The Journal of Experimental Biology 200, 633–642 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB0637

OCTOPAMINERGIC INNERVATION AND MODULATION OF A LOCUST FLIGHT STEERING MUSCLE

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Accepted 11 November 1996

Summary

We demonstrate that the meso- and metathoracic pleuroaxillary flight steering muscle (M85 mesothorax, M114 metathorax) of the migratory locust are each innervated by a single dorsal unpaired median neurone (DUM3,4,5a). The soma of this neurone can be localized by retrograde staining of the motor nerve with Neurobiotin, but not with cobalt salts. The primary neurite projects in the superficial DUM cell tract, and the axons run in nerve roots 3, 4 and 5 and in all their secondary branches. Other muscle targets include the second tergal remotor coxa (M120) and the posterior rotator coxae (M122, M123, M124), but not the first tergal remotor coxa (M119) and subalar (M129) flight muscles.

Octopamine-like immunoreactive varicosities occur on the pleuroaxillary muscles. Stimulation of DUM3,4,5a and octopamine (10^{-6} mol l^{-1}) superfusion increased the amplitude and the relaxation velocity of neurally evoked twitch contractions of this muscle. Octopamine also significantly reduced the tonic tension that this muscle develops when stimulated at flight frequency (20 Hz), while increasing the amplitude of each phasic twitch. A catch-like tension is also reduced in the presence of octopamine. Simulations of the motor pattern experienced by the pleuroaxillary muscles during roll manoeuvres suggest that transient changes in tension underlying corrective steering could be doubled in the presence of octopamine.

Key words: biogenic amine, octopamine, insect, flight, locomotion, dorsal unpaired median neurone, *Locusta migratoria.*

Introduction

Numerous aspects of the physiology and behaviour of insects are influenced by octopamine functioning as a neurotransmitter, neuromodulator or neurohormone (for reviews, see Evans, 1985, Agricola *et al.* 1988; Orchard *et al.* 1993). With the recent development of highly specific antisera (Eckert *et al.* 1992), the localization of octopaminergic neurones in the central nervous system of locusts and several other insects is now known (for a review, see Stevenson and Spörhase-Eichmann, 1995). However, although the function of this important cell class may be inferred from the numerous physiological responses to octopamine, our knowledge is limited to the handful of cells that have been uniquely identified.

Most information is available on the so-called efferent dorsal unpaired median (DUM) neurones and their effects on muscle tension (for a review, see Evans, 1985). These cells release octopamine, which can change the response of muscle to motor signals from one that favours the maintenance of posture to one that favours locomotion (Evans and Siegler, 1982), when the DUM cells are active (Ramirez and Orchard, 1990). In more general terms, the DUM cells are often considered to function collectively in the mechanism underlying arousal (Orchard *et al.* 1993).

Recent studies, however, suggest that individual DUM cells, far from only contributing to a general state of excitability, may also exert specific influences on locomotion and behaviour (Burrows and Pflüger, 1995). It is thus becoming increasingly important to unequivocally identify individual DUM cells and their specific targets. Although the central nervous morphology of many DUM cells is documented (e.g. Watson, 1984; Pflüger and Watson 1988; Bräunig, 1991; Campbell *et al.* 1995), their peripheral targets remain largely unknown. Moreover, essentially only three of approximately 120 locust efferent DUM cells (see Stevenson and Spörhase-Eichmann, 1995) have in fact been shown to exert the same influence on their peripheral targets as exogenously applied octopamine (DUMETi, Evans and O'Shea, 1977; O'Shea and Evans, 1979; OV1, Orchard and Lange, 1985; DUMDL, Whim and Evans, 1988).

Several retrograde staining studies of axons using cobalt salts suggest that some muscles may receive no DUM cell innervation (e.g. Pflüger *et al.* 1986; Kutsch and Schneider,

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1987). However, cobalt salts are highly toxic, so that backfill staining will often fail to reveal the nerve cell bodies of neurones with small axons, such as DUM neurones, particularly when long diffusion distances are involved. In the present study, we used the tracer Neurobiotin to show that, contrary to earlier investigations, the locust pleuroaxillary flight steering muscle is innervated by a single DUM-neurone (DUM3,4,5a). We demonstrate that this cell modulates neurally evoked contractions of the pleuroaxillary muscles. The possible role of this neurone in behaviour is discussed. A preliminary account of some of the data has been presented elsewhere (Meuser *et al.* 1995).

Materials and methods

Experimental animals

All experimental animals, mature adult migratory locusts (*Locusta migratoria* L.) of both sexes, were taken from an established crowded colony maintained under standard conditions. All experiments were performed at room temperature (20–24 °C).

Intra- and extracellular recordings

The thorax was opened by a dorsal longitudinal incision and the animal was pinned ventral side down onto a cork board. The thoracic ganglia were exposed by removing overlying muscles and connective tissue, and positioned over a wax-coated metal spoon for support. The preparation was superfused with hypotonic locust saline (Clements and May, 1974; without sucrose). Neuronal somata were impaled with glass microelectrodes filled with cobalt hexammine chloride (5 %) having a d.c. resistance of 60–90 MΩ. They were identified by the 1:1 correlation of intracellular spikes with spikes recorded from peripheral nerves.

Extracellular nerve recordings were obtained using suction electrodes, with an adjacent indifferent electrode. Nerves were stimulated *via* the suction electrodes, using current from a stimulus isolation unit (Digitimer) triggered by a programmable stimulator (Biologic, Master 8). All signals were amplified using conventional techniques and stored on magnetic tape. Hard copies were obtained with an electrostatic printer (AstroMed, Dash 4).

Muscle mechanograms and octopamine application

The metathoracic pleuroaxillary muscle (M114) was dissected free, together with a length of its motor nerve and the cuticular attachments to the pleural ridge and third axillary sclerite. In some experiments, both left and right M114s were dissected completely free from the animal together with the intact nerve supply to the otherwise fully isolated metathoracic ganglion. The preparation was then transferred to an organ bath (0.5 ml) fashioned from Sylgard resin and continuously superfused with aerated isotonic locust saline (Clements and May, 1974). Here, the pleural ridge was pinned down, and the axillary sclerite glued with acrylic tissue adhesive to a thread attached to a force transducer for almost isometric muscle tension measurements (Cambridge Instruments, model 400A). Values for contraction/relaxation velocities were obtained by electronic integration of the recorded force. Saline could be exchanged for saline containing DL-octopamine (Sigma) using a manually operated two-way tap. Before starting an experiment, isolated muscle preparations were induced to contract at 1 Hz for at least 5 min by nerve stimulation.

Histochemical techniques

Retrograde 'backfill' staining of nerves was performed using the tracer Neurobiotin (Vector Laboratories). The pleuroaxillary muscle and its nerve supply were exposed by removing a small flap of cuticle under the wing-hinge (Fig. 1A,B). The motor nerve was then cut and the proximal stump placed in a Vaseline pool filled with distilled water, which was replaced after 5 min with a solution of Neurobiotin (2.5 %) in locust saline. After sealing the wound with Vaseline, the animal was left overnight at room temperature in a humidity chamber for the dye to diffuse. The ganglia were then excised, fixed in formalin (4 %, 1 h), washed in buffer (0.1 mol l^{-1} Tris–HCl, pH 7.6) and treated with a mixture of the enzymes hyaluronidase (Sigma) and collagenase/dispase (Boehringer; 1 mg of each enzyme in 1 ml of buffer; 36 °C, 30 min) to permeabilize the ganglion sheath. Neurobiotin was detected using peroxidase-coupled avidin/biotin complex from a standard kit (1:200, 2.5 h; Vector Laboratories), following the manufacturer's recommendations, and using diaminobenzidine as chromogen as described elsewhere (Stevenson *et al.* 1994).

Intracellular staining of metathoracic neurones was achieved by injecting cobalt ions from impaled microelectrodes with depolarizing current pulses (5–15 nA, 200 ms, 2.5 Hz, 30 min). After allowing for diffusion (30 min), the ganglion was excised, together with the portion of the metathorax containing the coxal muscles and flight muscles innervated by nerve 4. Cobalt was then precipitated with ammonium sulphide (0.3 %, 1 min). After washing in saline, the preparations were fixed in formalin (10 %, 1 h) and the staining intensified with silver (Bacon and Altman, 1977).

Octopamine-immunoreactive processes were revealed in whole-mount preparations of isolated muscles using an established antiserum (Eckert *et al.* 1992) according to protocols given elsewhere (Stevenson *et al.* 1994). Bound antiserum was detected by the avidin/biotin method using diaminobenzidine as chromogen as described above for Neurobiotin staining. No staining was observed when the primary antiserum was omitted.

All stained material was dehydrated and cleared in methyl salicylate for microscopic viewing (Zeiss Axiophot). Drawings were obtained with the aid of a *camera lucida.*

Terminology

Muscles are numbered according to Snodgrass (1929): M85, mesothoracic pleuroaxillary wing twisting muscle; M114, metathoracic pleuroaxillary wing twisting muscle; M119, metathoracic first remotor coxa wing elevator muscle; M120, metathoracic second remotor coxa wing elevator muscle; M129,

metathoracic subalar wing depressor muscle (see Fig. 1A,B). Nerves are numbered after Campbell (1961). Dorsal unpaired median (DUM) neurones are designated by the nerve root numbers containing their axons, with a lower case character for identified cells having the same basic branching pattern.

Results

Neurobiotin staining

Retrograde nerve staining using the tracer Neurobiotin shows that the meso- and metathoracic pleuroaxillary muscles are each innervated by four different types of neurones (Fig. 1C,D). Only three of these categories are known from cobalt-staining studies (see Pflüger *et al.* 1986; Kutsch and Schneider, 1987): (1) two excitatory motoneurones with large ventral lateral somata (diameter 35–45 μm); (2) four smaller (diameter 8–12 μm), but similarly positioned, somata of unknown function; and (3) the common inhibitor-1 neurone, which has a large ventral midline soma (40–50 μm diameter). In addition, Neurobiotin regularly labelled a single, large dorsal midline soma in both the meso- (diameter 30–40 μm) and metathoracic ganglion (diameter 60 μm; Figs 1C,D,

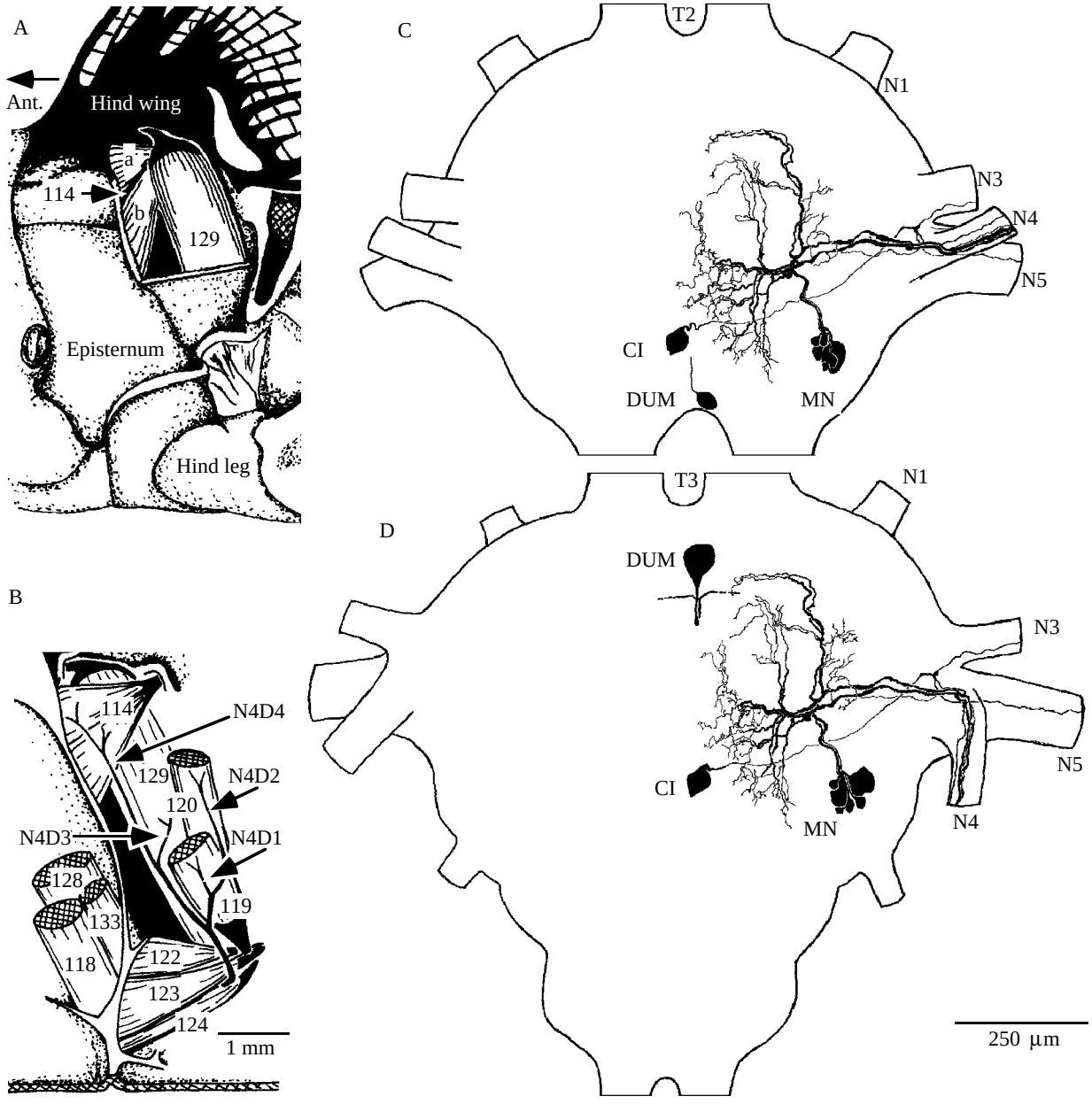

Fig. 1. The metathoracic pleuroaxillary muscle (114) and its innervation. (A) Drawing showing the position of M114 and the subalar wing depressor muscle (129) as seen through a flap cut in the epimeron. (B) Internal anatomy showing the four branches of nerve 4D to M114 together with a number of other flight muscles. (C,D) *Camera lucida* drawings of neurones innervating the mesothoracic (C, T2) and metathoracic (D, T3) pleuroaxillary muscle as revealed by Neurobiotin backfill staining of N4D4 in whole mount. MN, motoneurones; CI, common inhibitor neurone; DUM, dorsal unpaired median neurone; a,b, parts a and b of M114.

2A,B). The axon and dendrites of this cell were not visible. Nevertheless, the size and position of the soma suggest that it is a dorsal unpaired median (DUM) neurone. With the cobalt backfilling method, we were unable to stain a dorsal midline neurone in over 50 preparations using a range of different cobalt salt concentrations (0.5–5 %).

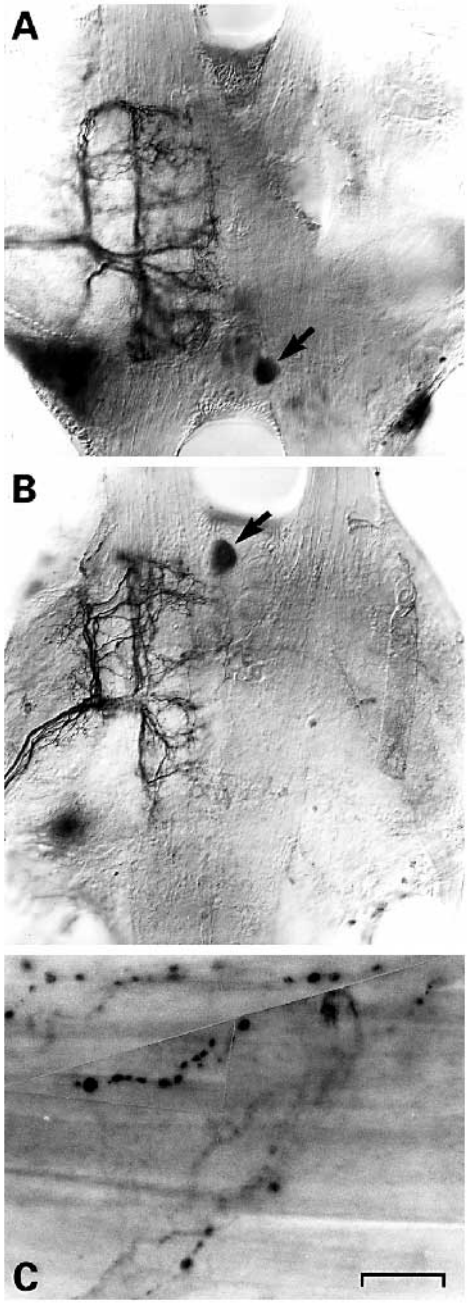

Fig. 2. Photomicrographs. (A) Dorsal view of the mesothoracic ganglion and (B) dorsal view of the metathoracic ganglion showing Neurobiocytin labelling resulting from backfilling N4D4 from the pleuroaxillary muscles (M85, M114; see Fig. 1C,D). The arrow points to the soma of the dorsal unpaired median neurone. The soma of the common inhibitor lies ventrally and is not in view. The neuropile projections stem from the motoneurones (cell bodies out of focus). (C) Octopamine-immunoreactive varicosities on the surface of M114 (whole mount). Scale bar: A,B, 100 µm; C, 30 µm.

Physiological recordings

Since DUM cells have bilaterally projecting axons, they can be physiologically identified by simultaneously recording and electrically stimulating contralaterally homologous nerves. In experiments of this type (*N*=4), stimulating the fine nerve branch N4D4 to M114 (6–10 V, 0.2 ms) evoked extracellular action potentials in the corresponding contralateral nerve branch after a constant delay of approximately 45 ms (Figs 3, 4D). The same stimulus also produced extracellular action potentials in the main root of contralateral N3, N5 and in nerve branch N4D2, which innervates the second remotor coxa wing elevator muscle (M120). However, no phase-locked spikes were evoked in nerve branches N4D1, which innervate the first remotor coxa wing elevator muscle (M119) or N4D3, which innervates the subalar wing depressor muscle (M129).

Intracellular recording/cobalt staining experiments verified that the pleuroaxillary muscle is innervated by a DUM cell

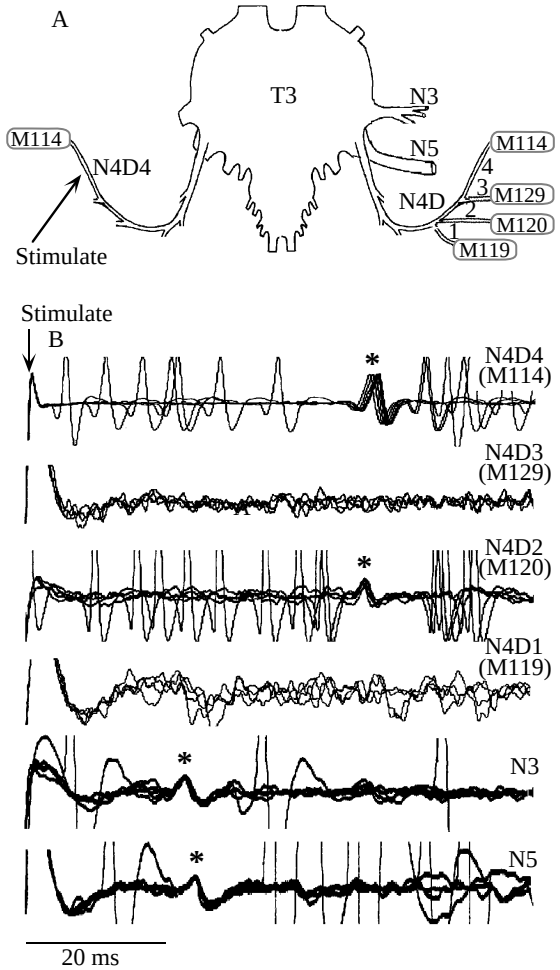

Fig. 3. Identification of the pleuroaxillary muscle DUM cell by extracellular nerve recordings. (A) The experimental arrangement. (B) The traces (each five superimposed sweeps) show that electrical stimulation of N4D4 to M114 on one body side evokes latency-locked spikes (*) in contralateral nerves: N4D4 to M114, N4D2 to M120, N3 and N5, but not N4D3 to M129 or N4D1 to M119. The peaks of some of the larger spikes are capped.

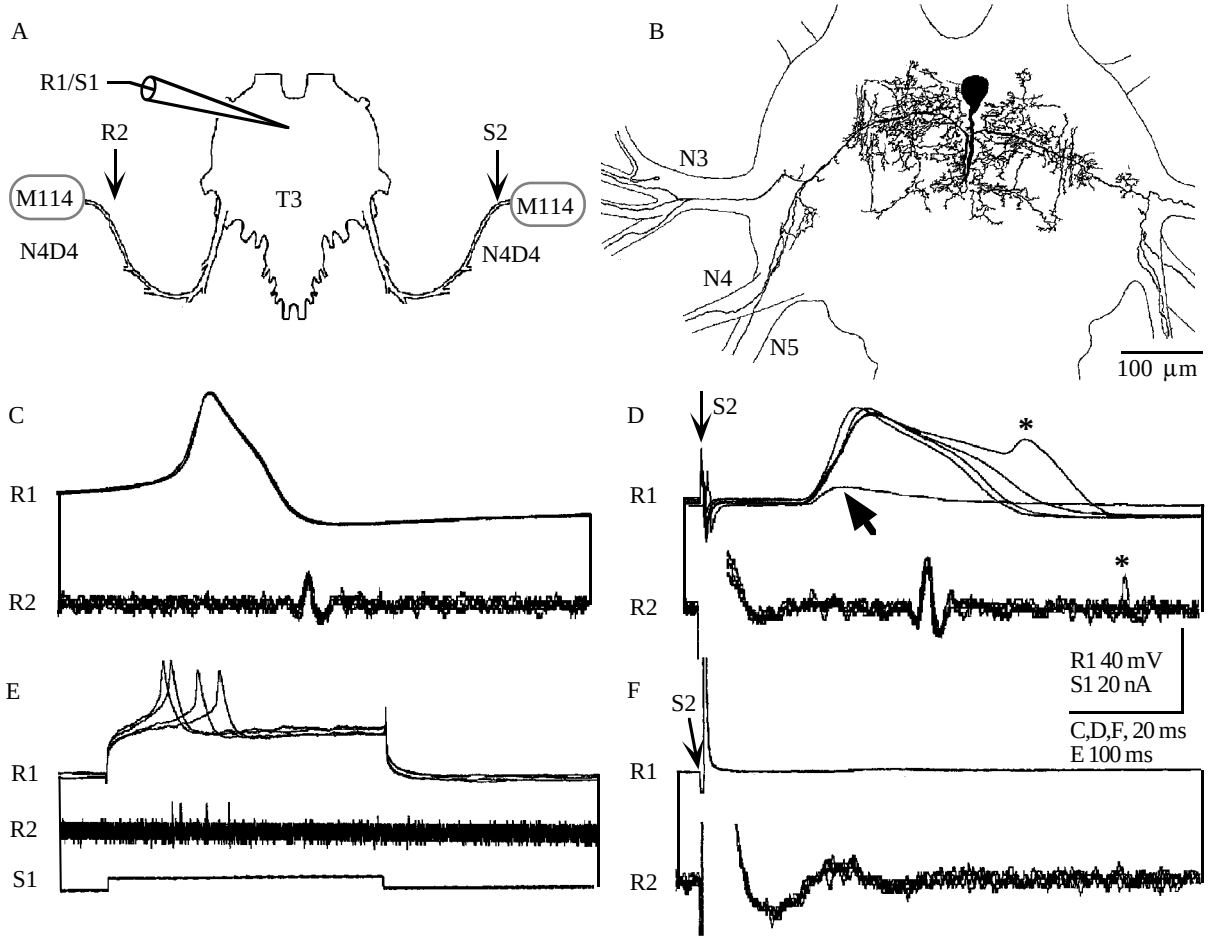

Fig. 4. Intracellular identification of the DUM neurone (DUM3,4,5a) innervating the metathoracic pleuroaxillary muscle (M114). (A) The experimental arrangement. (B) *Camera lucida* drawing of the cobalt-filled and silver-intensified metathoracic DUM3,4,5a neurone. (C–F) Intracellular soma recordings (R1) and extracellular recordings from the left N4D (R2) showing spontaneous action potentials (C), antidromic spikes evoked by stimulating the right N4D4 (S2) (D) and spikes evoked by intracellular current injection (S1) (E). (F) Failure to evoke spikes by stimulating N4D4 (S2) after killing the DUM cell by cobalt injection. Note that in one sweep in D, the spike conducted to the contralateral nerve failed to evoke an active soma response (arrow), and in another sweep a second soma spike arose from the shoulder of the first (*).

with axons in nerve roots 3, 4 and 5 (N=8). Soma spikes that occurred spontaneously (Fig. 4C), and spikes evoked either by unilateral stimulation of nerve N4D4 (Fig. 4D) or by intracellular current injection (Fig. 4E), produced action potentials in the nerve to M114 after a constant delay. As is typical for efferent DUM cells, the soma spikes were large and broad (45–48 mV, 20–25 ms). After intracellular injection of cobalt ions, which effectively killed the recorded cell, latency-locked spikes could no longer be evoked in the nerve to the M114 by stimulating the corresponding contralateral nerve branch (Fig. 4F). The pleuroaxillary muscles thus appear to be innervated by only one DUM neurone.

Intracellular cobalt staining

The morphology of the cobalt-stained DUM cell was revealed by subsequent silver intensification (Fig. 4B). Viewed from the dorsal aspect, the primary neurite of the cell projects posteriorly for 80–100 µm, before descending and turning to project anteriorly at a depth amounting to 16±4.2 % of the total thickness of the ganglion (mean ± S.D., N=10 ganglia). This DUM cell must therefore pass through the superficial DUM (SDT) tract rather than the deep DUM tract (DDT; relative depths: SDT 21.1±3.4 %; DDT 36.2±4.2 %, mean ± S.D., N=8 sagittal sections; data from Watson, 1984; Stevenson *et al.* 1992; Campell *et al.* 1995; P. A. Stevenson, unpublished observations). Peripheral axon projections were present in all branches of nerve 3 (N3A,B,C; Fig. 4B), nerve 4 (N4A,B,C,D) and nerve 5 (N5A,B) on both sides of the body. However, the resolution of the cobalt stains we produced was insufficient to reveal the full extent of these projections and their endings on specific targets. Nevertheless, in agreement with the results from extracellular recordings (Fig. 3), a single faintly labelled axon was observed in N4D4 to M114 and N4D2 to M120, but not in N4D1 to M119 or in N4D3 to M129. In addition, cobalt-labelled varicosities were observed on the surfaces of the posterior rotator coxae muscles (M122, M123, M124), which are innervated *via* N4B1 and N4C (Campbell, 1961). Since this

DUM neurone can now be uniquely identified, we name it DUM3,4,5a.

Octopamine immunocytochemistry

Previous studies have established that all locust DUM neurones with peripherally projecting axons exhibit octopamine-like immunoreactivity (Stevenson *et al.* 1992). Here, we show that both parts (a and b) of the pleuroaxillary muscles are covered with a loose network of octopamine-like immunoreactive varicosities (diameter 0.2–1 μm; Fig. 2C).

Modulation of M114 contractions by DUM3,4,5a

Using an isolated metathoracic ganglion–nerve–muscle preparation (Fig. 5A), we could show that the DUM3,4,5a neurone modulates neurally evoked isometric twitch tension of M114 (Fig. 5B,C) in the same way as bath-applied octopamine (cf. Fig. 6).

Stimulating the ipsilateral nerve to M114 with 0.8–1.2 V, 0.2 ms pulses at 0.5 Hz was generally just sufficient to produce a compound twitch contraction of both muscle parts a and b. At this stimulus intensity, recordings from the contralateral nerve showed that DUM3,4,5a is not activated. As shown in Fig. 5B,C, stimulating the contralateral nerve with 6–10 V, 0.2 ms pulses at 45 Hz for 1.5 s, to excite the DUM cell (cf. Figs 3, 4D), caused an approximately 30 % increase in the neurally evoked twitch tension, a marginal increase in contraction velocity and an almost 25 % increase in relaxation velocity. In these preparations, action potentials in the motor nerve and M114 contractions often occurred spontaneously. This appeared to be due to rapid deterioration of the isolated ganglion following the necessarily extensive dissection. The possible role of octopaminergic innervation of M114 was, therefore, further investigated by superfusing the isolated muscle with octopamine solutions.

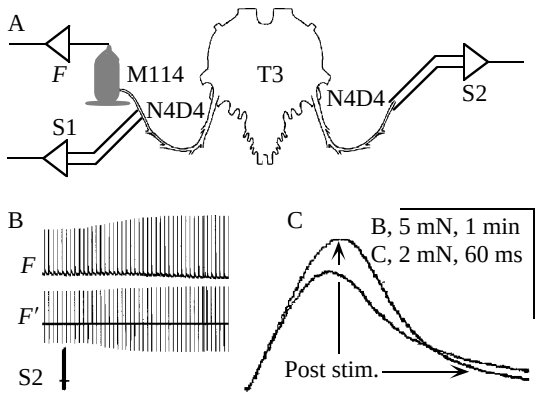

Fig. 5. Influence of DUM3,4,5a on neurally evoked twitch tension of M114 in an isolated metathoracic ganglion–nerve–muscle preparation. (A) The experimental procedure. (B) Twitch contractions of muscle M114 evoked by nerve stimulation (6 V, 0.2 ms, 0.5 Hz): *F* gives the recorded isometric twitch tension and *F′* the differentiated force (upward- and downward deflections from the baseline are proportional to the maximal contraction and relaxation velocities respectively); S2 shows stimulation of the contralateral N4D (10 V, 0.2 ms, 1.5 s, 45 Hz) used to excite the DUM cell. (C) Two superimposed single twitches, one before and the other after (Post stim. arrows) DUM cell stimulation.

Modulation of M114 contractions by octopamine

In the isolated muscle preparations, it was possible to activate each or both of the two motor units of M114 selectively by finely adjusting stimulus intensity and polarity (Fig. 6Ai). For compound contractions of both muscle parts, 10^{-6} mol l^{-1} DL-octopamine increased the twitch tension amplitude by 35±3.0 % (mean ± S.E.M., *N*=16), the contraction velocity by 16±2.5 % (mean ± S.E.M., *N*=16) and the relaxation velocity by 55±9.0 % (mean ± S.E.M., *N*=16) with little to no net change in baseline tension. Octopamine affected both motor units in essentially the same way (Fig. 6Aiii,iv); the responses were dose-dependent and could be reversed by washing with saline (Fig. 6B).

As shown in other studies (Evans and Siegler, 1982; Bräunig *et al.* 1994; Malamud *et al.* 1988; Whim and Evans, 1988), the net effect of octopamine on neurally evoked tension is dependent on both the activation frequency of the muscle (Fig. 6C) and the previous contraction record (Fig. 6D). In saline, stimulation frequencies of 5 Hz and above produce tonic tension in M114 with superimposed twitches (Fig. 6C, left), which tetanize at around 40 Hz (Fig. 6D, left; see also Elson and Pflüger, 1986). Superfusion with DL-octopamine (10^{-6} mol l^{-1}) causes a reduction in the peak tonic tension at stimulation frequencies below 40 Hz, while increasing the dynamic range (minimum to peak tension). Following brief periods of high-frequency stimulation, M114 also develops a catch-like tension (Ferber, 1986), known also as the Blaschko–Cattell–Kahn effect (Blaschko *et al.* 1931; see Hoyle, 1983; Günzel and Rathmayer, 1994). As shown in Fig. 6D, the catch-like tension decreases in the presence of octopamine.

In consideration of these various effects, we investigated the influence of octopamine on tension when the pleuroaxillary muscle is activated with the more complex patterns that it is thought to experience during corrective steering manoeuvres. In straight flight, both M114 motor units are activated once or twice during each wing downstroke. Following an imposed roll, the muscle on the downward-rotated body side is activated more often during each wingbeat so that the wing on this side produces more lift, whereas the muscle on the upward-rotated side is activated less often and hence less lift is produced by the wing (Elson and Pflüger, 1986; Wolf, 1990). The mechanical responses of the isolated pleuroaxillary muscle to stimulus regimes that simulate this behaviour are shown in Fig. 7. In Fig. 7A, the muscle is stimulated once every 50 ms, i.e. at flight frequency, with four interposed cycles of twin pulses 15 ms apart that produce a transient increase in tension. During superfusion of DL-octopamine (10^{-6} mol l^{-1}), the single-twitch amplitude increases, as does also the peak tension attained during the double-stimulus pulses, whereas both the maximum tension at 20 Hz and the catch-like tension following the twin pulses decrease. Conversely, in Fig. 7B, M114 is stimulated with twin pulses 15 ms apart every 50 ms with four interposed

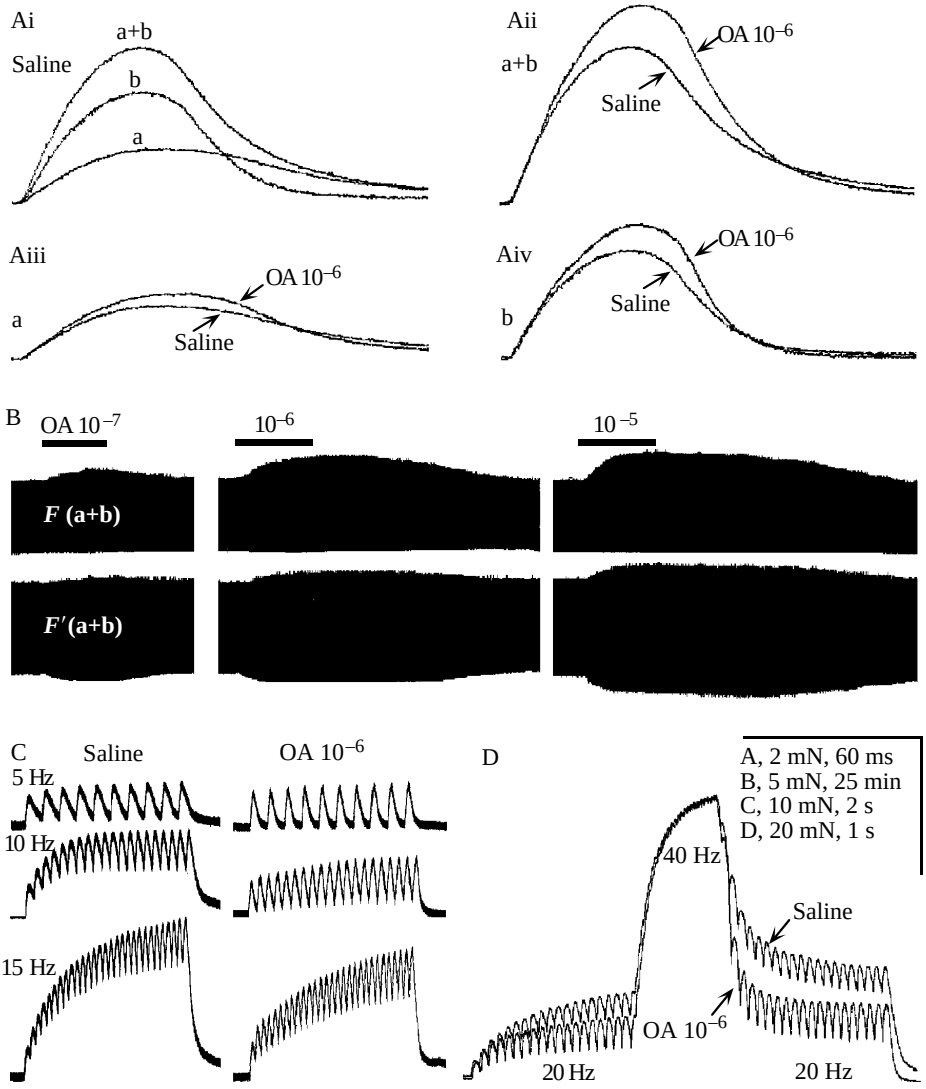

Fig. 6. Mechanograms showing the influences of DL-octopamine on M114 contractions evoked by motor nerve stimulation. (A) Superimposed single twitches: Ai, saline control showing the three different mechanical responses evoked by selective activation of each individual (a, b) or both (a+b) large motoneurones; Aii,iii,iv, these three mechanical responses in saline superimposed on the contractions recorded during octopamine superfusion (OA 10^{-6} mol l^{-1}). (B) Continuous recordings showing the influence of octopamine superfusion (OA 10^{-7}, 10^{-6}, 10^{5} mol l^{-1}) on twitch force F and contraction/relaxation velocity F' evoked by stimulating both motor units (a+b) at 0.5 Hz. (C) Left-hand traces control (saline) and right-hand traces during octopamine superfusion (OA 10^{-6} mol l^{-1}) showing the relaxing effect on tension for 5, 10 and 15 Hz stimulation. (D) Two superimposed traces, one during saline superfusion and the other during octopamine superfusion, where the muscle was stimulated at 20 Hz with an interposing period at 40 Hz. In the presence of octopamine, 20 Hz tension (compare with C) and the catch-like tension following 40 Hz stimulation are both reduced.

cycles of single pulses. Under these circumstances, octopamine causes an increase in maximum tetanic tension and more complete relaxation of the muscle during the four cycles of single pulses. All these effects were reversible by washing in saline.

Discussion

Insect muscle is innervated by relatively few neurones. In these compact motor systems, the need for the animal to achieve a wide dynamic range of effector movements is met by functional diversity of the neurone types (Hoyle, 1955) and of the muscle fibres they innervate (e.g. Müller *et al.* 1992), together with flexibility in the motor networks (Burrows, 1995). The pleuroaxillary muscle (M85, M114) studied in this paper is innervated by two large, putatively glutaminergic (see Bicker *et al.* 1988) motoneurones, four smaller neurones of unknown function and transmitter content, the common inhibitor-1 neurone (Kutsch and Schneider, 1987; Wolf, 1990), which is GABAergic (Watson, 1986), and a single octopaminergic dorsal unpaired median neurone (DUM3,4,5a; this paper Figs 1, 2). This list can be considered as complete, since the sum of eight neurones exactly matches the number of axons determined in nerve sections with the electron microscope (Kutsch and Schneider, 1987).

The DUM cell innervating the pleuroaxillary muscle is one of three such cells that have axons in nerves 3, 4 and 5 (Campbell *et al.* 1995). Since it is the first of these three that can be uniquely identified, we name it DUM3,4,5a in accordance with earlier conventions (Goodman, 1982; Stevenson and Spörhase-Eichmann, 1995). In contrast to Neurobiotin, cobalt backfills of N4D4 fail to reveal DUM3,4,5a, and the common inhibitor neurone stains unreliably (Pflüger *et al.* 1986; Kutsch and Schneider, 1987; this study). We conclude that Neurobiotin is more suitable for detecting somata with thin axons, especially when long diffusion distances are involved. There can be little doubt that DUM3,4,5a is octopaminergic. All efferent thoracic DUM neurones are octopamine-immunoreactive (Stevenson *et al.* 1992), octopamine-immunoreactive varicosities occur on

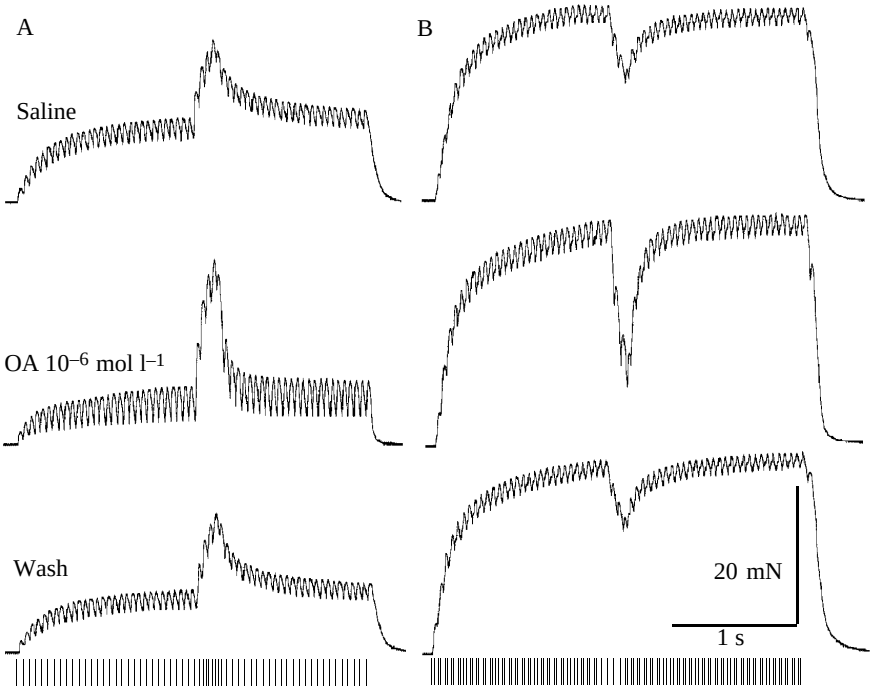

Fig. 7. Effect of octopamine on developed tension in M114 stimulated to simulate its motor activation during an induced roll in tethered flight. (A) Response to a 20 Hz train of single pulses with four cycles of interposed twin pulses 15 ms apart. (B) Response to a 20 Hz train of twin pulses 15 ms apart with four cycles of interposed single pulses. Examples are shown before (Saline), during (OA 10^{-6} mol l^{-1}) and after (Wash) octopamine superfusion. The stimulus marker is shown on the lower record.

M114 (Fig. 2C) and DUM 3,4,5a modulates M114 twitch contractions (Fig. 5) in the same fashion as octopamine (Fig. 6).

Considering that DUM3,4,5a projects in nerves 3, 4 and 5 on both body sides, as well as in all the secondary branches of these nerves, it is reasonable to assume that it innervates more than 10 different pairs of muscles. Despite this, several features of the functional organization of the DUM cell system support the hypothesis that even neurones such as DUM3,4,5a can have specific influences on locomotion. These include the following. (1) The activity of individual DUM cells is coupled to specific motor patterns (Burrows and Pflüger, 1995). How this applies to DUM3,4,5a is not known in detail at present. DUM3,4,5 neurones are active both during a kick (Burrows and Pflüger, 1995) and during fictive flight (C. Duch, personal communication), whereas DUM3,4 neurones are active during flight (Ramirez and Orchard, 1990) but not during kicking (Burrows and Pflüger, 1995). (2) Different DUM cell types innervate different muscle subsets. DUM3,4,5a projects in N4D to M114, which is involved in opening the wing and setting its attack angle (Pfau, 1977; Pfau and Nachtigall, 1981), and in M120, the only direct flight muscle activated during flight and walking (Duch and Pflüger, 1995), but not to the pure flight muscles M119 and M129 (Fig. 3) which are innervated *via* N4D by DUM3,4 cells (Kutsch and Schneider, 1987). Interestingly, all DUM3,4,5a targets identified so far (M114, M120, M122, M123, M124) are innervated by the common inhibitor-1 neurone (Duch and Pflüger, 1995), whereas M119 and M129 are not (Kutsch and Schneider, 1987). (3) Octopamine produces different effects on different muscles (direct flight muscle: Malamud *et al.* 1988; indirect flight muscle: Whim and Evans, 1988; visceral muscle: Orchard and Lange, 1985). This depends on the motor units activated (O'Shea and Evans, 1979) and the receptor types involved (for a review, see Evans and Robb, 1993). The effects of DUM3,4,5a and octopamine on M114 twitch contractions are qualitatively similar to those of DUMETi and octopamine on slow-motoneurone-induced tension of the extensor tibiae (Evans and O'Shea, 1977; O'Shea and Evans, 1979; Evans and Siegler, 1982): single-twitch tension and the contraction/relaxation velocities increase, whereas incompletely fused tetanic tension and a catch-like tension are both reduced (Fig. 6). The influence on contraction velocity is, however, not as pronounced as for the extensor tibiae muscle. (4) The net effect of octopamine on muscle tension depends on the motor activation pattern (Figs 6, 7).

As proposed by Pfau (1977) and Pfau and Nachtigall (1981) and later confirmed by Wolf (1990), the pleuroaxillary muscles exert force *via* the third axillary sclerite to decrease both downstroke pronation and upstroke supination of a wing, without altering its trajectory. Reducing downstroke pronation increases the aerodynamic angle of attack and can thereby increase lift and thrust, whereas reducing upstroke supination increases the *negative* angle of attack and the production of *negative* lift. Since the pleuroaxillary muscles are activated during the first half of the downstroke, the contractions are timed to enhance lift production (Elson and Pflüger, 1986). Nevertheless, when stimulated at flight frequency (20 Hz), the isolated muscle develops a considerable tonic tension (Elson and Pflüger, 1986; Wolf, 1990; this paper). Under the influence of octopamine, however, the tonic component at 20 Hz is considerably reduced, whereas the phasic increments in tension with each motor stimulus are greater, and these decay more rapidly (Figs 6, 7). Thus, activity of the octopaminergic

DUM3,4,5a neurone during flight could optimize lift production by focusing maximum pleuroaxillary muscle force on the wing downstroke phase to reduce pronation and thereby also to minimize negative lift generation during the upstroke. This may be of particular relevance during the take-off phase of flight. Interestingly, the basic tonus is also reduced by the common inhibitor neurone (Wolf, 1990). Thus, under the combined control of its octopaminergic and GABAergic innervation, the pleuroaxillary muscles could be tuned to produce only phasic contractions. This idea is in contrast to Pfau's original suggestion (Pfau, 1977; Pfau and Nachtigall, 1981) that the tension developed by the pleuroaxillary muscles during flight is mainly tonic.

DUM cells have independent spike-initiating zones distal to the bifurcation of the primary neurite (Heitler and Goodman, 1978). It has, therefore, been suggested that their spikes could be differentially shunted to the two body sides for more effective modulation of locomotion (Burrows and Pflüger, 1995). This need not be necessary and could even be counterproductive. The left- and right-side pleuroaxillary muscles, for example, are functional antagonists during corrective roll manoeuvres (Elson and Pflüger, 1986). Activation of the muscle on the downward-rotated side occurs both more often and earlier than on the upward-rotated side. Simulations of these activation patterns shows that octopamine can almost double the contraction force on the presumptive downward-rotated side, while enhancing relaxation on the presumptive upward-rotated side (Fig. 7). Furthermore, these transient changes in tension become almost fully reversible owing to the reduction in catch-like tension. The DUM cell, with its bilaterally projecting axons, is thus functionally well designed for optimizing steering reactions.

Clearly, the present paper can only indicate what DUM-3,4,5a may be capable of achieving. In order to resolve the issue, it will be necessary to determine exactly how this DUM cell is active during steering behaviour and to quantify the temporal dynamic relationship between the DUM cell spikes, the motoneurone spikes and muscle tension.

We thank Professor Dr H.-J. Pflüger, in whose laboratory these experiments were carried out, for lively discussions throughout this work and constructive comments on the manuscript. Supported by the Deutsche Forschungsgemeinschaft.

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