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THE PHYSIOLOGY AND MORPHOLOGY OF MEDIAN NERVE MOTOR NEURONES IN THE THORACIC GANGLIA OF THE LOCUST

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SUMMARY

Simultaneous intracellular recordings have been made from the two expiratory, and from the two inspiratory motor neurones which have their axons in the unpaired median nerves of the thoracic ganglia. Each motor neurone has an axon that branches to innervate muscles on the left and on the right side of one segment.

The expiratory neurones studied were those in the meso- and metathoracic ganglia which innervate spiracular closer muscles. The depolarizing synaptic potentials underlying the spikes during expiration are common to the two closer motor neurones in a particular segment. Similarly, during inspiration when there are usually no spikes, the hyperpolarizing, inhibitory potentials are also common to both motor neurones. The synaptic input to the neurones can be derived from four interneurones; two responsible for the depolarizing potentials during expiration and two for the inhibitory potentials during inspiration.

The inspiratory neurones studied were those in the abdominal ganglia fused to the metathoracic ganglion which innervate dorso-ventral abdominal muscles. During inspiration the two motor neurones of one segment spike at a similar and steady frequency. The underlying synaptic input to the two is common. During expiration, when there are usually no spikes, the hyperpolarizing synaptic potentials are also common to both neurones. In addition they match exactly the depolarizing potentials occurring at the same time in the closer motor neurones. The same set of interneurones could be responsible.

No evidence has been revealed to indicate that the two closer, or the two inspiratory motor neurones of one segment are directly coupled by electrical or chemical synapses.

The morphology of both types of motor neurone is distinct from that of other motor neurones in these ganglia. Both types branch extensively in both the left and in the right areas of the neuropile.

INTRODUCTION

Each segmental ganglion in an insect has, in addition to paired lateral nerves, an unpaired median nerve which contains the axons of neurosecretory cells, and motor neurones innervating muscles involved in ventilation. The purpose of this paper is to examine the mechanisms underlying the pattern of spikes in these motor neurones during ventilation.

The motor neurones are unusual in that their axons once within a median nerve, branch to innervate muscles on both the left and right side of the body (Case, 1957; Hoyle, 1959; Miller, 1960). The motor neurones themselves are nevertheless paired, so that the two corresponding muscles of one segment receive the same pattern of innervation from two motor neurones. In the thoracic segments the median nerves innervate only the muscles of the spiracles. These are paired structures which allow air access to the tracheae, through an aperture controlled usually by two muscles, an opener and a closer. The mesothoracic median nerve contains the axons of only two motor neurones to the spiracular muscles as these spiracles lack opener muscles (Hoyle, 1959; Miller, 1960). The prothoracic and metathoracic nerves contain four motor neurones, two each to the spiracular opener and closer muscles. This pattern is repeated for the first of the three abdominal ganglia which are fused to the metathoracic ganglion. In the other abdominal ganglia there is a different pattern of innervation by the median nerves (Lewis, Miller & Mills, 1973). They innervate the closer muscles of the spiracles and two dorso-ventral inspiratory muscles; the spiracular opener muscles are innervated by paired lateral nerves. This change in the innervation pattern is associated with a change in action of the spiracles; the anterior four pairs of spiracles open during inspiration to allow air to enter the tracheae, whilst the more posterior spiracles open during expiration to allow air to leave. In all median nerves, except the mesothoracic one, there are alternating bursts of spikes, one during the inspiratory and the other during the expiratory phase of ventilation. (For a diagram showing the arrangement of the median nerves, the muscles they innervate, and the timing of the bursts of spikes, see Fig. 1 in Burrows, 1975*b*.)

The ventilatory rhythmicity is derived from a dominant pacemaker in the fused metathoracic ganglion (Farley, Case & Roeder, 1967; Miller, 1960), which is not dependent upon, but can be modified by sensory feedback (Farley & Case, 1968). An abdominal ganglion in a locust may continue to generate a ventilatory rhythm when isolated from the rest of the central nervous system (Lewis, Miller & Mills, 1973), but a pro- or mesothoracic ganglion cannot (Miller, 1966, 1967). Intracellular recording from median nerve motor neurones in adult locusts (Burrows, 1974, 1975*b*, 1978) and in larval dragonflies (Komatsu, 1980) has already revealed some of the mechanisms controlling their output. In the locust, spiracular closer motor neurones in the thorax receive a depolarizing synaptic input during expiration which is shared with some flight motor neurones (Burrows, 1975*a*, *b*). During inspiration they receive an inhibitory input (Burrows, 1975*b*). From extracellular recordings of the median nerves it has been inferred that there is no direct coupling between the two closer motor neurones of one segment (Burrows, 1978). The inspiratory motor neurones with axons in the median nerve of the metathoracic or first unfused abdominal ganglion receive an inhibitory synaptic input during expiration (Burrows, 1974) which matches exactly the excitatory input to the closer motor neurones (Burrows, 1975*b*). During expiration they receive an excitatory input (Burrows, 1974). In dragonfly larvae there is no direct coupling between these inspiratory motor neurones (Komatsu, 1980).

Simultaneous intracellular recording in the locust from the two spiracular closer (expiratory) neurones of a particular segment and from the two inspiratory median nerve motor neurones has allowed the following three questions to be resolved.

unambiguously. First, is the excitatory synaptic input common to a segmental pair of expiratory or inspiratory motor neurones. Secondly, is the inhibitory input likewise common. Thirdly, is there direct coupling between a pair of motor neurones. Moreover by using dye-filled microelectrodes, the unusual morphology of these motor neurones has been revealed.

MATERIALS AND METHODS

Adult locusts, *Schistocerca americana gregaria* (Dirsh) (= *S. gregaria* (Forskal)) were obtained from our own crowded culture. Intracellular recordings were made from the somata of median nerve motor neurones in the pro-, meso- and metathoracic ganglia according to procedures described previously (Hoyle & Burrows, 1973). The electrodes were filled with 2 M potassium acetate (d.c. resistance 30–40 MΩ in saline) or with 0·4 M cobaltous chloride. To facilitate entry of the electrodes into a ganglion, the ganglionic sheath was treated with a 1% (w/v) solution of protease (Sigma Type VI) in saline for 2 min. The neurones were stained by the electrophoretic injection of cobalt (Pitman, Tweedle & Cohen, 1972) and subsequent intensification with silver in whole ganglia (Bacon & Altman, 1977). Drawings of the injected neurones were made with a cameria lucida attached to a compound microscope. All measurements of size were made after fixation and staining and are therefore subject to distortions introduced during these procedures. Descriptions of the morphology are based on ten successful stains of mesothoracic neurones and four each for the pro- and metathoracic neurones.

All electrical recordings were stored on magnetic tape using an FM tape-recorder, for later analysis and photography. Signal averaging was performed by a DL4000 signal processor (Data Laboratories Ltd, London). Descriptions of the physiology are based upon successful recordings from pairs of motor neurones in 27 locusts.

The terms 'left' or 'right' motor neurones refer to the position of their cell bodies within a ganglion.

RESULTS

Physiology of the motor neurones

Patterns of spikes in spiracular closer motor neurones

Intracellular recordings from the somata of spiracular closer motor neurones in any of the three thoracic ganglia reveal spikes with amplitudes as large as 16 mV and durations at half height as brief as 0·8 ms. The soma is not able to support an overshooting and propagated action potential, but is instead invaded by the decrementally conducted remnant of a spike that is presumed to have arisen in a distant region of the neurone within the neuropile. Nevertheless, the spike recorded in the soma is of an amplitude equalled by only one other thoracic motor neurone so far studied in the locust, the fast extensor of a metathoracic tibia (Hoyle & Burrows, 1973). The spike is also much briefer than any seen in other locust thoracic motor neurones.

When the central nervous system is intact and ventilation proceeding, the spikes of all the thoracic spiracular closer motor neurones occur in bursts in time with expiration (Fig. 1*a*). The pattern of spikes in the two motor neurones of any one segment is remarkably similar as revealed by simultaneous intracellular recordings

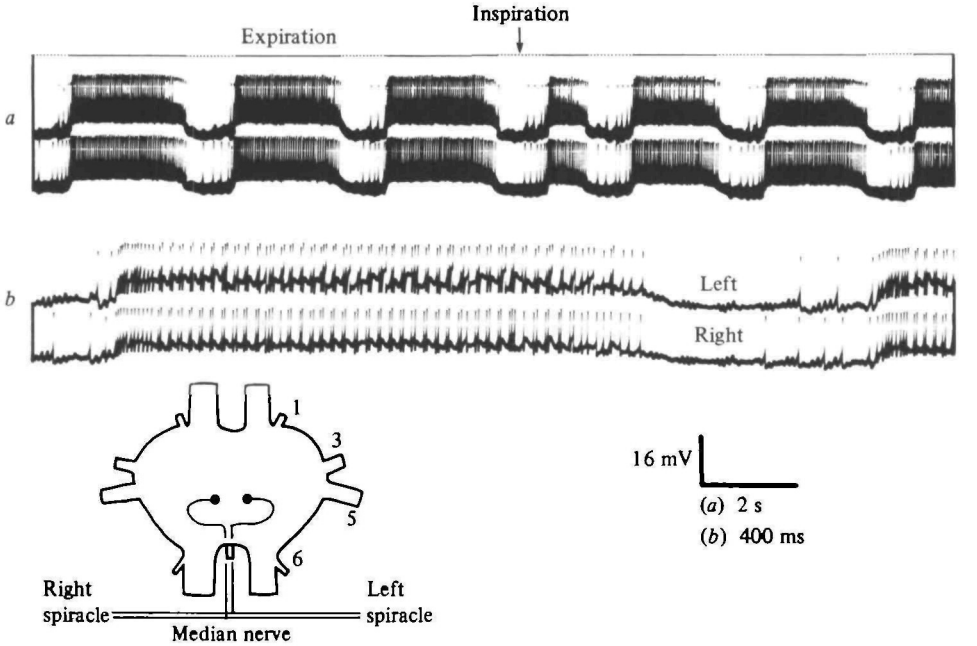

Fig. 1. Patterns of spikes in the two mesothoracic spiracular closer motor neurones during ventilation. The recordings were made simultaneously from the somata of the two motor neurones. (*a*) Both motor neurones produce bursts of spikes during the expiratory phase of ventilation and are inhibited during inspiration. (*b*) One cycle of ventilation on an expanded time scale to show the similar pattern of spikes in the two motor neurones. The diagram shows the position of the somata of the motor neurones and the paths the axons take to innervate the spiracular muscles from a ventral aspect.

(Fig. 1*a*). At the start of an expiratory burst, the spikes in both neurones occur at a high frequency. Thereafter, and for most of the expiratory phase the spikes tend to occur in groups of two or three, with each group separated from the next by longer intervals of approximately 50 ms (Fig. 1*b*). The groups occur at the same time in each motor neurone although in a particular group intervals between successive spikes and the number of spikes may not be the same in the two motor neurones. Towards the end of expiration the spikes occur at a lower frequency and are typically not in groups. During inspiration occasional spikes at still lower frequencies may occur.

Excitatory synaptic potentials

Synaptic potentials underlying the pattern of spikes during expiration in one of the two motor neurones innervating the mesothoracic spiracles can be seen more readily by applying a steady hyperpolarizing current through a microelectrode inserted into that motor neurone (Fig. 2*a*). Waves of synaptic potentials (EPSPs) are apparent during most of the expiratory phase. Spikes that arise from the peaks of one of these waves in the hyperpolarized neurone correspond to a group of spikes in the other, normal motor neurone (Fig. 2*a*).

When both motor neurones are hyperpolarized and the intracellular recordings examined on faster time scales, two points become clear (Fig. 2*b*, *c*). First, the waves of depolarization are caused by the summation of depolarizing synaptic potentials.

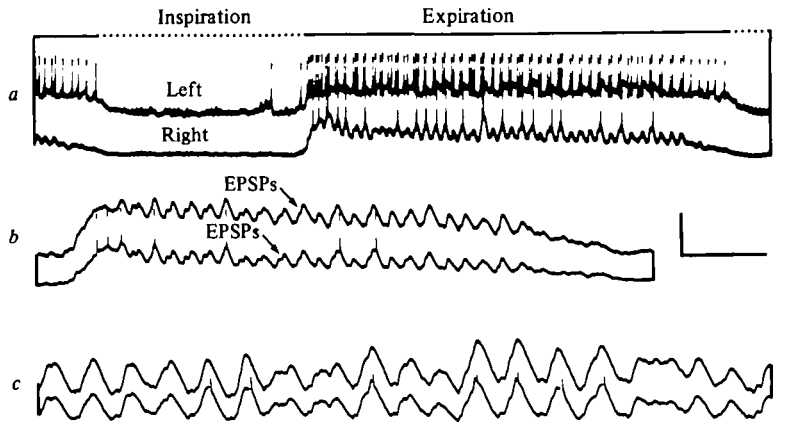

Fig. 2. Patterns of EPSPs in the two mesothoracic spiracular closer motor neurones during ventilation. (*a*) The right motor neurone was hyperpolarized by applying a steady current of 0·6 nA through the recording electrode. Only a few spikes now occur so that an underlying pattern of EPSPs is revealed which corresponds with the groups of spikes in the left motor neurone. (*b*, *c*) Both motor neurones are hyperpolarized with a steady current of 0·6 nA to reveal an exact match of the EPSPs during expiration in the two motor neurones. Calibration: Voltage (*a*, *b*) 16 mV, (*c*) 8 mV; time (*a*) 400 ms, (*b*) 200 ms, (*c*) 100 ms.

Typically two to four comprise each wave. Secondly, the pattern of synaptic potentials in the two motor neurones is the same. There is an exact correspondence between the depolarizing potentials in one neurone and those in the other. On none of five occasions have the synaptic potentials of a wave been observed in one neurone and not in the other.

This pattern of depolarizing synaptic potentials during expiration, here shown directly to be shared by the two motor neurones to the mesothoracic spiracular closer muscles, is also shared by the other thoracic spiracular closer motor neurones. Simultaneous recordings from a meso- and a metathoracic (first median nerve) spiracular closer motor neurone show that the excitatory potentials are precisely matched. Similarly, intracellular recordings from the two closer motor neurones with axons in the first metathoracic median nerve also reveal an exact matching of the synaptic potentials. Prothoracic spiracular closer motor neurones have already been shown to receive an excitatory input that is common to some flight motor neurones and other spiracular closer motor neurones (Burrows, 1975*a*, *b*). There is thus a common excitatory drive to all thoracic spiracular closer motor neurones provided by one set of interneurones, and not by segmentally repeated sets of interneurones.

Inhibitory synaptic potentials

The gradual end to the burst of spikes during expiration is attributable to a decline in the excitatory synaptic input and to the onset of a sequence of hyperpolarizing synaptic potentials which persist throughout inspiration and force the membrane potential to a value some 8 mV lower than the average during expiration (Fig. 3). Consequently few spikes occur during inspiration and those that do are an apparent result of rebound from a large hyperpolarization (Fig. 3*a*, *b*).

The hyperpolarizing potentials are common to both motor neurones innervating the mesothoracic spiracles (Fig. 3*b*). As for the depolarizing potentials during expira-

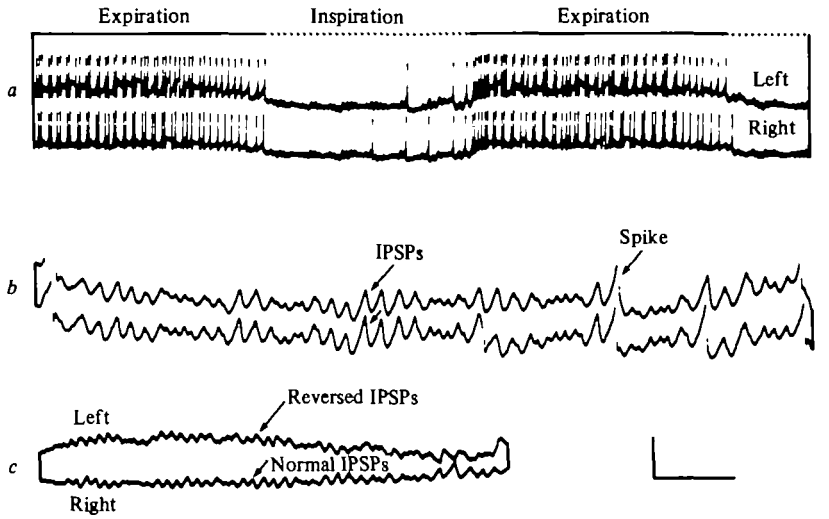

Fig. 3. Patterns of IPSPs in the two mesothoracic spiracular closer motor neurones during ventilation. (*a*) During inspiration both motor neurones are hyperpolarized by a rapid sequence of synaptic potentials. (*b*) A single inspiration on a faster time scale reveals an exact match of the synaptic potentials in the two motor neurones. There is a periodic fluctuation in the amplitude of the potential changes and an occasional spike may arise at the end of some of the larger excursions. (*c*) The left motor neurone is hyperpolarized with a steady current of 0·7 nA. The formerly hyperpolarizing synaptic potentials in this motor neurone are now depolarizing. Calibration: Voltage (*a*) 16 mV, (*b*) 4 mV, (*c*) 8 mV; time (*a*) 400 ms, (*b*) 100 ms, (*c*) 200 ms.

tion, an exact correspondence can be seen between the hyperpolarizing potentials in the two motor neurones. The failure to observe any occasions when there is not this exact matching during many hundreds of cycles of ventilation in any of the five locusts examined, suggests that the same presynaptic neurones evoke the potentials in the two motor neurones.

The application of a steady hyperpolarizing current to the soma of one of the motor neurones shows that the hyperpolarizing potentials have an apparent reversal potential, and that with the application of more current they can be reversed in polarity so that they are depolarizing (Fig. 3*c*). The apparent reversal potential is more negative than the average membrane potential during inspiration, so that the potentials are inhibitory postsynaptic potentials (IPSPs).

A characteristic feature of the hyperpolarization during inspiration is a waxing and waning of the amplitude of the potentials, often in a rhythmical fashion so that a pattern of beats occurs (Fig. 3*a*). The simplest explanation of the effect is that the IPSPs are derived from two sources. When the IPSPs from both putative sources occur at the same time, the amplitude of the hyperpolarization is large; when they occur at slightly different times the hyperpolarization is smaller (Fig. 3*b*). Any difference in frequency between the two sources is expressed as the frequency of the beats. A prediction based on this explanation is that if one source of the IPSPs is eliminated the pattern of beats should be abolished. This has been tested in experiments which identify the interneurones evoking the IPSPs (Burrows, in prep.).

The pattern of hyperpolarizing potentials during inspiration that is shared by the

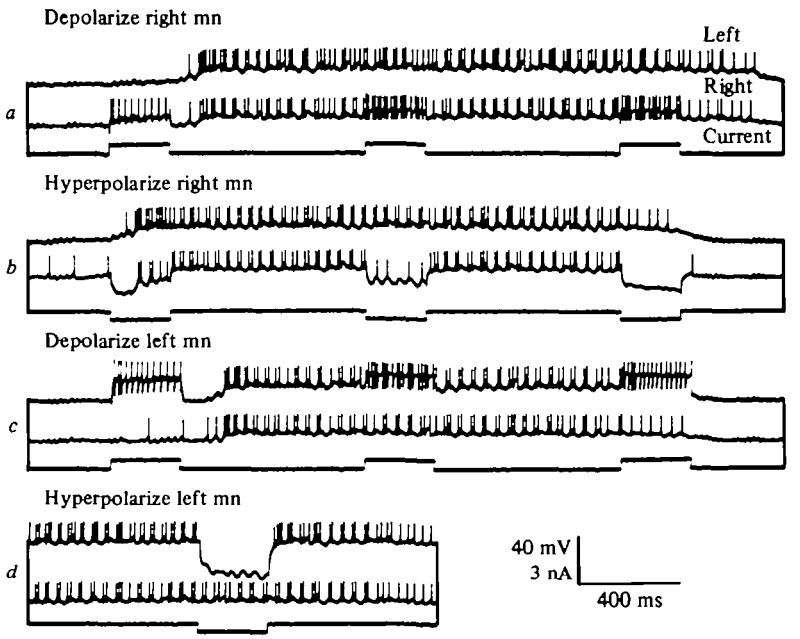

Fig. 4. Lack of evidence for electrical coupling between the two mesothoracic spiracular closer motor neurones. (*a*) Pulses of depolarizing current are injected through the recording microelectrode into the right motor neurone during both phases of ventilation. Evoking spikes during inspiration, or increasing their frequency during expiration, is without a detectable effect on activity in the left motor neurone. (*b*) Pulses of hyperpolarizing current injected into the right motor neurone interrupt the pattern of spikes during expiration, but again are without effect upon the left motor neurone. (*c*, *d*) Depolarizing (*c*) or hyperpolarizing (*d*) pulses of current injected into the left motor neurone are likewise without effect upon the right motor neurone.

two mesothoracic closer motor neurones is also present in the other thoracic spiracular closer motor neurones. Recordings made simultaneously from a metathoracic (first median nerve) and a mesothoracic closer motor neurone show the potentials to be common to both. Simultaneous recordings from the two closer motor neurones with their axons in the first metathoracic median nerve also show that the IPSPs are exactly matched. It would seem likely, therefore, that the hyperpolarizing potentials in all thoracic closer motor neurones are derived from the same interneurones.

Lack of evidence for direct coupling between closer motor neurones

To test whether direct electrical or synaptic coupling between the two closer motor neurones of one segment is an additional contributory factor to their similar patterns of spikes, two series of experiments were performed.

In the first, pulses of current were injected into one motor neurone in order to test for possible effects on the other motor neurone (Fig. 4). Such pulses do not affect the frequency of ventilation, or the timing of subsequent inspiratory or expiratory periods. Instead, their effects are limited to the time that the pulse is applied. Depolarizing pulses applied during inspiration evoke unpatterned sequences of spikes in the injected motor neurone (Fig. 4*a*, *c*). There is no observable change in the membrane potential of the other neurone evoked either by the sustained depolarization or by the

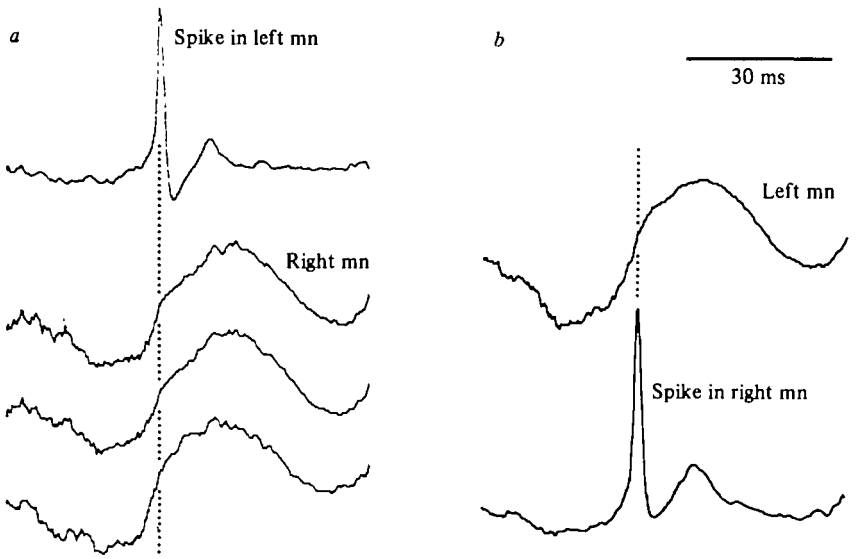

Fig. 5. Lack of evidence for direct synaptic coupling between the two mesothoracic spiracular closer motor neurones. (*a*) A spike in the left motor neurone, which occurs during expiration, is used to trigger a signal processor to average events that occur in the two motor neurones both before and after that spike. Three traces, each representing the average of 256 occurrences of a spike in the left motor neurone show a consistent depolarizing potential in the right motor neurone that precedes the spike. (*b*) A spike in the right motor neurone triggers the signal processor. A depolarizing potential in the left motor neurone now precedes this spike. The dotted lines indicate the peak of the spikes.

spikes. Pulses applied during expiration simply enhance the frequency of spikes in the injected motor neurone with no accompanying alteration of the pattern of spikes in the other motor neurone (Fig. 4*a*, *c*). Similarly, hyperpolarizing pulses that reduce the frequency of spikes, or even abolish them, do not alter the sequence of spikes in the other motor neurone (Fig. 4*b*, *d*).

A second test examined more closely the events in one motor neurone that might be associated with a spike in the other motor neurone. A spike triggered a signal averager and events that preceded or followed it were examined (Fig. 5*a*, *b*). A depolarization in one motor neurone was found to *precede* the spike in the other (Fig. 5*a*, *b*). The wave of depolarization is not smooth and in some traces (e.g. the first and second sweeps of Fig. 5*a* and Fig. 5*b*) there is a discontinuity coincident with the spike. An explanation of the depolarization is provided by the demonstrated presence of common synaptic potentials in the two motor neurones. The discontinuity is explicable not in terms of a potential linked to the occurrence of the spike, but by the fact that common potentials occur in groups of two or three (see Fig. 2*c*). The transition from one potential to the next within a group thus gives rise to the discontinuity. This test therefore does not provide evidence *against* electrical coupling but instead provides further evidence *for* common synaptic potentials. However, when the evidence from these two experiments is added to the more indirect evidence from other experiments (Burrows, 1978), there emerges no suggestion that the two closer motor neurones of one segment are electrically coupled.

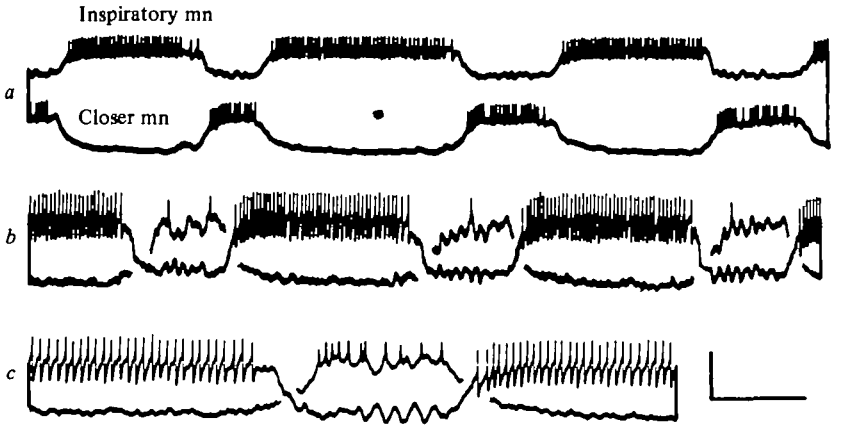

Fig. 6. Simultaneous recordings from an inspiratory motor neurone of the third median nerve (upper traces) and an expiratory, spiracular closer motor neurone of the second median nerve (lower traces) in the metathoracic ganglion. (*a*) The bursts of spikes in the two motor neurones alternate. (*b*) The onset of depolarization in one motor neurone coincides with the onset of hyperpolarization in the other. (*c*) The spiracular closer motor neurone is hyperpolarized with a steady current to reveal the similarity in its depolarizing potentials during expiration and the hyperpolarizing ones in the inspiratory motor neurone. Calibration: voltage (*a*) opener 16 mV, closer 8 mV, (*b*, *c*) opener 8 mV, closer 4 mV; time (*a*, *b*) 400 ms, (*c*) 200 ms.

Inspiratory motor neurones

In the prothoracic median nerve and in the first and second metathoracic median nerves, the opener motor neurones to the spiracles spike during inspiration when the closer motor neurones are inhibited. The mesothoracic median nerve has no opener motor neurones so that no spikes are present during inspiration. The third and fourth metathoracic median nerves contain the axons of motor neurones innervating inspiratory abdominal muscles (dorso-ventral muscles 177 and 192 respectively (Lewis *et al.* 1973)). The following description is concerned with these latter neurones. They spike during inspiration and therefore alternate with the closer motor neurones to the thoracic spiracles (Fig. 6*a*). At the beginning of expiration they are hyperpolarized at the time when the closer motor neurones are depolarized (Fig. 6*b*, *c*). There is usually, therefore, no overlap in the occurrence of spikes in the two types of motor neurone. By hyperpolarizing an impaled closer motor, the waves of EPSPs that underly its spikes during expiration can be seen to match the waves of hyperpolarizing potentials in an inspiratory motor neurone (Fig. 6*b*, *c*). The frequency of spikes in an inspiratory motor neurone remains steady at approximately 50 Hz throughout inspiration and shows no patterning (Figs. 6*c*, 7*b*). Occasional spikes at the beginning or end of the burst may, however, be of lower frequency. The spikes as recorded in the soma may have an amplitude as large as 15 mV and a duration at half height of 2–3 ms. Characteristically there is a large (5–8 mV) and prolonged repolarization after each spike.

Simultaneous intracellular recordings reveal similar patterns of spikes and synaptic potentials in two metathoracic inspiratory motor neurones with axons in the third median nerve (Fig. 7*a*, *b*). The frequency of spikes in the two may be different, so that in extracellular recordings from the median nerve beats are seen whose frequency is determined by the difference in frequency between the spikes in the two motor

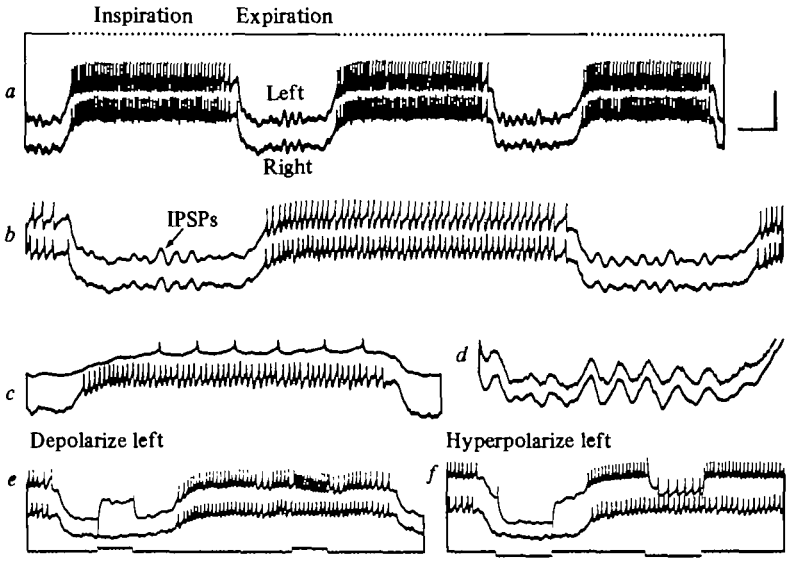

Fig. 7. Patterns of spikes and synaptic potentials in two inspiratory motor neurones with axons in the third median nerve of the metathoracic ganglion. (*a*, *b*) The left and the right motor neurones have similar patterns of spikes during inspiration and are both hyperpolarized during expiration. (*c*) During inspiration a steady hyperpolarizing current of 0·5 nA applied to the left motor neurone reveals a relatively smooth depolarization in which individual synaptic potentials are hard to discern. (*d*) During expiration, the prominent hyperpolarizing potentials are exactly matched in the two motor neurones. (*e*, *f*) Depolarizing (*e*) or hyperpolarizing (*f*) the left motor neurone with pulses of current reveals no direct coupling between these motor neurones. Calibration: Voltage (*a*, *b*) left motor neurone 16 mV, right 8 mV, (*c*) left 40 mV, right 8 mV, (*d*) left 8 mV, right 4 mV, (*e*, *f*) left 16 mV, right 8 mV; current 10 nA; time (*a*, *e*, *f*) 200 ms, (*b*, *c*) 100 ms, (*d*) 50 ms.

neurones. When one inspiratory motor neurone is hyperpolarized the underlying depolarization is seen to rise slowly at the start of inspiration, to be maintained throughout inspiration and then to fall rapidly at the end (Fig. 7*c*). When both neurones are hyperpolarized simultaneously, the time course of the depolarization and any fluctuations are seen to be the same in both. This observation indicates common synaptic driving of the two motor neurones, but as definite a conclusion as for the closer motor neurones cannot be drawn because the synaptic potentials are neither prominent nor patterned. By contrast during expiration it is obvious that the hyperpolarizing synaptic potentials are common to both neurones (Fig. 7*d*).

All observations and tests fail to provide evidence that there is direct electrical or chemical synaptic coupling between the two inspiratory motor neurones of one median nerve. A spike in one inspiratory motor neurone does not occur at a fixed time relative to a spike in the other motor neurone. No synaptic potential can be revealed in one motor neurone as a result of a spike in the other. Pulses of depolarizing or hyperpolarizing current injected into one motor neurone have no detectable effect upon the membrane potential or frequency of spikes of the other neurone (Fig. 7*e*, *f*).

Morphology of the motor neurones

Spiracular closer motor neurones

The somata of the two mesothoracic spiracular closer motor neurones lie within the ventral cortex of the ganglion some 100 μm to either side of the mid-line (Fig. 8*a*). A soma has a diameter of about 25–30 μm and gives rise to a single process, the primary neurite, 2–3 μm in diameter which enters the neuropile by running dorsally and medially (Fig. 8*a–c*). Within the central neuropile and 200 μm from the soma on the mid-line, the neurite expands to 6–7 μm. It is from this distinctive expanded segment that all the major processes of the neurone arise; the axon, the primary neurite itself and the large branches which in turn give rise to the plethora of smaller branches in the neuropile (Fig. 9*a*, *b*). In addition, some thinner branches may arise directly from this segment. The axon and the primary neurite form the lateral arm of the expanded segment on the side of the ganglion ipsilateral to the soma (Figs 8, 9). The axon does not take the shortest possible course through the neuropile, but instead loops laterally to the side of the ganglion ipsilateral to the soma, before emerging in the unpaired median nerve on the dorsal surface and towards the posterior of the ganglion. Of the four main neuropilar branches which arise from the expanded segment, two project anteriorly and dorsally and two posteriorly and dorsally (Fig. 8). The majority of smaller neuropilar branches emerge from these branches to form two main areas; one anterior and predominantly dorsal, the other posterior and dorsal (Fig. 8). In both areas there are branches on both sides of the antero-posterior mid-line.

The fine branches extend from the extreme anterior limit of the neuropile to the posterior one. Most branches are in the dorsal regions of the neuropile but some are ventral. This is more obvious in transverse sections than in the drawings of the whole ganglion in which it is not possible to represent accurately the extent of the neuropile.

The spiracular closer motor neurones in the prothoracic (Fig. 10) or metathoracic ganglion (Fig. 11) have some features in common with the mesothoracic ones. Their somata are in similar places in the ganglia, all the main neuropilar branches arise from a central expanded segment and their axons loop laterally before entering the median nerves on the dorsal surface of the ganglia.

Inspiratory motor neurones

The inspiratory motor neurones with axons in the third (Fig. 12) or fourth metathoracic median nerves also have a distinctive morphology. Their somata are about 30 μm in diameter and lie 50–70 μm from the mid-line in the ventral cortex of the ganglion. The basic structure of the neurone is Y-shaped, with two prominent lateral neuropilar branches forming the arms, and the axon the stem. The arms of the Y sweep around the lateral edges of the neuropile and give rise to lateral and medially projecting branches which in turn give rise to a multitude of smaller branches within the neuropile. The fine branches occur in both dorsal and ventral areas of the neuropile. The soma is linked to the arm of the Y on the ipsilateral side of the ganglion by a neurite some 3 μm in diameter.

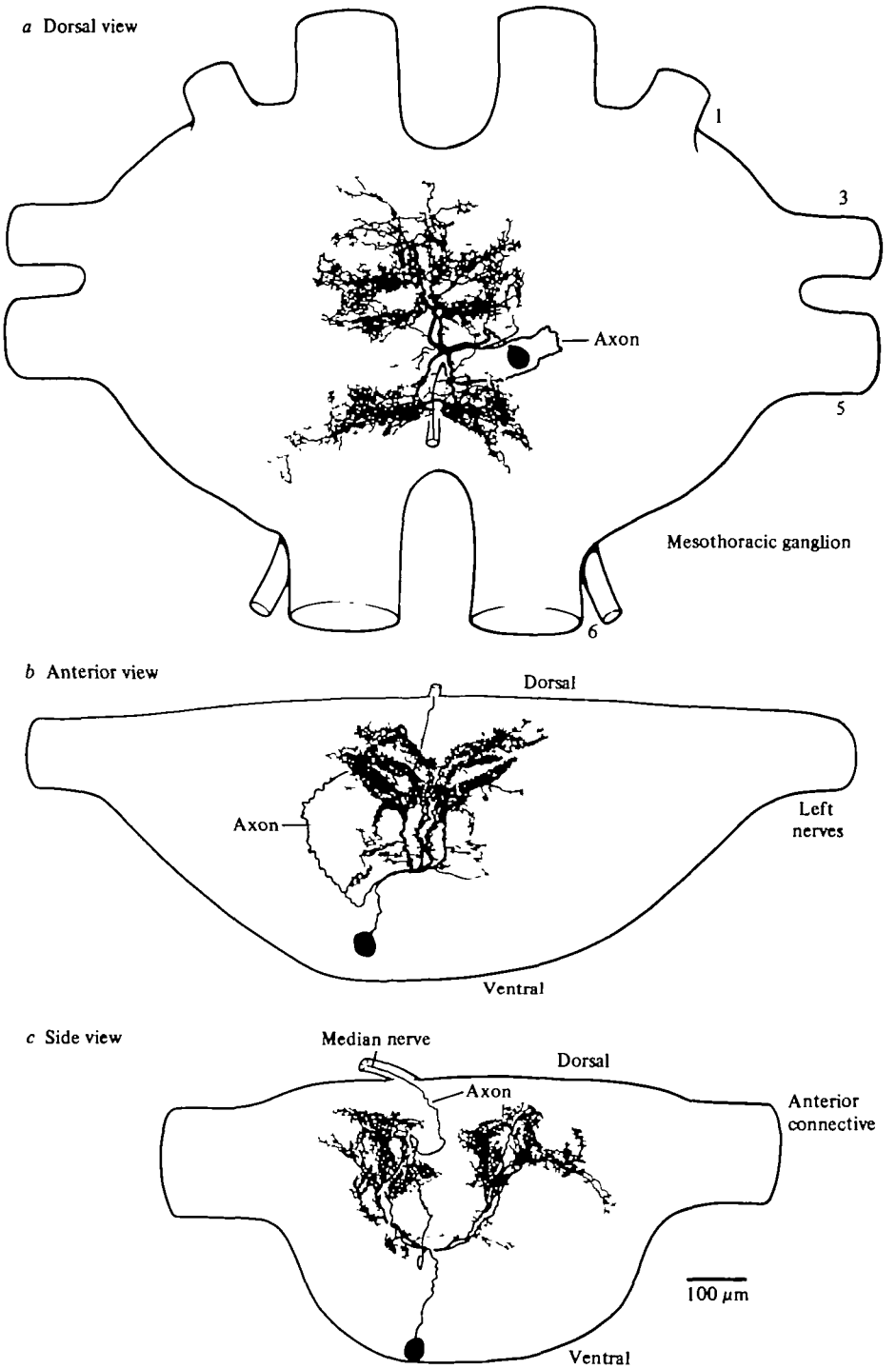

Fig. 8. The morphology of a *mesothoracic* spiracular closer motor neurone. The drawings were made from a whole-mount of the mesothoracic ganglion as viewed (*a*) dorsally, (*b*) anteriorly and (*c*) from the side. The lateral nerves are numbered in (*a*) with nerve two omitted. The axon of the spiracular closer motor neurone emerges in the unpaired median nerve from the dorsal surface of the ganglion.

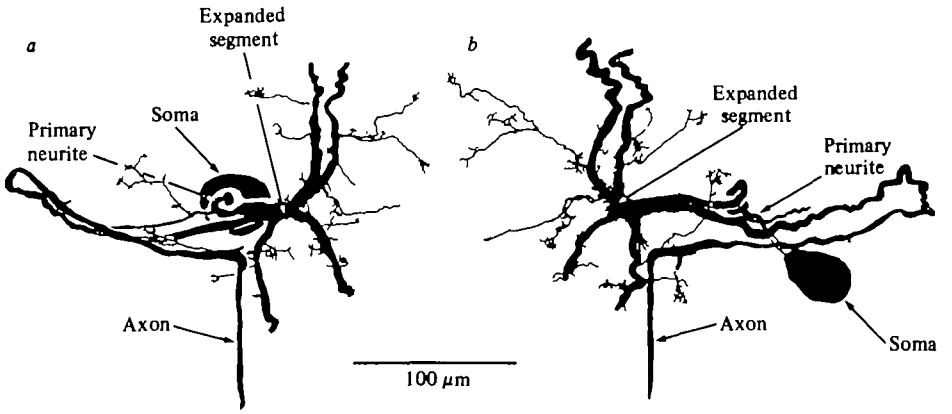

Fig. 9. The morphology of the main processes of a left (*a*) and a right (*b*) *mesothoracic* spiracular closer motor neurone stained in different locusts. Both the axon and the primary neurite emerge close together from an expanded segment. The axon then loops laterally before entering the median nerve. The main anterior and posterior branches also emerge from the expanded segment.

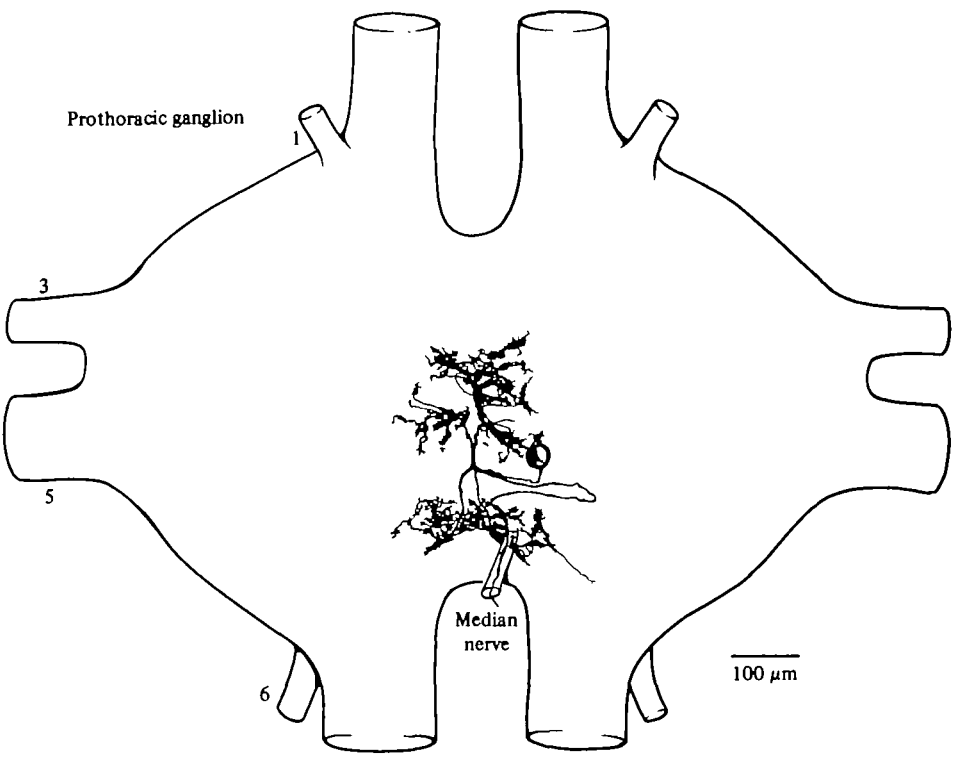

Fig. 10. The morphology of the right *prothoracic* spiracular closer motor neurone. The drawing was made from a dorsal view of a whole-mount of a prothoracic ganglion.

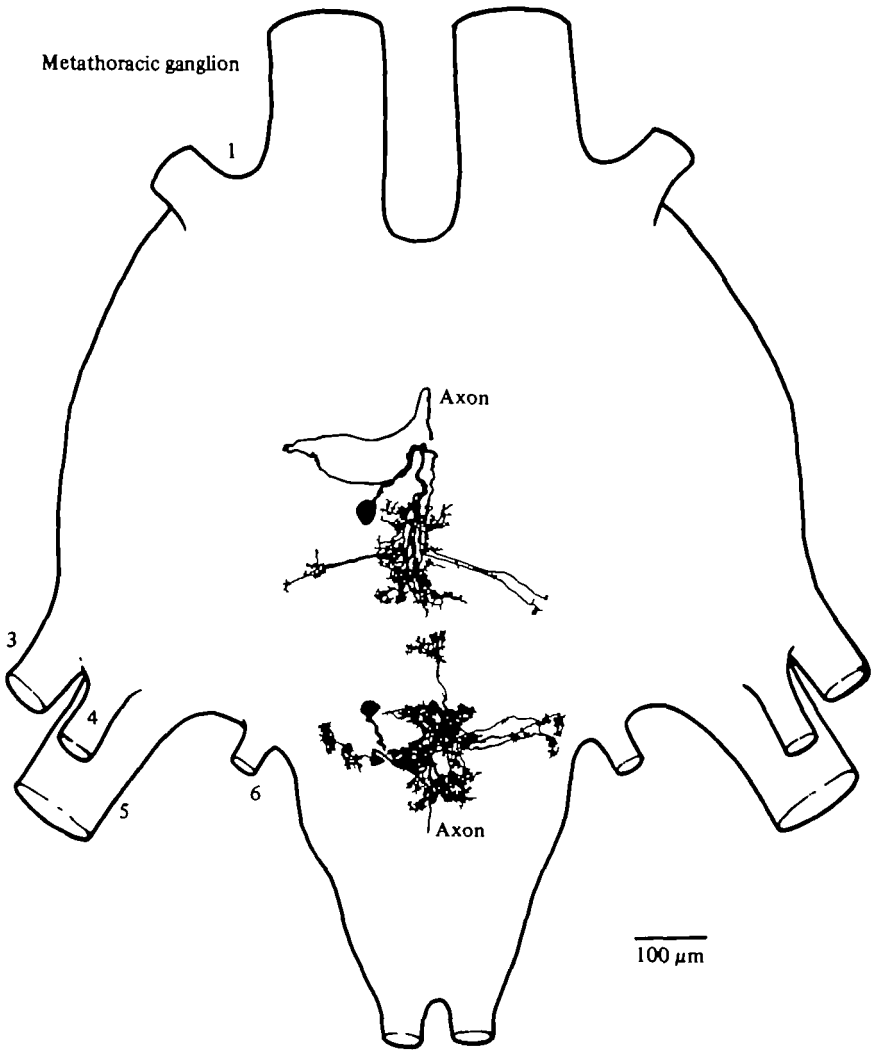

Fig. 11. The morphology of two *metathoracic* spiracular closer motor neurones. The drawing was made from a dorsal view of a whole-mount of the metathoracic ganglion. The anterior neurone has its axon in the first median nerve, the posterior one has its axon in the second median nerve. The anterior neurone was stained by Dr M. V. S. Siegler. The neurones are from different locusts.

DISCUSSION

Common synaptic driving is the method used by the locust to ensure that the two median nerve motor neurones innervating the same muscles of one segment produce similar bursts of impulses at the same time. An alternative method which might ensure the same result, coupling the motor neurones with electrical synapses, could not be demonstrated. In this respect these motor neurones resemble the majority of others examined in insects by showing no direct electrical coupling. The only evidence for electrical coupling between insect motor neurones is of an indirect nature for some flight motor neurones in crickets (Bentley, 1969). In fact electrical coupling between neurones is extremely rare in insect nervous systems as a whole. For limb

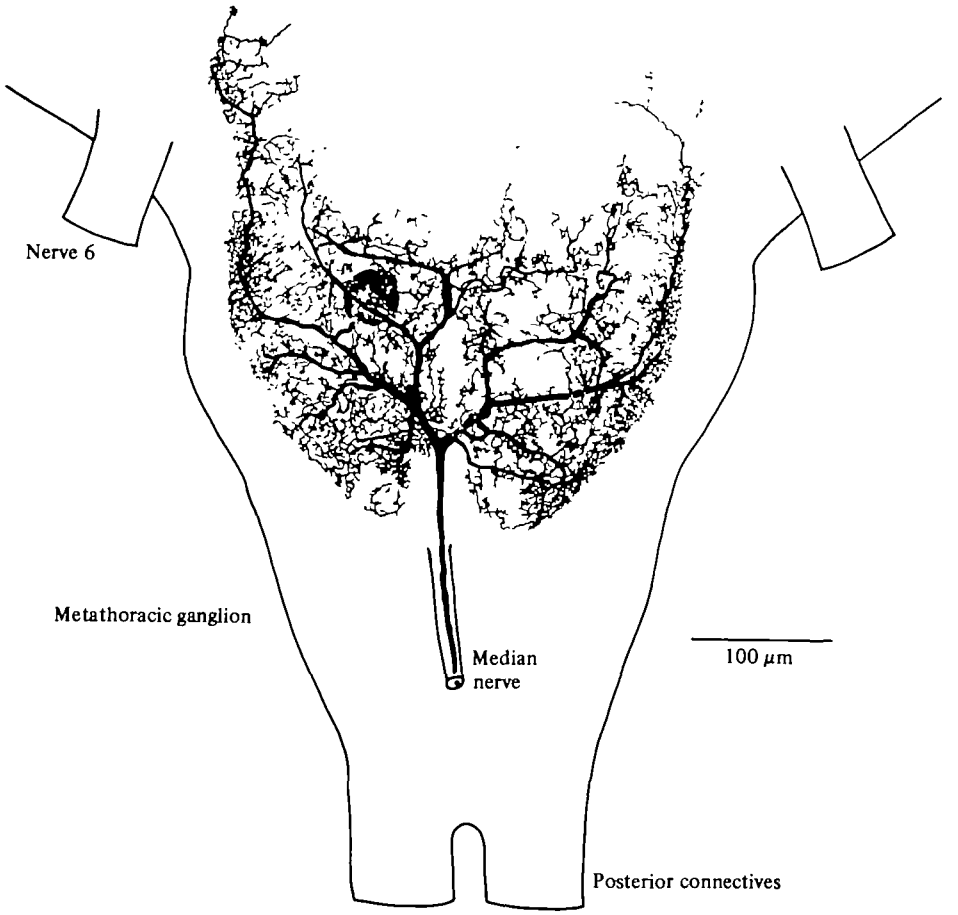

Fig. 12. The morphology of a *metathoracic* inspiratory motor neurone. The drawing is of a dorsal view of the ganglion posterior to the emergence of lateral nerves 5 to the hind legs. The axon of the motor neurone emerges in the third median nerve.

motor neurones the lack of electrical coupling can be most easily rationalized. Each muscle is innervated by few motor neurones, often only two, so that in order to allow an individual motor neurone independence of action in the wide range of movements in which it must participate, it is better that it is not coupled to other homonymous motor neurones.

For median nerve motor neurones, however, there is no evidence that an individual motor neurone shows any independence of action. During ventilation both neurones show similar patterns of activity, but no careful search has been made to see if local reflexes involve just one motor neurone. The overriding constraint on the control of the motor neurones would seem to be that they be coupled to the ventilatory rhythm by as small a number of interneurones as possible. The common synaptic potentials in the two closer, or the two inspiratory motor neurones of one segment indicate that the same interneurone innervates each member of a pair. From observations of these common synaptic potentials it is possible to estimate the numbers of presynaptic interneurones involved. During expiration, no more than two interneurones are

needed to explain the depolarizing potentials in the closer motor neurones and the inhibitory potentials in inspiratory motor neurones. These two interneurones synapse upon both closer motor neurones in each thoracic segment and upon some flight motor neurones (Burrows, 1975*a*, *b*). Similarly, during inspiration no more than two interneurones are needed to explain the inhibitory potentials in the closer motor neurones. The control of the inspiratory motor neurones during this period is, however, less well understood. The number of interneurones would seem to have been reduced to the minimum commensurate with the execution of as vital a function as breathing.

Correlating function and structure

The large and brief spike recorded in the soma of a spiracular closer motor neurone is perhaps explicable in terms of the unusual morphology of this neurone. All the insect motor neurones that have been stained previously conform to the same basic plan: a single process emerges from the soma and enters the neuropile where it expands in diameter and gives rise to numerous side branches. As this main neurite approaches the edge of the neuropile side branches no longer occur and it narrows to form the axon which enters a lateral nerve. Spikes are thought to originate in a region of the main neurite that bears side branches near to the edge of the neuropile (Gwilliam & Burrows, 1980). Thus the soma is distant from this spike initiating site and joined to it by a membrane that cannot support a propagated action potential. By contrast the soma of a closer motor neurone is at most only 200 μm away from the axon. The primary neurite from the soma joins the axon and is not separated from it by a long and broad region of neurite bearing the numerous side branches. Assuming similar membrane properties to other insect motor neurones, the spike should be larger in the soma of closer motor neurones.

In most insect motor neurones the neuropilar branches are restricted to the side of the ganglion which contains the soma and from which the axon emerges (Burrows, 1973). There are exceptions, most notably a dorsal longitudinal flight motor neurone whose soma is contralateral to its axon and whose branches occur on both sides of a ganglion, though predominantly on the side containing the axon (Tyrer & Altman, 1974). In contrast, the neuropilar branches of the median nerve motor neurones have a similar distribution on both the left and right sides of a ganglion. This feature accords with their physiological function of innervating muscles on both sides of the body. What remains to be explained is the considerable number and extent of the branches. The control of the motor neurones can be adequately explained by the presence of very few presynaptic interneurones. In order to offer further explanations about the morphology of these motor neurones and about their physiological control, it is clearly now necessary to identify the antecedent interneurones, inferred to exist from the common synaptic potentials, and to identify other inputs which might modulate their output.

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