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Free-Flight Ability in Locusts Recovering from Partial Deafferentation

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We tested whether there is functional recovery of the flight system in adult male locusts following ablation of the hindwing tegulae. We also ablated only the forewing tegulae, a treatment that changes the motor pattern only slightly, which we expected to have little effect on flight ability. Some locusts were able to fly unrestrained either immediately after or following recovery from ablation of either the hindwing or the forewing tegulae. Nevertheless, there was an overall deficit in free-flight ability when 3–4 weeks were allowed for recovery. This is inconsistent with studies which have demonstrated complete recovery of the motor pattern for flight following ablation of the hindwing tegulae. We also found that flight ability was impaired in locusts that were recovering from ablation of the fore-

wing tegulae. This is surprising as there is no known acute effect of ablating the forewing tegulae on the flight motor pattern. We suggest that the discrepancy between the apparent recovery seen in the motor pattern and that seen in free-flight ability is due to the generation of an altered flight motor pattern during tethered flight. This motor pattern probably reflects some low motivational state and is the same in intact and recovered locusts. Consistent with this idea was our observation that the average mean lift generated was less than sufficient to support the weight, and was the same in intact and recovered locusts. The extent to which nervous systems are able to compensate for the loss of neurons following injury is of wide interest and may have implications for the effective treatment of victims of accidents or strokes. In many instances recovery from injury is accompanied by sprouting of damaged

axons. Function of the hindlimbs can be restored after the spinal cord is severed in neonatal rats through a graft of embryonic spinal cord (Iwashita et al. 1994). Recovery can also be mediated by sprouting of remaining undamaged axons. In adult cats the loss of normal sensory input results in central axonal sprouting and functional reorganization of visual cortical areas (Darian-Smith and Gilbert 1994). Recovery after the loss of sensory input has also been demonstrated in the flight system of the locust (Büschges and Pearson 1991), which is particularly amenable to experimental manipulation. When there is loss of proprioceptive input to flight system neurons, the afferents from remaining proprioceptors are reorganized in a competitive fashion to compensate for the lesion (Wolf and Büschges 1997a, b).

The tegulae are proprioceptive organs necessary for the generation of a functional flight motor pattern (Wolf 1993). Ablating the hindwing tegulae increases the time interval between activation of wing depressor muscles and activation of wing elevator muscles, consequently decreasing wing-beat frequency (Wolf and Pearson 1988; Büschges and Pearson 1991). The motor pattern recovers in 2 weeks due to the forewing tegulae afferents which sprout and increase both the number and the strength of synaptic connections onto flight system neurons (Büschges et al. 1992a, b). In a previous study we used this model system to investigate whether the capacity for recovery, from ablation of the hindwing tegu-

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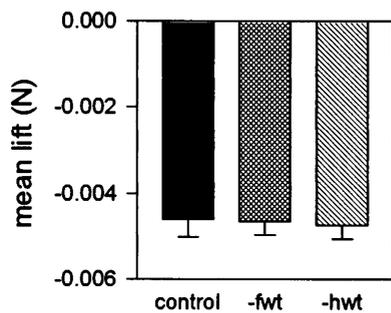


Fig. 1. The mean lift generated by intact locusts (control) was the same as the mean lift generated by locusts 3 weeks after ablation of the forewing (-fwt) or hindwing (-hwt) tegulae. Lift was recorded from tethered locusts flying in a wind tunnel using a flight balance. The average lift value was recorded for each wingbeat cycle and the mean lift calculated for each animal over a 1- to 2-min flight sequence. (mean \pm s.e.m; control $n=63$, -fwt $n=59$, -hwt $n=67$)

lae, is reduced as adult locusts mature. We found no difference in the rate or extent of recovery between immature (treated 1 day after imaginal ecdysis) and mature adult locusts (treated 14–16 days after imaginal ecdysis); however, when recovery was monitored by stroboscopic measurements of wingbeat frequency, the extent of the recovery appeared less complete than when recovery was monitored using electromyographic (EMG) recordings of flight muscle activity (Gee and Robertson 1996). This observation led us to investigate whether the recovery from ablation of the hindwing tegulae, which has been documented using tethered locusts, is functional. That is, we wished to determine whether recovered locusts can sustain free flight.

We chose to investigate the recovery resulting from two combinations of tegula ablations by comparing flight abilities of recovered and intact locusts. We ablated only the hindwing tegulae in some locusts to test whether the well-documented recovery mediated by sprouting of the forewing tegulae produced functional recovery. We expected to observe normal or close to normal flight ability in the locusts that had recovered from the hindwing tegula ablations. Alternatively, we ablated the forewing tegulae only. Our prediction was that the locusts that were recovered from

forewing tegulae ablations would demonstrate no deficit in flight ability as there is no known acute effect of forewing tegulae ablations on the generation of the flight motor pattern (Büschges and Pearson 1991).

Either the hindwing tegulae or the forewing tegulae were ablated from adult male locusts. The tegulae are external hair plates located on soft cuticle near the wing hinge (see Kutsch et al. 1980) and are easily removed by cauterizing, cutting or pinching off with forceps. Hindwing tegulae were removed using a fine pair of forceps while a locust was held ventral side down with one forewing at a time held open against a pin stuck in a piece of cork. Forewing tegula ablations were carried out in a similar manner except the tegulae were exposed by holding the locust on its side and gently pushing the pronotum forward against the pin. A third group of locusts were sham-operated by using a pin to make small holes in the cuticle near the wing hinge, similar in diameter to the tegulae. Each animal was numbered on the forewings using a permanent fine-tipped marker. A key was kept of the numbers and corresponding treatments of each locust, and testing was performed blind.

For one series of experiments a total of 210 locusts were treated in two trials (90 and 120, respectively), and all locusts from a given trial were kept in one cage. At the time of treatment approximately half the locusts in each trial were 1 day past the imaginal molt, and half were 14–15 days past adult ecdysis. More than 3 weeks after the ablations flight ability was tested in two ways. First each animal was affixed via the pronotum to a rigid tether with a drop of hot wax and suspended inside a wind tunnel (wind speed approx. 2.5m/s). A lift balance (described in Robertson and Johnson 1993) measured the lift generated by each animal throughout 1- to 2-min of tethered flight. The output from the force transducer was recorded to tape and analyzed by computer using a DataWave Technologies A/D board and software (Longmont, CO, USA). The output of the force transducer was calibrated so that a value of zero was returned with the tethering rod attached and no locust. During flight

the lift generated oscillates between negative and positive values as the wings move up and down. For each cycle the average lift produced was determined and used to calculate the mean lift for each locust.

There was no difference in the mean lift generated by the control locusts and the locusts recovered from ablation of the forewing or hindwing tegulae (Fig. 1; $H=0.226$, $df=2$, $P=0.893$; Kruskal-Wallis one-way ANOVA). The mean force produced by the weight of 87 locusts, which stopped beating their wings while suspended from the tether, was -0.016 ± 0.0004 N (equal to -16 mN). The mean lift produced by the tethered locusts to bring the mean lift measured to about -5 mN (Fig. 1), was therefore around 11 mN, and sufficient to support on average only about two-thirds of the locusts' weight. This amount of lift would be insufficient to maintain free-flight, which indicates that the locusts were not producing a normal flight effort. Net lift reported for eight locusts by Wolf (1993) was about 0.5 mN for intact locusts and around 0 mN immediately after tegula ablations (from inspection of Fig. 7 in Wolf 1993).

These values are considerably lower than those that we report (similar in magnitude to the standard error of our means), but they were obtained using a different method and were from much shorter sequences (32 wing-strokes vs. 1–2 min), making direct comparisons difficult. An additional difficulty in making a direct comparison with this previous study is that there is a discrepancy between the value of 0.5 mN and the statement elsewhere in Wolf (1993) that "net lift generation was between 0 and 25% of body weight when intact locusts were tested in still air." An upper limit of 1 mN for net lift would thus give a body weight of 4 mN, which corresponds to a mass of only 0.41 g. This means either that those locusts were considerably smaller than the locusts we used (mean mass 1.63 g), or that there is a calculation error somewhere in the previous paper. Moreover, the apparent decrease in net lift as a consequence of ablation of all tegulae (Fig. 7 in Wolf 1993) was shown for only 8 animals and not statistically

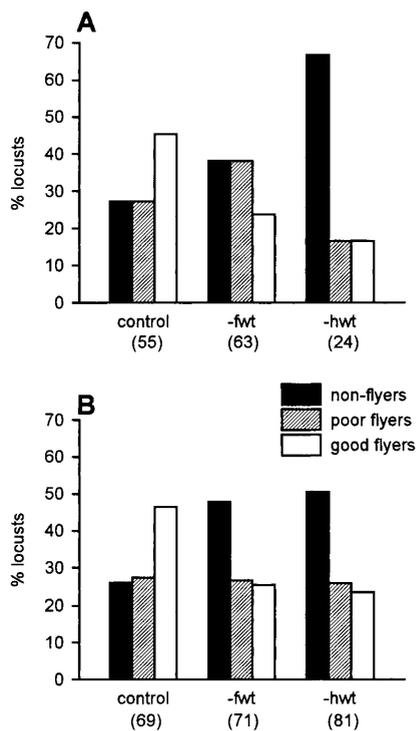


Fig. 2A–B. The free-flight ability of intact and/or sham-operated locusts (control) and locusts following ablation of either the forewing (*-fwt*) or hindwing (*-hwt*) tegulae. A) Free-flight ability was reduced by ablation of the hindwing tegulae when examined several hours after the treatments. ($H=11.91$, $df=2$, $P=0.0026$ Kruskal-Wallis one-way ANOVA on Ranks, $P<0.05$, Dunn's multiple comparison). B) Free-flight ability remained reduced in locusts that had either the forewing or hindwing tegulae ablated 3–4 weeks after the treatments ($H=13.49$, $df=2$, $P=0.0012$, Kruskal-Wallis one-way ANOVA on Ranks; $P<0.05$, Dunn's multiple comparison). Locusts were classified according to the time aloft and distance to landing after initiating flight from a 4-m high platform (see text; number of animals in brackets)

tested, whereas our comparisons are from numbers greater than 59 and not statistically different.

Free-flight ability was tested shortly after the lift determinations on a warm day (approx. 25°C) in an outdoor arena. Locusts were placed on a platform approximately 4 m above the ground and initiated flight by jumping either (usually) spontaneously or in response to a loud clap from behind. The time aloft and distance to landing were recorded for 175 individuals (others had died from undetermined causes) and used to classify locusts

into one of three categories that were ranked as follows: (a) nonflyers remained aloft fewer than 3 s, they essentially jumped and fell to the ground traveling horizontally fewer than 8 m; (b) poor flyers remained aloft for between 3 and 9 s, they beat their wings and controlled their descent but usually did not climb, landing fewer than 20 m from the start although the path was sometimes circuitous; (c) good flyers remained aloft for more than 9 s, distances to landing were usually greater than 30 m but were a poor indication of the much greater actual distances traveled. A two-way ANOVA on ranks indicated that there was no difference in flight ability due to age at the time of treatment (either 1 day or 14 days after imaginal ecdysis); therefore the age groups were combined. We found there was a difference in the distribution of free-flight abilities between the control locusts and the locusts that had recovered following ablation of either the forewing or hindwing tegulae even though there was no difference in the mean lift generation. We therefore performed some additional trials in an indoor arena (20×20×15m, with natural and artificial light) to determine whether there was an immediate effect of ablating the hindwing and/or forewing tegulae on free-flight ability. We also tested the free-flight ability of some of these locusts 3 weeks after the treatments and combined them with the locusts from the previous trials.

There was a significant reduction of free-flight ability due to ablation of the hindwing tegulae as compared to the control locusts (Fig. 2A, $H=11.91$, $df=2$, $P=0.0026$ Kruskal-Wallis one-way ANOVA; $P<0.05$ Dunn's multiple comparison). In one trial we pretested the flight ability of the locusts and removed all the non-flyers prior to the treatments; however, there was still a high proportion (approx. 25%) of control locusts that were nonflyers when retested. There was no significant difference in the free-flight ability following ablation of the forewing tegulae compared to either the control locusts or the locusts with the hindwing tegulae ablated ($P>0.05$, Dunn's multiple comparisons). In another study, which in-

cluded an examination of only the initial effect of ablating the forewing tegulae on free-flight ability, we found the effect was significant (Gee et al. 1998). Some of the locusts were able to fly following ablation of either the hindwing tegulae or the forewing tegulae when tested 3–4 weeks after the ablations (good flyers; Fig. 2B). This demonstrates that the locust flight system has a capacity for free-flight following loss of the tegulae.

Functional recovery was restricted however, and there was a significant difference in the distribution of flight abilities between the control (sham-operated) locusts and locusts recovered from hindwing tegula removal (Fig. 2B; Kruskal-Wallis one-way ANOVA on ranks $H=13.49$, $df=2$, $P=0.0012$; Dunn's multiple comparison, $P<0.05$). There were more good flyers than poor flyers or nonflyers among the control locusts, whereas the locusts allowed to recover from ablations of the hindwing or forewing tegulae were more often nonflyers or poor flyers than good flyers (Fig. 2). Thus it is apparent the ability to fly is not normally recovered in locusts following ablation of the hindwing tegulae. This contrasts with the mean lift produced during tethered flight, which did not differ between the intact and recovered locusts. The difference in flight abilities was unexpected because several studies have reported and confirmed that the timing of the flight motor pattern recovers completely following ablation of the hindwing tegulae (Büschges et al. 1991, 1992a,b; Gee and Robertson 1996).

We suggest that when intact and recovered locusts are suspended from a tether, the motor pattern produced represents some minimal effort that does not accurately reflect the motor pattern which is necessary to sustain flight. When wingbeat frequency is monitored using a stroboscope, however, there is a significant difference between the intact and recovered locusts (Gee and Robertson 1996), a finding in agreement with the differences in free-flight ability that we found. When wingbeat frequency is measured early in the flight sequence, likely when the locusts are startled by the stroboscope, the flight patterns

generated may represent a closer to maximal output (see Gewecke and Kutsch 1979). Our data can be explained if the *maximum effort* motor patterns that can be generated by intact and recovered locusts are different while the *minimum effort* motor patterns typically recorded electromyographically are the same. It has been documented that, when locusts are held by a rigid tether the *flight* generated differs in some respects from free-flight (e.g., Baker, Gewecke and Cooter 1981; see also Zarnack and Wortmann 1989). Additionally, short-term learning can occur in the locust flight system in response to an artificially imposed closed-loop situation in tethered locusts and this improves motor coordination (Möhl 1988). While making EMG recordings, locusts therefore adapt to being tethered and over time begin to generate flight patterns that would not keep them aloft, and which do not correspond to free-flight ability. The fact we observed average lift forces that would be insufficient to maintain flight, which were not different in the tethered control and recovered locusts, is consistent with this model. Surprisingly, we also found that the locusts recovered from ablation of the forewing tegulae could not fly. There was no difference in the flight ability of locusts recovered from ablation of either the forewing or the hindwing tegulae (Fig. 2B; Dunn's multiple comparison, $P < 0.05$). When the forewing tegulae are ablated in intact locusts, there is no effect on either the motor pattern (Büschges and Pearson 1991) or the wingbeat frequency measured with a stroboscope (Gee and Robertson 1996). Our data suggest, however, that intact forewing tegulae are indeed required for locust flight. Future studies will investigate what role the forewing tegulae play in flight generation. In conclusion, a subset of locusts were able to fly following recovery

from ablation of either hindwing or forewing tegulae, indicating for the first time that some locusts are capable of free-flight in spite of missing tegulae. Overall, however, a deficit remains in the locust flight system after recovery from ablation of either the forewing tegulae or the hindwing tegulae. This is the case despite both the apparently complete recovery of the flight motor pattern following hindwing tegula ablations and the apparent lack of effect on the flight motor pattern following forewing tegula ablations. We suggest that the effects of holding flying locusts with a rigid tether mask subtle differences in the motor patterns of intact and recovered locusts, an explanation consistent with the similar lift production of intact and recovered locusts in this study. Further investigations will determine whether changes to the flight motor pattern apart from the timing of activation of wing elevator and wing depressor muscles account for the decreased flight ability.

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