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DIFFERENTIAL ACTIVATION OF OCTOPAMINERGIC (DUM) NEURONES *VIA* PROPRIOCEPTORS RESPONDING TO FLIGHT MUSCLE CONTRACTIONS IN THE LOCUST

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Summary

The synaptic potentials generated in neuromodulatory octopaminergic dorsal unpaired median (DUM) neurones by afferents excited by twitch contractions of a dorso-ventral flight muscle were investigated in the locust. Responses to stimulation of the metathoracic wing elevator muscle 113 were obtained in locusts in which all sensory feedback from the thorax had been removed, except for feedback from the thoracic chordotonal organs, the axons of which enter *via* the purely sensory nerve 2. Afferents in nerve 2C, which originates from two chordotonal organs, responded reliably to twitch contractions of this flight muscle. Octopaminergic neurones innervating leg muscles (DUM5 neurones) received depolarising inputs and often spiked following stimulation of the muscle. In contrast, those innervating the wing muscles themselves (DUM3 and DUM3,4 neurones) received inhibitory inputs. The responses of DUM3,4,5 neurones, which project mainly to leg muscles, were more complex: most were excited by twitch contractions of M113 but some were inhibited. DUMDL, which innervates the dorsal longitudinal indirect flight muscles, showed no clear response. Direct stimulation of nerve 2C evoked depolarising inputs and spikes in DUM5 neurones and hyperpolarising inputs in DUM3 and DUM3,4 neurones. Our data suggest that sensory feedback from thoracic chordotonal organs, which are known to be activated rhythmically during flight, contributes to the differential activation of efferent DUM neurones observed during flight.

Key words: octopaminergic neurone, DUM neurone, neuromodulation, locust, flight muscle, sensory feedback, *Locusta migratoria*, *Schistocerca gregaria*.

Introduction

One of the most influential developments in neuroethology of recent years is the realisation that most aspects and components of behaviour are subject to continuous modulation by a host of neurochemicals (Kravitz, 1988; Bicker and Menzel, 1989; Harris-Warrick and Marder, 1991). The multiplicity of effects that neuromodulators can have at the synaptic, cellular, network and behavioural levels are well documented (e.g. Kupfermann et al., 1997; Harris-Warrick et al., 1997). Nonetheless, comparatively little is known of the circumstances leading to and governing the natural release of neuromodulators in an animal. Knowledge of how neuromodulatory neurones are synaptically embedded in neural networks is imperative for our understanding of the chemical control of behaviour.

Perhaps the most extensively studied population of modulator neurones in invertebrates are the octopaminergic dorsal unpaired median (DUM) neurones of insects (for an overview, see Evans, 1985; Stevenson and Spörhase-Eichmann, 1995). These neurones, numbering just over 100 identified individuals, are mostly efferent neuroparacrine cells. With their bilaterally projecting axons, each efferent DUM neurone forms a peripheral network of neurosecretory endings on the surface of several target muscles, and occasionally other tissues, on both sides of the body (Hoyle, 1974; Bräunig et al., 1994; Bräunig, 1997). They release the biogenic amine octopamine (Morton and Evans, 1984), which can then function as a neurotransmitter (Christensen and Carlson, 1982) and a neurohormone (Bräunig et al., 1994), but primarily as a neuromodulator (for reviews, see Orchard, 1992; Evans, 1985). For example, octopamine released by DUM neurones modulates the response of skeletal muscle to motor commands (Evans and O'Shea, 1977) in order to achieve the more rapid tension changes favourable for locomotion (Evans and Siegler, 1982; Whim and Evans, 1988; Stevenson and Meuser, 1997).

Early investigations suggested that the population of DUM neurones is activated collectively at the onset of motor activity and in response to sensory stimuli that tend to startle the quiescent animal (Hoyle and Dagan, 1978). Thus, DUM

neurones had been considered as having only a general function in the mechanisms underlying arousal (Orchard et al., 1993). It is now known, however, that DUM neurones are differentially activated during different behaviour patterns. In locusts, DUM neurones innervating leg muscles are preferentially excited during kicking and fictive walking, whilst other DUM neurones are inhibited (Burrows and Pflüger, 1995; Baudoux et al., 1998). In contrast, DUM neurones that project to wing muscles are phasically inhibited during restrained and fictive flight, whereas other types of efferent DUM neurones are excited (Duch and Pflüger, 1999).

Neurones directly presynaptic to DUM neurones have not yet been identified, so information about the mechanisms underlying their selective recruitment is limited accordingly. DUM neurones are depolarised by acetylcholine (Lapied et al., 1990), and they appear to receive glutamatergic and γ-aminobutyric acid (GABAergic) input synapses (Pflüger and Watson, 1995; Duch and Pflüger, 1999) and to possess autoreceptors for octopamine (Achenbach et al., 1997; Howell and Evans, 1998). Evidence from isolated ganglia suggests that the concerted activity of specific types of DUM neurone and their coupling to rhythmical motor patterns is at least partly under central control (Kalogianni and Theophilidis, 1993; Baudoux and Burrows, 1998; Baudoux et al., 1998; Johnston et al., 1999; Duch et al., 1999; Duch and Pflüger, 1999). However, DUM neurones that are hyperpolarised during flight are also inhibited by the activity of the tegula (Duch and Pflüger, 1999), a sense organ in the wing hinge involved in shaping the flight motor pattern (Wolf, 1993). Thus, the differential activation of DUM neurones may be dependent on sensory feedback of different modalities during the execution of specific behaviour patterns.

In the present study, we describe how different DUM neurones in the locust metathoracic ganglion are influenced by a distributed system of internal thoracic proprioceptors innervated by the lateral branches of the nerve root 2 (Bräunig et al., 1981). These receptors respond differentially to single contractions of different flight muscles and are responsible for evoking the delayed synaptic excitation of flight motor neurones (Stevenson, 1997) that follows electrical stimulation of flight muscles (Burrows, 1973). Our results support the idea that the selective activation of different types of DUM neurone can be under afferent control and, furthermore, indicate that the octopaminergic modulation of muscle may be coordinated and regulated interactively with motor commands by specific proprioceptors responsive to muscle contractions.

Materials and methods

Experimental animals and dissection

Fifty-three experiments were performed on adult male and female locusts (mostly *Schistocerca gregaria*, but *Locusta migratoria* was used in initial experiments) from our crowded colonies in Berlin and Cambridge. Animals were fixed dorsal side uppermost in modelling clay (Plasticine), an incision was made along the dorsal midline and the thorax was spread open by fixing the wings laterally. Following removal of the gut, salivary tissue and some ventral muscles, a wax-coated silver platform was slipped beneath the meso- and metathoracic ganglia to stabilise them for intracellular recording.

In most (49) preparations, all lateral nerves of the meso- and metathoracic ganglia were cut except for metathoracic nerve 2, a purely sensory nerve that innervates a number of thoracic chordotonal organs (Bräunig et al., 1981). Removal of the ventral diaphragm during dissection resulted in the destruction of nerve 2E to the metathoracic apodemal chordotonal organ, and insertion of the platform necessitated breaking nerve 2A to the anterior chordotonal organ. Thus, the only functioning chordotonal organs still connected to the metathoracic ganglion were the coxal chordotonal organ (cCO, innervated by nerve 2D), the posterior joint chordotonal organ (pjCO, innervated by nerve 2C in *Schistocerca gregaria*) and the myochordotonal organ (myoCO, also innervated by nerve 2C).

In a few preparations (four experiments), the connectives anterior to the mesothoracic ganglion and posterior to the metathoracic ganglion were also cut, isolating these ganglia from the rest of the ventral nerve cord.

Electrophysiological recordings and stimulation

Intracellular recordings were made using thick-walled glass microelectrodes filled with 6 % cobalt hexammine chloride (resistance 80–100 MΩ). Recordings of DUM neurones were made from their cell bodies, which produce characteristically large and long-duration action potentials with a prominent after-hyperpolarisation. Recordings from motor neurones were made from their main processes in the neuropile.

Extracellular recordings of metathoracic nerves 3A and 4 were made by sucking up their cut ends into suction electrodes. Recordings of metathoracic nerve 2C were made using either a paired hook electrode or *en passant* with a fine suction electrode. Recording and stimulation of nerve 2C was performed only in *Schistocerca gregaria* where this nerve contains afferent axons from the myoCO and pjCO.

A pair of 50 μm diameter steel wires, insulated except for the tips, were mounted on a manipulator and used for direct stimulation of the denervated metathoracic tergosternal muscle (M113). A diagram of the experimental arrangement is given in Fig. 1A.

Recordings were stored on an FM tape recorder (RACAL, store 7 DS) and later transferred to a computer using an analogue-to-digital conversion board (CED 1401 plus) and Spike 2 software (CED).

Identification of neurones

Efferent DUM neurones were stained with cobalt (Bacon and Altman, 1977) and assigned to different morphological types according to their axonal paths in the lateral nerves. Using this criterion alone, five different types were distinguished: DUM1 neurones, DUM3 neurones, DUM3,4 neurones, DUM3,4,5 neurones and DUM5 neurones. Further separation into individual, identified DUM neurones on the basis of their projections and targets (see Bräunig, 1997) was

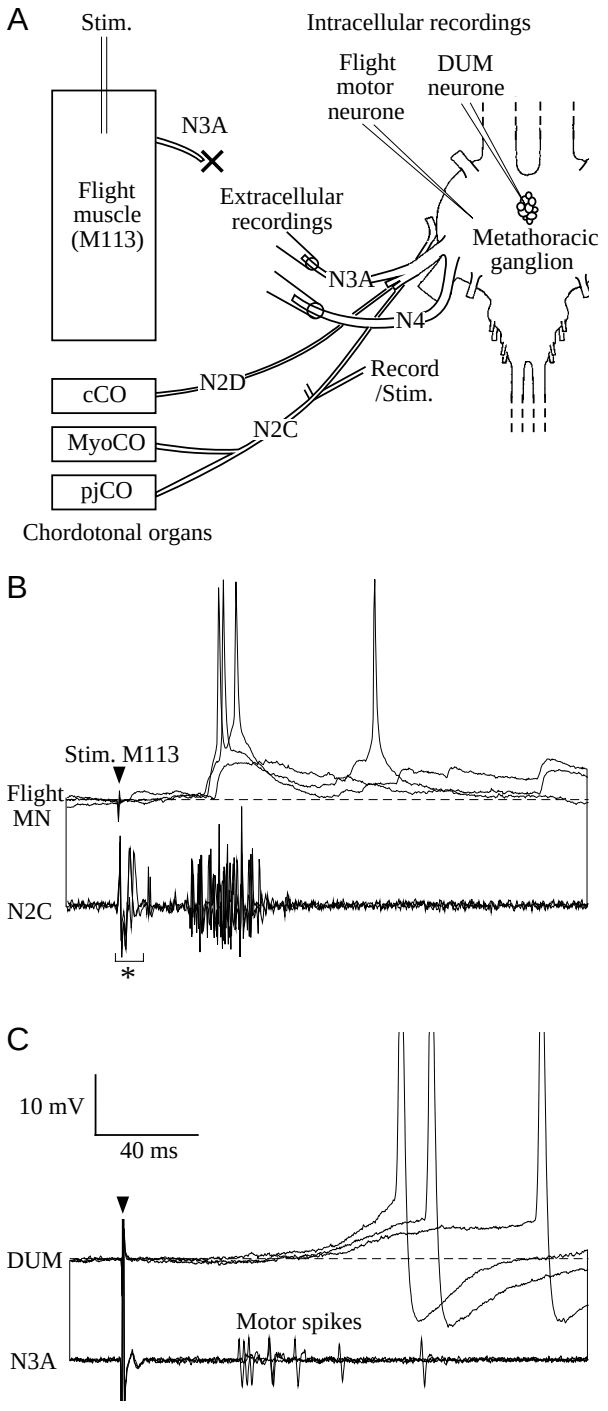

Fig. 1. The experimental arrangement and examples of the responses to contractions of muscle 113 (M113). (A) All nerves to the metathoracic ganglion were cut except for the purely sensory nerves 2D (N2D) and 2C (N2C), which innervate the coxal chordotonal organ (cCO), myochordotonal organ (MyoCO) and posterior joint chordotonal organ (pjCO). Single twitch contractions of muscle 113 were evoked by direct electrical stimulation (Stim.) of the denervated muscle. A paired hook electrode, or a suction electrode, was used for extracellular recording and stimulation of afferents in nerve 2C (Record/Stim.). Glass microelectrodes were used for intracellular recordings of flight motor neurones (MNs) and DUM neurones, and suction electrodes were used for extracellular recordings of nerves 3A and 4. (B) The twitch contraction produced by stimulation of muscle 113 evoked a burst of spikes in nerve 2C followed by excitatory postsynaptic potentials (EPSPs) and action potentials in a flight motor neurone (here, and in subsequent figures, the responses to three stimuli have been overlaid). The flight motor neurone continued to receive EPSPs long after the response in nerve 2C had ceased. (C) Contraction of M113 caused a motor neurone in nerve 3A to spike well before an unidentified DUM neurone received a depolarising input and produced an action potential (tops cut off). Black arrowheads mark the stimulus artefacts in the intracellular recordings, and the bar and asterisk indicate the stimulus artefact in the nerve 2C recording. The horizontal dashed line indicates the membrane potential prior to stimulation. The vertical scale bar applies to the intracellular recordings only.

beyond the scope of this study. The DUM neurone innervating the dorsal longitudinal muscles (DUMDL) was, however, identified as an individual by the one-to-one correspondence of its action potentials recorded extracellularly from the cut end of nerve 1D1 with those recorded intracellularly in the cell body.

Flight motor neurones were recognised by their spiking responses to wind blown at the head of the locust and subsequently by their morphology as revealed by cobalt staining.

Results

Responses of DUM neurones and motor neurones to twitch contractions of the tergosternal flight muscle

Twitch contractions of the metathoracic tergosternal muscle (M113) were evoked by direct electrical stimulation of the denervated muscle (Fig. 1A). Each contraction produced a burst of sensory spikes in nerve 2C followed by excitatory postsynaptic potentials (EPSPs) and action potentials in flight motor neurones (Fig. 1B). Excitation of motor neurones was also evident in the extracellular recordings of nerve 3A and nerve 4. The main finding of the present study was that twitch contractions of muscle 113 caused synaptic inputs in DUM neurones (Fig. 1C). Similar responses were recorded whether the ganglia were isolated from the rest of the ventral nerve cord or were still connected *via* the connectives.

Within each preparation, the response in nerve 2C occurred with a consistent delay following stimulation of muscle 113 and always preceded the responses in flight motor neurones and DUM neurones. Furthermore, the nature of the response observed in DUM neurones varied according to their morphological type. Thus, only those DUM neurones innervating leg muscles (DUM5 and DUM3,4,5) were excited by contraction of muscle 113, whilst those innervating flight muscles (DUM3 and DUM3,4) were nearly always inhibited.

Responses of DUM5 neurones to contractions of muscle 113 and stimulation of nerve 2C

The DUM5 neurones innervate muscles in the hindlegs *via* nerve 5 and are excited during a variety of motor outputs, including flight (Duch and Pflüger, 1999). In the present study, twitch contractions of muscle 113 caused depolarising inputs

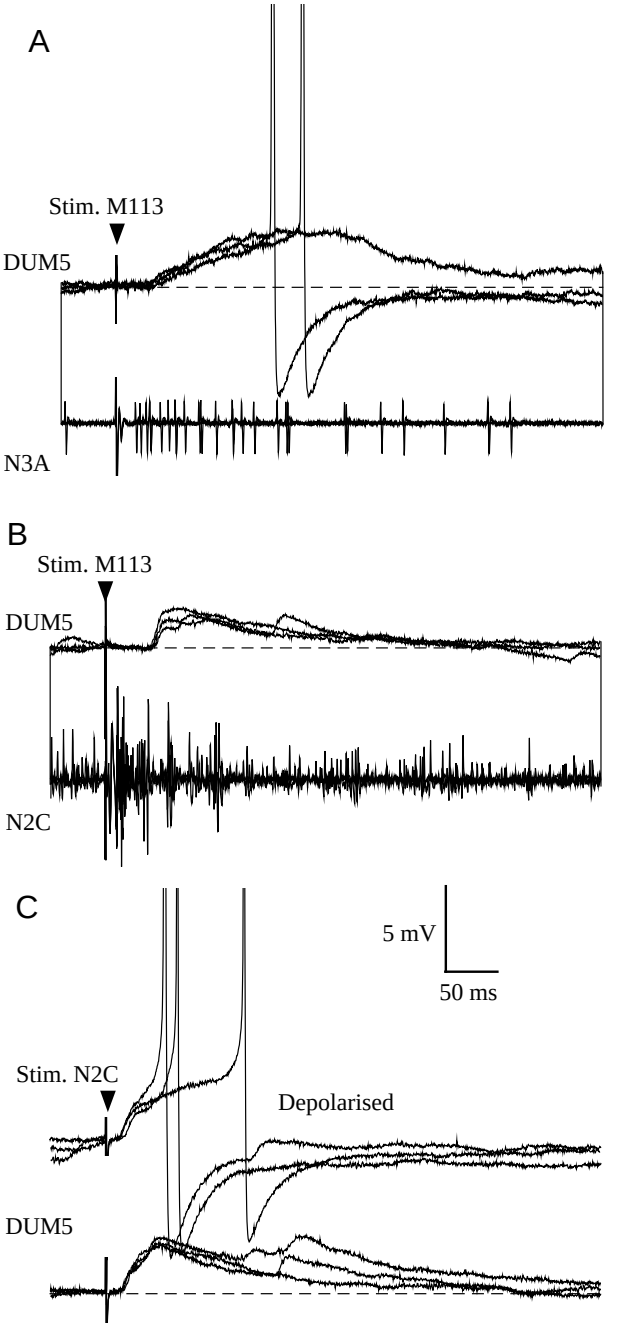

Fig. 2. DUM5 neurones were excited by contractions of muscle 113 (M113) and direct stimulation of nerve 2C (N2C). (A) Stimulation of muscle 113 evokes spikes in a motor neurone in nerve 3A followed by excitatory postsynaptic potentials (EPSPs) in a DUM5 neurone. In two of the three trials, the EPSPs led to an action potential (tops cut off). (B) In another preparation, stimulation of muscle 113 evoked spikes in nerve 2C followed by EPSPs in a DUM5 neurone. (C) Direct electrical stimulation of nerve 2C also evoked EPSPs in this DUM5 neurone, but at a shorter latency. EPSPs continued to arrive for up to 150 ms. When the DUM5 neurone was depolarised by current injection through the recording electrode, the EPSPs produced an action potential (tops cut off). For further details, see Fig. 1.

in most of the DUM5 neurones recorded (12 out of 15 recordings). In eight of these recordings, the depolarising inputs were sufficient to trigger action potentials (Fig. 2A) and were therefore clearly EPSPs. The EPSPs started to occur in the DUM5 neurone 45 ms after the stimulus, over 30 ms after the onset of the sensory response in nerve 2C, and continued to arrive for some 100 ms (Fig. 2B). When nerve 2C was stimulated directly from the same site, the DUM5 neurone received a depolarising input after only 15 ms (Fig. 2C). If the DUM5 neurone was depolarised towards threshold by current injection, the depolarising inputs caused by stimulation of nerve 2C evoked action potentials, confirming that they were indeed EPSPs.

Responses of DUM3 neurones, DUM3,4 neurones and DUMDL

The DUM3, DUM3,4 and DUMDL neurones, which innervate various flight muscles, are inhibited during a number of different motor outputs, including flight (Duch and Pflüger, 1999). In the present study, DUM3 and DUM3,4 neurones received hyperpolarising inputs following twitch contractions of muscle 113 (21 out of 24 recordings); depolarising inputs were never observed. Inhibitory postsynaptic potentials (IPSPs) began to arrive some 35–40 ms after stimulation of the muscle and held the DUM neurone hyperpolarised for over 100 ms (Fig. 3A). Thus, DUM neurones with axon paths in nerves 3A and 4 were inhibited at the same time as motor neurones travelling in these nerves were excited (Fig. 3B).

Direct stimulation of nerve 2C also evoked hyperpolarising inputs in DUM3 and DUM3,4 neurones (Fig. 3C). Twitch contractions of muscle 113 produced a burst of sensory spikes in nerve 2C followed by the arrival of IPSPs in the DUM3,4 neurone at a latency of approximately 40 ms (Fig. 3C, upper pair of traces). When nerve 2C was stimulated directly, the DUM3,4 neurone started to hyperpolarise at a much shorter latency of only 15 ms (Fig. 3C, lower traces).

Recordings were made from DUMDL, which innervates the indirect flight muscles (Hoyle, 1978), in three experiments, and in none of these was any response to stimulation of muscle 113 observed (Fig. 3D).

Responses of DUM3,4,5 neurones

The DUM3,4,5 neurones innervate leg muscles (Bräunig, 1997), with the exception of the flight steering muscles M85 and M114 (Stevenson and Meuser, 1997). They are excited during most motor activities, including flight (Duch and Pflüger, 1999). In the present study, stimulation of muscle 113 evoked depolarising inputs in seven out of 14 recordings of DUM3,4,5 neurones. In some preparations, these regularly evoked action potentials and were therefore clearly excitatory (Fig. 4A). Like DUM5 neurones, these DUM3,4,5 neurones were excited at longer latencies than flight motor neurones.

In three other preparations, neurones of the DUM3,4,5 category were hyperpolarised by twitch contractions of muscle 113 (Fig. 4B,C). These inputs were often small in amplitude but became more apparent if the membrane potential was

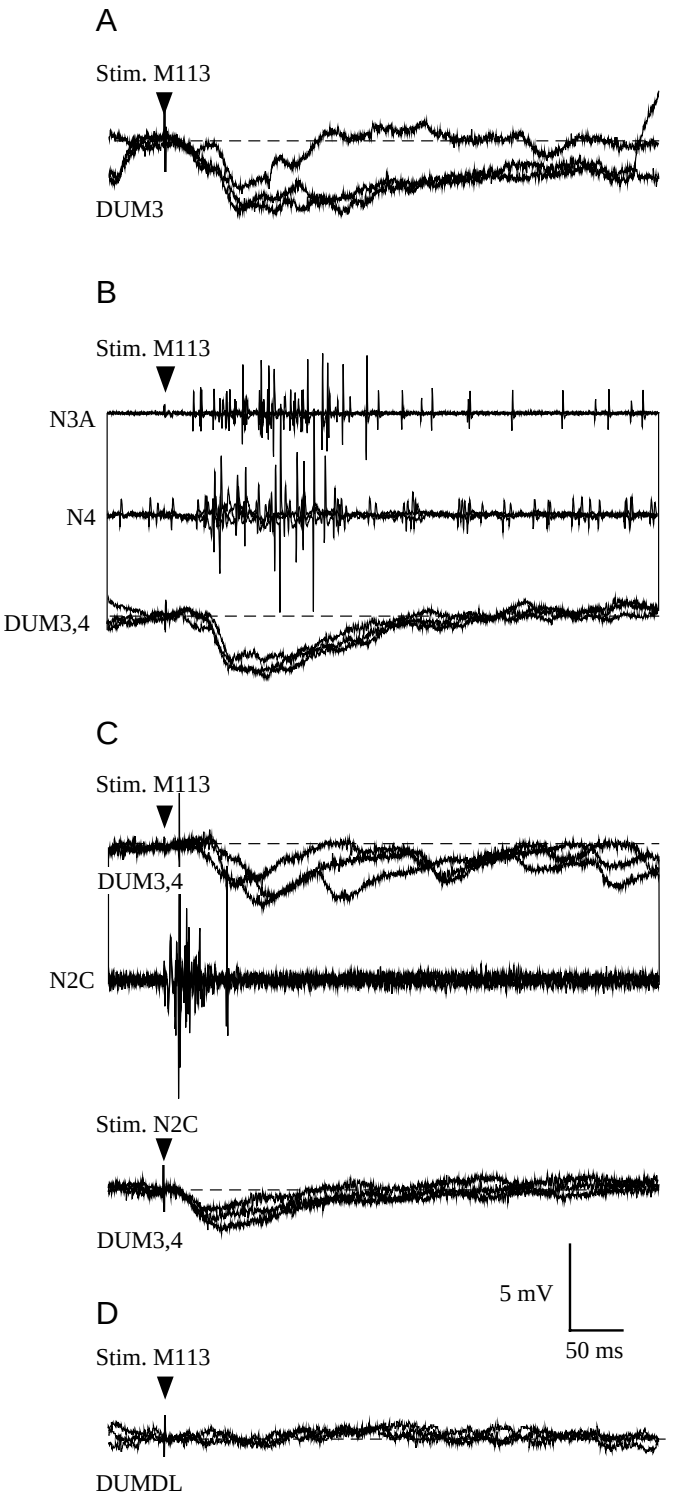

Fig. 3. DUM3 and DUM3,4 neurones were inhibited by stimulation of muscle 113 (M113) and nerve 2C (N2C). (A) Stimulation of muscle 113 evoked inhibitory postsynaptic potentials (IPSPs) in a DUM3 neurone, hyperpolarising it for over 100 ms. (B) Stimulation of muscle 113 caused hyperpolarisation of a DUM3,4 neurone at the same time as motor neurones in nerves 3A and 4 were excited. (C) Stimulation of muscle 113 produced spikes in nerve 2C followed by IPSPs in a DUM3,4 neurone. Direct stimulation of nerve 2C also evoked IPSPs. (D) DUMDL received no clear inputs following stimulation of muscle 113. For further details, see Fig. 1.

altered *via* current injection. Thus, the hyperpolarising input in a DUM3,4,5 neurone increased in size when the neurone was depolarised and reversed in polarity when the neurone was hyperpolarised, suggesting that it was due to IPSPs with a reversal potential close to the resting potential of the cell (Fig. 4B).

The inhibition of DUM3,4,5 neurones shown in Fig. 4B clearly contrasted with the excitation observed in other DUM3,4,5 neurones, raising the possibility that it was mediated *via* a different sensory pathway. However, DUM3,4,5 neurones which were inhibited by contraction of muscle 113 were also inhibited by direct stimulation of nerve 2C (Fig. 4C). This hyperpolarising input arrived 40 ms earlier than when muscle 113 was stimulated, indicating that the inhibition following the muscle contraction was mediated *via* afferents in nerve 2C.

The three different DUM3,4,5 neurones may be distinguished according to whether their primary neurite follows the superficial or the deep DUM tract (Watson, 1984), which in turn correlates with their more specific projection patterns and targets (Stevenson and Meuser, 1997). In the present study, however, there appeared to be no correlation between the kind of input a DUM3,4,5 neurone received following stimulation of muscle 113 and the tract followed by its primary neurite. Thus, it was not possible to assign the different responses of DUM3,4,5 neurones to different morphological types of DUM3,4,5 neurone.

Double recordings of DUM neurones

Simultaneous recordings from pairs of DUM neurones enabled a direct comparison of the responses of different DUM neurones to contraction of muscle 113. These recordings indicated that those DUM3,4,5 neurones that were depolarised by contraction of muscle 113 were excited by a common pathway with DUM5 neurones. A single twitch contraction of muscle 113 evoked the same pattern of EPSPs in a DUM5 neurone and a DUM3,4,5 neurone, with identical latencies (Fig. 5A). In contrast, simultaneous recordings in some preparations showed that the inhibition of DUM3 and DUM3,4 neurones caused by contractions of muscle 113 could precede the excitation of DUM5 and DUM3,4,5 neurones by as much as 50 ms (Fig. 5B). These differences in processing time confirmed that two independent pathways mediated the excitation and inhibition of different DUM subpopulations.

Repetitive stimulation of sensory nerve 2C

During flight, afferents in nerve 2 are stimulated repeatedly by the rhythmical contractions of the flight muscles (Stevenson, 1997). We investigated the effect that such repetitive activation of nerve 2 afferents might have on DUM neurones and motor neurones by applying short trains of stimuli to nerve 2C. When nerve 2C was stimulated

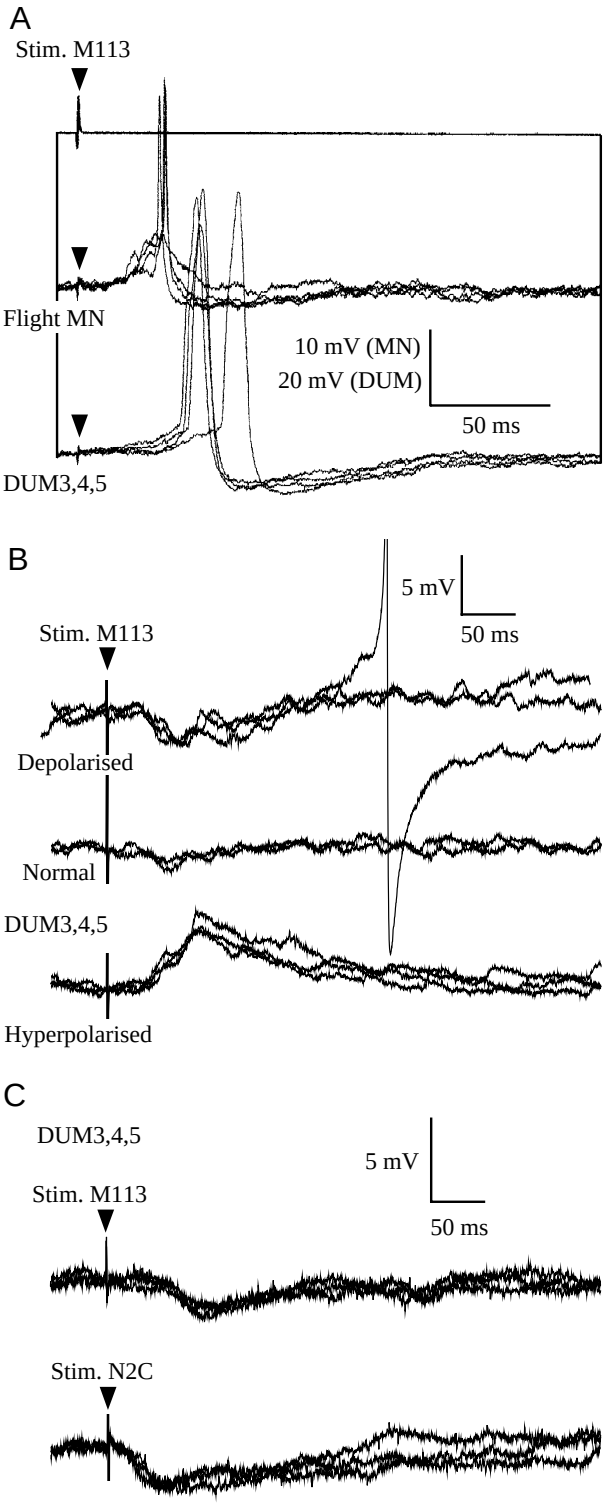

Fig. 4. Contractions of muscle 113 (M113) resulted in excitation of some DUM3,4,5 neurones and inhibition of others. (A) Stimulation of muscle 113 evoked excitatory postsynaptic potentials (EPSPs) and spikes in both a flight motor neurone (MN) and a DUM3,4,5 neurone. The flight motor neurone received EPSPs and spiked before the DUM3,4,5 neurone. Responses to four stimuli have been overlaid. (B) In another preparation, a DUM3,4,5 neurone received a small hyperpolarising input following stimulation of muscle 113 (middle traces). When the DUM3,4,5 neurone was depolarised by current injection (top traces), the amplitude of this hyperpolarising input increased, whereas hyperpolarising current injection caused reversal into a depolarising input (bottom traces). (C) Direct stimulation of nerve 2C evoked the same hyperpolarising response in a DUM3,4,5 neurone as stimulation of muscle 113, but at a shorter latency. For further details, see Fig. 1.

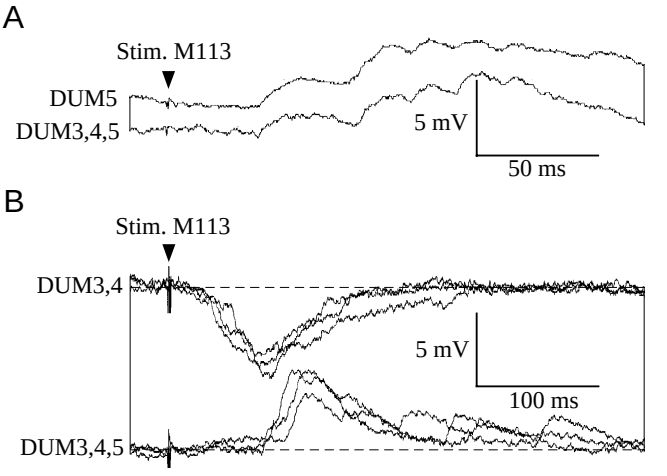

Fig. 5. Evidence for a common pathway mediating excitation of DUM5 and DUM3,4,5 neurones following stimulation of muscle 113 (M113), which is distinct from the pathway mediating inhibition of DUM3 and DUM3,4 neurones. (A) A DUM3,4,5 neurone and a DUM5 neurone received a similar pattern of excitatory postsynaptic potentials (EPSPs) with identical latencies following stimulation of muscle 113. (B) Contractions of muscle 113 often evoked inhibitory postsynaptic potentials (IPSPs) in DUM3 and DUM3,4 neurones at shorter latencies than the EPSPs in DUM5 and DUM3,4,5 neurones. In this example, a DUM3,4 neurone received IPSPs approximately 25 ms after stimulation of muscle 113, whilst the first EPSPs arrived in a DUM3,4,5 neurone 50 ms later. For further details, see Fig. 1.

repetitively at 10 Hz, summation of the individual IPSPs evoked in DUM3 and DUM3,4 neurones could be seen clearly, producing a prolonged hyperpolarisation that outlasted the period of stimulation by several hundred milliseconds (Fig. 6A). There was a simultaneous increase in the motor activity recorded in nerve 3A that, at least initially, appeared to be organised into bursts of spikes. At 20 Hz, the IPSPs following each stimulus fused to produce a rapid hyperpolarisation of both DUM neurones, reaching a steady-state hyperpolarisation of 2–3 mV after the first five or six stimuli (Fig. 6B). Excitation of motor neurones in nerve 3A was also much more rapid and prolonged when nerve 2C was stimulated at 20 Hz than at 10 Hz.

The effects of repetitive stimulation of nerve 2C on DUM5 and DUM3,4,5 neurones were less pronounced. Initially, these DUM neurones were excited, receiving EPSPs and producing spikes during the first few seconds of stimulation, but their responses adapted as stimulation continued (data not shown).

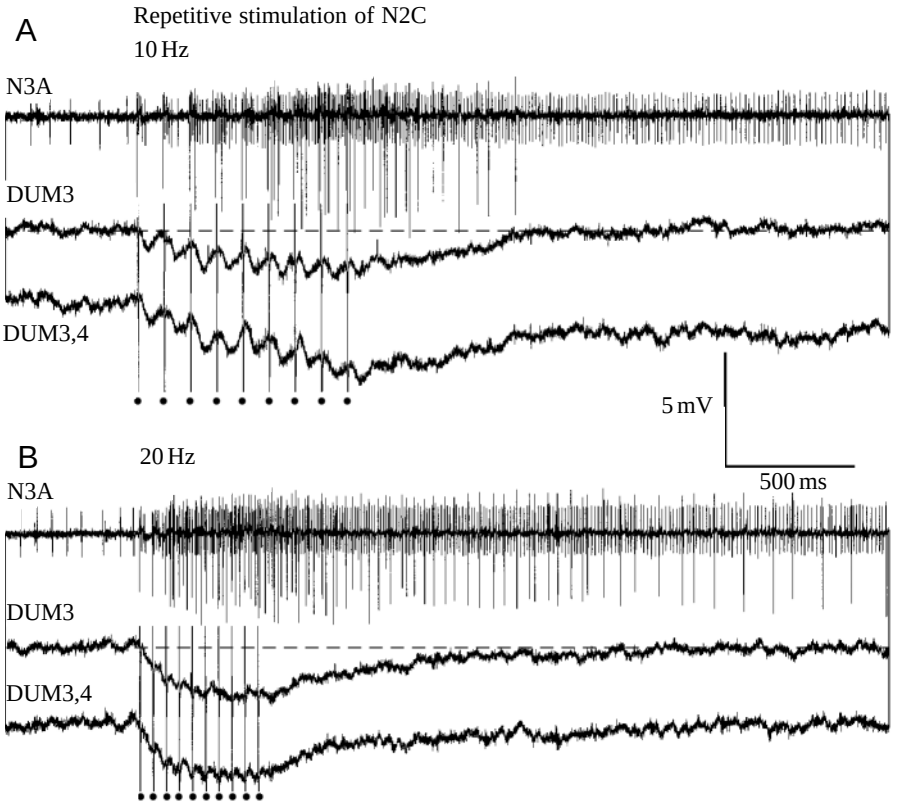

Fig. 6. Repetitive stimulation of nerve 2C (N2C) caused summating inhibitory postsynaptic potentials (IPSPs) in DUM3 and DUM3,4 neurones and prolonged excitation of motor neurones in nerve 3A. (A) At 10 Hz, summation of the individual IPSPs in the DUM3 and DUM3,4 neurones can be seen clearly. (B) At 20 Hz, summation of the IPSPs in the DUM3 and DUM3,4 neurones was much more rapid, reaching a steady-state hyperpolarisation of 2–3 mV after the first five or six stimuli. The increase in motor activity in nerve 3A is now more pronounced and prolonged. Neither neurone returns to its initial membrane potential for several hundred milliseconds following cessation of the stimulus. Dots indicate stimulus artefacts in the intracellular recordings. For further details, see Fig. 1.

Discussion

In this paper, we describe the synaptic inputs to efferent, octopaminergic DUM neurones that result from single, evoked twitch contractions of a representative flight muscle, the metathoracic tergosternal wing elevator muscle (M113). Care was taken to ensure that the only nervous connection between the ganglion and the periphery was *via* the purely sensory nerves 2C and 2D, which innervate three thoracic chordotonal organs (see Bräunig et al., 1981). Thus, as shown for the delayed excitation of flight motor neurones initiated by muscle twitches (Stevenson, 1997), the inputs to DUM neurones are mediated by internal proprioceptors responding to the mechanical movements produced by muscular contractions. Recordings of nerve 2C, which contains afferents of the myochordotonal organ and the posterior joint chordotonal organ, showed that these receptors produce a relatively consistent burst of action potentials involving numerous sensory units following each muscle twitch. The sensory response in nerve 2C preceded the synaptic responses in the DUM neurones, and direct stimulation of nerve 2C evoked corresponding synaptic inputs to DUM neurones, but at much shorter latencies (Figs 2, 3). Hence, proprioceptive information about muscle contractions is fed back not only to motor networks (Stevenson, 1997) but also to modulatory neurones, which are themselves renowned for modulating the contractile properties of insect muscle (Evans and O'Shea, 1977, 1978; Evans and Siegler, 1982; Whim and Evans, 1988; Stevenson and Meuser, 1997).

The long and variable delay from muscle stimulation until the arrival of synaptic potentials in DUM neurones and motor neurones indicates that the responses are mediated by polysynaptic pathways. Furthermore, the synaptic responses of DUM neurones and flight motor neurones continue for over 100 ms, far outlasting the afferent discharge (e.g. Figs 1B, 2B, 5), indicating a considerable degree of signal processing. Interneurones responsible for generating the flight motor pattern (Robertson and Pearson, 1985) are likely to be involved, since single twitch contractions of a dorso-ventral flight muscle can evoke alternate waves of excitation in wing depressor and elevator motor neurones, and stimulation of nerve 2 afferents can evoke flight motor activity (Stevenson, 1997).

The synaptic responses evoked by nerve 2C proprioceptors have different polarities in different subpopulations of DUM neurones (Figs 2–5). Taken together, our data suggest that there are at least two synaptic pathways from nerve 2 afferents to the DUM neurones (Fig. 7): an excitatory pathway to one group of DUM neurones, with a longer processing time than the pathways leading to excitation of motor neurones (Figs 1B, 4A, 7), and an inhibitory pathway to another group of DUM neurones (Figs 5B, 7). In some preparations, it was clear that the inhibitory pathway to DUM3 and DUM3,4 neurones had a shorter processing time than the excitatory pathway to DUM5 and DUM3,4,5 neurones (Fig. 5B), which is further evidence for the existence of two independent pathways controlling these subpopulations.

DUM neurones innervating leg muscles (DUM5 and DUM3,4,5 neurones) typically receive depolarising inputs

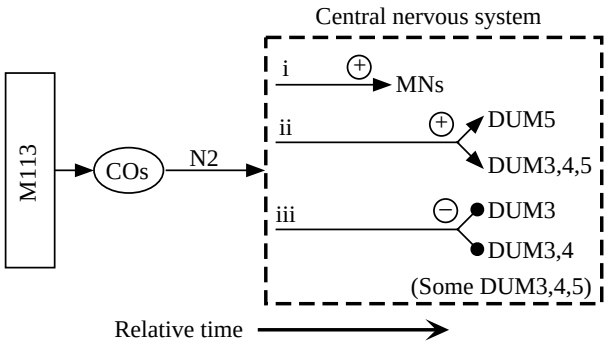

Fig. 7. Diagram of the relative time scales and different pathways mediating responses in motor neurones (MNs) and DUM neurones following twitch contractions of muscle 113 (M113). Because of the variation in latencies between preparations, time is presented in relative not absolute terms. Twitch contractions of muscle 113 excite thoracic chordotonal organs (COs) whose axons enter the central nervous system *via* nerve 2 (N2). The afferents in nerve 2 cause excitation of motor neurones and DUM5 and DUM3,4,5 neurones. The pathway exciting motor neurones (i) is shorter than the common pathway exciting DUM5 and DUM3,4,5 neurones (ii). Afferents in nerve 2 also cause inhibition of DUM3 and DUM3,4 neurones (and some DUM3,4,5 neurones) *via* a common pathway (iii).

which often lead to action potentials. Double intracellular recordings showed that matching synaptic potentials occur synchronously in DUM5- and DUM3,4,5-type neurones (Fig. 5A), which strongly suggests that they are driven *via* common presynaptic neurones. However, exceptions to this generalisation were observed in three of the 14 recorded DUM3,4,5 cells, which received hyperpolarising inputs (Fig. 4B,C). It is therefore possible that the three individual DUM3,4,5 neurones (Campbell et al., 1995), which differ with respect to some of their target muscles (Bräunig, 1997; Stevenson and Meuser, 1997), may also differ consistently with respect to their delayed synaptic inputs following muscle contractions. This argument has also been raised to explain the variation in the degree of coupling of synaptic potentials between different pairs of DUM5 and DUM3,4,5 neurones (Baudoux and Burrows, 1998). We therefore speculate that individual DUM3,4,5 neurones may be controlled by distinct pathways to achieve selective modulation of their specific targets. Further experimentation is required to verify this idea.

In contrast to the above, stimulation of the flight muscle or of nerve 2C caused delayed synaptic inhibition of all recorded DUM3 and DUM3,4 neurones (Fig. 3A,B), but had no clear effect on DUMDL (Fig. 3D). Double intracellular recordings indicate that these inhibitory inputs to DUM3 and DUM3,4 neurones also originate from a common presynaptic source (Fig. 6). The DUM3,4 neurones, of which there are six, innervate wing elevator and depressor muscles (Kutsch and Schneider, 1987), and the same probably applies to the five DUM3-type neurones (C. Duch, unpublished observations), whereas the single DUMDL neurone innervates the dorsal longitudinal indirect wing depressor muscle (Hoyle, 1978). Thus, the subpopulation of DUM neurones that innervate the major power muscles for flight are almost all inhibited *via* internal proprioceptors that respond to flight muscle contractions.

This distinction between DUM5- and DUM3,4,5-type neurones on the one hand and DUM3 and DUM3,4 neurones on the other corresponds to the recent categorisation of octopaminergic DUM neurones according to both their local (Baudoux and Burrows, 1998) and intersegmental (Duch et al., 1999) synaptic inputs. Furthermore, our finding that most DUM neurones innervating flight muscles are inhibited *via* nerve 2 afferents, whereas those innervating predominantly leg muscles are excited, mirrors the differential pattern of synaptic inputs that these octopaminergic neurones receive during flight motor activity (Duch and Pflüger, 1999). It therefore appears likely that the system of nerve 2 afferents will play a significant part in controlling the population of DUM neurones during flight behaviour. These afferents are rhythmically activated during flight (Stevenson, 1997), and stimulating them at a flight-like frequency hyperpolarised DUM3 and DUM3,4 neurones by more than 2 mV within a few cycles (Fig. 6). Taken together, current data suggest that DUM neurones innervating flight muscles receive phasic inhibition from three independent sources during flight: from the central rhythm-generating network, from the tegula exteroreceptor in the wing hinge (Duch and Pflüger, 1999) and from nerve-2-associated internal proprioceptors (this study). The excitation of DUM neurones innervating leg muscles (DUM5 and DUM3,4,5) was less robust during repetitive stimulation. Thus, although the nerve-2-associated internal proprioceptors would be expected to contribute to the excitation of these DUM neurones at the onset of flight, their role during sustained flight is less clear.

In conclusion, our finding that octopaminergic DUM neurones are differentially influenced by internal proprioceptors supports the emerging picture of different types of DUM neurone having specific functions in behaviour (Burrows and Pflüger, 1995; Stevenson and Meuser, 1997; Baudoux and Burrows, 1998; Baudoux et al., 1998; Duch et al., 1999; Duch and Pflüger, 1999), rather than simply underlying the general mechanisms of arousal (Hoyle and Dagan, 1978). In particular, the demonstration that DUM neurones innervating flight muscles are specifically inhibited *via* such afferents as a consequence of these muscles contracting is in line with the recent finding that these particular DUM neurones are inhibited during flight and lends additional weight to the call for a reconsideration of the role of octopamine and DUM neurones in insect flight (Duch and Pflüger, 1999). The inhibition of these DUM neurones during flight should result in a decrease in the octopamine levels of the flight muscles, and this could alter their metabolism in a manner appropriate to the requirements during flight (Duch and Pflüger, 1999).

Finally, our study points out the possibility that the octopaminergic modulation of insect muscle may be coordinated and regulated interactively with motor commands *via* feedback from internal proprioceptors responding to specific muscular contractions. An additional level of

integrative capacity in this system appears likely considering that chordotonal organs are themselves prone to modulation by octopamine (Büschges et al., 1993; Matheson, 1997). We conclude that internal proprioceptors may play a key role in regulating the peripheral release of octopamine *via* DUM neurones. Future studies must be devoted to unravelling the intricacies of the multiple interactions involved and determining their contributions to shaping motor performance.

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