ADAPTIVE MODIFICATIONS IN THE FLIGHT SYSTEM OF THE LOCUST AFTER THE REMOVAL OF WING PROPRIOCEPTORS

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Summary

Previous investigations on the flight system of the locust have found that removal of the wing tegulae in mature locusts (*Locusta migratoria*) results in an immediate change in the flight motor pattern: the wingbeat frequency (WBF) decreases, the interval between the activity of the depressor and the elevator muscles (the D–E interval) increases, and the phase of the elevator activity in the depressor cycle increases. Here we report the results of a detailed quantitative analysis of these changes. We also examined the flight motor pattern for up to 14 days after removal of the tegulae and found that the changes caused by this operation were not permanent. Beginning on the first day after the operation there was a time-dependent recovery of the WBF, the D–E interval and the phase towards their normal values. In about 80 % of the experimental animals the flight motor pattern recovered almost completely. Intracellular recordings from elevator motoneurones showed that this recovery was associated with changes in the pattern of excitatory input to these motoneurones. The modification of activity in elevator motoneurones was dependent on afferent input since complete deafferentation of recovered animals resulted in a motor pattern similar to that following deafferentation of normal animals.

Introduction

Animals have the capacity to adapt to injuries both to the nervous system and to other body structures (Cotman, 1978). In addition, constant adaptation of the central nervous system occurs during maturation to match the continuous changes in the body parts (Lisberger, 1988). This adaptive capacity has been demonstrated most clearly by changing the sensory input (Knudsen and Knudsen, 1990; Wiesel, 1982), by injuring the nervous system (Jenkins and Merzenich, 1987; Kaas *et al.* 1990) and by altering the motor apparatus (Optican *et al.* 1985). In insects, there are now several examples of animals adapting their behaviour to the loss of an appendage. For example, cockroaches adapt to the loss of one cercus or to the artificial rotation of it (Vardi and Camhi, 1982a,b; Comer and Camhi, 1984). After

an initial phase of misorientation in response to wind puffs the orientation response returns towards normal over a period of 4 weeks. Similarly, the phonotactic behaviour of female crickets adapts to the loss of an ear (Huber *et al.* 1984; Huber, 1987; Schildberger and Huber, 1988; Schildberger and Kleindienst, 1989; Schmitz, 1989). After recovery, the female cricket can maintain a stable course with respect to a sound source emitting the conspecific calling song of a male.

The present study focuses on the adaptive capabilities of the flight system in the mature locust. Two studies have already indicated that this system has adaptive properties: Kutsch (1974) showed that the wingbeat frequency (WBF) in adult mature locusts increases after its initial decrease following the removal of all wing stretch receptors; and Möhl (1988) showed that the steering system of the locust adapts to forced preset timing relationships between different steering muscles. In this study we investigated whether the fully developed pattern-generating system for flight is able to adapt to the removal of proprioceptive input, i.e. whether slow changes occur that return the motor pattern to normal. We chose to examine the effects of ablating the wing tegulae, because the function of these wing proprioceptors in the generation of the flight motor pattern is well understood: the phasic burst of activity of the tegulae during the downstroke of the wings induces a rapid depolarization in the elevator motoneurones and, as a consequence, initiates the onset of elevator activity and increases the wingbeat frequency (Pearson and Wolf, 1988; Wolf and Pearson, 1988). These previous studies on the flight system of the locust focused on the function of the hindwing tegulae and were largely qualitative. However, to assess the adaptive properties in the flight system after removal of the tegulae it was necessary to have detailed quantitative data on the changes that occur in the flight motor pattern immediately after ablation. Therefore, our initial aim was to confirm and expand previous data on the effects of removing the forewing tegulae, the hindwing tegulae or both groups of tegulae. We investigated whether the changes produced by removal of the tegulae were permanent. To do this we examined the motor pattern for 2 weeks after ablation of the tegulae by recording the electromyogram (EMG) activity in the flight muscles and by intracellular recordings of the patterns of synaptic input to flight motoneurones.

Materials and methods

Adult locusts (*Locusta migratoria* L.) from a laboratory culture at the University of Alberta were used for the experiments. The wing tegulae were removed more than 3 weeks after the final moult. This age was selected because it is known that the flight pattern of adult locusts changes systematically during the first 2–3 weeks of their adult life (Kutsch, 1974). Only animals with undamaged wings and the ability to beat their wings at high frequency when lifted by their pronotum were used. Experiments were carried out at room temperature (approx. 23°C).

Electromyograms and data analysis

Electromyograms (EMGs) were recorded either from forewing muscles 83 (tergosternal muscle) and 97 (first basalar muscle) or from hindwing muscles 113 and 127 (see Fig. 2A; recording from the hindwing muscles 113 and 127). The placement of the electrodes and the recording procedure were the same as those described in Pearson and Wolf (1987). The animals were tethered by the pronotum to a rigid rod and held in an upright position facing the opening of a wind tunnel. The tethering rod was free to rotate, enabling the animals to make yaw adjustments in the windstream. Flight was initiated and maintained by constant wind from the wind tunnel. The wind velocity was about $2.5\,\mathrm{m\,s^{-1}}$. The EMG recordings were first passed to a window discriminator with thresholds adjusted so as to generate pulses for all the muscle potentials. These pulses were then passed into monostable circuits that gave single pulses corresponding to the first action potential in each burst of elevator and depressor activity. The times of onset of elevator and depressor activity were stored and analyzed using a general purpose computer (DEC LSI 11/23). The time resolution for the analysis of the events was 0.1 ms. The following parameters of the flight motor pattern were evaluated: (i) the wingbeat frequency, calculated from the cycle period of the depressor activity; (ii) the time interval between the activity of the depressor and the elevator motoneurones (D–E interval), calculated from the first spike in the depressor EMG to the first in the elevator EMG; (iii) the phase of the elevator activity in the depressor cycle. WBF and D–E intervals for single trials were calculated as the average values for a continuous 30 s of stable flight starting 30 s after flight had been initiated (see Fig. 2B). The average phase value for each trial was calculated as the ratio of the average D–E interval to the average cycle period.

To compare the flight motor pattern of groups of animals in different experimental situations the average WBF and D–E interval were calculated as the arithmetic means of these parameters for all animals within a group. With respect to the phase of elevator activity, the average phases of elevator activity for each group of experimental animals were calculated using circular statistics after Horsmann *et al.* (1983). The phase value of each animal on a given day was taken as a unity vector in the circular period between successive depressor spikes. Then the average phase value (P) was evaluated using the formula given in Horsmann *et al.* (1983). The length (R) of the mean vector was also calculated to give a measure of the scatter. As R increases in length ($0 \leqslant R \leqslant 1$) the scatter decreases.

Intracellular recordings

Intracellular recordings from flight motoneurones during flight were carried out in the ventral preparation, as described by Wolf and Pearson (1987). This preparation allows the intracellular penetration of flight neurones without impairing the wing movements or the flight pattern. Recordings were taken from the cell bodies of elevator and depressor motoneurones in the metathoracic

ganglion. The neurones were identified by the location of their somata, by their activity pattern according to a reference EMG taken from the first basalar muscle, and by their morphology determined by injecting them with Lucifer Yellow dye. The soma recordings were very stable and allowed the transection of meso- and metathoracic nerves 1 and 6 while the recording was maintained.

Surgical removal of the wing tegulae and recording procedure during recovery

The animals were tethered by the pronotum to a rigid rod and placed above a cork platform. The forewings were unfolded and pinned to the platform. The exposed hindwing tegulae were ablated by slightly lifting each with forceps and then removing them with microscissors. To remove the forewing tegulae the animals were turned almost sideways to the dissection microscope and the junction between the meso- and metathoracic segments was enlarged to expose the forewing tegulae. The tegulae were then held with forceps and transected at their base. Recordings from nerve 1C showed that the wing nerve (1C1b) was not damaged by this operation (see also below). The procedures used to expose the tegulae had no significant effect on the flight ability and the flight pattern (see Fig. 6D–F, controls), as shown by sham-operated animals that were treated identically except for removal of the wing tegulae.

For the purpose of identification each animal was uniquely marked with white ink on the pronotum after the operation. The animals were kept together in a cage throughout the period of the investigation and the EMG electrodes were implanted for each recording session. The animals were tested in four different groups. (i) For the investigation of the hindwing flight motor pattern, one group of animals ($N=28$) was tested before and immediately after the removal of the wing tegulae. During the 2 weeks after the operation (see Figs 6,7) every animal in this group was tested 2–6 times, four times on average. These trials were randomly distributed over the group to avoid always testing the same animals on the same day. No other experiments were carried out with these animals. A control group ($N=8$) was only sham-operated and then treated in the same way as groups i and ii. (ii) A group of 14 animals was tested in the same way for the investigation of the forewing flight motor pattern. (iii) Another group of animals ($N=11$) was tested only once, 2 weeks after the removal of the wing tegulae. (iv) A fourth group of animals ($N=4$) was tested before and after removal of the tegulae and then every 2 h for the first day after the operation. A control group of animals ($N=4$) was only sham-operated and then treated in the same way.

More than 120 animals were operated on during these experiments (not all shown in the paper). About 20% of all operated animals did not recover their flight motor pattern and are not included in the following sections. These animals died, stopped flying during the 2 weeks after the operation or showed little or no change in the flight motor pattern for 2 weeks after the operation. We did not investigate whether this percentage of animals (20%) was comparable to the percentage of intact animals that died or showed poor flight activity during the same period.

Deafferentation of the flight system

In some experiments (see Figs 9, 10) the flight system was deafferented by severing the meso- and metathoracic nerves 1 distal to their bifurcations with nerves 6. This was done by cutting small holes in the ventral cuticle of the thorax and, after removing as little overlying tissue as possible, cutting the nerves. The holes in the cuticle were then closed with silicone grease.

Staining of tegulae and wing afferents

Afferents in nerve 1C were stained in intact control animals and in animals 14 days after removal of the wing tegulae by backfilling them with $CoCl_2$. Preparations were kept for about 18 h at 4 °C. After cobalt sulphide precipitation, the ganglia were fixed and intensified according to Bacon and Altman (1977). Staining of the metathoracic nerve 1C afferents was performed to confirm that removal of the tegulae caused degeneration of the afferents from these receptors. A comparison of seven stained preparations from intact animals with seven stained preparations from recovered animals revealed that the prominent dorsal lateral branches of nerve 1C afferents in the lateral neuropile, representing the tegula afferents (Tyrer and Altman, 1974; Bräunig *et al.* 1983), were absent ($N=4$, Fig. 1) or strongly reduced ($N=3$, a few arborizations in this region) in animals 14 days after removal of the tegulae (see arrowheads in Fig. 1). Furthermore, fewer axons were found to run in the fibre tract located dorso-medially in the ganglion (see arrowheads in Fig. 1). These data indicate that tegula afferents had degenerated by 14 days after removal of the tegulae. In addition, control experiments showed that no afferents in n1C could be activated by touching the former location of the tegulae. Thus, anatomical and electrophysiological data indicated that the flight motor system received no input from tegula afferents during the 14 days of the experiments.

Statistics

Depending on the sample sizes, significance was assessed with the Student's *t*-test (*t*-test) or with a modified Student's *t*-test (*mt*-test) after Dixon and Massey (1969). The latter test allows the comparison of sample sizes of less than 30 and with unequal variances. Samples were considered to be significantly different throughout this study at $P<0.02$.

Results

Effects of removal of the tegulae on the flight pattern

Previous studies on the flight system of the locust have shown that the phasic activity of the hindwing tegulae during the wing downstroke induces a rapid depolarization in elevator motoneurones and increases the WBF (Pearson and Wolf, 1988; Wolf and Pearson, 1987). The forewing tegulae are also excited during the downstroke and electrical stimulation advances elevator activity (Neumann,

Intact   14 days after removal of the tegulae
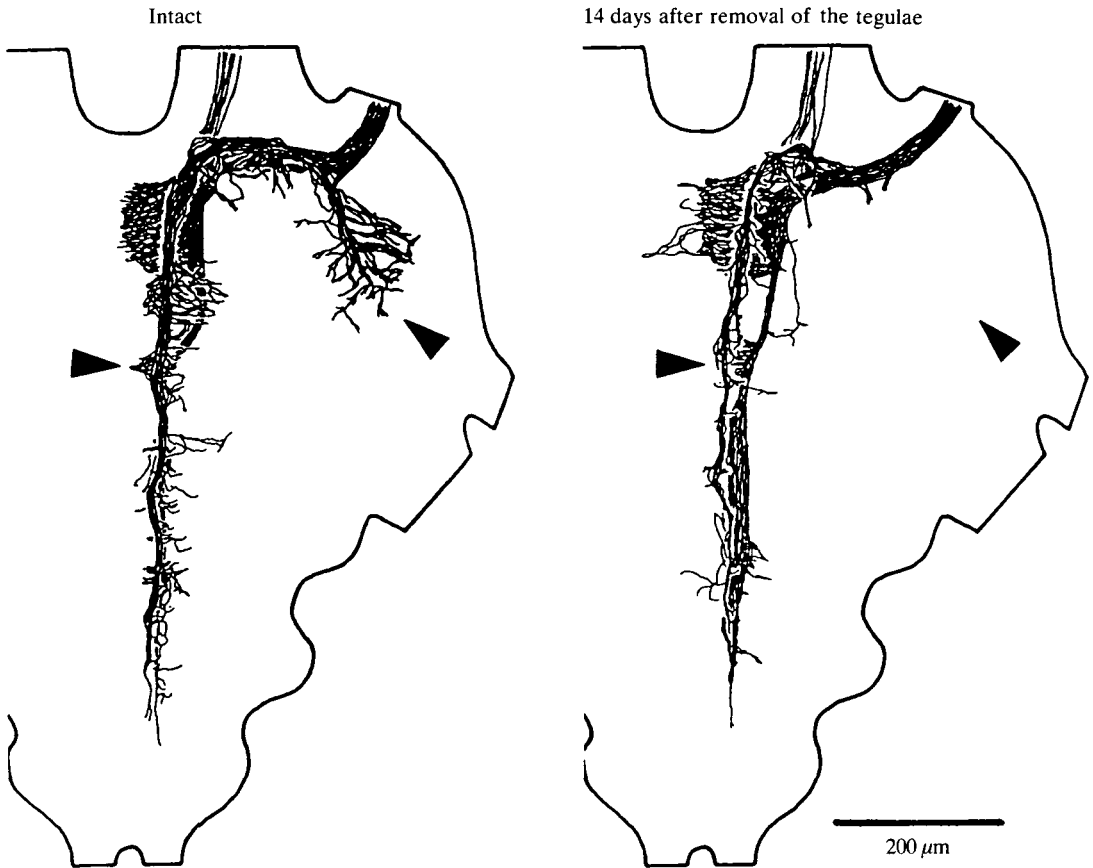

200 μm

Fig. 1. Projections of the afferents in nerve 1C in the metathoracic ganglion of an intact and a recovered animal (one preparation out of seven in each case), stained with $CoCl_2$ backfills. Note that the extensive arborizations in the lateral neuropile of the metathoracic ganglion are no longer visible in the recovered animal. In addition, the median dorsal branches are less dense in the recovered animal. The arrowheads mark these regions.

1985). Since a quantitative description of these effects is necessary for the assessment of adaptive capabilities in the flight system, we first established the changes in the flight motor pattern produced by removal of tegulae from the forewing, then from the hindwing and then from both wings.

Fig. 2B shows plots of WBF and D–E interval in the hindwing muscles for two flight sequences in an individual before and after removal of all wing tegulae. The average WBF in this animal decreased from 19.5 to 16.1 Hz and the hindwing D–E interval increased from 28.3 to 41.7 ms. The average WBF of all investigated animals decreased significantly from 20.0 Hz (see Fig. 3 for details and statistics) to 15.0 Hz (Fig. 3A, left column). The average D–E interval in the forewing increased from 19.8 to 32.0 ms (Fig. 3C, left column) and in the hindwing it increased from about 24.3 to 43.1 ms (Fig. 3B, left column).
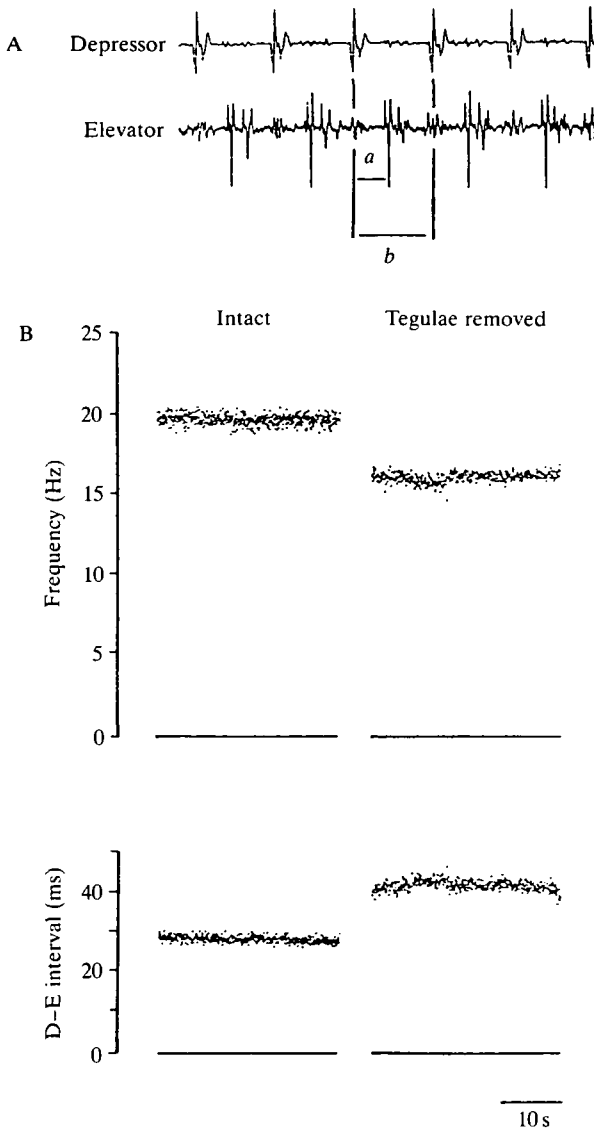
Fig. 2. (A) Electromyogram pattern recorded in the hindwing muscles (first basalar, 127 and anterior tergosternal, 113) in a tethered flying locust. (*a*, D–E interval; *b*, cycle period=1/wingbeat frequency). (B) Changes in the wingbeat frequency (WBF) and D–E interval of a tethered flying locust after removal of all wing tegulae. The plots on the left show the WBF and the D–E interval during a 30s flight before removal of the wing tegulae, recorded from the hindwing muscles. Removal of the wing tegulae caused a decrease in the WBF and an increase in the D–E interval (data shown in the plots on the right for 30s of flight immediately after the operation). Each point in the plots represents a single flight cycle.
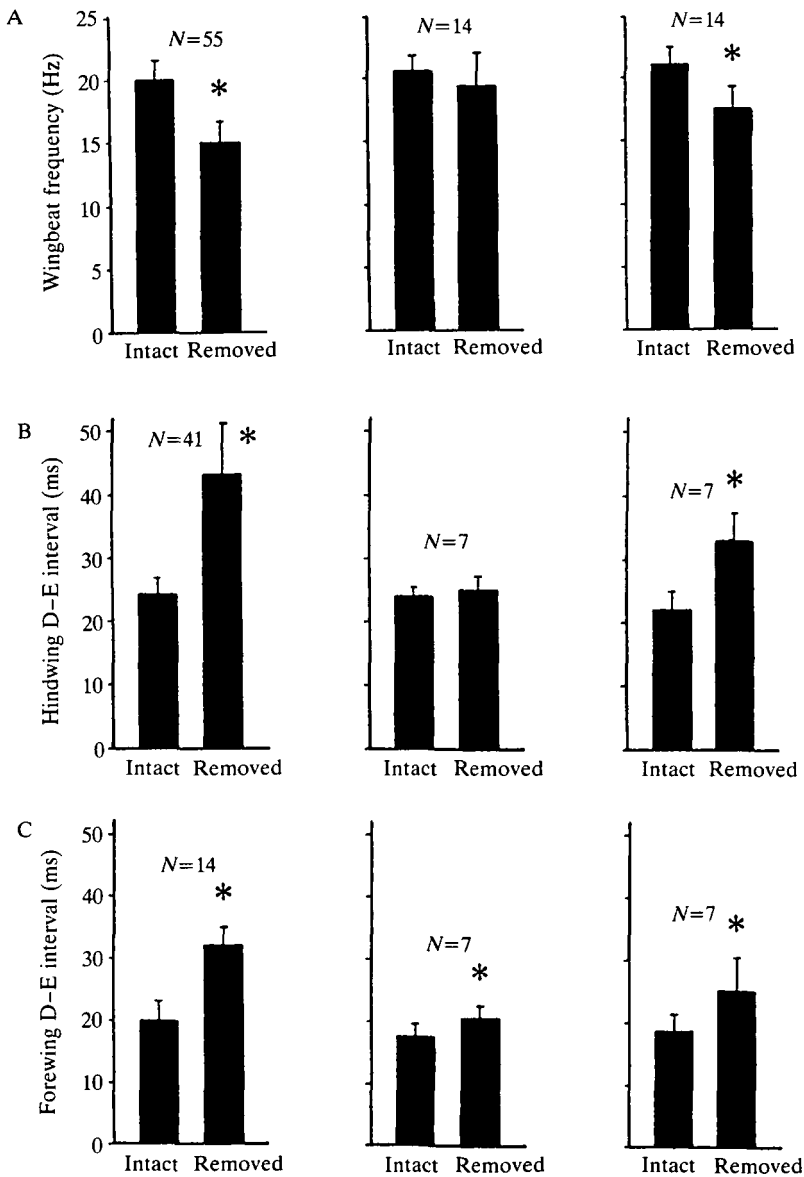
Fig. 3. Histograms showing the mean wingbeat frequencies (WBFs) and the mean
D–E intervals recorded in hindwing and forewing muscles before and after removal of
all wing tegulae (left column), the forewing tegulae (middle column) and the hindwing
tegulae (right column). (A) Average WBF; (B) average D–E interval in the hindwing
muscles; (C) average D–E interval in the forewing muscles. An asterisk (*) indicates
that the mean value after removal of the tegulae is significantly different from that
before removal.

These data are similar to those of Pearson and Wolf (1988), in which only the hindwing tegulae were removed, indicating that the forewing tegulae have a much weaker influence on the flight motor pattern than the hindwing tegulae. This was confirmed by recording the EMG patterns of the forewing and the hindwing elevator and depressor muscles following the removal of the forewing tegulae (Fig. 3, middle column). Removal of the forewing tegulae had almost no effect on the measured flight motor pattern of the fore- and hindwings. Neither the WBF nor the D–E interval of the hindwing muscles was significantly changed after removal of the forewing tegulae. Only the D–E interval of the forewing muscles was significantly influenced by the ablation of the forewing tegulae, increasing by 2.9 ± 1.8 ms. In contrast, the removal of the hindwing tegulae led to a significant decrease in the WBF and a significant increase in the D–E intervals in the forewing and in the hindwing muscles (Fig. 3, right column). Interestingly, the effects of the forewing and hindwing tegulae on the flight motor pattern appeared not to be simply additive, because removal of all the wing tegulae had a greater effect than the sum of the single effects caused by the removal of either the forewing or the hindwing tegulae. This was especially obvious for the D–E intervals (Fig. 3).

The increase in the D–E intervals after the removal of all wing tegulae was not simply a consequence of the decrease in WBF because the phase of the onset of the elevator activity in the depressor cycle increased after removal of the wing tegulae (for the effects of removal of the hindwing tegulae, see also Pearson and Wolf, 1988). Circular statistics showed that after removal of all tegulae, the value of the average phase of the elevator activity for the hindwing muscles changed from $P=0.49$ ($R=0.94$, $N=41$) to $P=0.61$ ($R=0.91$) (Fig. 4). For the forewing muscles the phase increased from $P=0.39$ ($R=0.97$, $N=14$) in intact animals to $P=0.49$ ($R=0.95$) in animals with all tegulae removed.

Recovery of the flight pattern following removal of the tegulae

As we have shown above, removal of all the wing tegulae resulted in an abnormal flight motor pattern (Fig. 3, left column): the WBF decreased markedly, the D–E intervals for both the forewing and the hindwing increased markedly and the phase of the elevator activity within the depressor cycle increased. The main aim of the present study was to determine whether these changes persisted after the operation, i.e. whether the flight system of the locust was able to recover from the loss of the wing tegulae. We found that from the first day after the operation throughout the following 2 weeks the flight pattern of most (80 %) of the operated locusts progressively returned to values close to normal (see Materials and methods and Figs 5, 6). For example, on the first day after the operation the WBF of the animal yielding the data in Fig. 2B and Fig. 6A–C had decreased to 61 % (11.9 Hz) of its intact value (Fig. 6A). On day 14 after the operation the WBF had recovered to 91 % (17.8 Hz) of its intact value. The D–E interval of this animal also returned towards its intact value: it increased to 185 % (52.4 ms) on the first day after the operation; 14 days later it was 109 % (30.8 ms) of the intact value. Other animals returned even closer to their intact values: for example, in one
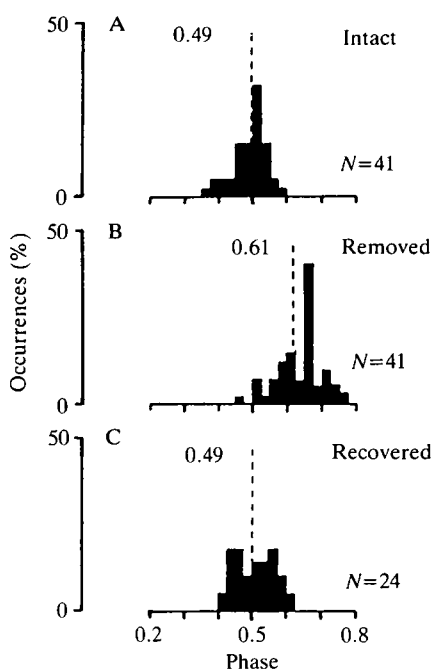
Fig. 4. Histograms of the phase values of the elevator activity in the depressor cycle recorded from the hindwing muscles. (A) Intact flying animals (N=41), R=0.94; (B) the same animals as in A after removal of the wing tegulae, R=0.91; (C) 24 of the 41 animals were tested 2 weeks after removal of the tegulae (tested on day 14, 15 or 16), R=0.94. The length R (given in the histograms) of the mean vector is shown by the dashed line, which is the length of the ordinate when R equals 1.

animal the average WBF initially decreased to 75 % (15.2 Hz) of its intact value and after 16 days it had recovered to 102 % (20.6 Hz); the D–E interval for the hindwing muscles was 190 % (48.7 ms) of its intact value after the operation; 16 days after the operation it had recovered to 100 % (25.7 ms).

The WBF and the D–E interval in 30 % out of a group of 24 animals (see also Fig. 5) recovered to values that were ±10 % of their intact values. Since the flight motor pattern of a locust can vary to some extent from day to day (Kutsch, 1973), we regard this recovery as complete. The other animals showed an incomplete return towards the intact values of the WBF and/or the D–E interval: 50 % of the animals returned to values within ±20 % of the intact values, while the remaining 20 % recovered only one of the two parameters to within ±20 % of the intact values.

The general effect of the recovery of the flight motor pattern is shown in Fig. 5. For a group of 24 animals, which were tested when intact, immediately after removal of the tegulae and 2 weeks (day 14–15) after the operation, the histograms of WBF and the D–E interval are shown. Each of the animals was tested up to seven times during the 2 weeks after the operation. Immediately after removal of the wing
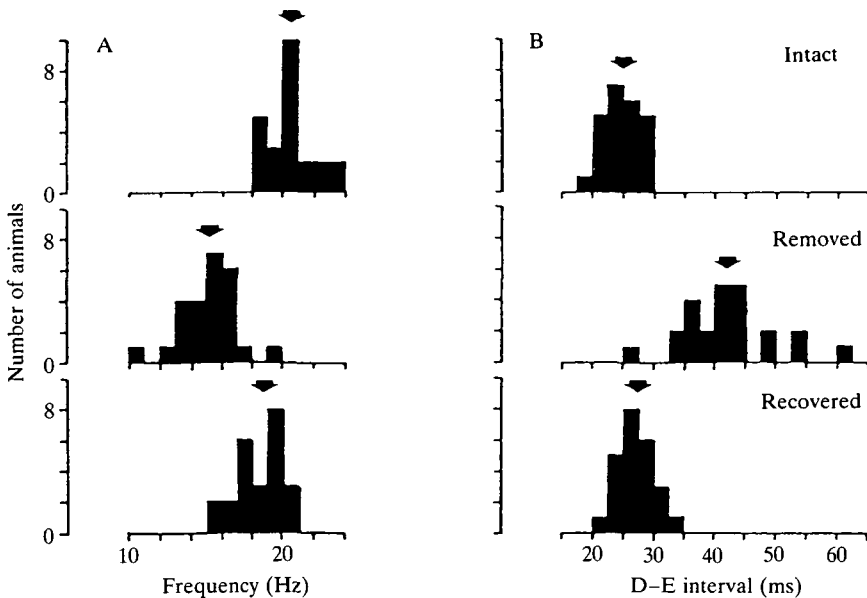
Fig. 5. Recovery of the wingbeat frequency (WBF) (A) and the D–E interval (B) of the hindwing muscles after removal of the wing tegulae. A group of 24 animals was tested before (upper histograms), immediately after (middle histograms) and 2 weeks (tested on day 14, 15 or 16) after removal of all the wing tegulae (lower histograms). The mean values of all tested animals are indicated by arrows (see also text).

tegulae the average WBF decreased from 20.4±1.6 Hz (*N*=24) to 15.2±1.7 Hz. Two weeks after the operation the average WBF of this group of animals had risen significantly to 18.5±1.6 Hz, but it remained significantly less than its values before removal of the tegulae (Fig. 5A). The average D–E interval also recovered to values close to normal (see Fig. 5B). For the hindwing muscles the average D–E interval was 24.6±2.7 ms (*N*=24) in intact animals. It was 41.9±7.6 ms (*N*=24) immediately after removal of the tegulae and 27.2±3.1 ms (*N*=24) 2 weeks later. Similarly the D–E interval in the forewing muscles returned towards normal values: in intact animals it was 19.8±2.1 ms (*N*=14), immediately after the operation it was 32.0±2.9 ms (*N*=14) and 14 days after removal of the tegulae it was 19.4±1.0 ms (*N*=5, the other animals were tested on days other than day 14). Finally, the phase of the onset of elevator activity in the depressor cycle returned completely to its intact value: for the hindwing muscles the phase was 0.49 (R=0.94) in intact animals, 0.61 (R=0.91) immediately after the operation and 0.49 (R=0.94) 2 weeks later (see Fig. 4A,B,C). This recovery of phase is shown in Fig. 6C (left side) for a single animal and in Fig. 4 for the group of tested animals.

   We also studied the recovery of the flight motor pattern of the hindwings in a group of animals (*N*=11) tested only once on day 13, 14 or 15 after the operation. The average WBF and the average D–E interval of this group were not
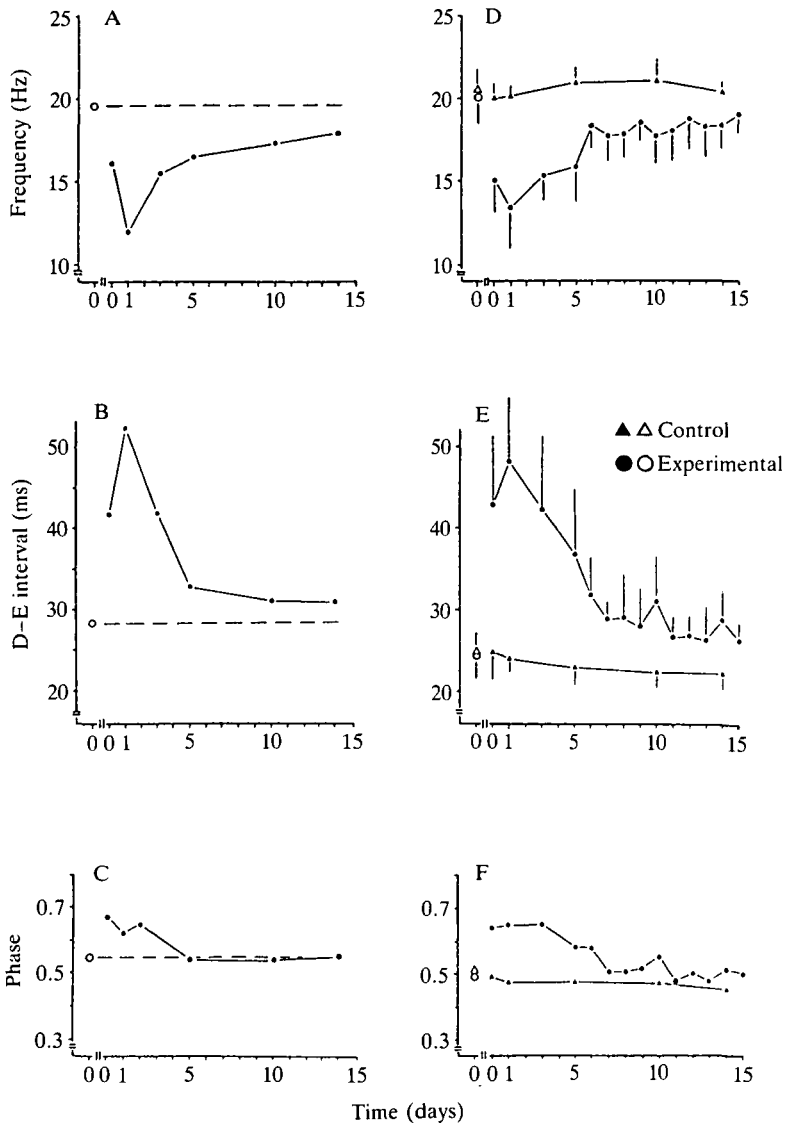
Fig. 6. The time course of the recovery of the flight pattern. (A–C) Plots of wingbeat frequency (WBF), D–E interval and phase of the elevator activity for an individual animal are shown over a period of 2 weeks following the removal of the wing tegulae. The initial values are shown by open circles and by the dashed line (see also data in Fig. 2B for the same animal). (D–E) Plots of average values for the WBF (D), the D–E interval (E) and the phase (F) for a group of 28 animals over 2 weeks after removal of the wing tegulae (●). A group of eight animals that had been sham-operated served as controls (▲). The open circle and the open triangle in each plot show, respectively, the mean values in the experimental group before removal of the tegulae and before the sham operation in control animals. Bars indicate s.d.

significantly different from the final values of the group of 24 animals (see Fig. 5) that were tested repeatedly during the recovery period.

Time course of the recovery of the flight pattern

Fig. 6A–C shows the values of WBF, D–E interval and phase in a single animal over the period of 2 weeks after the removal of all wing tegulae (EMG recording from hindwing muscles). The first point to note is that the WBF was lower and the D–E interval was larger on the first day after the operation than it was immediately after the operation (Fig. 6A,B). Starting on the second day after removal of the tegulae there was a progressive increase in the WBF and a progressive decrease in the D–E interval for the single animal as well as for a group of 28 animals (Fig. 6, compare left-hand and right-hand column).

Most of the recovery of the WBF and the D–E interval for single animals, as well as for the group of 28 animals, occurred within the first week after the operation (Fig. 6D,E). Thereafter, only small changes were detectable. These almost paralleled the slight changes in the control animals occurring during the same period. This is even more obvious when data from the tested animals are normalized. All values obtained for the WBF and the D–E interval throughout the 14-day period were therefore calculated as a percentage of the intact value and arithmetically averaged. This procedure showed that 6 days after the operation the experimental animals reached, on average, 90 % of their intact WBF and 110 % of their intact D–E interval. In addition, we found that the elevator phase in the depressor cycle showed a progressive return to its intact value. Starting on the first day after removal of the tegulae the phase value returned towards normal (Fig. 6C,F). On the seventh day after the operation the phase value had returned to its intact value.

The small changes in the average values of the WBF and the D–E interval detectable from day 6 to day 14 after the operation were partly due to individual differences in the recovery time course. Some animals recovered quickly, e.g. the WBF in one animal returned to 96 % of its intact value within 6 days, whereas others showed a much slower increase over the same time, e.g. another animal reached only 79 % of its intact value of WBF by the tenth day. After 2 weeks all animals had reached their final values and we did not observe any further significant time-dependent changes in the WBF, the D–E interval or the phase. Experiments on 14 animals showed that the time courses of recovery of the D–E interval and phase were similar for the forewing muscles.

As mentioned above, on the first day after the operation the WBF was lower and the D–E interval larger than immediately after the removal of the tegulae (Fig. 6). In four animals we investigated the time course of the changes in the WBF and the D–E interval for the hindwing motoneurones during the first 24 h after removal of the wing tegulae (Fig. 7). In all four animals the WBF decreased progressively during the first 12 h after the operation. At the same time the D–E interval increased progressively. A control group (sham-operated) of four animals did not show similar changes. Thus, these slow changes during the first 12 h after
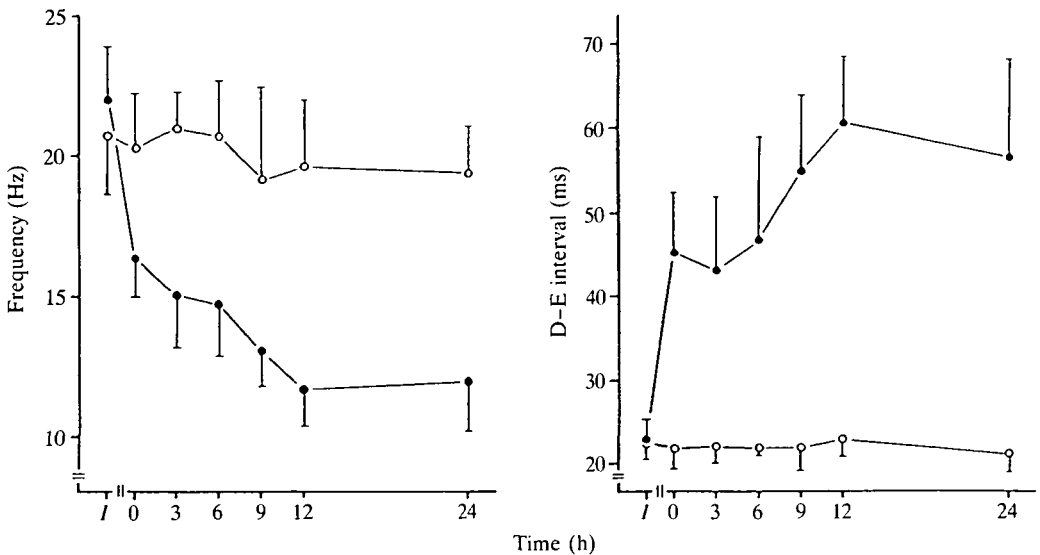
Fig. 7. Changes in the average wingbeat frequency (WBF) and the average D–E interval in the hindwing muscles during the first 24 h after removal of the wing tegulae. Bars indicate s.d. (●) Experimental animals (four animals); (○) controls (four animals).

removal of the tegulae are not due to the repeated testing procedure during this interval. This is consistent with the observation that most animals not flown repeatedly in the first 24 h showed a lower WBF and a larger D–E interval than did animals immediately after removal of the tegulae (Fig. 6). Between 12 and 24 h after the operation we observed no significant changes in the flight pattern.

Pattern of elevator activity in the recovered animals

The depolarization pattern of the elevator motoneurones and its dependence on proprioceptive input to the flight system have been investigated in detail by Wolf and Pearson (1988). They showed that the elevator motoneurones are rapidly depolarized following depressor activity, that this rapid depolarization is visible over a wide range of WBFs and that it is initiated by tegula activity. This initial rapid depolarization underlies the short D–E interval in the intact animal. Because recovered animals have a D–E interval close to normal, we wished to determine whether the pattern of synaptic input to elevator motoneurones in recovered animals resembled that in intact animals.

Intracellular recordings in the hindwing elevator motoneurones were made before and immediately after removal of the hindwing tegulae in animals in which the forewing tegulae had already been removed. (This was necessary because the forewing tegulae could not be removed while maintaining an intracellular recording.) We found no difference in the hindwing elevator depolarization with

and without forewing tegulae (see also Fig. 3, showing that removal of the forewing tegulae has no effect on the hindwing D–E interval). After removal of the hindwing tegulae the initial rapid depolarization was not generated and the onset of the first elevator spike was delayed, resulting in an increase in the D–E interval (Fig. 8A,B; Fig. 4 in Wolf and Pearson, 1988). At all WBFs the depolarization pattern showed only a burst of activity late in the flight cycle. In recovered animals the pattern of synaptic input to hindwing elevator moto-
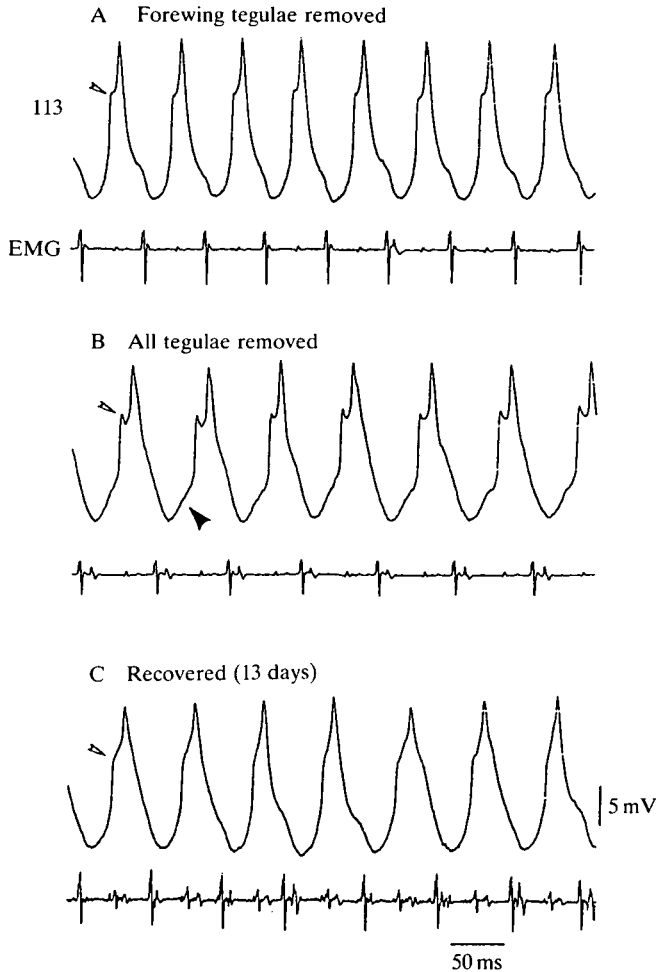

Fig. 8. Depolarization profile in an anterior tergosternal elevator motoneurone (113) before (A), immediately after (B) and 13 days after (C) removal of the tegulae. Note that the average phase of elevator activity in the depressor cycle is 0.51 in A, 0.61 in B and 0.52 in C. The first spike in each elevator depolarization is marked by an open arrowhead. The closed arrowhead marks the slope of the depolarization that decreased after the removal of the hindwing tegulae. The recordings in A and B were taken from the same animal, in which the forewing tegulae had been removed (see text). The bottom traces are EMG recordings from the hindwing first basalar muscle. The results were confirmed by recordings from 15 animals in each situation.

neurones (Fig. 8C) resembled that in animals with intact hindwing tegulae. The initial depolarization in elevator motoneurones was more rapid in recovered animals than in animals immediately after removal of the tegulae. In addition, at lower WBFs a second late component occurred in the elevator depolarization similar to that reported for intact animals (see Fig. 2 in Wolf and Pearson, 1988).

No obvious changes were found in the depolarization profile of depressor motoneurones immediately after removal of the tegulae, and this profile was similar to that in recovered animals.

Effects of complete deafferentation on the flight activity in recovered animals

A possible mechanism for the recovery of the flight motor pattern following removal of the tegulae could be alterations in the central elements of the motor pattern-generating network. In this case a total deafferentation of the flight system in a recovered animal should lead to a different flight pattern from that of an intact animal immediately after deafferentation. Contrary to this prediction, we found no significant differences in the average WBF and the average D–E interval in deafferented recovered animals (Fig. 9, left and middle histograms) compared with deafferented normal animals. Furthermore, the average elevator phase in the depressor cycle was similar for both situations (deafferented normal, 0.58; deafferented recovered, 0.59). We also found no significant differences in the flight duration (Fig. 9C) for the deafferented animals in both situations. Deafferentation of the flight system in the recovered animals led to a drastic change in the elevator activity pattern (Fig. 10), comparable to that occurring in normal animals after deafferentation. In both cases the rapid depolarization in the elevator motoneurones was abolished and bursts of elevator activity were generated following a relatively slow initial depolarization.
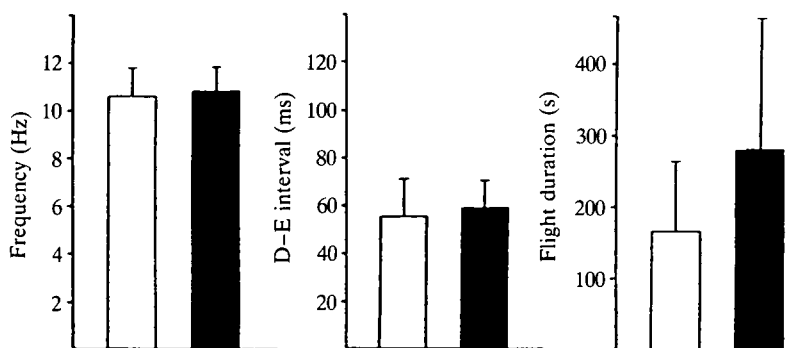
Fig. 9. Histograms of the average wingbeat frequencies (WBFs), D–E intervals and flight durations in animals immediately after deafferentation (open bars, $N=9$) and in recovered animals immediately after deafferentation (black bars, $N=11$). There is no significant difference in any of these parameters.

A Recovered

113

EMG
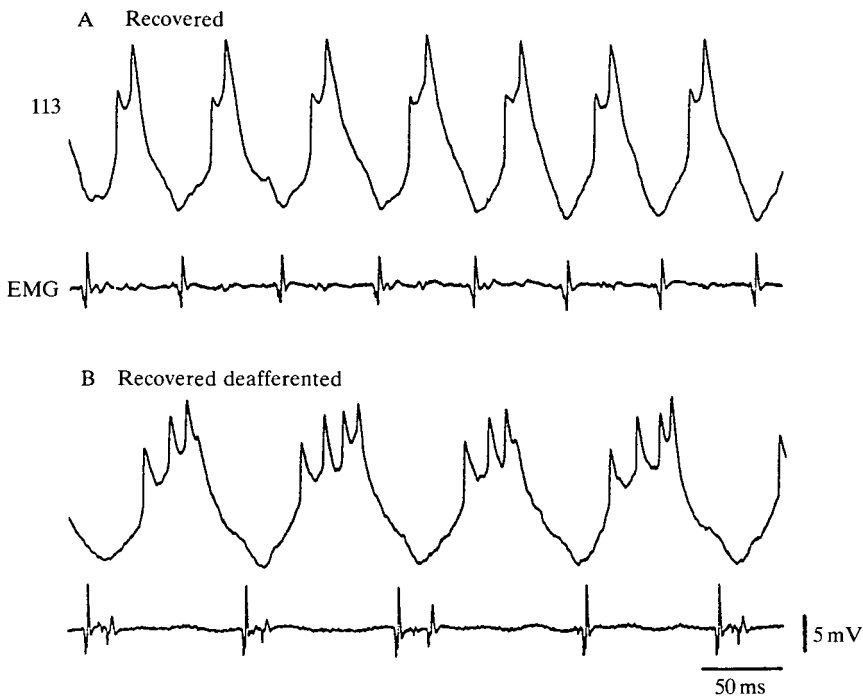
B Recovered deafferented

5 mV

50 ms

Fig. 10. Intracellular recordings from hindwing tergosternal motoneurones (113) of a recovered animal show that deafferentation of the flight system caused a drastic change in the depolarization profile. (A) Depolarization profile in a tergosternal elevator motoneurone during flight in a recovered animal. (B) Depolarization profile in the same motoneurone after deafferentation. The bottom traces in A and B show the EMG recording of a hindwing first basalar motoneurone. Results were confirmed in 10 preparations.

Discussion

Adaptive changes in the flight motor pattern following removal of the tegulae

We have shown that the removal of all wing tegulae resulted in an immediate change in the flight motor pattern: the average WBF decreased by about 25 % and the average D–E interval increased in the forewing muscles by about 62 % and in the hindwing muscles by about 78 % (Figs 3, 6). In addition, the elevator phase increased by 25 % in both the forewing and the hindwing muscles (Fig. 4). This change in the flight motor pattern was largely due to the loss of the hindwing tegulae: their removal significantly altered the WBF and the D–E interval in the forewing and the hindwing muscles, whereas the removal of the forewing tegulae only affected the D–E interval of the forewing muscles (Fig. 3). After the removal of all wing tegulae there was a progressive decrease in the WBF and an increase in the D–E interval (Fig. 7) during the first 12 h after the operation. These values were maximally different from those in intact animals on the first day after ablation of the tegulae (Figs 5, 7). We have no information on what mechanisms could be

responsible for the deterioration of the motor pattern during the 12 h after removal of the tegulae.

The main result of the present investigation was that the effects of the removal of the tegulae on the WBF, the D–E interval and the elevator phase were not permanent. Starting on the first day after the operation, all the parameters recovered towards their intact values. Some of the animals (30 %) recovered completely. The major part of this recovery occurred during the first 6 days after removal of the tegulae (Fig. 6) and final values were reached between 10 and 14 days. After 7 days the phase of the elevator activity in the flight cycle returned to its intact value (Fig. 6). The observed recovery in the motor pattern was not due to maturational processes because at the time of removal of the tegulae all animals had developed beyond the age (3 weeks) when maturation influences the flight motor pattern (Kutsch, 1973, 1974).

Because we measured only three parameters of the flight motor pattern, the question arises of whether other parameters are influenced by the removal of the wing tegulae and whether these also return towards normal during the recovery process. That is, is there a recovery of the whole flight motor pattern? In the absence of additional data we cannot answer this question. However, several behavioural observations indicate that the whole flight behaviour is influenced and does recover. First, when animals were flown on a roundabout the flight speed and the flight duration decreased immediately after the removal of the wing tegulae, but both returned to their intact values during the time course of the recovery (A. Büschges and K. G. Pearson, unpublished results). Second, most of the animals were able to fly freely across a room and generate lift 2 weeks after the operation. No animal was able to do this during the first days after removal of the tegulae (A. Büschges and K. G. Pearson, unpublished results).

An interesting aspect of the recovery process is that it occurred in the absence of active flight behaviour. This was shown most clearly for a group of 11 animals that were not flown during the 14-day recovery period. The average values of the parameters we measured were close to normal (average WBF, 18.7±1.7 Hz; average D–E interval of the hindwing muscles, 29.3±5.6 ms) and not significantly different from those in the group of animals that were flown repeatedly during the recovery period. Although this result demonstrated that flight activity is not required for the recovery, we have not yet ruled out the possibility that the rate of recovery could be influenced by active flight behaviour. The results of Möhl (1988, 1989) on short-term adaptations in the steering muscles of the flying locust indicate that this could occur.

Another important question is whether the described adaptive modifications of the motor pattern following removal of the tegulae have any functional relevance in the day-to-day life of the locust. Obviously the loss of all wing tegulae is a very unnatural situation. Nevertheless, the adaptive phenomenon following removal of the tegulae strongly resembles the adaptive events that occur after the loss of a cercus in the cockroach (Vardi and Camhi, 1982a) and the loss of an ear in the cricket (Schildberger and Huber, 1988; Schmitz, 1989). Thus, the phenomenon we

have observed is not unique and is similar to that found in systems where the functional relevance for recovery is clear. Since the generation of the flight motor pattern depends strongly on proprioceptive feedback (Wolf and Pearson, 1988), it is conceivable that the adaptation following removal of the tegulae is a reflection of the fact that the flight system is continuously using proprioceptive information to modify the motor pattern to make it appropriate for the current state of the flight apparatus (e.g. muscle strength, cuticular hardness, receptor transduction) and for body characteristics (e.g. weight, shape). All these factors change in the course of adult development and there is no *a priori* reason for expecting that sensory feedback would be utilized in the same manner throughout the adult life of an animal. It is quite conceivable that during adult development the strength and/or timing of tegula input is altered (owing to changes in wing mechanics and/or the properties of the tegulae themselves) and this must be compensated for by alterations in other elements of the pattern-generating network. It would be of interest, therefore, to examine the timing and strength of tegula input throughout adulthood. If these changes occur then it is to be expected that the immediate effects of removal of the tegulae may vary depending on the age of the animal.

Mechanisms underlying the adaptive properties in the flight system

Although the objectives of this study did not include the determination of the mechanisms underlying recovery, some data are relevant to this issue. The major change to occur during recovery was a change in the timing of the synaptic input to the elevator motoneurones. Immediately after removal of the tegulae the initial depolarization in the elevator motoneurones was slowed (Fig. 8B). In recovered animals the rate of depolarization in the elevator motoneurones was fast and comparable to that in intact animals (Fig. 8C). What causes this increase in the depolarization rate during the recovery?

There are potentially three general answers to this question: (i) there are alterations in the properties of the central network generating elevator activity; (ii) the excitatory effect of the wing tegulae is entirely replaced by input from other sense organs; (iii) the changes in the flight system underlying the recovery represent a combination of i and ii. In the third case, the sensitivity of the central elevator network would be altered so that input from the remaining afferents is sufficient to initiate a rapid depolarization in elevator motoneurones. Our results have excluded the first of these possibilities, as deafferentation of the flight system in recovered animals results in a slowing in the rate of depolarization in elevator motoneurones (Fig. 10). In addition, there was no significant difference in the measured parameters of the flight motor pattern (WBF, D–E interval, elevator phase, flight duration) between recovered deafferented and normal deafferented animals (Fig. 9). Preliminary data have also indicated that the second hypothesis is probably insufficient. So far we have not been able to mimic the effect of removal of the tegulae by ablating single wing receptors in recovered animals. Thus, our current belief is that the recovery of the flight motor pattern after removal of the

tegulae does involve alteration of some properties of the central network generating elevator activity, but that these alterations are only expressed in the presence of afferent input from the wing proprioceptors.

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