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

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SUMMARY

1. Some flight motoneurones receive two superimposed rhythms of depolarizing synaptic potentials when the locust is not flying; a slow rhythm which is invariably linked to the expiratory phase of ventilation, and a fast rhythm with a period of about 50 ms which is similar to the wingbeat period in flight.

2. By recording simultaneously from groups of motoneurones, the synaptic potentials which underly these rhythms have been revealed in 30 flight motoneurones in the three thoracic ganglia. The inputs occur in elevator motoneurones and some depressors but are of lower amplitude in the latter. The inputs have not been found in leg motoneurones.

3. The rhythmic depolarizations are usually subthreshold but sum with sensory inputs to evoke spikes in flight motoneurones at intervals equal to or multiples of the wingbeat period in flight.

4. Both rhythms originate in the metathoracic ganglion and are mediated by the same interneurones. They can be adequately explained by supposing that there are two symmetrical interneurones which each make widespread connexions with left and right flight motoneurones in the three ganglia.

5. The slow rhythm is coded in the overall burst of interneurone spikes during expiration and the fast rhythm in the interval between the spikes of a burst.

INTRODUCTION

The basic pattern generator for flight in the locust is in the thoracic ganglia (Wilson, 1961). In isolation these ganglia are capable of producing a patterned motor output in response to an unpatterned input (Wilson, 1961; Wilson & Wyman, 1965). Sensory feedback loops do exist, however (Burrows, 1975a), and they must influence flight (Wendler, 1974). It seems unlikely that the flight pattern generator consists only of interconnexions between the motoneurones as once suggested (Wilson, 1966). Intracellular recordings from motoneurones within the central nervous system have failed to reveal the appropriate connexions (Bentley, 1969; Burrows, 1973). There may be electrical coupling between motoneurones innervating the same muscle (Bentley, 1969) but there is no direct coupling between synergists, antagonists or bilateral

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homologues (Burrows, 1973). Two observations indicate the presence of interneurones. First, a spike in one motoneurone may be able to influence both that motoneurone and its contralateral partner by a delayed excitation mechanism mediated by interneurones (Burrows, 1973). Secondly, subthreshold rhythmic waves of depolarization at intervals similar to the wingbeat period in flight may occur in flight motoneurones in the absence of flight (Bentley, 1969; Burrows, 1973). It becomes imperative therefore to study interneurones in order to gain a further understanding of the way the flight pattern is produced and then controlled. By recording from several motoneurones at once the incidence of synaptic potentials in the neurones can be compared. Potentials which are common to each of the impaled neurones, and which occur with a constant latency, must be caused by the same antecedent neurone. Provided that long sequences of common synaptic potentials occur so that random coincidences can be eliminated, the pattern of some of the connexions of the antecedent neurones can be traced. I will show that at least two interneurones make widespread connexions with elevator and depressor motoneurones in all three thoracic ganglia. The interneurones impose two rhythms upon the flight motoneurones; a slow one in time with ventilatory movements and a faster one whose period is similar to that of the wingbeat in flight. A second paper (Burrows, 1975b) describes the connexions of these interneurones with ventilatory motoneurones.

MATERIALS AND METHODS

One hundred and three *Schistocerca gregaria* of either sex were obtained from our own cultures. Both fifth instar and adult locusts were used. The locust was mounted ventral surface uppermost and the three thoracic ganglia exposed. The meso- and metathoracic ganglia were stabilized on a single, wax-covered platform, the prothoracic ganglion on a second and separate platform. The thorax was perfused with a constant flow of saline (Usherwood & Grundfest, 1965) at 19–20 °C. The microelectrodes, filled with 2 M potassium acetate and with resistances of 50–80 MΩ, had to pass through the intact sheath of the ganglia before penetrating the somata of the motoneurones. Further details of the method are given by Hoyle & Burrows (1973a) and the criteria for identification of the motoneurones by Burrows (1975a). All intracellular recordings were made from somata. Neurones are called left or right on the basis of their location when viewed ventrally. Pairs of platinum hook electrodes were used to record extracellular spikes of sensory neurones or motoneurones. The numbering of the nerve trunks is taken from Campbell (1961) and of the muscles from Snodgrass (1929).

RESULTS

Slow rhythmic depolarization of flight motoneurones

Ventilation continues when the locust is lying on its back with its thoracic ganglia exposed and is indistinguishable from that in normal life. The thoracic spiracles open during inspiration, the abdominal ones during expiration and the abdominal muscles contract rhythmically to force air throughout the network of tracheae. Should ventilation become more laboured, then pumping movements of the head begin, brought about by contractions of the neck muscles. The somata of some of the motoneurones innervating these muscles are in the thoracic ganglia (Shepheard, 1974).
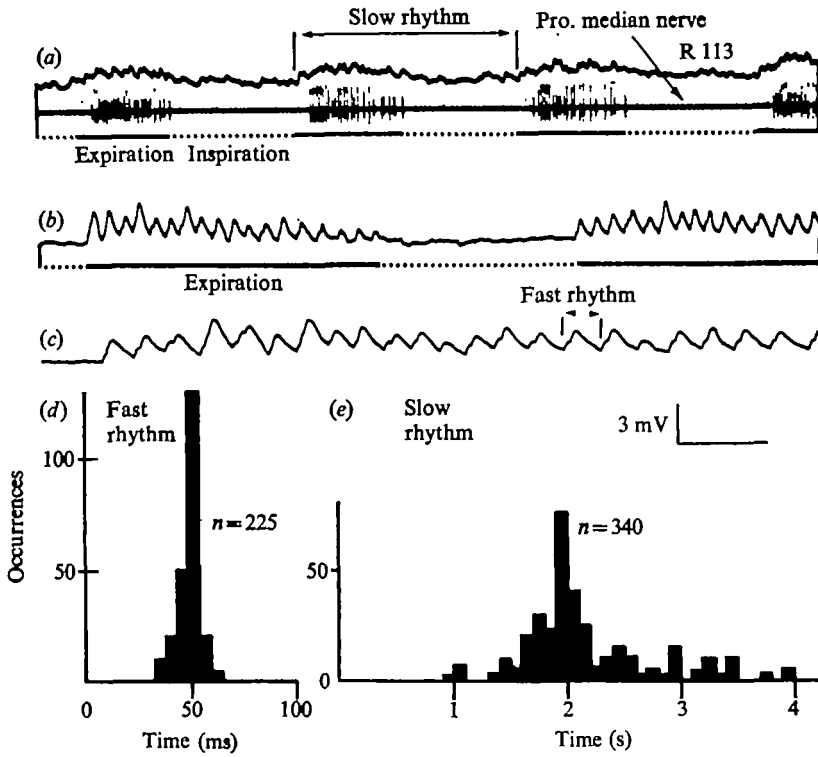
Fig. 1. Two rhythms of synaptic inputs to a flight motoneurone. (a) The right tergosternal motoneurone, an elevator of the hindwing, is depolarized in time with the expiratory burst of spikes in the median nerve to the closer muscles of the prothoracic spiracles (second trace). This is the slow, ventilatory rhythm. (b) During the expiratory depolarization the membrane potential of the motoneurone undergoes rapid oscillations. This rippling is the fast rhythm. (c) Each oscillation consists of a group of EPSPs. (d, e) Histogram of the period of the fast (d) and the slow rhythm (e) from the same 4-day-old adult male locust. Records (b, c) are from a 4-week-old adult female. In this and subsequent figures the solid horizontal bar indicates expiration, the dotted one inspiration. Calibration: horizontal (a, b) 400 ms, (c) 200 ms.

Those thoracic muscles which move the wings during flight are electrically silent and presumably contribute no force to the ventilatory movements. Nevertheless, the membrane potential of the hindwing tergosternal motoneurone, an elevator, undergoes a slow rhythmic depolarization in time with the expiratory phase of ventilation (Fig. 1a). The depolarization can be correlated with the expiratory movements of the abdomen, with the burst of spikes in motoneurones to expiratory muscles of the abdomen or most conveniently with the burst of spikes in the median nerve of the prothoracic ganglion which cause the prothoracic spiracles to close (Fig. 1a). The ventilatory rhythm and the slow rhythmic depolarization of the tergosternal motoneurone are always coupled. Should ventilation cease then the rhythmic depolarization also ceases. Should there be an expiratory pause so that the next inspiration is delayed, then the depolarization of the flight motoneurone is similarly prolonged. Stimuli which affect the ventilatory rhythm affect the slow rhythm in the flight motoneurone in the same way; for example, an increase in the concentration of carbon ⬤xide in the air causes an increase in the frequency of both rhythms. I have not

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observed the slow rhythm in the flight motoneurone in the absence of ventilation or vice versa and conclude that the ventilatory rhythm and the slow rhythm of depolarization in the flight motoneurone are one and the same rhythm. Its frequency can vary considerably from locust to locust and within one locust at different times, but a frequency of o·5 Hz is common (Fig. 1e). The amplitude of the depolarization is 1–4 mV, as recorded at the soma, and is typically subthreshold.

The tergosternal motoneurone is therefore continually depolarized in the ventilatory rhythm although it produces no spikes and contributes no force towards ventilation in the experimental situation used here.

Fast rhythmic depolarization of flight motoneurones

Rapid oscillations of the membrane potential of the tergosternal motoneurone occur during the expiratory phase of the slow ventilatory rhythm (Fig. 1b, c). The rippling of the membrane potential has a period of about 50 ms (Fig. 1d), varying from 45–70 ms in locusts of different age and sex. This is similar to the wingbeat period in flight. The locust will not fly when strapped on its back so that it is not possible to relate directly the fast oscillations with the wingbeat period. The locust will occasionally show periods of flight-like activity in which elevator and depressor muscles contract rhythmically and alternately. Then the spikes of the tergosternal motoneurone occur with the same periodicity as the oscillation (Burrows, 1973). The adult female used in Fig. 1a, d had a mean frequency of membrane oscillations of 50 ms while its wingbeat period in tethered flight was 70 ms, but the wingbeat period during flight of a tethered locust is always lower than that during free flight. When tethered on a flight balance a female of similar age and size (as measured by the length of the hind femur and fore wing) would be expected to have a wingbeat period of about 60 ms (Weis-Fogh, 1956).

The oscillations of the membrane potential during expiration represent a second, faster rhythm in this flight motoneurone. The individual oscillations are subthreshold so that no contractions of the flight muscles occur in this experimental situation. Both fast and slow rhythms occur in all locusts which are ventilating. It must now be asked how widespread these rhythms are. The connectivity pattern of those neurones responsible for the slow and fast rhythms can be determined by recording from two or more motoneurones at the same time and looking for common synaptic potentials.

Common synaptic inputs to elevator motoneurones of the hindwing

Simultaneous recordings from the left and right tergosternal motoneurones of the hindwings show that both are depolarized together in the slow ventilatory rhythm and in the fast rhythm (Fig. 2a–c). The synaptic potentials in the two motoneurones are matched. Each fast oscillation consists of a group of 1–3 synaptic potentials with the same number of potentials in each motoneurone (Fig. 2b). The exactness of the matching of the synaptic potentials in each motoneurone can be more clearly seen when the fast rhythm temporarily breaks down during expiration (Fig. 2c). No low resistance pathways are revealed between the two motoneurones when current pulses are injected into either one. The common synaptic potentials therefore do not result from electrical coupling between the two motoneurones. Synaptic potentials occur independently in the two motoneurones during inspiration (Fig. 2b) or when the slow ventilatory rhythm ceases (Fig. 2d). It is upon this background of independe
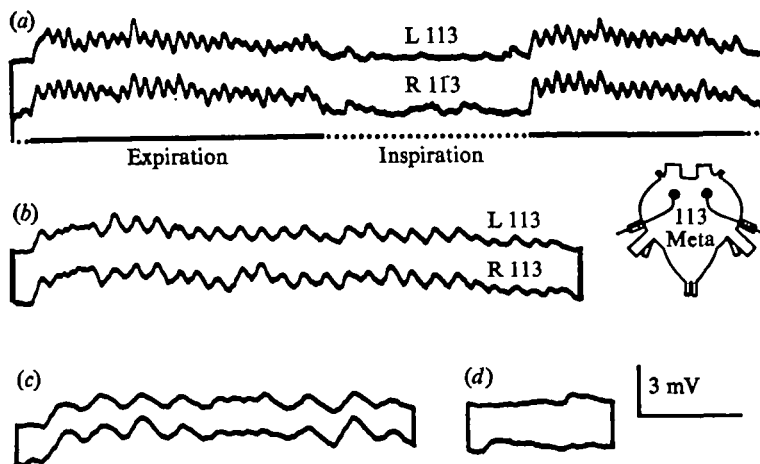
Fig. 2. Common synaptic inputs to the left and right tergosternal motoneurones (113) of the *hindwings*. (*a*) Both motoneurones are depolarized together in the slow rhythm. (*b*) The ripples of the membrane potential during the fast rhythm occur at the same time in both motoneurones. (*c*) EPSPs in the two motoneurones are matched during expiration, but synaptic potentials can occur independently (*d*). Records are from the same 4-week-old adult female. In this and subsequent figures the diagram on the right shows the position of the impaled somata in the thoracic ganglia. Calibration, (*a*) 400 ms, (*b*) 200 ms, (*c*, *d*) 100 ms.

synaptic potentials that the common potentials which cause the slow and fast rhythms are superimposed. Therefore during expiration there may be occasional potentials present in one motoneurone but not in the other. To show that the synaptic potentials which cause the slow and fast rhythms are always common in the two motoneurones it is necessary to choose those locusts in which the background synaptic potentials are of low amplitude and frequency. Then long sequences of synaptic potentials can be matched in the two motoneurones. There could be at least two explanations for the common potentials. First, both motoneurones could be innervated by the same interneurone(s) or secondly, each could be innervated by separate interneurones which are themselves electrically coupled. These two possibilities can be ultimately distinguished only by recordings from the interneurones, but because the synaptic potentials can be matched so well the first explanation is favoured. If the second explanation were correct, occasional failures of the synaptic potentials in one motoneurone would be expected, but these are not observed.

Common synaptic inputs to elevator motoneurones of the forewing

Slow and fast rhythmic depolarizations occur in the left and the right tergosternal motoneurones of the forewings (Fig. 3*a*). The slow rhythm is again correlated with ventilation, so that the depolarizing phase occurs at the same time as a burst of spikes in motoneurones to expiratory muscles of the neck (Fig. 3*a–c*). The synaptic potentials underlying the two rhythms can be matched, although potentials can occur independently at any time (Fig. 3*c*). Injecting pulses of current into either motoneurone reveals no low resistance pathways between the two motoneurones (Fig. 3*d, e*). The same conclusions can be drawn for these two forewing motoneurones as for the two hindwing ones. It must now be asked whether the synaptic inputs to the fore and hindwing motoneurones are derived from the same source.
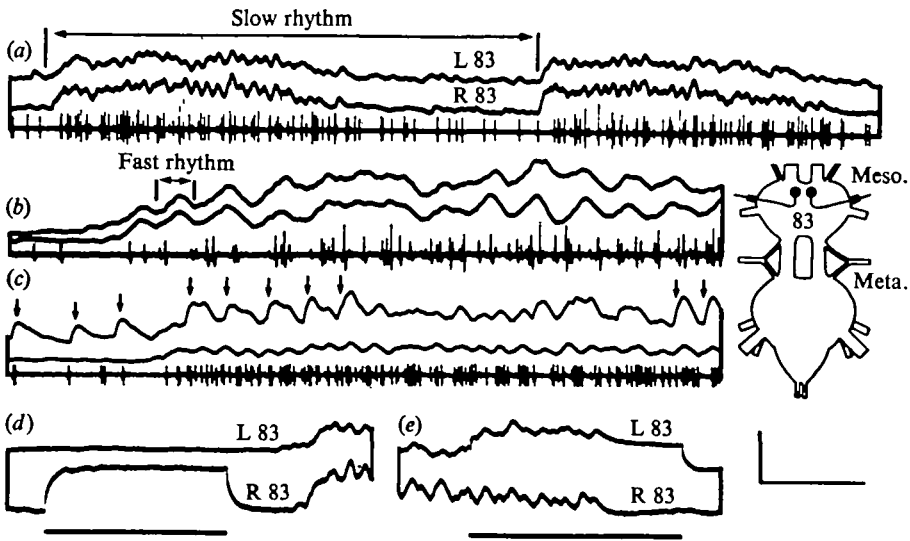
Fig. 3. Common synaptic inputs to the left and right tergosternal motoneurones (83) of the *forewings*. (*a*) Two rhythms are again present; a slow one in time with bursts of motoneurone spikes in the right N6 of the prothoracic ganglion to muscles of the neck (lower trace), and a fast one superimposed upon this. Some spikes of ventilatory motoneurones and of wing afferents occur during inspiration. (*b*) The synaptic potentials in the left and right moto-neurones are matched exactly. (*c*) Occasional large potentials (arrows) appear in one motoneu-rone but not the other. (*d*, *e*) A depolarizing pulse of current (horizontal bar) injected into the right (*d*) or left (*e*) motoneurone has no effect upon the other motoneurone. All records are from a 3-day-old adult male locust. Calibration: vertical (*a–c*) 4 mV, (*d*, *e*) 3 mV; horizontal (*a*, *d*, *e*) 400 ms, (*b*) 100 ms, (*c*) 200 ms.

Common synaptic inputs to elevator motoneurones of the fore and hindwings

To show that the common inputs to the two forewing tergosternal motoneurones are derived from the same source as those in the two hindwing motoneurones, record-ings must be made simultaneously from one of each. Ipsilateral fore and hindwing tergosternal motoneurones then are seen to be depolarized together in both the slow and the fast rhythms and the synaptic potentials in each can be matched (Fig. 4*a*, *b*). The common inputs also extend to contralateral fore and hingwind tergosternal motoneurones (Fig. 4*c*, *d*). Both are depolarized in the slow rhythm even when a normal expiration is followed quickly by a brief expiration (Fig. 4*c*). Finally, record-ings made simultaneously from the left and right hindwing, and left forewing, tergo-sternal motoneurones show that all three have common synaptic inputs (Fig. 4*e*, *f*). Therefore the synaptic potentials causing the slow and fast rhythms in both fore and hindwing motoneurones are apparently derived from the same sources.

Common synaptic inputs to elevator and depressor motoneurones

Motoneurones which innervate the dorsal longitudinal depressor muscles of the hindwing and which have their somata in the mesothoracic ganglion show the same slow and fast rhythmic depolarizations as the tergosternal motoneurones (Fig. 5*a*). The depolarization rarely exceeds 1 mV as recorded at the soma and in some moto-neurones cannot be distinguished from the background of other unpatterned synapti◀
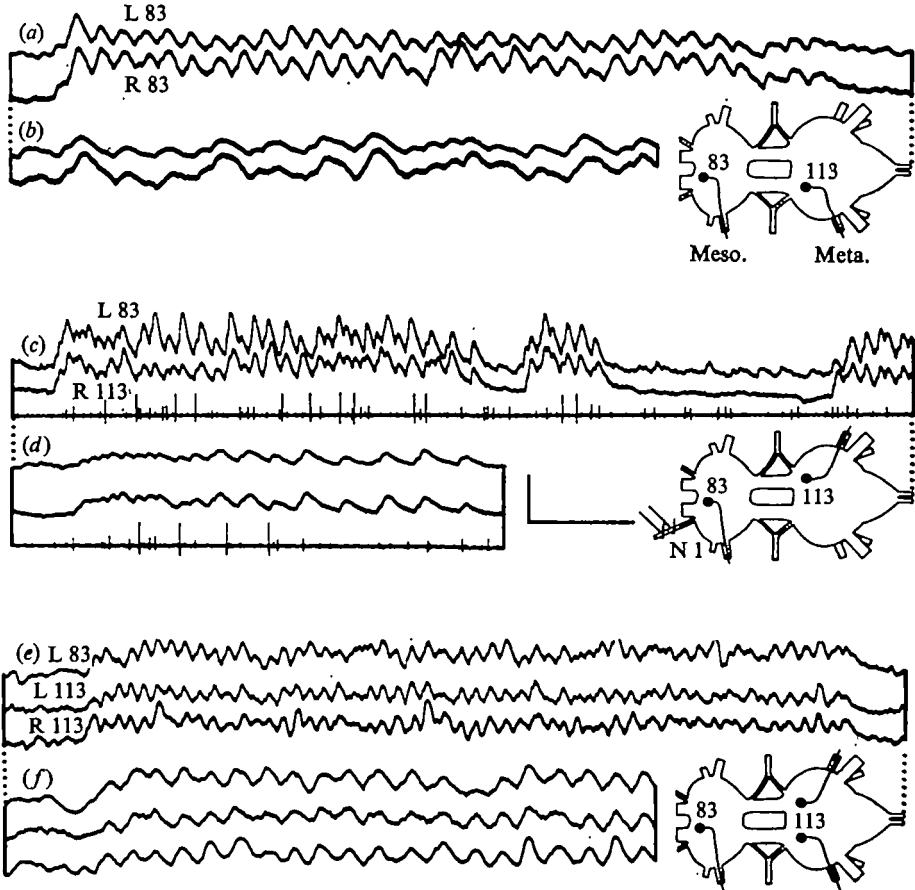
Fig. 4. Common synaptic inputs to the tergosternal motoneurones of both *fore* and *hindwings*. (a) *Ipsilateral* meso- (83) and metathoracic (113) tergosternal motoneurones are depolarized together in both the slow and fast rhythms. One complete depolarizing phase of the slow rhythm is shown. (b) The synaptic potentials can be matched exactly. (c, d) *Contralateral* meso- and metathoracic motoneurones also show both rhythms and their synaptic potentials can be matched exactly. The slow rhythm is in time with bursts of spikes in motoneurones of the left mesothoracic N 1 (third trace) which innervate neck muscles. (e, f) Simultaneous recordings from the left and right 113 motoneurones and the left 83 motoneurone show synaptic inputs common to each. Records (a, b) are from one male fifth instar locust, (c, d) from a second and (e, f) from a 3-day-adult female. Calibration: vertical (a, b) 5 mV, (c–f) 4 mV; horizontal (a, d, f) 200 ms, (b) 100 ms, (c, e) 400 ms.

potentials (Fig. 5b). The presence of the two rhythms can then be revealed by applying a d.c. hyperpolarizing current which accentuates the rhythmically occurring synaptic potentials (Fig. 5c). When depolarizing current is applied spikes occur during the expiratory depolarizing phase (Fig. 5d).

In other depressor motoneurones the rhythms are less clearly seen. In some locusts they can be observed, but in the homologous motoneurones in other locusts they appear to be absent. Where present, the depolarizations are always of lower amplitude than those seen simultaneously in elevator motoneurones. The left first basalar motoneurone, a depressor, and the left tergosternal, an elevator, both receive the common depolarizing inputs (Fig. 6a, b). The stretch receptor of the left forewing, however,
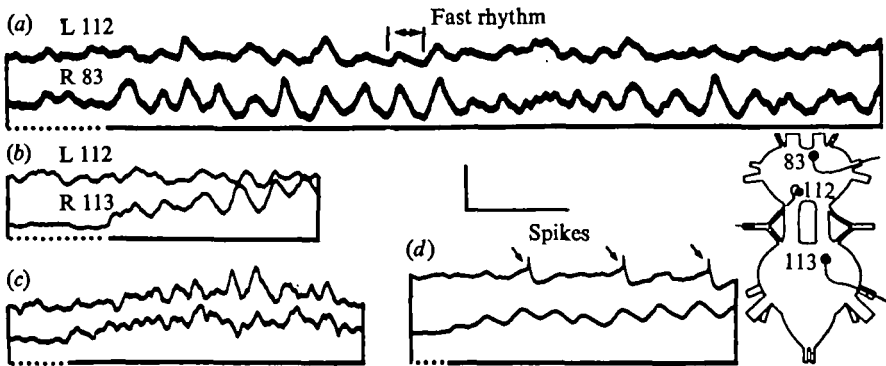
Fig. 5. Depressor and elevator motoneurones have common synaptic inputs. (a) Part of the depolarizing phase (solid bar) of the slow rhythm, in which the left dorsal longitudinal moto-neurone of the hindwing (112), a depressor, has inputs in common with the right tergosternal motoneurone of the forewing (83), an elevator. (b) Often no common synaptic potentials are apparent in 112. Their presence can be revealed by hyperpolarizing (c) the motoneurone with d.c. current or depolarizing it (d) so that the potentials evoke spikes (arrows) in time with the fast rhythm seen in a tergosternal motoneurone. Record (a) is from a 7-day-old adult female, (b–d) from a 4-day-old adult female. Calibration: vertical (a) 3 mV, (b–d) 5 mV; horizontal (a, b, d) 200 ms, (c) 400 ms.

evokes potentials of opposite sign in each (Fig. 6a). A single excitatory postsynaptic potential (EPSP) in the first basalar from the stretch receptor can be about twice the amplitude of the total depolarization during the slow rhythm (Fig. 6b).

The first basalar motoneurone of the hindwing apparently receives none of the potentials which cause the slow and fast rhythms in an elevator recorded at the same time (Fig. 6c). The EPSPs from the stretch receptor may occur continually, but are not modulated by either rhythm. It cannot, however, be said that the hindwing first basalar motoneurones do not receive these rhythmic inputs but merely that they cannot be observed. Similarly the presence of the rhythmic inputs could not be revealed in the subalar motoneurones of the forewing (Fig. 6d–f). When a subalar motoneurone is hyperpolarized beyond the reversal potential of the apparently un-patterned, inhibitory postsynaptic potentials (IPSPs), no rhythmic depolarizing inputs are apparent (Fig. 6e). The low frequency of spikes evoked by an applied depolarization is not changed during the depolarizing phase of the slow rhythm (Fig. 6f). It is therefore unlikely that the subalar motoneurones receive the synaptic potentials which cause the slow and fast rhythms.

The somata of the dorsal longitudinal motoneurones of the forewing are in the prothoracic ganglion, but they receive the synaptic inputs which cause the slow and fast rhythms (Fig. 7). Recordings from the meta- and mesothoracic tergosternal and a prothoracic dorsal longitudinal motoneurone show that all three have synaptic inputs in common (Fig. 7a, b).

Common synaptic inputs causing the slow and fast rhythms are thus found in motoneurones of all three thoracic ganglia. They affect most elevator motoneurones and some depressors on both sides of the ganglia. In all, 30 motoneurones receive these common synaptic inputs (Table 1).
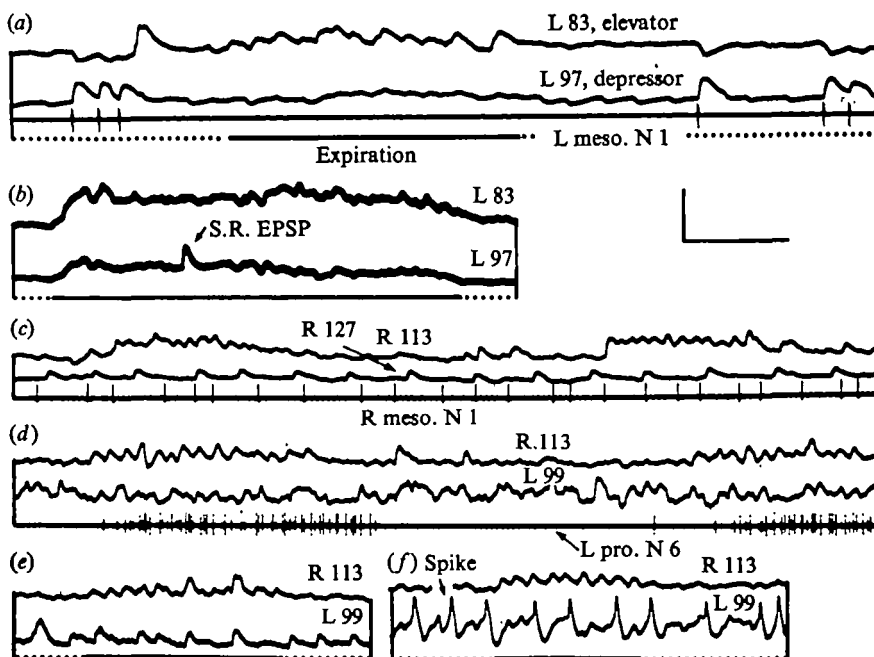
Fig. 6. Inputs to depressor and elevator motoneurones. (*a*) The stretch receptor of the left forewing (third trace) evokes EPSPs in the left forewing tergosternal (83) and IPSPs in the left first basalar (97). Both rhythms are clearly seen in the elevator but only a slight depolarization in the depressor. (*b*) The same two motoneurones in a different locust in which the rhythmic synaptic inputs can be matched. There is one EPSP (arrow) in the first basalar caused by the stretch receptor. (*c*) No common synaptic potentials are apparent in the right hindwing tergosternal (113) or first basalar (127) motoneurones during the slow rhythm. The EPSPs in the first basalar are caused by the stretch receptor of the right hindwing. The third trace monitors spikes of the right forewing stretch receptor. (*d*) No rhythms are apparent in a forewing subalar motoneurone (99), though present in the hindwing tergosternal and in the spikes of prothoracic N6 (third trace). (*e*) Hyperpolarizing the subalar beyond the reversal potential for most of the IPSPs still fails to reveal a rhythm. (*f*) Spikes (arrow) in the subalar evoked by a d.c. depolarization are not changed in frequency during the slow rhythm. Records (*a*), (*b*), (*c*), (*d–f*) are from different locusts during the first week of their adult life. Calibration: vertical (*a–c*) 10 mV, (*d–f*) 3 mV; horizontal (*a*) 200 ms, (*b–f*) 400 ms.

What causes the common synaptic inputs

The widespread occurrence of the common synaptic potentials makes it imperative to ask as to their nature. Are they true synaptic potentials or artefacts?

(1) They are not movement artefacts caused by ventilatory movements of the body because they persist when the abdomen is removed. They do not result from the movement of air in the tracheae of the ganglia because they persist in motoneurones of a ganglion from which all tracheae have been removed.

(2) They are not field potentials. The depolarizing potentials are accentuated by hyperpolarizing the motoneurone (Fig. 5*c*) and diminished by a depolarization (Fig. 5*d*). The reversal potential has not been determined but, because they lead directly to spikes (Fig. 11), they must be excitatory. The potentials do not occur in all motoneurones in one ganglion. Motoneurones innervating leg muscles, for example the
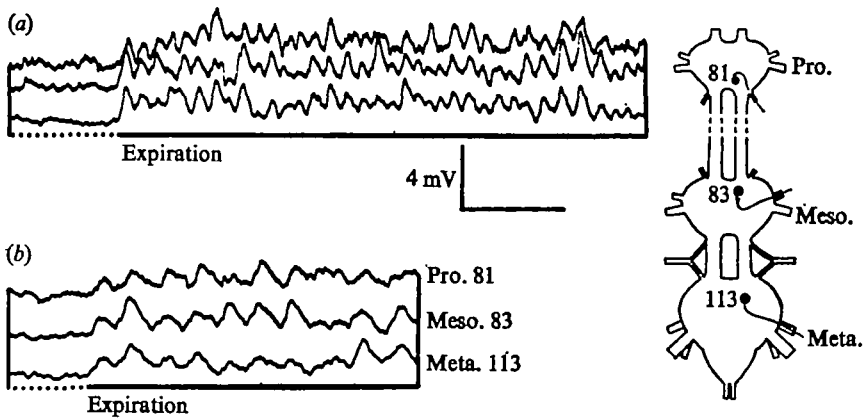
Fig. 7. Common synaptic potentials in motoneurones of three ganglia. (a, b) Simultaneous recording from a right prothoracic dorsal longitudinal motoneurone (81), a right mesothoracic tergosternal (83) and the right metathoracic tergosternal (113). All three are depolarized in the slow rhythm (horizontal bar) upon which is superimposed the oscillations of the fast rhythm. Records are from a 3-day-old adult male. Calibration: horizontal (a) 400 ms, (b) 200 ms.

Table 1. *Connexions of the interneurones with left thoracic flight motoneurones which have been revealed so far*

(Dashes indicate that no connexions were found, blanks that the observations were not made. The connexions are bilateral and extend to all three thoracic ganglia.)

| Motoneurone	Muscle number	Left interneurone	Right interneurone
Prothoracic ganglion			
Depressors			
Dorsal longitudinal, 1–4	81	EPSP	EPSP
Mesothoracic ganglion			
Elevators			
First tergosternal	83	EPSP	EPSP
Second tergosternal	84	EPSP	EPSP
Anterior tergocoxal, 1, 2	89	EPSP	EPSP
Tergotrochanteral	103	—	—
First posterior tergocoxal, 1	90	—	—
Depressors			
First basalar	97	EPSP	EPSP
Second basalar	98	—	—
Subalar, 1, 2	99	—	—
Dorsal longitudinal, 1–4	112	EPSP	EPSP
Mesothoracic ganglion			
Elevators			
Tergosternal	113	EPSP	EPSP
Anterior tergocoxal, 1	118	EPSP	EPSP
Tergotrochanteral	133		
First posterior tergocoxal	119		
Depressors			
First basalar	127	—	—
Second basalar	128	—	—
Subalar	129		
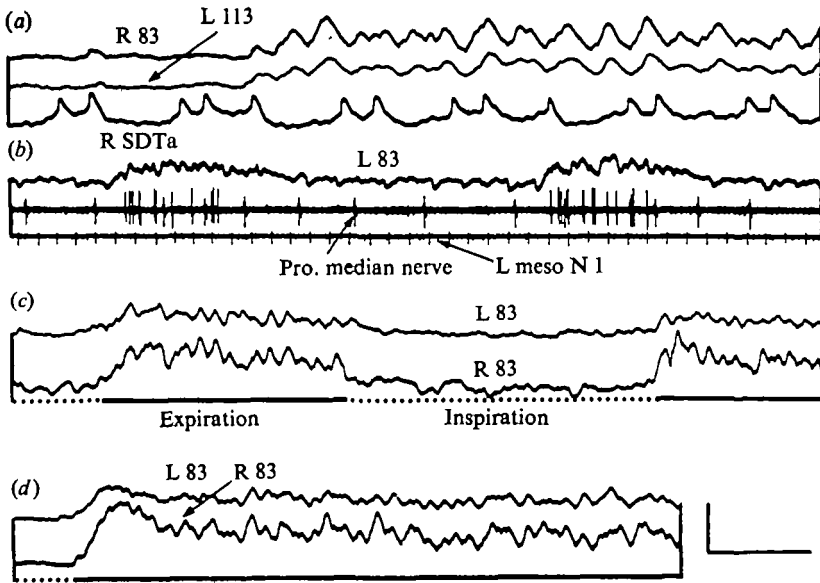
Fig. 8. The depolarizing rhythms in the motoneurones are not artefacts. (*a*) The rhythms are not seen in a metathoracic, slow tarsal depressor motoneurone (SDTa, third trace) but are seen in a right mesothoracic (83, first trace) and the left metathoracic (113, second trace) tergosternal motoneurones recorded at the same time. (*b*) The depolarization of a tergosternal motoneurone (first trace) occurs in the absence of bursts of spikes in mesothoracic N1 (third trace). (*c*) Common inputs to the left and right mesothoracic tergosternal motoneurones (83), present when the thoracic nervous system is intact, persist when the meso- and metathoracic ganglia are completely isolated (*d*). Note the increased duration of the slow rhythm in the isolated ganglia (horizontal bar). Records (*a*), (*b*) and (*c, d*) are each from different locusts. Calibration: vertical (*a*, trace 3) 5 mV, (*a*, traces 1 and 2, and *b–d*) 3 mV; horizontal (*a*) 200 ms, (*b–d*) 400 ms.

slow depressor of the tarsus, do not undergo rhythmic depolarizations but a meso- and a metathoracic tergosternal motoneurone recorded at the same time do (Fig. 8*a*). The fast flexor of the tibia, whose soma is next to a flight motoneurone which shows the rhythms, itself has no rhythms.

(3) There is a delay between the occurrence of the potentials in the different ganglia (Fig. 7*b*) which would not be expected of electrotonically conducted field potentials. Expt 2, 3 also exclude the possibility that the potentials derive from a common source of current picked up by the earth electrode. This is a large piece of silver wire placed in the abdomen, but its position has no effect on the appearance of the common synaptic potentials in the flight motoneurones.

(4) It is not necessary for the ventilation to be stressed in order that the rhythms be seen in the flight motoneurones. When ventilation is stressed, bursts of motor impulses occur in mesothoracic N1 (Fig. 4*c*) or prothoracic N6 (Fig. 3*a*) so that pumping movements of the head begin. Rhythmic depolarizations of the flight motoneurones may occur in the absence of such bursts (Fig. 8*b*).

(5) The potentials do not result from a rhythmic afference caused by the ventilatory movements. The frequency of spikes of wing afferents is not modulated by the ventilatory cycle in these experiments (Fig. 8*b*), but in an unrestrained and resting cricket e stretch receptor of a wing may spike during expiration (Moss, 1971). The stretch

receptor, however, cannot be the source of the potentials observed because it evokes IPSPs in elevators and EPSPs in depressor motoneurones and not EPSPs in both (e.g. Fig. 6a but for full description see Burrows, 1975a). Moreover, its spikes do not occur at the requisite frequency nor do they show the appropriate patterning. De-afferentation of the meso- and metathoracic ganglia, so that they are connected to the rest of the nervous system only by the abdominal and pro-mesothoracic connectives, does not abolish the slow or the fast rhythms. The common synaptic potentials still persist when the ganglia are completely isolated by cutting all the connectives (Fig. 8c, d). The frequency of the slow rhythm is initially drastically reduced when the pro-mesothoracic connectives are cut. The frequency gradually recovers but not to its initial level.

(6) The common synaptic potentials are not restricted to a particular time in the locust's life history. They occur in fifth (final) instar locusts (Fig. 4a–d), in young adults whose skeletons are still soft after the final moult (Fig. 3) or in sexually mature adults of both sexes (Figs. 2, 11). In recently moulted adults the ventilatory rhythm is more likely to consist of a prolonged expiration followed by several shorter ones.

Taken together these observations indicate that the common synaptic potentials are probably chemically mediated EPSPs caused by interneurones which make widespread connexions.

Pathways of the interneurones

The bursts of impulses in the prothoracic median nerve to the spiracles and venti-latory bursts in prothoracic N6, are abolished when both pro-mesothoracic connec-tives are cut. The slow and fast rhythms in the prothoracic dorsal longitudinal motoneurones are also abolished. Bursts of spikes continue in the appropriate nerves of the meso- and metathoracic ganglia, as do the rhythms in the flight motoneurones but at a reduced frequency (compare Fig. 8c with Fig. 8d). When both meso-metathoracic connectives are cut, ventilatory bursts in the mesothoracic median nerve and N1 are abolished but continue after a variable period of silence in the appropriate nerves of the metathoracic ganglion. Such surgery usually abolishes the rhythmic depolarizations in both a meso- and metathoracic tergosternal motoneurone recorded simultaneously. In time, however, the rhythmicity reappears in the metathoracic motoneurone but not in the mesothoracic one. The bursts of spikes in the ventilatory nerves and the rhythms in the flight motoneurones resume at the same time. There-fore the pattern generating mechanism for the slow ventilatory and fast rhythms appears to be within the metathoracic ganglion. The pro- or mesothoracic ganglia are incapable of producing these rhythms on their own; to do so they must receive ascend-ing commands from the metathoracic ganglion.

To determine the routes taken by the axons of the interneurones, recordings were made from two or more flight motoneurones which showed the rhythms and particular connectives were cut (Fig. 9). Common synaptic potentials still occur in the left and right mesothoracic tergosternal motoneurones when the right meso-metathoracic connective is cut (Fig. 9a). The result is the same if, in another locust, the left con-nective is cut, so that symmetrical pathways must be involved. There must be at least one axon in each connective which synapses upon both left and right motoneurones and the crossing-over must occur within the mesothoracic ganglion. The amplitu
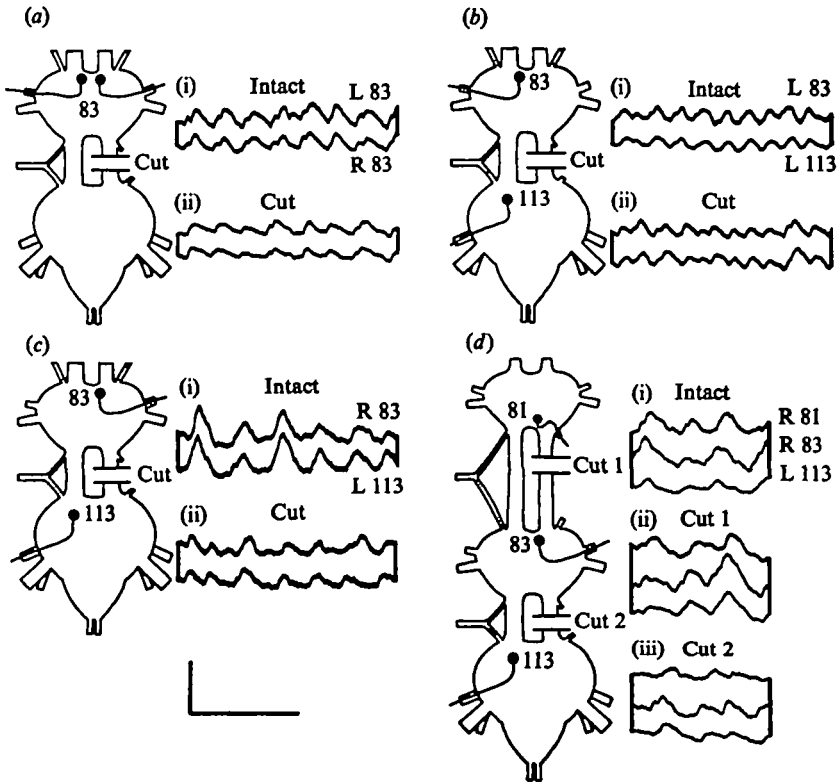
Fig. 9. Pathways of the interneurones mediating the common synaptic potentials. Recordings are made from two or three motoneurones simultaneously and one of the interganglionic connectives is cut as indicated. In each part of the figure, the top set of recordings (i) was made before, the lower set (ii) after cutting the connective. For full description see the text. Each experiment was performed on a different adult locust. Calibration: vertical (*a*, *b*, *d*) 4 mV, (*c*) 2 mV; horizontal (*a*, *b*, *c*) 250 ms, (*d*) 160 ms.

of each fast oscillation is reduced in both motoneurones when one connective is cut (Fig. 9*a* ii). In the intact nervous system most oscillations are composed of groups of two or three EPSPs (Fig. 9*a* i), but when one connective is cut only one or rarely two EPSPs are present. A similar reduction in the amplitude of the fast oscillations occurs in the left meso- and in the left metathoracic tergosternal motoneurones when the right meso-metathoracic connective is cut (Fig. 9*b*). The synaptic potentials in the two motoneurones remain matched. This is not the expected result on the assumption that the rhythms originate in the metathoracic ganglion; the oscillations in the meta-thoracic motoneurone should be unchanged. Undoubtedly cutting the nearly 8000 axons (Rowell & Dorey, 1967) in a connective has other effects, one of which may be to inhibit or damage the interneurone causing the common synaptic potentials which has its axon in that connective. The above two experiments (Fig. 9*a*, *b*) make it un-likely that the common potentials are derived from a single neurone with an axon in each connective. Such a neurone would need to have ipsilateral and contralateral branches of both of its axons in each ganglia; a somewhat bizarre arrangement. More-over, upon section of a connective there should be a difference in the pattern of synaptic potentials between a metathoracic and a mesothoracic motoneurone.
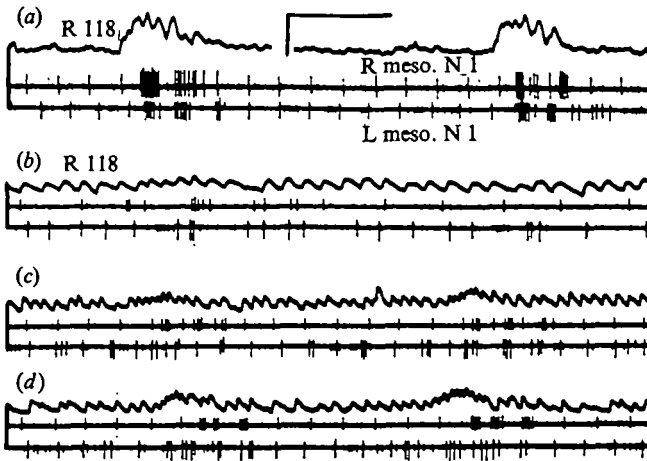
Fig. 10. Rhythmic synaptic inputs to a hindwing anterior tergocoxal motoneurone before and after a period of flight-like activity. (a) *Before*: both slow and fast rhythms are present. (b) *After*: the slow rhythm is absent and EPSPs occur continuously. There are no bursts of spikes in either of the mesothoracic N1s (second and third traces). (c, d) The progressive recovery of the slow rhythm and the diminution in the frequency of EPSPs during the inspiratory phase. The amplification of the extracellularly recorded N1 spikes is reduced in (b–d). Note the increased time scale of (b). Calibration: vertical, intracellular, 5 mV; horizontal (a, c, d) 400 ms, (b) 200 ms.

Section of the right connective when the right meso- and left metathoracic tergosternal motoneurones are impaled, causes a reduced amplitude of oscillations in both (Fig. 9c). Their synaptic inputs remain matched, confirming that the left interneurone makes connexions with both.

The same pathways are followed in the pro-mesothoracic connectives and within the prothoracic ganglion (Fig. 9d). Section of the right pro-mesothoracic connective causes a reduced amplitude of fast oscillations in the right prothoracic dorsal longitudinal motoneurone without apparently affecting meso- or metathoracic motoneurones impaled at the same time (Fig. 9d, i, ii). One source of the common synaptic potentials has therefore been removed from the prothoracic motoneurone. Inputs to this motoneurone are not changed by subsequent section of the right meso-metathoracic connective but the amplitude of the oscillations now falls in the meso- and metathoracic motoneurones (Fig. 9d iii).

These results imply that there are two symmetrically arranged interneurones or perhaps two sets of interneurones, one in each connective.

Conduction velocity of the interneurones

Some estimate of the conduction velocity of the interneurones can be gained from the delay between the appearance of the shared EPSPs in motoneurones of different ganglia. An EPSP in a prothoracic dorsal longitudinal motoneurone follows one in an ipsilateral mesothoracic tergosternal motoneurone with a delay of about 10 ms. The conduction velocity is about $1 \cdot 4 \, \mathrm{m \cdot s^{-1}}$. This assumes that a cable of constant diameter runs between the two motoneurones but within each ganglion the axon almost certainly gives off finer branches which then contact the motoneurones. The dangers inherent in such assumptions are illustrated by the occurrence in some locusts of
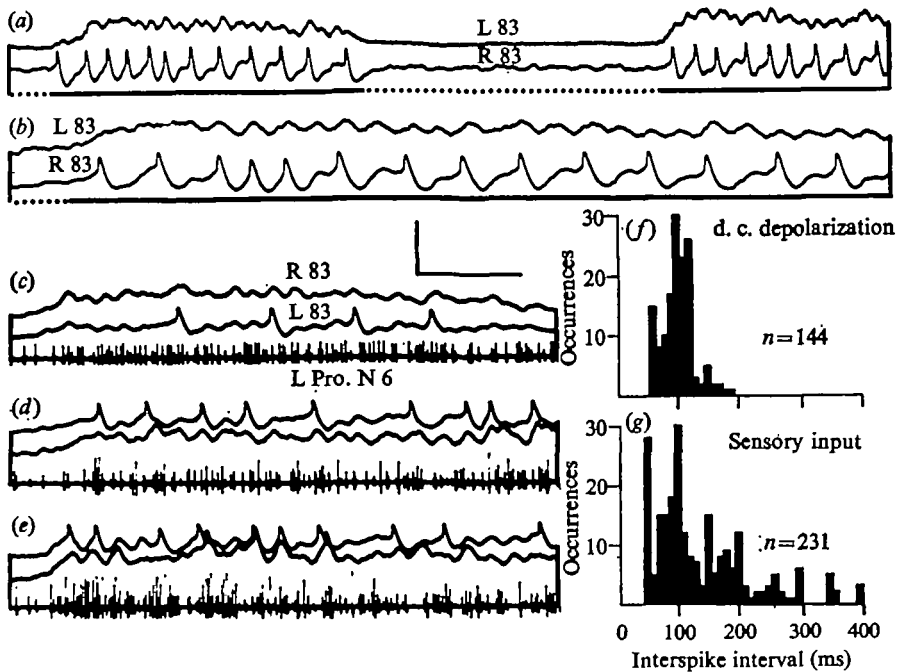
Fig. 11. The common depolarizing synaptic potentials can evoke spikes in the flight moto-neurones. (*a, b*) Two electrodes, one to record voltage the other to pass current, are placed in the right mesothoracic tergosternal motoneurone (83) and a single recording electrode in L83. When depolarized by a d.c. current, R83 gives bursts of spikes in time with the slow rhythm. Spikes occur on each or on alternate oscillations of the fast rhythm. (*c, d*) Blowing on the head causes a depolarization of the motoneurones which sums with the rhythmic depolarization to evoke spikes. (*e*) Stroking the abdomen depolarizes the motoneurones and evokes spikes only during the rhythmic depolarization. (*f*) Histogram of the interspike intervals in R83 when a d.c. depolarization is applied. (*g*) Histogram of interspike intervals in R83 when wind is blown at the head. Records (*a, b, f*) are from one adult male locust, (*c-e, g*) from another. Calibration: vertical (*a, b*) first trace 5 mV, second trace 10 mV, (*c-e*) 10 mV; horizontal (*a*) 400 ms, (*b-e*) 200 ms.

EPSPs in mesothoracic motoneurones before those in metathoracic ones, although the interneurones are thought to originate in the latter ganglion.

The rhythmic inputs will evoke spikes in motoneurones

In the majority of locusts the rhythmic depolarizations are subthreshold. On rare occasions they may lead to a few spikes on a slow depolarizing wave. Still more rarely, and always at times when neither the tape recorder nor the camera are running, they may cause apparently spontaneous sequences of spikes. The elevator motoneurones spike rhythmically in a pattern which closely resembles flight. For several minutes after the sequence of spikes no common synaptic potentials occur in any of the flight motoneurones. The interneurones must therefore be silent. It is assumed that they gradually resume activity since common EPSPs can be recorded in the appropriate flight motoneurones, but these EPSPs now occur tonically (Fig. 10*b*). There is no sign of the ventilatory rhythm in either the flight motoneurones or in nerves to venti-latory muscles (Fig. 10*b*). The ventilatory rhythm then gradually begins to re-emerge,

as shown by a periodic increase in the frequency of EPSPs in the flight motoneurones and bursts of spikes in nerves to ventilatory muscles (Fig. 10c). EPSPs occur throughout the inspiratory phase of the ventilatory cycle, but decrease in frequency with time (Fig. 10d). Eventually after some 5–10 min the patterns which were present before the evoked spikes resume (Fig. 10a). There are now no EPSPs during inspiration and clear oscillations of the membrane potential during expiration.

To show more clearly that the rhythmic synaptic input to the motoneurones could evoke a rhythmic output of spikes, two electrodes were inserted into a mesothoracic tergosternal motoneurone. One recorded the voltage, the other was used to pass current. Bursts of spikes are evoked during the depolarizing phase of the slow ventilatory rhythm when a d.c. depolarization is applied (Fig. 11a). The tergosternal muscle then contracts rhythmically in time with the expiratory phase of ventilation. Each spike arises from the rising phase of one of the fast oscillations of the membrane potential (Fig. 11b). Therefore both fast and slow rhythms are expressed in the spikes of the motoneurone. Some spikes occur on successive oscillations but most occur on alternate ones so that a histogram of the interspike intervals is bimodal, with peaks that correspond to the period and to twice the period of the fast oscillations (Fig. 11f). Changing the level of applied depolarization alters the proportion of spikes which occur on successive, alternate or subsequent oscillations.

The rhythmic synaptic inputs will sum with other unpatterned sensory inputs at the motoneurone to evoke spikes in either (Fig. 11c, d) or both left and right tergosternal motoneurones at the same time (Fig. 11e). An air stream directed at the head is the most effective input (Fig. 11c, d) but stroking the abdomen lightly with a paintbrush will also evoke spikes (Fig. 11e). The spikes occur during the depolarizing phase of the slow rhythm, though a blast of air at the head may evoke spikes at any time. The spikes occur on the rising phase of each fast oscillation: a few on successive oscillations, the remainder on the second or subsequent ones. The histogram of the interspike intervals therefore shows peaks at the basic period of the oscillations and at multiples of this (Fig. 11g). Both slow and fast rhythms can therefore be expressed as spikes in motoneurones when those stimuli are given which are likely to be encountered by the flying locust.

DISCUSSION

The observation of synaptic potentials common to several simultaneously impaled motoneurones has allowed the connexions of probably two interneurones to be defined. The limitation of the method is that some patterns of behaviour, in particular flight, do not usually occur while electrodes are in the thoracic ganglia. Consequently functional connexions can be revealed but their function in behaviour can only be surmized. The common potentials in 30 motoneurones innervating flight muscles with their somata in the three thoracic ganglia can be most easily explained by interneurones with the following properties (Fig. 12). Their somata are probably within the metathoracic ganglion because it is here that the rhythmicity arises. Common synaptic potentials can be recorded in motoneurones of an isolated metathoracic but not in isolated pro- or mesothoracic ganglia. The axon of each interneurone ascends to the prothoracic ganglion on the side that is assumed to be ipsilateral to its soma, though no conclusions are altered if it is contralateral. Within each ganglion the axons of the inter-
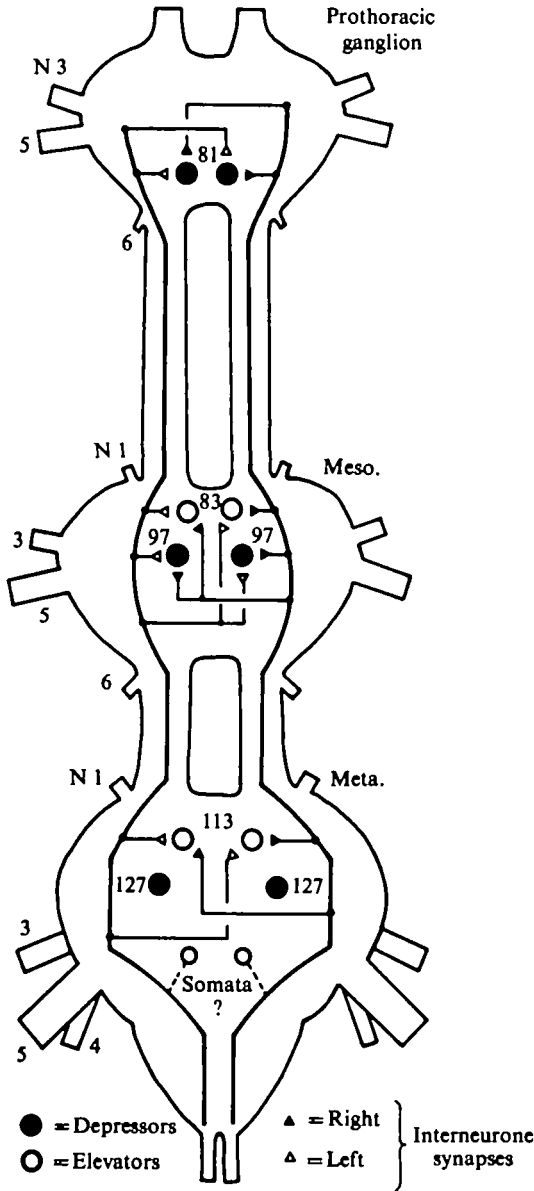
Fig. 12. Simplified diagram to indicate the pattern of synaptic connexions made upon some flight motoneurones by the inferred interneurones. One interneurone is shown in each connective with its soma probably in the metathoracic ganglion. Each interneurone makes bilateral connexions with flight motoneurones. 81, forewing dorsal longitudinal; 83, forewing tergosternal; 97, forewing first basalar; 113 hindwing tergosternal; 127, hindwing first basalar. The full set of connexions so far revealed is shown in Table 1.
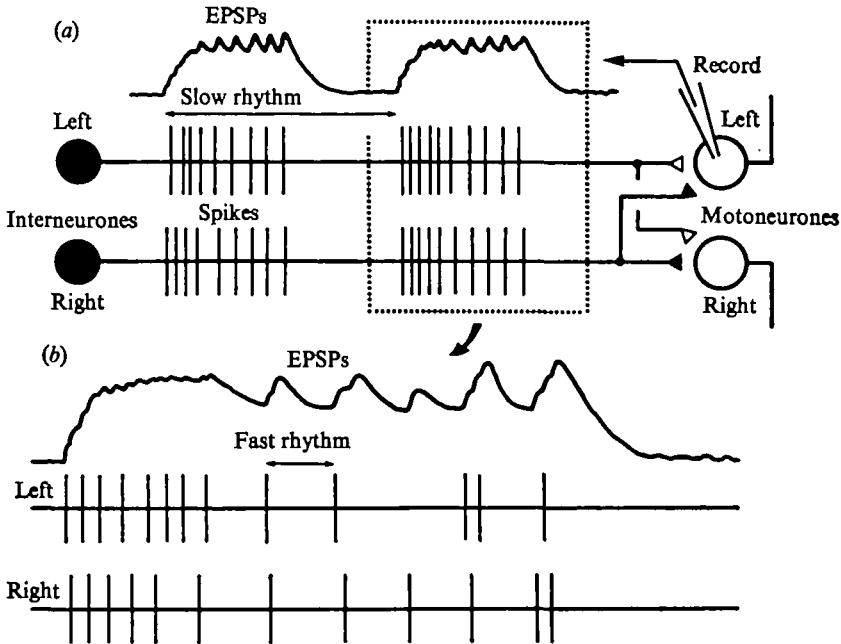
Fig. 13. Hypothetical patterns of spikes in two interneurones which could explain the common EPSPs in the flight motoneurones. The spikes have *not* been observed but are inferred. (a) *The slow rhythm.* The pattern of EPSPs recorded intracellularly in a left flight motoneurone is explained by the summation of the patterns of spikes in the left and right interneurones. The interneurones spike during expiration and are silent during inspiration. (b) *The fast rhythm.* One expiratory phase of the slow rhythm is shown. Each oscillation is caused by a few spikes in each interneurone which occur within a short time of each other.

neurones branch to synapse upon both left and right flight motoneurones. A particular motoneurone therefore receives two inputs, one from the ipsilateral and one from the contralateral interneurone.

If this is the correct interpretation of the way the interneurones are arranged then the patterns of EPSPs in the motoneurones can be derived from the following pattern of interneurone spikes (Fig. 13). At no time can the EPSP caused by one interneurone be clearly distinguished from that caused by the other. Each interneurone is assumed to produce a burst of spikes in time with the expiratory phase of the ventilatory cycle. During inspiration both are silent. At the start of expiration both interneurones must produce several spikes at a high frequency. There is apparently little or no coupling between the spikes of the two interneurones so that a tonic depolarization of large amplitude is produced in the motoneurones. A possible interpretation is that during inspiration the interneurones are inhibited and that the initial high frequency of spikes represents a postinhibitory rebound. Thereafter during expiration each interneurone spikes at intervals of about 50 ms. Occasionally one interneurone will miss a spike, or either may produce a closely spaced pair of spikes. The spikes in each interneurone usually occur at about the same time so that a definite rhythm is maintained, but sometimes they drift relative to each other so that the fast rhythm breaks down (Fig. 2c). This implies that any electrical coupling between the two interneurones, should it exist, is insufficient to synchronize their spikes. Each spike is assumed to evoke

EPSP in the motoneurones so that waves of usually one, two or sometimes three or four EPSPs occur at approximately 50 ms intervals. Each interneurone therefore conveys information about two rhythms. The slow rhythm is coded in the overall burst of spikes and the fast rhythm in the interval between each spike or each pair of spikes. The information garnered from the common synaptic potentials in motoneurones is insufficient to allow more than speculation as to how the interneurones themselves are driven.

Common synaptic potentials in motoneurones of different ganglia implies that the interneurones synapse directly upon the motoneurones and not upon intraganglionic premotor interneurones. The interneurones convey commands from the meta-thoracic ganglion to other ganglia of the thorax which are incapable of producing the rhythms. They are both co-ordinating and commanding the motoneurones of the different ganglia in the two rhythms.

What function do the rhythms have?

In the experimental conditions used here the rhythmic depolarizations of the flight motoneurones are usually subthreshold. Nevertheless the inputs are significant in that they can modulate the spikes of motoneurones in both rhythms; bursts of spikes in flight motoneurones occur at the ventilatory rhythm while the interval between each spike represents the fast rhythm. Perverse experimentation is not required to allow these rhythms to be expressed as motoneurone spikes. Stimuli, such as wind on the head, which the locust is likely to encounter in its everyday life allow both rhythms to be expressed.

For what purpose are the flight motoneurones depolarized in a ventilatory rhythm? I know of no examples in which flight muscles of the locust contribute force to venti-latory movements except during flight. In *Prionine* beetles, however, flight muscles may spike during expiration in the absence of flight and Miller (1971) has argued that the force so produced may be significant for ventilation. Apparently spurious coupling to the ventilatory rhythm of neurones other than those usually involved in ventilation has often been observed. Single impulses or even bursts of impulses may occur in leg motoneurones in time with ventilation although they cause no movement (Hoyle, 1964). Tarsi may wave in time with ventilation (Hoyle & Burrows, 1973b) and most spontaneously active neurones penetrated by Bentley (1969) in the neuropile of the mesothoracic ganglion of crickets spiked in phase with the ventilatory cycle. Both Hoyle and Bentley predicted that the irradiation was caused by neurones with wide-spread connexions, while Miller (1966) suggests a need to distinguish between the accidental and the functionally important features of a biological system, thereby implying that irradiation is accidental. The interneurones described here with their widespread connexions upon flight motoneurones go some way toward ex-plaining the widespread occurrence of ventilatory rhythms in the central nervous system. They are not, however, a complete explanation of all the coupling to ventila-tion which is observed. For example the interneurones do not appear to synapse upon tarsal motoneurones yet these may at times be coupled to the ventilatory rhythm, but explanations involving rhythmic afference has not been eliminated for these moto-neurones.

The similarity of the period of the fast rhythm to that of the wingbeat period in

flight leads to the tempting thought that the fast rhythm may be an expression of the endogenous pattern generator for flight. There is no direct evidence for this but circumstantial evidence is considerable. The fast rhythm occurs in flight motoneurones and can cause spikes at intervals similar to those which occur in flight. The periods of the two rhythms are thus similar. Could the fast rhythm be the fortuitous consequence of the necessity for the interneurones to spike at some frequency during expiration and that this frequency just happens to be similar to the flight period? The evidence suggests that the interneurones are themselves driven in a fast rhythm, producing a variable number of spikes on each cycle. Therefore the fast rhythm is a definite one superimposed upon the interneurones or possibly an inherent property of the interneurones themselves. Before the fast rhythm can be accepted as the flight rhythm the following apparent anomalies must be answered. These anomalies may not be real but may merely reflect ignorance about the flight pattern generator. First, hindwings move before the forewings in flight but motoneurones of both wings receive common synaptic inputs. The phase lag could result if the hindwing motoneurones spike earlier on the same wave of depolarization than the forewing ones. Secondly, elevator and depressor motoneurones of a particular wing alternate in flight but both may receive common synaptic inputs. The depolarization as recorded in somata of elevator motoneurones is of consistently larger amplitude than that in depressors, but this of course gives no indication of its likely effectiveness at the spike initiating zone. Wind on the head, however, which evokes spikes in elevators during expiration, may fail to do so in depressors. Not all depressors apparently receive inputs from these interneurones. They were not observed in the hindwing basalars or forewing subalars. Thirdly, the flight pattern is continuous but the fast rhythm occurs only during expiration under the experimental conditions used here. Common synaptic potentials occur in the homologous motoneurones of the Australian locust *Chortoicetes*, presumably mediated by similar interneurones (Burrows, 1973). In these smaller locusts the fast rhythm occurs continuously without the slow rhythm, perhaps because ventilation is more susceptible to the surgery. The *Schistocerca* interneurones can also spike continuously and be modulated by the slow ventilatory rhythm (cf. Fig. 10). Thus ventilation can proceed and the input to the flight motoneurones is continuous but modulated at the slow ventilatory rhythm. This is precisely the pattern of activity which would be necessary during flight. In crickets the end of flight may be marked by the appearance of bursts of spikes in flight motoneurones at the ventilatory rhythm (Bentley, 1969). During some of the ventilatory cycles the spikes may occur at the flight frequency, so that both flight and ventilatory rhythms are superimposed. Bentley (1969) interpreted this to indicate the summation of two pattern generators at the motoneurone, but the patterns of activity so closely parallel those of the *Schistocerca* interneurones that an explanation in terms of similar interneurones may be appropriate.

If the fast rhythm is nothing to do with flight then it is reasonable to ask as to its purpose. Why are there interneurones with wide fields of influence which cause the membrane potentials of flight motoneurones to ripple at the flight frequency and which can be shown to cause spikes in flight motoneurones at the flight frequency?

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