THE IDENTIFICATION OF MOTOR NEURONES INNERVATING AN ABDOMINAL VENTILATORY MUSCLE IN THE LOCUST

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SUMMARY

Motor neurones to abdominal ventilatory muscles, with their axons in nerve 6 of the metathoracic ganglion of the locust, have been identified by intracellular recording and staining. Three muscles are innervated by the larger branches of this nerve: nerve 6a contains six motor neurones innervating the ventral diaphragm; nerve 6b contains four motor neurones innervating the median internal ventral muscle, and nerve 6d contains five motor neurones innervating the longitudinal dorsal muscle. All motor neurones innervate muscles on one side of the body only. Both the median internal ventral and the longitudinal dorsal muscles contract during the expiratory phase of ventilation. Three excitatory motor neurones to the median internal ventral muscles spike during expiration whilst the fourth, an inhibitory motor neurone, is active during both expiration and inspiration. Two of the excitatory motor neurones have cell bodies in the half of the ganglion ipsilateral to the muscle they innervate. Their neuropilar branches, however, are in both left and right halves of the ganglion. The third excitatory motor neurone has its cell body close to the midline and has most of its neuropilar branches in the half of the ganglion ipsilateral to its axon. The inhibitory motor neurone has its cell body just to the contralateral side of the midline, and three distinct areas of neuropilar branches, two contralateral and one ipsilateral to its axon.

INTRODUCTION

Ventilation in the locust is accomplished by the circulation of air within a complex network of tracheae. The force that propels the air through the body is generated by the rhythmical contraction of muscles in the abdomen. The paired spiracles on each of the thoracic and abdominal connectives open and close in time with this rhythm to allow the passage of air into or out of the body.

The ventilatory rhythm is generated in the metathoracic ganglion (Miller, 1960, 1966). When isolated from this ganglion by severing the linking connectives, the more

posterior abdominal ganglia are able to sustain a slower rhythm, but the more anterior thoracic ganglia are unable to do so. The first three abdominal ganglia are, however, fused to the neuromere of the third thoracic segment to form the metathoracic ganglion and thus cannot be isolated by simple surgical procedures. The network of neurones responsible for generating the ventilatory rhythm could therefore reside in any of the fused ganglia, or be distributed between them.

Few of the neurones involved in the production or execution of the ventilatory rhythm have been characterized. Only two interneurones are known, one that conveys the rhythm to abdominal ganglia (Pearson, 1980), and the other to thoracic ganglia (Burrows, 1982b). Of the many motor neurones, only those innervating the closer muscles of the thoracic spiracles have been characterized in any detail (Burrows, 1975, 1982a). For the remaining motor neurones, the characterization is less complete; some of the rhythmical synaptic input to motor neurones in the metathoracic ganglion has been revealed (Burrows, 1974), as has the distribution of cell bodies of motor neurones in the first unfused abdominal ganglion (Lewis, Miller & Mills, 1973).

The object of this paper is to identify the ventilatory motor neurones that have their axons in nerve 6 of the metathoracic ganglion and innervate muscles in the first abdominal segment. Attention is focused upon four motor neurones innervating one particular muscle that contracts during expiration. The motor neurones are identified by their morphology and by their action during ventilation.

MATERIALS AND METHODS

Adult male and female locusts, *Schistocerca americana gregaria* (Dirsh) [= *S. gregaria* (Forskal)] were obtained from our crowded laboratory culture. The metathoracic ganglion was prepared for intracellular recording from the cell bodies of motor neurones in a way that has been described in detail elsewhere (Hoyle & Burrows, 1973). Electrodes were filled with 2 M potassium acetate, with 0·4 M-cobaltous chloride (Pitman, Tweedle & Cohen, 1972) or with 0·1 M hexamine cobaltic chloride (Brogan & Pitman, 1981). Neurones were stained by the intracellular injection of cobalt, or by infusing the cut ends of their axons with cobalt, and then by intensifying the stain with silver (Bacon & Altman, 1977). Drawings of the stained neurones were made with the aid of a drawing tube attached to a compound microscope. The numbering of the muscles in the abdomen is based on Albrecht (1953).

RESULTS

Nerve 6 emerges from the metathoracic ganglion just posterior to nerve 5 to the hind leg (Fig. 1). Its larger branches are lettered sequentially. The first branch, 6a, innervates the thin sheet of muscle that forms the ventral diaphragm. Nerve 6 then continues posteriorly passing between the two sternal apophyses before branching again. The second branch, nerve 6b, innervates muscle 143, the median internal ventral muscle which lies just beneath the ventral cuticle, close to the midline. This muscle arises from the posterior of the antecosta of the first sternum and inserts on the

posterior surface of the second sternum. Nerve 6 then runs dorsally with a third branch, nerve 6c, innervating the tympanum. The fourth branch, nerve 6d, innervates muscle 141, the longitudinal dorsal muscle. This muscle lies dorsal to the tympanum, arising from the third phragma of the first abdominal tergum and inserting onto the anterior edge of the second abdominal tergum.

Infusing cobalt chloride into the cut end of individual branches of N6 reveals the cell bodies of neurones that have axons in that branch (Fig. 2). The number and location of the cell bodies were determined on a minimum of five occasions for each branch. All the cell bodies revealed are on the ventral surface of the ganglion. Staining nerve 6a to the ventral diaphragm reveals six cell bodies, four in the half of the ganglion ipsilateral to the stained nerve and two contralateral (Fig. 2A). The ipsilateral cell bodies occur in two groups, one more posterior and lateral than the other, and each with two cell bodies. Staining nerve 6b to the median internal ventral muscle reveals four cell bodies, two ipsilateral to the stained nerve, one close to the midline, and one contralateral (Fig. 2B). Staining nerve 6d to the longitudinal dorsal muscle
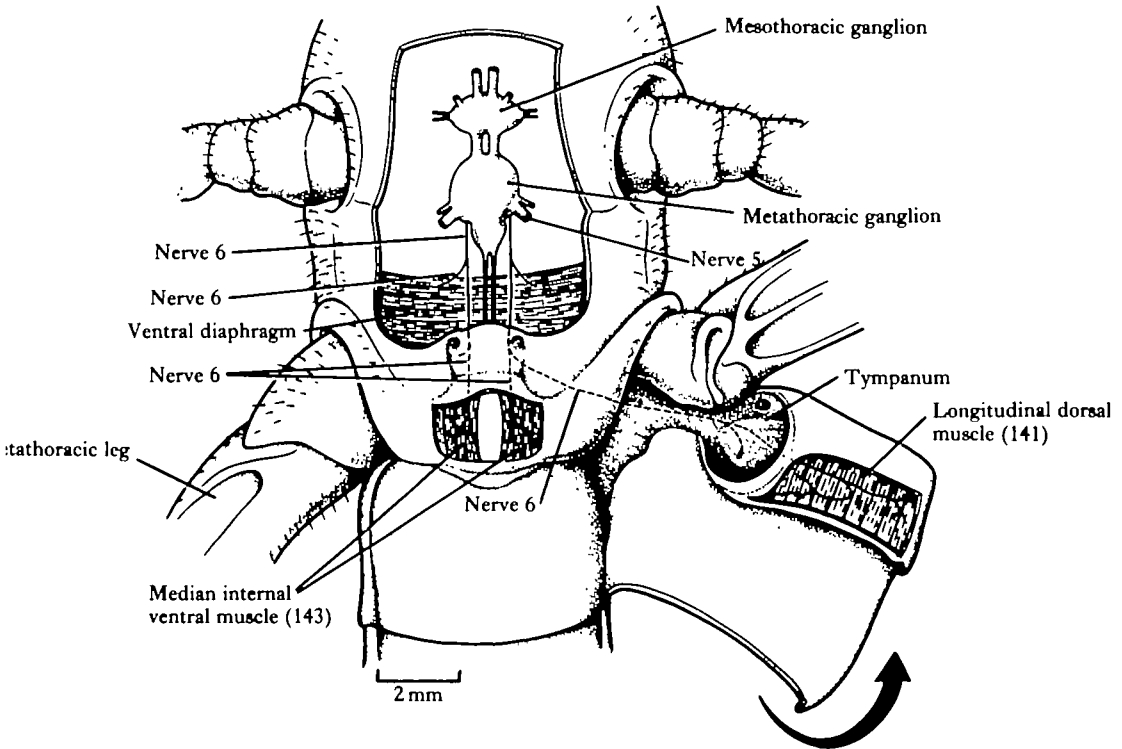

Fig. 1. A drawing of a ventral dissection of a locust to show the muscles innervated by nerve 6 of the metathoracic ganglion. The thorax is opened to show the meso- and metathoracic ganglia and the innervation of the ventral diaphragm by branch 6a. The abdomen has been cut along the dorsal midline and the dorsal part of the first segment deflected to one side (as indicated by the arrow). This allows the median internal ventral muscle (number 143) and its innervation from nerve 6b to be seen at the same time as the longitudinal dorsal muscle (number 141) and its innervation from nerve 6d. Both muscles are viewed through windows cut in the cuticle. The tympanum, innervated by nerve 6c can also be seen.
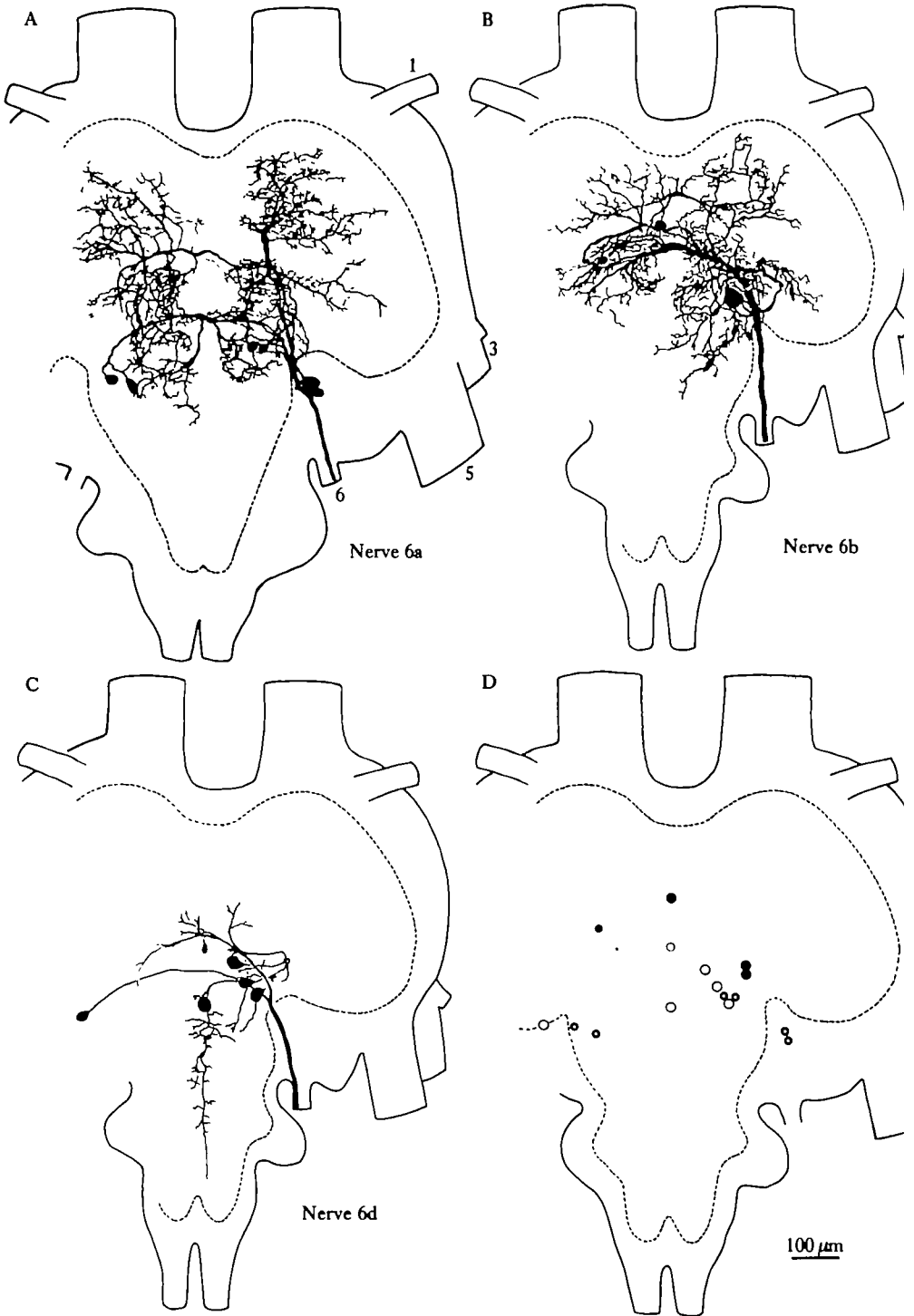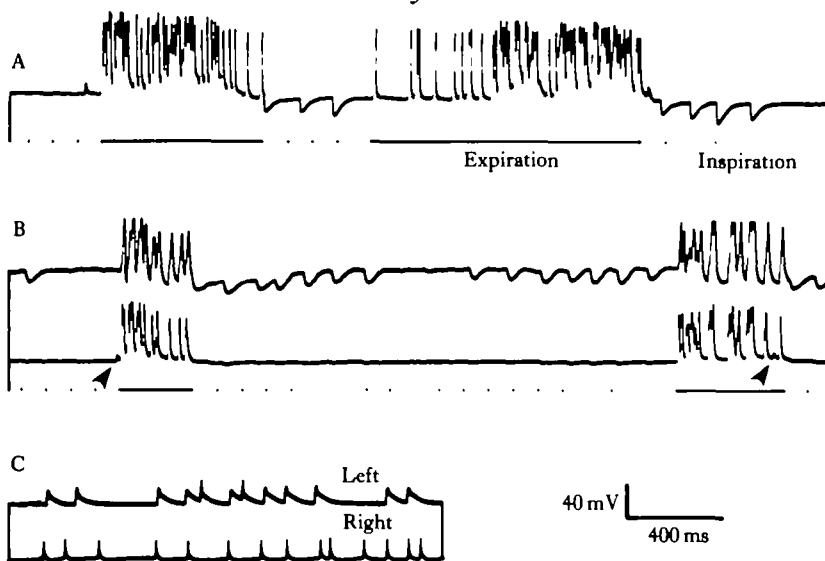
Fig. 2

Fig. 3. Intracellular recordings from the median internal ventral muscle. (A) During expiration, excitatory motor neurones evoke depolarizing junctional potentials, and during inspiration there are hyperpolarizing junctional potentials. (B) A simultaneous recording from two muscle fibres that are both innervated by two excitatory motor neurones, and by an inhibitory motor neurone. A third excitatory motor neurone evokes small depolarizing potentials only in the fibre on the second trace (arrows). (C) A simultaneous recording from a fibre in the muscle on the left (first trace) and on the right (second trace) side of the body, indicates that the two muscles are innervated by separate motor neurones. In this and other Figures, expiration is indicated by a continuous line, inspiration by a dotted one.

typically reveals five cell bodies, four ipsilateral and one contralateral (Fig. 2C). Sometimes a sixth neurone is also revealed with its cell body close to the midline and more anterior than the other cell bodies of this branch.

Combining the stains of the individual branches in one drawing shows that the cell bodies of the various branches of N6 are intermingled, and are not distributed according to the spatial distribution of the muscles they innervate (Fig. 2D).

Action and innervation of the median internal ventral muscle

During ventilatory movements of the abdomen, the median internal ventral muscle contracts rhythmically in time with expiration. Intracellular recordings from its fibres reveal bursts of excitatory junctional potentials (EJPs) during expiration and inhibitory junctional potentials (IJPs) during inspiration (Fig. 3A, B). Occasional IJPs may also occur during expiration. One muscle fibre can be innervated by several motor neurones. During expiration, EJPs of three different amplitudes can be recognized in

Fig. 2. The distribution of cell bodies and main arborizations of motor neurones with axons in nerve 6. Individual branches were infused with cobalt, and the ganglia were then intensified with silver. (A) Staining nerve 6a (ventral diaphragm) reveals six cell bodies; (B) staining nerve 6b (median internal ventral muscle) reveals four cell bodies; (C) staining nerve 6d (longitudinal dorsal muscle) reveals five cell bodies; (D) the average positions, based on five stains of each branch, of the cell bodies with axons in nerve 6. Filled circles represent nerve 6b, thin circles nerve 6d and thick circles nerve 6a. The dashed line indicates the extent of the neuropile. Lateral nerves 1, 3, 5 and 6 only are indicated. Not all the fine branches of the stained neurones are drawn.

some fibres. No more than this number has ever been observed in one fibre. Stimulat-
ing nerve 6b with current pulses of gradually increasing strength causes three distinc■
increases in the amplitude of the evoked potential in a muscle fibre, suggesting that
three excitatory motor neurones are recruited sequentially. The muscle was also
sampled extensively with two microelectrodes, one remaining in a particular fibre as
a reference, whilst the second sampled fibres from other regions. This test shows that
three excitatory motor neurones are sufficient to explain the pattern of EJPs, and that
they do not innervate all fibres equally (Fig. 3B).

A similar series of tests was carried out to reveal the number of inhibitory motor
neurones. Only one size of IJP is seen in any one muscle fibre, although the size of
the IJPs varies considerably from fibre to fibre (Fig. 3B). The pattern of IJPs can be
explained by the existence of just one inhibitory motor neurone.

Simultaneous intracellular recordings from fibres in the median internal ventral
muscle in the left and the right halves of the body show no overlap in their innervation.
Each muscle is innervated by a separate and bilaterally symmetrical set of motor
neurones (Fig. 3C).

Identification of the motor neurones

The motor neurones innervating the median internal ventral muscle were charac-
terized both physiologically and morphologically.

Excitatory motor neurones

To characterize neurones physiologically, simultaneous intracellular recordings
were made from a cell body within the ganglion and from a muscle fibre. Fig. 4
illustrates this procedure for one of the excitatory motor neurones. Spikes recorded
in a particular cell body are correlated with one class of EJPs in a muscle fibre (Fig.
4A). Triggering the oscilloscope from the spikes shows that EJPs follow without
failure and with a constant latency (Fig. 4B). Stimulating nerve 6b evokes an
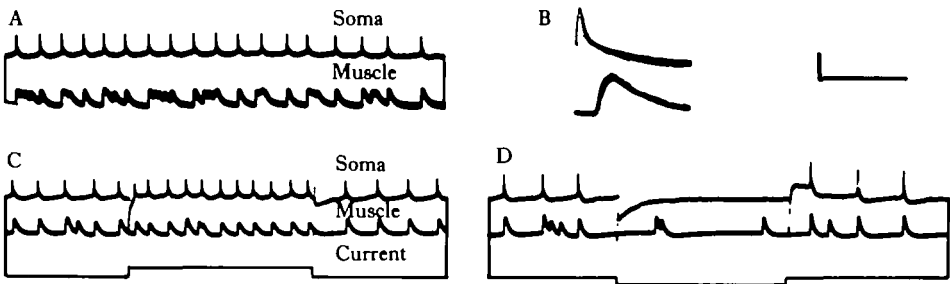

Fig. 4. Physiological identification of an excitatory motor neurone whose morphology is shown
in Fig. 5. Simultaneous intracellular recordings were made from the cell body of the motor neurone
and from a fibre in the median internal ventral muscle. (A) Spikes in the cell body correspond with
one class of junctional potential in the muscle. (B) Multiple sweeps triggered by the spikes of the
motor neurone show the occurrence, after a constant delay, of junctional potentials in the muscle. (C)
A pulse of depolarizing current injected into the cell body increases the frequency of spikes and
junctional potentials. (D) Hyperpolarizing current abolishes the spikes and abolishes one size of
junctional potential. Calibration: voltage, (A, C, D) 20 mV, (B) 10 mV: current 10 nA: time (A, C,
D) 400 ms, (B) 20 ms.

antidromic spike in the cell body and at the same time an EJP in a muscle fibre. This indicates that no other neurone is interposed between that penetrated in the ganglion and the muscle fibre. The neurone is therefore a motor neurone.

Depolarizing the motor neurone directly with current injected through the recording microelectrode increases the frequency of EJPs in a muscle fibre (Fig. 4C). The frequency increases by approximately 1 Hz for each nA of current injected, up to values of 20 nA. An imposed hyperpolarization of the motor neurone that is sufficient to abolish spikes, also abolishes EJPs in a muscle fibre (Fig. 4D).

Altering the membrane potential of this, or other, motor neurones to the median internal ventral muscle has no disruptive effect on the ventilatory rhythm. Increasing or decreasing the frequency of spikes in one motor neurone likewise has no effect on the frequency of EJPs or IJPs evoked by the other motor neurones. These tests therefore provide no evidence for the existence of electrical or synaptic coupling between members of this pool of motor neurones. Furthermore, examination of the sequence of EJPs during expiration indicates that no preferred time relationships are maintained by the spikes of the different excitatory motor neurones.

Following physiological characterization, a motor neurone was injected with cobalt to reveal its morphology (Fig. 5). The motor neurone described above has a cell body
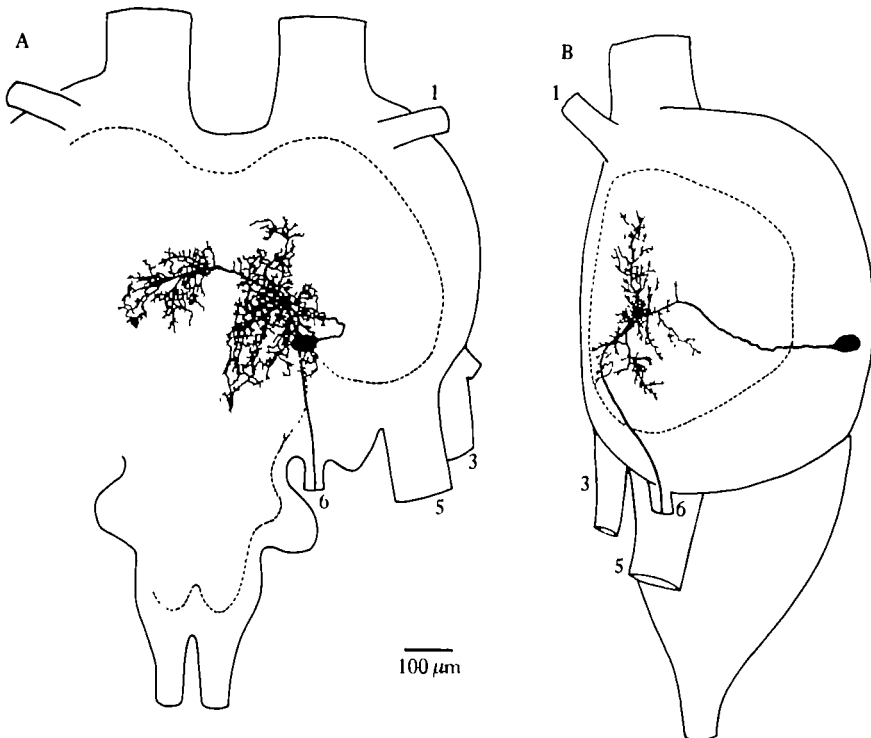

Fig. 5. A drawing of an excitatory motor neurone to the median internal ventral muscle that has its cell body ipsilateral to its axon. There are two motor neurones with this morphology. The motor neurone shown was stained by the intracellular injection of cobalt into its cell body. (A) A drawing from a whole-mount of the ganglion as viewed dorsally. The cell body is ventral and is thus partially obscured by the more dorsal branches. (B) A side view of the same motor neurone. Nerves 1, 3, 5 and 6 only are indicated. This motor neurone, or its partner of similar morphology, has been stained on eight occasions.
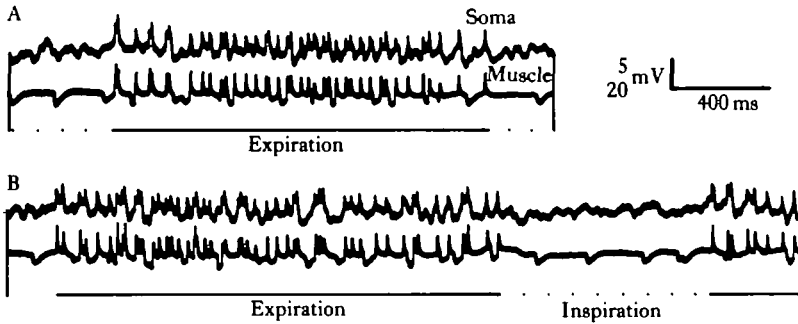
Fig. 6. The action during ventilation of the excitatory motor neurone whose morphology is shown in Fig. 7. Simultaneous recordings were made from the cell body of the motor neurone and from a fibre in the median internal ventral muscle. The muscle fibre was innervated by this excitatory motor neurone and the inhibitor. (A, B) Continuous record to show two complete expiratory phases of ventilation. During both inspiration and expiration there is a complex excitatory and inhibitory synaptic input that during expiration results in an uneven frequency of spikes.

of approximately 40 μm in diameter. It lies within the ventral cortex of the ganglion at co-ordinates defined by an antero-posterior line passing through the centre of the ipsilateral anterior connective, and by a line linking the anterior point of emergence of lateral nerves 3. The primary neurite runs dorsally from the cell body to give rise to two distinct areas of branches in the more dorsal regions of the neuropile. The first area is contralateral to the axon. Branches from it extend anteriorly and posteriorly but do not cross the midline. The second area is less extensive and is ipsilateral to the axon. It arises from a single process that crosses the midline in a dorsal commissure. No branches emerge from this process for approximately 20 μm on either side of the midline, thus providing a clear separation of the two areas of branches.

Two motor neurones with these physiological and morphological characteristics have been found. In some ganglia in which nerve 6b is infused with cobalt, the two cell bodies can be seen to lie close together and their major processes to follow parallel courses. In other experiments, the two motor neurones have been stained in the same locust by the intracellular injection of cobalt. Again both appear to have the same shape, with their branches superimposed and forming distinct fields in the left and right halves of the ganglion.

The third excitatory motor neurone has similar physiological characteristics, but a quite distinct morphology when compared with the above motor neurones. Recordings from its cell body reveal a barrage of predominantly depolarizing synaptic potentials during expiration, and predominantly hyperpolarizing potentials during inspiration (Fig. 6A, B). The depolarization during expiration is sufficient to evoke spikes at an unsteady frequency, with larger depolarizations associated with groups of two or three spikes.

The cell body of this third motor neurone is approximately 20 μm in diameter and lies close to the ventral midline (Fig. 7). The primary neurite enters the more dorsal areas of the neuropile and gives rise to two branches ipsilateral to the cell body. These are the only branches in this half of the ganglion. The primary neurite then crosses the midline in a commissure that is further anterior than that containing the primary neurites of the two preceding excitatory motor neurones. Once across the midline, a
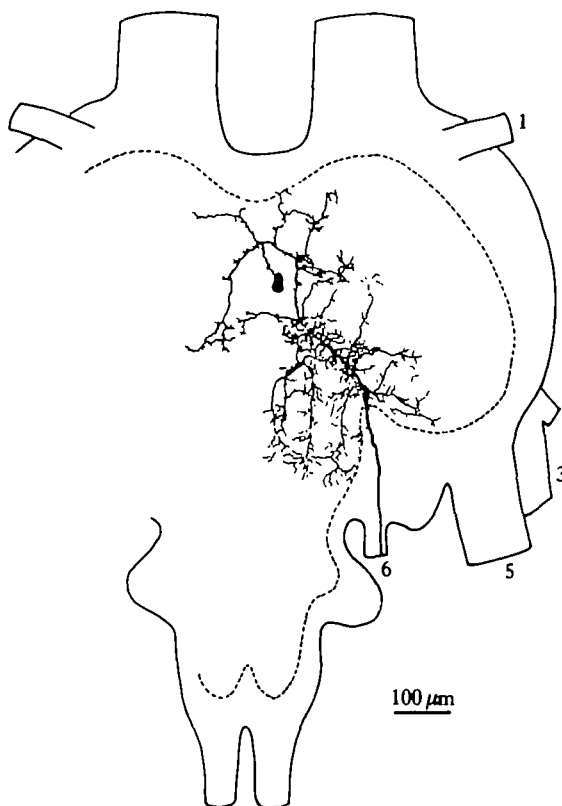
Fig. 7. The morphology of the third excitatory motor neurone to the median internal ventral muscle. The motor neurone was stained and then drawn in the same way as the one in Fig. 5. It has been stained four times.

few branches run anteriorly to approach the anterior limits of the neuropile, but the majority of branches are more posteriorly directed. These branches are in both the metathoracic neuropile and that belonging to the first abdominal ganglion.

Inhibitory motor neurone

Spikes in the cell body of one motor neurone with an axon in nerve 6b evoke IJPs in the median internal ventral muscle (Fig. 8). The pattern of spikes frequently shows no obvious modulation in time with the ventilatory rhythm. Often, however, there are more spikes and hence more IJPs during inspiration. The evidence accumulated so far indicates that the inhibitory effect on the muscle is brought about by a postsynaptic conductance mechanism (Fig. 8B). A long series of recordings from one muscle fibre shows many different time relationships between an IJP and an EJP. When the IJP follows an EJP, it abolishes the tail of the EJP and reduces the expected initial height, depending on how closely it follows. When the IJP precedes the EJP, it reduces the depolarization that would normally be caused by the EJP.

These effects can make it difficult to measure the frequency of EJPs in a muscle fibre during ventilation, as some will be obscured by the occurrence of the IJPs. This effect can be demonstrated by altering the frequency of spikes in the inhibitor by

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Fig. 8. The action of the inhibitory motor neurone during ventilation. (A) A simultaneous recording from the cell body (first trace) and from fibres in muscles on the left (second trace) and right (third trace) halves of the body. The frequency of spikes changes little during the two phases of ventilation. The inhibitor innervates muscles on one side of the body only. (B) The inhibitory action. A selected series of spontaneous depolarizing and hyperpolarizing junctional potentials to show the reduction in the EJP when an IJP occurs. Calibration: voltage (A), motor neurone 2 mV, muscle fibres 20 mV, (B) 10 mV; time (A) 400 ms, (B) 200 ms.

Fig. 9. The morphology of the inhibitory motor neurone to the median internal ventral muscle. The motor neurone was stained by the intracellular injection of cobalt into its cell body following physiological characterization. This motor neurone has been stained four times.

passing current into its cell body. When depolarized, the frequency of EJPs appears to decrease because more IJPs occur. When hyperpolarized, the frequency of EJPs appears to increase as fewer are obscured by the IJPs.

When stained with cobalt, the motor neurone with these inhibitory effects is seen to have a cell body contralateral to its axon (Fig. 9). It is about 25 μm in diameter and lies in the ventral cortex of the ganglion at a position given by an antero-posterior line passing close to the medial edge of the anterior contralateral connective and a line just anterior to the emergence of lateral nerves 2. There are three distinct areas of neuropilar branches, two contralateral to the axon and one ipsilateral. The primary neurite from the cell body runs dorsally and then turns towards the midline, giving off a series of branches which form the first and more posterior contralateral area. The second contralateral area arises from one anteriorly directed process. After its emergence from the primary neurite, this process is devoid of side branches for about 100 μm before it branches profusely at the anterior edge of the neuropile. The primary neurite crosses the midline in a dorsal commissure, before giving rise to a more restricted area of branches ipsilateral to the axon. This area is at the same level as the posterior ipsilateral area, but the branches do not extend so far laterally.

DISCUSSION

Two of the large branches of metathoracic nerve 6 of the locust innervate two muscles in the abdomen, the median internal ventral muscle and the longitudinal dorsal muscle, both of which are active during the expiratory phase of ventilation. This is not their only function, for during flight the motor neurones to these muscles spike in bursts in time with the movements of the wings (Camhi & Hinkle, 1974). During flight, but not during normal ventilatory movements, these motor neurones respond with changes in the frequencies of their spikes to wind blown at the head. They thus contribute to the postural adjustments made by the abdomen as a means for controlling the stability of flight, in addition to their continuing role in ventilation.

Camhi & Hinkle (1974) also examined the innervation of some abdominal muscles. They traced a nerve from the metathoracic ganglion which they called nerve 4, but which would appear to be the nerve examined in this paper, namely nerve 6. They describe the nerve as innervating the longitudinal dorsal muscle, 141, and a ventral longitudinal muscle, 113. It would appear that the latter muscle was given an incorrect number and is in fact the one described in this paper, the median internal ventral muscle, 143. In cross sections of the nerves innervating these muscles viewed with the light microscope, Camhi & Hinkle (1974) found approximately 15 axons in the branch to the longitudinal dorsal muscle, of which five were 10 μm and the others less than 5 μm in diameter. Assuming that the large diameter axons belong to motor neurones, this result accords well with the stains reported here in which typically five motor neurones are found. The branch to the median internal ventral muscle contained two axons of 3 μm and five of 1 μm diameter (Camhi & Hinkle, 1974). Their recordings from this branch revealed two units with large spikes that were readily distinguishable from the others. Our results, however, based on infusing cobalt into the axons of this branch, on intracellular recordings from the muscle and on the identification of the

motor neurones in the ganglion, indicate that four motor neurones innervate this muscle; three are excitatory and one is inhibitory.

Morphology of the motor neurones

The cell bodies of neurones with axons in nerve 6 are scattered in the middle region of the metathoracic ganglion. Some are clearly in the metathoracic neuromere, although they innervate muscles in the first abdominal segment. Those that innervate the longitudinal dorsal muscle show a distribution characteristic of the cell bodies of motor neurones to the homologous muscle in the thoracic segments of this (Neville, 1963) and other insects (see Kondoh & Obara, 1982 for most recent review): four cell bodies are ipsilateral to the muscle innervated, and one is contralateral.

Of the three excitatory motor neurones to the median internal ventral muscle, two have ipsilaterally placed cell bodies whilst the third has its cell body at the midline close to some of the inhibitors to leg muscles (Burrows, 1973). No other excitatory motor neurones to the legs, wings or abdomen are yet known with such medially placed cell bodies. The inhibitory motor neurone, by contrast has its cell body just contralateral to the midline. No functional correlate of this distribution has yet been found. Unlike leg motor neurones, those which innervate the median internal ventral muscle have distinct areas of branches in both the left and the right halves of the ganglion. An area contralateral to the axon of the inhibitory motor neurone extends to the anterior limits of the metathoracic neuropile. The reason for these extensive anterior, or bilateral projections is not known, and an explanation must await a more detailed knowledge of the inputs which control the action of these motor neurones. Only one other motor neurone in the metathoracic ganglion of the locust, a thoracic dorsal longitudinal motor neurone (Tyrer & Altman, 1974) is yet known to have branches in the left and right halves of the ganglion, and this has a cell body on the side of the ganglion contralateral to its axon. The reason for this bilateral distribution of branches is again unknown.

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