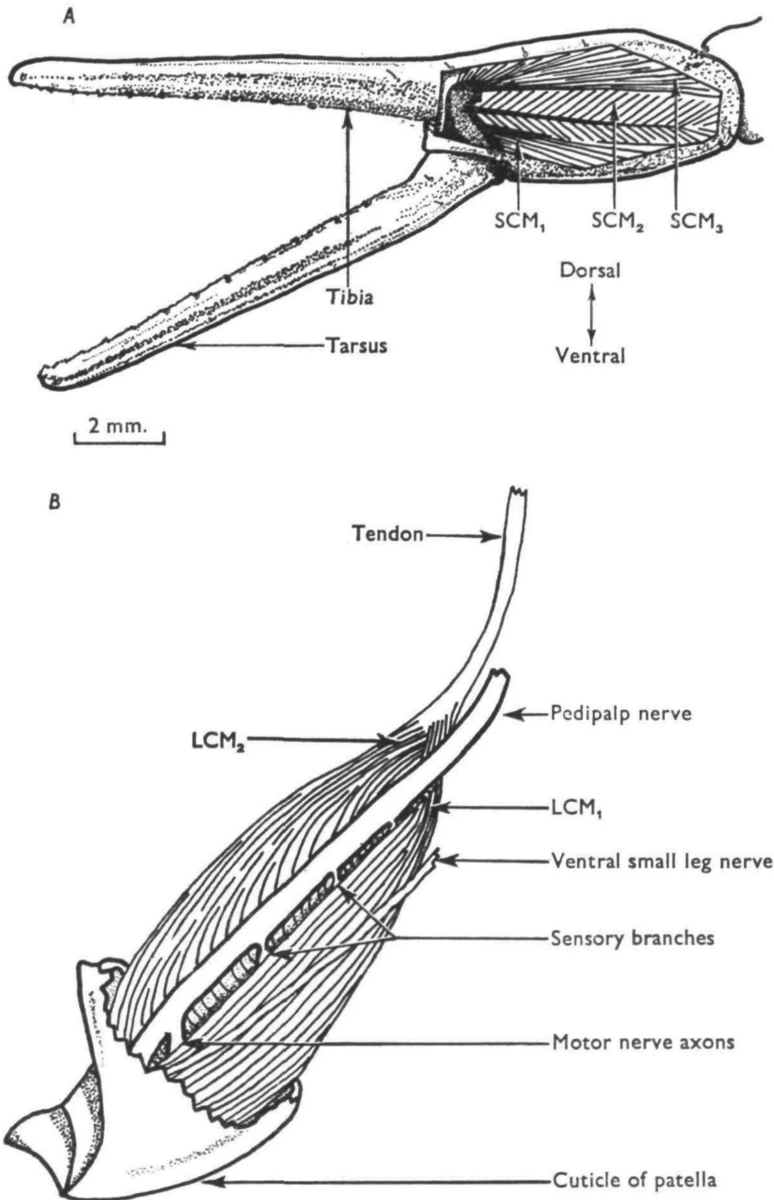
Text-fig. 2. Left pedipalp of the scorpion, ventral aspect. The cuticle was removed in order to show the main pedipalp nerve and the tarsus closer muscles. LCM, Long closer muscle. SCM, Short closer muscle.

RESULTS

Anatomical and histological observations

The muscular apparatus for closing of the claw in *Leiurus quinquestriatus* has not been previously described. In order to clarify the physiological data a short account of the anatomy and histology of these muscles is given. The closer system in the scorpion's pedipalp is composed of two agonistic muscles. One muscle is situated in the patella and the second in the tibia (Text-figs. 1, 2). The fibres of the muscle located in the patella are connected proximally directly to the cuticle. The fibres extend approximately the whole length of the patella and converge on a long tendon which passes through the tibia to connect to the tarsus. This muscle, which was termed the long closer muscle (LCM), is divided into two main bundles: the LCM_1 and LCM_2 (Text-fig. 3*B*). It was thus possible to cut the long tendon from its attachment and to connect it directly to the mechanical transducer to record tension produced by the LCM alone. In such preparations recording of tension from the tarsus represents tension contributed only by the muscle located in the tibia. The second muscle (Text-fig. 3*A*), which was called the short closer muscle (SCM), is divided into three

main bundles: SCM_1, SCM_2 and SCM_3. All of the fibres are connected at the proximal side to the cuticle. The upper and lower bundles (SCM_1 and $_3$) connect directly to the tarsal lever. The fibres of the central bundle are arranged in a pinnate form and are connected through a tendon to the tarsus (Pl. 1).



Text-fig. 3. (*A*) The tibial and tarsal segments of a left pedipalp. The lateral cuticle is removed to show the arrangement at the short closer muscle (SCM) bundles. The fibres are connected at the proximal side to the cuticle and at the distal side to the tarsus. (*B*) Schematic drawing of the long closer muscle of the tarsus which is situated in the patella. The tendon is cut at the tibia level. Two main bundles of muscle fibres (LCM_1; LCM_2) are connected to the same tendon. The main pedipalp nerve branches along the muscle.

The main nerve (pedipalp nerve in Text-fig. 2) passes ventrally to the long closer muscle. At the base of the patella the motor axons to the LCM branch off and penetrate the muscle. Nerve branches composed exclusively of sensory fibres also penetrate the muscle along its length (Text-fig. 3*B*). This anatomical arrangement made it possible to cut the nerve distally to the motor branch to the LCM, so that each muscle could be stimulated separately (Text-fig. 1).

Previous work on crustacean muscles (Cohen, 1963; Dorai-Raj, 1964; Atwood, 1967*a*) has shown that individual fibres in the same muscle can differ in appearance or sarcomere length. It was suggested that tonic fibres have longer sarcomeres (8–12 μ) and the filaments are arranged in a 'Felderstruktur' while the phasic fibres have short sarcomeres (2–4 μ) and the contractile material is densely packed (Atwood & Dorai-Raj, 1964; Kennedy & Takeda, 1965; Parnas & Atwood, 1966*a*; Hoyle, 1969). Such diversity in fibre structure was not found in the closer muscles of the scorpion. The fibres appeared uniform in both longitudinal and transverse sections. In Pl. 1 *B*, *C* longitudinal and cross sections of peripheral fibres of the LCM are presented. The fibres are tubular, their diameter varying between 50 and 70 μ. The filaments are arranged as a ring in the circumference. The nuclei are packed in the centre. Sarcomere length was determined as 3–4 μ at rest. Although fibres with long sarcomeres were looked for, they were not found. The fibres of the SCM showed generally the same features; their pinnate organization is shown in Pl. 1, *A*. It can also be seen from Pl. 1, *A*, *C* that the cell boundaries were clearly defined and no regions of close contacts or oppositions between adjacent cells (as in the lobster: Parnas & Atwood, 1966*a*) could be observed. Because of the clear boundaries of the cells no difficulty was encountered in the insertion of two microelectrodes into the same fibre.

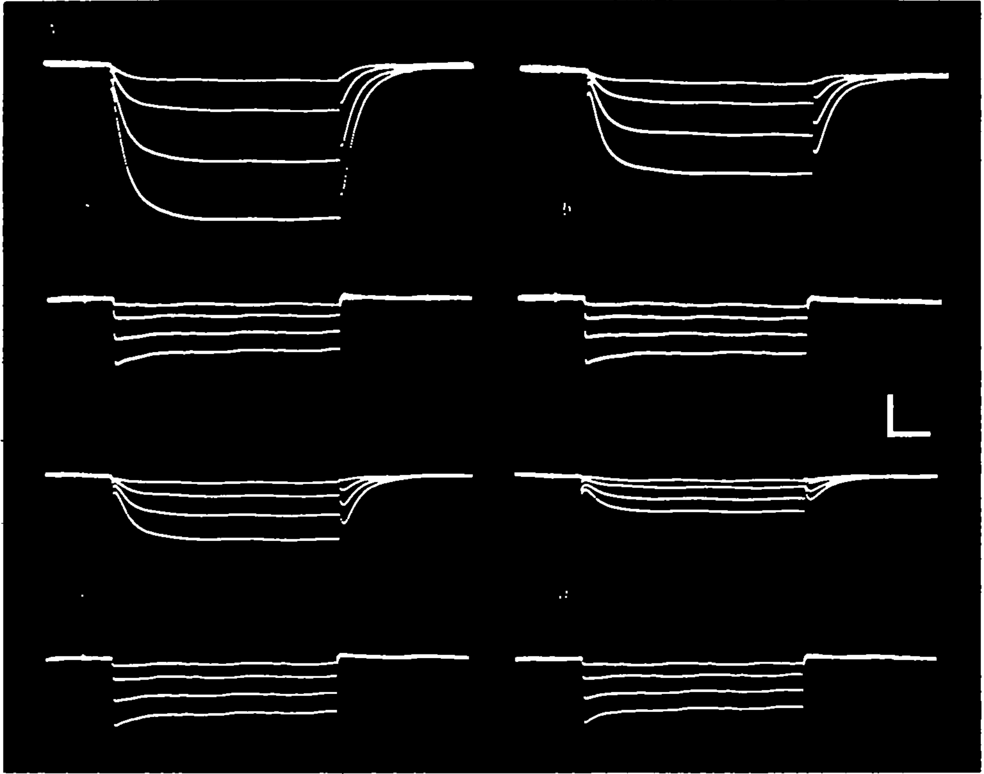
Determination of cable constants

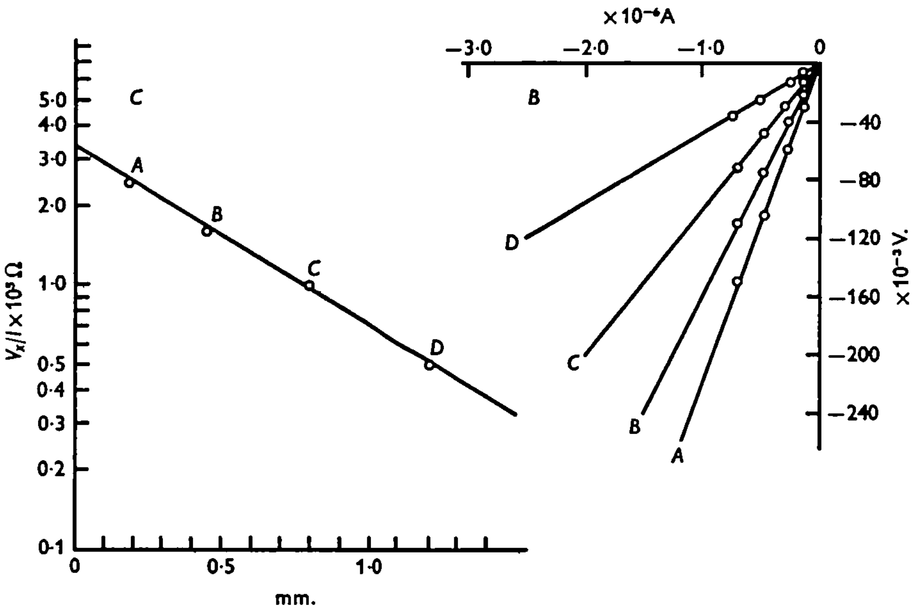
Two microelectrodes were inserted into the same muscle fibre. One electrode served for passing current, the second for recording membrane potential. Text-fig. 4*A* shows the membrane responses (upper traces) and the respective currents (lower traces) recorded at different distances from the current-passing electrode. At each recording point the responses to four hyperpolarizing current intensities were recorded. The space constant was calculated by the method described in detail by Bureš, Petráň & Zachar (1967) and using the equation:

$$\frac{V_x}{I_0} = \tfrac{1}{2}\sqrt{(r_i\, r_m)}\, \exp - \frac{x}{\lambda},$$

where V_x is the voltage recorded at the distance x for a current I_0; r_i is the internal resistance in Ω/mm.; r_m is the membrane resistance in Ω mm., and λ is the space constant in mm. $\frac{1}{2}\sqrt{(r_i r_m)}$ is the input resistance at the distance $x = 0$. The input resistances for Text-fig. 4*A* were plotted in Text-fig. 4*B* and λ was calculated from the semi-log plot of Text-fig. 4*C*. Plotting the input resistance at each point against the distance (Text-fig. 4*C*) gave a straight line from whose slope λ could be calculated.

The specific internal and membrane resistances could be calculated from $Ri = r_i \pi a^2$ and $R_m = r_m\, 2\pi a$, when a is the measured fibre diameter.





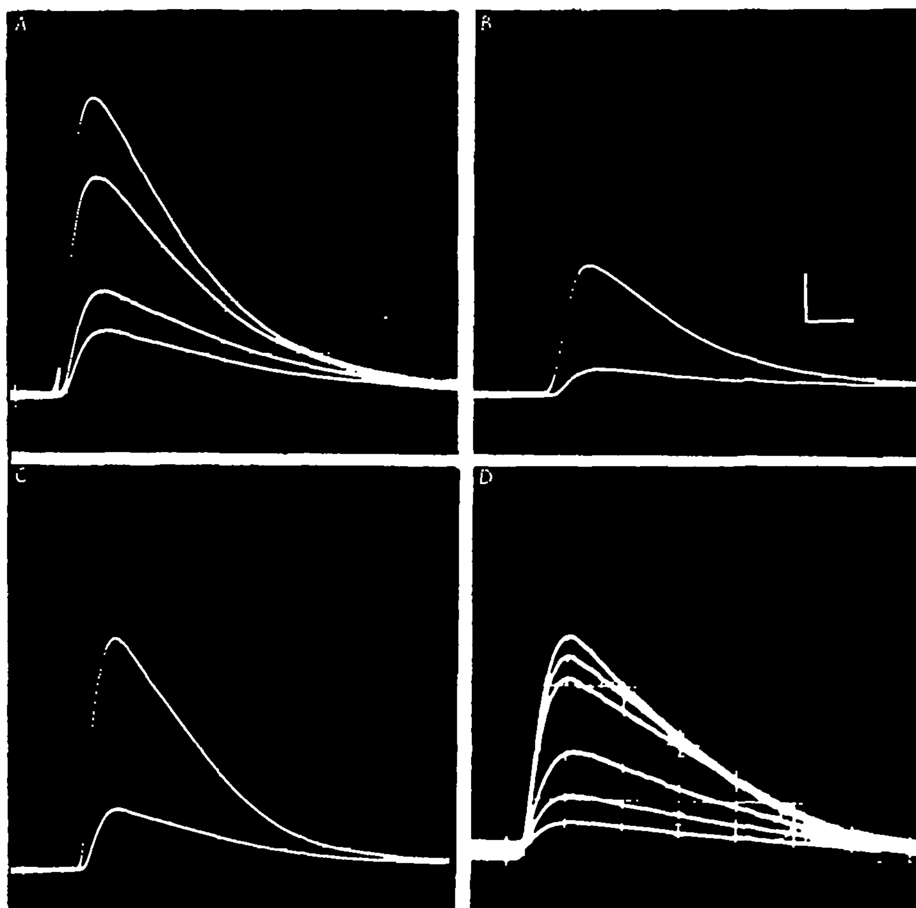
Text-fig. 4. (*A*) Membrane electronic responses (upper traces) to hyperpolarizing rectangular pulses (lower traces). Recordings made at the following distances from the stimulating electrode: (*a*) 190 μ; (*b*) 450 μ; (*C*) 800 μ; (*d*) 1100 μ. Calibration: 40 mV; 0·5 μamp; 10 msec. (*B*) Voltage–current curves for the above-mentioned results. (*C*) The dependence of the input resistance upon the distance between stimulating and recording electrodes.

The results (mean ± s.d.) of such calculations are given in Table 1. In different fibres the space constant varied between 0·5 and 0·7 mm., a result which agrees closely with the space constant found by Atwood (1963) for fast fibres in *Carcinus*.

Table 1. *Membrane electrical constants* of the scorpion's claw closer muscle fibres*

Input resistance 10^5 Ω	Length constant mm.	Internal resistance 10^5Ωmm.$^{-1}$	Specific internal resistance Ωcm.	Membrane resistance 10^5Ωmm.	Specific membrane resistance Ωcm.^{2}	Membrane capacity μF.cm.$^{-2}$	Membrane time constant msec.	Diameter μ	Length mm.
3·3 ± 0·4	0·59 ± 0·10	9·9 ± 0·7	280 ± 15	4·4 ± 1·5	830 ± 1·06	3·6 ± 0·2	3·0 ± 0·9	60 ± 8	7 ± 1·6

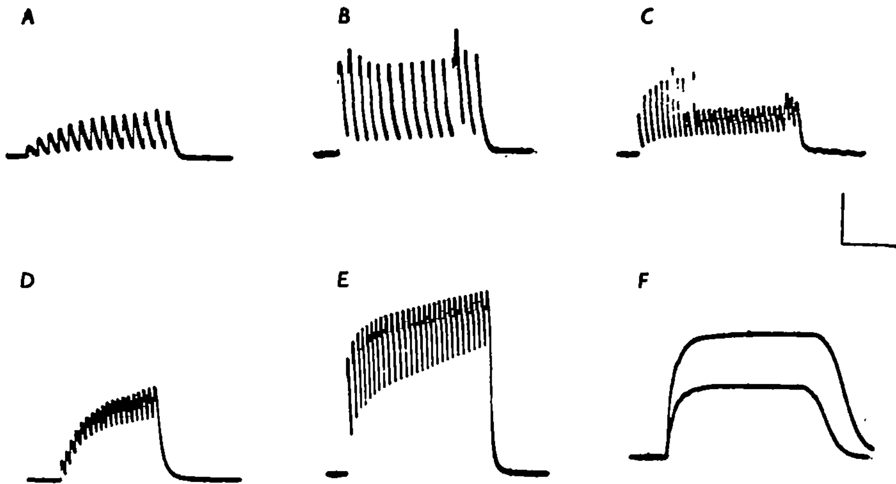
* Mean ± s.d.



Text-fig. 5. Mechanical activity of the closing muscles (superimposed recordings) to single nerve stimuli with increasing intensity. (*A*) Four levels of mechanical tension recorded from LCM. Note the longer latency of the two smaller levels (type 'A' responses) and the similarity of time courses. (*B*) Two mechanical levels (type 'A' and type 'B' responses) recorded from LCM_1 after LCM_2 was cut. (*C*) Recording from LCM_2 after LCM_1 was cut. (*D*) Mechanical tension recorded from SCM. The six mechanical steps are divided into two groups according to their maximum twitch tension response. Calibration: 50 msec. *A*, *B*, *C*, 50 mg; *D*, 100 mg.

Mechanical responses of the LCM and SCM

Even without separation of the nerve into bundles there were indications that at least four axons innervate the LCM, as evidenced by the discrete mechanical levels seen with increases of the stimulus intensity (Text-fig. 5 *A*). Moreover, when LCM_2 was cut so that only LCM_1 was stimulated, only two mechanical steps were observed (Text-fig. 5 *B*) and vice versa for the LCM_2 (Text-fig. 5 *C*). Thus each of the bundles LCM_1 and LCM_2 developed two mechanical steps. Each of the four twitches could be obtained to a single indirect stimulus and each response showed the same time course.



Text-fig. 6. Mechanical responses to repetitive stimulation recorded from the LCM. (*A*, *D*) Mechanical facilitation of type 'A' responses. Frequency 5/sec. (*B*, *E*) Type 'B' mechanical responses. Frequency 5/sec., no marked facilitation. (*C*) Type 'A' and 'B' responses of a 'fatigued' LCM preparation. Note the rapid fatigue of the 'B' response. (*F*) Tetanic responses at 20/sec of type 'A' (lower trace) and type 'B' (upper trace). Calibration: *A*, *B*, *C*, 0·5 sec; *D*, *E*, 1·0 sec; *F*, 2·0 sec. *A*, *C*, *D*, 0·5 g.; *B*, *E*, 0·1 g.; *F*, 1·0 g.

The duration of a twitch response varied between 400 and 500 msec. in different preparations at room temperature, with a rise time of 35–40 msec. and a half-decay time of 180–200 msec. The tensions at the tip of the tarsus ranged between 0·1 and 0·3 g.

The SCM showed six discrete mechanical steps to increase of stimulus intensity (Text-fig. 5 *D*), apparently two steps per bundle as with the LCM. The twitch tensions from SCM stimuli varying from 0·1 to 0·4 g., the rise time of the twitches varied from 40 to 50 msec., the half-decay time being 160–170 msec.

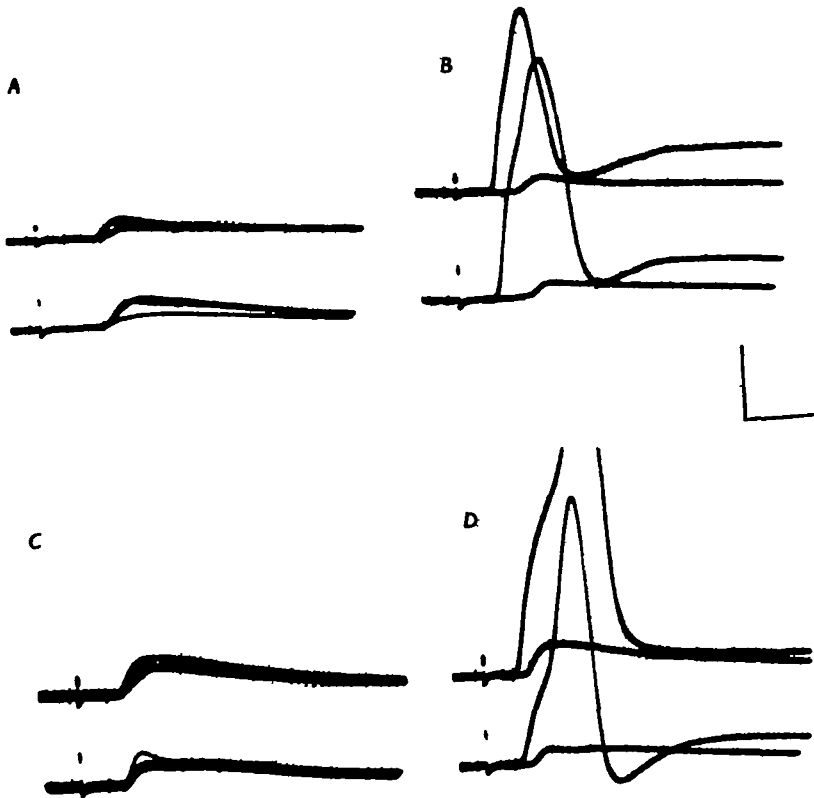
The four mechanical responses shown in Text-fig. 5 *A* could be divided into two types. The first two responses, induced at the lower thresholds, were classified as a type 'A' response; the third and fourth mechanical responses as type 'B'. This classification is preferred to a 'fast' and 'slow' distinction (Wiersma, 1961) because the tension steps have the same duration. The two groups differed in properties. Thus, type 'A' responses showed marked mechanical facilitation at a stimulation rate of

5/sec (Text-fig. 6*A*, *D*), while type 'B' responses showed no facilitation (Text-fig. 6*B*, *E*). Type 'A' responses fatigued less rapidly and could sustain tension for long periods. Text-fig. 6*C* shows that when the nerve was stimulated at 10/sec in a 'fatigued' preparation the 'B' response dropped after several beats, while the 'A' response sustained tension. In both 'A' and 'B' types fusion occurred at 20/sec, when the 'B' response developed almost twice as much tension (Text-fig. 6*F*). It should be noted that the 'B' response always included the lower threshold 'A' responses. In the SCM it was not possible to separate individual bundles, but by analogy the responses could again be classified as 'A' and 'B' (Text-fig. 5*D*).

Even the more resistant tetanic 'A' responses in the LCM could not maintain tension for more than a few seconds, while SCM tetanic responses could be evoked for periods of several minutes.

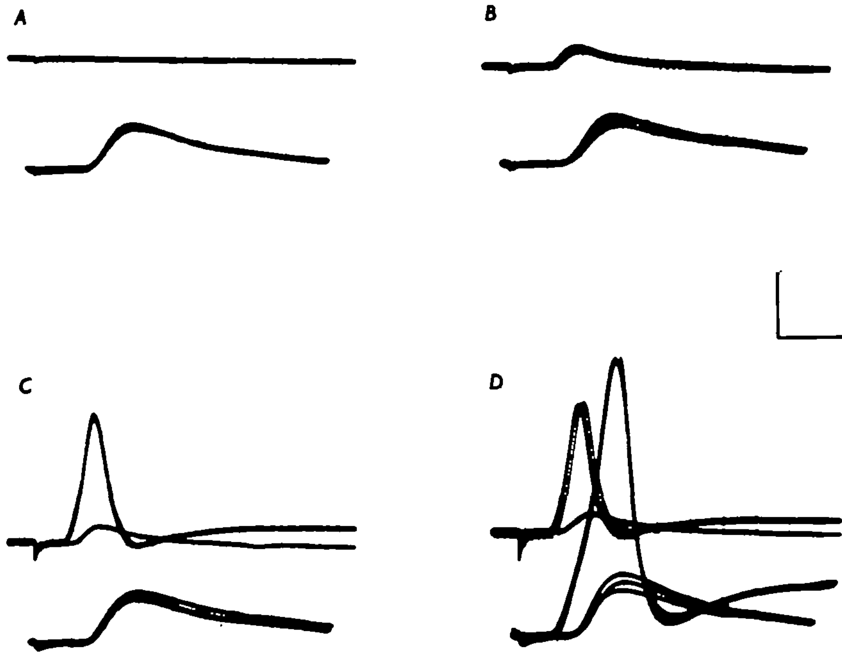
Electrical responses in muscle to nerve stimulation

The muscle-fibre responses to nerve stimulation were recorded simultaneously with two microelectrodes inserted into different cells. Text-fig. 7 shows the responses recorded when the two microelectrodes were in LCM_1 (Text-fig. 7*A*, *B*) or in LCM_2



Text-fig. 7. Electrical responses of muscle fibres to indirect stimulation. Superimposed recordings from two cells of the same bundle. (*A*) Local responses (JP's) recorded in two fibres of LCM_1. Note the JP's with slower rise time. (*B*) JP's and spike-like response (SLR) induced at different thresholds in fibres of LCM_1. (*C*) Local responses (JP's) recorded from two fibres of LCM_2. (*D*) JP and SLR recorded from fibres of LCM_2. Calibration: 10 msec.; 8 mV.

(Text-fig. 7*C*, *D*). At low stimulus intensity small junction potentials (JP's) were recorded (Text-fig. 7*A*, *C*). The junction potentials recorded at different points in a cell many length-constants apart, show similar magnitudes and time courses, indicating a pattern of multiterminal innervation. The rise time of the JP in both bundles was 5–7 msec. and the half-decay time was 14–16 msec. The amplitude varied between 2 and 6 mV. It can also be seen that when the nerves were stimulated many times the rise time of the JP was often quite slow, suggesting initiation of the JP at a distant terminal.



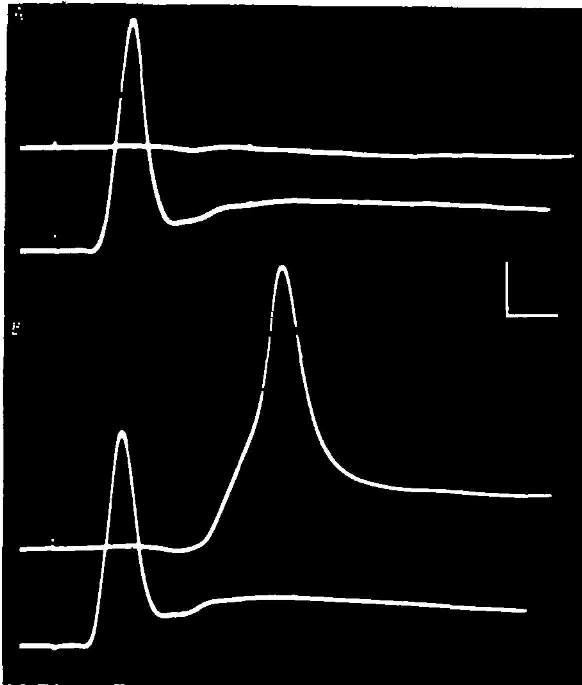
Text-fig. 8. Electrical responses recorded from LCM fibres. Superimposed responses recorded from LCM_2 (upper trace) and LCM_1 (lower trace) to stimulation of the whole nerve. (*A*) Stimulation of a type 'A' axon induces a local JP only in LCM_1. (*B*) A small increase in the intensity of the stimulus elicits a similar response also in LCM_2. (*C*) A further increase of the intensity activates type 'B' axon which innervates only the LCM_2 and causes an SLR. (*D*) Stimulating at higher intensity activates another type 'B' axon which innervates only the LCM_1. Calibration: 10 msec.; upper beam 20 mV.; lower beam 8 mV.

Increase of the stimulus intensity resulted in a second response in the same cells. These responses looked like spikes and will be called spike-like responses (SLR) (Text-fig. 7*B*, *C*). The level of depolarization required to initiate an SLR was 15 ± 5 mV. The variations in threshold were of the same order as the variations in resting potential (62 ± 5 mV.) but no attempt was made to relate these findings. The axons inducing the SLR, although only responding to stronger stimuli than those that produced the JP's, appeared to conduct much faster, exhibiting a briefer latent period (Text-fig. 7*B*, *D*). A more remote possibility is that there exists a great difference in synaptic delays, the lower-threshold fibres having a much longer delay.

Thus each muscle cell of the LCM is innervated by two excitor axons, one inducing a JP, the second an SLR. The JP-inducing axons were termed type 'A' axons, the

SLR-inducing axons were termed type 'B'. This is in accordance with the mechanical activity that they evoked.

The experiments described thus far show that each muscle cell is innervated by at least two excitor axons. The mechanical measurements also indicate that each of the LCM bundles is innervated by only two excitor axons. Text-fig. 8 represents typical results of an experiment determining the number of axons innervating a muscle. One microelectrode was inserted in LCM_1 and the second in LCM_2. It can be seen that each of the two bundles is innervated by different axons, and with gradual increase in the stimulus intensity four steps of initiation of electrical responses (two per bundle) were observed in accordance with the mechanical activity. Thus, it appears that each of the two axons to each bundle innervates each fibre of that bundle.



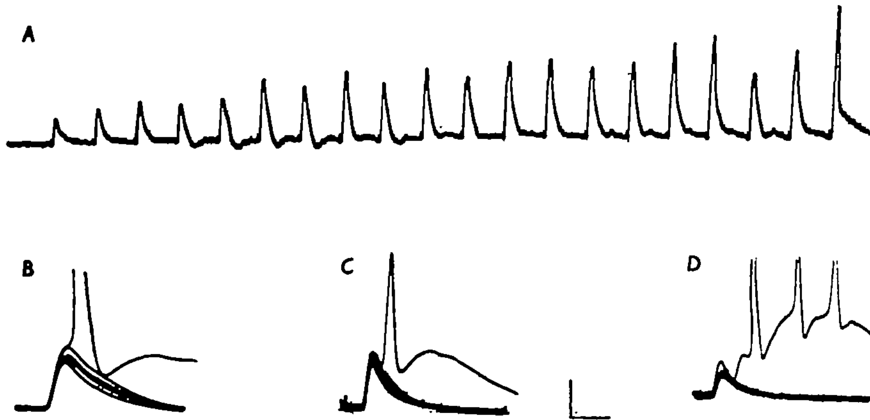
Text-fig. 9. Simultaneous intracellular recording of responses from LCM (lower trace) and SCM (upper trace) to nerve stimulation with increasing intensities. (*A*) At the low stimulus intensity only fibres of the LCM are activated. (*B*) Increasing the intensity activates fibres of the SCM. The longer delay is due to the greater distance of the SCM from the stimulating electrode. Calibration: 5 msec.; 10 mV.

In a similar way it was found that each cell in the SCM is dually innervated by axons inducing a JP and SLR. Each of the three bundles of the SCM is innervated by two axons, and these are anatomically distinct from the four innervating the LCM. Text-fig. 9 shows an example of the results where one microelectrode was placed in a bundle of SCM, the second microelectrode in a bundle of LCM. It is clear that the 'B' responses in the SCM and LCM were initiated by different axons. In the SCM the electrical responses could also be classified as 'A' and 'B'. It should be emphasized that the electrical and mechanical responses were alike and that each cell

is innervated by two axons, one producing a JP (type 'A'), the second an SLR (type 'B').

Type 'A' responses

Activation of type 'A' axons always caused a JP of 2–6 mV. associated with a small twitch response (Text-fig. 14*A*). As with the mechanical activity (Text-fig. 6*A*), stimulation of type 'A' axons produced marked electrical facilitation with repetitive stimulation at 5/sec (Text-fig. 10*A*). Stimulation at 10/sec caused spike initiation at the peak of the JP (Text-fig. 10*B*). Spikes sometimes appear during the falling phase of the JP (Text-fig. 10*C*, *D*). Such responses might indicate that not all of the terminals were



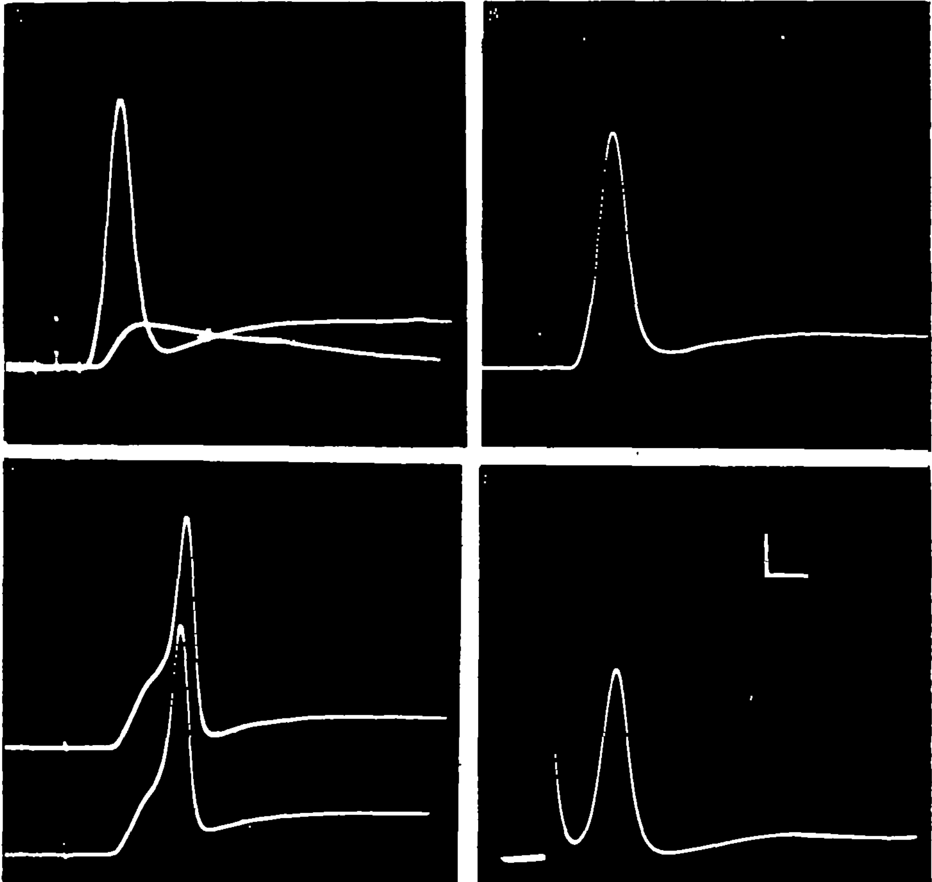
Text-fig. 10. Electrical responses recorded from LCM fibres to repetitive stimulation of type 'A' axon. (*A*) Facilitation at 5/sec. (*B*) Stimulation at 10/sec causes spike-like responses (SLR) at the peak of the JP. (*C*) Stimulation at 10/sec. The SLR appears on the descending phase of the JP, indicating propagation from a distant junction. (*D*) Facilitation and generation of repetitive SLR following a single indirect stimulus. Calibration: *A*, 100 msec., 8 mV; *B*, 10 msec., 4 mV.; *C*, 20 msec., 4 mV.; *D*, 20 msec., 8 mV.

facilitated or invaded at the same rate (Atwood & Parnas, 1968; Bittner, 1968) and that the spike was initiated at a distant terminal and propagated (see below) to the recording site. Indeed, a spike could be evoked by a short intracellular pulse of 0·5 msec. (Text-fig. 11*D*) which was propagated with a conduction velocity of 0·5–1·0 m/sec. Sometimes, after facilitation, a single stimulus of an 'A' axon could evoke a train of repetitive spikes (Text-fig. 10*D*). Facilitation was quite similar in time course and extent in different cells and in different specimens.

Type 'B' responses

The type 'B' response always occurred at a higher threshold than the type 'A' response. It is, therefore, possible that the type 'B' axon induces only a JP, and with the higher stimulus intensity both type 'A' and 'B' axons were stimulated and their responses summated to the level of depolarization needed for the initiation of a spike. The experiments described in Text-fig. 11*A*, *B* show that this is not the case. Text-fig. 11*A* shows the JP and SLR responses induced at two stimulus strengths

(note the stimulus artifact). Axon 'A' was then completely fatigued at the lower threshold at 200/sec., and after no response was obtained at the low threshold, a stimulus at the higher strength caused a spike similar to that of the controls (Text-fig. 11 *B*). It can be concluded that axon 'B' induces SLR in the muscle fibres independently of the activity of axon 'A'.

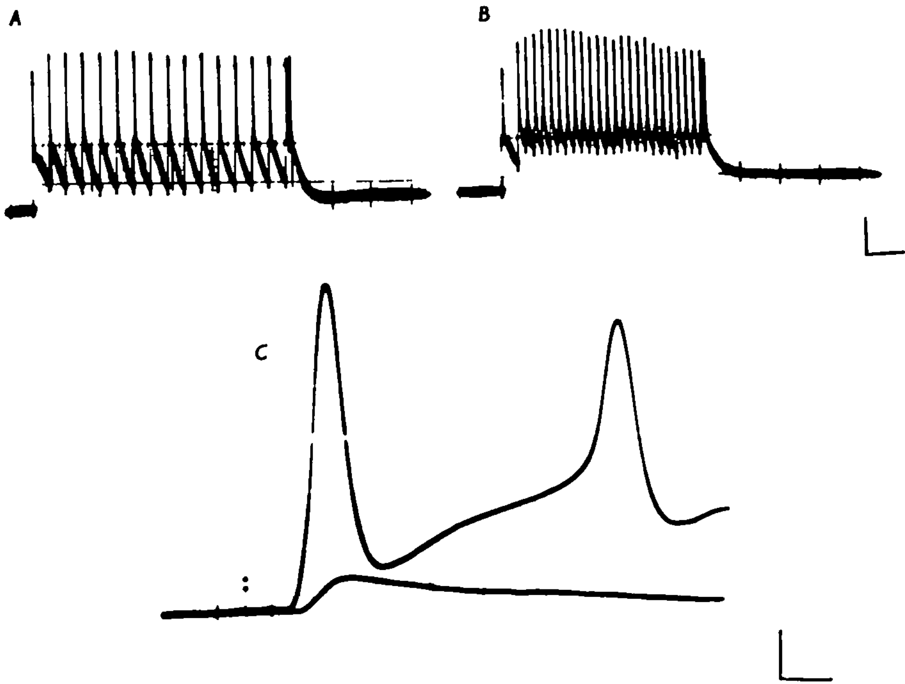


Text-fig. 11. (*A*) A JP and SLR recorded from an LCM fibre in response to indirect stimuli with increasing intensity. (*B*) After axon 'A' was stimulated at the lower intensity at 200/sec. and the JP completely abolished, a single pulse at the higher intensity induced a characteristic SLR. (*C*) SLR recorded with two microelectrodes inserted in the same muscle fibre in response to indirect stimulation. Note the similarity of the latent period, and the shape of SLR's. (*D*) Muscle-membrane response to direct intracellular pulse of 0·5 msec. duration. The velocity of propagation is 0·5 m/sec. Calibration: *A*, *B*, 5 msec., 4 mV.; *C*, *D*, 5 msec., 10 mV.

The difference in latency between the 'A' and 'B' responses in Text-fig. 11 *A* is well correlated with a difference in latency of the mechanical responses seen earlier in Text-fig. 5 *A*, *B*, *C*.

The spikes of response 'B' were almost always all-or-none. They varied between 25 and 50 mV. with a rise time of 5–10 msec. and exhibited durations of 9–16 msec.; when two microelectrodes were inserted in the same cell, spikes with the same latent

periods were recorded from both electrodes (Text-fig. 11 *C*). This indicates that the spike was initiated at more than one receptor region, or that the two electrodes were at the same distance from the same terminal. However, since this result was typical of several experiments, the probability of positioning the electrodes equidistant from the same point by chance is very low.

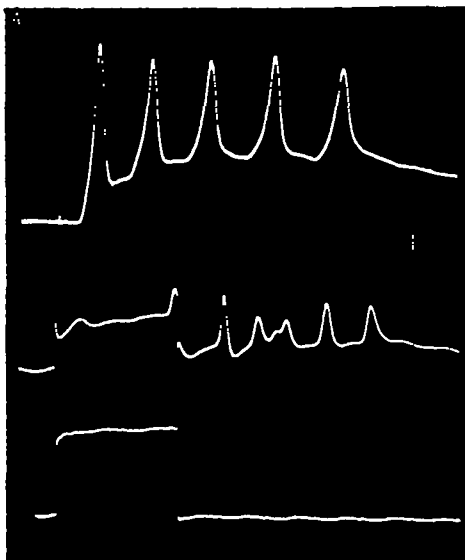


Text-fig. 12. Electrical responses of LCM muscle fibres to repetitive stimulation of axon type 'B'. (*A*) Repetitive stimulation at 5/sec.; neither facilitation nor fatigue can be observed. (*B*) Stimulation at 10/sec. The level of active responses is reduced due to fatigue. (*C*) A low stimulus intensity elicits only a JP, while with increased intensity a single pulse induces repetitive SLR. Note the typical reduction in peak height of the second response. Calibration: *A*, *B*, 0·5 sec., 10 mV.; *C*, 5 msec., 4 mV.

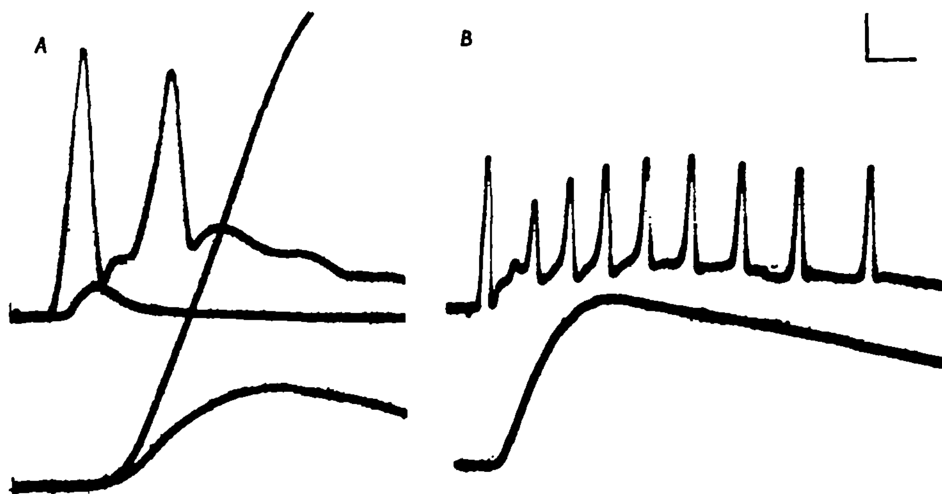
The type 'B' electrical responses never showed marked facilitation (Text-fig. 12 *A*, *B*), which is not surprising in view of their large size and the apparent involvement of electrogenic non-synaptic activity. After repetitive stimulation the 'B' responses fatigued to give smaller responses which, in turn, recovered after rest. In many cases a single pulse to the nerve caused a train of SLR's recorded in the muscle fibre (Text-fig. 12 *C*). This was clearly a non-synaptic event and resulted from the properties of the electrogenic non-synaptic membrane. Text-fig. 13 *A* shows one other example, at low sweep speed, of the repetitive SLR to a single neural stimulus. In *Limulus* such oscillatory responses can be associated with the repetitive firing of the nerve (Hoyle, 1958; Parnas *et al.* 1968). In the scorpion this repetitive firing is not associated with oscillations of the nerve, and an intracellular pulse induces a repetitive spike response after the intracellular current is over (Text-fig. 13 *B*).

Electrical and mechanical activity

Although the electrical activity was recorded from single cells and the mechanical tension from the whole muscle, there is a good correlation between the two. Text-fig. 14*A* shows that activation of the type 'A' axon generates a JP which is associated



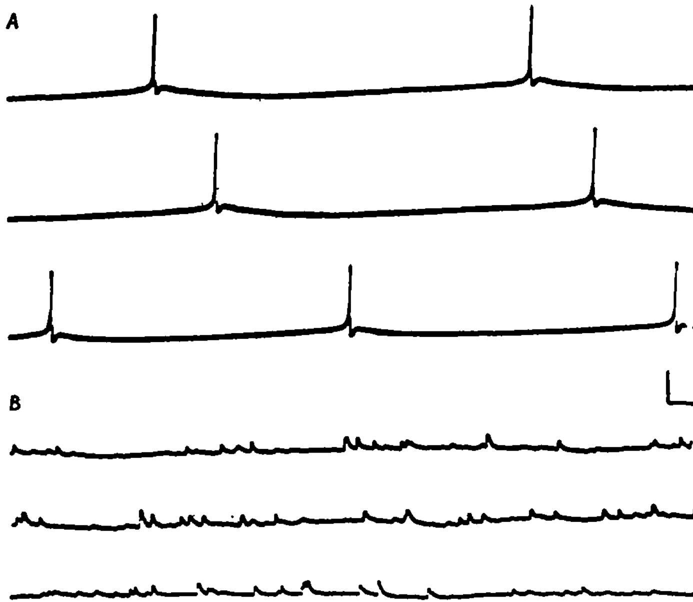
Text-fig. 13. Repetitive spike-like responses recorded in a muscle fibre to a single stimulus applied to the nerve. Note that the first spike is the highest. (*B*) Repetitive SLR (upper trace) initiated by a direct intracellular pulse (lower trace). Note that the repetitive response appears after the pulse has ended. Calibration: *A*, 10 msec., 10 mV.; *B*, 20 msec., 20 mV., 0·5 μamp.



Text-fig. 14. Electrical and mechanical response of the closing muscles. (*A*) LCM superimposed responses to axons 'A' and 'B'. (*B*) Repetitive SLR and prolonged tension evoked with a single pulse to the nerve. Calibration: *A*, 10 msec., 10 mV.; *B*, 20 msec., 20 mV. Tension: *A*, 50 mg; *B*, 100 mg.

with a small tension, compared to the greatly increased tension generated by the type 'B' axon.

With a repetitive train of spikes generated at the muscle-fibre membrane one observes summation and prolongation of the tension (Text-fig. 14*B*). At times, even when only a single spike was recorded, the tension was also prolonged. A probable explanation is that in this case other cells fired repetitively.



Text-fig. 15. (*A*) Spontaneous SLR discharge recorded from an LCM muscle fibre with a resting potential of 60 mV. (*B*) Miniature junction potentials recorded in a central region of an LCM fibre. Note MJP's with different rise times. Calibration: 50 msec., *A*, 4 mV.; *B*, 1 mV.

Spontaneous events

Spontaneous spike discharges (Text-fig. 15*A*) were occasionally observed from muscle fibres in several cases. These probably resulted from injury to the nerve or damage to the muscle cell. In the latter case the resting potential of the cell was seen to decrease slowly.

Miniature JP's (MJP's) were recorded from any place along the muscle fibre (Text-fig. 15*B*). As in other arthropods one can observe several groups of MJP's, differing in rise times and descending phases (Atwood & Parnas, 1968). This is probably also a reflexion of multiterminal innervation.

Inhibitory axons and the effect of GABA, picrotoxin and glutamate

There were no indications of peripheral inhibition in the preparations studied. Stimulation of the nerve with different intensities never showed reduction in tension. Dividing the main pedipalp nerve into bundles failed to show inhibitory responses

upon stimulation of the individual bundles. Inhibitory hyperpolarizing potentials were not found.

Application of GABA up to 10^{-2} gm./ml., picrotoxin 10^{-3} gm./ml., or glutamate up to 10^{-3} gm./ml. showed no effect on electrical or mechanical activity of the closing muscles.

At first it was postulated that the lack of effect might be due to the presence of permeability barriers, or the location of nerve terminals deep inside clefts. Even after treatment of the preparation with 1 % and 2 % pronase (Willows, 1967; Spira, 1968), no effect of GABA, picrotoxin or glutamate was observed, even when damage to superficial muscle fibres resulted.

DISCUSSION

The neuromuscular physiology of the closer muscles in the pedipalp of the scorpion was studied and compared with similar systems in other arthropods. The general pattern of innervation of arthropod muscles is polyneural and multiterminal (Wiersma, 1961; Atwood, 1967*a*; Usherwood, 1967; Rathmayer, 1965; Parnas *et al.* 1968; Hoyle, 1969). In crustacea there is a marked diversity in the properties of the neuromuscular and the mechanical responses of cells in the same muscle. Thus, the 'slow' and 'fast' responses (Wiersma, 1961; Hoyle, 1969) were both attributed to differences in properties of axon terminals in regard to quantal content (Parnas & Atwood, 1966*b*; Atwood, 1967*b*; Atwood & Parnas, 1968; Bittner, 1968) and to the non-homogeneity of the innervation and of the constituent fibres of a single muscle (Atwood, 1963; Dorai-Raj, 1964). The properties of the neuromuscular system of the scorpion, although polyneural and with multiterminal innervation, differ from those of other arthropods and have some features in common with those of *Limulus*.

Electrical response

The constancy of the electrical responses recorded in the scorpion muscles was remarkable. All of the observed cells were innervated by two neurones with different characteristics. These differences are manifested by different post-junctional responses, one producing a JP, the second a post-synaptic spike-like response. All of the cells composing a single bundle are innervated by the same two axons, neither of which innervates other bundles. In crustaceans, insects, spiders and *Limulus* the cells are innervated by one, two or three axons, and two or more muscle bundles share a common motor axon. Even in the deep abdominal extensors, where three main bundles were found, there is cross innervation between the bundles (Parnas & Atwood, 1966*a*). Thus, a bundle of muscle fibres does not correspond to a motor unit. In the scorpion closer muscles each of the bundles is an anatomically distinct motor unit in the sense that it is activated only by its own axons, without any cross innervation, and each cell is dually innervated.

The shapes of the junction potentials and of the spikes were quite constant, whether they were recorded in cells of the same or different preparations. The JP's behaved similarly to repetitive stimulation, all showing facilitation more or less at the same rate. These JP's were often close to threshold for initiation of an SLR and only slight facilitation was necessary to produce an SLR. Approximately the same level of depolarization appeared to be required in a given cell to cause a spike-like response

induced by the second (type 'B') axon. This constant behaviour suggests that the different terminals of a given axon behave in a similar way, and probably the diversity of properties found in different nerve terminals of the same axon in crustaceans (Atwood, 1967*b*; Atwood & Parnas, 1968; Bittner, 1968) and *Limulus* (Parnas *et al.* 1968) is not present in the closer muscles of the scorpions.

But, even with such constancy of responses, it was found that the spike could at times be induced in turn in different terminals, and propagated actively along the cell (Text-fig. 10). The space constant of the cells varied between 0·5 and 0·7 mm., while cells are 5–9 mm. long, allowing measurement of conduction velocity which is 0·5–1·0 m./sec. The reason why the different terminals activate a spike in turn is not known, but the advantage of such behaviour is obvious and enables persistent contraction even in a system in which some of the junctions are more susceptible to fatigue than others.

Mechanical response

Both the JP-producing axon and the spiking axon induced a twitch to a single pulse. Depolarization of 5 mV. was sufficient for the excitation–contraction coupling, and a small increase of extracellular K^+ concentration was sufficient to cause a rapid and transient contraction. The time course of the twitches obtained by both axons, in all of the bundles studied, was similar. Thus, the distinction between 'slow' and 'fast' responses is not applicable to this scorpion system. The same was found to be true for *Limulus* (Parnas *et al.* 1968). All of the muscle fibres probably give fast twitches. Such behaviour would be in accord with their histological appearance. The cells have short sarcomeres of 4–5 μ and responded with a transient tension to potassium depolarization.

Membrane electrical properties

Atwood (1967*a*) showed that crustacean muscles are composed of three classes of fibres with different cable properties, and ascribed the different mechanical and electrical responses to these differences. In the scorpion, the fibres constituting a muscle bundle have similar cable properties. The input resistance, space constant and membrane capacity are much less variable. It should be noted that, while in other arthropods membrane capacity varies between 30 and 50 μF./cm.^{2}, in the scorpion the capacity is in the range of 3–4 μF./cm.^{2} and is similar to the values found for frog sartorius fibres. It is thus possible that the cell membrane is less convoluted. The very short space constant of 0·6 mm. necessitates a very dense multiterminal innervation or a propagated response in order to provide synchronized contraction of the long fibre. Some crustacean muscles also show similar properties (Abbott & Parnas, 1965).

Inhibition

We were unable to demonstrate peripheral inhibition in the closer system of the scorpion. Peripheral inhibition has been found in crustaceans (for review cf. Atwood, 1967*a*), insects (for review cf. Usherwood, 1967) and *Limulus* (Parnas *et al.*

1968). However, in systems without antagonistic muscles such as the *adductor scutorum* of the barnacle (Hoyle & Smyth, 1963) no peripheral inhibition has been found. As in spiders (Rathmayer, 1965), our studies of scorpions show that there is likewise no peripheral inhibition in systems where antagonistic muscles are absent.

In crustaceans and insects motor units are not clearly defined and the fibres show overlapping polyneuronal innervation. In such muscles effective peripheral integration takes place. The richness of innervation, the diversity of the properties of terminals, the frequent absence of conducted spikes and the interposition of inhibitory synapses all aid in integration at the level of the muscle fibres.

In the claw closers of scorpions control is probably purely central. There is no inhibition, and motor units are clearly defined anatomically and physiologically. Moreover, the properties of the axon terminals and the muscle membrane appear to be relatively invariant.

SUMMARY

1. The closing system of the pedipalp claw in the scorpion is composed of two agonistic muscles situated in both the patella (the longer closer muscle) and tibia (the short closer muscle).

2. Membrane electrical constants of the muscle fibres were found to be: $\lambda = 0{\cdot}6$ mm.; $r_i = 1 \times 10^6$ Ωmm.$^{-1}$; $R_i = 280$ Ωcm.; $r_m = 4{\cdot}4 \times 10^5$ Ωmm.; $R_m = 830$ Ωcm.^{2}; Cm. = $3{\cdot}6$ μF. cm.$^{-2}$; $\tau = 3{\cdot}0$ msec.; input resistance = $3{\cdot}3 \times 10^5$ Ω.

3. Each muscle fibre is multiterminally innervated by two motor axons, one initiating a junction potential and the second inducing a post-synaptic spike-like response.

4. The long closer muscle is composed of two anatomically distinct motor units, the short closer muscle of three motor units. Each unit is innervated by its own pair of specific motor axons.

5. No distinction between 'slow' and 'fast' axons or muscle fibres could be observed. Both muscles responded with a twitch to a single stimulus applied to the nerve.

6. No peripheral inhibition was observed.

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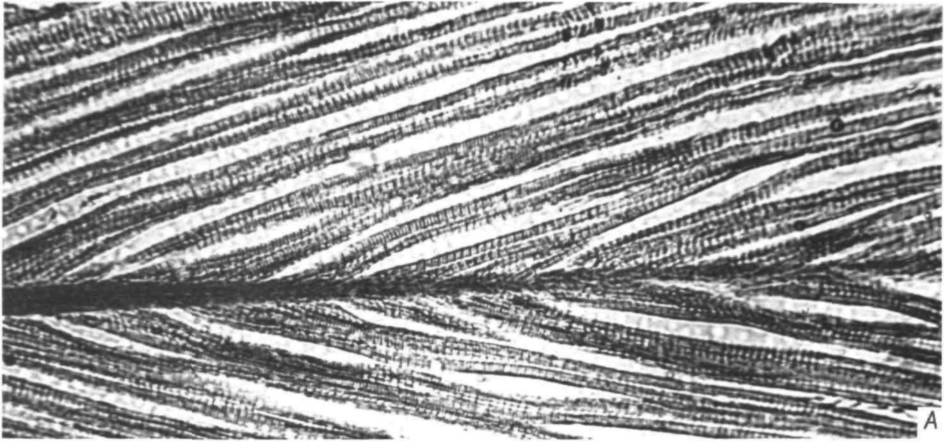
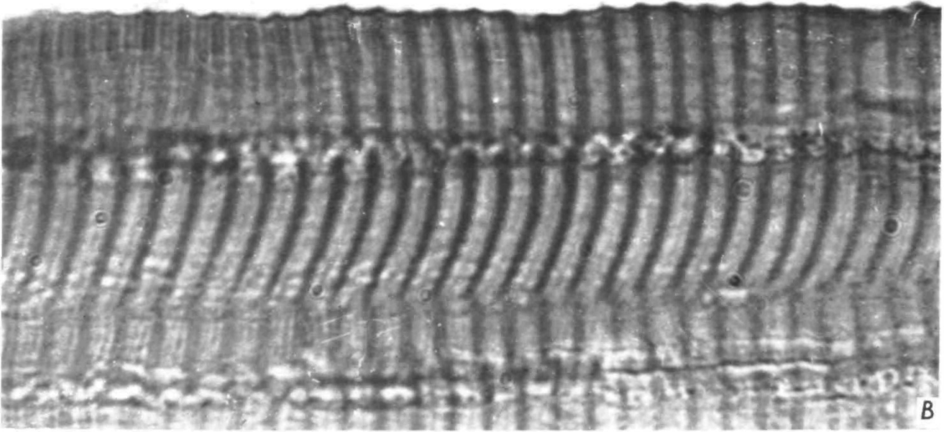
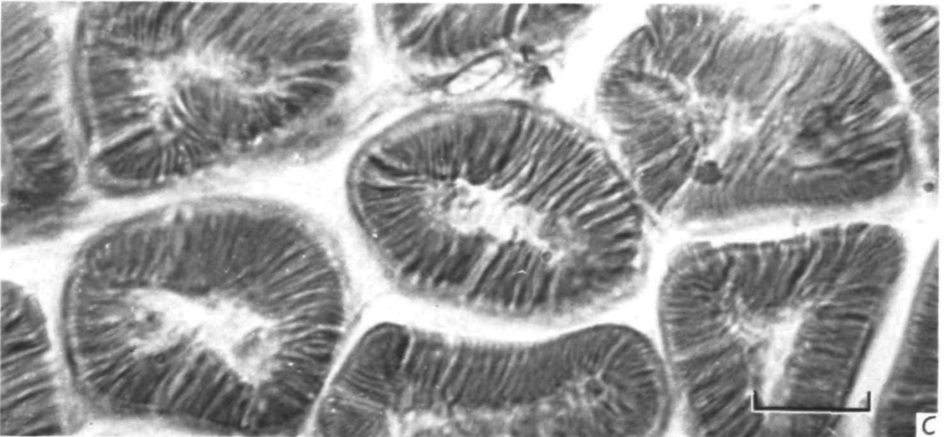
EXPLANATION OF PLATE

(*A*) Longitudinal section of the short closer muscle. The tubular cells are arranged in a pinnate form with a sarcomere length of 4μ. (*B*) Longitudinal section of LCM fibres. Sarcomere length, 3–4μ. (*C*) Cross section of LCM fibres. The fibres are tubular; the filaments are arranged in the circumference, and the canal in the centre contains sarcoplasm and nuclei. Calibration: *A*, 50 μ; *B*, *C*, 10 μ.







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