

THE ACTIVITY OF A STEERING MUSCLE IN FLYING LOCUSTS

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SUMMARY

1. The pleuroaxillary muscle of a forewing (M85) or hindwing (M114) in the locust is supplied by two motor neurones. Each of the two motor neurones innervates a different part of the muscle. Single impulses in these motor neurones produce small twitches in the muscle which tetanize at about 30 Hz. At the wingbeat frequency they show considerable tonic tension upon which ripples are superimposed, 1:1 for each stimulus pulse.

2. During sustained, straight, tethered flight, the motor neurones spike rhythmically, producing one (less often two) spike(s) per wingbeat in the first half of each downstroke. At the end of flight, when the wing is folded, a high-frequency, unpatterned burst of spikes occurs.

3. During flight-like motor activity where rhythmic sensory feedback is reduced, the pleuroaxillary muscle of a hindwing spikes throughout the 'wingbeat' cycle, with little sign of rhythm.

4. The forewing muscle, M85, responds to imposed rolling during flight by advancing the timing of its spike, increasing the number of spikes at each wingbeat, and recruiting a second motor unit on the side which is rotated downwards; converse changes occur on the side that is rolled upwards. The magnitude of the time-shift response in M85 depends on the angular position of the locust about the roll axis. The hindwing muscle shows similar changes in the number of spikes and in recruitment.

5. Motor neurones of both the forewing and hindwing muscles can spike in response to imposed rolling in locusts that are not flying. Excitation increases on the side that is rolled down. The response to angular movement about the roll axis is primarily phasic and is dependent on visual cues.

6. It is concluded that these muscles take part in steering behaviour during corrective reactions. Activation is increased on the side where more lift must be produced. Similar changes of activity in these muscles may play a role in active steering manoeuvres.

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INTRODUCTION

Locust flight provides a system in which one may study the modification of a basic locomotor pattern during active manoeuvres and compensatory reactions. The motor pattern underlying flight consists of a stereotyped alternation of activity in elevator and depressor muscles with a small time difference of 5–10 ms between the hindwings and the forewings (Wilson & Weis-Fogh, 1962). Each spike in the principal flight muscles elicits a discrete twitch at flight frequency (Neville & Weis-Fogh, 1963) so that the detailed pattern of motor spikes exerts a determining influence on the movements of the wings. Steering forces are thought to result from changes in the kinematics of the beating wings and from ruddering movements of the abdomen and legs (Dugard, 1967; Camhi, 1970; Baker, 1979*a*; Zarnack & Möhl, 1977; Taylor, 1981*a,b*). Increasing the angle of attack of the wings leads to increased production of lift and thrust. Regulation of this angle should be particularly effective during the downstroke of the wing, when the major aerodynamic force is produced (Jensen, 1956; Nachtigall, 1981*a,b*). Differential pronation of the forewings is the only parameter of wing motion which is always correlated with the performance of turns in manoeuvring locusts (Baker, 1979*a*). (Pronation corresponds to downward twisting of the leading edge of the wing in the downstroke, and decreases the angle of attack.)

It is not yet clear how the locust uses its flight musculature to effect these changes of angle of attack. During steering behaviour phase shifts can occur in the timing of motor unit spikes in all direct depressor muscles and in those elevator muscles which have been studied (Zarnack & Möhl, 1977; Baker, 1979*b*; Taylor, 1981*b*). Of the depressor muscles, the basalars should pronate the wing while depressing it, and the subalar muscle, once considered a downstroke-supinator (i.e. antagonizing pronation, Wilson & Weis-Fogh, 1962), may usually (in the forewing at least) also contribute to pronation as it depresses the wing (Pfau, 1977; Pfau & Nachtigall, 1981). In contrast, the small pleuroaxillary muscle should exert fine control over the amount of pronation (decreasing it in the downstroke) but should have no elevator or depressor action (Pfau, 1976, 1977). Previously this muscle had been ascribed a role in folding the wing (Snodgrass, 1929; Pringle, 1968). In the resting animal its contractions alter the angular setting of the forewing (Heukamp, 1984), but its function during flight is still unknown. Pfau & Nachtigall (1981) proposed that the forewing pleuroaxillary muscle is tonically active, and that its suggested wing-twisting action enables it to function as a steering muscle. Bilateral activation would permit a symmetrical increase in lift and thrust, whereas differential activation would generate asymmetrical forces needed for steering. The locust pleuroaxillary muscles have therefore been compared with the non-fibrillar flight-steering muscles of Diptera (Nachtigall, 1967; Nachtigall & Wilson, 1967; Heide, 1968).

In this paper we investigate the activity of these previously unstudied muscles in flying locusts during straight tethered flight and during imposed rolling movements. The locusts were not free to make active turns. Motor responses to imposed rotations were presumed to be corrective reactions. We show that during sustained flight their motor neurones spike rhythmically and at a particular phase of the wingbeat. During

imposed rolling their activity is modulated in a way appropriate to produce a compensatory response of the wings.

MATERIALS AND METHODS

Mature, adult locusts of the species *Schistocerca gregaria* (Forskål) and *Locusta migratoria* (L.) were obtained from crowded colonies maintained at Cambridge and Konstanz.

Muscle physiology

The pleuroaxillary muscles of the fore- and hindwings (M85 and M114, respectively, in the numbering of Snodgrass, 1929) are each innervated by nerve 4D4 of their respective segmental ganglion. This nerve has been shown in the preceding paper to contain the axons of the two motor neurones which innervate each muscle (Pflüger, Elson, Binkle & Schneider, 1986). The nerve was cut proximally and stimulated by bipolar silver electrodes. Forces produced by a muscle were measured by clamping its distal end to a force-displacement transducer (Grass FT03C). Three sets of experiments were carried out, both at room temperature and at 30°C.

(i) The amount of current was first set to stimulate only one of the two motor neurones and the force measured. The second motor axon was recruited by a slight increase of the stimulus amplitude and the total force measured. The results are based on five *Locusta* in which M85 and M114 were each tested.

(ii) One part of a muscle (a or b) was removed from its insertion on the third axillary sclerite, so that it did not contribute to the force (two *Locusta*).

(iii) Intracellular recordings were made from various muscle fibres during electrical stimulation of the motor nerve. Electrodes were filled with 2 mol l⁻¹ potassium acetate and had resistances of 20–30 MΩ (two *Locusta*).

Flight and rolling experiments

Adult locusts of both sexes were used, but males performed tethered flight more readily. Locusts were placed centrally in the airstream of an open-jet wind-tunnel, at a distance from the mouth where the airflow had uniform velocity and was non-turbulent. The temperature of the air-jet was maintained at 30°C. Wind speeds of 3–4 ms⁻¹ were used. In most of the experiments all three pairs of legs were amputated at the coxotrochanteral joint and the wounds sealed with wax. The thoracic sternum was waxed to a small platform at the end of a thin rod. This was mounted at the end of a rotatable axle, situated behind the locust so that turbulence was minimized. The attitude of the body relative to the wind was adjusted to be in the range 5–10°, i.e. close to the preferred body angle. In the Cambridge experiments the axis of rotation passed close to the animal's centre of gravity, thus reducing to a minimum side-to-side translations during rolling. In the Konstanz experiments the locust was mounted eccentrically (7 cm from the axis of rotation) and therefore experienced translatory movements (cf. Zarnack & Möhl, 1977). The results on M85 were gained in Cambridge, those on M114 in Konstanz, unless

otherwise stated. Rolling was imposed by rotations of the axle, driven by a servomotor under the control of a function generator.

A horizon-display consisting of a screen divided horizontally into an upper, white portion and a lower, matt black portion was provided. The artificial 'horizon' so formed was adjusted to be in the horizontal plane of the animal and was illuminated by a distant lamp. All other sources of light were excluded from the laboratory.

Electromyograms were recorded using up to three pairs of 20 μm or 50 μm steel wires, insulated except at the tip, inserted into the desired muscle through the thoracic pleura or the third axillary sclerite. They were waxed in place and did not disturb wing movements.

Spikes of M85 motor units were selected by window circuits. Histograms of the number of spikes in successive bins of selected width were generated by computer (Datalab DL4000). To measure the time difference between the occurrence of spikes in two muscles, their spikes were first captured by separate window circuits; the pulse outputs of these circuits were combined and fed into a circuit measuring instantaneous interval. This generated pulses whose amplitudes were related to the size of the preceding interval. As the intervals between two spikes occurring in different muscles in the same wingbeat (e.g. the spikes in the left and right M85s) were always much shorter than the intervals between spikes in the same muscle occurring at the wingbeat period, the pulses corresponding to the former class of intervals could be easily discriminated by their amplitude and displayed separately. The size of these intervals was plotted against time, showing the duration of these intervals at successive wingbeats and the change in interval duration with time as rolling stimuli were applied (Fig. 7).

RESULTS

Physiology of pleuroaxillary muscles

The pleuroaxillary muscles of the fore- and hindwing (muscles 85 and 114, respectively, in Snodgrass, 1929) are each innervated by two motor neurones (Pflüger *et al.* 1986). To study the contractile response of a pleuroaxillary muscle, and, in particular, its response to motor neurone impulses at flight frequencies (about 20 Hz), the force produced by a muscle (M85) was measured while stimulating its motor neurones selectively at a range of frequencies (Fig. 1). Stimulating the axon of a single motor neurone (Fig. 1A) at 1 Hz produced discrete small twitches. At 10 Hz, the twitches were still separate so that no tonic tension developed. Between 10 and 20 Hz, summation of twitches began, but discrete ripples were superimposed on the tonic contraction. A smooth tetanus was reached between 20 and 40 Hz. When both motor axons were stimulated (Fig. 1B) the muscle behaved very similarly, but with increased force at each stimulus frequency. The recruitment of the second motor neurone approximately doubled the size of single twitches, but at higher stimulus frequencies the increase in tension was smaller (40–80 %, cf. Fig. 1, 10–40 Hz). The twitch:tetanus ratio when stimulating both motor neurones was

about 1:6. M114 behaved similarly in response to stimulation of both its motor neurones (Fig. 2B).

Each pleuroaxillary muscle consists of two bundles, termed parts a and b (Pflüger *et al.* 1986). The two motor neurones of a muscle could conceivably each innervate muscle fibres in both parts, or in one part only. Cutting away one muscle part (part b of M114, Fig. 2A) from its distal insertion, so that it no longer contributed to the measured force, showed that the single remaining muscle part was innervated by only one motor neurone. The ablation reduced the force by about 50% (cf. Fig. 2A,B), and only one size of twitch could be recruited in the remaining part as the stimulus intensity was increased from a subthreshold value. The converse experiment, in which part a was ablated, yielded similar results. This indicates that each part is supplied by only one motor neurone and that the two motor neurones innervate different parts. Accordingly, intracellular recordings from individual fibres showed only one class of excitatory junctional potential (EJP). This was true of fibres in parts a and b of both muscles. No inhibitory junctional potentials (IJPs) were seen.

Activity of pleuroaxillary muscles in flying locusts

Electromyograms of pleuroaxillary muscles in flying locusts showed two sizes of muscle potentials (cf. also Heukamp, 1984). These were recognized as the potentials

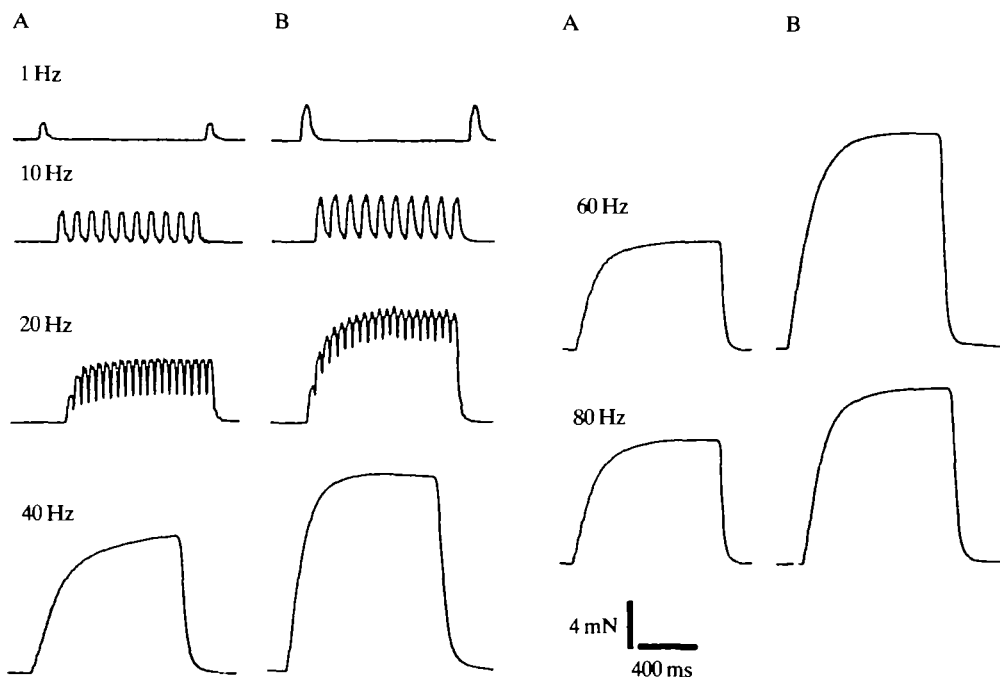


Fig. 1. Force produced by contraction of M85 in response to electrical stimulation of its motor nerve at 1–80 Hz (1, 10, 20, 40, 60, 80). (A) Only one unit is activated. (B) The stimulus amplitude is increased by 0.1 V, both units are activated.

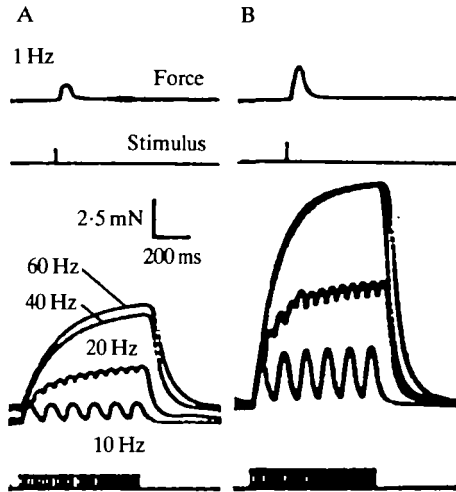


Fig. 2. Force produced by M114 in response to electrical stimulation of its motor nerve at 1–60 Hz. Upper, 1 Hz; lower, 10, 20, 40, 60 Hz. (A) Response of part a only (part b removed). (B) Response of both parts a and b.

produced by the two motor units, and were termed the '*large*' and '*small*' units respectively, on the basis of their size.

Straight, tethered flight

The pleuroaxillary muscles of both the fore- and the hindwing were rhythmically active at the wingbeat frequency in straight, tethered flight (Fig. 3). The large motor unit fired one spike (more rarely two) for each wingbeat. The small unit apparently fired at the same time as the large, but was often obscured by it. However, when the large unit dropped out, as in the spontaneous changes in excitation described below, the spikes of the small unit were revealed at the time of the wingbeat previously occupied by the large unit (Fig. 3A, arrowhead; cf. also Fig. 4C).

The muscles fired at a characteristic time in the wingbeat, close to the depressors (Figs 3, 4). Spikes occurred after the firing of the direct depressor muscles of the forewing and the hindwing (Fig. 3A,B). The spike in M85 followed a spike in the metathoracic subalar muscle with a latency of 10–20 ms; a spike in M114 followed one in the metathoracic subalar by 8–30 ms (these values were for wingbeat periods of 60–70 ms). On occasions, therefore, spikes in M85 and M114 could coincide (Fig. 4B). During episodes of regular, straight flight the timing of pleuroaxillary spikes in the wingbeat was precise. An example is shown in Fig. 4C of the spikes in M114 following those of a hindwing depressor (subalar) within a narrow range of latencies (9–18 ms). The spikes in pleuroaxillary muscles on opposite sides of the body maintained a constant difference in timing, typically in the range 0–10 ms, during straight flight. Occasional spontaneous changes in this motor pattern occurred during episodes of regular flight. The large unit would drop out of rhythmic firing, becoming silent, and then later resume spiking in the normal rhythmic pattern

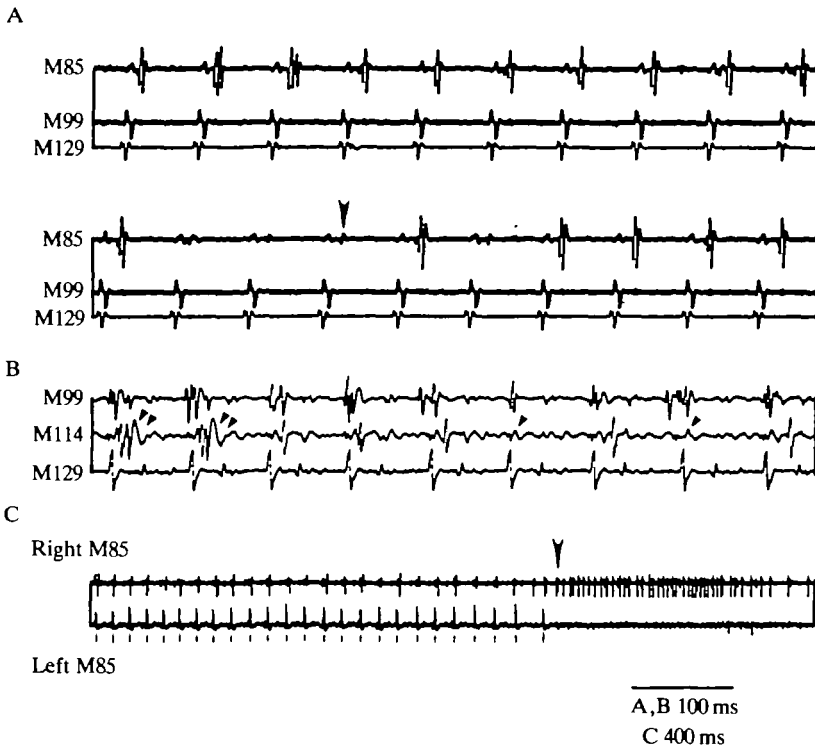


Fig. 3. Activity of the forewing (A) and hindwing (B) pleuroaxillary muscles in sustained, straight, tethered flight, and the activity of the forewing muscle at the end of flight (C). (A) Simultaneous recording by electromyograms of the left forewing pleuroaxillary muscle (M85), the left forewing subalar muscle (M99) and the left hindwing subalar muscle (M129). Large spikes in the M85 trace are those of the large unit. The lower example is from later in the same flight. Arrowhead: small unit. (B) Simultaneous recordings from a hindwing pleuroaxillary muscle (M114), M99 and M129. Single arrowhead: no large unit spikes. Double arrowhead: two large spikes in M114. (C) End of flight. Recordings from right and left M85s. Note the burst of spikes in right M85 (arrowhead).

(e.g. Fig. 3A). The number of spikes produced at the characteristic time in each wingbeat could also fluctuate between one and two spikes per wingbeat. These changes occurred in the absence of imposed external stimuli and without any accompanying change in depressor activity monitored in the subalar muscles (e.g. Fig. 3A).

In contrast to this pattern of activity, at the start of flight the pleuroaxillary muscles could fire more than one or two spikes per wingbeat and spikes could occur at other times in the wingbeat. As flight continued the regular pattern emerged. However, during irregular wing movements the regular rhythmical pattern disappeared, and the muscles would fire at other times of the wingbeat and produce additional spikes.

At the end of flight episodes, M85 (Fig. 3C) and M114 would sometimes give a high-frequency burst of spikes as the locust folded its wings.

Activity of M114 during reduced feedback

In a dissected preparation where the meso- and metathoracic ganglia were isolated from the periphery by cutting the nerve roots (except nerves 1 and 4 in both segments), and the pleura and wing-stumps pinned out (thus greatly reducing phasic sensory feedback), flight-like rhythmical activity was evoked in the remaining flight power muscles by blowing air over the head (cf. Robertson & Pearson, 1982). The metathoracic subalar muscles were rhythmically active, producing one or two spikes per cycle. The small unit of M114, however, fired throughout the whole cycle and at random (Fig. 5A,B). Accordingly, the spike of the small unit no longer followed the spikes in the subalar muscle at constant latency (Fig. 5C), in contrast to the activity in straight, tethered flight (Fig. 4C). The large unit still spiked near to the time of the subalar spikes (Fig. 5A), but the constant-latency following characteristic of straight, tethered flight was lost.

Responses to imposed rolling during flight

The motor units of M85 responded to imposed rolling during flight by changes in the following three parameters at each wingbeat: (i) the time of firing (time-shifting); (ii) the number of spikes produced by single units; and (iii) the number of units active (changes in recruitment).

A correction for unintended roll requires differential activity on opposite sides of the locust. Therefore only differential changes in M85 activity (either unilateral

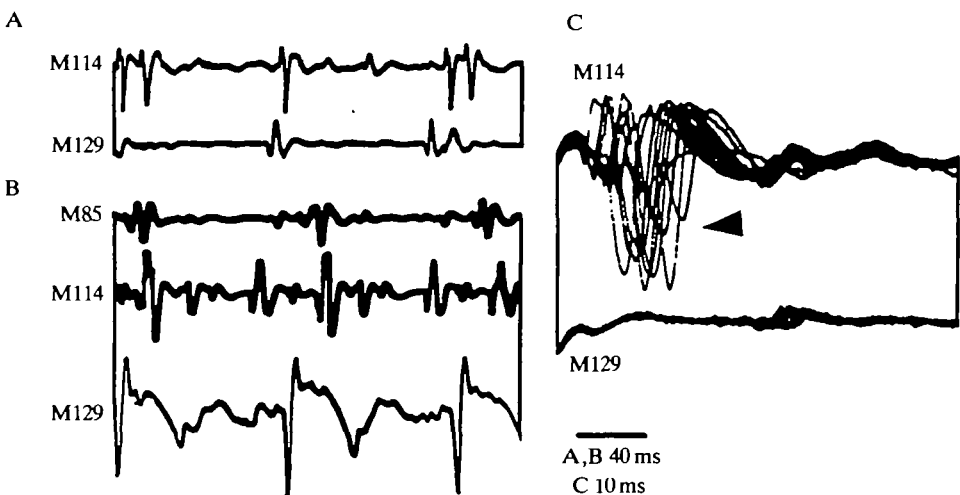


Fig. 4. Timing of pleuroaxillary muscle spikes during sustained, straight flight. (A) M114 firing one or two spikes following the spike(s) in M129. (B) M85 and M114 producing spikes at about the same time. The mainly upward-going potentials in the M114 trace, occurring before M129, are those of an elevator muscle. (C) Spikes of M114 occurring at nearly constant latency after the spike in M129 (from which the oscilloscope is triggered). Many sweeps overlaid. Arrowhead marks the amplitude of smaller potentials occurring at the same time as the large potentials. All recordings are from muscles on the same side of the animal.

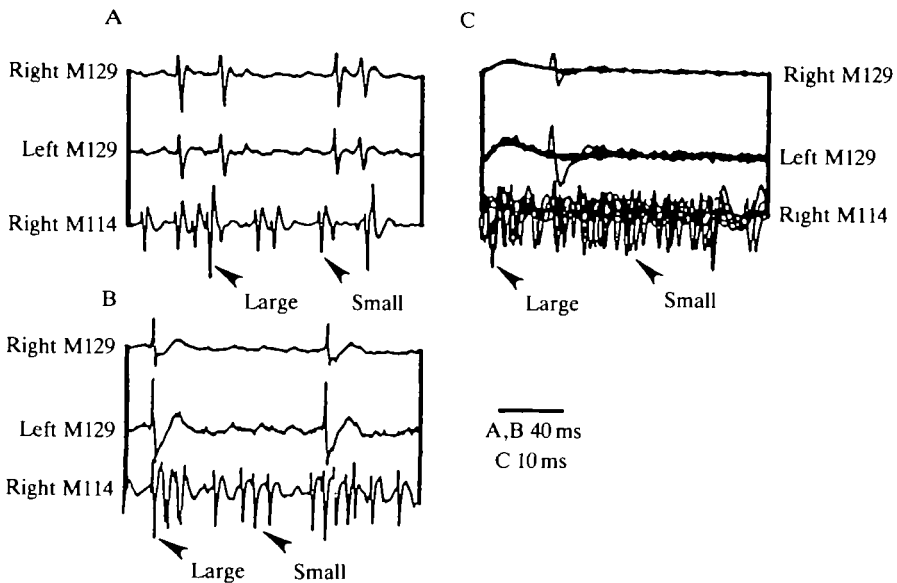


Fig. 5. During fictive flight M114 fires irregularly while the hindwing subalar muscles fire rhythmically (A,B). (C) The small unit has no constant-latency relationship to the subalar spikes (from which the oscilloscope is triggered; several sweeps overlaid). The amplitudes of spikes attributed to the small and large units are marked.

changes, or opposite changes on both sides) were considered to be flight corrections. Such responses took place under open-loop conditions because the locusts were rigidly fixed to the stimulus device.

Time-shifting (M85)

Time-shifting appeared as a change in the relative timing of pleuroaxillary motor spikes on opposite sides of the body (Figs 6, 7). During imposed rolling the time-difference between left and right muscles (constant during straight flight, see above) was modulated, although the wingbeat period remained constant. The shift in relative timing of the motor spikes therefore corresponded to a shift in relative phase. A typical response to imposed roll is shown in Fig. 6. In this locust the spike of the right M85 preceded that of the left M85 in straight flight. An imposed roll to the *left* (rotating the locust's left side downwards) resulted in a *decreased* interval between the two sides (Fig. 6A, Δt_1). Conversely, rolling to the *right* (Fig. 6B) evoked an *increased* interval (Δt_2). This modulation of the time difference was due to time shifts of the spikes of individual motor units on both sides of the thorax (Fig. 6). Rolling to the left (Fig. 6A) advanced the timing of the left muscle spike and delayed the timing of the right muscle spike, relative to the spike of a hindwing subalar muscle (which was used as a reference). Opposite changes occurred during rolling to the right (Fig. 6B). This and other experiments showed that the timing of pleuroaxillary motor spikes was advanced on the side which was rotated downwards by the imposed rolling, and delayed on the side which was rotated upwards.

Ramp-hold roll stimuli indicated that the response to a maintained change in angular position was a maintained time-shift, with little apparent dynamic response to the movement to the new angular position. To study further the relationship of time-shift to angular position and movement, sinusoidal (Fig. 7A–C) and triangular (Fig. 7D) oscillations in the roll plane were imposed. The left–right time difference was clearly modulated and followed the waveform of the imposed stimulus (Fig. 7C,D). The magnitude of the time-shift was dependent on the angular position, with maxima and minima coinciding with the peaks of the stimulus (Fig. 7). With different frequencies of sinusoidal roll (Fig. 7A,B), the phase of the response relative to the stimulus did not change, suggesting the lack of a dynamic sensitivity.

Changes in the number of spikes (M85 and M114)

Motor units responded to imposed rolling in flight by firing more spikes per wingbeat on the side of the animal that was rolled downwards (Fig. 6B). Changes in the number of spikes occurred more often in the small unit. The number of spikes per wingbeat altered within the range of 1–3 spikes in the small unit, 1–2 in the large. The roll deviation from horizontal that was required to evoke a response was less for the small unit.

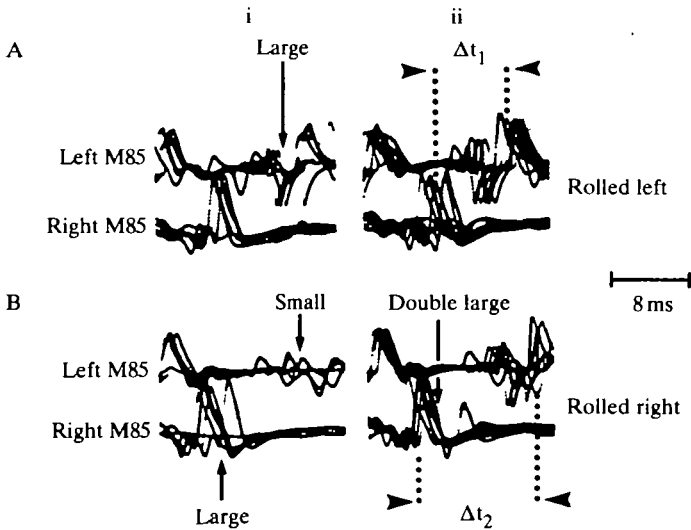


Fig. 6. Responses of the left and right forewing pleuroaxillary muscles to imposed rolling in a flying locust. Each pair of traces shows the potentials in the two muscles occurring at the wingbeat. The oscilloscope is triggered from the left subalar muscle of the hindwing (M129), which is used as a reference point, and several wingbeats are overlaid. The spike of the large motor unit is identified in both traces, as is the spike of the small unit in the left M85. Double large indicates a double firing of the large unit in the right M85. (A) The locust is rolled 23° to the left (i.e. left side rotated down). Two examples are given (i, ii). In (ii) the interval between right and left spikes is marked, Δt_1 . (B) The locust is rolled 23° to the right. Note that the spike of the right muscle is earlier, and the spike of the left muscle later than in A. Thus the interval, Δt_2 , is larger. In (ii) the large unit of the right muscle fires twice. In (i) the large unit of the left muscle drops out, leaving the small unit.

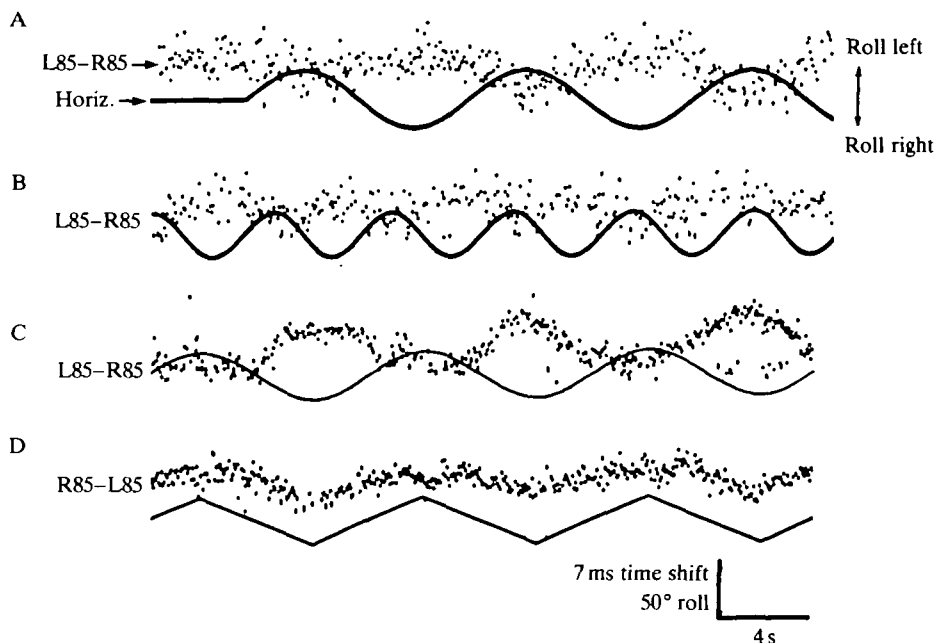


Fig. 7. Time-shifting by the spikes of the right and left forewing pleuroaxillary muscles in response to sinusoidal (A–C) and triangular (D) roll stimuli. In A–C the interval between the spikes of the right and left M85s at each wingbeat is plotted for successive wingbeats in the upper traces (L85–R85; the right muscle was leading). Each point represents the magnitude of this interval at one wingbeat. The height of the point is proportional to the interval magnitude (for more details see Materials and Methods). The lower traces indicate angular position of the locust about the roll axis. Rolling is symmetrical about the horizontal, Horiz. Roll left and Roll right indicate rolling to the left and right (left side and right side down) respectively. (A) Rolling at 0.1 Hz. During a roll to the left, the interval from the right spike to the left spike decreases (cf. Fig. 6A). (B) Rolling at 0.2 Hz. Note that the phase of the response relative to the stimulus is unchanged from A. (C) Another example of sinusoidal rolling. Note the sinusoidal modulation of the interval. (D) Modulation of the interval between the spikes in left and right pleuroaxillary muscles (R85–L85; the left spike was leading) during triangular rolling. The modulation of the interval parallels the stimulus wave-form.

Changes in the number of motor units active (M85 and M114)

Changes could also occur in the number of motor units active at each wingbeat. Early in a flight episode both motor units often fired at every wingbeat and responses were expressed only as time-shifts. However, later in a flight, or when the locust was flying less strongly, spikes of the large unit of M85 and M114 could drop out (Fig. 3A,B). During sinusoidal roll stimuli (Fig. 8A) the probability of the large unit firing at each wingbeat increased on the side that was rotated down, and decreased when that side was rotated up (Fig. 8A; also Fig. 6Ai,Bi). When the large unit did fire, it produced one spike per wingbeat with the appropriate timing. During a maintained roll deviation (Fig. 8B,C) the large unit of M85 fired once at each wingbeat for the duration of the stimulus on the side that was rolled down (Fig. 8B).

Maintained roll deviations in the opposite direction silenced previous sporadic spiking in this motor unit (Fig. 8C).

Responses in non-flying locusts

In some locusts that were not flying but remained tethered in the warm airstream, responses to imposed rolling were expressed in both M85 and M114 as a frequency-modulation of continuous spiking (Fig. 10A), or the recruitment of spikes (Fig. 9) in the small unit. These reactions could persist for many minutes after the end of flight, sometimes with a slow decline in the rate of tonic firing while the locust was horizontal and a decrease in the intensity of the response to rolling. Wind was not necessary for the response but had a potentiating effect. However, the presence of visual cues was necessary, as described below.

Imposed rolling excited the small unit of the pleuroaxillary muscle (initiating spiking, Fig. 9, or causing it to fire at higher frequency, Fig. 10A) on the side that was rolled down. Conversely, spiking was switched off, or reduced in frequency, when that side was rolled upwards (Figs 9, 10A). Ramp-hold or step stimuli produced a phasic increase in spike frequency on the side rotated downwards (Figs 10B, 11A) and a silencing of the spikes on the side rotated upwards (Fig. 11B). The spike frequency was not maintained at any new level, but instead gradually returned

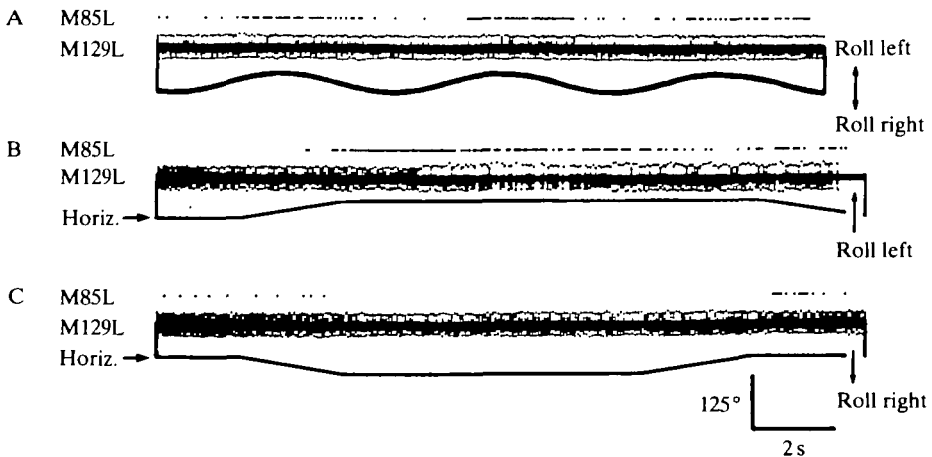


Fig. 8. Modulation of the occurrence of rhythmic spiking in the large unit of the left M85. When the unit fires, it produces one spike per wingbeat. Rolling to the left (Roll left) increases the probability of the large unit of the left muscle (M85L) firing at each wingbeat; rolling to the right (Roll right) produces a decrease. Top traces: each dot marks the occurrence of a single spike in the unit. Middle traces: myogram of the subalar muscle of the left hindwing (M129L), used here as a marker of the wingbeat. Bottom traces: angular position of the locust about the roll axis. (A) Sinusoidal rolling, symmetrical about the horizontal. (B),(C) Maintained changes in the occurrence of rhythmic spiking are elicited by maintained angular displacements of the locust about the roll axis. Horiz. indicates the horizontal position. (B) A roll to the left (locust's left side down) switches spiking on when the unit was previously silent. (C) A roll to the right silences previous sporadic spiking.

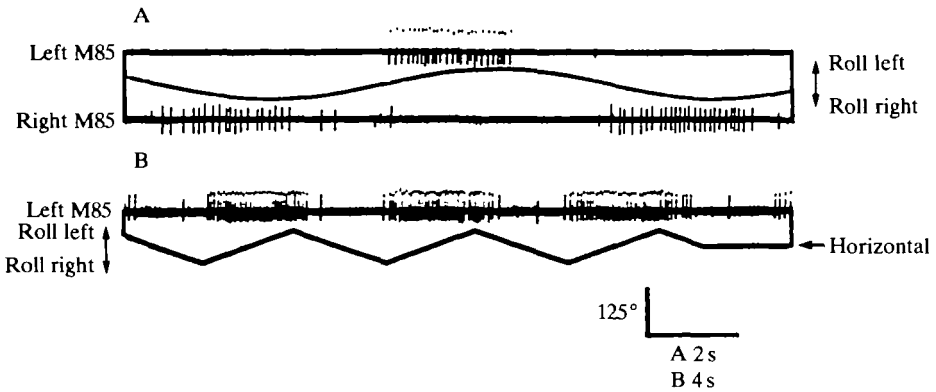


Fig. 9. Responses of the motor units of M85 to imposed rolling in a non-flying animal (tethered in the airstream; illumination on). Left M85: the potentials of the identified small unit of the left pleuroaxillary muscle. Right M85: an unidentified unit in the right pleuroaxillary muscle, probably the small. Rolling is symmetrical about the horizontal position. (A) Sinusoidal rolling. Spiking is elicited on the side that is rolled down. (B) Triangular roll stimuli.

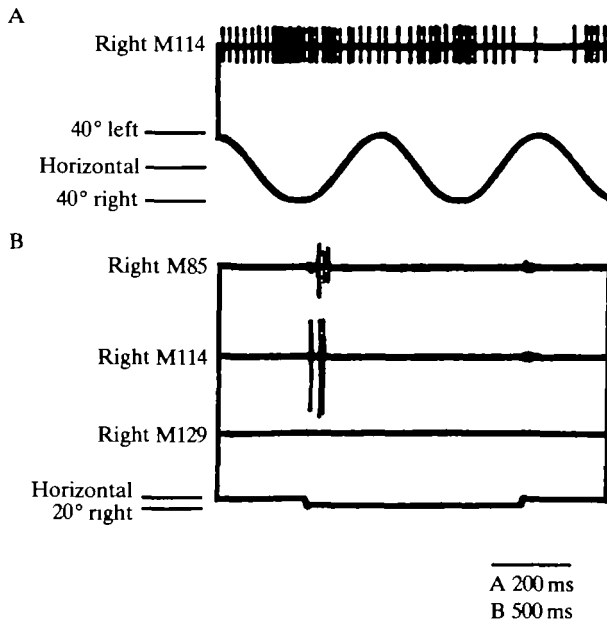


Fig. 10. Frequency-modulation of pleuroaxillary spikes (A) and phasic responses to roll movements (B). (A) An unidentified unit of M114, probably the small. Frequency-modulation of continuous firing. (B) Phasic excitation of units of the right M85 and M114, but not the right M129, when a sudden angular shift to the right is imposed. (Rolling movement included lateral translations; Konstanz experiments, see Materials and Methods.)

towards the average value it had at the horizontal (Fig. 11A). A dynamic response occurred, however, during angular movement (Fig. 11, arrowheads). During a

triangular roll stimulus (i.e. rolling at constant angular velocity) the frequency of spikes in the small unit of M85 increased as its side of the locust was rolled down, and decreased during the roll in the opposite direction (Fig. 12A; cf. Fig. 9B). During the roll down the frequency of spikes rose quickly at first, but then more gradually, and finally fell off rapidly as the direction of rolling was reversed. This indicated that the frequency of motor spikes was not proportional to the imposed roll angle. The frequency was higher in one direction of rolling than the other, showing that the response was directionally-selective and a function of the angular velocity. During sinusoidal roll (Fig. 12B; cf. Fig. 9A) maximal and minimal excitation preceded the turning points of the imposed movement (Fig. 12B, arrow and arrowhead). This again suggests a role for angular velocity in determining the frequency of spikes. However, the progressive increase in spike frequency that does occur during downward rolling at constant velocity (Fig. 12A) indicates some dependence on angular position also.

These motor unit responses to imposed rolls in non-flying animals depended crucially on the presence of visual cues. When the locust was kept in total darkness and subjected to continuous roll oscillations, the small motor unit either stopped spiking or fired spontaneously with no relationship to the imposed movement (Fig. 13A). When the illumination of the horizon display was switched on again, spiking reappeared in the characteristic pattern (Fig. 13B, arrowheads).

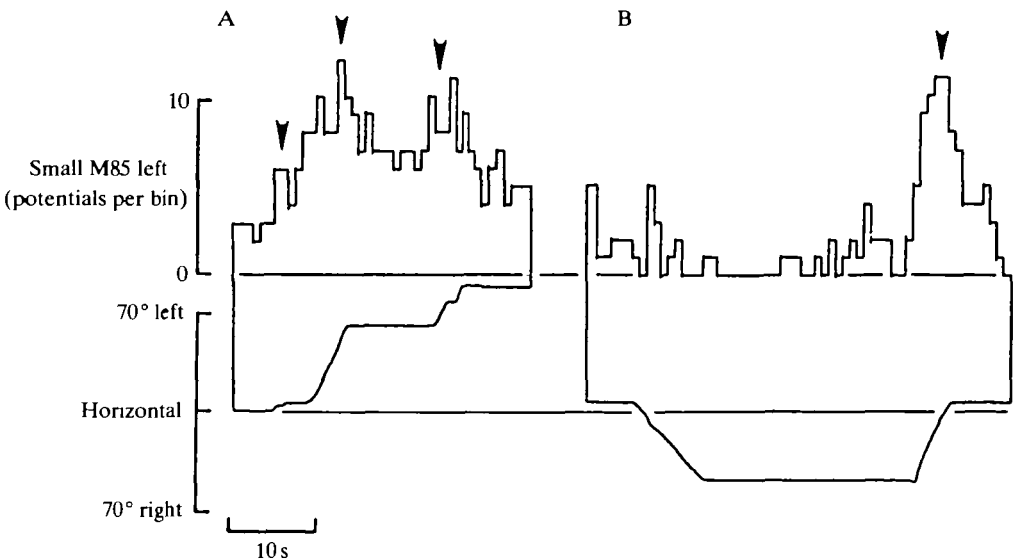


Fig. 11. Imposed roll in a non-flying locust produces frequency changes in the firing of the small unit of the *left* M85. Histograms were generated by counting the number of potentials in successive bins 800 ms wide. Ordinate, number of potentials per bin. Lower traces: angular position of locust. The output of the function-generator which controlled the servo-motor was adjusted manually. (A) Ramp roll to the left; arrowheads, dynamic responses. (B) Ramp roll to the right, followed by a rapid return to horizontal; arrowhead, dynamic response.

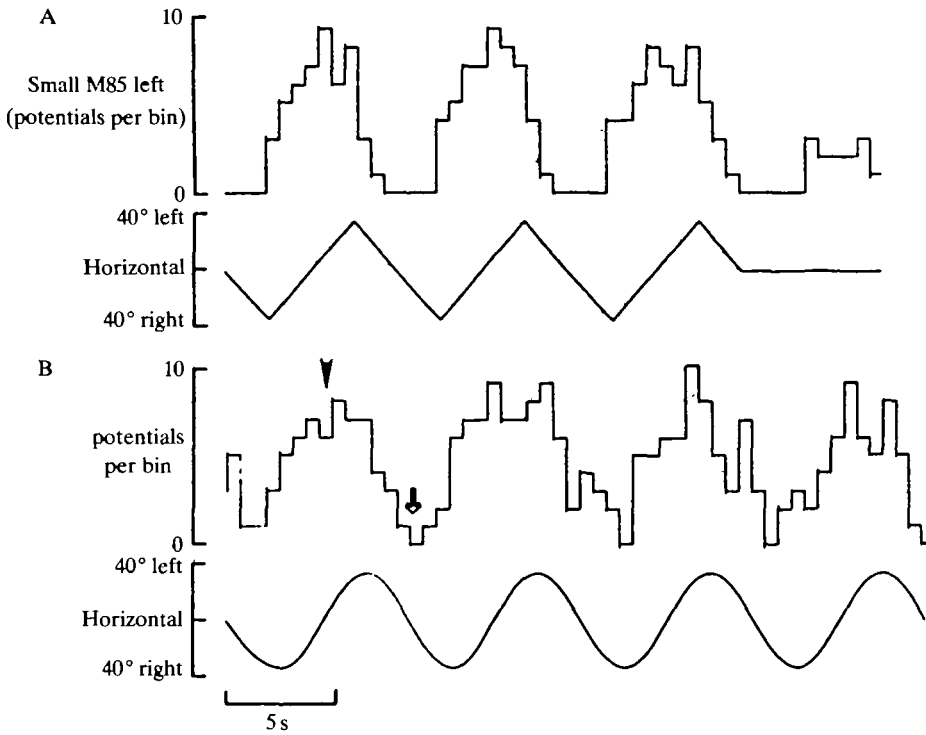


Fig. 12. Frequency-modulation of the potentials in the small unit of the *left* M85 during roll oscillations in a non-flying locust. Histograms show the number of potentials in successive bins 600 ms wide. Ordinate, number of potentials per bin. Lower traces show the angular position of the locust about the roll axis. (A) Triangular stimulus. (B) Sinusoidal stimulus. Filled arrowhead, maximum excitation; open arrow, minimum excitation.

DISCUSSION

Action of pleuroaxillary muscles in straight flight

The pleuroaxillary muscles are activated by single spikes at wingbeat frequency during straight flight. When stimulated at 20 Hz (close to the wingbeat frequency), the muscles produce phasic twitches superimposed on considerable tonic tension. Thus in flight a pleuroaxillary muscle will exert a tonic force on the third axillary sclerite, with an additional phasic force modulated in time with the wingbeat. Therefore although their rhythmic motor pattern does not correspond neurally to the tonic activity suggested by Pfau (1976), their contractile response does include a large tonic component, as proposed by Pfau (1976) and Pfau & Nachtigall (1981). In their mechanical properties they differ from the principal flight muscles such as the tergothoracic and dorsal longitudinal muscles, which produce discrete twitches at these frequencies (Ewer & Ripley, 1953; Neville & Weis-Fogh, 1963). The twitch:tetanus ratio in the principal muscles does not exceed 1:2 whereas that in the pleuroaxillary muscles is about 1:6.

The forewing muscle, M85, typically fires some 20 ms after the ipsilateral subalar muscle. The onset of its phasic contraction will therefore occur after the upper

turning point of the forewing and within the first half of the downstroke (based on a comparison with the data of Wilson & Weis-Fogh, 1962). M114 fires at about the same time as M85. A similar comparison places its phasic contraction near the middle of the hindwing downstroke, as the hindwings lead the forewings by some 10 ms. What are the consequences of the tonic and phasic contractions for the wing movements? From anatomical studies Pfau & Nachtigall (1981) proposed that a tonic contraction of M85 during the wingbeat would diminish both pronation in the downstroke and supination in the upstroke. Reducing downstroke pronation would increase the angle of attack, and hence the amount of lift and thrust. The additional phasic contraction in the downstroke would produce an extra phasic reduction of pronation and an additional increment of lift and thrust. The flying locust generates lift and thrust in the downstroke (Jensen, 1956; Nachtigall, 1981*b*), so that the phasic contraction is appropriately timed to influence that generation. In sustained, tethered flight, M85 is not phasically active in the upstroke. Spikes occur in this phase only in 'irregular' flight. The function of reduced upstroke supination, which would increase the negative angle of attack and the production of negative lift in this

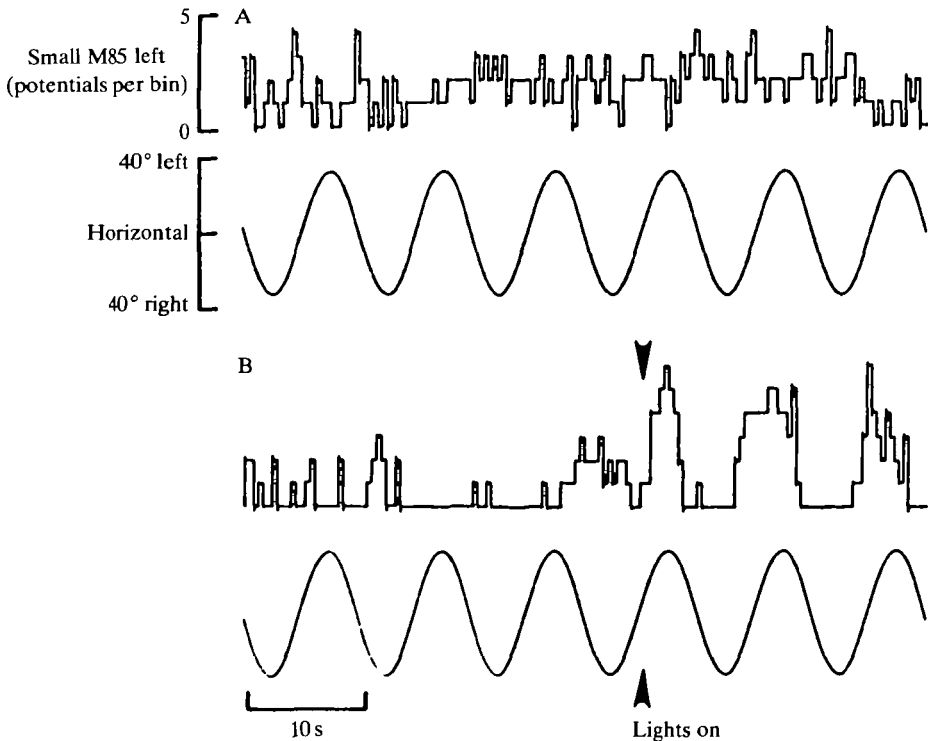


Fig. 13. Dependence of the response in non-flying locusts on visual cues. Frequency of occurrence of the potentials of the small unit of the *left* pleuroaxillary muscle. Histograms as in Figs 11 and 12. Ordinate, number of potentials per bin. Bin width, 400 ms. The two records are continuous. (A) In complete darkness there is no modulation of spikes. (B) The illumination of the horizon is switched on at the arrowheads, and the frequency-modulated response reappears.

phase, is unclear. The occurrence of spikes in the upstroke might reflect attempts to glide or sink. The action of M114 on the stroke of the hindwing is unknown because the hindwing joint has not been studied. However, the similarity between the activity of the fore- and hindwing muscles in flight suggests that M114 may influence the wingstroke in a similar way.

The other proposed function of the pleuroaxillary muscles is in folding the wing (Snodgrass, 1929; Pringle, 1968). This role is supported by the occurrence of a high-frequency burst of pleuroaxillary motor spikes when the locust folded its wings at the end of flight.

Rhythmical activity of pleuroaxillary motor neurones

In a preparation where the central nervous system expresses a flight rhythm but lacks most of the rhythmic feedback, depressor motor neurones fired rhythmically but the small unit of M114 was active during the whole wingbeat cycle, with little apparent relationship to the rhythm. This contrasts with the rhythmical motor pattern in tethered flight and suggests that phasic sensory inputs during flight may be especially significant in the generation of the timing and rhythm of spikes in the pleuroaxillary motor neurones. Peripheral feedback may comprise part of this phasic input. For example, pleuroaxillary motor neurones receive a powerful, supra-threshold excitatory input when the campaniform sensilla of their own wing are stimulated (Heukamp, 1984; R. Elson, in preparation). The irregular activity in fictive flight probably also results from the constant turbulent wind stimulus which has to be directed to the front of these animals to maintain rhythmical activity. The motor neurones of M114 are easily excited to unpatterned, tonic spiking by such stimuli in non-flying locusts (H.-J. Pflüger, in preparation).

The steering function of pleuroaxillary muscles

During presumed compensatory reactions evoked by imposed rolling, changes in the activation of pleuroaxillary muscles occurred in both flying and some non-flying animals. Excitation was increased on the side of the animal that was rotated downwards. In non-flying locusts, increased excitation appeared as an increase in the frequency of spiking of the small motor units. In flight, the motor neurones and premotor interneurones will receive rhythmical drive at flight frequency: increased excitatory input would cause the neurones to reach threshold earlier (a time shift to earlier firing) and to produce more spikes per cycle (cf. Waldron, 1967). Recruitment is explained by different thresholds of the two motor neurones of a muscle.

To compensate for an imposed roll, steering muscles must be activated asymmetrically. The pleuroaxillary muscles therefore show an appropriate activity. Moreover, excitation is increased on the side that experiences the downward rotation. This will cause greater production of lift on this side by reducing downstroke pronation. The results suggest that when the locust performs a compensatory reaction during flight, it uses the pleuroaxillary muscles to increase the amount of lift. Similar motor activity may underlie active manoeuvres as well. [Changes in lift are considered here, because formally these constitute the appropriate response to rotation in the roll

plane. However, an asymmetrical activation of the pleuroaxillary muscles will cause asymmetrical thrust as well, leading to yawing unless counter movements are made (e.g. ruddering by the abdomen, cf. Baker, 1979*a*, Introduction). Actively turning locusts do not isolate roll and yaw, performing banked turns (Baker, 1979*a*), and course deviations in free flight may often involve a combination of rotations about more than one axis. The tethered flight and imposed roll conditions of our experiments are therefore artificially restricted, allowing inferences about lift production only. Nevertheless, from these considerations it is likely that the pleuroaxillary muscles are also used when an increase in thrust is effected. A direct demonstration of how these muscles affect the production of aerodynamic force is still lacking, however, and requires the measurement of torques in a flight balance.]

Increases in the number of spikes produced, and the number of motor units active, will cause an increase in the magnitude of the pleuroaxillary muscle contraction. In this case the amount of downstroke pronation may differ on the two sides of the animal. However, in some reactions these parameters remained constant while shifts in timing nearly always occurred. This will leave the size of the contraction unchanged, but will affect the timing of the phasic component within the wingbeat cycle. This component produces a phasic reduction of pronation in the downstroke. Advancing the time of the phasic contraction (which occurs on the side that the locust tries to lift in the compensatory reaction) will cause an earlier reduction of downstroke pronation, an earlier increase in lift and a later development of full pronation. Baker (1979*a*) found that locusts making active turns showed forewing movements which differed in the relative timing of pronation, full downstroke pronation developing later (i.e. reduced in the early downstroke) on the side which the locust lifted in the turn, and which was presumably generating more aerodynamic lift (cf. Taylor, 1981*b*).

It is unlikely that steering is under the exclusive control of the pleuroaxillary muscles. Some of the principal thoracic flight muscles can also influence the angle of attack of the wing (Wilson & Weis-Fogh, 1962; Pfau, 1977; Nachtigall, 1981*a,b*) and undergo similar changes in the recruitment of motor units, the number of spikes per wingbeat and their time of firing (Dugard, 1967; Zarnack & Möhl, 1977; Möhl & Zarnack, 1977; Baker, 1979*b*; Taylor, 1981*b*; Pollack & Hoy, 1981). These muscles could likewise effect flight steering by controlling pronation (amount and timing) or – also – wingbeat amplitude (Taylor, 1981*b*). It is more likely that the pleuroaxillary muscles work in concert with phasic changes in other thoracic flight muscles to produce flight manoeuvres. However, their ability to produce small, graded twisting movements of the wing (Heukamp, 1984) may suit them for a role in the fine, graded control of the production of aerodynamic force, either for symmetrical changes (control of speed and climb) or asymmetrical changes (steering) (Nachtigall, 1981*a,b*; Pfau & Nachtigall, 1981).

Sensory control of steering muscles

The pleuroaxillary muscles are powerfully affected by sensory inputs, which can sometimes evoke overt spiking responses in the motor neurones of a non-flying

animal. The non-flying responses are dependent upon visual input, and are abolished when visual cues are removed. The responses are primarily phasic and may be an expression of an optomotor reaction to roll, similar to that studied by Thorson (1966*a,b*). Rind (1983) found that an identified descending optomotor interneurone connects directly to pleuroaxillary motor neurones in the moth, *Manduca*, another neurogenic flier.

In flying animals, however, the amplitude of the time-shift response has a tonic relationship to the imposed angular displacement. What is the source of the tonic drive? The apparent position of the visual surround may be the only cue that flying locusts can use to measure their angular deviation about the roll axis. Wind-sensitive head hairs probably cannot provide this information in these conditions, because of the radial symmetry of the wind-stream. Also it is not known whether locusts can estimate their position relative to gravity. The reaction of the pleuroaxillary muscles would effect a dorsal light response, the direction of the motor response tending to realign the locust with the horizon so that the dorsum would be oriented toward the illuminated area. The ocelli may participate in the flying responses because they are able to detect the apparent position of the horizon and can mediate dorsal light responses (D. M. Wilson, 1968; M. Wilson, 1978; Stange & Howard, 1979; Taylor, 1981*a*). A tonic signalling could result from combining visual information with excitation from wind-hairs (e.g. Simmons, 1980; Reichert, Rowell & Griss, 1985). Similar tonic time-shifting reactions can be induced visually in depressor and elevator motor neurones by rotation of an apparent horizon (Taylor, 1981*b*).

Comparison with flight-steering muscles in Diptera

The locust pleuroaxillary muscles have been likened (Pfau & Nachtigall, 1981) to the accessory, non-fibrillar flight muscles of Diptera which perform a flight-steering function (reviewed in Heide, 1983). Both the locust pleuroaxillary and the fly accessory muscles are activated differentially in steering reactions that require differential control of wingbeat on opposite sides (locust: this paper; flies: see Heide, 1983). In flies this differential activation distinguishes the steering muscles from the fibrillar 'power' muscles (cf. Heide, 1968; Nachtigall, 1967), but in locusts no such strict segregation of function is found: elevator and depressor muscle activity is also differentially regulated during steering (Zarnack & Möhl, 1977; Baker, 1979*b*; Taylor, 1981*b*). The motor neurones of both locust pleuroaxillary and fly accessory muscles are powerfully affected by visual stimuli (cf. Heide, 1983; Spüler, 1980). When turned on during steering behaviour, fly steering muscles fire at wingbeat frequency and the spikes are phase-locked, occupying a narrow phase-band of the wingbeat cycle (Heide, 1971), parallelling the behaviour of locust pleuroaxillary muscles. Time-shifts of muscle spikes can occur in at least one fly steering muscle (Heide, 1983).

However, the significance of the time of firing and of time shifting in these fly muscles remains unclear because, when stimulated at the high wingbeat frequencies of dipteran flight (about 150 Hz in *Calliphora*), the phasic ripple superimposed on the tetanic contraction is very small (Heide, 1971). In contrast, the phasic ripple of

locust pleuroaxillary muscle occupies a larger proportion of the total contraction, and meaning can be attached to the phase of the wingbeat in which it occurs and to the time shifts that take place during steering. Thus many similarities appear between locust pleuroaxillary muscles and the accessory muscles of flies, in their adjustment of the wing-setting without affecting the power of the stroke (Pfau & Nachtigall, 1981) and in their differential control during steering (this section). However, the regulation of the timing of their action in the wingbeat may be more significant in the locust.

This study has shown that the pleuroaxillary muscles are activated differentially during steering reactions and that the direction of the response is appropriate for a compensatory reaction. They are powerfully affected by visual inputs, and other studies have shown their sensitivity to inputs from the wind-hairs of the head (Heukamp, 1984) and the campaniform sensilla of the wings (Heukamp, 1984; R. Elson, in preparation). This suggests that they may exercise an important role in the fine control of steering movements. Whether their motor neurones are controlled by different interneurones from the other flight motor neurones, or whether their sensitivity to sensory inputs merely reflects a lower threshold, deserves investigation.

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