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# ELECTRICAL COUPLING BETWEEN SUPERNUMERARY MOTOR NEURONES IN THE LOCUST

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## SUMMARY

1. While recording from a slow tarsal levator motor neurone of the locust metathoracic ganglion, a second neurone of this type was discovered in the same half of the ganglion. In thirty animals from which recordings were subsequently made, the additional neurone was found in only one.

2. Since two motor neurones could not be found routinely, other tests were made to determine how many motor neurones normally innervated the levator muscle. The tibial nerve containing the motor axons was stimulated whilst intracellular recordings were made from the levator muscle fibres; recordings were made simultaneously from the cell body of a levator motor neurone, from the tibial nerve and from the levator muscle fibres; and the composition of fine nerve branches that terminate on the levator muscle was examined by electron microscopy.

3. It is concluded that normally the levator muscle is innervated by one excitatory motor neurone and two inhibitory ones. The single excitatory motor neurone has the synaptic inputs ascribed in earlier studies to the 'slow' motor neurone. It can, however, evoke movements of the tarsus ranging from slow smooth ones, to rapid twitchlike ones. No evidence was found for a second, 'fast' motor neurone previously reported (Hoyle & Burrows, 1973).

4. In the two locusts found to possess a supernumerary levator motor neurone, the two motor neurones were electrically coupled by non-rectifying junctions that were strong enough to ensure that the pair invariably spiked in 1 : 1 fashion.

5. The coupled levator motor neurones received different synaptic inputs.

6. One pair of coupled motor neurones was stained intracellularly with cobalt-silver. The cell bodies were some 50 $\mu$m apart, but their neurites converged as they entered the neuropil. The neurites and major side branches followed similar courses in the neuropil, and the two neurones shared the major anatomical features of the single levator motor neurones stained in other animals.

## INTRODUCTION

One advantage of using insects for the electrophysiological study of motor patterns is that many of their central neurones are distinctive in form and function, and can be found routinely in different conspecific animals. Of such 'identifiable' neurones, motor neurones have been most studied. Compared with other central neurones they

are easy to locate using anatomical and electrophysiological means, their activity can be readily related to the animal's movements, and intracellular recordings from them can yield information about the activity of presynaptic neurones.

In locusts, the morphology and physiology of many leg and wing motor neurones have been described (e.g. Bentley, 1970; Burrows, 1973; Burrows & Hoyle, 1973; Burrows & Horridge, 1974; Hoyle & Burrows, 1973; Tyrer & Altman, 1974; Wilson, 1979*a*, *b*; Phillips, 1980, 1981). The leg and wing muscles are typically innervated by a small (usually 2–4) and apparently constant number of excitatory motor neurones. These are now sufficiently known that it is feasible to choose particular motor neurones for study, and with intracellular microelectrodes, trace the presynaptic interneurones that co-ordinate their activity. Nonetheless, information about the number, type, and peripheral distribution of the neurones innervating skeletal muscles in locusts is not complete. Given the advantages that accrue from working with identified neurones, it is important to continue to examine the innervation of skeletal muscles, and to document normal and variant patterns of innervation.

The present study concerns the innervation of the metathoracic tarsal levator muscle. It was prompted by the chance finding, in one animal, of two 'slow' tarsal levator motor neurones within one half of the metathoracic ganglion. They were electrically coupled, and spiked in synchrony. This finding was of interest for two reasons. Firstly, the levator muscle is reported to be innervated by only one 'slow' motor neurone, in addition to a 'fast' motor neurone (Burrows & Hoyle, 1973; Hoyle & Burrows, 1973). Secondly, in insects, no direct evidence has been presented for electrical coupling between the motor neurones that innervate a given muscle, although this has been well-documented for animals of other invertebrate phyla, including crustaceans, annelids and molluscs. Indeed, the only known example of an electrical synapse in the central nervous system of an adult insect is that between two identified interneurones, the LGMD and the DCMD, in the visual systems of grasshoppers and locusts (O'Shea & Rowell, 1975).

Subsequently, it was possible to penetrate two such levator motor neurones in only one more animal of thirty tested. This raised the possibility that normally there is only one slow levator motor neurone within each half of the metathoracic ganglion, though occasionally there are two. Alternatively, there might normally be two motor neurones, which are electrically coupled and spike in synchrony, but the probability of penetrating both of them with intracellular microelectrodes is low.

An aim of the present study, therefore, was to determine the normal innervation of the tarsal levator muscle. The electrophysiological and anatomical tests used to do so are described in the first section of the Results. The second section of the Results will describe the electrophysiological properties of the coupled motor neurones, and the third, the morphology of these motor neurones.

## MATERIALS AND METHODS

Experiments were performed on adult male and female locusts, *Schistocerca americana gregaria* Dirsh, obtained from our own crowded culture.

For intracellular recording from motor neurones, animals were mounted ventral

side uppermost, and dissected to expose the meso and metathoracic ganglia. The ganglia were stabilized on a wax-covered platform, and associated tracheae were pinned aside to expose the region of the metathoracic ganglion containing the cell bodies of tarsal motor neurones. Just prior to intracellular recording, the surface of the thoracic ganglia was treated with a 0·2% (w/v) solution of protease (Sigma Type VI) in saline, for 4 min. Thereafter, the body cavity was perfused continuously with saline (Siegler, 1981). Micro electrodes for intracellular recording contained 2 M-potassium acetate, and had DC resistances in saline of about 50 MΩ. All neurones were penetrated in their cell bodies.

The electrical activity in tarsal muscles was recorded either intracellularly or extracellularly. In experiments where the tibia and tarsus were left intact, the tibia of one hind leg was attached to a servo-motor that was driven by a function generator. This allowed imposed changes to be made in the angle of the femoral-tibial joint. For intracellular recordings from the tarsal muscles, and when extracellular recordings were to be made from nerves in the tibia, one hind leg was immobilized ventral surface uppermost, in a fully extended position. The ventral cuticle of the tibia was removed to expose the nerves and the tarsal levator and depressor muscles. The depressor muscle was deflected to one side, and any fibres over the levator muscle were removed. Axons of the motor neurones that supply these two muscles are contained within the more anterior of the two main nerves in the tibia (Pflüger, 1980). Paired silver hook electrodes were placed on this nerve for extracellular recording and stimulation of the motor axons. When the nerve was stimulated to determine the number of motor axons innervating the levator muscle, the nerve was cut proximal to the stimulating electrodes.

For intracellular staining of motor neurones, microelectrodes were filled with a 5 or 10% (w/v) solution of $CoCl_2.6H_2O$ in distilled water. Stain was injected intracellularly using depolarizing pulses of 5–10 nA, 300 ms long at a frequency of 1 Hz for 15–30 min. Ganglia containing cobalt-filled neurones were subsequently treated with ammonium sulphide to precipitate cobalt sulphide (Pitman, Tweedle & Cohen, 1972). Ganglia were fixed for 1 h in 5% formaldehyde buffered with calcium carbonate, and containing 20 g/l of sucrose. The CoS stain was intensified with silver (Bacon & Altman, 1977).

For electron microscopy, the exposed levator muscle was fixed *in situ* for 10 min in saline containing 2·5% glutaraldehyde. The muscle and overlying nerves were then dissected from the tibia and fixed for a further 1 h in 2·5% glutaraldehyde in 0·05 M-phosphate buffer (pH 7·4) containing 6·8% (w/v) sucrose. After washing in several changes of sucrose phosphate buffer, the tissue was treated in 1% osmium in buffer for 1 h, and in 2% uranyl acetate for 1 h. It was then rapidly dehydrated in ethanol, rinsed in propylene oxide, and embedded in Spurr's resin. Thin sections were stained with 2% uranyl acetate and Reynold's lead citrate. They were examined in a Jeol 200 CX electron microscope. Dr Alan Watson kindly sectioned, stained, and photographed the embedded tissue.

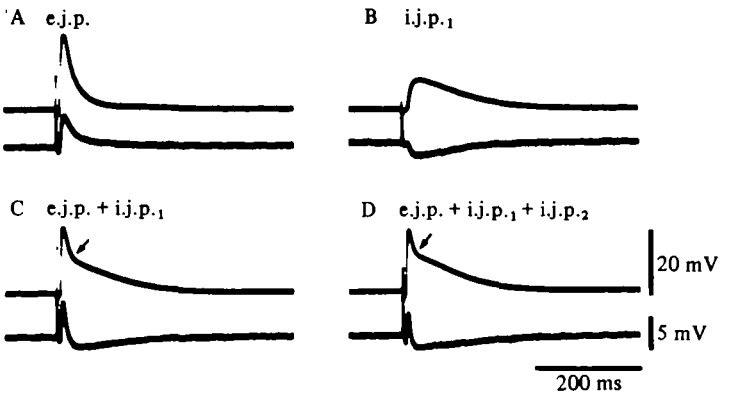



Fig. 1. Recruitment of an e.j.p. and two i.j.p.s in the tarsal levator muscle by electrical stimulation of the tibial nerve. Simultaneous intracellular recordings are made from adjacent muscle fibres. The i.j.p. with the lower threshold is designated i.j.p.$_1$ and that with the higher, i.j.p.$_2$. (A) E.j.p.s are elicited in both fibres when the nerve is stimulated. (B) When stimulus duration and polarity is altered, only i.j.p.s are evoked. I.j.p.$_1$ is depolarizing in one fibre and hyperpolarizing in the other. (C) The stimulus strength is increased to elicit e.j.p.s and i.p.j.s together. (D) The stimulus is again increased to elicit e.j.p.s, i.j.p.s, and i.j.p.$_2$s. In C and D an arrow indicates an inflection in the falling portion of the compound junction potential.

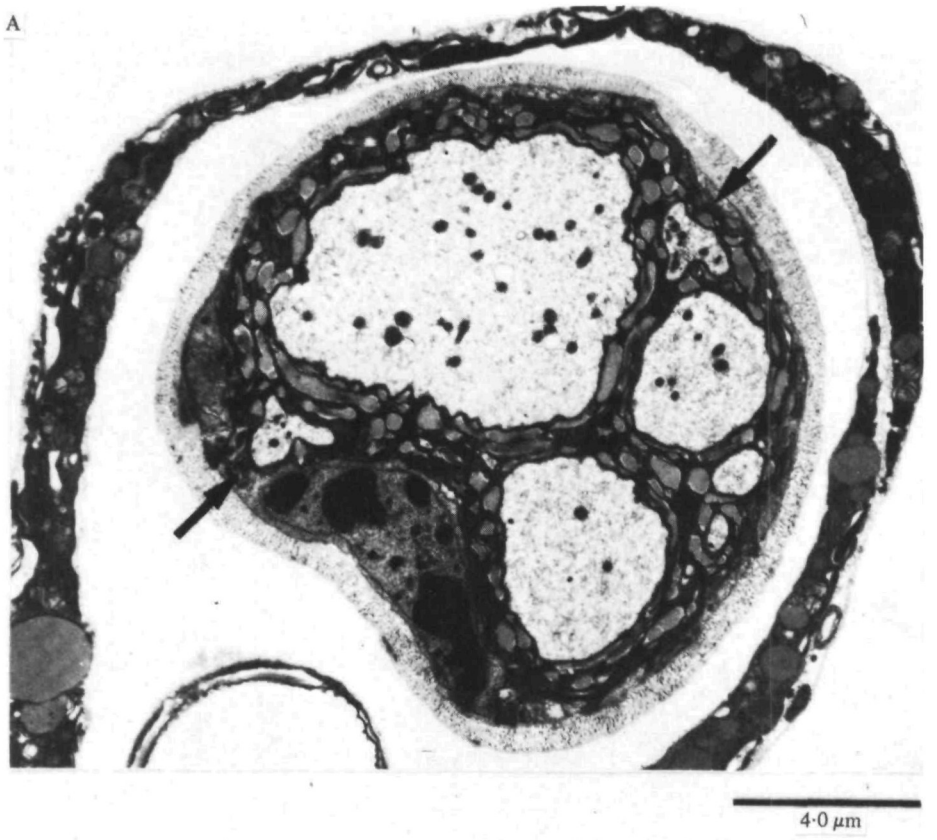
## RESULTS

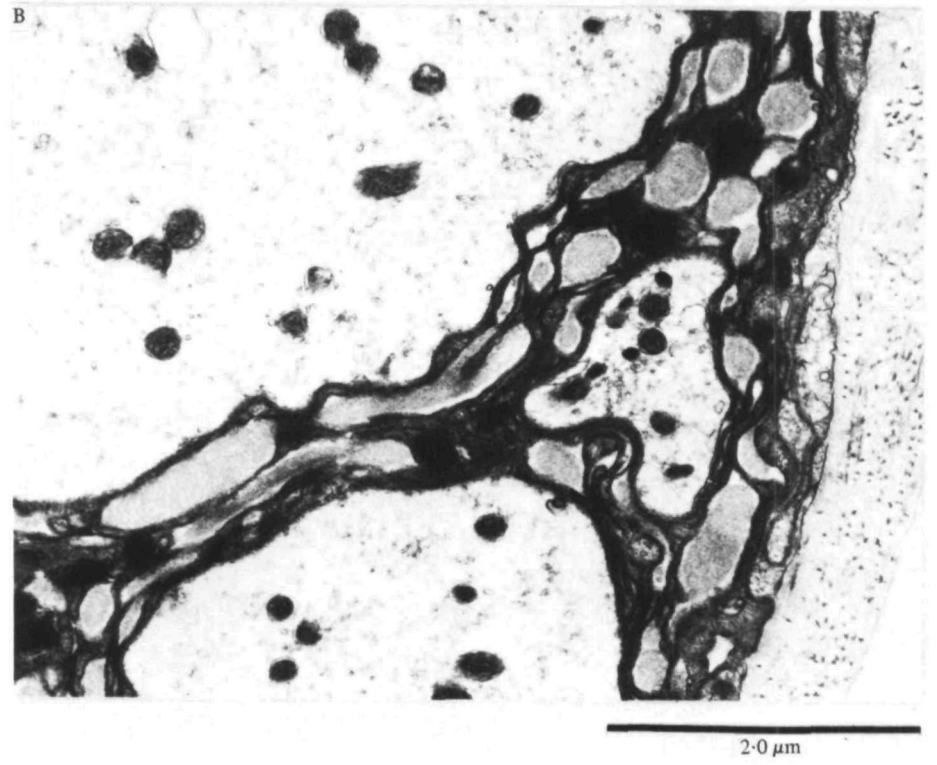
### *Normal innervation of tarsal levator muscle*

#### *Recruitment of junction potentials by nerve stimulation*

As one test of the innervation of the tarsal levator, the tibial nerve containing the levator motor axons was stimulated, while recordings were made extracellularly more distally on the same nerve, and intracellularly from fibres of the levator muscle. The number of discrete thresholds for the recruitment of junction potentials in the muscle fibres should correspond to the number of motor axons supplying the muscle.

By this test, evidence was obtained that the levator muscle was innervated by one excitatory axon, and two inhibitory axons. The results from one locust are illustrated in Fig. 1. In Fig. 1 A, a suprathreshold shock to the nerve was followed by an excitatory junction potential (e.j.p.) in the two muscle fibres penetrated. In Fig. 1 B, when the stimulus was altered slightly in duration, a suprathreshold shock was followed by an inhibitory junction potential (i.j.p.$_1$) in the same two fibres. In one fibre, i.j.p.$_1$ was depolarizing, whilst in the other it was hyperpolarizing. In Fig. 1 C, when the stimulus intensity was increased, shocks to the nerve elicited the e.j.p., and i.p.j.$_1$ together. In the fibre where i.j.p.$_1$ was depolarizing, the peak of the compound potential was slightly less than that of the e.j.p. alone, and the decline from peak had an inflection point (arrow). After this point the shape of the compound junction potential was about the same as that of i.j.p.$_1$ alone. In the fibre where i.j.p.$_1$ was hyperpolarizing, the depolarizing portion of the compound potential was briefer than the e.j.p. alone, and it was followed by a hyperpolarizing deflexion. In Fig. 1 *D*, a second inhibitory junction potential, i.j.p.$_2$, was recruited by further increasing the stimulus. As a result, in the fibre where i.j.p.$_1$ was depolarizing, the inflexion point in the falling phase of the compound junction potential occurred earlier and was more pronounced. In the


A
4·0 μm


B
2·0 μm

fibre where i.j.p.$_1$ was hyper-polarizing, the depolarizing portion of the compound junction potential was reduced in duration and amplitude. Increasing the intensity of the stimulus produced no further change in the size or shape of the compound junction potential in either fibre.

By moving first one microelectrode, then the other, it was possible to compare the thresholds for e.j.p. and i.j.p. recruitment in a large proportion of fibres in the levator muscle. All fibres sampled received e.j.p.s, and for all of these fibres, the strength of nerve stimulation necessary to recruit the e.j.p. was the same. In all fibres where the two i.j.p.s were also seen, the thresholds for their successive recruitment were likewise identical from fibre to fibre. I.j.p.s were not seen in all fibres, however. Some fibres may lack an inhibitory innervation, or alternatively, have a resting membrane potential the same as that of the equilibrium potential for the i.j.p.s.

In all, 8 male and 8 female locusts were tested. In none was there evidence for more than one excitatory axon to the levator muscle. Most commonly, the single excitatory axon had the lowest threshold for nerve stimulation, with the inhibitory axons each having discrete and successively higher thresholds. The preparation illustrated in Fig. 1 was unusual, in that either the excitatory axon, or one of the inhibitors, had the lowest threshold for recruitment, depending upon the duration and polarity of the stimulus to the tibial nerve. In 3 locusts, only two rather than three thresholds could be detected; the shapes of the junction potentials suggested, however, that the excitatory axon and one inhibitory axon had identical thresholds to extracellular stimulation.

*Cross sections of peripheral nerve branches*

In 5 of the preparations just described, where physiological evidence was obtained for one excitatory and two inhibitory axons, the composition of the fine nerve branches that terminate at the levator muscle was examined by electron microscopy. Cross-sections revealed three larger profiles, as well as a variable number of smaller ones. In the cross-section shown in Fig. 2A, for example, the three largest profiles are roughly 7, 4 and 3 $\mu$m across. Of these, the largest is probably that of the excitor, whilst the two smaller ones are probably those of the inhibitors. This would be in accord with their relative thresholds for recruitment by extracellular stimulation (see above). Although the sizes of the three largest profiles were different in different cross sections, their relative diameters were about the same. On the average, the two larger axons were 2·7 and 1·2 times the diameter of the smaller.

In Fig. 2A, there are also 4 smaller profiles, between 0·6 and 1·3 $\mu$m in diameter. Two of these contain dense-cored vesicles (arrows) and one is shown at higher magnification in Fig. 2B. Cross-sections from the other preparations revealed 2–5 smaller profiles, with 1 or 2 of these containing dense-cored vesicles.

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Fig. 2. (A) Cross section of a side branch of the tibial nerve innervating the tarsal levator muscle. Physiological tests indicate that the three largest profiles are those of the excitor, and two inhibitors. The smaller profiles, some containing dense-cored vesicles (arrows), are of unknown origin. (B) Higher magnification of one profile in A that contains dense-cored vesicles.

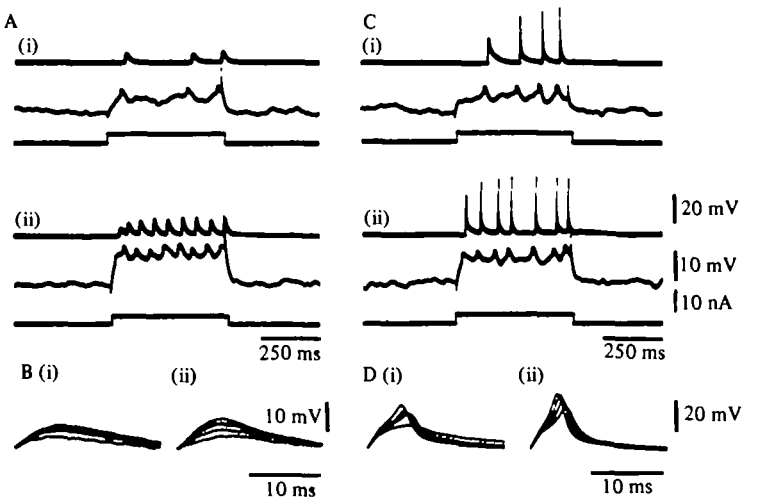


Fig. 3. Facilitating e.j.p.s and active graded membrane responses in levator muscle fibres. A levator motor neurone is made to fire repetitively during 500 ms long depolarizing pulses, injected every 2 s. In A and C the traces are: first, intracellular record from a muscle fibre; second, intracellular record from the motor neurone; third, depolarizing current to motor neurone. In B and D only the intracellular responses of the muscle fibres are shown. (A) Records from a short distally-originating fibre. (i) and (ii) show responses to different frequencies of motor neurone firing. (B) Same fibre as in A. (i) and (ii) show superimposed e.j.p.s from two more 500 ms pulses, from the same series as those in A. (C) Records from a longer, proximally-originating fibre. (i) and (ii) show active membrane responses to different frequencies of motor neurone firing. The active responses increase in amplitude with successive impulses. (D) Same fibre as in C. (i) and (ii) show superimposed active responses from two more 500 ms pulses, from the same series as those in C.

*Simultaneous recordings from levator motor neurone and muscle*

In 6 other animals, recordings were made simultaneously from the cell body of a levator motor neurone, and from the tibial nerve, and/or intracellularly from levator muscle fibres. Invariably, a spike in the cell body of the motor neurone was followed at a constant latency by a single spike in the nerve, and/or a single e.j.p. in a muscle fibre (Fig. 3). There was no evidence that firing in the one penetrated motor neurone recruited any other motor neurone to the levator muscle, and all e.j.p.s recorded in the muscle corresponded to spikes in the penetrated motor neurone. In the shorter fibres, which originate most distally along the tibia, facilitating e.j.p.s were recorded (Fig. 3A, B) whilst in the longer fibres, which originate most proximally, the e.j.p.s could give rise to graded and active membrane responses (Fig. 3C, D). The sizes of the e.j.p.s and the active responses depended upon the recent history of motor neurone spikes, with larger responses occurring when the motor neurone had been firing rapidly.

Results consistent with the above were obtained in some 20 more animals where recordings were made extracellularly from the levator muscle, with the tibia and tarsus intact (not shown). When a levator motor neurone was activated, for example during imposed flexion of the tibia about the femur, the potentials recorded extracellularly from the levator muscle followed the spikes in the motor neurone in a 1:1 fashion. If the motor neurone was hyperpolarized with injected current to

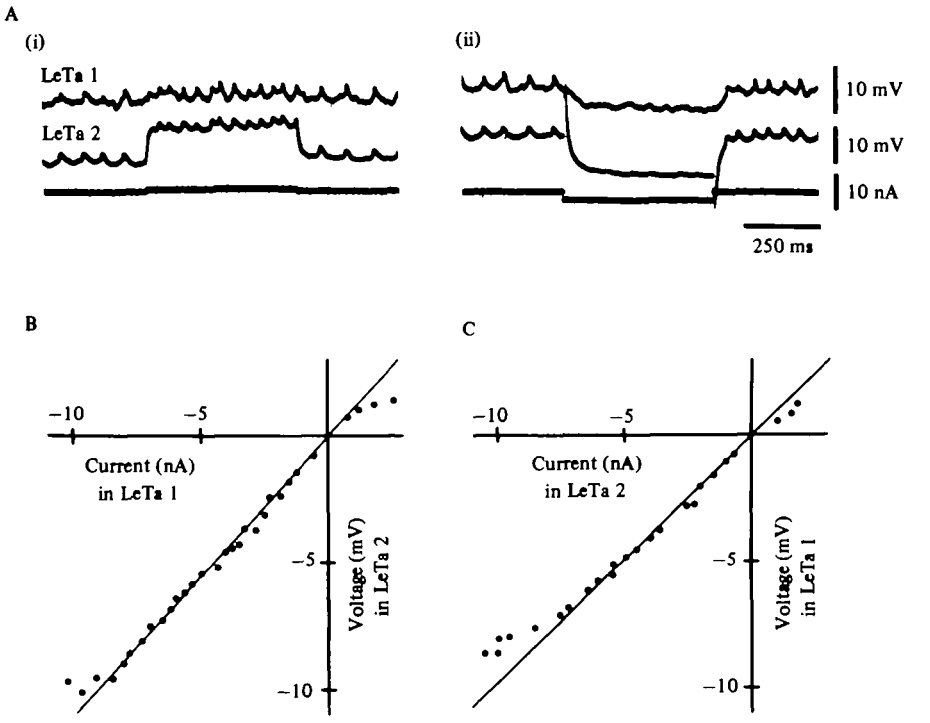


Fig. 4. Non-rectifying electrical coupling between LeTa 1 and LeTa 2. (A) LeTa 2 is depolarized (*i*) and hyperpolarized (*ii*) by injecting current through a bridge circuit. LeTa 1 is depolarized or hyperpolarized accordingly. (B) Plot of steady change in voltage in LeTa 2 when rectangular current pulses 500 ms long are injected into LeTa 1. The line is the best fit for points from 0 to −7 nA. (C) As for B, except current is injected into LeTa 2.

abolish spikes, then the muscle potentials were also abolished. All of the tarsal levator motor neurones examined in this way had the synaptic inputs previously ascribed to the 'slow' motor neurone (Burrows & Horridge, 1974), and no evidence was obtained for the 'fast' motor neurone reported by Hoyle & Burrows (1973) and Burrows & Hoyle (1973).

The way in which the tarsus moved in response to spiking in the levator motor neurone varied, however, for a given animal. At one extreme, a series of spontaneous or evoked spikes in the levator motor neurone resulted in a slow, and smooth levation of the tarsus. Individual twitches corresponding to each spike were not apparent. At the other extreme, each spike in the levator motor neurone evoked a distinct and fast levation of the tarsus. The extracellularly recorded muscle potentials were small when the levation was slow, and large when levation was fast, evidence that the amplitude of the movement was related to the amplitude of the muscle membrane response. The slower response of the tarsus more often occurred when the animal had been quiescent, and the levator motor neurone firing infrequently, whereas the faster response more often occurred when the animal had been struggling or aroused, and the motor neurone firing repeatedly.

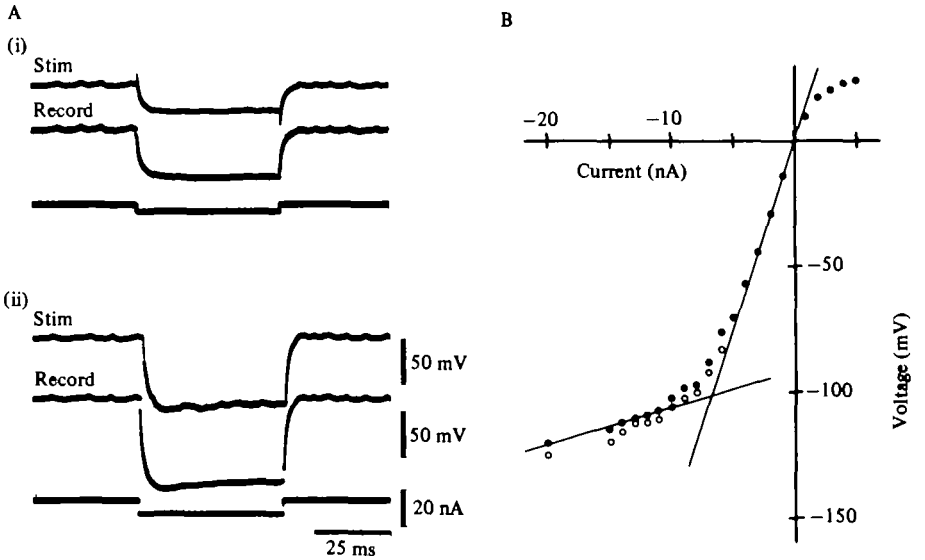


Fig. 5. Current-voltage relationship in a single levator motor neurone. Two microelectrodes were inserted into the cell body. (A) Current (third trace) is injected into one electrode (first trace) via bridge circuit and voltage change is measured at other electrode (second trace). (i) At currents less than about −6 nA, the voltage change in response to a rectangular current pulse reaches a steady level after about 100 ms. (ii) For larger hyperpolarizing currents the voltage declines to a plateau after an initial peak. (B) Plot of the relationship between current and voltage. (●) Steady voltage; (○) peak voltage. One line is the best fit for steady voltage values at currents up to −5 nA, the other for −12 nA to −20 nA.

## *Properties of partner motor neurones*

### *Electrical coupling*

Following the chance discovery of two tarsal levator motor neurones in one locust, a second neurone was sought in thirty more locusts. In only one of these could two motor neurones be found. Both pairs studied were electrically coupled. When depolarizing or hyperpolarizing current was injected into the cell body of one motor neurone of a pair, LeTa 1 (levator tarsus 1), there was a change in membrane potential of the same polarity in the other, LeTa 2 (levator tarsus 2) (Fig. 4A). When hyperpolarizing currents of up to about −7 nA were injected into either LeTa 1 (Fig. 4B) or LeTa 2 (Fig. 4C), the voltage response of the coupled neurone was linear. The slope of this response was the same, about 1 mV/nA, whether the current was injected into LeTa 1 or into LeTa 2; therefore the junction between the two neurones was non-rectifying.

In three other animals, where the cell body of a single levator tarsus motor neurone was penetrated with independent stimulating and recording electrodes, the voltage changes produced over the same range of currents were considerably larger (Fig. 5). Hyperpolarizing currents of up to about −5 nA produced a steady hyperpolarization (Fig. 5A(i)) and resulted in a linear change in voltage (Fig. 5B). In one motor neurone, the slope of this relationship 14 mV/nA (Fig. 5B). In the two other motor neurones tested, slopes of 12·5 mV/nA and 13 mV/nA were obtained for the linear portion of the steady response. For hyperpolarizing currents above −5 nA, the change in

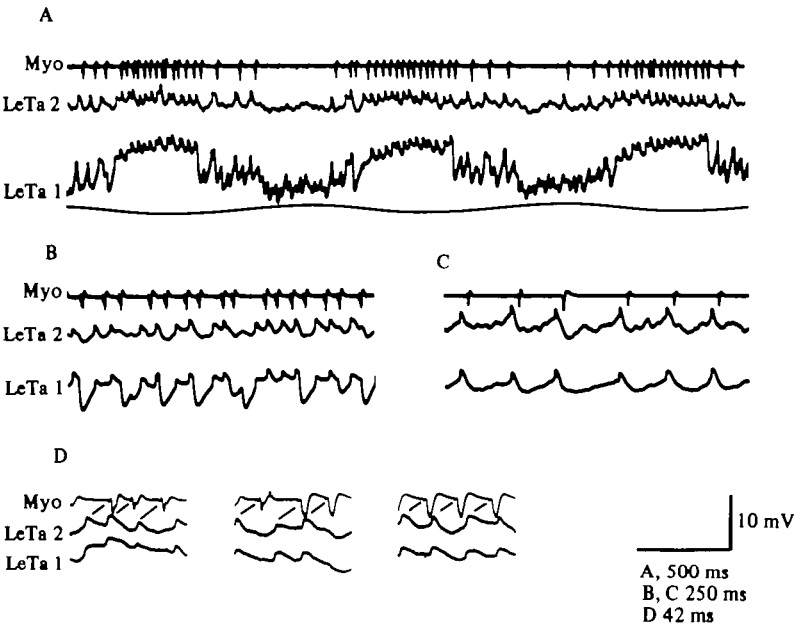


Fig. 6. Intracellular recordings from the cell bodies of LeTa 1 and LeTa 2. Myogram potentials (Myo) recorded from the levator muscle correspond 1:1 with the motor neurone spikes. (A) The motor neurones are excited and inhibited when the angle of the femoral-tibial joint of the ipsilateral hindleg is altered sinusoidally. In the fourth trace, which monitors movement, extension is up, flexion down. (B) IPSPs, marked by vertical bars, occur 1:1 in LeTa 1 and LeTa 2 but are much larger in LeTa 1. IPSPs are from the same source as are those elicited by flexion of the femoral-tibial joint. (C) LeTa 2 receives a steady barrage of PSPs that are either not apparent or much smaller in LeTa 1. (D) LeTa 2 is injected with depolarizing current to evoke rapid spiking in the two cells. The corresponding myogram potentials, which occur with a latency of about 12 ms, vary in size and shape. Lines indicate corresponding spikes and myogram potentials. The three frames were photographed at intervals of about 1 s.

membrane potential reached a peak initially, then declined to a plateau (Fig. 5A(ii). Increasing amounts of current produced successively smaller increments in both the plateau and the peak values, with the slope of the steady current-voltage relationship declining to about 1·4 mV/nA (Fig. 5B). This rectification would account for the non-linearity of the current-voltage relationship between coupled motor neurones, at the larger currents tested (Fig. 4B, C).

*Synaptic inputs and firing pattern*

The coupled neurones fired in synchrony, and their action potentials occurred in a 1:1 fashion with potentials recorded extracellularly from the levator muscle (Fig. 6). They depolarized and spiked several times during imposed flexions of the femoral-tibial joint, and conversely, hyperpolarized during imposed extensions of the joint (Fig. 6A). In one of the neurones, LeTa 1, each hyperpolarization was the result of the summed activity of large IPSPs (inhibitory postsynaptic potentials). This corresponds to the pattern of synaptic inputs previously reported for the slow levator motor neurone (Burrows & Horridge, 1974). In the other neurone, LeTa 2, IPSPs that

corresponded 1 : 1 with those in LeTa 1 resulted in a considerably smaller summed hyperpolarization during each imposed extension of the tibia. When these IPSPs occurred singly, against a background of steady spiking in the motor neurones, they were some 5–6 mV in LeTa 1, but only about 1 mV in LeTa 2 (Fig. 6B).

When hyperpolarizing current was injected into LeTa 1 during imposed movements of the tibia, the IPSPs could be reversed simultaneously in the two neurones, with the smaller IPSPs in LeTa 2 reversing at the same current, about −2 nA, as the larger IPSPs in LeTa 1. By contrast, the IPSPs could not be reversed in either neurone when LeTa 2 was injected with the same, or much larger hyperpolarizing currents (−20 nA). Since the junction between the two neurones is non-rectifying (Fig. 4), the failure to reverse the IPSPs via LeTa 2 cannot be explained by an asymmetry in electrical properties. It is concluded instead that the neurone(s) responsible for the IPSPs synapsed directly only upon LeTa 1, and that the IPSPs recorded in LeTa 2 were due to electrotonic spread. The failure to reverse the IPSPs indirectly, by injecting current into LeTa 2, is explained by two related factors. Firstly, far more current would have to be injected into LeTa 2 than into LeTa 1 to change the membrane potential of LeTa 1 by a given amount. Secondly, the effect that current injected into LeTa 2 could have upon LeTa 1 would be considerably limited by membrane rectification (Fig. 5). As higher currents were injected into LeTa 2 its input resistance would drop, and most added current would be shunted across the membrane of LeTa 2 rather than flowing to LeTa 1. It might not be possible, therefore, to hyperpolarize LeTa 1 to the IPSP reversal potential by injecting current into LeTa 2.

Other smaller IPSPs, and EPSPs (excitatory postsynaptic potentials), which occurred whilst the animal was quiescent, were larger in LeTa 2 than in LeTa 1 (Fig. 6C), but they were not sufficiently regular or distinctive to permit a comparison of their reversal potentials in the two neurones. It was not possible, therefore, to judge whether they resulted from common but unequal inputs to the two neurones, or from the electrotonic spread of inputs to one of the neurones only.

Although action potentials in the cell bodies of the two motor neurones occurred virtually in synchrony, there was variability in the form of the corresponding potential recorded extracellularly from the levator muscle (Fig. 6D). At some times there was a large single peak and at other times, two smaller peaks. Attempts to evoke spikes in only one of the two neurones were unsuccessful, so it was not possible to examine their individual contributions to the muscle potential. Nonetheless, the variability in the form of the potential suggests that both neurones had axons that terminated at the levator muscle. Differences in the conduction velocities of the two axons, and in the relative times at which spikes were initiated centrally in the two neurones could have contributed to the variations observed in the muscle potentials. Action potentials have been considerably broadened by electrotonic spread when they reach the cell bodies, however, and it is not possible to judge from such recordings the exact times of their initiation.

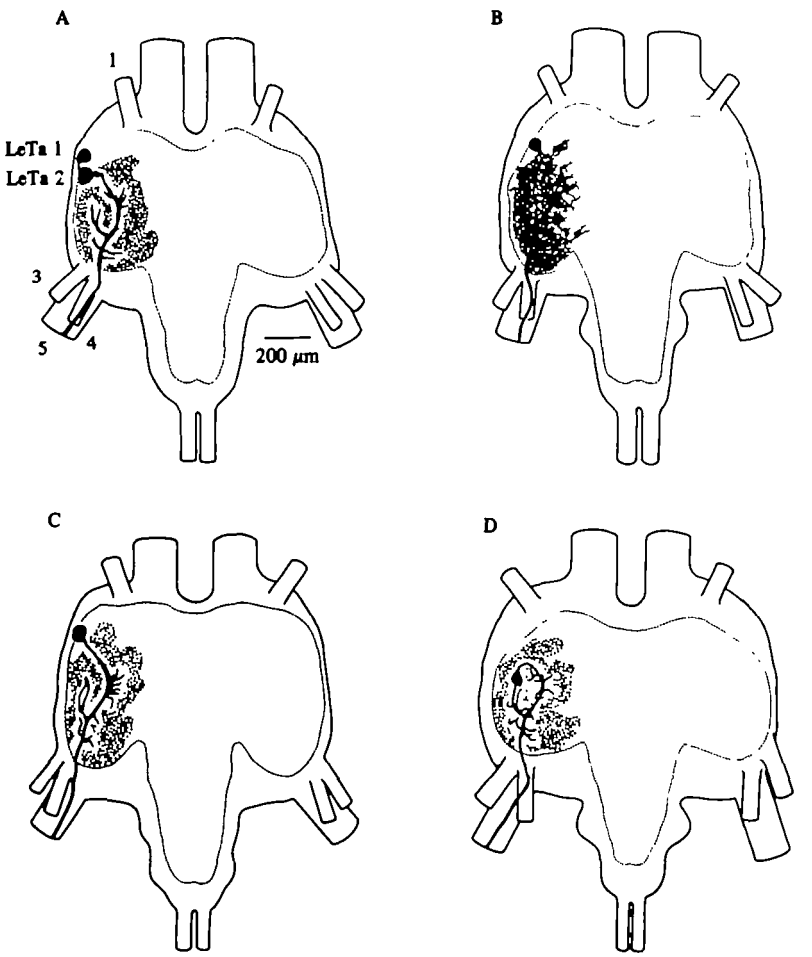


Fig. 7. Morphology of levator motor neurones stained with cobalt and silver, shown in camera lucida drawings made from whole mounts of metathoracic ganglia. Nerve 2 and nerves posterior to Nerve 5 are omitted from the drawings. The dotted line indicates the border of the neuropil. (A) LeTa 1 and LeTa 2, showing cell bodies and major neuropilar processes. The extent of fine branching in the neuropile is indicated by stippled region. (B, C, D) Levator motor neurones from animals that each had only one such motor neurone per side. In B, the entire neurone is drawn; in C and D only major branches are shown, and the extent of branching is indicated by the stippled region.

### *Morphology of levator motor neurones*

In one animal, after the coupled levator neurones were characterized physiologically, they were repenetrated with microelectrodes containing cobaltous chloride instead of potassium acetate, and stained. The cell bodies, major neuropilar processes, and axons of the motor neurones are drawn in Fig. 7A. The cell body of LeTa 1 was about 50 $\mu$m anterior, and about 50 $\mu$m dorsal to that of LeTa 2. The neurite of LeTa 1 travels ventrally, and that of LeTa 2 dorsally, from their respective cell bodies, to converge as they enter the neuropil. The two neurites followed almost

identical courses through the neuropil, and then left the ganglion in Nerve 5. The major side branches likewise followed similar courses. It was not possible, however, to determine whether finer processes also had branching patterns similar to each other. The neuropil was very darkly stained, and in tracing the two neurones, it was often impossible to decide first, whether there was one, or two closely apposed processes, and second, to which neurone a given process belonged.

The two stained motor neurones shared the major anatomical features of levator motor neurones from other animals where only one such neurone was found (Fig. 7B–D). The neurites from their respective cell bodies travelled medially and posteriorly from the cell body region for about 100–150 $\mu$m, then turned laterally, at an angle of 90°–135°, and continued posteriorly to Nerve 5. The distance from the cell body to this turn, and angle of it, appeared to depend upon the position of the cell body. A major branch arose from the main neurite about 150 $\mu$m anterior to the point where the neurite left the neuropil to enter Nerve 5. This branch curved laterally, anteriorly, and slightly ventrally, branching repeatedly to give rise to the majority of the anterior processes that were lateral to the main neurite. In all the levator motor neurones, several other fine branches arose more anteriorly from the main neurite. These varied in number and dimensions, so it could not be judged which branches were 'equivalent' from neurone to neurone. Each neurone had two other major regions of proliferation, likewise comprised of branches variable in size and number. One was between the origin of the major lateral branch, and the point where the main neurite left the neuropil. Along here, branches arose in all directions from the main neurite. The other region was more anterior, where several medially directed branches arose near the most medial turn of the main neurite.

The position of the cell body of single levator motor neurones varied considerably from animal to animal, but did not fall into distinct groups, corresponding, say, to the positions of LeTa 1 and LeTa 2 in Fig. 7A. This variation was not due to distortion during histological procedures, for it was also apparent in fresh tissue. Therefore, even if a cell body was penetrated in an 'unusual' location, it did not mean that there would be another one in a more 'normal' position.

## DISCUSSION

### *What is the normal innervation of the levator muscle?*

There is little question that the levator muscle is normally innervated by two inhibitory axons. Evidence for an inhibitory input was first provided by Ripley & Ewer (1951), for *Locusta migratoria*. Studies independent of my own (J. Hale, personal communication) have shown that in *Schistocerca* the levator muscle is innervated by branches of the anterior and posterior inhibitor. These neurones have been described briefly by Burrows and Horridge (1974). The present results indicate that they constitute the entire inhibitory innervation of the levator muscle.

The tarsal levator muscle has been reported to be innervated by two excitatory motor neurones, one slow and one fast (Hoyle & Burrows, 1973). The results of the present study indicate instead that there is normally only a single excitatory motor neurone. In animals where a supernumerary excitatory motor neurone was found

activity in either produced similar peripheral effects, not surprisingly since the neurones were electrically coupled and spikes always occurred in a 1:1 fashion in the two. The response of the muscle fibres, and the movement of the tarsus itself could vary considerably in a particular animal, however, which may account for the earlier report that there were both slow and fast motor neurones. In line with the present findings, Wilson (1979*b*) reported only one excitatory motor neurone to the tarsal levator muscles in the pro- and mesothoracic segments. By contrast to the metathoracic levator muscle, the antagonistic tarsal depressor muscle has excitatory innervation from a fast motor neurone, and at least three slow motor neurones. They are not electrically coupled, and can be recruited sequentially by stimulating the same tibial nerve that contains the levator motor neurones. (M. V. S. Siegler, unpublished observations; Burrows & Hoyle (1973) report one slow and one fast motor neurone).

The excitatory and inhibitory motor axons probably do not constitute the entire input to the levator muscle, for the nerve branches also contain a few smaller axons. Some of these contain dense-cored vesicles, and are similar in appearance to the peripheral profiles of DUMETi, (Hoyle *et al.* 1980), a neurosecretory cell to the extensor tibiae muscle. DUMETi is only one of two cells of the dorsal medial (DUM) cluster whose peripheral target is known. The other is 'DUMDL', to the dorsal longitudinal muscles (Hoyle, 1978). At least one cell of the DUM group may innervate the tarsal levator muscle. Like DUMETi, it could modulate neruomuscular transmission (Evans & O'Shea, 1977; O'Shea & Evans, 1979) and alter muscle contraction and relaxation (Evans and Siegler, 1982).

### *Supernumerary neurones*

Supernumerary neurones have been found previously in studies of identified ocellar interneurones in the locust *Schistocerca nitens* (Goodman, 1974; 1976; 1977); motor neurones and sensory neurones in the leech (Kuffler & Muller, 1974); giant neurones in the sea hare *Aplysia* (Treistman & Schwartz, 1976; Treistman, 1979; Dagan & Adams, 1981); and sensory neurones in the nematode *Caenorhabditis elegans* (Chalfie, Horvitz & Sulston, 1981).

In the studies of the leech, and of *Aplysia*, the electrical interconnections of the supernumerary neurones, and their synaptic connections, were examined. Unfortunately, upon comparison with the results for the locust, few common features emerge. This is not surprizing perhaps; the number of examples of supernumerary neurones is small, and in none is the cause of the variation in cell number known.

Although electrical junctions connect the supernumerary motor neurones in locust and leech, and the supernumerary giant neurones in *Aplysia*, the strength and the reciprocity of coupling is different. Coupling is strong for the locust motor neurones. Spikes invariably occur synchronously in partner neurones and their electrical connection is non-rectifying. By contrast, supernumerary motor neurones in the leech appear to be only weakly coupled. Impulses are not synchronous, and each motor neuron can exert its motor effect independently (Kuffler & Muller, 1974). In *Aplysia* supernumerary pleural giants and duplicated giants R2 (Treistman & Schwartz, 1976) and R15 (Treistman, 1979) are electrically coupled in a non-recti-

fying way. A spike in one giant neurone is accompanied in the other either by a spike or by a subthreshold coupling potential. In triplicated R15 giants, however, electrical coupling between cells is rectifying, endogenous bursting in the neurones is asynchronous, and spikes in one neurone produce little effect in the other two (Dagan & Adams, 1981).

Synaptic inputs are distributed differentially to partner neurones in the locust; therefore the neurones are not 'duplicates' in the strictest sense. By contrast, in *Aplysia*, the supernumerary giant neurones appears to receive identical spontaneous and evoked synaptic inputs (Treistman & Schwartz, 1977; Dagan & Adams, 1981), and in the leech all supernumerary sensory and motor neurones receive normal synaptic inputs (Kuffler & Muller, 1974).

### *Developmental aspects*

In adult locusts, motor neurones normally are not electrically coupled. The coupling between supernumerary levator motor neurones in the metathoracic ganglion may, however, be a remnant of embryonic development. In the same ganglion, the progeny of at least one neuroblast are originally electrically coupled, but become uncoupled during embryonic development (Goodman & Spitzer, 1979). The origin of the supernumerary motor neurones is unknown. One possibility is that a neurone survives that ordinarily would die; cell death during embryogenesis is important in determining the final complement of neurones within each ganglion (C. M. Bate and C. S. Goodman, personal communication). Another possibility is that extra neurones are produced. In the nematode *C. elegans* single gene mutations have been found that lead to cell lineage changes, resulting in supernumerary neurones (Chalfie *et al.* 1981). In the locust, genetic factors favour the occurrence of supernumerary ocellar interneurones (Goodman, 1977) and could likewise contribute to the occurrence of supernumerary motor neurones.

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