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ON THE FUNCTION OF A LOCUST FLIGHT STEERING MUSCLE AND ITS INHIBITORY INNERVATION

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Summary

1. In tethered flying locusts, the pleuroalar (or pleuroaxillary) muscle of the forewing (M85) was stimulated *via* its efferent nerve. The effect on the angular setting of the wing was observed using photogrammetry. Maximal tetanic contraction of the muscle reduced downstroke pronation and upstroke supination by more than 25°. A more physiological stimulus regime resulted in angular changes of about 7°, which is near the range observed during steering manoeuvres. This confirms that the pleuroalar muscle plays an important role in adjusting the wing's aerodynamic angle of attack, as proposed in anatomical studies by Pfau (1978).

2. Unit *a* of the pleuroalar muscle was found to be innervated by the common inhibitor neurone 1 (CI) of the segmental ganglion. IJPs with amplitudes between 2 and 10 mV were elicited by action potentials in CI.

3. A basic tonus was observed in the pleuroalar muscle in the absence of activity in excitatory motoneurones. CI input reduced this basic contracture but did not affect EJPs or muscle twitches elicited by excitatory input.

4. Activity of the common inhibitor was recorded intracellularly and with nerve electrodes in tethered flying locusts. Tonic discharges were observed with spike frequencies ranging from 5 to 35 Hz, 25 Hz being a typical value.

5. EMG recordings from the two units of the pleuroalar muscle showed that only unit *a* was active during most horizontal flight sequences. While its discharge was modulated in response to imposed roll movements, unit *b* was recruited primarily during ipsilateral roll.

These results indicate functional specialization between pleuroalar muscle units *a* and *b* and suggest that the inhibitory innervation of unit *a* functions in the fine adjustment of wing pronation.

Introduction

Locust flight, a well-studied rhythmic locomotory behaviour, is modulated either for stabilization against external perturbations or to allow active changes of the flight trajectory. Although the first aspect, corrective steering, has received more attention (reviewed by Rowell, 1988), the production of torques is required

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for both kinds of steering activity. The locust creates torques during flight by using its hindlegs and abdomen as rudders (e.g. Arbas, 1986) and by differentially regulating lift and thrust generated by the wings. There are three principal means of altering lift and thrust: changes in wingbeat amplitude; changes in the phase relationship between forewing and hindwing movements; and, most important, changes in wing pronation (the term pronation designates a downward twisting of the leading edge of the wing, which decreases the aerodynamic angle of attack during the downstroke; supination designates the counter-rotation). All three parameters are regulated by the principal flight muscles, regarding their recruitment and the timing and magnitude of their activation (e.g. Rowell, 1988; Thüring, 1986; Zarnack, 1988). With respect to wing pronation, however, an additional, specialized flight steering muscle – the pleuroalar – is active. It is assumed to regulate the wing's angular setting without affecting the powerstroke. The function of the pleuroalar (or pleuroaxillary) muscle (M85) in flight steering has been inferred from studies of the functional anatomy of the locust forewing hinge (Pfau, 1978). Physiological properties of this muscle, the patterns of its activity during flight, its innervation and input from sense organs have been studied (Elson, 1987; Elson and Pflüger, 1986; Ferber, 1986; Heukamp, 1984; Pflüger *et al.* 1986). Also, the aerodynamic effects of changes in wing pronation are well established and correlations between pronation and steering manoeuvres have been analysed (Baker, 1979; Schmidt and Zarnack, 1987; Reuse, 1987; Waldmann and Zarnack, 1988; Zarnack, 1988). One important question, however, has not yet been answered: to what extent do changes in pleuroalar muscle contraction actually affect the angular setting of the forewing during flight? This question is of crucial significance since wing pronation is assumed to be regulated not only by the pleuroalar muscle but also by a mechanism involving a number of principal flight muscles. Thus, the role, if any, of the pleuroalar muscle in adjusting the wing's angular position, and its contribution relative to that of the principal wing muscles, remains uncertain.

In the present investigation, the force output of the pleuroalar muscle was manipulated in tethered flying locusts and the effect on forewing pronation was observed. It was further demonstrated that the muscle is supplied by a branch of CI_1, one of the three mesothoracic common inhibitors previously known to innervate a large number of leg muscles (Hale and Burrows, 1985). As yet, inhibitory innervation of the pleuroalar muscle has been regarded as unlikely (Pflüger *et al.* 1986), despite some contradictory evidence (Kutsch and Schneider, 1987). The inhibitor's activity during flight and its effect on pleuroalar muscle force suggest that it may indeed function in the fine control of wing pronation.

Materials and methods

Animals

Adult male and female *Locusta migratoria*, aged from 3 to 5 weeks after the imaginal moult, were obtained from a crowded breeding colony at the University

of Konstanz. Males were preferred in tethered flight experiments because they flew more readily and for longer periods. No other differences were noted with respect to gender. All experiments were performed at temperatures of 23–28 °C.

Preparation technique and electrode placement

Chronically implanted electrodes

Fig. 1 is a diagram of the mesothoracic anatomy showing the muscles and nerves described in this section. Bipolar stimulation electrodes (stainless-steel wire, 30 μm in diameter, insulated except for the inner surfaces of the hooks) were attached to mesothoracic nerve 4D4 (nomenclature after Campbell, 1961) which supplies the pleuroalar muscle of the forewing (M85 after Snodgrass, 1929).

The locusts were cooled and mounted on plasticine. A window was cut into the

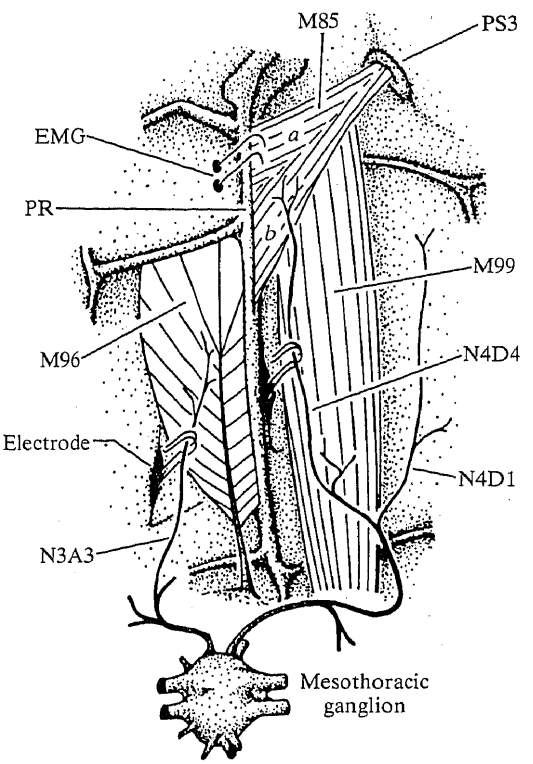

Fig. 1. Schematic inside view of the pleural region of the locust mesothorax. Anterior is to the left. Only cuticular structures, the muscles mentioned in the text and the nerve branches innervating them are shown. The sites of hook electrode placement on nerves 4D4 and 3A3 are indicated, as is a bipolar EMG electrode inserted into pleuroalar muscle unit *a* (insertion from the anterior is shown, although electrodes were usually inserted from the caudal direction). Numbering of the muscles is according to Snodgrass (1929): M85, pleuroalar; M96, abductor coxae; M99, subalar. N4D1, nerve 4D1; PS3, third pleuroaxillary sclerite; PR, pleural ridge.

mesothoracic pleura above the subalar muscle (M99). Without damaging trachea or muscle, the subalar muscle was gently displaced caudally to expose nerve 4D4. The electrode was slid down along the pleural ridge, hooked onto the nerve, and secured to the pleura with wax resin (beeswax/collophonium mixture: 1/2). Silicone grease was squeezed into the opening of the hooks for additional insulation. The subalar muscle was then released and the cuticle lid replaced and sealed with wax resin.

The mesothoracic common inhibitor neurone 1 (CI_1, Hale and Burrows, 1985) was stimulated antidromically by placing a bipolar electrode on the branch of nerve 3A3 which supplies muscle 96 (Fig. 1). The nerve was crushed distal to the stimulation site to avoid activation of the muscle by the stimulus. Experiments were completed within 2 days of electrode implantation, i.e. before nerve degeneration occurred.

The animals were glued to a steel rod by their thoracic sternites and placed in front of a wind tunnel. The holder did not impair movements of wings or abdomen. In most experiments, the animals' legs were severed in the coxotrochanteral joint to avoid the undesired interruption of flight sequences by tarsal contact with the holder.

Acute preparations of M85

Animals were left intact and unanaesthetized, except for brief cooling. They were mounted on plasticine with the thoracic pleura of one side of the body facing upwards. Thorax and legs were immobilized with metal clasps glued to the cuticle to achieve stable muscle force recordings. Abdomen and stigmata were left free, however, to allow normal ventilation of the tracheal system. Bipolar hook electrodes (non-insulated minuten steel pins) were placed on nerves 4D4 and 3A3, as described for the chronic implantation (Fig. 1). However, the subalar muscle was removed to gain easier access to the pleuroalar muscle and nerve 4D4. Recording sites were insulated against the haemolymph with silicone grease. In some experiments, the electrode for stimulating CI was placed on nerve 4D1 (instead of 3A3), which supplies muscle 90 (see Fig. 7A). The third axillary sclerite (attachment site of M85) was excised from the wing and the wing removed. The sclerite was connected to a force transducer (force-displacement transducer, Grass FTO3C) and the pleuroalar muscle stretched to a length corresponding approximately to the upstroke position of the wing. Intracellular recordings from single muscle fibres were made with 20–50 MΩ glass electrodes filled with 3 mol l^{-1} potassium chloride.

In one set of experiments (see Fig. 11) the animals were not mounted on plasticine but tethered on a steel holder as described above. This permitted the recording of pleuroalar muscle force and neural activity in locusts flying with three intact wings. The wing of the muscle under investigation and all legs were removed. The wing hinge with the insertions of the flight muscles, except the subalar and the pleuroalar, were left in place.

CI recordings

A detailed description of the preparation employed for the intracellular recording of common inhibitor activity in tethered animals with an intact flight system is provided by Wolf and Pearson (1987). In chronically implanted locusts, CI_1 activity was monitored by the electrodes placed on nerves 4D4 and 3A3 (see Fig. 9).

Electromyography

In tethered animals, electromyograms served as a reference for flight activity. Bipolar electrodes (copper wire, 100 μm diameter, insulated except for the tips) were inserted into the hind- or forewing first basalar muscles (M127 or M97) at their sternal attachment sites.

Electromyograms of the two units of the forewing pleuroalar muscle (M85) were recorded with bipolar electrodes (30 μm steel wire insulated except for the cut end). The electrodes were inserted through prepared holes in the cuticle just posterior to the suture of the pleural ridge. Unit M85*b* was recorded in the ventralmost region of the muscle's attachment at the pleural ridge, unit M85*a* in the dorsalmost area. In the middle region, the two units overlap and selective electromyographic recordings are impossible (compare Pflüger *et al.* 1986). To confirm successful electrode placement, current was injected into the electrodes on completion of physiological experiments (approx. 100 mA for 20 s in each polarity). After dissection, Fe^{2+} released by the current was precipitated with a crystal of potassium hexacyanoferrate(II) added to the haemolymph (Berlin Blue reaction). Activity of the subalar muscle was inevitably picked up by the EMG electrodes because the subalar is one of the large principal downstroke muscles and lies immediately caudolateral to the pleuroalar. These subalar potentials were conveniently used as reference points to determine the timing of pleuroalar muscle activity (see Fig. 10).

Stimulation technique

Electrical stimuli (0.1 ms voltage pulses of up to 2 V) were delivered to the bipolar electrodes on mesothoracic nerves 4D4 or 3A3 to excite the motor axons supplying M85 or the ipsilateral CI_1 of the segment, respectively. The stimuli applied to nerve 4D4 were sometimes triggered with an adjustable delay by the flight muscle electromyogram, i.e. delivered time-coupled to the flight rhythm (see Fig. 4B).

The branch of nerve 3A3 that supplies M96 contains only efferent fibres (R. Hustert, personal communication). Thus, if the nerve is severed distal to the electrode, a stimulus will produce neither a reflex activation (*via* an induced movement) nor a direct excitation of sensory afferents, but will exclusively excite the efferent fibres in that nerve. And since CI_1 is the only neurone to bifurcate several times on its way from the CNS to the periphery, this arrangement permits a selective activation of the inhibitor's axon in the other ipsilateral, segmental

nerves (compare Hale and Burrows, 1985). Successful activation of the inhibitor and spike transmission across the bifurcations was monitored with the electrode on nerve 4D4.

Nerve 4D4 also contains only efferent fibres, and the two excitatory motor axons innervating the two units of M85 have much larger diameters than the remaining fibres (Pflüger *et al.* 1986). Thus, through careful adjustment of the stimulus voltage, the excitatory axons were selectively activated without interfering with signal transmission in the smaller units, particularly CI_1.

Stimulation of nerve 4D4 with the chronically implanted electrodes specifically activated M85 and did not spread to neighbouring muscles, even the closely overlying subalar (M99). This could easily be determined after moving the forewing of the resting animal into the stroke position. A contraction of M85 resulted in a clear supination of the wing, while activation of the subalar produced a pronounced downward twitch and coxal movement.

Experimental situation, data acquisition and evaluation

Flight sequences were initiated by removing a styrofoam ball from the animal's tarsi or by directing a wind stream (2.5–3.0 $m\,s^{-1}$) onto its head. For photogrammetry experiments and some intracellular CI recordings, the wind stimulus was terminated after the initiation of flight because the pattern of wing movements proved to be more stable without it (Wolf and Pearson, 1987). For photogrammetric recordings only, compound eyes and ocelli were covered to avoid orientation of the animal to the stroboscope light. In all other experiments, an illuminated artificial horizon was displayed in front of the animal, covering about 110° of its visual field. Roll stimuli were applied by rotating the animal around its longitudinal axis, i.e. under open-loop conditions. The holder was coupled to a position-sensing transducer that monitored the degree of rotation. Stimuli were delivered by hand.

For photogrammetry experiments, two thin white lines were drawn across the upper and lower surfaces of the forewing, at about the point where the posterior cubital vein meets the caudal wing margin. The animals were illuminated by a stroboscope with a flash frequency of 400 Hz, the marker lines thus producing images of the wing profile at 2.5 ms intervals. A camera (100 mm telephoto lens) was centred on the wing hinge with the optic axis perpendicular to the sagittal plane of the animal. Exposure times of 1/15 or 1/30 s recorded approximately a single wing stroke (see Fig. 3). Exposures were made alternately with and without electrical stimulation of N4D4 after the animal had settled into stable flight performance. Positions and angles of the wing profiles were digitized from prints on a digitizing pad (Hewlett Packard 9874A) and further calculations performed on a desktop computer (Hewlett Packard 9000/226). Distortions due to lens aberration and perspective were disregarded. Prismatic distortions resulting from the parallel projection of the wing movement onto the plane of the film were corrected using basic spatial geometry (Figs 2, 3). The *true supination angle* β of the wing profile was calculated by reading the x and y coordinates (origin in wing

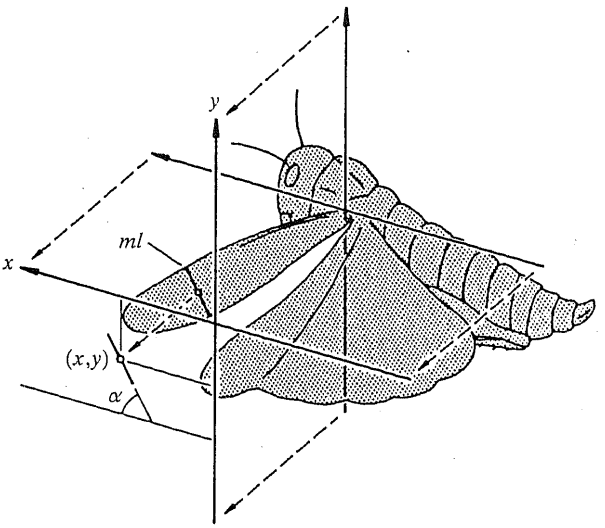

Fig. 2. Evaluation of photogrammetry experiments. Photographic exposures (camera centred on forewing hinge, perpendicular to the animal's sagittal plane) represent parallel projections of the wing movement onto the film plane (dashed lines). The image of the wing hinge was taken as the origin of the coordinate system for evaluation (abscissa horizontal) and the image of the marker line (*ml*) on the forewing was evaluated. Centre coordinates (x,y) and supination angle α of the line's image were determined for computer-aided calculation of the angular setting of the wing.

hinge) and the 'apparent' supination angle α of the wing profile from the photograph (Fig. 2) and using the formula:

$$\beta = \arctan\left[\tan\alpha\left\{\cos\arcsin\left(x/z\right)/\cos\arcsin\left(y/z\right)\right\}\right],$$

where z is the distance between wing hinge and white marker line.

Electrophysiological recordings were amplified conventionally and stored on magnetic tape (Racal store 4DS) for later display on a Gould chart recorder (ES 1000). Parameters (time constants, amplitudes, etc.) were determined on a digitizing storage oscilloscope with plotting facility (Tektronix 5223).

Results

Effect of pleuroalar muscle contraction on forewing pronation

To determine the effect that pleuroalar muscle activity has on wing pronation during tethered flight, stimulation electrodes were chronically implanted on the nerve supplying this muscle (Fig. 1, N4D4). Wing movements were monitored using photogrammetric techniques (Figs 2, 3). In the initial experiments (eight animals), the muscle was maximally activated, i.e. both units were stimulated at frequencies that produced smooth tonic contractions (50 Hz or above, Elson and Pflüger, 1986). From a comparison of wing movements with (Fig. 3B) and without (Fig. 3A) stimulation of nerve 4D4, it is immediately evident that a contraction of

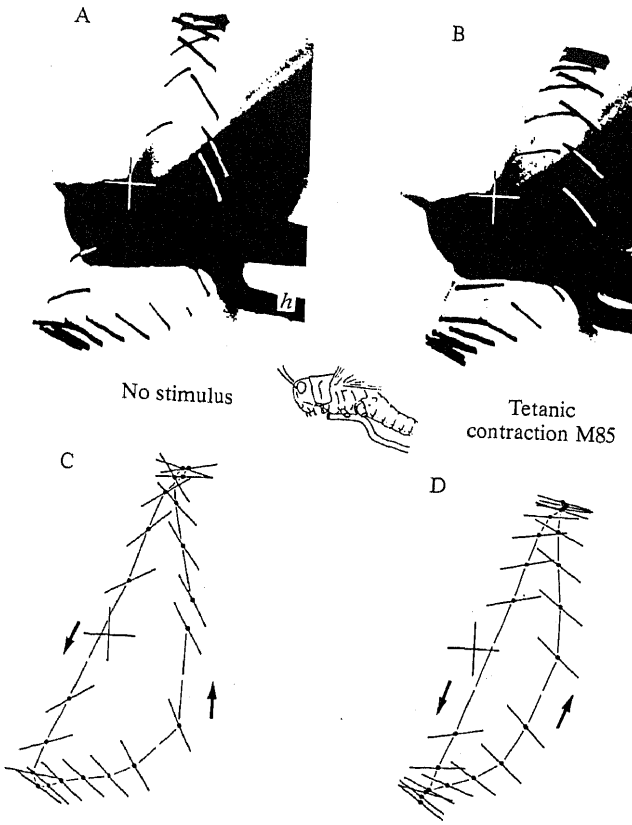

Fig. 3. Raw data of photogrammetry experiments, with (B, D) and without (A, C) tetanic stimulation of the pleuroalar muscle. (A, B) Slightly retouched original photographs (see centre sketch for outline of animal; *h*, holder for tethering). (C, D) The data extracted from the photographs by correcting prismatic distortions (see Materials and methods); note the steeper inclination of wing profiles, particularly close to the reversal points. Arrows indicate direction of wing movement, crosslines mark position of forewing hinge. Exposures were taken with 400 Hz stroboscopic illumination and roughly a single wing stroke was recorded. Note that in B and D downstroke pronation and upstroke supination are distinctly smaller than in A and C.

the pleuroalar muscle reduced downstroke pronation and upstroke supination (i.e. increased upstroke pronation), besides effecting smaller changes in wing trajectory. The correction of prismatic distortions (see Materials and methods) yielded the actual pronation angles of the wing profiles. In Fig. 3C, D these profiles are superimposed on a parallel projection of the wing trajectory onto the animal's sagittal plane (compare Fig. 2). Such data allowed a quantitative description of changes in wing pronation.

The supination angle of the wing profile (negative values indicate pronation) was plotted against the up-and-down movement of the wing (*y*-axis in Fig. 2) (Fig. 4A). In normal tethered flight (open circles) the wing was pronated by a relatively constant angle, in this case about 35°, throughout most of the

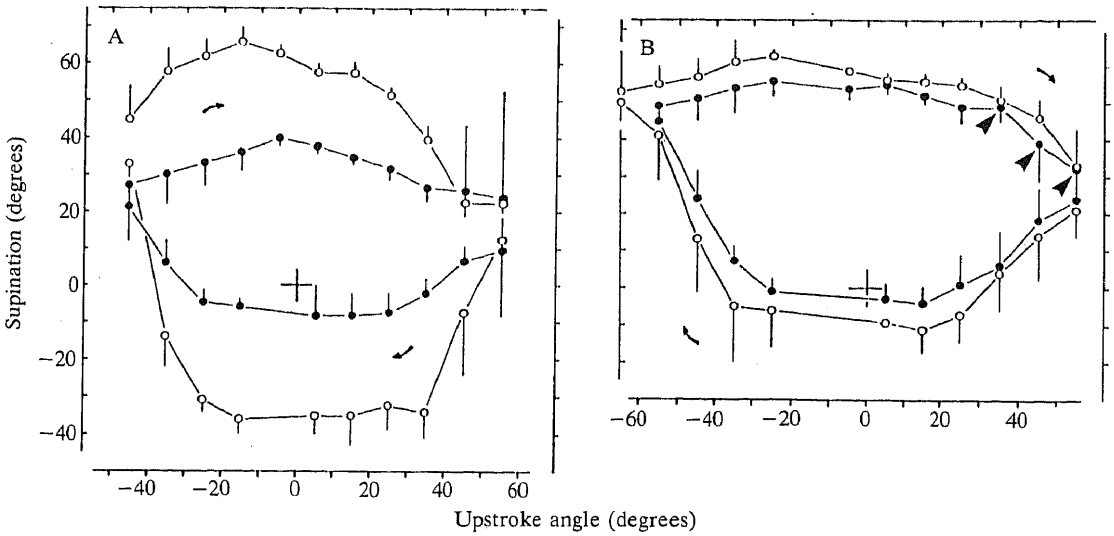

Fig. 4. Pleuroalar muscle contraction decreases downstroke pronation and upstroke supination. (A) Tetanic stimulation. The relationship between forewing supination (ordinate, zero corresponds to horizontal position of wing profile, negative values to pronation) and the up-and-down movement of the wing (abscissa, angle between wing's longitudinal axis and horizontal plane) is shown for normal tethered flight (open circles) and for tetanic stimulation of the pleuroalar muscle *via* N4D4 (filled circles). Arrows indicate the direction of the wing movement. Data points for the middle downstroke are missing, since the marker line was invisible at nearly horizontal wing positions (see Fig. 3A, B). 328 wing profiles of 22 wingbeat cycles were evaluated; error bars represent standard deviations. (B) Physiological stimulation. Same diagram as in A is presented but data are from a different individual. N4D4 was severed proximally and stimulated with single voltage pulses 10 ms after a discharge in the first basalar EMG (arrowheads on graph indicate approximate timing of stimulus). Both units of the muscle were activated and 362 wing profiles from 20 wingbeat cycles were evaluated.

downstroke. Pronation was reduced only near the upper and lower reversal points. Standard deviations are large near the stroke reversal because the wing is turned from the pronated downstroke into the supinated upstroke position, and *vice versa*, at these positions (see Fig. 3). By contrast, upstroke supination was largest – in this animal up to 65° – in the first half of the upstroke and then gradually decreased as the wing approached the upper reversal point. The general shape of the curve was similar in different animals although the average angular settings of upstroke and downstroke varied.

Tetanic contraction of the pleuroalar muscle (filled circles) had a marked effect on wing pronation. Mid-downstroke pronation was reduced by about 25° and mid-upstroke supination by about 20° compared with straight tethered flight. Changes in wing trajectory were minor compared with these dramatic effects. The trajectory as a whole was moved backwards by a maximum of 5°, the paths of up- and downstrokes ran closer together, and the amplitude of the wing stroke was

slightly reduced as a result of tetanic pleuroalar muscle contraction (quantitative data not shown, compare Fig. 3A,C with 3B,D).

A tetanic contraction of the pleuroalar muscle never occurs during flight in intact locusts. In tethered flying animals, about one muscle potential is generated per wingbeat cycle, according to electromyographic records. The force produced by the muscle if activated at such a rate (about 20 Hz) ranges from about 15 to 25 % of the tetanic force output, depending on which of the muscle's units are actually activated and considering individual variability (Elson and Pflüger, 1986; see also Fig. 11). Assuming a linear relationship between muscle force and effect on wing pronation, as a first approximation, a physiological activation of the muscle should reduce downstroke pronation and upstroke supination by about 3–6°.

In the experiment shown in Fig. 4B, both units of the pleuroalar muscle were stimulated with one impulse per wingbeat cycle, delivered at a physiological latency of 10 ms time-coupled to the spike in a depressor muscle EMG (first basalar 97). This stimulus regime mimics pleuroalar muscle activity during moderate steering action (Elson and Pflüger, 1986, see also Fig. 10). Nerve 4D4 was severed proximal to the stimulation site to avoid normal, efferent activation of the muscle. The stimulus reduced downstroke pronation and upstroke supination by an average of 7° during about the last three-quarters of the downstroke and the first half of the upstroke. During the rest of the wingbeat, near the upper reversal point, stimulus-related differences in the angular setting of the wing were distinctly smaller (average of 3°). Similar observations were made in the four other animals examined. Changes in wing stroke trajectory were negligible in this experiment.

In a quiescent animal with its forewings clicked into the stroke position, application of a 20 Hz stimulus train resulted in a supination of the respective wing (see also Heukamp, 1984). Angular changes of 5–6° (i.e. comparable to those recorded during flight) were observed.

Inhibitory innervation of M85

Electrophysiological analysis

Pflüger *et al.* (1986) reported that backfill experiments with cobalt ions, when performed on mesothoracic nerve 4D4, sometimes stained a neurone with a ventromedial cell body and axonal branches in the roots of nerves 3, 4 and 5. This was recognized as an indication that the pleuroalar muscle may be innervated by the mesothoracic common inhibitor 1 (CI_1). In the present study, backfill experiments confirmed that CI_1 is stained in about 50 % of the animals if the cobalt ions (1.5 % $CoCl_2$) are allowed to diffuse for about 5 days (see also Kutsch and Schneider, 1987). However, Elson and Pflüger (1986) reported that 'intracellular recordings from single muscle fibres do not show inhibitory junction potentials' and Pflüger *et al.* (1986) suggested in their detailed anatomical study that 'innervation of the pleuroaxillary muscle by CI_1 may be variable or non-functional'. They proposed that the inhibitory innervation might be present in larvae but retract in the course of the larval–adult transition.

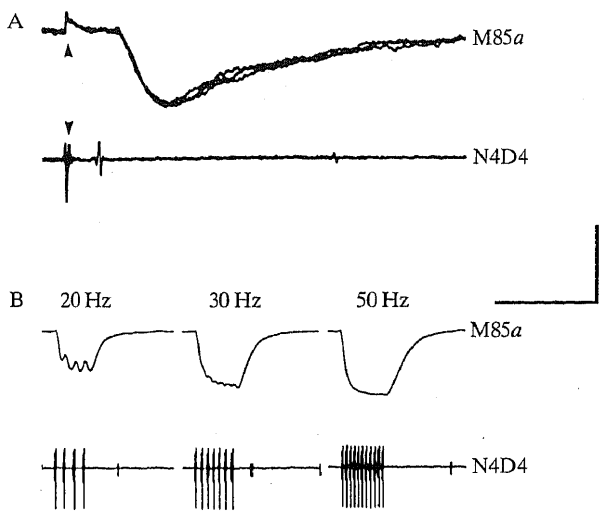

Fig. 5. Inhibitory innervation of M85 by CI_1. Intracellular recordings from unit *a* muscle fibres are shown in the top traces, *en passant* recordings from N4D4 in the bottom traces. CI_1 was stimulated in N3A3, stimulus artefacts are marked by arrowheads in A. (A) Potentials recorded during three stimulus presentations are superimposed. CI spikes in N4D4 and resulting IJPs in the muscle fibre are clearly visible. Note the small spike about half-way along the N4D4 record. Comparing its biphasic potential to that of the CI spike shows that it is from an efferent fibre. (B) Stimulus trains (200 ms duration) were presented with repetition rates of 20, 30 and 50 Hz and summation of IJPs was observed. Scale bars, 20 ms, 10 mV in A, 500 ms, 15 mV in B.

The consistent observation of inhibitory junction potentials (IJPs) in intracellular pleuroalar muscle fibre recordings was therefore unexpected. Stimulation of the ipsilateral CI_1 with hook electrodes on mesothoracic nerve 3A3 (see Materials and methods, Fig. 1 and Hale and Burrows, 1985) revealed that the IJPs represented input from this inhibitory neurone (Fig. 5). The IJPs occurred at latencies of about 10 ms with regard to the stimulus and had amplitudes ranging from 2 to 10 mV, rarely 12 mV. *En passant* recordings from nerve 4D4 demonstrated that most of this latency (approx. 7 ms) was due to the conduction time required for spike propagation from nerve 3A3 to nerve 4D4 *via* the mesothoracic ganglion.

Only one of the two units of the pleuroalar muscle, unit *a*, was found to receive inhibitory innervation. It was often clearly visible to which of the two units the impaled muscle fibre belonged (for anatomy see Pflüger *et al.* 1986 and Fig. 1). However, since the two units overlap, particularly in the middle region, the procedure shown in Fig. 6 was applied for an unequivocal identification. The excitatory axons in nerve 4D4 were stimulated in addition to CI (which was activated in N3A3). At low stimulus intensities only one of the two motor axons was excited, usually that of unit *a* (Fig. 6B). The activated unit could be identified

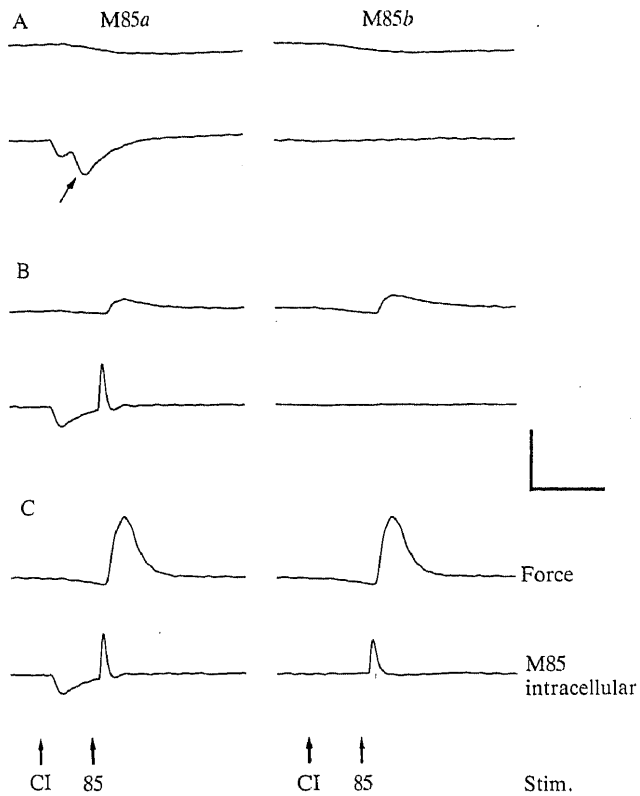

Fig. 6. Only unit *a* of M85 receives inhibitory input. Top traces are M85 force recordings, bottom traces are intracellular muscle fibre recordings. Intracellular recordings on the left are from one unit *a* fibre, those on the right from one unit *b* fibre, both impaled in the same muscle. CI_1 (in N3A3) and excitors of M85 (in N4D4) were stimulated at times indicated at the bottom of the figure. Activation of CI was always provided ('spontaneous' IJP superimposed on stimulus-related one in A, arrow). (A) Stimulus intensity to N4D4 was subthreshold; (B) unit *a* was activated; (C) both units were excited. Note amplitudes and durations of JPs (see Ferber, 1986, for detailed electrophysiological characterization of M85). Scale bars, 100 ms, 0.4 mN (force record), 60 mV (unit *a*) and 25 mV (unit *b*).

visually and by a simultaneous force record (unit *a* always produced much smaller twitch forces). The occurrence of stimulus-related excitatory junction potentials (EJPs) in the intracellular recording then permitted the identification of the impaled muscle fibre. As a control, subthreshold stimuli (Fig. 6A) or higher intensities that activated both units (Fig. 6C) were applied. With this procedure, inhibitory input from CI_1 was observed in more than 90 % of the unit *a* fibres (27 animals examined). In the remaining cells, the presence of CI innervation was not ruled out but the lack of apparent inhibitory potentials may have been due to membrane potentials that were close to the reversal potential of the IJPs. No

inhibitory junction potentials were observed in the muscle cells of unit *b*, even in high-potassium saline (15 mmol l^{-1}).

The membrane potentials determined in unit *a* fibres ranged from −30 to −52 mV (average −40±7 mV, N=126 in 17 animals), those recorded in unit *b* fibres from −31 to −50 mV (average −39±6 mV, N=40 in 16 animals). These values are comparatively low, but within the range previously reported for locust muscle fibres (Hoyle, 1966; Usherwood and Grundfest, 1965). Although the tracheal supply of the muscle was intact and unobstructed, and saline (modified after Usherwood and Grundfest, 1965, or Clements and May, 1974) was applied only in small amounts or not at all, a control experiment was carried out to ensure that the membrane was not depolarized (and excitation–contraction coupling threshold reached, see following section) due to an unphysiological ionic environment or to poor oxygenation of the muscle (Yamaoka and Ikeda, 1988). The membrane potentials of several fibres were determined in seven minimally dissected animals shortly (5–10 min) after the locusts had been captured from the breeding cage and without adding saline. The potentials measured under these conditions were similar to those determined in the above experiments.

Inhibitory junction potentials recorded in unit *a* muscle fibres were of comparatively long duration (half-widths ranging from 20 to 30 ms; compare with much shorter – and larger – EJPs in Fig. 6). Accordingly, summation of the IJPs occurred at frequencies above 10 Hz (Fig. 5B). A plateau hyperpolarization of 10–20 mV, depending on membrane potential, was reached above 30 Hz. This means that the IJP reversal potential was usually between −50 and −60 mV.

Anatomical evidence further supported the finding that only unit *a* of the pleuroalar muscle is innervated by CI_1. In one locust, a particularly stable CI_1 soma recording was obtained. Lucifer Yellow was injected into the neurone with 10 nA hyperpolarizing current for 30 min and the dye allowed to diffuse for 36 h. Subsequent inspection of the dehydrated and cleared pleuroalar muscle under an epifluorescence microscope revealed terminal branches of CI on the muscle. Stained axonal ramifications were detected only on unit *a*.

Effects of CI activity on pleuroalar muscle force

To assess a possible role of inhibitory M85 innervation, the muscle's force output was monitored during the stimulation of CI (Fig. 7A, see also Fig. 6A). In the resting muscle – that is, in the absence of activity in the two excitatory motor axons – spikes of the inhibitor usually (in 33 out of 36 animals) produced distinct, transient relaxations. These relaxations were of long duration with half-widths of 100 ms or above. The pleuroalar muscle thus obviously has a basic tonus (Hoyle, 1983) in the absence of input from the two excitatory motoneurones.

Along with the two large excitatory motoneurones and CI, a number of small axons run in nerve 4D4. *En passant* recordings (note bipolar potentials in the N4D4 recordings, Fig. 5) and morphology (Pflüger *et al.* 1986) indicate that these are efferent fibres. Thus, the question was raised of whether these small axons provided a permanent excitation to the pleuroalar muscle that was responsible for

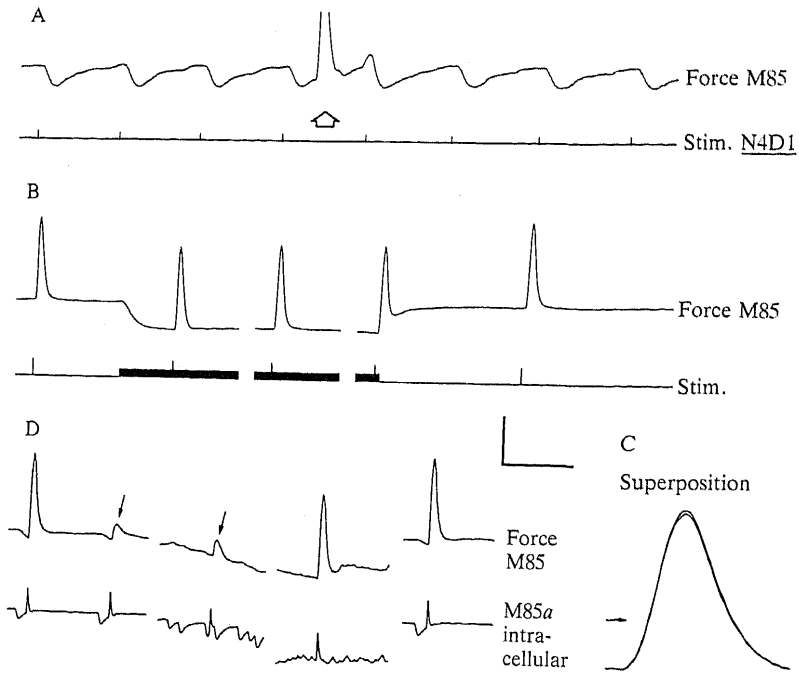

Fig. 7. CI activity reduces basic tonus. (A) Mesothoracic CI_1 was stimulated (in N4D1, bottom trace) and the force output of M85 recorded (top trace). Each stimulus, i.e. spike of CI, caused a transient relaxation of M85 (see also Fig. 6A). The effect persisted after transection of N4 root (arrow and movement artefact in the middle). (B) Both excitors of M85 were stimulated in N4D4 (stimulus frequency about 1 Hz, bottom trace), CI_1 was activated in N3A3 (stimulus frequency 50 Hz, black bar in bottom trace) and M85 force output was recorded (top trace). CI stimulation reduced basic tonus but left muscle twitches almost unaffected. (C, inset) One muscle twitch elicited with simultaneous CI stimulation (smaller twitch) and one without (larger twitch) are superimposed to demonstrate the minute effect of CI activity on twitch amplitude (approx. 2 % decrease; time scale 10 times, amplitude two times expanded, arrow indicates level of basic tonus without CI stimulation). (D) During the experiment described in Fig. 6 spontaneous struggling of the animal occurred, accompanied by strong CI_1 discharges (two middle segments, IJP rate 25–35 Hz). Reductions in membrane potential and basic tonus and constancy of EJPs and twitch responses are visible. Recordings from the quiescent animal are presented in the first and last segment, only unit *a* was activated in the second and third muscle twitch shown (arrows). Scale bars, 500 ms, 0.1 mN in A, 0.3 mN in B; 400 ms, 35 mV or 0.25 mN in D.

the observed basic contracture. This appeared not to be the case, however, because the relaxation in response to stimulation of CI persisted after long periods of silence in *en passant* recordings of nerve 4D4 and even after transection of the root of nerve 4 (Fig. 7A, CI_1 was stimulated in N4D1 in this case).

Repetitive stimulation of CI (compare Fig. 5) caused a considerable decrease in the basic contracture. In response to high-frequency stimulation of the inhibitor the force declined by up to 0.2 mN, which is about one-third of the M85 twitch

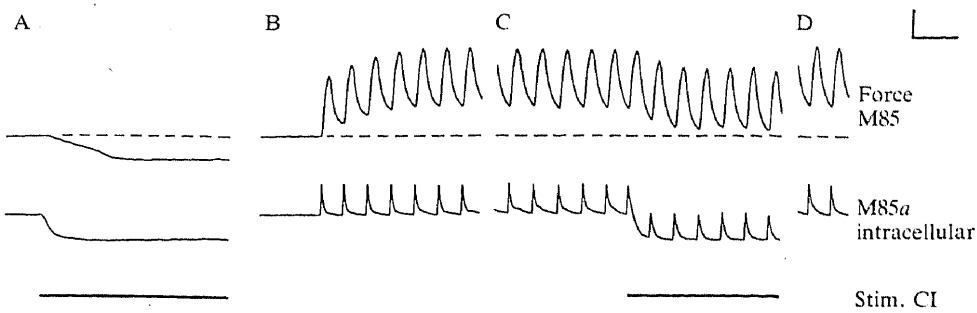

Fig. 8. Interaction of excitatory and inhibitory input to M85. CI_1 was stimulated in N3A3 at 50 Hz (bars at bottom), the two excitatory motoneurones in N4D4 at 20 Hz (stimulus trace not shown). The effects were monitored in an intracellular unit *a* fibre recording (lower trace, slightly unstable due to muscle contractions) and a muscle force recording (upper trace). (A) CI_1 was initially stimulated alone to demonstrate the resulting decline in basic tonus. (B) The start of the stimulus train to N4D4. Note accumulation of tetanic tension. (C) CI was activated during ongoing N4D4 stimulation. (D) The restoration of basic tonus after CI stimulation had been terminated. Broken reference line indicates force level without N3A3 or N4D4 stimulation. Scale bars, 100 ms, 20 mV or 0.25 mN.

force (Fig. 7B,D). Contractions elicited by excitatory input, in contrast, remained almost unaffected by CI discharges. Twitch amplitudes were reduced by less than 5 % during CI activity and no changes were noted in twitch relaxation (Fig. 7C). Similar constancy was noted with regard to the EJPs recorded in unit *a* muscle fibres (see Figs 7D, 8).

The same held true for twitch trains elicited in M85 (Fig. 8) which mimicked the activity pattern observed during flight (Elson and Pflüger, 1986). Initially, CI was activated alone to show the resulting decreases in membrane potential and basic tonus (Fig. 8A). When both units of M85 were subsequently stimulated at a rate of 20 Hz, summation of the individual muscle twitches occurred and a gradual accumulation of residual tetanic tension was observed during the initial contractions. Stimulation of CI at a frequency of about 50 Hz then resulted in a general decline of the force level. This decline was equal to the reduction in basic tonus caused by the CI discharge alone. Simultaneous intracellular recording from a unit *a* muscle fibre demonstrated that the EJPs remained unaffected by the hyperpolarization resulting from CI stimulation.

CI activity during flight

Considering both the function of the pleuroalar muscle in flight steering and the influence CI discharges have on the muscle's contraction, two questions are immediately evident: is CI_1 active during flight and, if so, what is the pattern of its activity? Intracellular soma recordings were performed in intact, tethered flying locusts using the method of Wolf and Pearson (1987). In these recordings, CI activity was characterized by a steady depolarization that lasted throughout the

flight sequence and gave rise to a tonic spike train (Fig. 9A). Spike rates between 5 and 35 Hz were observed during flight but, despite considerable variability, the rate rarely dropped below 20 Hz. The lower rates occurred mainly towards the end of longer flight episodes. The average rate was close to 25 Hz and a modulation of the spike discharge in the flight rhythm was weak or absent. At the beginning of a sequence, a distinct burst of action potentials was regularly observed. Spike frequencies of 70 Hz or above were recorded in these initial bursts which always commenced before rhythmic flight motor oscillations (marked by EMGs in Fig. 9). A similar, though less pronounced, burst of spike activity often occurred at the end of a flight sequence. CI activity could outlast rhythmic flight motor oscillations by several hundred milliseconds. Between flight episodes, CI activity was absent or single spikes occurred at irregular intervals.

To determine the origin of CI activity during flight, sensory input was gradually reduced in the preparation. Mesothoracic CI_1 receives input from a large number of receptors throughout the body but in particular from the mesothoracic segment (Schmidt and Rathmayer, 1988). Therefore, all nerves of the mesothoracic ganglion were severed in a first set of experiments (Fig. 9B). This did not result in any apparent changes in CI activity, although flight episodes were generally shorter after this operation. In a second step, the nerves of all three thoracic ganglia were cut (including abdominal connectives), compound eyes and ocelli were covered with black lacquer, and all cuticular structures of the thorax immobilized with wax resin. Only metathoracic nerve 3, which supplies the EMG muscle and the anterior connectives, was left intact. After this operation, the activity of CI_1 still remained virtually unchanged (Fig. 9C). The main sensory input remaining under these conditions was from the wind sensilla on the head. Input from these sensilla was essential for eliciting flight motor activity but appeared to make only a small contribution to the pattern of CI discharge. In the rare event that flight motor activity commenced spontaneously in deafferented animals (always shortly after a sequence elicited by wind stimulation) CI discharges resembled those recorded in intact locusts, even with regard to the initial spike burst. In contrast, when a wind puff failed to elicit flight motor activity, only weak and often subthreshold depolarizations were observed in CI_1 (inset Fig. 9C).

Neurograms obtained with chronically implanted hook electrodes on nerve 4D4 substantiate the results of intracellular recordings. In two of the chronic hook electrode recordings (experiments described in initial Results section), CI activity was clearly discernible, showing typical features of the neurone's spike discharge (Fig. 9D): an initial burst and a fairly steady spike train of, in this case, about 30 Hz during flight. The final spike burst was either weak (as in Fig. 9B) or was obscured by the much larger spikes of the excitatory motoneurones.

Activity of the two units of the pleuroalar muscle during flight

Since only unit *a* of the pleuroalar muscle receives inhibitory innervation (Fig. 6), knowledge of the discharge patterns of both units during flight is required

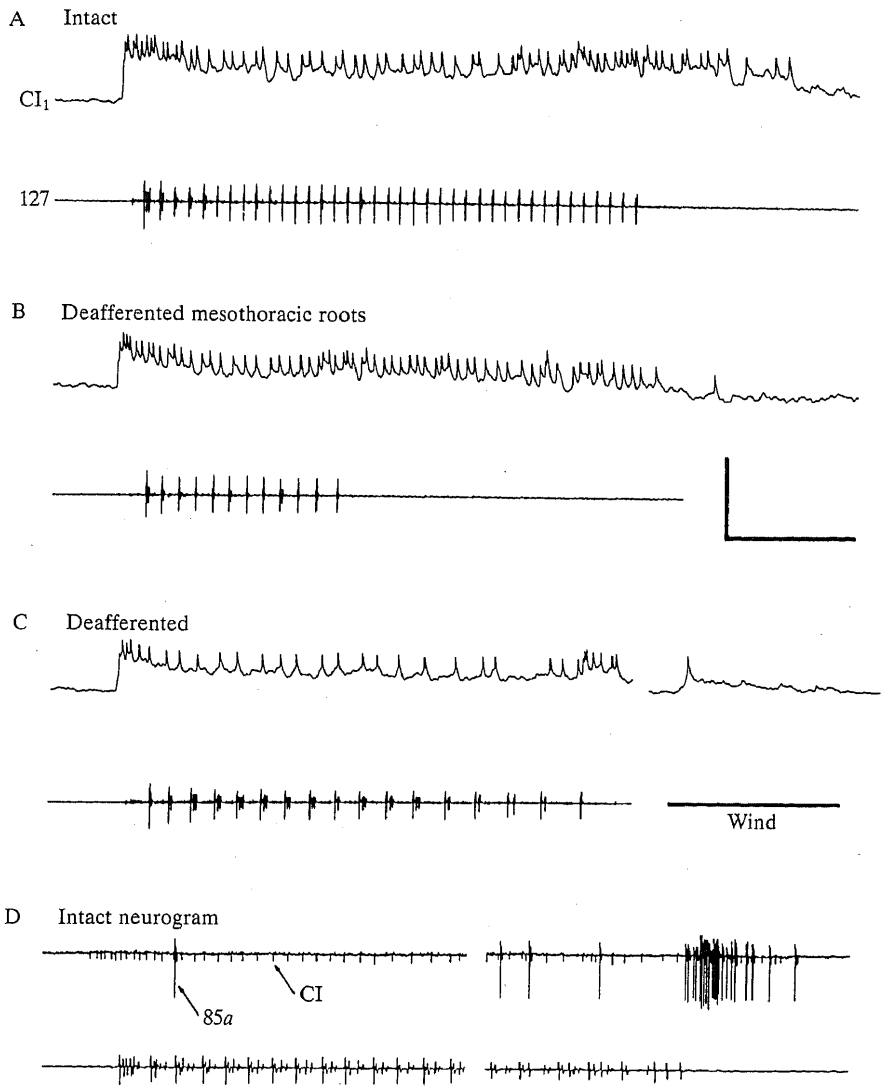

Fig. 9. CI_1 activity during flight. Intracellular soma recordings (A–C) and a neurogram (D) demonstrate the patterns of CI_1 depolarization and spike discharge during tethered flight (top traces; EMGs from hindwing first basalar, 127, monitor flight activity in bottom traces). (A) Sequence from an intact, tethered flying animal (particularly short flight episode selected to match records in B and C). The same individual had all mesothoracic nerve roots severed in B and the thoracic nerve cord isolated from the periphery in C [only metathoracic nerve 3 (EMG) and neck connectives intact]. In addition, the response to a wind stimulus that did not elicit flight motor activity is shown (bar marks duration of stimulus). (D) Neurogram recorded on N4D4 in an intact, tethered animal. Small action potentials are from CI_1, large ones from unit *a* motoneurone (85*a*) (final burst contains also unit *b* potentials). An unusual flight episode was selected, in which excitatory motoneurones of M85 were rarely active, to provide a clear picture of CI activity. Scale bars, 10 mV, 500 ms (A–C) or 250 ms (D).

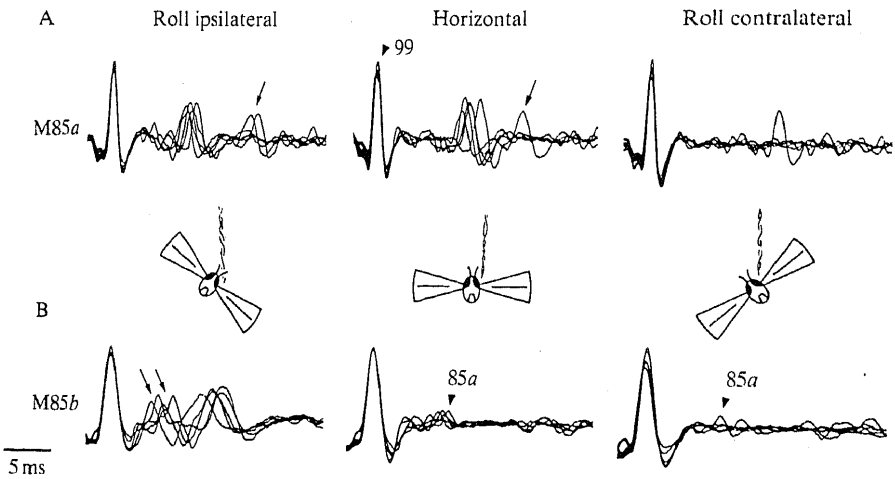

Fig. 10. Activity of motor units M85*a* (A) and M85*b* (B) during horizontal flight (middle) and imposed roll movements (approx. 45°, contralateral side down in right and up in left diagram, see sketches). Recordings from five trials are superimposed taking pick-up of M99 potentials as reference (arrowhead, top middle, 99). During horizontal flight or ipsilateral roll the units sometimes fired doublets (arrows). Note pick-up of unit *a* potentials in B (arrowheads, 85*a*).

for a functional interpretation. Elson and Pflüger (1986) and Reuse (1987) have already presented detailed electromyographic studies of pleuroalar muscle activity during horizontal flight and steering manoeuvres. However, selective recordings of the two motor units were not made and, hence, an identification of their contribution to muscle activity was not possible.

Selective electromyographic recordings from pleuroalar muscle units *a* and *b* (in different individuals, $N=12$) yielded the following results (Fig. 10). In accordance with the report by Elson and Pflüger (1986), usually one but sometimes both of the muscle's units were active after the animals had settled into stable flight. In the horizontal position, unit *a* regularly discharged one (less often two) potential per wingbeat cycle. The potentials occurred with latencies of about 5–15 ms with regard to a spike in the ipsilateral subalar muscle (large pick-up potentials in Fig. 10). Unit *b* was rarely active during horizontal flight and was usually recruited only by imposed roll movements towards the ipsilateral side. This presumably reflected compensatory steering action – that is, the production of a countertorque (Elson and Pflüger, 1986). An ipsilateral roll also decreased the latency of unit *a* potentials and increased the probability of the unit firing doublets. A roll movement to the contralateral side increased the latency and decreased the probability of a discharge in both units. Unit *b* was thus mainly recruited for the production of a presumed corrective roll torque to the contralateral side, whereas unit *a* was active during horizontal flight.

Additional evidence for this conclusion was provided by an experiment in which one forewing of the locust was removed and a force gauge attached to the

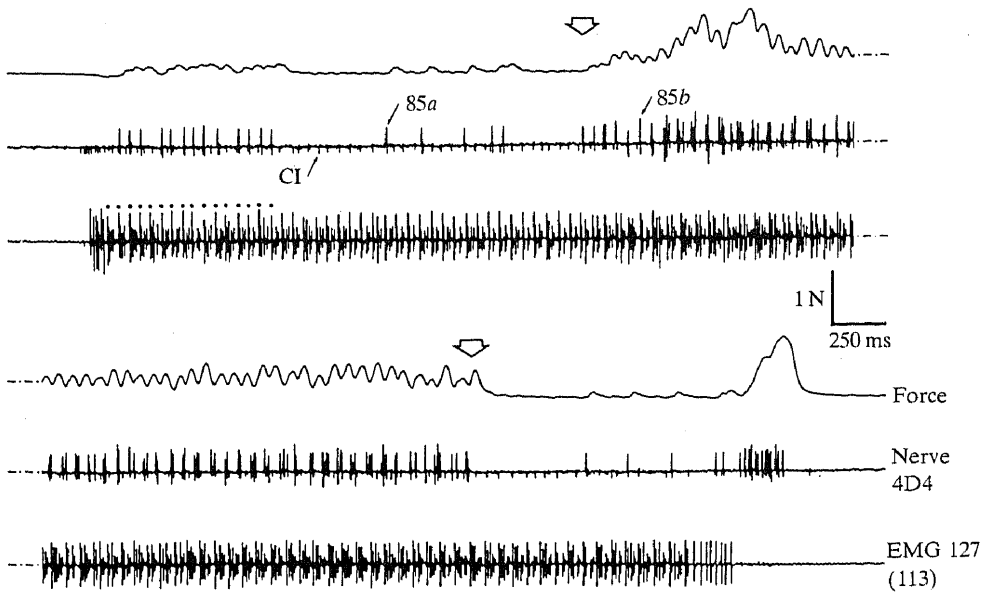

Fig. 11. Activity of motor units M85*a* and M85*b*. In a tethered animal with one forewing removed, pleuroalar muscle force (top trace), activity in N4D4 (middle trace) and a hindwing first basalar EMG (bottom trace; plus pick-up from M113, initial M127 potentials marked by dots for identification) were recorded. The smallest spikes in the neurogram are from CI_1. Unit *a* produced the middle-sized action potentials, unit *b* the largest ones. The animal flew undisturbed at the beginning and end of the sequence. Arrows mark start and termination of a visual roll stimulus.

pleuroalar muscle (Fig. 11, upper trace). A bipolar hook electrode on nerve 4D4 recorded neural input to the muscle (Fig. 11, middle trace) and an EMG electrode monitored flight activity (Fig. 11, bottom trace). The animal was otherwise intact. Single motoneurone spikes elicited by visual stimuli in the quiescent animal indicated that the motoneurones of units *b* and *a* produced the largest and second-largest spikes in the neurogram, respectively, while the smallest class of action potentials was from CI_1. As mentioned above, unit *a* produced smaller muscle twitches than unit *b*. At the beginning of the flight sequence, unit *a* showed a quite typical pattern of activity, discharging about one action potential per wingbeat cycle, but after about 1 s its activity decreased. The open arrow marks the onset of a visual roll stimulus, produced by switching on a light contralateral to the operated side. This resulted in the recruitment of unit *b*, discernible by the occurrence of larger spikes in the neurogram and muscle twitches of more than double the amplitude of the unit *a* twitches in the force record. The second open arrow marks the dimming of the light source, which resulted in a return to the original pattern of activity. The flight sequence terminated soon afterwards and the typical burst of pleuroalar activity associated with wing backfolding was observed (Snodgrass, 1929; Pringle, 1968). It is notable that the neurogram provides further support for the pattern of CI activity during flight reported in the

previous section. The average rate of CI spikes during the flight sequence was about 20 Hz. A frequency of 75 Hz was measured in the initial spike burst (note initial, negative deflection in force record).

Discussion

The present study confirms through nerve stimulation in tethered flying locusts that the pleuroalar muscle of the forewing (M85) reduces downstroke pronation and upstroke supination (Figs 3, 4), as proposed by Pfau (1978) in his anatomical investigations. It was further demonstrated that the same common inhibitor (CI_1) which supplies a large number of the locust's intrinsic and extrinsic leg muscles also innervates M85 (Figs 5, 6). The effect this inhibitory innervation has on the muscle's contractile response was analysed (Figs 7, 8) and CI activity during flight was recorded (Fig. 9).

Effect of pleuroalar muscle activity on forewing pronation

Pfau (1977, 1978, 1983) carried out a number of detailed investigations of the functional anatomy of the locust forewing hinge. From these studies he concluded that the pleuroalar muscle serves the specific function of regulating the angular setting of the wing without influencing the stroke trajectory. He proposed that activity of the muscle reduces downstroke pronation and upstroke supination, and presumed the muscle to be tonically active during flight. The present data provide the first direct confirmation of these assumptions. A smooth tetanic contraction of the pleuroalar muscle was observed to reduce downstroke pronation and upstroke supination by about 25°. By comparison, only small changes were recorded in wing trajectory (Fig. 3), even with this strong, unphysiological activation of the muscle. A more physiological stimulus regime (Fig. 4B) produced no noticeable changes in wing trajectory. It should be noted that efferent nerve stimulation with chronically implanted electrodes proved to be essential for these experiments. Muscle electrodes inflict considerable damage to the small pleuroalar muscle and stimuli easily spread to neighbouring muscles.

The pleuroalar muscle is not the locust's only means of changing the aerodynamic angle of attack of the forewing: the muscle is one component of a synergism that also involves a number of principal downstroke muscles, namely the first and second basalar and subalar muscles. With these muscles it is the timing of their contractions, relative to each other and to the wing movement, that influences the course of wing pronation during the downstroke. Correlations between the activity of these muscles and several parameters of the wing stroke (Zarnack, 1988; Reuse, 1987; Schmidt and Zarnack, 1987) or roll torques generated by the animal (Waldmann and Zarnack, 1988; Reuse, 1987) are relatively well studied. Changes in wing pronation in the range of 5° – sometimes of up to about 10° – were recorded during steering activity (Zarnack, 1988; Reuse, 1987). However, correlations between forewing pleuroalar activity and wingstroke parameters or roll torques have not yet been analysed (although correlations between stroke

parameters and roll torque are well established by Waldmann and Zarnack, 1988; Reuse, 1987), nor have the relative contributions of the pleuroalar and the principal downstroke muscles to changes in wing pronation been assessed.

One experiment represented a first step towards determining the pleuroalar's contribution to the adjustment of wing pronation, relative to that of the principal flight muscles. After severing nerve 4D4, the pleuroalar muscle was stimulated with a pattern similar to that recorded in electromyograms during moderate steering action (Elson and Pflüger, 1986). Under such conditions, decreases in downstroke pronation and upstroke supination of about 7° were observed as a result of muscle activation. This indicates that the pleuroalar muscle contributes significantly to the adjustment of wing pronation, since the observed changes are of the same magnitude as those recorded during steering manoeuvres in tethered flight (Zarnack, 1988; Reuse, 1987). Changes of this magnitude were recorded only during about the last three-quarters of the downstroke and the initial half of the upstroke. Near the upper reversal point, stimulus-related decreases in wing pronation were distinctly smaller (Fig. 4B). This may indicate that phasic activation of the pleuroalar muscle has, in part, a phasic effect on the angular setting of the wing – contradicting Pfau's assumption of purely tonic M85 activity during flight.

Innervation of the pleuroalar muscle by CI_1

Electrophysiology

The results presented in Figs 5 and 6 provide convincing evidence that most, if not all, of the unit *a* fibres in the pleuroalar muscle are innervated by the mesothoracic CI_1, while the fibres of unit *b* lack inhibitory innervation. Two points need to be discussed, however. First, why did Elson and Pflüger (1986) fail to record IJPs in M85? This led to the assumption that there was no inhibitory innervation of M85 (Pflüger *et al.* 1986; compare Kutsch and Schneider, 1987). The probable reason is that the muscle was studied after severing nerve 4D4 proximal to the stimulation site. When the nerve is stimulated, the excitatory motoneurones are excited at lower intensities than CI owing to their much larger diameters (Pflüger *et al.* 1986). This means that stimulation of CI is impossible without synchronous activation of the excitors. Consequently, IJPs will be difficult to observe in intracellular muscle fibre recordings, particularly since the IJPs commence slightly later than the EJPs owing to the lower conduction velocity of the small CI axon. In addition, CI activity has little or no effect on EJP size (Figs 7D, 8). Only separate stimulation of the CI axon *via* a different nerve (e.g. N3A3) or spontaneous activity clearly reveal the inhibitory input and, in both cases, an intact connection of nerve 4D4 to the CNS is essential.

Second, it should be mentioned that the pleuroalar muscle is an unusual target for CI_1. The locust's inhibitor, as a rule, innervates only leg muscles recruited during walking and only those fibres that also receive input from a 'slow' motoneurone (Hale and Burrows, 1985). M91 is the only exception known: it acts

not only as coxa remotor but is also involved in wing elevation. In other insects, too, inhibitory innervation of flight muscles is uncommon (e.g. Ikeda and Boettiger, 1965).

Effects of CI activity on pleuroalar muscle force

The main effect of CI activity on the force developed by the pleuroalar muscle is a reduction in basic tonus (Figs 7, 8). The observation of a basic muscle contracture in the absence of discharges in excitatory motoneurones is unusual but not unprecedented (Hoyle, 1968*a*,*b*). The membrane potentials determined in M85 were remarkably low (average near −40 mV). Experimental conditions (proper oxygenation through intact tracheal supply, little or no saline added, muscle undamaged) and controls (membrane potentials determined in minimally dissected animals) suggest that these low membrane potentials are of functional significance and not an artefact. Apparently, excitation–contraction coupling (EC) threshold is reached in the absence of excitatory motoneurone discharges, leading to the basic contracture of pleuroalar muscle fibres (see Hoyle, 1983).

Whether the low membrane potentials are due to tonic release of excitatory transmitter (demonstrated to be effective at some inhibitory junctions in crustaceans; Parnas *et al.* 1975) or are caused by differences in membrane permeability to sodium (as has recently been shown for crustacean tonic muscle fibres; Hammelsbeck and Rathmayer, 1989) remains to be investigated. Miniature junction potentials were not recorded in pleuroalar muscle fibres. The reduction of basic tonus caused by CI activity persisted after transection of the nerve 4 root, i.e. after the abolition of any possible efferent input (Fig. 7A), as well as after long periods of silence in nerve 4D4. This establishes that spike activity in the group of small N4D4 axons with unknown function (Pflüger *et al.* 1986) is not necessary for the maintenance of basic tonus in M85.

CI activity during flight

CI_1 is tonically active during flight with a discharge rate adequate to affect the contraction of M85 (compare Figs 9, 5B). In intracellular and neurogram recordings, discharge frequencies between 20 and 35 Hz were consistently observed. In view of the results discussed in the previous paragraphs, it appears clear that under physiological conditions the level of basic contracture in unit *a* is regulated by CI activity (Figs 7B,D, 8). Contractions elicited by excitatory input are superimposed on this basic force level. Deafferentation experiments further suggest that CI depolarizations are under strong central nervous control during flight, despite the manifold and powerful sensory input demonstrated in quiescent locusts by Schmidt and Rathmayer (1988). This may indicate that CI innervation is employed in the fine control of flight performance. The initial, high-frequency spike burst observed in intracellular CI recordings (Fig. 9A) is probably associated with the unfolding of the wings. This spike burst may serve to relax M85 completely, which otherwise might counteract wing unfolding in its function as a retractor of the wing (Snodgrass, 1929; Pringle, 1968).

Activity of the two M85 units during flight

The above interpretation is substantiated by the finding that unit *a* of the pleuroalar muscle – which receives CI innervation – is active during normal, horizontal flight, whereas unit *b* is recruited mainly for steering manoeuvres (Figs 10, 11). If a muscle with two units is activated below or within the frequency range where an accumulation of residual, tetanic tension begins, the force output can be regulated only coarsely – that is, in increments whose size is determined by the twitch amplitudes of the two units. At flight frequency, i.e. in the working range of the pleuroalar muscle, only partial tetanic fusion of the individual muscle twitches was observed (Figs 8, 11; Elson and Pflüger, 1986). Thus, regulation of muscle force *via* the two excitatory motoneurones might be too coarse to permit a sufficiently subtle adjustment of wing pronation as required during 'idling' horizontal flight. CI activity could provide the more gradual regulation necessary. This task might also be accomplished, however, by steady changes in the timing of the principal flight muscles' activity (see above).

Another, though not alternative, interpretation is as follows. By reducing the basic tonus of the pleuroalar muscle, CI activity regulates the force level upon which the contractions resulting from the excitors' activity are superimposed. A marked decrease in basic contracture will enhance the relative effect of the phasic component of M85 contractions and impart particular importance to the timing of muscle activity. The normal timing of excitatory motoneurone spikes in nerve 4D4 is such that the resulting muscle twitch will peak during the first half of the downstroke. A delay of the muscle contraction, as occurs during active roll manoeuvres to the ipsilateral side, will lead to a delayed reduction of downstroke pronation and, thus, to a decrease in lift produced by the wing. Accordingly, an advanced muscle contraction will increase lift production during the downstroke. Upstroke supination may be affected to a much smaller extent than downstroke pronation in the same wingbeat cycle if the M85 twitch force has decayed by the time the wing has passed the lower reversal point (see also data in Fig. 4B discussed above). This may reduce or eliminate generation of negative lift during the upstroke. In fact, variability of wing pronation was clearly more pronounced during the downstroke (Fig. 4A). This may indicate that downstroke pronation and upstroke supination are indeed regulated independently.

A selective stimulation of CI_1 after elimination of its normal activity (as shown in Fig. 4B for the excitatory motoneurones) would be necessary to test these hypotheses. As yet, however, this experiment has not been possible. If CI_1 were involved in corrective steering, its discharge during flight would be expected to be dependent on roll stimuli. Visual input to CI_1 has been reported by Burrows and Rowell (1973) and Schmidt and Rathmayer (1988). This input is bilaterally symmetrical, however. Visual stimulation of the locust when recording CI activity during tethered flight (Fig. 9) revealed a marked phasic response of the inhibitor to a dimming of the illumination. The response to a brightening of the illumination was less pronounced. Bilateral asymmetrical responses, although sometimes observed, were not reproducible. This may indicate that CI is mainly involved in

the overall adjustment of lift and thrust; for instance, when switching from climbing to horizontal flight. It may also indicate that visual stimulation during intracellular neural recording in tethered animals was inadequate.

Another question raised by the present study concerns the activity of the leg muscles during flight. Many of them are known to receive inhibitory input from CI_1 (Hale and Burrows, 1985). The reported CI discharge should affect tonic contractions of the leg musculature, contractions one would expect to be responsible for the maintenance of the locust's flight posture. The question is of particular significance with regard to the hindlegs, which are used as rudders in flight steering (Arbas, 1986).

The present study has demonstrated that the pleuroalar muscle plays an important role in adjusting the angular setting of the forewing during flight. Inhibitory innervation of pleuroalar muscle unit *a* was determined and a function in the fine adjustment of wing pronation has been proposed. Establishing whether CI activity serves to regulate overall lift and thrust or is involved in compensatory steering and whether the phasic component of M85 activity is actually of importance for flight steering requires experiments under closed-loop conditions, combined with more accurate methods of measuring wing pronation (Zarnack, 1978). Only such experiments could account for the locust's ability to adapt to external and internal perturbations (e.g. Möhl, 1988) and may reveal how the pleuroalar muscle and CI activity are actually employed during flight.

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