CO-ORDINATING INTERNEURONES OF
THE LOCUST WHICH CONVEY TWO PATTERNS OF
MOTOR COMMANDS: THEIR CONNEXIONS WITH
VENTILATORY MOTONEURONES

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SUMMARY
1. The interneurones which make widespread connexions with flight
motoneurones also synapse upon ventilatory motoneurones so that in all 50
motoneurones receive synapses. They influence three aspects of ventilation;
(a) the closing and opening movements of the thoracic spiracles, (b) some
aspects of abdominal pumping movements and (c) the recruitment of some
motoneurones controlling head pumping.

2. The two closer motoneurones of a particular thoracic spiracle receive
the same excitatory synaptic inputs (EPSPs) during expiration. The EPSPs
match those in appropriate flight motoneurones.

3. The closer motoneurones of each thoracic spiracle whose somata are in
the pro-, meso- or metathoracic ganglia all receive the same excitatory
synaptic inputs. These inputs are an adequate explanation of the pattern of
spikes in the closer motoneurones. Both the slow ventilatory and fast
rhythms of synaptic potentials are expressed as spikes; the slow as the over-
all expiratory burst of spikes and the fast as the groups of spikes within that
burst. This establishes a ventilatory function for the interneurones. All
thoracic closer motoneurones therefore receive the same excitatory com-
dands which will tend to synchronize the movements of each spiracle.

4. Spiracular opener motoneurones are inhibited during expiration, their
IPSPs matching the EPSPs in flight or closer motoneurones. Therefore the
interneurones have reciprocal effects on the antagonistic motoneurones of the
spiracles.

5. The interneurones synapse upon some motoneurones which control the
pumping movements of the abdomen and which have their somata in the
metathoracic or first unfused abdominal ganglion. Motoneurones in four
separate ganglia therefore receive inputs from these interneurones.

6. The interneurones also synapse upon motoneurones which control an
auxiliary form of ventilation, head pumping.

INTRODUCTION
At least two interneurones make widespread connexions with some of the moto-
neurones innervating the flight muscles of the locust (Burrows, 1975). They depolarize

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the motoneurones in two rhythms; a fast rhythm which has a period similar to that of
the wingbeat in flight and a slow rhythm in time with ventilation. The slow rhythmic
depolarization is inexorably linked to the expiratory phase of the ventilatory cycle but
in the experimental conditions used is subthreshold so that it is not expressed as
behaviour. The purpose of this paper is to establish a function for these interneurones
by showing that they directly control the pattern of spikes in a group of motoneurones.
Its strategy is to make simultaneous intracellular recordings from flight motoneurones
and from those motoneurones which innervate ventilatory muscles, to look for any
common synaptic potentials. It will be shown that these interneurones synapse upon
three groups of motoneurones; those mediating the normal ventilatory movements of
the abdomen, those mediating auxiliary ventilatory movements such as head pumping
and those which control the aperture of the spiracles. The pattern of synaptic input
from the interneurones on to the motoneurones innervating the closer muscles of the
thoracic spiracles is an adequate explanation of the pattern of motor spikes.

MATERIALS AND METHODS

Thirty six adult locusts, Schistocerca gregaria, were obtained from our own culture.
The surgical and recording techniques were as in the preceding paper (Burrows,
1975). The meso- and metathoracic ganglia were stabilized on one platform, the pro-
thoracic or the first unfused abdominal ganglion on a second. Suction electrodes were
used to record extracellular spikes from the cut ends of connectives, and hook elec-
trodes to record from the median nerves. The numbering of the muscles is taken from
Snodgrass (1929). Muscles or motoneurones are labelled left or right as they appear
when viewed ventrally.

RESULTS

General features of ventilation

Air is moved throughout the network of tracheae by pumping movements of the
abdomen. The main powerstroke is expiration; inspiration is accomplished mostly by
elastic recoil with the assistance of a few inspiratory muscles. The somata of the moto-
neurones innervating these abdominal muscles are in the metathoracic or abdominal
ganglia. Pumping movements of the head occur when ventilation is laboured and are
mediated by motoneurones with their somata in the prothoracic and suboesophageal
ganglia. Access to the tracheal network from the outside is afforded by a series of ten
spiracles, one pair on each of the thoracic and abdominal segments. Spiracles 1–4
close during expiration while spiracles 5–10 open. During inspiration the sequence is
reversed so that air may move in one direction throughout the tracheae. The aperture
of the second thoracic spiracle is controlled only by a closer muscle, which acts
against the elasticity of the cuticle. All other spiracles have both a closer and an opener
muscle. The muscles of spiracle 1 on the prothorax are innervated by motoneurones
whose somata are in the prothoracic ganglion and whose axons run in the median
nerve (Fig. 1). This is an unpaired nerve which arises from the dorsal midline of the
ganglion. There are two closer and two opener motoneurones whose axons bifurcate
within the median nerve to innervate the muscles of both the left and the right
spiracles (Miller, 1960). Therefore, each spiracle receives the same pattern of moto
impulses. The closer muscle of spiracle 2 is innervated by two excitatory motoneurones from the mesothoracic ganglion. Spiracles 3 and 4 are innervated by the first two median nerves of the metathoracic ganglion in the same way as spiracle 1 (Fig. 1). The metathoracic ganglion consists of the segmental metathoracic fused with the first three abdominal ganglia, so that there are four median nerves. The posterior two median nerves innervate spiracles 5 and 6 on the third and fourth abdominal segments respectively. The first unfused (and therefore really the fourth) abdominal ganglion innervates spiracle 7 on the fifth abdominal segment. The following description is for this spiracle though it is applicable to any of the spiracles 5–10. There are two closer motoneurones with axons in the median nerve, which, as for the thoracic spiracles, branch to innervate the left and the right spiracles. The opener motoneurones, of which there are at least 2, are in the next posterior ganglion and have axons in the lateral nerve 1 of that ganglion (Lewis, Miller & Mills, 1973). They innervate only one
Fig. 2. Closer motoneurone of the mesothoracic spiracle (spiracle 2). (a) Bursts of spikes in the soma which occur during expiration (solid bar) can be correlated with spikes in the median nerve (second trace). Within each burst the spikes tend to occur in groups. (b, c) Progressively hyperpolarizing the soma reveals a patterned synaptic input from which the spikes arise. The groups of EPSPs occur at approximately 50 ms intervals. (d) During inspiration (dotted horizontal line) the soma is hyperpolarized by a barrage of IPSPs (d, i), which can be reduced in amplitude (d, ii) and then reversed (d, iii) by an applied hyperpolarization. In this and subsequent figures the diagram on the right shows the position of the impaled soma within its ganglion. The horizontal solid bar indicates expiration, the dotted one inspiration. Hook electrodes record from the median nerve after it has branched to either side of the body. Calibration: horizontal, (a) 400 ms (b–d) 200 ms.

spiracle, so that the left and the right spiracles of the same segment are capable of opening independently. Two other axons in the median nerve of the first abdominal ganglion branch to innervate a dorso-ventral inspiratory muscle on either side of the body.

Patterns of activity in closer motoneurones of the mesothoracic spiracles

The somata of the two closer motoneurones are about 30 μm in diameter and lie on the ventral surface of the ganglion on either side of the midline at the level of emergence of nerve 5. They can be identified anatomically by allowing cobalt chloride to enter the cut ends of the axons of the median nerve. Two prominent somata are filled. Physiologically they can be identified by correlating spikes recorded intracellularly in the soma with those recorded extracellularly from the median nerve (Fig. 2 a). Spikes of both closer motoneurones are always present at whatever point extracellular recordings are made from the median nerve. The pattern of their spikes is similar so that in extracellular recordings the spikes of both may be summed. It is therefore impossible to identify correctly a single closer motoneurone in extracellular recordings and difficult to correlate intra- and extracellularly recorded spikes (Fig. 2 a). The spikes occur in bursts during the expiratory phase of the ventilatory cycle.
Within each burst the first spikes in one closer motoneurone occur at a high frequency but subsequently tend to be arranged in groups at intervals of about 50 ms. Two rhythms of synaptic inputs are seen to underly the spikes when a d.c. hyperpolarization is applied to the soma (Fig. 2b, c). There is a slow rhythmic depolarization which evokes the overall expiratory burst of spikes, superimposed upon which is a faster rhythm of rapid oscillations of the membrane potential (Fig. 2c). Each ripple consists of a group of synaptic potentials which evoke one or a group of spikes (Fig. 2b). The potentials are increased in amplitude by an applied d.c. hyperpolarization and reduced by a depolarization. They are probably chemically mediated excitatory post-synaptic potentials (EPSPs).

The expiratory burst of spikes is terminated by hyperpolarizing potentials which continue throughout inspiration (Fig. 2d i). At the start of inspiration they occur at high frequency and are of low amplitude. Later they occur at a lower frequency and have a larger amplitude despite the now higher membrane potential. This can be interpreted by supposing that the potentials come from two sources which become phase locked towards the end of inspiration. The large potentials thus result from the summation of two smaller potentials. Progressively hyperpolarizing the motoneurone with an applied d.c. current reduces the amplitude of the potentials until they are barely visible (Fig. 2d ii) and then appears to reverse their polarity so that they are now depolarizing (Fig. 2d iii). The potentials are thus chemically mediated inhibitory postsynaptic potentials (IPSPs).

The patterns of depolarizing synaptic inputs in the closer motoneurones are similar to those which occur in flight motoneurones (Burrows, 1975); there is a slow periodic input in time with the expiratory phase of ventilation, superimposed upon which is a faster rhythm with a period similar to the wingbeat period in flight. To determine whether the same interneurones cause these rhythms in the flight motoneurones and the closer motoneurones, recordings were made simultaneously from both.

**Common synaptic inputs to the mesothoracic spiracular closer and flight motoneurones**

The slow rhythmic depolarization of the left mesothoracic tergosternal (83) motoneurone, an elevator of the forewing, occurs at the same time as the expiratory burst of spikes in the left, mesothoracic spiracular closer motoneurone (Fig. 3a). The groups of spikes in the closer motoneurone within the expiratory burst also coincide with the ripples in the flight motoneurone. Hyperpolarizing the closer motoneurone with an applied d.c. current to block most of its spikes shows that the waveforms in the two motoneurones are matched (Fig. 3b). The individual EPSPs can also be matched and thus it is probable that they are caused by the same interneurones. Therefore an elevator motoneurone of the forewing and a closer motoneurone of the mesothoracic spiracle receive excitatory synapses from the same interneurones. There are occasions when the EPSPs do not seem to match exactly but it must be remembered that any common synaptic potentials are superimposed upon a background of other synaptic potentials which occur independently in the two motoneurones. The EPSPs which cause the slow and fast rhythmic depolarization in the left tergosternal (113) motoneurone, an elevator of the hindwing, also match those in the mesothoracic closer motoneurone (Fig. 3c). The same interneurones synapse upon all three motoneurones.
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Fig. 3. Common synaptic inputs to a mesothoracic spiracular closer motoneurone and flight motoneurones. (a) The pattern of closer motoneurone spikes (first trace) resembles the pattern of depolarization in the left mesothoracic tergosternal (83) motoneurone (second trace). Both are depolarized during expiration and the groups of closer spikes correspond with the ripples in the flight motoneurone. (b) The closer motoneurone is hyperpolarized to block most of the spikes and reveal that the EPSPs in the two motoneurones are matched. (c) EPSPs in the left metathoracic tergosternal (113) motoneurone (second trace) are matched with those in the closer motoneurone when the latter is hyperpolarized. All records are from the same adult male locust. Calibration: vertical, closer motoneurone 25 mV, flight motoneurone (a, b) 5 mV, (c) 3 mV; horizontal, (a) 400 ms, (b, c) 200 ms.

and are therefore those interneurones which make widespread connexions with flight motoneurones (Burrows, 1975). In the flight motoneurones the synaptic potentials are usually subthreshold in the experimental situation used here, but in the spiracular closer motoneurone they are suprathreshold. They impose both the slow ventilatory and fast rhythms upon the pattern of spikes of the closer motoneurone.

The connexions of these interneurones with other ventilatory motoneurones can be traced by simultaneously recording from any of the 30 flight motoneurones upon which they have previously been shown to synapse, and the appropriate ventilatory motoneurone.

Metathoracic spiracular closer motoneurones

Of the four spiracles innervated by the metathoracic ganglion only spiracles 3 and 4 have been studied here. Their closer motoneurones have similar patterns of spikes and synaptic inputs (Burrows, 1974) so that although the following description is of spiracle 4 it is equally applicable to spiracle 3. The expiratory burst of spikes corresponds with the slow depolarization of the left mesothoracic tergosternal (83) or right metathoracic anterior tergocoxal motoneurone (Fig. 4a). There is initially a high frequency of spikes which coincides with a large amplitude depolarization in the flight motoneurones. The burst of spikes is terminated by EPSPs which may then be followed by a slow repolarization which can evoke a group of spikes during inspiration (Fig. 4b). The source of this additional depolarization is not known but it does not occur in the flight motoneurones. The IPSPs apparently do not persist throughout inspiration as do those in the mesothoracic closer motoneurones, so that the membrane potential rises gradually before the onset of the next expiratory depolarization.

The EPSPs during expiration in the closer motoneurone with its soma on the right side of the ganglion are revealed when the spikes are abolished by an applied d
Fig. 4. A **metathoracic** closer motoneurone of spiracle 4. (a) The expiratory burst of spikes in the closer motoneurone (third trace) occurs at the same time as the slow rhythmic depolarization in the left mesothoracic tergosternal (83) (first trace) and the right metathoracic anterior tergocoxal (118) motoneurone (second trace). (b) The burst of closer spikes (second trace) is terminated by IPSPs. There is then a rebound during inspiration after the occurrence of three common EPSPs (dotted lines), which leads to a few spikes. (c) The closer motoneurone is hyperpolarized to block most of the spikes and the waveforms in each motoneurone are matched during expiration. (d) Faster sweep to allow the matching of the EPSPs in each motoneurone. Each fast ripple consists usually of two EPSPs, the second of which may occur progressively later relative to the first so that the fast rhythm temporarily breaks down. All records are from the same adult female locust. Calibration: vertical, (a, c, d) traces 1 and 2 3 mV, trace 3 10 mV; horizontal (a) 400 ms, (b, c) 200 ms (d) 100 ms.

hyperpolarization (Fig. 4c). They exactly match those seen in the flight motoneurones innervating muscles on either side of the body, from which simultaneous recordings are made (Fig. 4c, d). Similarly, the EPSPs in the closer motoneurone with its soma on the left side of the ganglion match those in left and right flight motoneurones. Therefore it may be inferred that the two closer motoneurones of spiracle 4 receive the same synaptic inputs during expiration and thus receive synapses from the same interneurones. The experiment of recording simultaneously from the two closer motoneurones has not been achieved but there seems to be no reason to doubt the logic of the inference based on symmetry. The same argument can be used for the two closer motoneurones of each thoracic spiracle.

Each ripple of the membrane potential during expiration consists usually of two EPSPs which occur at approximately the same time so that the fast rhythm is maintained for many cycles. Sometimes the second EPSP may occur progressively later with respect to the first so that the fast rhythm disappears (Fig. 4d). The fast rhythm then reappears suddenly and not gradually as would be expected of a phase progression of one EPSP relative to the other.
The spikes of both closer motoneurones recorded in the median nerve occur during expiration. Within the burst the spikes are grouped at intervals which correspond with the ripples in the left mesothoracic tergosternal (83) motoneurone (Fig. 5a). Recordings from the soma of one closer motoneurone also show that the spikes during expiration are grouped (Fig. 5b, c). They can with difficulty be matched with those in the median nerve. Expiration is terminated by a high frequency burst of EPSPs which continue throughout inspiration. The pattern thus resembles that seen in the mesothoracic closer motoneurones even to the extent that the EPSPs become larger at the end of inspiration, presumably due to summation.

The spikes during expiration arise from a depolarizing synaptic input (Fig. 5d). Occasionally an EPSP may fail to evoke a spike in the impaled neurone but one is still recorded in the median nerve (Fig. 5d). It must have come from the other closer motoneurone.
Fig. 6. A metathoracic opener motoneurone of spiracle 4. (a) There is a burst of spikes at a steady frequency throughout inspiration and groups of IPSPs during expiration. (b) The IPSPs are reduced in amplitude by an applied hyperpolarization but a slow rhythmic undulation of the membrane potential at the frequency of ventilation remains. (c) The IPSPs in the opener motoneurone (first trace) correspond with EPSPs in the right mesothoracic tergo-sternal (83) motoneurone (second trace). (d) The inspiratory burst of spikes is rapidly terminated when the slow expiratory wave of depolarization appears in the flight motoneurone. Occasional opener spikes during expiration occur upon rebound from a group of IPSPs. (e) A faster sweep to show the exact correspondence of IPSPs and EPSPs in the two motoneurones. In (d, e) the prothoracic median nerve showing spikes of closer motoneurones is on the third trace. Records (a, b) are from one adult female locust, (c–e) from another. Calibration: vertical, opener 10 mV, tergosternal motoneurone 5 mV; horizontal, (a–d) 400 ms, (e) 200 ms.

motoneurone and is further evidence that the two closer motoneurones of one spiracle receive the same depolarizing synaptic inputs. When the closer motoneurone is hyperpolarized by an applied d.c. current, the EPSPs during expiration match those in the left or right mesothoracic tergosternal motoneurones (Fig. 5e, f). A delay of 10 ms between the EPSP in the prothoracic motoneurone and that in the mesothoracic one indicates that the interneurones have a conduction velocity of about 1.4 m.s⁻¹ (cf. Burrows, 1975).

**Metathoracic spiracular opener motoneurones**

An opener motoneurone of spiracle 4 spikes during inspiration (Fig. 6a). Within the burst the spikes occur at a steady frequency with no apparent patterning, but there may be a slight increase of the frequency at the beginning and a reduction at the end of a burst. When the spikes are abolished by an applied d.c. depolarization the membrane potential regularly falls during inspiration and rises during expiration (Fig. 6b). This may indicate that an excitatory synaptic input underlies the spikes during inspiration, but alternatively the spikes may result from inhibitory rebound. The burst
Fig. 7. Spikes in the connectives between the metathoracic and first unfused abdominal ganglion can be modulated by the slow ventilatory and fast rhythms. (a) Suction electrode recordings from the cut ends of the right (first trace) and left (second trace) connectives. Some of the descending spikes show the two rhythms; a burst of spikes during expiration (horizontal bar) which are arranged in groups at the fast rhythm. (b) The spikes correspond with the depolarization in the left metathoracic anterior tergocoxal (118) motoneurone. (c, d) At faster sweeps there is good correlation between the groups of spikes and the ripples in the ipsilateral mesothoracic tergosternal (83) motoneurone. (e, f) Stimuli applied to the left (e) or right (f) connectives evoke EPSPs in the right mesothoracic tergosternal (83) and in the left metathoracic tergosternal (113) motoneurones. Single sweeps are shown in parts (i), multiple ones in (ii). (g) Stimulation of the left connective (dots) evokes EPSPs in the left mesothoracic spiracular closer and left metathoracic tergosternal motoneurone. The inputs sum with the slow ventilatory rhythm. Each line of records is from a different adult female locust. Calibration: vertical, (a, b) 9 mV, (c-f) 3 mV, (g) spiracular closer 10 mV, tergosternal 3 mV; horizontal (a, b) 400 ms, (c, g) 200 ms, (d) 100 ms, (e, f) 40 ms.

of spikes is clearly terminated by IPSPs which occur in waves at intervals of about 50 ms throughout expiration (Fig. 6a). The IPSPs are reduced in amplitude by an applied hyperpolarization (Fig. 6b).

The IPSPs occur at the same time as the wave of EPSPs upon flight motoneurones (Fig. 6c). The opener starts to depolarize as soon as the EPSPs cease in the flight motoneurones and is hyperpolarized when the EPSPs of the next expiratory cycle reappear. The EPSPs in the right mesothoracic tergosternal motoneurone exactly match the IPSPs in the right opener motoneurone and they must be caused by the same interneurones (Fig. 6d, e). These interneurones therefore have a reciprocal effect on the spiracular motoneurones, exciting the closers during expiration and inhibiting the openers. Occasionally an opener may spike during inspiration but does so on rebound from a wave of IPSPs (Fig. 6d, e).
**Dual action locust interneurones**

Recordings from the connectives between the metathoracic and first unfused abdominal ganglion reveal bursts of descending spikes during expiration (Fig. 7a). The bursts occur in both connectives with the same intraburst patterning in each. The spikes typically occur as groups at intervals of about 50 ms although at the end of the burst there may be no patterning but a higher frequency. The patterned bursts of spikes occur at the same time as the expiratory depolarization in the left metathoracic anterior tergocoxal motoneurone (118) but the later high frequency of spikes occurs only when this depolarization is over (Fig. 7b). The groups of spikes correspond with and precede the fast ripples in the membrane potential of a flight motoneurone (Fig. 7c, d). Stimulating either of the connectives consistently evokes EPSPs in meso- or metathoracic elevator motoneurones on both sides of the body (Fig. 7e, f). An EPSP is also evoked in a mesothoracic spiracular closer motoneurone (Fig. 7g).

The spikes in the connectives could come from at least two sources. First they could be spikes of motoneurones. There are motoneurones which have somata in the metathoracic ganglion and axons which travel in the connectives before emerging from the central nervous system in lateral nerves of the first abdominal ganglion (Lewis et al. 1973). Secondly they could be interneurones responsible for conveying information about the slow ventilatory and fast rhythms to motoneurones in the abdomen. Interneurones which spike during expiration and which run the length of the abdomen are known (Lewis et al. 1973). Whatever the origin of the spikes recorded here they do suggest that abdominal ventilatory motoneurones may be influenced by both rhythms. To see whether the same interneurones which synapse upon thoracic flight and venti-
Abdominal ventilatory motoneurones. (a) An expiratory motoneurone (second trace) with its soma in the metathoracic ganglion spikes at the same time as the closer motoneurones in the prothoracic median nerve (third trace). Most spikes occur at the same time as the fast ripples in the left mesothoracic tergosternal motoneurone (first trace). (b) The expiratory motoneurone is hyperpolarized to reveal the correspondence between its depolarizing synaptic inputs and those of the flight motoneurone. (c) A second metathoracic expiratory motoneurone (first trace) whose synaptic potentials are similar to those in the flight motoneurone (second trace) but they cannot easily be matched. (d) An expiratory (first trace) and an inspiratory motoneurone (second trace) with their somata in the first unfused abdominal ganglion. The third trace shows the right mesothoracic tergosternal (83) motoneurone and the fourth an extracellular recording from the right metathoracic-first abdominal connective. Records (a, b, c) and (d) are each from different locusts. Calibration: vertical (a, b) trace 1 5 mV, trace 2 2 mV, (c) trace 1 5 mV, trace 2 3 mV, (d) trace 1 8 mV, trace 2 3 mV, trace 3 15 mV; horizontal (a-c) 200 ms, (d) 400 ms.

Ventricular motoneurones also synapse upon motoneurones in the first unfused abdominal ganglion, recordings were made simultaneously from both. One unidentified left abdominal motoneurone is depolarized during expiration at the same time as a right mesothoracic and the left metathoracic tergosternal motoneurone (Fig. 8a). During inspiration it is hyperpolarized. At the normal membrane potential of the ventilatory motoneurone the fast oscillations are not apparent. Spikes, however, tend to occur at the same time as the rising phase of a fast ripple in the flight motoneurone (Fig. 8b). That fast ripples are present in the ventilatory motoneurone which match those in the flight motoneurone is revealed when a d.c. hyperpolarization is applied (Fig. 8c). The inhibitory input during inspiration is now reversed in polarity but the ripples are still depolarizing and are therefore excitatory. The exact matching of the ripples in the three motoneurones indicates that they are caused by the same interneurones. Therefore the sphere of influence of these interneurones extends not only throughout the thoracic nervous system but also into the abdomen.

The interneurones also synapse upon motoneurones innervating expiratory abdominal muscles whose somata are in the abdominal region of the metathoracic
Fig. 10. Prothoracic ventilatory motoneurones which cause pumping movements of the head. 

(a) An inspiratory motoneurone (first trace) which spikes at low frequency during inspiration and is hyperpolarized during expiration (horizontal bar). The hyperpolarization occurs at the same time as the depolarization of the right mesothoracic tergosternal (83, second trace) and the left metathoracic tergosternal (113, third trace). (b) The IPSPs in the ventilatory motoneurone correspond with the EPSPs underlying the fast ripples in the metathoracic anterior tergocoxal motoneurone (118, second trace). (c) An expiratory motoneurone whose bursts of spikes correspond with the depolarization of the left mesothoracic tergosternal motoneurone (87, second trace). (d) Hyperpolarizing the ventilatory motoneuron reveals a correspondence between the depolarizing ripples in it and the flight motoneuron. Each line of records is from a different adult female locust. Calibration: vertical (a) trace 1 8 mV, traces 2, 3 3 mV, (b) trace 1 5 mV, trace 2 2 mV, (c) 8 mV, (d) trace 1 8 mV, trace 2 3 mV; horizontal (a, c) 400 ms, (b, d) 200 ms.

One unidentified motoneurone spikes a few times during inspiration but produces a definite burst of spikes during expiration (Fig. 9a). Its synaptic input during expiration, when revealed by an applied d.c. hyperpolarization, matches that in a left mesothoracic tergosternal motoneurone (Fig. 9b). In other motoneurones the method of inferring the connexions of interneurones by matching synaptic potentials is no longer possible. For example, an expiratory motoneurone is depolarized at the same time as the flight motoneurone and the overall waveform of the depolarization is similar but EPSPs cannot be matched exactly (Fig. 9c). Similarly an expiratory motoneurone in the first, unfused abdominal ganglion is depolarized and an inspiratory motoneurone hyperpolarized at the same time as the slow wave of depolarization appears in a flight motoneurone (Fig. 9d). The synaptic potentials cannot be matched but the waveforms, in particular that in the inspiratory motoneurone, are similar. It would thus seem likely that at least some of the synaptic inputs driving these motoneurones in the ventilatory rhythm are caused by those interneurones which also synapse upon the flight and spiracular motoneurones.
Table 1. Connexions of the left and right interneurones with left motoneurones in the three thoracic and in the first unfused abdominal ganglia

(Blanks indicate that the observations were not made. The same connexions are made with the right motoneurones.)

<table>
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<th>Motoneurone</th>
<th>Muscle number</th>
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<th>Right interneurone</th>
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<td>EPSP</td>
<td>EPSP</td>
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Prothoracic motoneurones involved in head pumping

Within the prothoracic ganglion are motoneurones innervating neck muscles which, during laboured ventilation, spike either during inspiration or expiration. The IPSPs in an unidentified inspiratory motoneurone occur at the same time as the expiratory wave of depolarization in the flight motoneurones (Fig. 10a). The IPSPs in the inspiratory motoneurones match the EPSPs in the flight motoneurones, indicating they come from the same sources (Fig. 10b). The burst of spikes in expiratory motoneurones coincides with the expiratory depolarization of flight motoneurones but often EPSPs in the two cannot readily be matched (Fig. 10c). In other expiratory motoneurones, clear ripples of the membrane potential are present and match those in a flight motoneurone (Fig. 10d). Therefore these interneurones are involved with the recruitment of motoneurones to assist laboured ventilation.

DISCUSSION

Connectivity patterns of the interneurones

The two interneurones which make widespread connexions with 30 flight motoneurones and depolarize them in a slow ventilatory rhythm and in a fast rhythm (Burrows, 1975) are shown to synapse upon 20 ventilatory motoneurones (Table 1).
They have reciprocal effects on antagonistic ventilatory motoneurones, depolarizing those which spike during expiration and hyperpolarizing those which spike during inspiration. They synapse upon ventilatory motoneurones in each of the three thoracic ganglia and upon those in the first unfused abdominal ganglion. I expect that they also make connexions in the other abdominal ganglia. The ventilatory motoneurones from which recordings have been made are only a small, but hopefully representative, sample of the whole population. Many more connexions of these interneurones may be expected, and some may already be inferred. For example, the opener motoneurones of spiracle 4 have similar patterns of activity as opener motoneurones of other spiracles so that it is not unreasonable to assume that they all have the same synaptic inputs. In other ventilatory motoneurones there is the suggestion that inputs from these interneurones are present but the method of attempting to match synaptic potentials does not allow a definite conclusion to be drawn.

The pattern of connexions with ventilatory motoneurones can best be described by reference to spiracle 4 (Fig. 11). There is probably one interneurone in each of the two connectives and each makes bilateral synaptic connexions with motoneurones. A particular spiracular closer or opener motoneurone therefore receives two inputs; one from the ipsilateral and one from the contralateral interneurones. The opener and closer motoneurones of one spiracle receive reciprocal inputs during expiration; the two closers receiving the same excitatory inputs, the two openers the same inhibitory inputs. The symmetry extends even further in that both closer and both opener moto-
neurones innervate the left and the right spiracles. The two spiracles of one segment must therefore both follow the commands of these interneurones. To summarize: each interneurone is believed to have its soma in the metathoracic ganglion and an axon which branches. One branch ascends to the meso- and prothoracic ganglia, the other descends to the first unfused abdominal ganglion. In each of the four ganglia the interneurones make bilateral connexions with ventilatory and flight motoneurones, if the latter are present.

Co-ordination of the thoracic spiracles

Previous explanations of the central control of spiracular and ventilatory movements in the locust have been derived from observations of peripherally recorded sequences of motor impulses (Miller, 1967; Lewis et al. 1973). The closer motoneurones of the thoracic spiracles were thought to be controlled by a pair of interneurones which spiked during expiration and which synapsed either on premotor, intraganglionic interneurones or upon the closer motoneurones themselves. The observation that the synaptic potentials can be matched in the closer motoneurones of different ganglia during expiration implies that the interganglionic interneurones synapse directly upon them. The interneurones convey information about two rhythms to other ganglia which have no innate rhythmicity. The results could explain why sequences of impulses to the closer muscles of spiracles 1 and 2 often appear to be similar (Miller, 1966). Control by the same interneurones does not mean that all the spiracles must behave in the same way, but merely that under some conditions there will be a tendency to do so. The implication of this method of controlling spiracles will be considered in a later paper (M. Burrows, in preparation).

Abdominal ventilatory movements were also thought to be co-ordinated by a pair of autoactive interneurones originating in the metathoracic ganglion whose spikes were inhibited during inspiration (Lewis et al. 1973). Spikes of such a putative interneurone were recorded in the abdominal connectives but the function and connexions of these interneurones were unresolved. They were thought to synapse upon intraganglionic interneurones which then distributed the excitation to the pool of expiratory motoneurones and inhibition to the inspiratory ones. The interneurones were also thought to have a direct but weak effect on the inspiratory motoneurones (Lewis et al. 1973). It was not known if these interneurones were the same as those which co-ordinated the thoracic spiracles. The matching of synaptic potentials during expiration in abdominal motoneurones and any of the thoracic spiracular closer motoneurones implies that the same interneurones run between all of these ganglia, synapsing directly on the motoneurones. This, of course, does not preclude the existence of interneurones similar to those predicted by Lewis et al. (1973) but implies that they must act in parallel with those already observed. It should also be remembered that isolated abdominal ganglia have an innate rhythmicity. The interneurones described here could be responsible for the co-ordination of the ventilatory output of the abdominal ganglia.

What aspects of ventilation do the interneurones affect?

The interneurones exert some control over three aspects of the ventilatory movements. First, they directly affect the pattern of spikes in motoneurones innervating abdominal muscles and therefore influence the major force producing mechanism fo
ventilation. Secondly, they depolarize or hyperpolarize motoneurones innervating muscles of the neck which cause pumping movements of the head. This is an auxiliary movement recruited only when ventilation is laboured. The synaptic input to these motoneurones is always present but is subthreshold during normal ventilation. These motoneurones are therefore recruited to ventilation when their membrane potentials are lowered sufficiently by a variety of other inputs to allow the ever present ventilatory input now to exceed threshold. The recruitment does not require that a new set of commands from other interneurones be issued. This interplay between an ever present motor command and other synaptic inputs to the motoneurone may be an important way in which particular elements of a behaviour or even different patterns of behaviour are recruited.

Thirdly, the interneurones control the output of spikes in closer and opener motoneurones of the spiracles. The pattern of synaptic potentials from these interneurones is an adequate explanation of the overall expiratory burst of spikes and the intraburst patterning in closer motoneurones. It is therefore reasonable to suppose that a causal relationship between the two exists. Miller (1967) concluded from extracellular recordings of sequences of impulses that there are two distinct patterns during an expiratory burst in a closer motoneurone, each caused by a separate interneurone. Pattern 1 is the high frequency of spikes often seen at the start of expiration and which closes the spiracle and is followed by pattern 2 which maintains the closure. The latter pattern was subdivided in three types, none of which correspond to the grouping of impulses at intervals of about 50 ms (the fast rhythm) which is described here. Miller's patterns differ in degree and not cause so that there is no need to invoke other interneurones. For example, pattern 1 results from a high frequency of EPSPs from the same source as that which causes the grouping of the spikes later in the burst.

The above observations establish that these interneurones can affect, and sometimes appear to be, the sole cause of the patterns of spikes in groups of motoneurones. Both the slow and the fast rhythms may be expressed as spikes in motoneurones. A definite behavioural role for these interneurones is thus established; they assist in the coordination of ventilatory movements.

Why the fast rhythm in ventilatory motoneurones?

It must now be asked why the ventilatory motoneurones are depolarized or hyperpolarized in the fast rhythm. Clearly this can affect the patterning of spikes during expiration, particularly in spiracular closer motoneurones, and must therefore have a behavioural consequence. The closer muscle of the spiracle produces larger twitch contractions when pairs of electrical stimuli separated by as little as 3-4 ms are delivered to the median nerve as compared with single stimuli at the same frequency (Hoyle, 1959). This does not imply that the neuromuscular junction of the closer muscle is pattern sensitive, because the total number of stimuli in the two situations are not comparable. The tetanus fusion frequency of the muscle is 60 Hz so that groups of 1-3 impulses at a frequency of about 100 Hz separated by intervals of 50 ms (the period of the fast rhythm) would be expected to cause fluctuating amounts of tension and hence fluttering movements of the spiracular valves. These partial opening and closing movements which do occur, may have an effect upon the entry of air into the tracheae but the magnitude of this effect is unknown. On the other hand,
it would seem unlikely that any functional significance can be attached to the patterned inhibition of the opener motoneurones during expiration.

Crickets which are not flying may also have ventilatory bursts of motor spikes subdivided at intervals of 30 ms which is the same as their wingbeat period in flight (Bentley, 1969). As for bursts in ventilatory motoneurones of the locust, other than spiracular closers, there seems no adequate explanation, in terms of increased mechanical efficiency, for the intraburst patterning.

During flight the frequency of impulses in abdominal motoneurones of the locust may be modulated by both ventilatory and flight rhythms (Camhi & Hinkle, 1972; Hinkle & Camhi, 1972). In the first few seconds of flight the abdomen vibrates at the flight frequency but the ventilatory rhythm is absent. Gradually this returns so that the number of spikes in the bursts which are in time with the wingbeats rises and falls with the ventilatory cycle. The vibration of the abdomen is caused by active muscular contraction and not by mechanical effects spread from the thorax, the patterning of which is derived from interneurones within the thoracic nervous system. The interneurones described in this and the preceding paper (Burrows, 1975) could explain these results but offer no further insight as to their function.

**Interaction of pattern generators**

Interactions between fast (flight) and slow (ventilatory) pattern generators in the insect central nervous system have been indicated before (cf. Huber, 1960; Kutsch, 1969). For example the song of crickets is modulated by both rhythms, the slow rhythm determining the sequence of chirps, the fast the sequence of pulses (Kutsch, 1969). Moreover auditory interneurones which respond to the song are modulated by ventilation. Chirps of the male are more likely to evoke spikes in the auditory interneurones of the female if they occur during one phase (expiration?) of the female's ventilatory cycle (Stout & Huber, 1972).

The results in this and the preceding paper (Burrows, 1975) indicate that the interaction could take place at an interneurone which then distributes information about both patterns to a wide variety of motoneurones.

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**REFERENCES**


