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K. L. Shoemaker · R. M. Robertson

Flight motor patterns of locusts responding to thermal stimuli

Abstract Correctional and intentional steering manoeuvres in locusts differ in several important respects. The most profound difference between the two is the production of large forewing asymmetries in angle of elevation during the downstroke in intentional steering that are not obvious in correctional steering. We investigated the flight motor patterns during intentional steering responses to a radiant heat source. We found asymmetries in the timing of forewing first basalar (m97) activity on the left and right sides that were strongly and positively correlated with forewing asymmetries. Timing asymmetry in the second basalar (m98) and pleuroalar (m85) muscles was not significantly different from the changes observed in m97. The hindwing first basalar (m127) shifted its asymmetry in the opposite direction. The forewing subalar muscle (m99) did not shift its asymmetry with the same magnitude as m97, but instead was phase-shifted relative to m97 on the left and right sides, suggesting its role as a supinator. We conclude that large asymmetries in the elevation angle of the forewings during the downstroke, as are evident in intentional steering, are generated by bulk shifts in the activation times of forewing depressor muscles to cause a relative shift in the time of stroke reversals of the two forewings.

Key words *Locusta migratoria* · Wing kinematics · Motor pattern · Sensorimotor · Insect flight

Abbreviations *EMG* electromyography · *FWA* forewing asymmetry · *HWA* hindwing asymmetry · *WBF* wingbeat frequency

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Introduction

Two types of steering during flight have been described in locusts: *correctional steering* and *intentional steering* (for review see Kammer 1985; Rowell 1988). Correctional steering allows the locust to maintain a straight course in the face of environmental perturbations or motor errors. In simplistic terms, correctional steering can be thought of as an "autopilot" by which reflexes act to compensate for involuntary deviation from course. Intentional steering occurs when the locust turns in response to internal motivation or external stimuli, such as those which may be dangerous (e.g. an obstacle in the flight path) or alluring (e.g. a potential food source). Intentional steering, often described as a tendency to turn, must involve eliminating correctional reflexes, i.e. disabling the autopilot (Robert and Rowell 1992).

Superficially, the behaviours associated with intentional and correctional steering are similar. The hindleg ipsilateral to the turn extends, and the abdomen deflects in the direction of the turn (Camhi 1970; Arbas 1986; Robert 1989). The abdominal deflection is a reliable indicator of steering direction. In terms of wing kinematics, several asymmetries become apparent, including changes in the angle of attack on left and right sides, changes in stroke amplitude, and phase changes in the timing of depression (for review see Rowell 1988).

A closer look at these behaviours reveals that correctional and intentional steering are different. Camhi (1970), in a comparison of the two types of steering, noted that different abdominal segments are involved in the two steering types. Both Camhi (1970) and Robert and Rowell (1992) noted that head movements are different between the two types of steering. Robert and Rowell (1992) found that during correctional steering, head movements are directed into the turn, while in an intentional steering manoeuvre, the head moves in a direction opposite that of the turn.

A further difference between intentional and correctional steering is that the former results in the genera-

tion of profound forewing asymmetries (FWAs) of elevation angle during the downstroke that are not obvious in the latter (Robertson and Reye 1992). For example, during an intentional turn to the right, the right forewing is more depressed than the left during the downstroke. Often, this asymmetry is so large that during the downstroke, the forewing is more depressed than the hindwing on the side of the body ipsilateral to the turn. FWA changes have been observed during attempted steering manoeuvres in response to objects approaching in the flight path (Robertson and Reye 1992; Robertson and Johnson 1993), laterally placed heat stimuli (Robertson et al. 1996), and auditory stimuli mimicking the calls of echolocating bats (Dawson et al. 1997).

There is considerable information available about correctional motor patterns (Zarnack and Möhl 1977; Thüring 1986; Waldmann and Zarnack 1988), but relatively little about the motor patterns that underlie intentional steering manoeuvres, particularly the FWA. The general goal of this study was to describe the motor pattern for the FWA to facilitate future studies of the mechanisms by which sensory information is integrated with the activity of the flight circuitry.

Dawson et al. (1997) have suggested that FWA during the downstroke can be generated by changing the relative timing of stroke reversals at the top of the upstroke. This would effectively cause one forewing to lead the other during the downstroke. To change the timing of stroke reversal, the time of contraction of muscles controlling stroke reversal must be modified and it was suggested that "bulk shifts" in depressor muscle activity may be required to generate FWA. These bulk shifts refer to the advancement of timing of all forewing depressor muscles (m97, m98, m99, m85) on one side of the body relative to their corresponding contralateral muscles.

Bulk shifts have been reported in correctional steering manoeuvres (Thüring 1986; Waldmann and Zarnack 1988; Zarnack 1988), yet these bulk shifts have only been loosely correlated with phase shifts of stroke reversal timing for left and right sides. Additionally, despite these bulk shifts, Zarnack (1988) and Waldmann and Zarnack (1988) stress that changing the angle of attack is the crucial element for steering manoeuvres. Our specific goal was to test the hypothesis that the FWA is strongly correlated with bulk shifts in the timing of forewing depressor muscle activity.

Using a heat source to provide stimuli to induce steering, we simultaneously recorded flight muscle activity from two contralateral muscle pairs and videotaped postural and wing kinematic behaviour. Thermal stimuli were used because it has already been established that they generate intentional steering manoeuvres including the FWA (Robertson et al. 1996), and that the responses observed are several seconds in duration. We found that forewing asymmetries and m97 activity shifts are strongly and positively correlated. An earlier firing of m97 is associated with a greater wing depression during the downstroke. Additionally, we found that shifts in timing of other forewing depressor muscles are similar in direction and magnitude to those observed in m97. These findings support the contention that large asymmetries in the elevation angle of the forewings during the downstroke, as are evident during intentional steering, are generated by bulk shifts in the activation times of forewing depressor muscles to cause a relative shift in the time of stroke reversals of the two forewings.

Materials and methods

Animals

Adult male *Locusta migratoria* L. at least 2 weeks past the adult moult were used. Locusts were collected from a crowded colony (light:dark cycle of 16:8 h) maintained at Queen's University. Males were selected for these experiments to reduce variability in the magnitude of manoeuvres associated with the size difference between males and females.

### Animal preparation and experimental procedure

Each animal was blinded and deafened before a flight sequence to ensure that the attempted steering responses were not to visual or auditory cues. Compound eyes and ocelli were covered with opaque ceramic paint, taking care not to cover surrounding areas of cuticle. To deafen the animal, both tympanic membranes were destroyed using an insect pin. Additionally, all six legs were removed at the coxa-trochanter joint so that the animal could not grasp either the electrodes or the tether. Removing the legs is a treatment that has been used by various researchers of locust flight and steering (Wilson and Weis-Fogh 1962; Camhi 1970; Taylor 1981; Baader 1990) and has no noticeable effects on steering behaviour.

Animals were dorsally tethered by fixing a rod to the pronotum with wax, and were then suspended in a windstream (air speed approximately 3 m/s). Care was taken during tethering to ensure that the animal was straight, to reduce potential steering biases to asymmetrical wind stimulation of the head. Four electromyography (EMG) electrodes were implanted (see below). The animals were allowed to fly uninterrupted until the abdomen was in line with the dorso-longitudinal axis of the thorax, which usually took about 2 min. Once steady tethered flight was achieved, a 250-W heat lamp, placed 7.5 cm to the left or right of the animal, was turned on. Thermal presentations usually lasted between 9 and 14 s, but could be shorter or longer depending on the response time of the animal. The timing of thermal presentations was monitored with a Sensortek thermometer (model BAT-12, Clifton, NJ) with a copper/constantan thermocouple placed 1 cm above the locust's head. Thermocouple output was an indication of stimulus timing only, and for this reason, it was not converted to degrees Celsius from the voltage change produced by the device.

Although a second stimulus was usually presented, only responses to first stimuli were analysed to avoid analysing smaller, habituated responses.

EMG analysis

Four EMG electrodes, consisting of fine copper wire (100 μm in diameter) and insulated except at the tip, were inserted into two downstroke muscle pairs through small holes made in the cuticle. These insertion points were on either the sternal or pleural surface of the body, depending on the muscles selected (Fig. 1A, B). Each electrode was held in place with a small drop of wax. A fifth indifferent silver electrode was inserted beneath the pronotum behind the head.
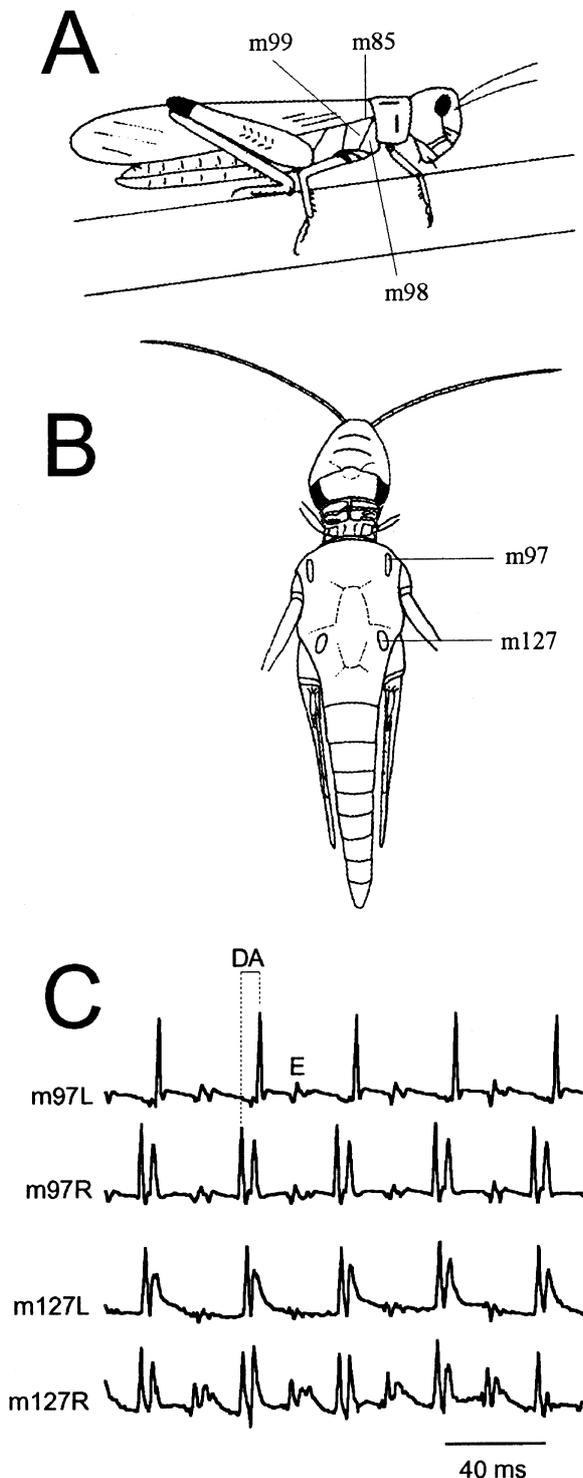
Fig. 1A–C Drawings of a male locust illustrating electrode placement. **A** Side view of a locust showing electrode positions for *m85*, *m98* and *m99*. Electrode placement for *m85* is just beneath the edge of the forewing. **B** Ventral view of a locust showing electrode positions for *m97* and *m127*. Note that these insertion points are on the callosities, which are regions of bare cuticle. The length of the male locust, from top of head to tip of abdomen, is between 4 and 5 cm. **C** Sample EMG trace showing flight motor pattern activity of muscles *m97* and *m127*. The large events in each trace indicate the activity of the muscle of interest, while the small transients (*E*) indicate crosstalk from elevator muscles. A positive depressor asymmetry (*DA*) for m97 is shown (*L* left, *R* right)

From every animal in the dataset, we recorded the activity of left and right forewing first basalar muscles (m97). The second pair of electrodes recorded from either the forewing pleuroalar muscles (m85), the forewing second basalar muscles (m98), the forewing subalar muscles (m99) or the hindwing first basalar muscles (m127) (nomenclature according to Snodgrass 1929). A sample trace of m97 and m127 depressor activity is shown in Fig. 1C.

Signals from the EMG electrodes were first amplified, and then recorded onto VHS videotape using a Neuro-corder (model DR-886, Delaware Water Gap, Pa.). The instantaneous timing of muscle activity was analysed using hardware and software from Datawave Technologies (Longmont, Colo.). Three separate elements of the flight motor pattern were calculated from the digitized time values. (1) We measured depressor asymmetry, which is the difference in the activation time of corresponding contralateral motor units (e.g. Fig. 1C). It is calculated by subtracting the right muscle activation time from that of the left. A positive depressor asymmetry indicated that the right muscle was firing earlier in the wingbeat cycle than the left. (2) We measured the muscle timing relationships on a single side of the body by subtracting m85, m98, m99, or m127 timing from m97 timing. For example, a negative relationship between m85 and m97 indicated that m97 fired first. (3) We determined the number of spikes per burst of m97 during thermal presentations (e.g. Fig. 1C).

Following each experiment, electrodes were cut, leaving their end wires within the animal. Electrode placement was confirmed by post-mortem dissection. This was done by first injecting the animal with 1 ml fixative (1:4 95% ethanol:99.7% acetic acid). The following day, animals were dissected by first removing the head, and completely bisecting the animal along the dorso-longitudinal axis to separate left and right sides. Without disrupting the natural curve of the pleural cuticle, the flight muscles were then exposed. The elevator muscles were removed to reveal the underlying depressor muscles, and electrode placement was determined by careful dissection until the tip of the electrode was seen.

Videotape analysis

During flight sequences, animals were videotaped from behind (Hitachi 5200A video camera, electronic shutter speed 1/1000 s) so that we could later calculate abdomen position and wing elevations. Using hardware and software manufactured by PEAK Performance Technologies Inc. (Englewood, Colo.), we conducted a frame-by-frame analysis of approximately 350 frames encompassing a stimulus response. We refer to a stimulus response as one occurring either to the lamp turning on or off (hereafter referred to as either *lamp on* or *lamp off*). Details of the analysis using the PEAK system have been previously described (Robertson and Johnson 1993). Using the PEAK system, we measured wing angles and the horizontal displacement of the abdomen in every frame. The horizontal position of the tip of the abdomen is reported in screen pixels which represent the number of image pixels to the right (plus) or left (minus) of a zero position directly beneath the tether. These values have not been converted to actual distances because such a conversion would be relatively meaningless in terms of postural changes of the abdomen. The abdomen curled as it deflected and thus the tip was not confined to a vertical plane. Moreover, the angle that the abdomen made with the anterior/posterior axis could not be measured because of the vantage point of the video camera. Nevertheless, the measure we obtained was adequate to obtain relative magnitudes and directions of abdominal deflections.

Usually, in response to a stimulus, the abdomen reached its full deflection in less than 2 s. We monitored 350 frames (5.6 s) to ensure that we captured the entire pre- and post-stimulus wing and abdomen response.

From the calculated wing angles we determined FWA, the difference between the angles of the forewings during the downstroke. FWA was calculated by subtracting the angle of the left wing from the angle of the right wing. Since 0° indicates a position directly above the animal and 180° is directly below, a positive

FWA indicated that the right wing was more depressed than the left during the downstroke. Since hindwings lead forewings during the downstroke (Wilson and Weis-Fogh 1962), downstroke frames were determined when the hindwing was more depressed than the forewing on the outside of the turn. Because we wanted to correlate muscle activity with wing movements, and because the depressor muscles fire at the top of the downstroke (Wilson and Weis-Fogh 1962), we selected for analysis only those downstroke frames in which the forewing on the outside of the turn was between 0° and 80°. For those frames in which FWA was measured, we also measured hindwing asymmetry (HWA) by subtracting the angle of the left hindwing from the angle of the right.

Dataset

Recordings were taken from 91 male locusts; however, 57 were removed from the dataset due to excessive crosstalk between recording electrodes, subsequent determination that the wrong muscle had been recorded, or because the animals did not fly continuously. Of the remaining 34 locusts, 20 were used for analysis of activity in m97 and m127 muscle pairs. Four locusts were used to analyse activity in m97 and m85 muscle pairs. Five were used to analyse activity in m97 and m98 muscle pairs. Five were used to analyse activity in m97 and m99 muscle pairs.

Statistics

We used SigmaStat statistical software (Jandel Corporation, San Raphael, Calif.) to conduct appropriate parametric and non-parametric tests. Significance was assumed when $P < 0.05$.


Results

Behaviour

When the wind tunnel was turned on, most locusts began to beat their wings. Others required additional stimulation, and a quick pinch of the abdomen with forceps usually sufficed. At the beginning of these flight sequences, the abdomen position was not constant, and only after approximately 2 min did the abdomen finally settle in a position in line with the dorso-longitudinal axis of the thorax. Once the locust was maintaining straight and steady flight, the thermal stimulus was presented. The abdominal response for 33 locusts to thermal stimuli varied in latency, magnitude, duration and direction. Abdominal deflections are reliable indicators of steering by deflecting into the turn (see Introduction), and we analysed the directionality of the abdominal responses to determine the direction of steering attempts. Ten of 33 animals (30.3%) deflected their abdomens away from the heat lamp in response to lamp on. Ten of 33 (30.3%) did not respond to lamp on, but did respond to lamp off by deflecting their abdomens towards the lamp. Another 10 animals (30.3%) performed both of these manoeuvres: they deflected their abdomens away from lamp on, and towards the heat lamp at lamp off. Only 3 animals (9.1%) deflected their abdomens towards an illuminated heat lamp. Two of these three responded only when the heat lamp was turned on. One animal responded towards the heat lamp both at lamp on and at lamp off.

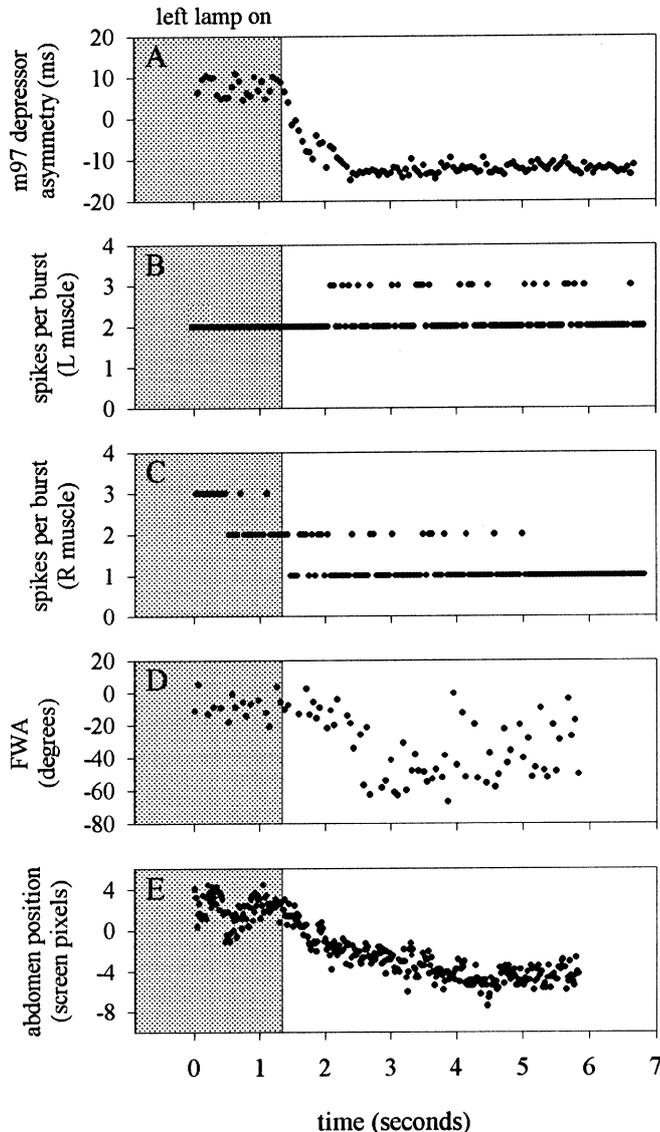### Muscle m97 activity, abdomen position, and wing angles

Muscle activity of m97 was monitored from every animal, and the asymmetry in m97 activity between left and right sides shifted in response to thermal stimuli. These m97 asymmetry shifts were sometimes gradual, progressing over several seconds, or they could be abrupt. Often the abrupt responses occurred at lamp off (e.g. Fig. 2). This individual showed little change in m97 asymmetry at lamp on, but at lamp off, a large positive m97 asymmetry shift of more than 10 ms occurred. Abdominal deflections indicated that in this trial, the locust attempted to steer right at lamp off (not shown, see below for correlation of motor pattern changes and abdominal deflections). For 34 responses to either lamp on or lamp off, there was a statistically significant change in wingbeat frequency (WBF), indicating that the stimulus was detected at both of these times (two-tailed paired t-test, $t = -4.8$, $P < 0.05$, $n = 34$). Twenty-eight locusts (82%) showed increases in WBF in response to lamp on or lamp off. The average WBF
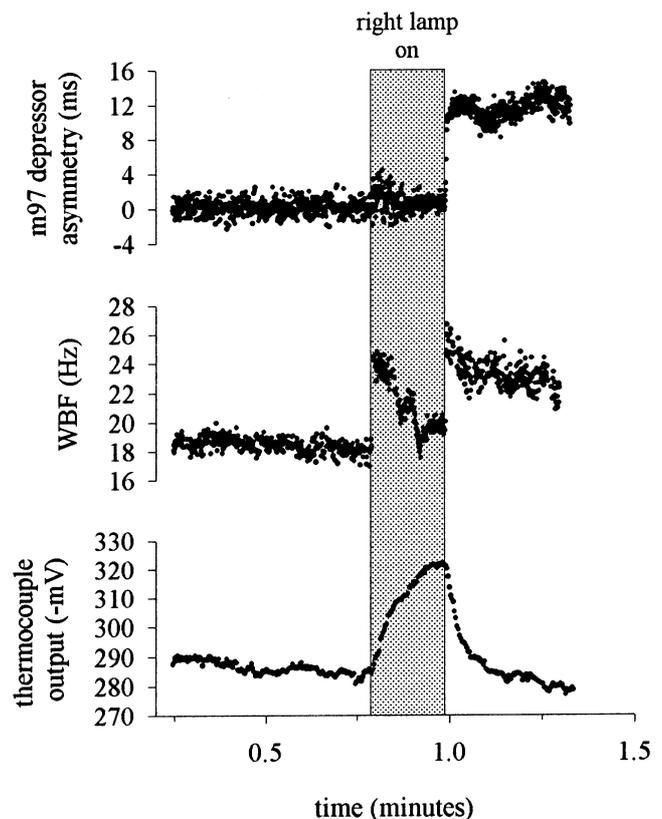
Fig. 2 Changes in m97 depressor asymmetry and wingbeat frequency (*WBF*) in response to a thermal presentation. These data are from an individual locust that attempted a right turn to lamp off. The *shaded bar* indicates presentation of the thermal stimulus based on thermocouple output (*bottom trace*). In subsequent figures, the shaded bar alone indicates stimulus presentations. A positive depressor asymmetry shift occurred when the heat lamp was turned off (*top trace*), indicating that the right m97 was firing before the left. Note also that at both lamp on and lamp off, there was an increase in wingbeat frequency (*middle trace*)

before a steering attempt was 20.0 ± 0.4 Hz (mean $\pm$ SE). The average increase in WBF during the steering manoeuvre was 1.3 ± 0.3 Hz.
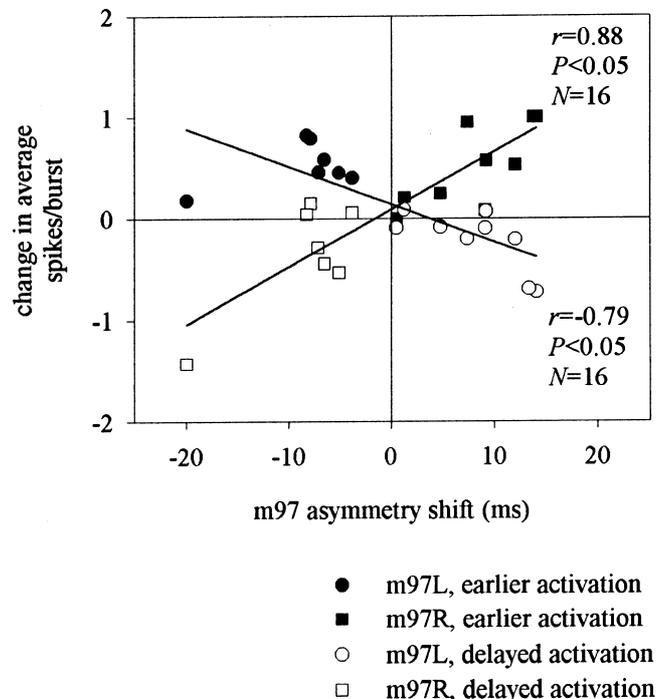
For 25 of the locusts, we determined the wing angles and abdomen positions during the steering response. 350 video frames (5.6 s) were analysed from each flight sequence. The calculated FWA (right wing angle minus left wing angle) was time-aligned with abdomen position and EMG-recorded m97 activity (L−R asymmetry and spikes per burst). In Fig. 3, in response to lamp off, there

was a negative m97 asymmetry shift, and an increased number of spikes per burst in the left m97 and a decreased number of spikes per burst in the right m97. Concurrently, there was a negative FWA shift (only the FWA at the top of the downstroke is shown) and an abdomen deflection to the left. From time-aligned muscle activity, abdomen movement and wing elevations, we computed and correlated the magnitudes of their shifts. Magnitudes of shift were calculated by subtracting the pre-stimulus average from the post-stimulus average. The pre-stimulus average was based on a 1-s time period at the beginning of the trace. The post-stimulus average was based on a 1-s time period at a time when the abdomen appeared to have reached full deflection.

We found significant correlations between the change in spikes per burst of muscle m97 and the magnitude and direction of the m97 asymmetry shift (Fig. 4). When m97 asymmetry shifted positively (right muscle firing first), the right muscle fired more spikes per burst, while the contralateral muscle fired fewer spikes per burst. When the asymmetry shift was negative (left muscle firing first), the spikes per burst increased in the left muscle and decreased in the right muscle. Although m97 is capable of firing one, two or three spikes per burst

Fig. 3A–E Coincident changes in m97 depressor asymmetry, spikes per burst (*L* left, *R* right), *FWA*, and abdomen position for an individual locust at lamp off. **A** When the left heat lamp was turned off, there was a negative m97 depressor asymmetry shift, indicating that the left muscle shifted to fire before the right. **B** The average spikes per burst of the left m97 increased at lamp off. **C** The average spikes per burst of the right m97 decreased at lamp off. **D** There was a negative shift in FWA, indicating that the left forewing was more depressed that the right during the downstroke. **E** The abdomen deflected to the left at lamp off

- ● m97L, earlier activation
- ■ m97R, earlier activation
- ○ m97L, delayed activation
- □ m97R, delayed activation

Fig. 4 There were significant correlations between the direction and magnitude of m97 asymmetry shift and changes in m97 spikes per burst. The spikes per burst of the right (*R*) m97 increased during positive asymmetry shifts and decreased during negative asymmetry shifts (*filled and open squares*, Pearson product moment correlation, $r = 0.88$, $P < 0.05$, $n = 16$). The spikes per burst of the left (*L*) m97 increased during negative asymmetry shifts and decreased during positive asymmetry shifts (*filled and open circles*, Pearson product moment correlation, $r = -0.79$, $P < 0.05$, $n = 16$)

(Waldron 1967), the change in average spikes per burst was not always a whole number. This is because often, m97, instead of firing only singlets, doublets or triplets, fired a combination of singlets and doublets, or a combination of doublets and triplets.
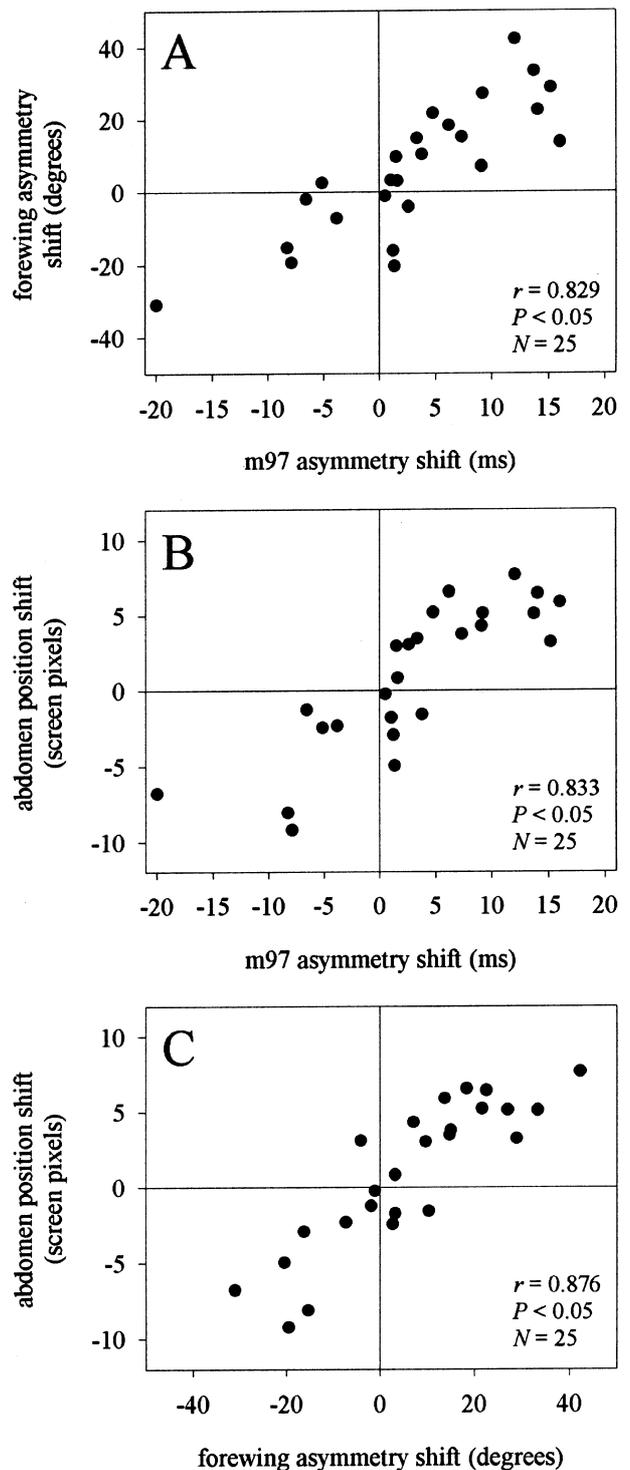
We found significant positive correlations between the magnitudes of m97 asymmetry shift, abdomen deflection and FWA shift (Fig. 5). A positive asymmetry shift in m97 was significantly correlated with a positive FWA shift (Fig. 5A) and a rightward abdomen deflection (Fig. 5B). A positive FWA shift was correlated with a rightward abdomen deflection (Fig. 5C). A linear regression drawn through the data points naturally passes through the origin in all comparisons (not shown). The magnitude of the m97 depressor asymmetry shift was sometimes large: the average magnitude of shift, without regard to direction, was 7.2 ± 5.5 ms. Six of 25 animals had m97 asymmetry shifts greater than 10 ms, and the largest shift we observed was 19.9 ms (pre-stimulus average, 7.5 ms; post-stimulus average, −12.3 ms).

We found no relationship between the generation of HWA and the usual correlates of steering (Fig. 6). There was no correlation between the direction and magnitude of HWA and FWA (Fig. 6A), or between HWA and abdomen position (Fig. 6B).

Activity of other downstroke muscle pairs

Four EMG electrodes were implanted into each animal. Two of these monitored activity of left and right m97 muscles. The other two electrodes monitored activity from either m85, m98, m99, or m127 muscle pairs. We found that the downstroke muscles of the forewing, in response to thermal stimuli, showed asymmetry shifts in the same direction and often with a similar magnitude as m97 asymmetry shifts (Fig. 7). The m85 asymmetry shifts that occurred in response to lamp on or lamp off were similar in direction and magnitude to those that occurred in m97 (Fig. 7A top and middle traces). On the left side of the body, a negative relationship between m97 and m85 indicated that m85 fired later than m97 during the wingstroke (Fig. 7A bottom trace), and on average, for four animals, m85 fired later than m97 by 4.4 ± 0.5 ms. A small deviation is noticeable in the relationship between m97 and m85 at lamp on. This indicates that the asymmetry between left and right m97 muscles developed earlier or faster than the asymmetry between left and right m85 muscles. In the other individuals from which m97 and m85 activity were recorded, the asymmetry shifts developed simultaneously.

Muscle m98 and m97 showed synchronous and similar shifts in asymmetry, both in direction and magnitude (Fig. 7B). Muscle m98 fired later than m97 during the downstroke (Fig. 7B bottom trace). On average, for five animals, m98 fired later than m97 by 3.7 ± 0.2 ms.

Fig. 5A–C Correlations between the magnitudes of m97 asymmetry shift, FWA shift, and abdomen deflection during tethered flight. **A** There was a significant positive correlation between m97 asymmetry shift and FWA shift. **B** There was a significant positive correlation between m97 asymmetry shift and abdomen deflection. **C** There was a significant positive correlation between FWA shift and abdomen deflection
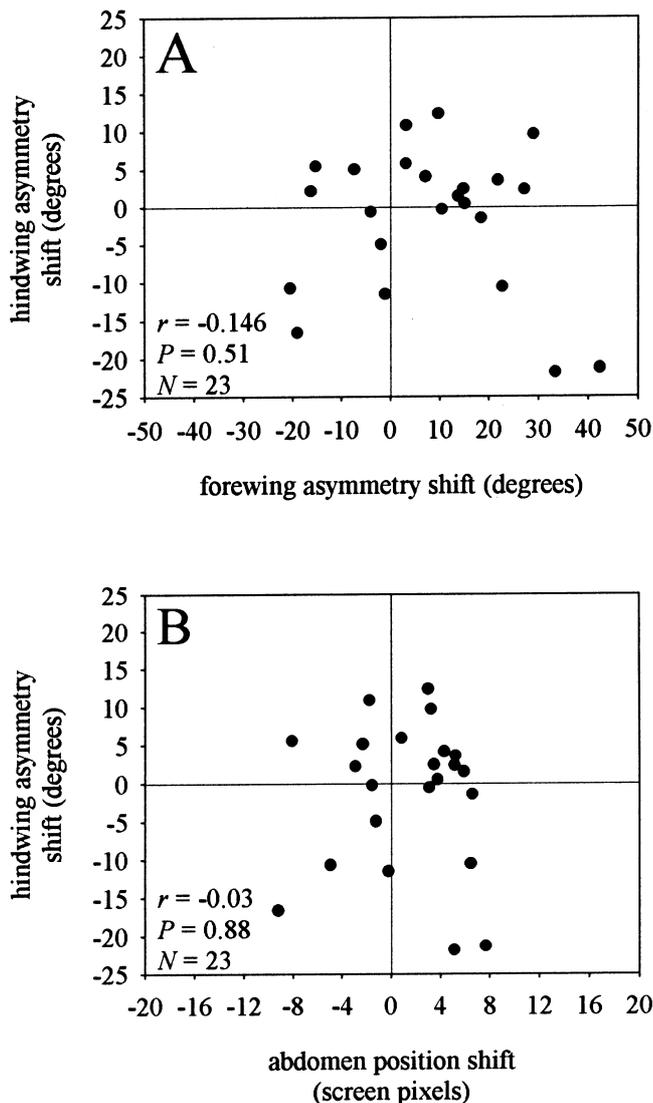
Fig. 6A, B Correlations between the magnitudes of HWA shift, abdomen position shift, and FWA shift. **A** There was no significant correlation between the FWA shift and HWA shift. **B** There was no significant correlation between abdomen position shift and HWA shift

Muscle m97 and m99 showed similar shifts in the direction of asymmetry (Fig. 7C). Muscle m99 fired later than m97 during the downstroke and a small shift in this relationship was observed at lamp off in this trial (Fig. 7C bottom trace).

In a correlation analysis, we found significant positive relationships between the asymmetry shifts of m97 and m85, between those of m97 and m98, and between those of m97 and m99. This means that for an asymmetry shift in m97, there is a corresponding asymmetry shift in m98, m99, and m85. From the slope of a model II regression analysis (principal axis slope) and the equation for a t-test, we determined whether the slope of the principal axis for each correlation was significantly different from a slope of 1 which would indicate identical shifts in asymmetry. The slope of the correlation between m97 and m85 was not significantly different from a slope of 1

(t-test, $t = 3.15$, $n = 4$, $P > 0.05$). The slope of the correlation between m97 and m98 was also not significantly different from a slope of 1 (t-test, $t = 0.006$, $n = 5$, $P > 0.05$). The slope of the correlation between m97 and m99 was significantly different from a slope of 1 (t-test, $t = 7.9$, $n = 5$, $P < 0.05$), indicating that the magnitudes of shift of m97 asymmetry and m99 asymmetry are not the same.

Muscles m97 and m127 shifted in opposite directions during a thermal presentation (Fig. 8A top and middle traces). The magnitude of m127 shift that occurred in this animal was similar to the shift in m97, but this is atypical. Typically, the size of m127 shifts were smaller than the shifts observed in m97. Based on the slope calculated from a model II regression, the shifts in m127 were only 0.4 times the size of m97 asymmetry shifts. The relationship between m127 and m97 on the left side of the body varied during the flight sequence (Fig. 8A bottom trace) although m127 usually fired first. A correlation analysis of m97 asymmetry shifts and m127 asymmetry shifts for 20 animals was negative (Fig. 8B), indicating that a positive m97 asymmetry shift was coincident with a negative m127 asymmetry shift. We also found a significant positive relationship between m127 asymmetry shift and HWA shift (Fig. 8C), indicating that when m127 was advanced in its timing, the corresponding hindwing was more depressed than the opposite hindwing during the downstroke.

Discussion

A previous study of steering behaviour in response to radiant heat found that locusts perform avoidance manoeuvres (Robertson et al. 1996); however, the attempted steering responses we have described above were variable. Although it is true that some animals attempted to turn away from the heat lamp, equally as many responded only at lamp off by attempting to turn towards it, and a few animals attempted to turn towards an illuminated heat lamp. From these behaviours, it seems reasonable to speculate that the locusts were attempting to thermoregulate by steering towards a preferred temperature. Whether or not this is correct, it is clear that the stimulus induced intentional steering manoeuvres comprising abdominal deflections correlated with asymmetries in the angles of elevation of the forewings during the downstroke. The following discussion of the associated changes in the motor pattern supports our primary conclusion that these forewing asymmetries were generated by bulk shifts in the activation times of forewing depressor muscles to cause a relative shift in the timing of stroke reversals of the two forewings.

Forewing asymmetry and abdomen deflection

During intentional steering manoeuvres, we observed abdomen deflections as well as forewing asymmetries in
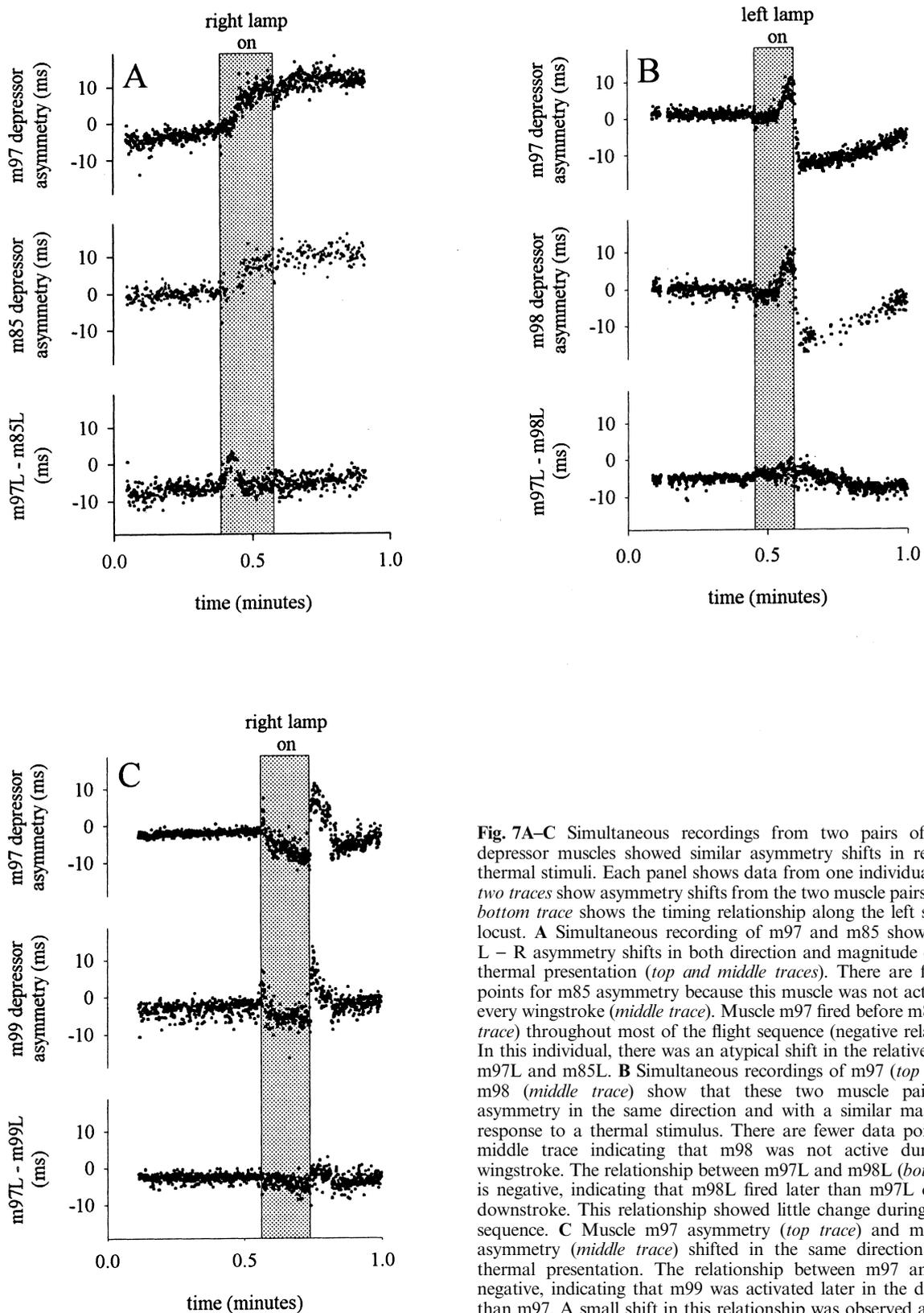
Fig. 7A–C Simultaneous recordings from two pairs of forewing depressor muscles showed similar asymmetry shifts in response to thermal stimuli. Each panel shows data from one individual. The *top two traces* show asymmetry shifts from the two muscle pairs, while the *bottom trace* shows the timing relationship along the left side of the locust. **A** Simultaneous recording of m97 and m85 showed similar L − R asymmetry shifts in both direction and magnitude during the thermal presentation (*top and middle traces*). There are fewer data points for m85 asymmetry because this muscle was not active during every wingstroke (*middle trace*). Muscle m97 fired before m85 (*bottom trace*) throughout most of the flight sequence (negative relationship). In this individual, there was an atypical shift in the relative timing of m97L and m85L. **B** Simultaneous recordings of m97 (*top trace*) and m98 (*middle trace*) show that these two muscle pairs shifted asymmetry in the same direction and with a similar magnitude in response to a thermal stimulus. There are fewer data points in the middle trace indicating that m98 was not active during every wingstroke. The relationship between m97L and m98L (*bottom trace*) is negative, indicating that m98L fired later than m97L during the downstroke. This relationship showed little change during the flight sequence. **C** Muscle m97 asymmetry (*top trace*) and muscle m99 asymmetry (*middle trace*) shifted in the same direction during a thermal presentation. The relationship between m97 and m99 is negative, indicating that m99 was activated later in the downstroke than m97. A small shift in this relationship was observed at lamp off

which the forewing ipsilateral to the turn was more depressed than the contralateral wing during the downstroke. In a correlation analysis, we found that the magnitudes of shift of these two variables were significantly and positively correlated with one another, as well as with corresponding m97 asymmetry shifts.

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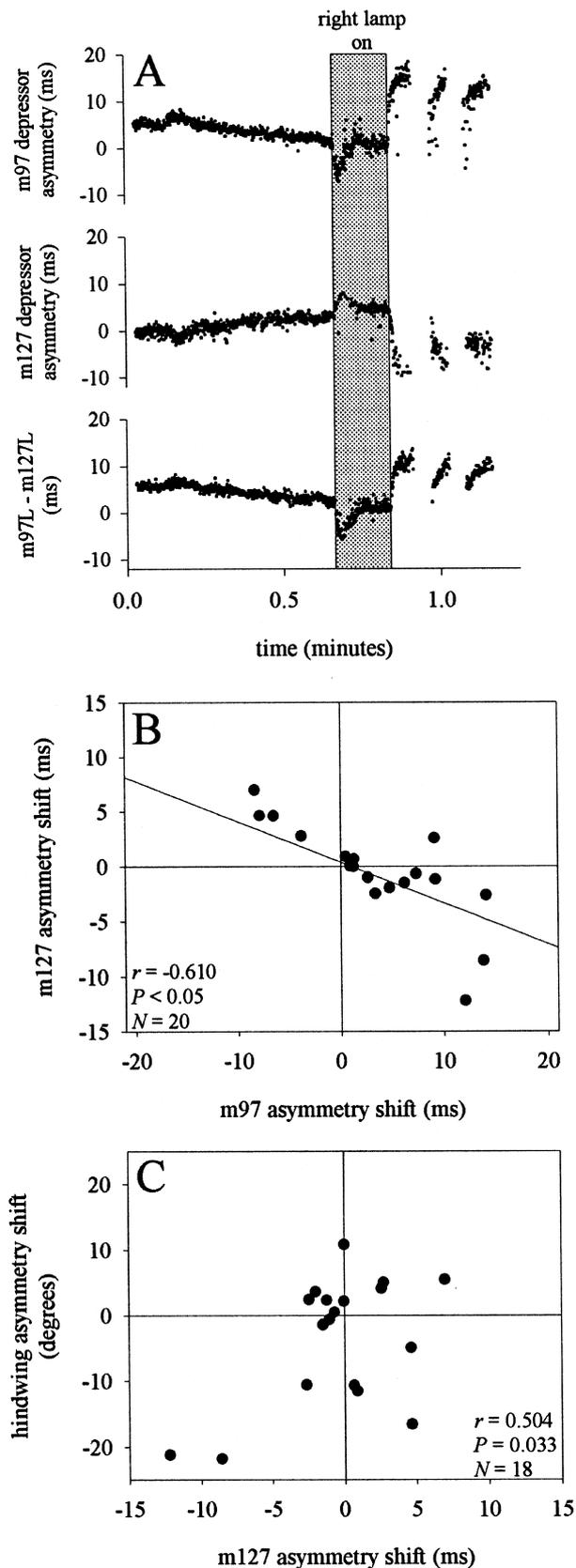
Fig. 8A–C Simultaneous recordings from pairs of forewing (m97) and hindwing (m127) depressor muscles showed opposite asymmetry shifts in response to thermal presentations. **A** Muscle m97 asymmetry (*top trace*) and m127 asymmetry (*middle trace*), recorded from an individual locust, showed that m127 shifted in the opposite direction from m97 and with a smaller magnitude during a thermal stimulus. The relationship between m97L and m127L (*bottom trace*) was variable. Note that following the thermal presentation, the locust twice stopped and restarted flight. Although the animal restarted without depressor asymmetry, it was quickly reestablished within the first few wingbeats. **B** Summarized data from twenty animals. There was a significant negative relationship between m97 and m127 asymmetry shift during thermal presentations. **C** Summarized data from time-aligned HWA measurements and m127 asymmetries. There was a significant correlation between the magnitude of HWA shift and m127 asymmetry shift. Note that 2 of 20 animals were removed from this analysis due to an insufficient number of frames to calculate HWA

Abdomen deflection has long been a correlate of steering direction (Camhi 1970) and it contributes to the generation of steering torques by increasing drag on the side of the intended turn, and by shifting the centre of mass upon which the aerodynamic forces act. The role that asymmetries in forewing elevation angles play in steering is less clear. Phase shifts in the timing of forewing depression during correctional manoeuvres have been described (Baker 1979a; Thüring 1986; Zarnack 1988; Waldmann and Zarnack 1988) but often discounted as contributing to steering torques. During intentional steering manoeuvres, however, forewing asymmetries are larger (Robertson and Reye 1992; Robertson and Johnson 1993; Robertson et al. 1996; Dawson et al. 1997), and the forewing ipsilateral to the turn can sometimes be more depressed than the ipsilateral hindwing during the downstroke.

Although the correlations between m97 asymmetry shifts, abdomen deflection and forewing asymmetry shifts are very tight, it is difficult to ignore those data points that indicate large negative FWA shifts but small positive m97 asymmetry shifts (Fig. 5A), or a positive m97 asymmetry shift with an abdomen deflection to the left (Fig. 5B). We believe that this scatter is partly an artefact of the technical limitations of our experiments. The first limitation is the rigid tether. The animal does not generate 100% lift, exteroceptive input (e.g. direction of wind) is abolished during steering behaviours and motor pattern biases may occur (Möhl 1985). A second limitation is our videotaping procedure. A filming speed of 60 frames/s only captures the wings in a downstroke position on approximately every third frame. Even fewer frames captured the wings at the top of the downstroke.

Despite these limitations, we are confident that the correlations we describe are valid for the following reasons. First, we saw coordinated steering behaviour involving the wings, muscles and abdomen. Second, these steering behaviours were coincident with the onset or end of the thermal presentation. Third, the animals steered both to the left and the right, suggesting that tethering biases, if present, were minimal.

Spikes per burst

During steering manoeuvres, we found relationships between the timing activity of m97 and the number of spikes it fired per burst. Generally, when m97 advanced its timing in the wingbeat cycle, its spikes per burst increased. A similar trend has been described by Baker (1979b), Dugard (1967), and Waldron (1967). Sometimes, the m97 that was delayed in its timing fired fewer spikes per burst, and in this way, the spikes per burst of m97 on opposite sides of the body experienced a "push-pull" relationship. However, the m97 spikes per burst on the outside of the turn did not always decrease. We offer two explanations for why the muscle on the inside of the intended turn *always* increased its spikes per burst while the muscle on the outside only decreased its spikes per burst sometimes. (1) During stimulus presentation, the wingbeat frequency increases (see below), which is associated with increased spikes per burst (Wilson and Weis-Fogh 1962). Thus, on the inside of the turn, an increased spikes per burst due to m97 timing changes was compounded by the increase due to WBF. On the outside of the turn, however, increases in spikes per burst due to WBF could cancel any decrease associated with timing delay. (2) During straight flight, the m97 muscles often fire singlets. During a steering manoeuvre, the m97 on the inside of the turn increases its spikes per burst, but on the outside of the turn, unless it stops firing completely, the muscle activity cannot decrease.

Wingbeat frequency

During steering manoeuvres we observed increases in WBF. Since it is known that temperature influences WBF in locusts (Foster and Robertson 1992), it might be argued that the change in WBF was due to non-specific effects of an increase in thoracic temperature rather than being a component of the manoeuvre. For two reasons, we feel that this is unlikely. First, the increase in WBF was brief relative to the length of the thermal presentation. Shortly after the stimulus onset, it returned to pre-stimulus values, even though the temperature continued to rise. Secondly, this increase in frequency occurred when temperatures increased at lamp on and when temperatures *decreased* at lamp off. Based on these observations, we propose that the increase in WBF is part of a startle response associated with relatively abrupt changes in radiant heat.

Motor patterns and proposed model

During thermal presentations, we observed large asymmetry shifts in muscle activity in response to both lamp on and lamp off. Although we recorded from only two muscle pairs (four electrodes) at once, we can, based on the relationships between m97 and the other recorded muscles, construct a model of how all the muscles interact during a thermal steering manoeuvre (Fig. 9). Before the stimulus is presented, during perfectly straight flight, all contralateral muscles fire synchronously (Fig. 9A). Hindwing first basalars (m127) are activated first. This is to be expected since the hindwings lead the forewings during straight flight (Wilson and Weis-Fogh 1962). Approximately 3.5 ms later, the forewing first basalars (m97) fire. Following m97, in sequence, are the activity of m99, m98, and m85. Every 50 ms, at a wingbeat frequency of 20 Hz, this pattern of activity is repeated. The sequence of muscle firing for m97, m98, m99, and m127 is similar to that found by Wilson and Weis-Fogh (1962) and Möhl (1985).

In Fig. 9B, we show the modification of muscle activity in response to a thermal stimulus. The muscle activity is based on the positive m97 asymmetry shift of 10 ms, which corresponds to a right-hand turn. We chose a 10-ms shift because it is within the range of observed responses, and it generates a model in which the changes in muscle activation times are more obvious. Across the body, the right m97 fires 10 ms before the left m97. The WBF increases during a stimulus, and in Fig. 9B is shown to be 21 Hz. The hindwing first basalars (m127) undergo an opposite shift to the forewing first basalars (m97). This occurrence is supported by Thüring (1986) and Möhl (1985). Muscle m127L fires approximately 8.5 ms earlier than m97L. On the right side of the body, m127 fires in time with some of the forewing muscles, and fires about 4 ms after m97.

In the forewings, for both the left and right sides of the body, the timing relationship between m97, m98 and m85 maintains their pre-stimulus relationship. In other words, these muscles on the right side of the body all fire 10 ms before their corresponding contralateral muscles (Fig. 9B). Muscle m99, however, although maintaining its place in the sequence of forewing depressor activity, is asymmetrically phase-shifted in its timing. On the left side of the body, m97 and m99 fire almost synchronously, with m99 firing only about 0.5 ms after m97. On the right side of the body, m99 fires more than 3 ms after m97.

The phase difference in timing in m99 across the body could affect the angle of attack during steering. Wilson and Weis-Fogh (1962) state that the changes in angle of attack of the forewings is due to changes in the phase of activity of the subalar muscle. Muscle m99 (supinator) controls the angle of attack by counteracting the effects of m97 (pronator). In our model, the synchronous firing of m97 and m99 on the left side of the body would lead to decreased pronation and an increased angle of attack. On the right side of the body, the long latency between m97 and m99 would cause increased pronation and a decreased angle of attack. Since an increased angle of attack is associated with increased lift, the right-hand side of the body (inside of the turn) would generate less lift, and result in a banked turn to the right. This prediction of wing kinematics is consistent with the obser-
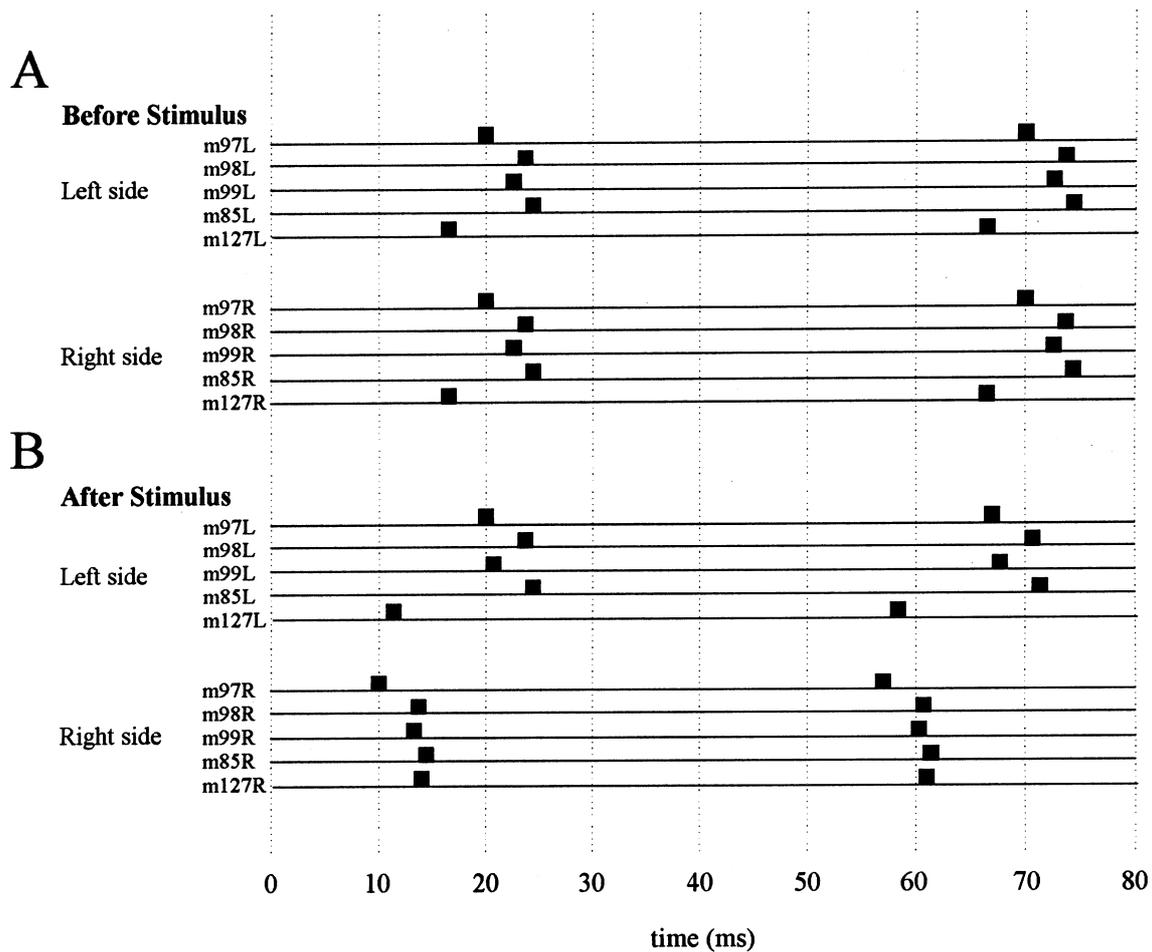
Fig. 9A, B Schematic diagram of motor pattern modulation of depressor muscles during a thermal presentation. Forewing muscles m97, m98, m99, and m85 were included in this study, along with hindwing muscle m127. **A** In the absence of a stimulus, during straight flight, homologous muscle pairs fire synchronously. Muscle m127 of the hindwing is activated before the muscles of the forewing. **B** When attempting to steer, the wingbeat frequency increases (shorter period). Here we show an m97 asymmetry shift of 10 ms. In both wings, the relationship between m97, m98 and m85 remains constant. The sequence of firing remains constant between m97, m98, m99 and m85. Although not included here, there are also changes in m97 spikes per burst during steering (see Results)

vations made during both correctional and intentional steering (for review see Kammer 1985; Rowell 1988).

Muscle m85 is different from the other muscles studied in this report because it is not a direct depressor muscle. Instead, evidence suggests that it exerts fine control over the degree of pronation of the forewings by decreasing pronation during the downstroke (Elson and Pflüger 1986; Wolf 1990). It may act by increasing its activity (spikes per burst) or recruiting a second motor unit. Our data cannot support or refute the observations of Elson and Pflüger (1986) or Wolf (1990) because we did not analyse number of spikes per burst or the traces for motor unit recruitment. We can predict, however, that if m85 is taking part in counteracting pronation during the downstroke, it acts together with m99.

Conclusion

The bulk shifts in forewing muscle activity described above offer an explanation for FWA. During a right turn, in which the right forewing is more depressed than the left forewing, the right forewing depressor muscles are all advanced in their timing. An earlier contraction would lead to an earlier stroke reversal, and thus phase-shifting of wing depression. This explanation is additionally convincing since the magnitude of the bulk shift is strongly correlated with the magnitude of FWA shift.

Many researchers maintain that pronation and supination are the key elements generating the aerodynamic forces for turning (Baker 1979a; Waldmann and Zarnack 1988; Zarnack 1988). Increased pronation (decreased angle of attack) on the side of the intended turn would decrease lift generated on that side. On the side contralateral to the intended turn, decreased pronation (increased angle of attack) would increase lift on that side. This differential lift would generate a steering manoeuvre in the same direction as the forewing asymmetry. We did not, in our experiments, investigate the occurrence of pronation or supination, but we believe that these changes occur in addition to FWA, and that their aerodynamic forces augment the effects of FWA. From our model, it is likely that pronation and supi-

nation do occur based on the phase-shifting of m99 on left and right sides.

The role of the hindwings during intentional steering manoeuvres in response to radiant heat remains unclear. It has been suggested that the hindwings generate asymmetry in angle of elevation opposite to that of FWA (Möhl 1985; Thüring 1986), and indeed, the significant negative relationship between the asymmetry shifts of m97 and m127 supports this prediction. However, two other lines of evidence suggest that HWA plays no role in intentional steering. One line of evidence is that no significant relationship was observed between HWA and the standard correlates of steering, FWA and abdomen position. Additionally, although m127 asymmetry shifts and HWA shifts are significantly correlated, this association is weak (Fig. 8C). In fact, 2 of 18 data points at the extreme negative values of m127 asymmetry shift appear to drive this relationship. There is no significant correlation between the remaining 16 data points (Pearson product moment correlation, $r = -0.08$, $P = 0.77$, $n = 16$), suggesting that the role of m127 in intentional steering is *not* to generate HWA. We suggest that m127 is involved in generating asymmetries in hindwing angle of attack that contribute to asymmetrical flight forces during steering. Further examination of hindwing kinematics or a study of hindwing motor activity would help clarify this issue.

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