Locust flight behavior after hemisection of individual thoracic ganglia: evidence for hemiganglionic premotor centers

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Summary. The flight behavior of locusts with hemisected mesothoracic or metathoracic ganglia was observed in unrestrained animals and monitored electromyographically in tethered animals. Animals with hemisected mesothoracic ganglia were able to initiate and carry out free flight. Hemisection of the mesothoracic ganglion caused no significant changes in the pattern of flight muscle firing; both intra- and intersegmental coordination of flight muscle activity were retained (Figs. 3, 4). Additional transection of one meso-metathoracic connective altered the pattern of flight muscle firing but did not abolish rhythmic activity (Fig. 8). Deafferentation of the thoracic ganglia in animals with hemisected mesothoracic ganglia resulted in rhythmically coordinated motor activity (Fig. 5) which was indistinguishable from that shown by deafferented animals with all ganglia intact. Hemisection of the metathoracic ganglion resulted in an abnormal pattern of flight muscle firing. However, a basic rhythmicity of motor activity was still present (Fig. 6). The implications of these results for rhythm generation and motor coordination in the flight control system of the locust are discussed.

Introduction

Locomotor control in arthropods involves multiple interacting neural mechanisms. Central circuits are involved in generating rhythmic motor output (e.g. Delcomyn 1980). Proprioceptive feedback interacts with and stabilizes the operation of these central circuits (Wendler 1974, 1983; Pearson 1985). Exteroceptive sensory control circuits enable the proper orientation of the locomoting animal in its

environment (Reichert and Rowell 1985). Since arthropod locomotion usually involves multiple segmental appendages such as legs or wings, additional control circuits are needed to coordinate the action of all of these segmentally homologous appendages into an adaptive motor act (e.g. Bush and Clarac 1985). Since all of these control circuits interact, an analysis of any individual control network is difficult.

In locust flight, a classical system for investigations of rhythmic locomotor control (Wilson 1961), the movements of the four wings are controlled by four homologous sets of flight muscles and motoneurons (Snodgrass 1935; Tyrer and Altman 1974). The bilateral and segmentally homologous organization of the effector and motoneuron systems would seem to predict a hemiganglionic organization of basic premotor control circuitry. This would correspond to the unit central pattern generator concept developed in studies on other invertebrate and vertebrate locomotor control systems (Grillner 1985; Weeks 1981; Paul and Mullen 1986). However, in cellular analyses of the neuronal oscillator for locust flight no evidence for hemiganglionic premotor centers has as yet been found. Rather, a distributed oscillator system composed of intersegmental interneurons in the three thoracic and at least three abdominal ganglia has been postulated (Robertson and Pearson 1982, 1983, 1984, 1985a, b; but see Stevenson and Kutsch 1987).

As a first step towards uncovering a possible hemiganglionic organization in the locust flight control system, we studied the operation of the flight system in animals in which individual thoracic ganglia had been surgically hemisected (cf. Huber 1960; Ronacher 1987). In this report we show that the normal flight behavior in such animals can be retained to a remarkable degree. As is shown in the following report, this is also true

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Fig. 1. Limited extent of degeneration following ganglion hemisection. Transverse section of a previously hemisected mesothoracic ganglion at the level of Nv5. The control preparation was carried out at the 9th day after hemisection. Schematic on right indicates major tracts (after Tyrer and Gregory 1982). *MDT* median dorsal tract; *LDT* lateral dorsal tract; *DIT* dorsal intermediate tract; *VIT* ventral intermediate tract; *MVT* median ventral tract; *VMT* ventral median tract; *Nv5* root of nerve 5

for the characteristics of premotor drive to the flight motoneurons. Since ganglion hemisection impairs the function of many of the intersegmental oscillator interneurons believed to be responsible for the generation of the flight rhythm, we think that a reconsideration of present models for flight pattern generation in the locust is warranted.

Material and methods

Adult males and females of *Locusta migratoria* or of *Schistocerca gregaria* (Acrididae) were obtained from a commercial supplier. No obvious differences in experimental results were seen between the two species. Stridulatory behavior was investigated in detail with another acridid, *Chorthippus biguttulus* (Ronacher 1987). Hemisections were carried out either on the mesothoracic ganglion or on the metathoracic ganglion or on both. Hemisection of the metathoracic ganglion also involved hemisection of the first three abdominal ganglia, which are fused onto the metathoracic ganglion. In some experiments, sensory nerves and/or thoracic connectives were cut as well. Transection of mesothoracic nerves 1 eliminated proprioceptive input from the tegulae, the campaniform sensilla and the windhairs of the mesothoracic wings. Additional transection of mesothoracic nerves 6 and metathoracic nerves 1 abolished all direct sensory input from wing proprioceptors including the wing stretch receptors.

Prior to surgery, animals were anaesthetized by cooling or brief exposure to CO₂. The thorax was then opened from the ventral side and the desired thoracic ganglia exposed. A ganglion was hemisected mediosagittally with a razor blade. Care was taken to minimize damage to the tracheal system. Completeness of the transection was inspected immediately by carefully separating the two halves of the transected ganglia until the ventral aspect of the gut could be seen. The excised piece of cuticle was then replaced and attached with wax colophonium.

After recovery from surgery the overall motor behavior of the animal was noted. At times ranging from 3 h to 15 days after the operation, the animal's flight behavior was tested in two ways. In the first test the animals were allowed to escape freely into a large room and their flight trajectory was observed. In the second test the animals were tethered and electromyograms from meso- and metathoracic flight muscles were recorded. For this, thin steel wires (40 µm diameter, with lacquer coating except at the tip) were inserted into flight muscles of the fore- and hindwings. Flight was elicited in tethered animals by loss of tarsal contact and by wind directed toward the head from a 1 cm diameter tube located at a distance of 2–3 cm or by light on/off stimuli. In some cases animals flew more regularly without application of wind. During flight behavior, muscle potentials were monitored with conventional amplification and recording techniques. Data evaluation was performed with a Nova 4X computer after digitalization by a spike detector (Zarnack and Möhl 1977).

The localization of the recording electrodes as well as the quality of the surgical hemisection were confirmed by postmortem inspection. In all cases tested the hemisection of the ganglia remained complete. Hemisected ganglia retained an intact overall appearance (Fig 1). Only minor signs of neuronal degeneration in the midline neuropil caused by surgical intervention were observed. Loose connective tissue arising from the ganglion sheath often superficially rejoined the two ganglion halves. However, upon removal of this connective tissue covering, the two ganglion halves separated with no linking interconnections.

Results

Flight behavior after hemisection of the mesothoracic ganglion

Most of the mesothoracic motoneurons are hemisegmental in their structural organization and are thus not affected by ganglion hemisection. This

Fig. 2. Morphology of selected flight interneurons according to Robertson and Pearson (1983, 1985a, b, and unpublished). Mesothoracic and metathoracic ganglia are shown. 301, 401, 501 and 504 are interneurons with reset properties; 401, 501, 504, and 503 are interneurons that have segmental homologues in the metathoracic and the first three abdominal ganglia

is different for most of the known interneurons of the flight control system. To date, 85 different types of thoracic flight interneurons have been identified (Robertson and Pearson 1982, 1983, 1985a, b, and unpublished data; Reichert 1985; Reichert and Rowell 1985, and unpublished data). The structure of some of these interneurons is shown in Fig. 2. Each interneuron type is representative of at least two individual cells since bilateral homologues exist. In some cases an interneuron type is representative of four or more individual cells since homologues within a given hemiganglion also exist. Of these interneuron types, 46 are mesothoracic and of these 37 have extensive bilateral dendritic arbors in the mesothoracic ganglion. Hemisection of the mesothoracic ganglion is thus likely to impair the functioning of roughly one half of the known interneuron types which comprise the flight control system.

Hemisection of the mesothoracic ganglion was carried out on 57 animals. Approximately one quarter of the operated individuals did not show flight motor activity. The rest did. Among these were individuals which were able to walk, jump, kick, stridulate and fly in a manner which was indistinguishable from unoperated controls. In walking behavior the coordination of all six legs and especially the movements carried out by the mesothoracic legs appeared normal. Individual an-

imals could jump, initiate flight and fly through the room. Some individuals were able to steer through curves, to avoid obstacles and to land softly.

Tethered animals usually produced bouts of flight activity of up to 1 min duration. One individual flew uninterrupted for over 12 min. The mean wingbeat frequency ranged between 17.8 and 23.2 Hz (at 27–33 °C for 8 different animals tested) and was thus similar to that observed in intact animals. The coordination between forewing and hindwing muscles on any one side of the locust and the coordination between muscles on opposite sides of the mesothoracic and metathoracic segments was normal (Figs. 3, 4), as was the coordination between elevator and depressor muscles. As in intact animals, the different depressor muscles did not fire in perfect synchrony. In the flight sequence presented in Fig. 3A the spikes of the right M129 lead those of the left M129 by approximately 2 ms, those of the left M97 by approximately 7 ms.

A quantitative analysis of the time intervals between the firing of several flight muscles is shown in Fig. 4. The results are consistent with those reported for the tethered flight of intact, unoperated animals (Möhl 1985; Pearson and Wolf 1987). In one case, the kinematics of wing movements were analysed with miniature coils mounted on the wings according to Zarnack (1981). They were the

Fig. 3A, B. Normal motor coordination persists following mesothoracic ganglion hemisection. Electromyograms from flight muscles in a tethered locust during flight activity. Recordings are from depressor muscles of the hindwing (M129) and forewing (M97). In some of the traces, activity of antagonistic elevator muscles is seen in attenuated form. In this and all subsequent figures 'l' and 'r' denote left and right side units. Schematic diagrams of the meso-, and metathoracic ganglion are given. Transections of nerves or connectives are symbolized by solid bars. **A** Animal with a hemisected mesothoracic ganglion. Experiment carried out 12 days after hemisection. **B** Intact animal

Fig. 4. A quantitative analysis of flight muscle firing times following hemisection of the mesothoracic ganglion reveals normal coordination. Temporal relationship of spikes in different flight muscles of an animal with a hemisected mesothoracic ganglion. The animal is the same as in Fig. 3A. M129 is a hindwing depressor, M97 is a forewing depressor, M119 is a hindwing elevator, M83 is a forewing elevator. The firing of M129r is used as temporal reference point (arrows) since this unit usually fired first in the wingbeat cycle. The asterisk in the uppermost diagram on the left indicates the mean period duration of 47.7 ± 3.9 ms. Histograms represent firing times of each muscle in reference to M129r (bin width 1 ms). 243 wingbeat cycles were evaluated. The electrode used to record the EMGs from muscle M97l partially inserted into muscle M83l and the electrode used to record the EMGs from muscle M129l partially inserted into muscle M119l (as confirmed by postmortem inspection) thus permitting the recording of the firing times of 4 muscles with only two extracellular electrodes. Temperature 31 °C. Error bars above histograms for M129l, M97l and M97r indicate the values obtained by Möhl (1985) for the firing times of these muscles in intact animals

same as seen in intact controls. The two abnormalities that were consistently observed in animals with a hemisected mesothoracic ganglion were a longer period of muscle activity associated with wing unfolding during flight initiation and a shorter average duration of flight activity. During ongoing

flight no significant differences in flight muscle activity were observed in animals with hemisected mesothoracic ganglia as compared to unoperated controls.

We did not observe a progressive deterioration of either the flight rhythm or the intersegmental

Fig. 5A–E. Persistence of patterned motor activity following mesothoracic ganglion hemisection is not due to a compensatory role of wing proprioceptors. **A** Electromyograms from depressor muscles in an animal with a hemisected mesothoracic ganglion and with both mesothoracic nerves N1 transected. M97 is a forewing depressor, M129 is a hindwing depressor. Scale bar 200 ms. **B** Electromyograms of depressor muscles in an animal with a hemisected mesothoracic ganglion and with both mesothoracic nerves N1, both mesothoracic nerves N6 and both metathoracic nerves N1 transected. M97 and M98 are forewing depressors, M129 is a hindwing depressor. Scale bar 200 ms. **C** Temporal relationship of spikes in different flight muscles of the same animal as in **B**. Histograms generated as in Fig. 4. **D** Dependence of intervals between elevator and de-

pressor firing (**E–D**) and of intervals between depressor and elevator firing (**D–E**) on wingbeat cycle length. Data from the same animal as in **B**. The top part shows data (152 wingbeat cycles) from a mesothoracic depressor (M98l) and a mesothoracic elevator (M103l). The bottom part shows data (207 wingbeat cycles) from a metathoracic depressor (M129r) and a metathoracic elevator (M119r). Intervals were measured from the first elevator (depressor) spike to the first depressor (elevator) spike in each wingbeat cycle. Error bars: standard deviations. **E** Electromyograms from depressor muscles in the same animal as in **B** taken during the initial period of flight activity. Arrow indicates beginning of wind stimulation. At onset of flight activity a transient period of abnormally long bursts of flight muscle firing occurs. Scale bar 200 ms

coordination in animals with a hemisected mesothoracic ganglion. Animals were able to fly freely as early as 2–3 h after the operation and normal rhythms and coordination of depressor muscles were recorded between 3 h and 15 days after the operation.

Effects of deafferentation on animals with hemisected mesothoracic ganglia

Sensory feedback from numerous wing proprioceptors is involved in the production of the locust flight motor pattern (Wilson and Gettrup 1963; Wendler 1974, 1983; Pearson et al. 1983; Wolf and Pearson 1987a, 1988). The persistence of an apparently normal flight pattern in animals with hemisected mesothoracic ganglia might thus be due to a synergistic and compensatory role of phasically active wing proprioceptors minimizing the effects of central nervous deficits. However, this does not seem to be the case. Elimination of proprioceptive input by transection of mesothoracic nerves 1 did not markedly perturb the coordination between the different wings (Fig. 5A). Animals retained the ability to perform free flight. Occasionally phase shifts in the firing of some of the flight muscles were seen and in some cases a reduction in the wingbeat frequency (range: 14.5–20.7 Hz) was observed. Additional transection of mesothoracic nerves 6 and metathoracic nerves 1 abolished all direct sensory input from wing proprioceptors to the mesothoracic and metathoracic ganglion. A flight motor pattern was produced which is characteristic of deafferented animals (Fig. 5B, C). The mean wingbeat frequency decreased to about 10 Hz and the interval between depressor firing and elevator firing was dependent on flight cycle duration (Fig. 5D). (For comparable data on intact animals see Pearson and Wolf 1987; Stevenson and Kutsch 1987.) Sometimes sequences of abnormally long bursts of flight muscle firing were observed (Fig. 5E). These bursts generally occurred during the flight initiation period. They were transitory and usually reverted spontaneously to the normal deafferented firing pattern. In other respects, the type of motor pattern observed under the conditions of deafferentation was the same regardless of whether the mesothoracic ganglion was hemisected or not.

Similar results were observed if segmental sensory nerves 1 and 6 in the meso- and metathoracic ganglia as well as segmental sensory nerves 6 in the prothoracic ganglion were transected (data not shown). Under such conditions, no known wing proprioceptive input to the central nervous system persists. Yet no obvious difference between the centrally generated rhythmic

activity in such deafferented animals with hemisected mesothoracic ganglia as compared to similarly deafferented animals with all ganglia intact was seen (cf. Wolf et al. 1988). To exclude the possibility of exteroceptively detected phasic activity resulting from the air currents generated by wingbeat in these animals (Horsmann 1985) the wings were removed as well. Once again, the resulting deafferented motor pattern was similar in animals with hemisected mesothoracic ganglia and in animals with all ganglia intact.

Flight behavior after transection of the metathoracic ganglion

The adult metathoracic ganglion is a mass composed of 4 embryologically discrete neuromeres, the metathoracic neuromere and the first three abdominal neuromeres. All four of these neuromeres contain important neurons of the flight control system. 39 of the to date identified flight interneuron types are found in these four ganglia. 34 of these have bilateral metathoracic arbors and are thus likely to be impaired by hemisection of the ganglion (Robertson and Pearson 1982, 1983, 1985a, b, and unpublished data; Reichert 1985; Reichert and Rowell 1985, and unpublished data; see also Fig. 2).

Hemisection of the metathoracic ganglion was carried out on 61 animals. The success rate of the surgical procedure was less than for mesothoracic hemisection. 6 animals died shortly after the operation. Among the surviving animals were individuals that walked, kicked and jumped using their metathoracic legs in a coordinated manner. 24 of the animals were able to beat both pairs of wings rhythmically. However, none of the animals was able to sustain free flight. Electromyograms from flight muscles during flight motor activity revealed a rhythmic activity of motoneurons in both hindwings and forewings at a mean frequency of approximately 10 Hz. However, this activity was more variable in its temporal organization and in its coordination than that in intact animals. The wingbeat activity of the front wings was generally less affected by ganglion hemisection than that of the hindwings. Wingbeat sequences with clear alternating firing of elevator and depressor muscles were observed (cf. Wolf et al. 1988; their Fig. 6). Yet even for the forewings, the wingbeat cycle duration often showed a high degree of temporal variation. For example, in one animal a standard deviation of ± 17 ms for the firing time of flight muscle 99 was observed.

In one and the same animal both relatively well coordinated sequences and poorly coordinated sequences of flight motor activity were seen. Poor sequences of flight activity were characterized by: (i) the failure of some of the flight muscles during

Fig. 6. Rhythmic activity is maintained but coordination is abnormal after hemisection of the metathoracic ganglion. Electromyograms from depressor muscles in an animal with a hemisected metathoracic ganglion. M97 is a mesothoracic depressor, M128 and M129 are metathoracic depressors. Experiment carried out 6 days after hemisection. Note the bursts in the recordings of M97r and M128l. Also note that M129 fires out of phase with the other depressors in these recordings. Such loss of coordination was often seen in animals with a hemisected metathoracic ganglion towards the end of a flight activity sequence. Scale bar 100 ms

Fig. 7. Apparent loss of rhythmic activity following hemisection of mesothoracic and metathoracic ganglia. Electromyograms from depressor muscles in an animal with both mesothoracic and metathoracic ganglia transected. M97 is a mesothoracic depressor, M129 is a metathoracic depressor. Scale bar: 500 ms. Arrow: onset of wind stimulation

several consecutive cycles, (ii) a tendency of elevator and especially depressor muscles to produce spike bursts, (iii) periods in which muscles on one side of the animal fired rhythmically whereas contralateral muscles discharged in a more tonic manner. Finally, in animals with hemisected metathoracic ganglia the initiation and termination phases of the flight motor activity were perturbed.

An example of the type of motor activity recorded in animals following metathoracic ganglion hemisection is shown in Fig. 6. Note that in this example the firing time of the right flight muscle 129 is phase shifted as compared to its normal firing pattern. This is one example of the type of variable motor output seen in such animals. However, it is not a necessary result of ganglion hemisection since in other animals with a hemisected metathoracic ganglion this type of phase shift did not occur.

In 22 animals both the metathoracic and the mesothoracic ganglia were hemisected. The success

rate of this surgical operation was low. Only 7 animals survived for more than 3 days. Some individuals were able to walk using all 6 legs, however, their walking posture was abnormal. None of the animals were able to beat their wings in a coordinated and rhythmical manner although occasionally a low amplitude wing 'shivering' was seen. In response to wind stimulation 3 animals retracted their legs to form the typical flight posture. None could perform free flight. Electromyograms from flight muscles revealed no clear rhythmic activity. However, from electromyograms alone the existence of remnants of a flight rhythm at about 10 Hz cannot be ruled out (Fig. 7).

Effects of connective transection on flight behavior

Transection of one of the connectives between mesothoracic and metathoracic ganglia carried out in animals with intact mesothoracic and metathoracic ganglia did not abolish free flight capability.

Fig. 8A, B. Transection of a mesothoracic-metathoracic connective in addition to hemisection of the mesothoracic ganglion alters coordination but does not abolish rhythmic motor activity. **A** Control experiment in which the left mesothoracic-metathoracic connective was transected in an animal with all ganglia intact. Electromyograms from depressor muscles are shown. **B** Electromyograms from depressor muscles in an animal with a hemisected mesothoracic ganglion and a transected right meso-metathoracic connective. M97 and M99 are forewing depressors, M128 and M129 are hindwing depressors. Scale bars 100 ms

In these animals motor coordination, amplitude of wingbeat movements and wingbeat frequency (16.2–21.6 Hz) were close to normal (Fig. 8A). This indicates that in an otherwise intact animal, sufficient descending and ascending information is carried in one of the connectives between mesothoracic and metathoracic ganglia for the expression of functional flight behavior.

Does transection of one of the connectives between mesothoracic and metathoracic ganglia impair the flight motor activity in animals in which a ganglion hemisection had been carried out? To investigate this, a transection of one of the connectives between mesothoracic and metathoracic ganglia and an additional hemisection of the mesothoracic ganglion was carried out in 32 animals. Although individuals were able to walk, kick and jump in a normally coordinated way, none were able to fly freely. The amplitude of wingbeat movements was usually reduced, especially ipsilateral to the transected connective. Electromyograms showed a more variable rhythmic activity (Fig. 8B). For example, the wingbeat periods as measured for 4 animals with hemisected mesothoracic ganglia and transected mesothoracic-metathoracic connectives were 59.5 ± 9.8 ms, 57.1 ± 9.2 ms, 50 ± 9.6 ms and 41 ± 7.5 ms. Moreover, the coordination between different muscles was often impaired. Standard deviations for the mean firing times of flight muscles in these animals were in the range of ± 10 ms to ± 13 ms. An increased tendency to fire tonically, notably in the muscles of the forewings, was also observed. However, the frequency of wingbeat activity (14–24 Hz) was fairly normal.

Thus, transection of a mesothoracic-metathoracic connective in addition to hemisection of the mesothoracic ganglion does not abolish rhythmic motor activity but does alter motor coordination. This suggests that information transfer in both of the connectives between mesothoracic and metathoracic ganglia contributes to the persistence of flight behavior after ganglion hemisection.

Discussion

A summary of the major findings presented in this paper is shown in Table 1. These results indicate a previously unexpected functional *independence* of hemisegmental motor centers involved in flight control. Although no quantitative analyses were carried out, similar results seem to apply to other motor behaviors like walking, jumping, kicking and stridulation. The most surprising finding is the fact that no obvious deficits in flight behavior were observed after hemisection of the mesothoracic ganglion. This has implications for our understanding of the mechanisms of rhythm generation and motor pattern coordination in the flight control system.

The persistence of a normal flight rhythm after hemisection of the mesothoracic ganglion

In the flight control system of the locust only very few cases of local interneurons (interneurons with all of their processes restricted to a given ganglion or hemiganglion) have been described (Reichert et al. 1985; Elson 1987) in contrast to other motor control systems in the locust and in other arthro-

Table 1. Survey of results after different surgical interferences (TG2 mesothoracic ganglion, TG3 metathoracic ganglion)

Type of operation	Free flight	Wingbeating in tethered animals	Wing beat frequency (Hz)	Coordination of wing muscles
Hemisect. TG2	Y	Y	17.8–23.2	Normal
Hemisect. TG2 + cut N1 TG2	Y	Y	14.5–20.7	More or less normal
Hemisect. TG2 + cut N1 TG2, N6 TG2, N1 TG3	No	Y	6.7–12.3	More or less normal
Hemisect. TG2 + cut N6TG1, N1TG2, N6TG2, N1TG3	No	Y	9–13	More or less normal
Hemisect. TG3	No	Y	9.2–14	Impaired
Hemisect. TG2 + TG3	No	No (only wing shivering)	–	–
Connective TG2/TG3 cut	Y	Y	16.2–21.6	Normal
Hemis. TG2 + Connective TG2/TG3 cut	No	(Y) (small amplitude)	14–24	Impaired

Pods (Burrows and Siegler 1983; Siegler 1984). The majority of the known premotor and oscillator interneurons for flight are intersegmental and have axons or major dendritic processes that pass through the commissural pathways (Robertson and Pearson 1982, 1983, 1985a, b; Reichert 1985; Reichert and Rowell 1985). These interneurons must be functionally impaired by ganglion hemisection.

Given that many flight interneurons can be functionally impaired without affecting the flight rhythm, a reinterpretation of current models for flight generation seems necessary. To date, the most extensive circuit diagram of interconnected premotor and oscillator interneurons (Robertson and Pearson 1985b) is composed of 6 identified mesothoracic interneurons and 13 identified metathoracic interneurons. 17 of these interneurons would suffer major disruption by ganglion hemisection. For example, interneuron 301 is considered to be of central importance in the neural circuitry for flight rhythm generation. The cell body and dendrites of interneuron 301 are located in the mesothoracic ganglion and its axon arises from a midline process, descends in the contralateral connective and eventually branches extensively and bilaterally in the flight neuropil of the metathoracic ganglion (cf. Fig. 2). Hemisection of the mesothoracic ganglion severs the axon from the dendritic arbors of this cell thus making functional impairment highly likely. Yet flight behavior is unimpaired in animals with mesothoracic hemisections, suggesting that a functional interneuron 301 is not necessary for the production of rhythmic flight behavior.

Similarly, the interneuron group termed 404 which are thought to function in initiation and maintenance of flight activity in response to wind

are also transected by mesothoracic hemisection. Interneurons 404 have extensive bilateral and midline arborizations in the mesothoracic ganglion (cf. Fig. 2) and are thus likely to be functionally perturbed by hemisection of the mesothoracic ganglion. Although tethered animals with hemisected mesothoracic ganglia did have some difficulties in unfolding their wings, they clearly were able to jump, unfold their wings and initiate flight when free.

The retention of normal coordination among the flight muscles in all segments after hemisection of the mesothoracic ganglion

Despite the interruption of the direct pathways through commissures in the mesothoracic ganglion, the timing among the flight muscles of both forewings as well as the timing among the muscles of fore- and hindwings is unimpaired. This coordination persists after extensive deafferentation and thus cannot be due to a synergistic and compensatory action of proprioceptive feedback. It follows that coordinating interactions between separated right and left hemiganglia must be possible through other central pathways involving the remaining intact ganglia (see also Wolf et al. 1988).

It is conceivable that some of this coordination is due to intersegmental interneurons such as 301, 401, 501 or 504. These interneurons all have reset properties and are considered to be important members of the central flight rhythm generator. However, beyond their role in rhythm generation, they might also function as intra- and intersegmental *coordinating* interneurons. A possible role of these interneurons in the coordination of basic oscillator subunits in each pterothoracic hemisegment might explain why their activity can affect

the entire flight rhythm generating system. Moreover, the pronounced discoordination observed following hemisection of the metathoracic ganglion could be due to the fact that most of these interneurons (301, 501, 504 and to some degree 401) are impaired by the metathoracic split.

The neurobehavioral experiments on surgically manipulated animals reported in this study present constraints which any hypothesis for flight production must satisfy. However, conclusions concerning the neural organization of the rhythm generating network for flight are rather limited especially in cases where abnormal patterns are found. This is because an analysis of motoneuron firing allows only a limited insight into the function of the premotor network. Similar firing patterns in motoneurons could be brought about by different types of synaptic premotor drive (e.g. Wolf and Pearson 1987b). More precise and detailed information about the organization of the flight rhythm generator can be gained by carrying out intracellular recordings from flight motoneurons in animals with hemisectioned thoracic ganglia. This is the subject of the following report which corroborates and extends our findings.

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