

RECOVERY OF THE FLIGHT SYSTEM FOLLOWING ABLATION OF THE TEGULAE IN IMMATURE ADULT LOCUSTS

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Accepted 6 March 1996

Summary

The capacity of the flight system to recover from ablation of the tegulae was studied in immature adult *Locusta migratoria* and compared with recovery in mature adults. We ablated the hindwing tegulae or all tegulae in adult locusts either 1 day after the imaginal moult (immature locusts) or 2 weeks after the imaginal moult (mature locusts). We monitored recovery throughout the recovery period by using a stroboscope to measure the wingbeat frequency of tethered locusts. In addition, we measured other parameters of the flight motor pattern using electromyographic electrodes implanted into recovered locusts. Both methods of monitoring recovery yielded the

same results. There was no reduction, during adult maturation, in the capacity of the locust flight system to recover from the loss of these proprioceptors. Plasticity of the locust flight system was therefore maintained in the mature adult locust. This suggests that the flight system is not fixed and simply implemented when the locust reaches adulthood, but that the circuitry can be remodelled throughout the animal's life to produce behaviour adapted to the needs and constraints of the individual.

Key words: insect, proprioceptor, functional recovery, development, plasticity, locust, *Locusta migratoria*.

Introduction

Injuries or manipulations sustained during critical periods of development are often compensated for more rapidly and more completely than injuries sustained in mature animals. For example, immature but not mature barn owls can recover completely from the effects of monaural occlusion on their ability to localize sounds in space (Knudsen *et al.* 1984). Also, functional recovery after spinal cord transection in the chick embryo is limited to a period that ends concurrently with the onset of myelination in the spinal cord during development (Hasan *et al.* 1991). Most recovery from injury is mediated by regeneration of severed axons and/or growth of neurones that escaped damage. Development is a period of neuronal growth and, in the rat, similar mechanisms appear to promote sprouting of the corticospinal tract both during development and during regeneration in the adult (Schnell *et al.* 1994).

The flight system of *Locusta migratoria* matures during the 2 weeks following adult ecdysis (Kutsch, 1973). Concurrently, there is growth (Altman *et al.* 1978) and reorganization (Gray and Robertson, 1996) of sensory afferents, the thoracic ganglia expand (Sbrenna, 1971) and flight system interneurones increase in size (Gee and Robertson, 1994). It has also been shown that activity of the forewing stretch receptor increases with maturation (Gray and Robertson, 1994). We were interested in determining whether this period of maturation constitutes a critical period of increased plasticity in the locust

when neural parameters can be better adjusted to conform to an individual's idiosyncratic morphology and physiology.

The locust flight system can recover from the loss of the wing proprioceptors (Kutsch, 1974), specifically of the tegulae (Büschges and Pearson, 1991). The tegulae are external proprioceptors found at the base of each wing that, along with other sensory organs, monitor wing kinematics and modulate the phasic firing of flight motoneurones during flight (Wolf and Pearson, 1988). Removal of the hindwing tegulae causes an immediate decrease in wingbeat frequency and affects the phase relationship of antagonistic wing muscle groups (Kutsch, 1974; Büschges and Pearson, 1991). During the 2 weeks following removal of the hindwing tegulae in mature locusts, the flight motor pattern returns towards normal. This recovery is mediated by the forewing tegulae which sprout and form new connections onto flight interneurones, taking over the role of the missing hindwing tegulae (Büschges *et al.* 1992a,b).

A previous study by Kutsch (1974) investigated whether wing sense organs influenced maturation of the locust flight system. The hindwing tegulae were ablated in nine male locusts on days 5–7 after imaginal ecdysis. The wingbeat frequency (WBF) increased from the ablated level but did not appear to recover fully (see Fig. 2 of Kutsch, 1974). The small number of animals and the relatively late time during maturation that Kutsch ablated the tegulae in the immature locusts did not allow us to determine whether the capacity for

recovery of the flight system demonstrated in mature locusts (Büschges and Pearson, 1991) was also present in immature locusts. We tested this by ablating the tegulae in immature adult locusts and monitoring recovery of the flight system using parameters of the motor pattern. We also repeated the experiments of Büschges and Pearson (1991) with mature animals to serve as the appropriate and necessary control for our investigation.

Materials and methods

Adult male *Locusta migratoria* L. were collected on the day of imaginal ecdysis from the colony maintained at Queen's University. Ablations were performed 1 day after emergence (immature group) or after 2 weeks of maturation (mature group). The locusts were numbered by writing with a permanent marker on their forewings and were housed together during each experiment in a cage at 30 °C.

Ablations

To ablate a hindwing tegula, the locust was held in one hand, dorsal-side-up, and the forewing was extended by placing it against a pin stuck in a cork base under a dissecting microscope. This exposed the hindwing tegula, which was then cauterized using a fine-tipped soldering iron under visual control. In one experiment, the tegulae were also removed by pulling them off with fine forceps or cutting them off with fine scissors. The forewing tegulae were ablated in a similar fashion by holding the locust on its side and gently pushing the pronotum forward to expose the tegulae. Locusts in control groups were handled in the same way as the experimental animals, including manipulation of the wings to expose the tegulae. The locusts in the sham-operated groups were handled, the tegulae exposed, and the cuticle was burned below the wing hinge.

Measuring wingbeat frequency

To measure wingbeat frequency (WBF), a locust was removed from the warm cage (approximately 30 °C), tethered dorsal-side-up in a wind-tunnel (air speed approximately 2.5 m s⁻¹) and illuminated with a stroboscope. The frequency of the stroboscope at which the wings appeared motionless was taken to be the WBF. Immature locusts will only beat their wings for a short period. To remain consistent, we recorded WBF as soon as a stable wingbeat was evident, within the first minute of tethered flight. Care was taken to ensure that we did not record fractions or multiples of WBF.

We chose to use a stroboscope for measuring WBF because it requires very little manipulation of the animal and the WBF can be determined rapidly. It required 1–2 min total time per animal to obtain a measure of WBF, allowing us to record daily from a large number of individuals.

Monitoring the flight motor pattern

To monitor other parameters of the flight motor pattern, electromyographic (EMG) recordings were taken from

hindwing and forewing depressor and elevator muscles on one side of the animal. The prothoracic and mesothoracic legs were removed by cutting at the autotomy plane and the metathoracic tarsi were removed to prevent the locusts from interfering with the electrode wires. The locusts were then attached to a tether by the pronotum using hot wax. Fine (100 µm) Teflon-coated copper wires were inserted through small holes in the cuticle and waxed into place. A silver indifferent electrode was placed under the pronotum just behind the head. Muscles 83 and 97 in the forewing and muscles 113 and 127 in the hindwing were used to record elevator and depressor muscle activity from the fore- and hindwings, respectively (nomenclature according to Snodgrass, 1929; for electrode placement, see Pearson and Wolf, 1987). The locust was then placed dorsal-side-up in front of a wind-tunnel (air speed approximately 2.5 m s⁻¹) and the activity of the flight muscles was recorded.

The records were digitized and the time of activation of each muscle was extracted from at least 100 cycles (usually 500 cycles), collected at different times during a flight sequence, using DataWave Technologies A/D conversion and software. The time values were used to calculate the average depressor–depressor and depressor–elevator intervals for each wing and the phase of elevator activity within the depressor cycle for each locust. We also calculated mean phase values for some individuals using circular statistics (according to Horsmann *et al.* 1983) and found no difference between these means and the arithmetic means because of the low variability of the phase values within individuals. The time delay from activation of the hindwing depressor to activation of the forewing depressor was also calculated, as was the frequency of hindwing depressor activation.

Tests of methodology

Two preliminary tests were carried out to investigate aspects of our methodology. Waldron (1968) has shown that locust wingbeats can become coupled to a flashing stroboscope, indicating that measuring WBF using a stroboscope may be problematic. To address this, an EMG electrode was implanted into a forewing depressor muscle of an adult male locust and the locust was suspended in the wind-tunnel. A digital oscilloscope that recorded frequency (Gould DSO 420 or DSO 640) was used to view the EMG records and the depressor frequency was recorded to obtain a measure of WBF. The stroboscope was then switched on and the WBF was measured using the stroboscopic method. When adjusting the frequency of the flashes to match the wingbeat, we were unaware of the frequency of the stroboscope so the measurements were effectively blind until the wings appeared stationary.

Our usual method of ablating the tegulae using cautery was different from that of Büschges and Pearson (1991), who cut away the tegulae. We ablated the tegulae using three different methods to determine whether the ablation method affected the initial decrease in WBF and the subsequent recovery. Immature locusts had their hindwing tegulae ablated by cauterization ($N=8$), by pulling them off with forceps ($N=8$) or by cutting them off with fine scissors ($N=8$). WBF was then

monitored daily with the stroboscope for 9 days. There were no differences between the WBFs of these three groups (results not shown). We were therefore confident that cauterizing the tegulae did not affect the subsequent recovery.

Comparing the time course of recovery of wingbeat frequency between immature and mature locusts

For the first experiment, we collected male locusts that were either 2 weeks past the imaginal moult or male locusts that had ecdysed the day before. WBF was measured using the stroboscope for each animal, and the animal was given a number and marked. Within each age class, the locusts were segregated into three groups with the same mean WBFs. One group then had the hindwing tegulae ablated, while the other two groups were sham-operated or handled (controls). The WBF was again recorded for each animal and during the next 2 weeks WBF was monitored daily or every second or third day. At the end of the 2 week period, WBF was recorded, the forewing tegulae were ablated from all locusts and WBF was measured again. The WBF was monitored several times over the next week and the trial was ended.

Any locusts that died or became too damaged to beat their wings (usually due to cannibalism) during the trial were removed from the analysis. We also removed from the final analysis any locusts with their hindwing tegulae ablated that did not show a decrease in WBF of more than 2 Hz following ablation of the forewing tegulae. This was carried out to ensure that we were comparing recovery due to functional substitution of the forewing tegulae as described previously (Büschges and Pearson, 1991; Büschges *et al.* 1992a,b).

This protocol was repeated and the results of the trials were pooled.

To compare recovery after ablation of all the tegulae, newly ecdysed and mature adult male locusts were collected and divided into groups as above. WBF was again monitored with the stroboscope before and after the ablations and over the next 3 weeks. This experiment was performed in a single trial.

Comparing parameters of the motor pattern in immature and mature recovered locusts

This experiment essentially repeated the previous experiment in addition to using four EMG electrodes to examine other parameters of the flight motor pattern in the recovered locusts. Immature and mature locusts were collected but the collection was staggered by a few days so that the EMG recordings were made at about the same length of time after the ablations. The locusts were again divided into three groups based on their initial WBFs. One group was halved, and half the locusts were sham-operated while the other half were handled. There were no differences between the results for these locusts, so their results were combined as the sham group. The remaining two groups had either the hindwing tegulae ablated or all tegulae ablated. WBF was monitored with the stroboscope several times during a 3–3.5 week recovery period. Over the next several days, EMG recordings were taken from all individuals; then, without removing the

EMG wires, the forewing tegulae were pulled off all locusts that had forewing tegulae and a second set of EMG recordings were stored from all individuals.

The acute effects of ablating the tegulae on motor pattern parameters were recorded from mature locusts. We also attempted to do this in the immature (1 day post-ecdysis) locusts; however, it was practically impossible to induce 1-day-old adult locusts to beat their wings after inserting the four EMG electrodes and especially after ablation of the tegulae.

Statistical analyses

All data are expressed as mean \pm standard error of the mean (S.E.M.). The numbers of locusts used in each comparison are given in the figure legends. Statistical analyses were performed with the aid of SigmaStat software.

Results

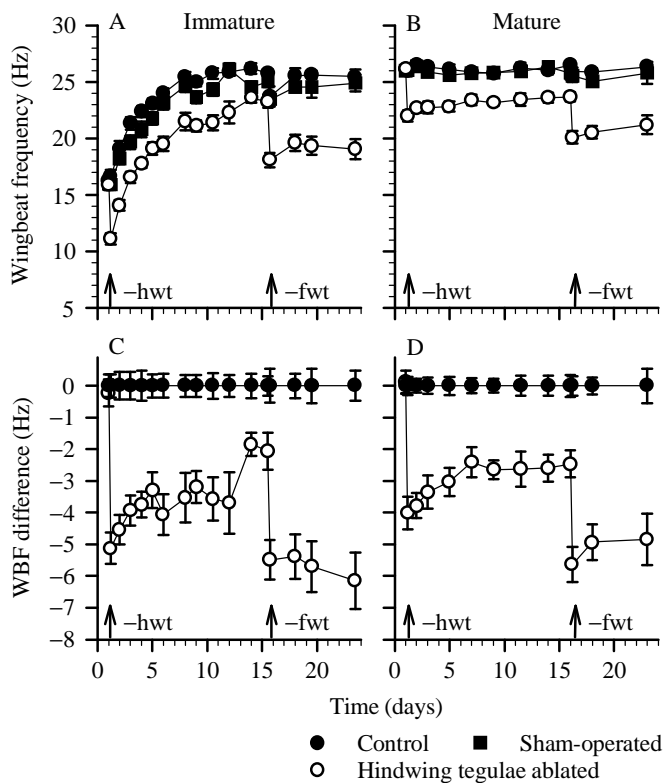
Effect of the stroboscope on wingbeat frequency

Wingbeat frequency (WBF) was measured both electromyographically without the stroboscope and in the same individuals using the stroboscope. There was no difference in the WBF measured with and without the flashing stroboscope (20.75 ± 0.88 Hz, 21.24 ± 0.90 Hz, respectively; $P=0.07$, $t=-2.0$; paired t -test). We therefore decided to continue using the stroboscope for measuring WBF, particularly when it was necessary to record repeatedly from the same individuals.

Recovery of wingbeat frequency in immature and mature locusts

Ablation of the hindwing tegulae caused the WBF to decrease in both immature and mature adult locusts, whereas the WBF did not decrease after treatment in the control and the sham-operated locusts (Fig. 1A,B). After ablating the hindwing tegulae, the WBF decreased by 5.03 ± 0.45 Hz in the immature locusts and by 4.15 ± 0.42 Hz in the mature locusts. There was no difference in the effect of ablating the hindwing tegulae between the immature and mature adult locusts ($P=0.2$, $t=-1.4$; t -test). Throughout the 2 week recovery period, the average WBF of the locusts with their hindwing tegulae ablated was always lower than the average WBFs of the control and sham-operated locusts. The WBFs of the control and sham-operated mature locusts remained constant throughout the experiment (Fig. 1B), while the immature locusts showed an increase in WBF (Fig. 1A). The increase in WBF during the first 2 weeks after the imaginal moult defines the period of maturation of the flight system (Kutsch, 1973, 1974).

To facilitate comparison of the amount of recovery between immature and mature locusts, the control and sham-operated groups were combined and the differences between the means of the non-ablated and the ablated groups were plotted (Fig. 1C,D). Most recovery of the WBF occurred in the first 6 days after the ablations. By day 15/16 of the experiment, there was no significant difference between the recovered WBFs of the immature and mature locusts, all of which were fully mature by the time of the test (Fig. 1; $P=0.6$, $t=-0.5$; t -test).



When the forewing tegulae were ablated in the locusts that had recovered from ablation of the hindwing tegulae, the WBF decreased by 5.21 ± 0.41 Hz in the immature group and by 3.60 ± 0.33 Hz in the mature group (Fig. 1C,D). The decrease in WBF after ablation of the forewing tegulae in the immature ablated locusts was significantly greater than the decrease in WBF of the mature ablated locusts (Fig. 1A,B, $P=0.006$, $t=2.96$; t -test). This suggested that there was a greater capacity of the forewing tegulae to take over from the hindwing tegulae in immature adult locusts, which is inconsistent with the similarity between the recovered WBFs. However, comparison of the differences in mean WBF between the intact locusts and the locusts that had their hindwing tegulae ablated (Fig. 1C,D) indicated that ablation of the forewing tegulae had a similar effect in the immature and mature groups. The relative effects of ablating the forewing tegulae were therefore the same in the immature and mature groups, which is congruous with their similar recovery of WBF. The sham-operated and control groups recovered quickly after the forewing tegulae ablations, but the WBF of the locusts lacking their hindwing tegulae did not recover (Fig. 1).

The extent and speed of recovery of WBF from ablation of all four tegulae was the same in immature and mature locusts (Fig. 2). The WBFs of the ablated groups at the end of the recovery period were not different (Fig. 2A,B; $P=0.3$, $t=-1.1$; t -test). After recovery from the ablation of all four tegulae, the WBFs of the immature and mature ablated locusts were, on average, 3.4 Hz and 3.0 Hz lower than the respective intact WBFs (Fig. 2C,D). Immature and mature adult locusts therefore have the same capacity for recovery of WBF either

after ablation of the hindwing tegulae only (Fig. 1) or after ablation of all four tegulae (Fig. 2).

Motor pattern parameters in recovered locusts

Parameters of the motor pattern were determined from EMG records taken from recovered locusts that were sham-operated, had their hindwing tegulae ablated or had all four tegulae ablated. WBF was monitored stroboscopically for approximately 3 weeks after the ablations and the recovery of WBF was essentially the same as shown in Figs 1 and 2 (data not shown). The final, recovered WBFs were the same for the locusts that had their hindwing tegulae ablated either when immature or when mature and were also the same between the two groups that had all four tegulae ablated (Fig. 3). The WBFs of the control groups were different from each other, however, so that the mature locusts that had their hindwing tegulae ablated appeared to recover more completely than the group ablated while immature (Fig. 3).

Table 1 presents the motor pattern parameters measured from immature and mature recovered locusts, including the parameters measured from acutely ablated (measured on the day of the ablation) mature locusts. There was no consistent

after ablation of the hindwing tegulae only (Fig. 1) or after ablation of all four tegulae (Fig. 2).

Motor pattern parameters in recovered locusts

Parameters of the motor pattern were determined from EMG records taken from recovered locusts that were sham-operated, had their hindwing tegulae ablated or had all four tegulae ablated. WBF was monitored stroboscopically for approximately 3 weeks after the ablations and the recovery of WBF was essentially the same as shown in Figs 1 and 2 (data not shown). The final, recovered WBFs were the same for the locusts that had their hindwing tegulae ablated either when immature or when mature and were also the same between the two groups that had all four tegulae ablated (Fig. 3). The WBFs of the control groups were different from each other, however, so that the mature locusts that had their hindwing tegulae ablated appeared to recover more completely than the group ablated while immature (Fig. 3).

Table 1 presents the motor pattern parameters measured from immature and mature recovered locusts, including the parameters measured from acutely ablated (measured on the day of the ablation) mature locusts. There was no consistent

Table 1. Comparison of EMG parameters of the flight motor pattern in immature and mature locusts after recovery from tegulae ablation and the response to acute ablations

Parameter	Immature	Mature	Significance, <i>t</i> -test
Hindwing-forewing delay (ms)			
Sham recovered	4.7±0.6 (37)	5.2±0.6 (28)	NS (<i>P</i> =0.5)
Sham recovered/-fwt	4.7±0.6 (37)	4.7±0.8 (16)	NS (<i>P</i> =1)
-hwt acute ablation	–	4.8±1.6 (9)	
-hwt recovered	7.0±0.5 (22)	8.2±0.7 (20)	NS (<i>P</i> =0.2)
-hwt recovered/-fwt	7.0±0.8 (22)	5.1±1.3 (14)	NS (<i>P</i> =0.2)
-all teg acute ablation	–	0.96±1.8 (9)	
-all teg recovered	7.5±0.9 (15)	6.7±0.9 (14)	NS (<i>P</i> =0.5)
Forewing depressor-depressor interval (ms)			
Sham recovered	52.2±1.3 (37)	58.2±1.8 (28)	<i>P</i> =0.008
Sham recovered/-fwt	55.6±1.5 (37)	53.5±4.0 (16)	NS (<i>P</i> =0.5)
-hwt acute ablation	–	74.8±3.4 (9)	
-hwt recovered	54.4±1.8 (22)	59.7±2.9 (20)	NS (<i>P</i> =0.1)
-hwt recovered/-fwt	64.8±2.6 (22)	70.8±2.8 (14)	NS (<i>P</i> =0.1)
-all teg acute ablation	–	83.3±3.9 (9)	
-all teg recovered	67.8±2.6 (15)	75.0±3.8 (14)	NS (<i>P</i> =0.1)
Forewing depressor-elevator interval (ms)			
Sham recovered	22.9±0.9 (37)	23.6±1.0 (28)	NS (<i>P</i> =0.6)
Sham recovered/-fwt	26.2±0.9 (37)	24.8±1.1 (16)	NS (<i>P</i> =0.4)
-hwt acute ablation	–	33.3±2.4 (9)	
-hwt recovered	21.7±0.8 (22)	24.8±0.9 (20)	<i>P</i> =0.01
-hwt recovered/-fwt	32.7±1.5 (22)	38.4±2.2 (14)	<i>P</i> =0.04
-all teg acute ablation	–	50.1±4.3 (9)	
-all teg recovered	35.1±1.8 (15)	39.3±3.0 (14)	NS (<i>P</i> =0.2)
Forewing elevator phase, <i>E/D</i>			
Sham recovered	0.439±0.011 (37)	0.408±0.014 (28)	NS (<i>P</i> =0.07)
Sham recovered/-fwt	0.472±0.009 (37)	0.441±0.016 (16)	NS (<i>P</i> =0.09)
-hwt acute ablation	–	0.466±0.027 (9)	
-hwt recovered	0.403±0.060 (22)	0.428±0.018 (20)	NS (<i>P</i> =0.3)
-hwt recovered/-fwt	0.516±0.018 (22)	0.544±0.024 (14)	NS (<i>P</i> =0.3)
-all teg acute ablation	–	0.518±0.015 (15)	
-all teg recovered	0.518±0.015 (15)	0.523±0.020 (14)	NS (<i>P</i> =0.9)
Hindwing depressor-depressor interval (ms)			
Sham recovered	52.1±1.3 (37)	58.2±1.8 (28)	<i>P</i> =0.007
Sham recovered/-fwt	55.7±1.5 (37)	56.8±1.8 (16)	NS (<i>P</i> =0.7)
-hwt acute ablation	–	74.8±3.4 (9)	
-hwt recovered	54.4±1.8 (22)	59.4±3.0 (20)	NS (<i>P</i> =0.2)
-hwt recovered/-fwt	65.0±2.6 (22)	70.9±2.8 (14)	NS (<i>P</i> =0.1)
-all teg acute ablation	–	83.1±4.0 (9)	
-all teg recovered	67.9±2.7 (15)	75.0±3.8 (14)	NS (<i>P</i> =0.1)
Hindwing depressor-elevator interval (ms)			
Sham recovered	25.8±0.9 (37)	27.1±1.0 (28)	NS (<i>P</i> =0.3)
Sham recovered/-fwt	28.3±0.9 (37)	27.0±0.9 (16)	NS (<i>P</i> =0.4)
-hwt acute ablation	–	42.2±2.7 (9)	
-hwt recovered	27.8±1.0 (22)	33.0±1.1 (20)	<i>P</i> =0.0008
-hwt recovered/-fwt	37.5±1.8 (22)	41.5±2.8 (14)	NS (<i>P</i> =0.2)
-all teg acute ablation	–	52.3±5.4 (9)	
-all teg recovered	42.1±1.9 (15)	45.8±3.3 (14)	NS (<i>P</i> =0.3)
Hindwing elevator phase, <i>E/D</i>			
Sham recovered	0.495±0.009 (37)	0.471±0.014 (28)	NS (<i>P</i> =0.2)
Sham recovered/-fwt	0.508±0.008 (37)	0.483±0.016 (16)	NS (<i>P</i> =0.1)
-hwt acute ablation	–	0.567±0.021 (9)	
-hwt recovered	0.525±0.013 (22)	0.566±0.021 (20)	NS (<i>P</i> =0.1)
-hwt recovered/-fwt	0.581±0.019 (22)	0.588±0.030 (14)	NS (<i>P</i> =0.8)
-all teg acute ablation	–	0.622±0.046 (9)	
-all teg recovered	0.623±0.017 (15)	0.611±0.021 (14)	NS (<i>P</i> =0.7)

–hwt, hindwing tegulae ablated; –fwt, forewing tegulae ablated; –all teg, all four tegulae ablated; *D*, depressor; *E*, elevator; NS, no significant difference.

Acute ablation, value measured on day 1 immediately following ablation; recovered, value measured 23–26 days following ablation.

Values are means ± S.E.M. (*N*).

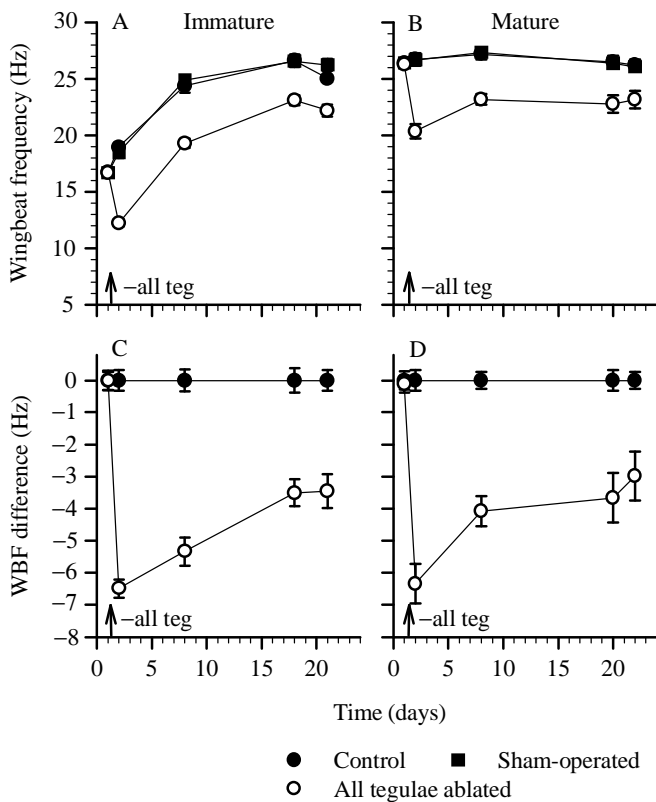


Fig. 2. Recovery of wingbeat frequency (WBF) following ablation of all tegulae (-all teg) in immature and mature adult locusts. (A) Immature locusts were treated 1 day after imaginal ecdysis. The locusts were handled (control), sham-operated or had all four tegulae ablated. Control group $N=11$; sham-operated group $N=12$; ablated group $N=23$. (B) Mature locusts were treated 16–18 days after the imaginal moult in the same manner as the immature locusts. Both the immature and mature locusts appeared to recover slightly from ablation of all four tegulae. Control group $N=11$; sham-operated group $N=11$; ablated group $N=20$. (C,D) To compare the extent of recovery, the differences in the mean WBFs were plotted (see Fig. 1 legend). There was no difference in the rate or extent of recovery between the immature (C) and mature locusts (D). Values are means \pm S.E.M.

significant difference between the parameters obtained from locusts that recovered from tegula ablations performed when they were immature or when they were mature (Table 1).

Fig. 4 shows the immediate effects of ablating either the hindwing tegulae or all four tegulae on key parameters of the flight motor pattern of mature locusts. The changes are the same as those described by Wolf and Pearson (1988), and we have shown these to illustrate that our ablations had the expected effects on the flight motor pattern. The cycle frequency of hindwing depressor activity decreases after the ablations (Fig. 4A), corresponding to the decrease in WBF seen in Figs 1 and 2. The delay in the time of activity in the forewing depressor relative to activity of the hindwing depressor did not change significantly in response to the ablations (Fig. 4B). The phase of forewing elevator activity within the forewing depressor cycle increased after ablation of all the tegulae (Fig. 4C) and the phase of hindwing elevator

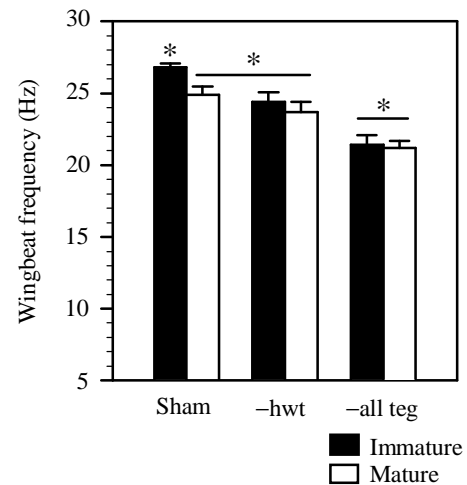


Fig. 3. Wingbeat frequencies of recovered locusts measured stroboscopically 22 or 24 days after handling or sham-operating (sham), ablating the hindwing tegulae (-hwt) or all tegulae (-all teg) in immature or mature locusts, respectively. Other flight parameters from the same animals are shown in Table 1 and Figs 5 and 6. Values are means \pm S.E.M., see Table 1 for values of N : $F=14.9$, $P < 0.001$; one-way ANOVA; asterisks indicate significant differences between groups, horizontal bars indicate no significant difference using multiple comparisons, $P < 0.05$, Student–Newman–Keuls test.

activity within the hindwing depressor cycle increased after all the tegulae were ablated (Fig. 4D).

Figs 5 and 6 show the same parameters of the flight motor pattern from control and recovered locusts that were ablated when immature and mature, respectively. Immature adult locusts that had only their hindwing tegulae ablated on day 1 showed recovery of most parameters of the flight motor pattern; however, the motor pattern remained significantly different in the locusts that had all four tegulae ablated (Fig. 5). The apparent lack of recovery of the hindwing-to-forewing depressor delay (Fig. 5B) must be viewed cautiously as there was no effect on this parameter of the acute hindwing tegula ablations (Fig. 4B). The motor pattern in the immature locusts with intact tegulae (sham-operated) was not affected by ablating the forewing tegulae (Fig. 5). In contrast, ablation of the forewing tegulae in locusts that had recovered from hindwing tegula ablations did affect the motor pattern (Fig. 5A,C,D). This flight motor pattern was the same as that recorded from locusts that had 'recovered' from ablation of all four tegulae (Fig. 5A,C,D).

Mature locusts showed the same pattern of flight parameter recovery as the immature locusts (Fig. 6). The hindwing elevator phase did not appear to recover from the hindwing tegula ablations; however, as the timing of elevator activity is coupled between the wings (e.g. Pearson and Wolf, 1987), this is a reflection of the longer hindwing-to-forewing delay in this group (Fig. 6C,D, -hwt recovered group). Ablating the forewing tegulae did not change the motor pattern in the sham-operated, intact locusts but did change the motor pattern of locusts that had recovered from ablation of the hindwing

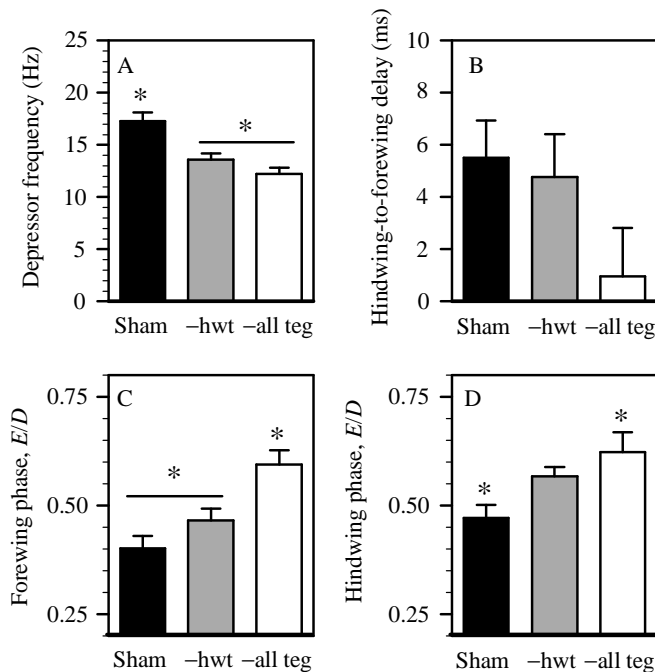


Fig. 4. The acute effects immediately after ablation of the tegulae on key parameters of the flight motor pattern in mature locusts. Electromyographic (EMG) electrodes were used to monitor activity in four flight muscles on one side of each locust. One depressor and one elevator muscle were monitored from the forewing (M97 and M83) and the hindwing (M127 and M113). (A) Ablation of the hindwing tegulae (-hwt) or all four tegulae (-all teg) resulted in a significant decrease in the frequency of hindwing depressor activation (EMG frequency). Sham, sham-operated. (B) Ablation of the tegulae had no significant effect on the delay of activation of the forewing depressor from activation of the hindwing depressor. The activation of elevator activity is coupled between the two wings. The phase of elevator (*E*) activation within the depressor (*D*) cycle of the forewing (C) and hindwing (D) increases after ablation of the tegulae. The phase values after ablation of all four tegulae are significantly different from the phase values measured when the locusts were intact. Values are means + S.E.M.; *N* values are given in Table 1: (A) $F=15.7$, $P<0.0001$; (B) $F=2.22$, $P=0.13$; (C) $F=11.4$, $P=0.0003$; (D) $F=5.0$, $P=0.02$; one-way ANOVA; asterisks indicate significant differences between groups at $P<0.05$, horizontal bars indicate no significant difference using multiple comparisons, Student–Newman–Keuls test.

tegulae (Fig. 6). Again, after the forewing tegula ablations, the motor pattern of the group with ablated hindwing tegulae changed to resemble that of locusts that had all four tegulae ablated (Fig. 6).

Discussion

The recovery after ablation of the hindwing tegulae that we found for mature adult locusts was essentially the same as has been reported previously (Büschges and Pearson, 1991). We found that WBF measured using the stroboscope recovered, although it remained significantly lower in the locusts that had their tegulae ablated. When the forewing tegulae were also

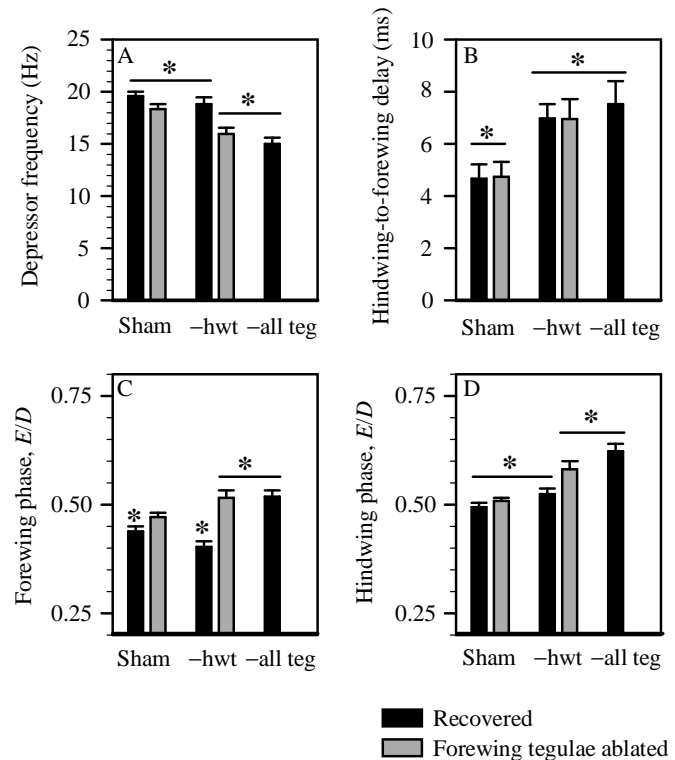


Fig. 5. Recovery of select parameters of the flight motor pattern measured 24–26 days after ablation of the tegulae in immature adult locusts. The same parameters were also measured immediately after ablation of the forewing tegulae in the same locusts (complete data set in Table 1). Ablation of the forewing tegulae in sham-recovered locusts had no effect on (A) the hindwing depressor frequency, (B) the hindwing-to-forewing delay of depressor activation, or (C) the phase of elevator (*E*) activity within the depressor (*D*) cycle of the forewing and (D) the phase of elevator activity within the depressor cycle of the hindwing. The locusts that recovered from ablation of all four tegulae showed significant differences in all parameters when compared with the control motor pattern. Recovery from ablation of the hindwing tegulae was apparently complete and there were no differences from the control parameters except for the hindwing-to-forewing delay. After ablation of the forewing tegulae, the motor pattern of the locusts recovered from ablation of the hindwing tegulae was no different from the pattern in the locusts that had all four tegulae ablated. Values are means + S.E.M., see Table 1 for values of *N*: (A) $F=11.8$, $P<0.0001$; (B) $F=4.44$, $P=0.002$; (C) $F=12.6$, $P<0.0001$; (D) $F=15.7$, $P<0.0001$; one-way ANOVA; asterisks indicate significant differences between groups at $P<0.05$, horizontal bars indicate no significant difference, Student–Newman–Keuls test.

subsequently ablated, the WBF decreased in the locusts that had recovered from ablation of the hindwing tegulae but did not change in the control and sham-operated locusts. When we used other flight system parameters to monitor recovery, the same conclusions could be drawn. In general, all parameters of the flight motor pattern showed recovery after ablation of the hindwing tegulae and incomplete recovery after ablation of all four tegulae. The hindwing-to-forewing depressor delay did not change in a consistent way immediately after the tegula ablations or after the recovery period, possibly indicating this

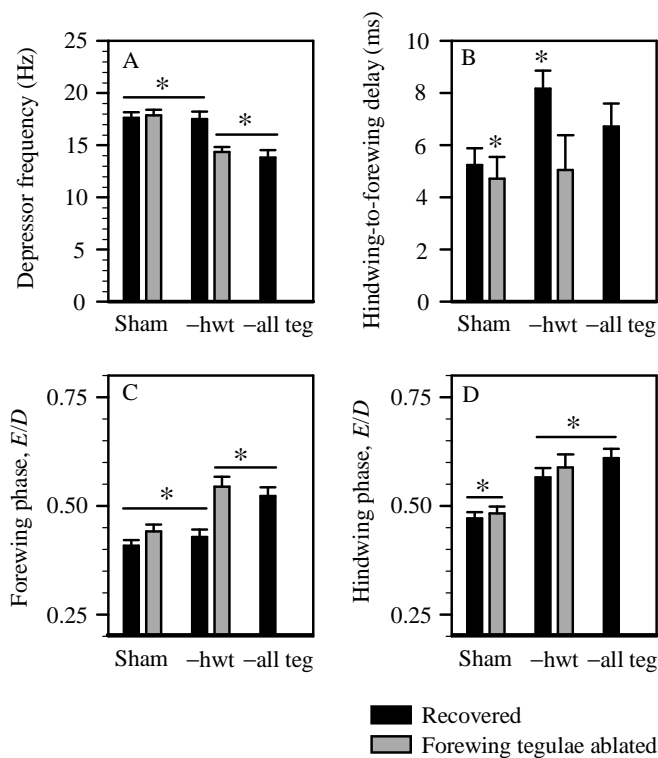


Fig. 6. Recovery of select parameters of the flight motor pattern measured 23–26 days after ablation of the tegulae in mature adult locusts. The same parameters were also measured immediately after ablation of the forewing tegulae in the same locusts (complete data set in Table 1). Ablation of the forewing tegulae in sham-recovered locusts had no effect on (A) the hindwing depressor frequency, (B) the hindwing-to-forewing delay of depressor activation, or (C) the phase of elevator (E) activity within the depressor (D) cycle of the forewing and (D) the phase of elevator activity within the depressor cycle of the hindwing. The locusts that recovered from ablation of all four tegulae showed significant differences in all parameters when compared with the control motor pattern. Recovery from ablation of the hindwing tegulae was apparently complete and there were no differences from the control parameters except for the hindwing-to-forewing delay. After ablation of the forewing tegulae, the motor pattern of the locusts recovered from ablation of the hindwing tegulae was no different from the pattern in the locusts that had all four tegulae ablated. Values are means + S.E.M., see Table 1 for values of N : (A) $F=8.38$, $P<0.0001$; (B) $F=2.99$, $P=0.02$; (C) $F=11.1$, $P<0.0001$; (D) $F=10.2$, $P<0.0001$, one-way ANOVA; asterisks indicate significant differences between groups at $P<0.05$, bars indicate no significant difference, Student–Newman–Keuls test.

is a less critical parameter of the tethered flight motor pattern. Ablation of the forewing tegulae also had a greater effect on the motor pattern in locusts that had their hindwing tegulae removed than in controls. In the mature locusts, the forewing tegulae were therefore taking over the role of the hindwing tegulae during recovery of the flight system, presumably using the same mechanisms that have been described elsewhere (Büschges *et al.* 1992a,b).

We found that recovery of the flight system after ablation of the hindwing tegulae in immature locusts was similar to

recovery in the mature locusts and, in addition, that subsequent ablation of the forewing tegulae had the same effect in the immature locusts. This suggests that recovery in immature adult locusts is mediated by the same mechanisms as recovery in mature locusts, although this has not been directly tested (Büschges *et al.* 1992a,b).

Following ablation of all four tegulae, we also saw some recovery of WBF. The recovery was again similar in locusts that were either immature or mature at the time of the ablations. The partial recovery after ablation of all four tegulae in the mature locusts confirms other work (Büschges and Pearson, 1991). The mechanism of this recovery has not been elucidated, although it seems likely that other proprioceptors mediate this recovery (Büschges and Pearson, 1991).

The fact that both the mature and immature flight systems are capable of recovering from ablation of the tegulae indicates one of two possibilities. If the mechanisms underlying maturation do reach a less flexible state with maturity, then there must be different mechanisms mediating recovery in mature animals. Alternatively, the mechanisms underlying maturation and recovery are the same and the flight system cannot be considered as reaching a fixed mature state. In either case, the capacity for recovery is unaltered with maturation.

The apparent amount of recovery of the flight system was somewhat greater when we used EMG recordings to monitor recovery than it was when we measured WBF using the stroboscope. Our average values for WBF in the intact mature locusts are also higher than have been reported by others (e.g. Kutsch, 1974; Büschges and Pearson, 1991). The locust flight system has been reported to couple to a stroboscope that is flashing at the frequency at which the wings are beating (Waldron, 1968). We found no difference between the depressor frequency recorded with EMG electrodes before turning on the stroboscope and the WBF measured with the stroboscope.

When measuring WBF with the stroboscope, we recorded the frequency as quickly as possible during the flight sequence, whereas the parameters recorded with the EMG electrodes were averages taken from several minutes of tethered flight. This difference in the time when the measurements were taken may account for the difference in the apparent amount of recovery found using the two methods. Tethered locusts do not need to support their own weight and are known to beat their wings on average more slowly than do locusts in free flight (Baker *et al.* 1981). Locusts are also able to adjust their flight motor pattern to maintain what they sense as straight flight when the parameters required to keep them oriented are artificially imposed under closed-loop conditions (Möhl, 1988). By recording WBFs early in each flight sequence, the WBF may have been fast both because the locusts were startled by the abrupt onset of wind stimulation and because the flight motor pattern had not yet settled into the lower-frequency operating range characteristic of tethered flight (see Gewecke and Kutsch, 1979). This could account for the difference in average cycle frequencies using the two methods. It is possible that the motor pattern produced during free flight is most similar to the motor pattern produced during initiation and the

early part of tethered flight. If this is the case, then the measurements made early in the flight sequence (e.g. using the stroboscope) may provide a more sensitive indication of the amount of recovery. Related to this, it has not been determined whether locusts that have recovered from hindwing tegula ablations are actually capable of free sustained flight. It would be interesting to examine this issue and to determine the extent to which recovery of the flight motor pattern permits flight.

We have found that immature adult locusts are able to recover after ablation of the hindwing tegulae and, to a lesser extent, after ablation of all the tegulae. The extent and the speed of recovery were the same as in mature adult locusts. We conclude that there is no reduction in the capacity of the adult locust flight system to recover from ablations of the hindwing tegulae during maturation. There does not appear to be a critical period of increased plasticity during adult maturation of the locust flight system. In contrast, the ability to remodel the flight circuit continues throughout the mature life of the insect, in a similar fashion to other circuits in arthropods and invertebrates (Murphey, 1986). The maturation period can thus be thought of as the time it takes for the flight system, including the neural circuitry, to reorganize in preparation for a new adult-specific behaviour. It has been suggested that maturation of the locust flight motor pattern involves activation of a preformed neuronal circuit around the time of imaginal ecdysis (Stevenson and Kutsch, 1986, 1988). Given the increasing information on growth in the nervous system during maturation, and the fact that it takes about 2 weeks for the reorganization to achieve equilibrium, our ideas about the extent to which the adult flight circuitry can be considered to be preformed during pre-adult stages may need to be modified.

Victoria Russell collected preliminary data for this study. We would like to thank W. Bendena, J. Gray, K. Shoemaker, H. Xu and the referees for constructive criticism of a previous version of this manuscript. We would also like to acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and the Advisory Research Council of Queen's University.

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