Flight motor patterns recorded in surgically isolated sections of the ventral nerve cord of *Locusta migratoria*

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Summary. In the locust, *Locusta migratoria*, the pairs of connectives between the three thoracic ganglia and in the neck were transected in all possible combinations. Each of these preparations was tested for the production of rhythmic flight motor activity, with sensory input from the wing receptors intact and after deafferentation. The motor activity elicited in these preparations was characterized by intracellular recordings from motoneurons and electromyographic analyses.

The motor patterns observed in locusts with either the neck or the pro-mesothoracic connectives severed (Figs. 2, 3, and 4) were very similar to the flight motor pattern produced by animals with intact connectives. The activity recorded in mesothoracic flight motoneurons of locusts with either only the meso-metathoracic connectives cut or both the meso-metathoracic and the neck connectives transected were similar to each other. Rhythmic motor activity could be observed in these preparations only as long as sensory feedback from the wing receptors was intact. These patterns were significantly different from the intact motor pattern (Figs. 5, 6, and 7). Similar results were obtained when the mesothoracic ganglion was isolated from the other two thoracic ganglia, although the oscillations produced under these conditions were weak (Fig. 8 upper). In the isolated metathorax no rhythmic flight motor activity could be recorded (Fig. 8 lower), even when wing afferents were intact.

Considering the differences between the motor patterns observed in the various preparations these results suggest that the ganglia of the locust ventral nerve cord do not contain segmental, homologous flight oscillators which are coupled to produce the intact flight rhythm. Instead they support the idea that the functional flight oscillator network is distributed throughout the thoracic ganglia (Robertson and Pearson 1984). The results also provide further evidence that sensory feedback from the wing sense organs is necessary for establishing the correct motor pattern in the intact animal (Wendler 1974, 1983; Pearson 1985; Wolf and Pearson 1987a).

Introduction

Studies on a large number of rhythmic motor systems have revealed that patterned motor activity can be generated in the absence of afferent input (Delcomyn 1980). The neuronal network responsible for generating the motor pattern in a deafferented preparation is now referred to as a ‘central pattern generator (CPG)’ (Grillner 1985; Grillner and Wallen 1985). One of the major aims over the last decade has been to localize CPGs in the nervous systems of numerous animals. A general approach has been to progressively reduce the nervous system by a series of selective lesions and so establish the minimal portion of the central nervous system which is still capable of generating a rhythmic motor output. For behaviors involving rhythmic movements of a number of serially homologous appendages or body segments, for instance walking, swimming, or swimmeret beating, CPGs have been found to be localized to either a few spinal cord segments in vertebrates (e.g. Grillner and Wallen 1985) or to single segmental ganglia in the nerve cords of invertebrates (Weeks 1981; Paul and Mulloney 1986). Coordination of the overall motor pattern for these behaviors is

Abbreviations: CPG central pattern generator; EMG electromyogram
considered to depend on central coupling of the individual CPGs (Stein 1978; Pearce and Friesen 1985; Grillner and Wallen 1985).

One exception to this general finding of separate CPGs being attributed to each appendage or each body segment appears to be the flight system of the locust (Robertson and Pearson 1984). The analysis of the neuronal organization of this system has shown that many flight interneurons are polysegmental and distributed over the ganglia belonging to six different body segments: the three thoracic ganglia and the three abdominal neurones fused to the metathoracic ganglion. The main observations suggesting the existence of a single distributed oscillator in the locust flight system are (i) that there is little homology of flight interneurons in the meso- and metathoracic ganglia (the two ganglia associated with the segments which bear the two pairs of wings), (ii) that most interneurons are polysegmental and have output projections in both the meso- and metathoracic ganglia, and (iii) that the centrally generated rhythm can be reset by current injection into single neurons in the meso-, the metathoracic, or the three fused abdominal ganglia.

This view of a distributed central oscillator for locust flight is contrary to the conclusion drawn by Wilson (1961) that separate oscillators exist in the meso- and metathoracic ganglia. Wilson, in his original study on central patterning in the locust flight system, reported that rhythmic contractions could be generated independently in forewing and hindwing muscles following transection of the connectives between the ganglia of these two segments. Wilson's proposal of segmental oscillators has recently received support from the observation that rhythmic motor patterns resembling flight activity can be elicited by the application of octopamine to the isolated metathoracic ganglion (Stevenson and Kutsch 1987). In the isolated mesothoracic ganglion the existence of an oscillator has not been demonstrated with this method but rhythmic activity in forewing muscles was observed after severing the meso-metathoracic connectives in otherwise intact locusts. The issue is whether these observations of flight motor oscillations in isolated meso- and metathoracic segments are inconsistent with the hypothesis of a distributed flight oscillator. Two possible explanations might account for the above results. (i) There exist homologous segmental oscillators in the two pterothoracic ganglia which in the intact animal are coupled to produce the functional motor pattern. Such oscillators would be expected to generate a more or less correct motor pattern in isolation. (ii) The rhythmic activities produced in meso- and metathorax after the ganglia of these segments have been disconnected are produced by fragments of one distributed oscillator. A precedent for this is the lobster pyloric oscillator in which fragments are still able to produce rhythmic activity (Miller and Silverston 1982). If this second explanation is correct then the activity in single isolated ganglia would be expected to be different from the motor pattern produced by the entire nerve cord and probably also different in individual isolated ganglia. What is clearly needed for a judgement on this issue is a better knowledge of the oscillator properties of isolated segments of the locust nerve cord. It was the scope of the present study to provide some of necessary information by (i) a systematic analysis of the distribution of oscillator properties in segments of the locust ventral nerve cord and (ii) a characterization of the observed oscillations by intracellular motoneuron recordings and electromyographic analyses.

Methods

Adult Locusta migratoria from a laboratory culture, at ages between two and three weeks after the imaginal moult and mainly females, were used for the experiments. Only animals that flew steadily for at least ca. 30 s when held by the pronotum were used.

To allow intracellular recordings from motoneurons during flight the animals were mounted (wax-resin mixture 1:2) upside down on a special holder which did not impair the movement of the wings (see Wolf and Pearson 1987a, b). Usually the legs were removed but otherwise the locust was intact. A small window was cut into the ventral cuticle of the thorax above one of the thoracic ganglia leaving all apodemes and muscle attachment sites uninjured to allow normal flight performance. The ganglion was exposed and supported by a stainless steel spoon. Recordings were taken from the cell bodies of flight motoneurons (40–60 MΩ glass microelectrodes) and the neurons were injected with Lucifer yellow dye for identification. The recordings obtained from the cell bodies were essentially the same as those from the neuropil region, except that the action potentials were smaller (Wolf and Pearson 1987a). Cell body recordings were very stable, however, and allowed the transection of connectives or meso- and metathoracic nerves 1 while recording from a certain cell. In this way the activity of a single neuron could often be recorded subsequently in the intact animal and after severing connectives and sensory nerves. The neck connectives could be exposed for transection by simply cutting a small slit into the soft cuticle of the thorax.

In parallel with intracellular recordings wing depressor activity was monitored electromyographically as a reference for flight activity. Electromyograms were taken from the first basilar muscles (97 in the meso-, 127 in the metathoracic ganglion according to Snodgrass 1935) and sometimes also from the subalars (99 and 129, respectively), by inserting two copper wires (diameter 0.1 mm with lacquer coating, insulated except for) the tips into prepared holes in the sternum or pleural cuticle above the muscle (Pearson and Wolf 1987).

For a quantitative characterization of flight motor activity (see data in Figs. 3, 6, and 7) electromyograms were recorded
from elevator (tergesternal, 84/84 and 113) and depressor (1st basalar, 97 and 127) muscles. Surgical manipulations were performed in the same way as described above but because no intracellular recordings were necessary in these experiments the ganglia did not have to be exposed completely and damage to the tracheal system was minimal. The EMG recordings were digitized by a window discriminator and fed into a general purpose computer (DEC LSI 11/23) for further evaluation.

Intracellular recordings were made from a variety of meso- and metathoracic flight motoneurons. Usually the tergesternal and first basalar motoneurons were impaled because they innervate pure flight muscles and are not involved in walking (Snodgrass 1935). Their activity was recorded in intact animals and in locusts which had one or two pairs of connectives cut. In addition, the preparations could be deafferented by cutting the four nerves 1 of the meso- and metathoracic ganglia. The pairs of connectives between the three thoracic ganglia plus the neck connectives were cut in all possible combinations. In order to disconnect two ganglia completely often the two nerves 6 of the more rostral ganglion were severed in addition to cutting the connectives; this was always done when disconnecting meso- and metathoracic ganglion. Considering the intact situation and the possibilities of deafferentation this resulted in 14 different preparations (see Fig. 1 for a diagram showing the thoracic ventral nerve cord with sensory nerves and connectives labeled).

A variety of methods were used in attempts to elicit flight, according to the particular preparation: wind pulls directed onto the animal's head, abdomen, or thorax; loss of tarsi; or hot wire; stimulation of the wing receptors by imposed movements and bending of the wings; dimming of ambient light; and finally by electrical stimulation of connectives or connective stumps adjacent to the thoracic ganglia (3 to 5 V pulses of ca. 0.2 ms at approximately 100 Hz).

In those cases in which a reproducible pattern of flight motor activity could be recorded only between 4 and 15 animals were tested. In these preparations rhythmic motor activity could be elicited with a probability of 94% on average (n = 62). Negative results, i.e. the lack of a rhythmic motor response to the stimuli described above, were confirmed in more than 20 different animals for any particular preparation and only those animals were considered which exhibited good flight performance immediately prior to the final transection of nerves or connectives.

Results

Motoneuron activity in intact animals and animals with pro-mesothoracic or neck connectives cut

The potentials recorded in intact animals and in locusts which had their pro-mesothoracic or their neck connectives transected were almost indistinguishable. The few consistent differences which could be observed did not concern the time course and shape of the motoneuron depolarizations or the intra- and intersegmental muscle coordination but rather the duration of flight episodes and the wingbeat frequency. Flight sequences in animals with the pro-mesothoracic or the neck connectives cut usually lasted only about 30 s or less whereas intact animals would fly for several minutes in response to pinching of the abdomen or a wind stimulus delivered to the head. The wingbeat frequency was reduced from the normal value of between 17 and 23 Hz to frequencies ranging from ca. 12 to 18 Hz in locusts which had the neck connectives or the pro-mesothoracic connectives severed (Fig. 3, graphs on the left). In addition, these animals often had difficulties in opening their wings at the start of flight. To allow normal flight performance the forewings had to be placed in the open position by the experimenter.

Intra- and intersegmental coordination of flight motor activity and synaptic input to the motoneurons in intact and in deafferented locusts have been described in detail elsewhere (Pearson and Wolf 1987; Wolf and Pearson 1987a). Therefore the following description shall be confined to the basic features as determined in intact locusts and observed in the two preparations just mentioned (Figs. 2, 3, and 4). Wing elevator and wing depressor motoneurons alternated in their activities. As the most distinctive feature, the latency between the first spike of a depressor burst and the first spike of the subsequent elevator burst remained relatively constant with changes in wingbeat frequency (Fig. 3 left graphs). Consequently, changes in the elevator-depressor latency accounted almost completely for variations in wingbeat cycle duration. The depolarizations in depressor and elevator motoneurons were short, with
steep and smooth rise and decay phases. One, or more commonly two, spikes were discharged per wingbeat cycle (Fig. 2 top traces, neck connectives cut).

Deafferentation caused no major changes in the shape of depolarizations and the number of spikes per wingbeat cycle as far as depressor motoneurons were concerned (Fig. 2 bottom right, pro-mesothoracic connectives cut). The shape of elevator depolarizations, in contrast, was changed considerably by deafferentation. The depolarizations commenced with a gentle rising slope, culminated in a pronounced final depolarization, and terminated with a repolarization that was much steeper than the rising slope. Usually the number of spikes per wingbeat cycle increased due to the broader shape of the depolarizations (Fig. 4 top) and the elevator spikes were delayed with respect to the preceding depressor burst due to the initial slow rise of the depolarizations. As the electromyographic analysis presented in Fig. 3 (right graphs) shows, the result was that the interval between the first depressor spike and the following elevator spike became clearly dependent on wingbeat frequency. This dependency was such that the phase of the onset of elevator activity in the depressor cycle remained roughly constant. While the motor patterns produced by deafferentated locusts with intact ventral nerve cords or with severed neck connectives resembled each other closely (top and middle right graphs in Fig. 3), the patterns produced by animals with transected pro-mesothoracic connectives were different (bottom right graph in Fig. 3). These different patterns were produced despite the fact that the underlying motoneuron depolarizations were very similar in all three of these preparations (e.g. Fig. 4). In deafferented animals with transected pro-mesothoracic connectives spikes were generated only during the pronounced, final part of the elevator depolarizations whereas in deafferented locusts with an intact nerve cord or with severed neck connectives spikes were usual-

![Fig. 2. Patterns of activity recorded in flight motoneurons after transection of the pro-mesothoracic or the neck connectives, before (examples on the top, recordings made from animals with neck connectives cut) and after (bottom examples, recordings in animals with pro-mesothoracic connectives cut) deafferentation (see Methods). The diagrams on the left indicate the transections. The solid lines mark the actual transections which were performed, the broken lines mark alternative transections which yielded a similar pattern of intracellularly recorded activity (for details see text). The potentials recorded in elevators (mesothoracic tergosternal 83/84) are shown on the left, those observed in depressors (mesothoracic 1st basalars 127) on the right, respectively. Electromyograms were taken from the metathoracic (lower left, 127) and mesothoracic (other recordings, 97) 1st basalars. The motoneuron activity in locusts with the pro-mesothoracic or the neck connectives severed were both almost indistinguishable for the depolarizations which could be recorded after deafferentation with the exception that in deafferented animals with severed pro-mesothoracic connectives consistent changes in spike initiation could be observed (details in Figs. 3 and 4, a typical pattern as produced by a deafferented but otherwise intact animal is presented in the upper part of Fig. 4).](image-url)
Motoneuron activity in the mesothorax after disconnection from the metathoracic ganglion or after additional transection of the neck connectives

The potentials which could be recorded in mesothoracic flight motoneurons after transection of the meso-metathoracic connectives and after additional cutting of the neck connectives were very similar to each other and are therefore presented together. Rhythmic motor activity could be reliably elicited (in 31 out of 33 animals) as long as sensory feedback from the forewings receptors was present. This rhythmic activity was accompanied by clear and often almost full-amplitude movements of the forewings at about half the normal wingbeat frequency. Most animals were unable, however, to unfold their forewings and these had to be drawn into the stroke position by the experimenter. The sequences of rhythmic motor activity lasted no longer than 30 to 40 s, even with continuous wind stimulation.

The activity pattern observed in mesothoracic motoneurons after transection of the meso-metathoracic connectives, when compared to the motor pattern of intact locusts, appeared much more variable and showed different characteristics as far as the coordination of elevators and depressors, the number of spikes per wingbeat cycle, and the shape and amplitude of motoneuron depolarizations are concerned. The elevator activity presented in Fig. 5 (upper left) gives one of the most regular examples that could be recorded. Depressor (shown as EMG) and elevator motoneurons alternated in their discharges and usually produced two and three spikes per wingbeat cycle, respectively. However, the activity of depressor neurons in particular was usually much less regular than in this episode. The number of spikes discharged per cycle varied widely within a single flight sequence and many of the depressors showed only subthreshold oscillations (Fig. 5 upper right). This variability in spike generation reflected a similarly variable pattern of motoneuron depolarizations. Generally the shape of depressor depolarizations changed from one wingbeat cycle to the next (Fig. 5 upper middle); these depolarizations were broad with gentle but
uneven rise and decay slopes. The shape of elevator depolarizations seemed more constant but was also broad with a gentle rise and decay. Rapid depolarization slopes as occurred in intact animals or steep repolarizations as occurred in intact and in deafferented (but otherwise intact) locusts could never be observed. When compared to the recordings made in intact animals, the amplitude of the oscillations in membrane potential was clearly reduced in both depressor and elevator motoneurons. Whereas in intact locusts a typical amplitude of the motoneuron depolarizations was 15 mV an amplitude of more than 10 mV could never be observed in preparations with severed meso-metathoracic connectives.

Figure 6 gives a quantitative description of this motor pattern, similar to the data provided by Fig. 3, top, for intact and deafferented locusts. The pattern appeared roughly phase-constant and variable (note size of standard deviations). When compared to the graphs in Fig. 3 the closest resemblance is perhaps to the deafferented pattern at the top right. However, elevator activity commenced much sooner after a depressor burst in locusts with transected meso-metathoracic connectives, this decreasing the depressor-to-elevator interval and prolonging the elevator-to-depressor interval. The above examination of the intracellularly recorded potentials (Fig. 4 upper as compared to Fig. 5 upper left) has shown that these two motor patterns, although superficially alike, are produced by quite dissimilar motoneuron depolarizations.

The histograms presented in Fig. 7 (bottom) once more illustrate the variability of the motor pattern produced in the mesothorax after being disconnected from the metathoracic ganglion. All three histograms appear very broad when compared to the respective histograms for the deafferented or intact patterns. Notably, the histograms for the depressor-to-elevator interval (bottom left) and for the interval between the last elevator spike and the first spike of the subsequent depressor burst (bottom right) extend to negative values.
Fig. 5. Flight motoneuron activity in the mesothorax after disconnection of the meso- and metathoracic ganglion. After additional transection of the neck connectives no significant changes could be observed in the depolarization patterns of the motoneurons (broken lines in the diagrams on the left indicate this additional transection which did not further affect the motor pattern). The potentials recorded in elevators (tergosternal 83/84) are given on the left, those observed in depressors (1st basalar 97 in the upper example, dorsal longitudinal 112 at the bottom) on the right hand side, respectively. The rhythmic activity observed with afferents from the wing sense organs intact is shown in the top examples. The lower recordings were made after deafferentation and no rhythmic activity could be observed. EMGs: basalar 99 in the upper left and upper far right (here marked by asterisks), tergosternal 83/84 (marked by arrows) in the upper middle, and tergosternal (83/84) in the bottom examples.

This means that an elevator burst could commence before the 'previous' depressor burst (in this case defined as such only by the still discernible alternating elevator-depressor activity, depressor bursts were much shorter than the 'subsequent' elevator burst in these cases) and that an elevator burst could extend beyond the start of the subsequent depressor burst. Neither event could ever be observed in intact or deafferented locusts (middle and top histograms in Fig. 7).

After deafferentation, i.e. after cutting of the metathoracic nerves 1, no rhythmic motor activity could be observed in metathoracic motoneurons. This result was confirmed in 32 animals. Depressor motoneurons were completely silent and showed only weak hyperpolarizations following, for instance, a wind stimulus (Fig. 5 lower right). In elevators wind stimulation elicited very reliably a tonic spike burst of several 100 ms duration (Fig. 5 lower left). This activity caused a distinct upward movement of the forewings or, at low spike frequencies toward the end of the burst, single upward wing twitches. This activity slightly resembles the spike bursts observed in elevators at the start of normal intact flight during unfolding of the wings – as do the hyperpolarizations in depressors (see e.g. Wolf and Pearson 1987a, their Fig. 5; Hedwig and Pearson 1984).
Motoneuron activity in the isolated mesothoracic ganglion

After the mesothoracic ganglion had been disconnected from the other two thoracic ganglia, rhythmic motor activity was rarely observed. In two out of 32 animals, a few oscillations in membrane potential, occasionally initiating single spikes, could be recorded. This motoneuron activity produced weak vibrations of the wings. The upper part of Fig. 8 presents two examples which were recorded in a wing depressor motoneuron. The two to four oscillations in membrane potential were irregular in shape and small in amplitude. The simultaneously recorded elevator muscle potentials show some rudimentary signs of alternating depressor-elevator activity (the arrow heads point to elevator bursts occurring during hyperpolarizations of the depressor). Intracellular recordings from elevator motoneurons were not obtained due to the rare occurrence of flight oscillations in this preparation.

After deafferentation no rhythmic or pronounced tonic activity could be recorded in the flight motoneurons of the completely isolated mesothorax (34 animals).

Motoneuron activity in the isolated metathoracic ganglion

In the metathoracic ganglion no rhythmic flight motor activity could be observed after the connectives to the mesothorax and the mesothoracic nerves 6 had been severed (32 animals). Instead, tonic bursts of elevator activity could be elicited quite reliably (Fig. 8 lower), sometimes accompanied by weak hyperpolarizations in depressors (similar to Fig. 5 lower right). These recordings resembled those made in the deafferented mesothoracic ganglion with the meso-metathoracic connectives transected (Fig. 5 bottom left). The elevator bursts were several 100 ms in duration and accompanied by upward movements of the hindwings. Again, this activity is reminiscent of the elevator spike bursts observed at the start of intact flight.

Following deafferentation, neither rhythmic motor activity nor tonic spike bursts could be elicited in the metathoracic flight motoneurons.

Discussion

Methodological remarks

The rhythmic motor activity which could be elicited in intact locusts and in a number of preparations with different segments of the thoracic nerve cord isolated was characterized by intracellular motoneuron recordings. The intracellular recording of neural activity provides a considerable advantage compared to electromyographic or visual observations (as e.g. by Wilson 1961). Not only can the different motor patterns be characterized more precisely – by means of the time course of synaptic input to motoneurons – but it is possible to recognize the rhythmic character of weak and even subthreshold oscillations (see Fig. 5, upper right or Fig. 8 upper). Furthermore, it allows the distinction between a tonic spike train in a motoneuron and a similar spike train produced by synthetically induced oscillations in membrane potential. The former situation could easily give rise to single, rhythmically succeeding muscle twitches and wing movements (similar to the situation in Fig. 8 lower) – which is not an uncommon observa-
Fig. 8. Flight motoneuron activity recorded in the mesothorax (top) and metathorax (bottom) after the ganglia of these segments had been disconnected from the adjacent thoracic ganglia (the diagrams on the left indicate the transections). Nerves 1 were always intact. The mesothoracic 1st basalar (97) was recorded in the upper two examples (both from the same animal), the activity of the metathoracic tergosternal (113) is shown in the lower recording (ca. 250 ms of the central portion of the spike burst omitted). The electromyograms represent elevator activity (top: tergosternal 83/84, arrows indicate elevator bursts occurring in alternation with the depolarizations in the intracellular depressor recording; bottom: tergosternal 113).

...tion in an animal with an injured nerve cord and could thus be interpreted as oscillatory activity in EMG recordings. Finally, very similar patterns of rhythmic electromyographic activity, distinguished only by more or less subtle differences, could be generated by quite different patterns of synaptic input to motoneurons. Vice versa, motoneuron depolarizations of similar shape can produce quite different discharge patterns (see examples above, compare Figs. 3 and 4).

The negative results reported in this study, i.e. the consistent absence of rhythmic motor activity in a number of preparations, should be treated with some caution. First, this is because negative statements in general have restricted validity (if something has not been observed it remains possible that it occurs only rarely or observation methods or experimental conditions were inappropriate) and, secondly, because for some of these preparations, in particular the isolated and deafferented mesothoracic ganglion, the stimulus repertoire which was available for attempts to evoke flight activity was obviously restricted. It is conceivable therefore that under different conditions rhythmic motor activity might be observed in preparations which did not exhibit such activity in the present study. For example, Sombati and Hoyle (1984) reported that injections of octopamine directly into the thoracic ganglia elicited flight activity in the locust. Recently Stevenson and Kutsch (1987) confirmed this observation for the isolated metathoracic ganglion. Because the effects of such transmitter injections are not yet completely understood, and may be unphysiological, such techniques were not applied in the present study.

Despite these restrictions some of the negative results appear very significant, particularly when compared to a corresponding preparation which still generated rhythmic motor activity. For instance, flight motor oscillations could be readily elicited by abdominal stimulation after severing the pro-mesothoracic connectives (see Fig. 2 bottom) but the additional severing of the meso-metathoracic connectives abolished rhythmic activity in the metathoracic ganglion even though the hitherto effective stimulus pathway was not disrupted. Similarly, wind puffs directed onto the animal's head could evoke oscillations in the mesothoracic ganglion after transection of the meso-metathoracic connectives (see Fig. 5 upper) but these were abol-
ished by deafferentation which presumably did not influence the previously effective input to the ganglion.

**Distribution of oscillator properties throughout the thoracic nerve cord**

The existence of segmentally homologous flight oscillators in the locust meso- and metathoracic ganglia is suggested by the strictly homologous organization of flight muscles and motoneurons in meso- and metathorax (Snodgrass 1935), by the homologous segmental organization of sensory structures (such as stretch receptors and tegulae) which form important elements of the intact flight oscillator (e.g. Wendler 1974, 1983; Pearson et al. 1983), and by the results of Wilson (1961) and Stevenson and Kutsch (1987) cited in the Introduction. On the other hand, the known interneurons of the flight rhythm generator network show very little segmental homology and neurons capable of resetting the centrally generated rhythm in the deafferented nerve cord are found in the meso-, meta-, and fused abdominal ganglia (Robertson and Pearson 1984). Considering the present knowledge about the locust flight interneurons and their interactions, the basic burst-generating network appears not to involve segmentally homologous neurons of both meso- and metathoracic ganglia (Robertson 1986). Instead the central components of the flight oscillator appear to be distributed over five ganglia (meso-, meta- and three fused abdominal). How then are we to view the fact that oscillations can be observed in several isolated segments of the ventral nerve cord, particularly those occurring after disconnecting the two pterothoracic ganglia (see upper parts of Figs. 5 and 8, Wilson 1961 and Stevenson and Kutsch 1987)?

First it should be noted that a wide variety of motor patterns could be produced in flight motoneurons depending on which connectives were cut and whether or not the preparation was deafferented. The motoneuron activity observed in the meso- and metathoracic ganglia, when together they were disconnected from the more anterior ganglia, were very similar to the intact motor pattern (see Fig. 2). Deficiencies could be observed only with respect to start and termination of flight and regarding flight frequency and the duration of flight episodes. This indicates that the pterothoracic segments contain most of the elements necessary for generating a complete flight motor pattern. One should bear in mind, however, that in a complex oscillator like the locust flight system the removal of single elements may produce only minor changes of the output pattern (Horsmann 1985). For instance, by cutting the neck connectives afferent input from the wind hairs on the head was abolished. Although these hairs form part of the flight oscillator (Bacon and Möhl 1983) no changes in the flight motor pattern could be observed on the level of our analysis. Following deafferentation the meso- and metathoracic ganglia when isolated together were still able to generate rhythmic motor activity (Figs. 2 and 3), with the depolarizations in depressors but not in elevators resembling those in the intact animal. Thus the two pterothoracic ganglia, when connected, certainly contain a strong central oscillator which is capable of generating oscillations with some features being similar to those observed in the intact flight pattern. In contrast, the rhythmic activity observed in the mesothorax when the pro- and mesothoracic ganglia together were isolated from the rest of the ventral nerve cord bore little resemblance to the intact flight motor pattern. Almost all specific characteristics of these two patterns were different, except the alternating activity in depressor and elevator motoneurons (see Fig. 5 upper). As far as it can be judged from the few weak oscillations observed in the isolated mesothoracic ganglion, the same holds true for this preparation (see Fig. 8 upper). Finally, the metathoracic ganglion, following the transection of the connectives to the mesothorax, did not show any rhythmic motor activity in the present experiments even with afferents intact (see Fig. 8 lower).

The large changes in the pattern of motor activity in meso- and metathoracic neurons following severance of the meso-metathoracic connectives does not provide support for the hypothesis of independent segmental oscillators, each capable of generating a more or less correct motor program for the segment and the individual oscillators being coupled in the intact animal to produce the metachronous flight pattern. For example, the oscillations which were observed in the mesothorax when the meso-metathoracic connectives were cut or when the pro- and mesothoracic ganglia together were isolated from the remaining ventral nerve cord (Figs. 5 top, 6, and 7) were dissimilar from the intact motor pattern to such an extent that they could be interpreted as the residual activity of a fragment of a distributed oscillator network. These oscillations were weak, extremely variable and could not be sustained for long periods. In addition, the isolated metathoracic segment was never observed to generate rhythmic motor activity. Thus the neuronal networks in the isolated meso- and metathoracic segments do not appear
to be homologous with regard to their ability of generating flight motor oscillations. This difference in the oscillatory capacity of the two segments is consistent with the finding that little homology exists among the flight interneurons of the meso- and metathoracic ganglia (Robertson and Pearson 1984). Nevertheless, within each ganglion a basic mutual inhibition between elevator and depressor neurons may exist which accounts for the reciprocal pattern of activity (even when this activity is not rhythmic). For the metathoracic ganglion this may provide the basis for the generation of rudimentary oscillations but is clearly insufficient for the production of the correct physiological motor pattern. The capacity of fragments of complex oscillators to oscillate has been demonstrated in other systems such as the pyloric oscillator in the lobster stomatogastric ganglion (Miller and Selverston 1982).

It is a noteworthy observation that the generation of rhythmic motor activity by the mesothoracic ganglia after transection of the meso-metathoracic connectives was dependent on the presence of wing afferents (Fig. 5), as were the oscillations produced by the pro- and mesothoracic ganglia when isolated together from the rest of the ventral cord. Sensory feedback thus appears as a constitutive element of the oscillator in the mesothoracic ganglion when disconnected from the metathoracic ganglion. This necessity of sensory feedback for the generation of motoneurone oscillations in, as we just concluded, a fragment of a distributed pattern generator is consistent with previous findings of the important role of wing sense organs in the generation of the intact flight pattern (Wendell 1983; Möhl 1985; Pearson 1985; Pearson et al. 1983; Wolf and Pearson 1987a).

In summary, the existence of a distributed flight oscillator, spanning at least the meso- and metathoracic ganglia (including the three fused abdominal neuromeres) is suggested by the observation that meso- and metathorax together, when isolated from the anterior ganglia, are still able to produce an almost normal motor pattern whereas this pattern changes dramatically after transection of the meso-metathoracic connectives. Even the altered motor activity generated by the deafferented nerve cord is much more reminiscent of the intact pattern than is the activity produced by the preparations with the meso-metathoracic connectives severed. Together with previous observations concerning the interneuronal organization of the flight oscillator we conclude that the central nervous portion of the locust flight oscillator is distributed throughout the thoracic and fused abdominal ganglia.

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References

Note added in proof. Recently we carried out an intracellular analysis of the rhythmic motor activity elicited in the isolated metameric ganglion by topical application of octopamine (see Stevenson & Kutsch 1987). The depolarizations observed in the flight motoneurons, and in particular the elevators, of this preparation differed significantly from the depolarizations recorded in locust with an intact nerve cord and from any of the patterns described above. This observation indicates that the octopamine-activated oscillator in the isolated metameric ganglion has different characteristics than the oscillator active in the mesothorax after transection of the meso-metathoracic connectives or the (distributed) oscillator active in the connected thoracic ganglia.