Patterned synaptic drive to locust flight motoneurons after hemisection of thoracic ganglia

Harald Wolf^{1*}, Bernhard Ronacher^{2**}, and Heinrich Reichert³

¹ Department of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7

² Department of Zoology II, Universität Erlangen-Nürnberg, D-8520 Erlangen, Federal Republic of Germany

³ Department of Animal Biology, Université de Genève, CH-1211 Genève, Switzerland

Accepted May 12, 1988

Summary. Intracellular recordings were carried out on locust flight motoneurons after hemisection of individual thoracic ganglia. With the exception of minimal surgical manipulations, the animals were intact and able to perform tethered flight. Analysis of the synaptic drive recorded in the motoneurons during flight motor activity revealed the extent to which ganglion hemisection influenced the premotor rhythm generating network.

1. Hemisection of the mesothoracic ganglion (Fig. 2) as well as hemisection of both the mesothoracic and the prothoracic ganglia (Fig. 3) had no significant effects on the pattern of synaptic input to the flight motoneurons. Thus the rhythm generating premotor network does not depend on commissural information transfer in the mesothoracic and the prothoracic ganglia. This conclusion was supported by experiments in which more extensive surgical isolations of thoracic ganglia were carried out (Fig. 5).

2. Removal of input from wing receptors (deafferentation) in addition to hemisection of the mesothoracic ganglion (Fig. 4) resulted in rhythmic and coordinated oscillations of the motoneuron membrane potential which were indistinguishable from those observed in deafferented animals with all ganglia intact.

3. Hemisection of the metathoracic ganglion had more pronounced effects on the patterns of synaptic drive to the flight motoneurons and their spike discharge. Rhythmic activity which was often subthreshold could, however, still be recorded following a metathoracic split (Fig. 6).

4. No rhythmic synaptic input was observed after hemisection of both mesothoracic and metathoracic ganglia (Fig. 7).

Introduction

In the locust flight control system the motoneurons are output elements and not part of the rhythm generating system (Robertson and Pearson 1982). Their activity is determined by a premotor interneuron network located predominantly in the mesothoracic and metathoracic ganglia and by direct sensory input (e.g. Burrows 1975; Pearson and Wolf 1988). Many elements of this premotor network have been studied using intracellular recording techniques and some of the connectivity of this rhythm generator network has been established (Robertson and Pearson 1983, 1985a, b).

This direct neurophysiological approach to the operation of a rhythm generator network is one of the most precise and informative methods of analysis. However, since it is often difficult to identify all the neurons in a given oscillatory circuit (Selverston 1980), a hypothesis concerning circuit organization may be founded on incomplete cellular data. Indeed for the central oscillator for locust flight, we have shown in the neurobehavioral analysis presented in the preceding report (Ronacher et al. 1988) that current models for oscillator circuit function are incomplete (Robertson and Pearson 1985a).

An indirect method of analyzing neuronal oscillators is the characterization of the oscillatory synaptic potentials in the motoneurons. The synaptic drive to the motoneurons reflects the input from *all* interneurons, appropriately weighted, and provides information on oscillator activity even if the potentials in the motoneurons remain subthreshold. In the locust flight system such an analysis is possible since (i) the motoneurons involved in flight behavior have been identified (Burrows 1973; Tyrer and Altman 1974; Hedwig and Pearson 1984) and since (ii) intracellular recordings

* Present address: Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany

** To whom offprint requests should be sent

from these motoneurons can be carried out in different preparations, ranging from almost intact to surgically reduced (Robertson and Pearson 1982; Wolf and Pearson 1987a, b; Stevenson and Kutsch 1987). In this report, we examine the oscillatory synaptic drive to motoneurons in locusts with hemisected thoracic ganglia. The results of this study have implications for understanding the organization of the pattern generating circuitry of locust flight.

Materials and methods

Adult *Locusta migratoria* (Acrididae) from a laboratory culture were employed for the experiments. Both males and females were used although females were preferred because of their large body size. Experiments were carried out at room temperature (ca. 23 °C). Hemisections of different thoracic ganglia or transections of connectives or thoracic nerves were carried out either 1 day prior to testing as described in the preceding report (Ronacher et al. 1988) or acutely during the preparation procedures for intracellular recording. Acutely operated animals were allowed to rest for 30 to 120 min prior to testing. No differences in experimental results were found between animals subjected to the two procedures.

Intracellular recordings were made from a variety of meso- and metathoracic motoneurons. Usually the tergosternal (M83/84, M113; Snodgrass 1935) and first basalar motoneurons (M97, M127; Snodgrass 1935) were impaled since these are monofunctional flight motoneurons. For intracellular recordings from the somata of flight motoneurons the animals were mounted ventral side up on a holder which did not impair the wing movement (Wolf and Pearson 1987c). The legs were removed and small holes cut into the ventral cuticle of the thorax in order to expose the thoracic ganglia. Care was taken to leave all apodemes and muscle attachment sites intact. Peripheral nerves of the thoracic ganglia were accessible in this preparation and could be transected if desired. In particular, deafferentation with respect to the wing sense organs (tegulae, stretch receptors, campaniform sensilla and wing hairs) was achieved by cutting meso- and metathoracic nerves 1 distal to the point where the nerves 6 of pro- and mesothoracic ganglion branch off (see Fig. 1 B, diagram on left). This nerve contains all afferents from the wing and the wing base. For intracellular recordings from the neuropil of motoneurons (Fig. 5B) the animals were mounted dorsal side up. The thoracic ganglia were exposed by removing the overlying gut and leg muscles. Nerves 3, 4, and 5 of the mesothoracic and metathoracic ganglia, which innervate the direct flight muscles were cut in order to reduce movement artifacts. If desired, other peripheral nerves and/or the intersegmental connectives could be severed. The soma recordings were very similar to the recordings from neuropil regions, except for the smaller amplitude of the action potentials. A stainless steel spoon was used as support in order to stabilize ganglia for intracellular recording. Recordings were carried out with 40–80 M Ω glass microelectrodes. Microelectrodes were filled with a 5–7% aqueous solution of Lucifer yellow in order to allow the morphological identification of the recorded motoneurons. Following physiological characterization of the neurons, the ganglia were fixed in 4% paraformaldehyde, dehydrated and cleared in methyl salicylate. Stained neurons were drawn from wholemounts of the thoracic nerve cord using an epifluorescence microscope immediately following this procedure.

Electromyograms of wing depressor muscles were recorded along with intracellular activity of selected motoneurons. The electromyograms were usually taken from the first basalar muscles by inserting electrodes into prepared holes in the cuticle above the muscle (Pearson and Wolf 1987). Electromyogram recording electrodes consisted of 100 μ m copper wires insulated except for the tips. Recordings were amplified conventionally and stored on magnetic tape.

Flight motor activity was usually initiated by a wind puff directed onto the animal's head. In cases where an anterior pair of connectives had been cut, flight could be initiated by brief tactile stimulation of the pleurae, ears or cerci. In certain cases (Fig. 5B), flight motor activity was induced by bathing the thoracic cavity in a 0.1 M solution of octopamine in locust saline (see Stevenson and Kutsch 1987).

Results

Synaptic drive to flight motoneurons in animals with intact thoracic ganglia

The activity of flight motoneurons in animals with an intact nervous system has been described previously. The effects of transecting connectives in different parts of the ventral nervous system as well as different types of deafferentation have also been studied and evaluated in detail (Hedwig and Pearson 1984; Wolf and Pearson 1987a, b; Stevenson and Kutsch 1987). For reference, a typical example of the intracellularly recorded flight motor activity of a depressor and an elevator motoneuron in an intact animal is shown in Fig. 1 A. Recordings from the same motoneurons but after deafferentation of the animal are shown in Fig. 1 B.

Some of the distinctive features of the motoneuron depolarizations will be briefly described. In the intact locust, both elevator and depressor motoneuron depolarizations were usually short and characterized by a steep rise and decay. Toward lower wingbeat frequencies it was predominantly the duration of the elevator depolarizations that was increased (right part of Fig. 1 A). The characteristic steep rising slope was maintaining and the elevator depolarizations were prolonged by a shoulder or a separate small depolarization inserted into the repolarization phase (arrows in Fig. 1 A). The depressor depolarizations were increasingly spaced out with decreasing wingbeat frequency but there were only minor changes regarding the duration or their rise and decay slopes.

Following deafferentation the depolarizations recorded in depressor motoneurons looked much like those observed in the intact locust (cf. Fig. 1 B left and 1 A right). In contrast, the shape of the elevator depolarizations was changed substantially by deafferentation. The constant, steep rising phase disappeared and instead the elevator depo-

Fig. 1. Flight motoneuron depolarizations recorded in an intact locust (A) and following deafferentation with respect to the wing proprioceptors (B). Simultaneous intracellular recordings were taken from the somata of a wing elevator (top traces, tergosternal, E113) and a wing depressor (middle traces, first basalar, D127) motoneuron in the metathoracic ganglion. The electromyogram (bottom traces) was recorded from the contralateral metathoracic first basalar muscle. In order to illustrate some distinctive features of the depolarizations, episodes recorded at two different wingbeat frequencies are shown. (Indicated by arrows: shoulder and prolonged depolarization in repolarization phase and shoulder in rising phase of the elevator depolarizations recorded in intact and deafferented animals; details in text). Schematic diagrams of the thoracic nerve cord are given on the left (from top: pro-, meso-, and metathoracic ganglion). Filled circles in this and the following figures indicate the recording sites; transections of nerves or connectives are symbolized by solid bars. *E* elevator motoneuron; *D* depressor motoneuron. Scale bars in this and all following figures 10 mV and 100 ms, except in Fig. 5B where they represent 10 mV and 400 ms

larizations commenced gradually and the rising slope was typically interrupted by a shoulder (arrows in Fig. 1B). Furthermore, the rising slope decreased with decreasing wingbeat frequency (right part of Fig. 1B). The decay slope was much less affected by changes in wingbeat frequency. In summary, the elevator depolarizations were more affected by deafferentation and changes in cycle duration. For this reason we focus mainly on elevator motoneurons in the following description.

Synaptic drive to flight motoneurons in animals with hemisected mesothoracic ganglia

Hemisection of the mesothoracic ganglion had little if any effects on the flight behavior and the timing of flight muscle activity (see Ronacher et al. 1988). To investigate if this was due to an unaltered synaptic input to the flight motoneurons, intracellular recordings were carried out on mesothoracic and metathoracic motoneurons in 15 animals with hemisected mesothoracic ganglia. In all cases, a depolarization pattern was observed in both mesothoracic and metathoracic motoneurons that was

similar to the synaptic drive recorded in animals with intact thoracic ganglia (Fig. 2). In these recordings the shape of the depolarizations, their temporal characteristics and the number of spikes produced per cycle were comparable to those in intact animals. In particular, the characteristic, steep rising slope of the elevator depolarizations was observed as was the appearance of a shoulder or a small separate depolarization in the repolarization phase at lower wingbeat frequencies (arrows in Fig. 2, lower left).

Given that these depolarizations reflect the input the motoneurons receive from the premotor interneuron network (and from sensory afferents), this indicates that the premotor network is able to produce a normal motor output after the elimination of commissural information transfer in the mesothoracic ganglion. If the mesothoracic commissural pathways are dispensable, where are the essential elements for the generation of the depolarization pattern localized? Can a set of necessary elements be defined unequivocally at all? Candidates for centers of pattern generation are the two remaining thoracic ganglia, the prothoracic and

Fig. 2. Depolarization patterns recorded in meso- (top records) and metathoracic (bottom records) motoneurons after hemisection of the mesothoracic ganglion. Elevators (anterior tergoxal E89 and tergoxal E113) are on the left and depressors (first basalars D97 and D127) are on the right hand side. Electromyograms are from the metathoracic first basalar muscles. Lower left two sections recorded at different wingbeat frequencies; note prolonged elevator depolarizations (arrows, compare Fig. 1 A, upper right)

Fig. 3. Depolarization patterns recorded in a mesothoracic elevator motoneuron (top record, tergoxal E83/4) and in a metathoracic elevator motoneuron (bottom record, anterior tergoxal E118) after hemisection of the pro- and mesothoracic ganglia. Electromyograms are from the first basalar muscle in the upper and from the tergoxal muscle in the lower recording. Smaller electromyogram potentials from the first basalar (marked by small arrows) are also apparent in the lower recording but were not generated in each wingbeat cycle. Note low-amplitude depolarizations at the beginning of the upper record and shoulders in the repolarization phase in the lower record (large arrows, compare Fig. 1 A, upper right)

Fig. 4. Activity of an elevator motoneuron (mesothoracic tergoxal E83/4) after hemisection of the mesothoracic ganglion and additional deafferentation. The electromyogram was recorded from the mesothoracic first basalar muscle

the metathoracic ganglia. Also, since afferent input from wing receptors is important for determining the depolarization pattern in elevator motoneurons (Wolf and Pearson 1988), sensory feedback might be crucial for maintaining a normal depolarization pattern in the motoneurons after hemisection of the mesothoracic ganglion. These possibilities were investigated in the following experiments.

Hemisection of the prothoracic ganglion in addition to hemisection of the mesothoracic ganglion did not affect the salient characteristics of premotor drive to mesothoracic motoneurons (5 animals tested; Fig. 3). Again, the characteristic steeply rising slope of membrane depolarizations was observed at all wingbeat frequencies and the shoulder in the repolarization phase prolonged the elevator depolarisations at lower wingbeat frequencies. However, the motoneuron activity was much more variable and sometimes small and gradually developing elevator depolarizations were recorded (e.g. first depolarization in upper record of Fig. 3). Such depolarizations were not observed in intact, tethered flying locusts.

Removal of all afferent input from the sensory structures associated with the wings (deafferentation) in locusts with hemisected mesothoracic ganglia (13 animals tested) resulted in a synaptic drive to the motoneurons which was similar to that of deafferented animals with intact thoracic ganglia (Fig. 4). In elevator motoneurons gradually rising depolarizations could be observed, the rising slope being interrupted by a shoulder. The wingbeat frequency was decreased following deafferentation to values around 10 Hz. This pattern of elevator activity is characteristic of deafferented locusts with an intact nerve cord (compare Fig. 1 B, upper right). The only notable difference in comparison

Fig. 5. **A** Activity in an elevator motoneuron (mesothoracic tergosternal E83/4) after hemisection of the mesothoracic ganglion, deafferentation, and transection of the pro-mesothoracic connectives. Electromyogram recorded from the contralateral mesothoracic first basalar muscle (D97). **B** Oscillatory activity of an elevator motoneuron in a completely isolated mesothoracic hemiganglion after application of octopamine. (Although the elevator motoneuron was filled with dye and on structural criteria was probably E89, an unambiguous identification based on morphology alone was not possible; cf. Hedwig and Pearson 1984)

to locusts with an intact ventral nerve cord was that during the period preceding flight initiation, unusual periods of tonic depolarizing drive to motoneurons were seen in deafferented animals with hemisected mesothoracic ganglia. Once flight was initiated this drive was, however, replaced by regular phasic oscillations (cf. Ronacher et al. 1988; Fig. 5). Thus sensory feedback, although necessary for the generation of the normal depolarization pattern, does not account for the fact that the flight motor pattern survives hemisection of the mesothoracic ganglion.

These results obtained by intracellular recordings on acutely operated animals agree well with the electromyogram patterns recorded in animals after long term recovery from surgery (see Ronacher et al. 1988). In all cases, hemisection of the mesothoracic ganglion affected neither the timing of motoneuron discharge nor the shape and time course of the synaptic drive to the flight motoneurons. In the experiments presented in Fig. 5 more

extensive surgical manipulations were carried out on animals with hemisected mesothoracic ganglia in acutely operated preparations. In contrast to the experiments described above it was not possible to study the effects of these more extensive manipulations on animals after recovery from surgery in behavioral experiments or electromyogram studies.

In a first set of experiments (7 animals tested), both connectives between the prothoracic and mesothoracic ganglion were severed and a complete deafferentation was carried out. This effectively isolated the pterothoracic ganglia from the anterior nervous system and from proprioceptive feedback from the wing receptors. In this preparation flight motor activity was evoked with more difficulty than in animals which had their connectives left intact. Despite hemisection of the mesothoracic ganglion, synaptic drive patterns reminiscent of the depolarizations recorded in deafferented preparations could be generated by the animal (Fig. 5A). Again, the elevator depolarizations showed the gradual rising slope typical of the deafferented situation (compare Fig. 1B, upper right). It is noteworthy that the motoneuron activities in the right and left halves of the hemisected mesothoracic ganglion were correctly coordinated; the intracellular elevator recording was obtained in one and the electromyogram in the other half of the ganglion. With the prothoracic-mesothoracic connectives being severed bilaterally the two halves of the ganglion were connected only via the metathorax.

In a different set of preparations (3 animals tested), intracellular motoneuron recordings were carried out in a completely isolated half of a mesothoracic ganglion: both ascending and descending ipsilateral connectives as well as all peripheral nerves had been transected. In such preparations no sign of rhythmic activity was seen under normal conditions. Since bath application of a 0.1 M solution of octopamine has been shown to release rhythmic flight motor activity in surgically reduced preparations (Stevenson and Kutsch 1987), we also applied octopamine to such an isolated hemiganglion preparation. As a result, oscillatory synaptic drive to flight motoneurons was occasionally observed (Fig. 5B). These oscillations were supra-threshold and caused bursts of action potentials. However, such oscillations had extremely long cycle durations (500 ms) and showed none of the characteristic features described for the depolarizations seen in flight motoneurons of intact animals. Thus it is doubtful whether these oscillations represent flight related activity.

Fig. 6 A, B. Depolarization patterns in motoneurons after hemisection of the metathoracic ganglion. **A** Activity recorded in a mesothoracic elevator motoneuron (top records, tergosternal E83/4) and in a mesothoracic depressor motoneuron (bottom records, first basalar D97). **B** Activity in a metathoracic depressor motoneuron (first basalar D127). In **A** and **B** electromyograms were recorded from the mesothoracic first basalar muscles but smaller potentials from the adjacent tergosternal muscles are also apparent. Note the variability of the elevator depolarizations and the subthreshold oscillations in membrane potential of the mesothoracic depressor (single action potentials are marked by arrows in **A** and **B**)

Synaptic drive to flight motoneurons in animals with hemisected metathoracic ganglia

In contrast to hemisections of the mesothoracic ganglion, hemisection of the metathoracic ganglion had more pronounced effects on the pattern of synaptic drive to flight motoneurons and their spike discharge (12 animals tested). This was true for the potentials recorded in both meso- and metathoracic ganglia. The elevator depolarizations recorded in the mesothoracic ganglion were sometimes quite similar to the depolarizations recorded in intact locusts (Fig. 6A, top left). They showed the characteristic, steep initial depolarization and at lower wingbeat frequencies a shoulder in the repolarization phase. At other times the depolarizations were reminiscent of the deafferented situation in that the rising slope was gradual and variable (Fig. 6A, top right). It should be noted as a consistent observation that the depressor motoneurons frequently showed only subthreshold oscillations in membrane potential (Fig. 6A, bottom, see also electromyograms in Fig. 6).

In the metathoracic motoneurons the depolarizations usually appeared quite irregular and were of small amplitude. In particular, the characteristic

rising or repolarization periods were not observed. For instance, the beginning of a depolarization phase could be interrupted by a small hyperpolarization (arrowhead in Fig. 6) and the depolarizations often remained completely below the threshold for action potential generation. Consequently spikes, if generated, were much less precisely coordinated with the discharges of other motoneurons than in the intact locust (see Ronacher et al. 1988). Figure 6B gives an example of a relatively well structured motoneuron activity.

If in addition to hemisection of the metathoracic ganglion, one of the connectives between mesothoracic ganglion and metathoracic ganglion was severed (9 animals tested), rhythmic activity could still be recorded in mesothoracic flight motoneurons and the depolarization pattern observed in elevator motoneurons could be reminiscent of the pattern recorded in intact locusts. This was true even for mesothoracic motoneurons ipsilateral to the site of connective transection (Fig. 7A; compare with Fig. 1 upper right). It is noteworthy that the depolarizations recorded in mesothoracic motoneurons ipsilateral to the severed connective were always more pronounced and had a more regular shape than the depolarizations recorded in

Fig. 7. **A** Elevator motoneuron activity (anterior tergocoxal E89) in the mesothoracic ganglion after hemisection of the metathoracic ganglion and additional transection of the ipsilateral mesothoracic-metathoracic connective. Electromyogram was recorded from the mesothoracic tergosternal muscle (E83/4) contralateral to the severed connective. **B** Activity in mesothoracic elevator motoneurons after hemisection of the meso- and metathoracic ganglia (tergosternal E83/4 in upper traces, anterior tergocoxal E89 in lower traces). The spikes in the electromyogram recordings are from the contralateral tergosternal muscle (E83/4)

the motoneurons in an intact mesothoracic ganglion after the transection of both meso-metathoracic connectives (see Wolf and Pearson 1987b). This indicates that in this type of preparation the metathoracic hemiganglion still contributes to pattern generation.

No rhythmic oscillations in synaptic drive to motoneurons were seen if both metathoracic and mesothoracic ganglia were hemisected. The firing of motoneurons under these conditions was due to a long lasting tonic depolarization (Fig. 7B). We have not been able to elicit rhythmic motor activity in completely isolated metathoracic hemiganglia even with the application of octopamine, in contrast to the situation described in Fig. 5B for the mesothoracic ganglion.

Discussion

The degree to which the neuronal oscillator for locust flight can survive the hemisection of the

thoracic ganglia, the resulting disruption of the ganglion sheath and loss of ionic regulation, the destruction of commissural structures and midline neuropil and the transection of many of its component interneurons is remarkable. It is equally remarkable that a similar resistance to ganglion hemisection apparently applies to other motor systems of the locust such as walking, jumping and kicking. We do not know what the cellular effects of the transection of the interneurons are. It is possible, that both halves of a transected neuron can continue to function for days after separation (e.g. Hoy et al. 1967; Clark 1976a, b; Krasne and Lee 1977). It is clear, however, that information transfer in such neurons is disrupted.

The fact that hemisection of the mesothoracic ganglion leaves the characteristics of the membrane potential oscillations in the flight motoneurons virtually unaffected means that the synaptic drive to the motoneurons is very much like in the intact locust. This implies that the premotor (interneuronal) network is still functional after these surgical manipulations. This finding is relevant for concepts concerning the organization of the neural oscillator for flight. Here we consider the implications of two (not necessarily mutually exclusive) interpretations of our results.

(i) The commissural pathways of the mesothoracic ganglion (and to a certain degree, those of the metathoracic ganglion) are relatively unimportant for rhythm generation because the central oscillator is composed of hemisegmental subunits. In this case, coordination of the rhythm production would depend on coupling of the hemisegmental subunits by a distributed *coordinating* network. The coupling would be primarily central, since elimination of proprioceptive feedback does not abolish correct coordination. This type of organization would be analogous to many other cases in which rhythmic behaviors involve movements of a number of serially homologous appendages or body segments (Stein 1978; Pearce and Friesen 1985; Grillner and Wallen 1985; Paul and Muloney 1986).

Potential candidates for the postulated, distributed coupling network are interneurons with reset properties (26 identified to date by Robertson and Pearson 1983, 1985a, and unpublished). If these interneurons coordinate the oscillations produced by hemisegmental premotor networks they should be able to influence the timing of the oscillations in one subunit according to the input they receive either directly or via external feedback loops from the other subunits. This effect would be compatible with the reported reset characteristics of these in-

terneurons. These neurons would be considered members of the locust flight oscillator by virtue of their reset properties even if their function is mainly a coordinative one.

It is interesting that most of the mentioned interneurons with reset properties have their integrative segments and somata in the metathoracic ganglion (see Fig. 2 in Ronacher et al. 1988). This may be relevant to the finding that the oscillatory activity in the two separated halves of the mesothoracic ganglion can be coordinated via the metathoracic ganglion. This is especially clear when the connectives to the prothoracic ganglion have been severed in addition to mesothoracic hemisection as was the case in the example given in Fig. 5A. In contrast, following hemisection of the metathoracic ganglion, the recorded flight motor activity was much less regular and well coordinated, especially as concerns the metathoracic motor units.

(ii) The central oscillatory circuit is a distributed network. However, this network is redundantly organized in the sense that it is composed of numerous mutually reinforcing interneurons, which share similar or identical functions and interconnect the pterothoracic ganglia through different pathways. In this case, ganglion hemisection would impair the function of only a small subset of these interneurons. The overall shape of the synaptic potentials caused by the ensemble of interneurons left unimpaired would still resemble that produced by the intact oscillator system. This is because in a redundantly organized system the drop-out of some elements may cause only small changes in overall systems function (Pavlidis 1973). There is some evidence for this type of organization since families of flight interneurons with similar but not identical physiological and anatomical properties have been found (Robertson and Pearson 1983). It is not known, however, how large these families are or to what extent the branching patterns of the interneurons in one family can differ.

When comparing the effects of hemisecting the meso- and the metathoracic ganglia it appears that the metathoracic ganglion has a greater overall importance for pattern generation than the mesothoracic ganglion. Hemisection of the metathoracic ganglion always produced more severe effects than mesothoracic hemisection. We do not know if this is due to the fact that four neuromeres are involved in the metathoracic ganglion mass or if the metathoracic neuromere incorporates a greater share of the oscillator circuitry than does the mesothoracic ganglion. Although an isolated metathoracic ganglion can produce rhythmic motor activity if

treated with octopamine (Stevenson and Kutsch 1987), the metathoracic ganglion is not the exclusive site of rhythm generation, since rhythm generation persists after the functional elimination of this ganglion (e.g. Wolf and Pearson 1987b).

To decide between the two organization principles mentioned above, further cellular studies must now follow. As our experiments show, current tentative models for flight oscillator function are incomplete. Furthermore, the input and output connections of many of the identified flight interneurons have not yet been established. The role of families of similar flight interneurons remains unclear. Moreover, it appears likely that local interactions have not been adequately incorporated. The oscillator circuit (Robertson and Pearson 1985a, b) has a conspicuous lack of the local interneurons seen in other motor systems of the locust (Burrows and Siegler 1983). This might be because local interneurons are often small and difficult to study and successful intracellular recordings may have been biased towards the larger intersegmental interneurons. Finally the possibility of intersegmental interneurons having distributed input and output sites and thus participating in intrasegmental interactions remains to be investigated.

Acknowledgements. We thank K.G. Pearson for helpful comments on the manuscript. This work was supported by grants from the Alberta Heritage Foundation for Medical Research (to H.W.) and the Schweizerische Nationalfonds (to H.R.).

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