The Journal of Experimental Biology 201, 3333–3338 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1581

LOCUST DORSAL UNPAIRED MEDIAN (DUM) NEURONES DIRECTLY INNERVATE AND MODULATE HINDLEG PROPRIOCEPTORS

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Accepted 14 September; published on WWW 17 November 1998

Summary

A subgroup of the large efferent octopaminergic dorsal unpaired median (DUM) neurones of the third thoracic ganglion, the DUM3,4,5 neurones, directly innervates the tendons of certain proprioceptors of the locust hindleg, the so-called strand receptors. The terminals of the DUM neurones occur in regions of the strands that also contain the dendrites of the mechanoreceptive sensory cells. Both stimulation of the DUM3,4,5 neurones and bath application of octopamine change the responses of strand receptor units to mechanical stimulation. In both situations, most single strand receptor units show an increased response to mechanical stimulation. Some units, however, decrease their sensitivity to mechanical stimulation in response to octopamine application or DUM neurone stimulation.

Key words: *Locusta migratoria*, proprioceptor, mechanoreceptor, neuromodulation, octopamine, biogenic amine, strand receptor.

Introduction

The most intensely studied neuromodulatory cells of insects are the dorsal unpaired median (DUM) neurones. Small groups of these large, bilaterally projecting efferent neurones are located in every ganglion of the ventral nerve cord. Several of these neurones have been shown to innervate skeletal or visceral muscles, where they modulate neuromuscular transmission by releasing octopamine (for reviews, see Agricola *et al.* 1988; Stevenson and Spörhase-Eichmann, 1995). In insects and crustaceans, octopamine has also been shown to modulate the responses of mechanoreceptors (Pasztor and Bush, 1989; Pasztor and Macmillan, 1990; Ramirez and Orchard, 1990; Ramirez *et al.* 1993; Matheson, 1997). Since there is no indication of any direct innervation of insect proprioceptors such as stretch receptors and chordotonal organs by octopaminergic neurones, a humoral action has to be postulated. It is known that octopamine acts as a neurohormone in insects, but neurohaemal release sites from octopaminergic neurones were not identified until one metathoracic DUM neurone of the locust (DUM1B) was shown to form extensive terminal networks on the surface of peripheral nerves (Bräunig *et al.* 1994). To determine whether all locust DUM neurones form such neurohaemal terminals in the periphery, we have recently studied the anatomy of other individual DUM cells, including the so-called DUM3,4,5 neurones of the third thoracic ganglion (DUM3,4,5 neurones were named after their axons in peripheral nerves 3, 4 and 5; Watson, 1984). We found that metathoracic DUM3,4,5 neurones innervate almost all skeletal muscles of the hindleg and form neurohaemal terminals only in a specific and restricted area of the peripheral nervous system (Bräunig, 1997).

Much to our surprise, during the course of this study we noticed that DUM3,4,5 neurones also directly innervate a special class of proprioceptive sense organ in the locust hindleg, the strand receptors. These receptors consist of connective tissue strands innervated by the dendrites of one or more mechanoreceptive cell able to sense elongation of the strand during leg joint movement. In contrast to the great majority of arthropod sensory neurones, the somata of strand receptor cells are located within the central nervous system (CNS) (Bräunig and Hustert, 1980, 1985; Bräunig, 1982, 1985; Pflüger and Burrows, 1987). As we will show here for one of these receptors, both stimulation of the DUM3,4,5 cells, which innervate the receptor strand, and bath application of octopamine modulate the responses of its sensory units to mechanical stimuli.

Materials and methods

The methods for cobalt staining the peripheral ramifications of DUM neurones have been described in detail in previous publications (Bräunig *et al.* 1994; Bräunig, 1997). For detailed descriptions of the location and innervation of strand receptors, see Bräunig *et al.* (1981) and Bräunig (1982, 1985). For physiological experiments, we chose the larger of the two coxo-trochanteral strand receptors (cxtrSR1) since, of all these sense organs, it is the easiest to stimulate and to record from.

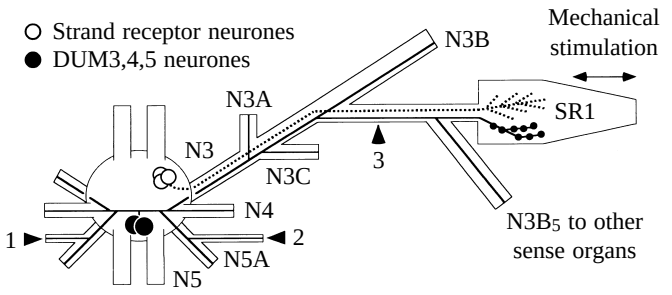


Fig. 1. Schematic representation of the experimental arrangement that also shows the morphology of the DUM3,4,5 neurones (solid lines) and the sensory cells (dotted lines) of coxo-trochanteral strand receptor 1 (SR1). The DUM neurones were electrically stimulated at site 1 (contralateral nerve 5A; N5A), while a recording at site 2 (ipsilateral nerve 5A) served as a monitor for suprathreshold stimulation. Strand receptor units were recorded at site 3 (ipsilateral nerve $3B_5$).

A schematic drawing of the experimental arrangement is provided in Fig. 1. The receptor strand of cxtrSR1 was exposed and fixed to a piezoelectric stimulator using a small droplet of Histoacryl (Braun) adhesive. Sensory units were recorded from the receptor nerve (nerve $3B_5$) using small metal hook or suction electrodes.

Mechanical stimuli were adjusted such that each sinusoidal or ramp stimulus was just above threshold, i.e. causing only one impulse per unit and stimulus cycle in one or two of the most sensitive units. Stimulus presentation rate ranged between approximately 0.5 and 1 Hz. At such rates of repetition, strand receptor units respond consistently to each stimulus for long periods, in exceptional cases for up to 2 h. For evaluation, intervals of 60 s of continuous stimulation were chosen for the calculation of mean impulse frequencies. The number of events per 60 s interval accordingly varies between different experiments.

The DUM3,4,5 neurones were activated antidromically by stimulation of nerve 5A on the side contralateral to the strand receptor recording. Since action potentials from DUM3,4,5 neurones are difficult to detect in the recording from the strand receptor nerve, a control recording of the ipsilateral nerve 5A served as a monitor for suprathreshold DUM cell stimulation. The DUM neurones were activated for 1–4 s at 10–15 Hz. These parameters are within the range of activity bursts of DUM3,4,5 neurones observed during natural behaviour

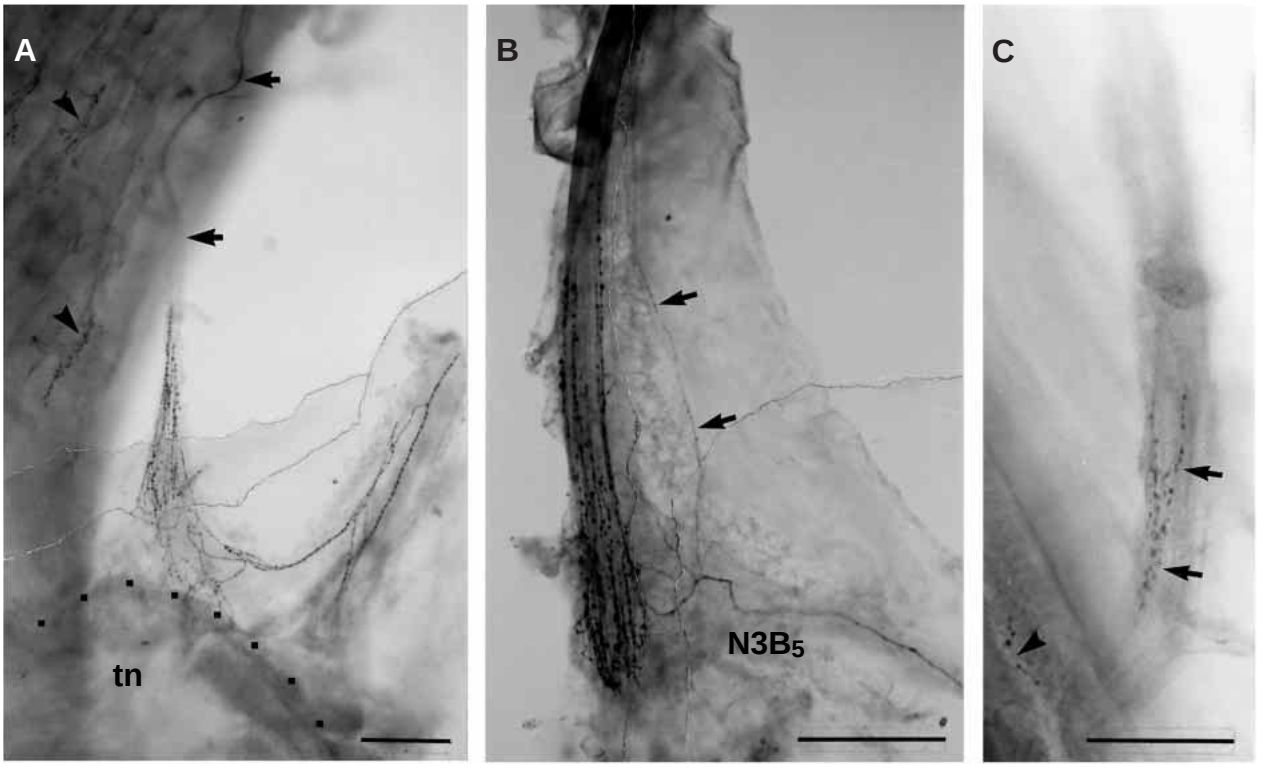


Fig. 2. Peripheral ramifications of a DUM3,4,5 neurone after intracellular cobalt staining. (A) Terminal ramifications in the strand of the pleuro-trochantinal strand receptor located at the subcoxal joint (distal to the top). This receptor is attached to the pleural wall distally and to the trochantin (tn; rim indicated by dots) proximally. The distal part of the receptor strand, which is devoid of sensory dendrites, is indicated by arrows. Note that DUM neurone ramifications are located in the proximal region only. Note also the DUM neurone terminals in nearby muscle 125 (arrowheads). (B) DUM neurone terminals in the strand of the larger coxo-trochanteral strand receptor originating from an axon collateral in nerve $3B_5$ ($N3B_5$). Note the additional collateral in the distal part of nerve $3B_5$ (arrows). (C) DUM neurone ramifications in the strand of the smaller coxo-trochanteral strand receptor (arrows) and trochanteral levator muscle 132 (arrowhead). Scale bars, 100 μm in A and B, 50 μm in C.

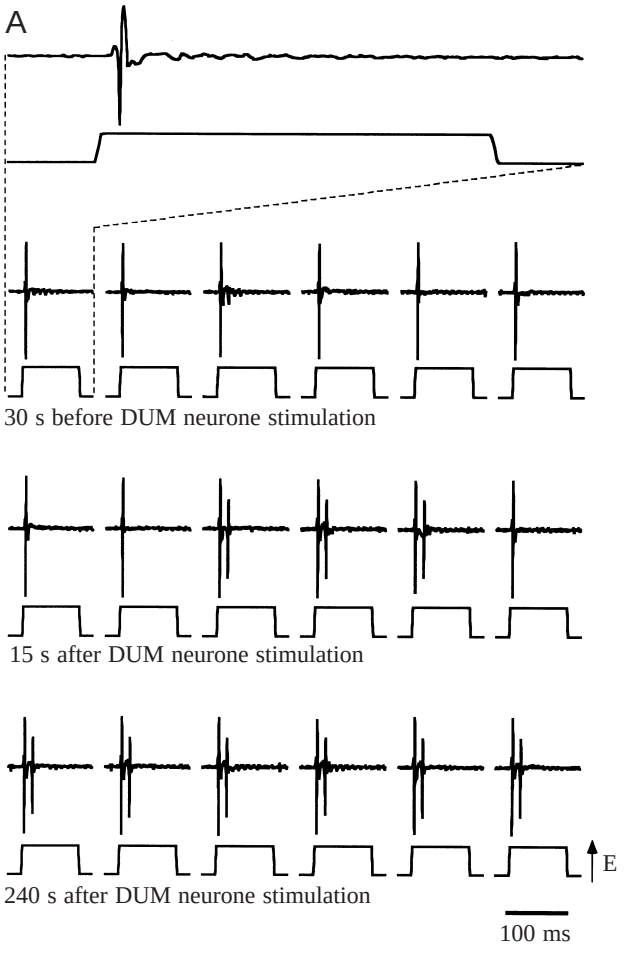
patterns (Burrows and Pflüger, 1995). Since cxtrSR1 is embedded in the musculature of the coxa and since DUM3,4,5 neurones also innervate all skeletal muscles of the coxa *via* nerves $3B_6$, 4A and 5A (Bräunig, 1982, 1997), these nerves were cut to prevent indirect influences on the sense organ of octopamine released from terminals within nearby muscles.

In a second set of experiments, strand receptor responses were tested during bath application of octopamine. Stock solutions were prepared by dissolving DL-octopamine (Sigma) in locust saline. Appropriate volumes of freshly prepared stock solutions were added to and mixed with the saline of the bath to yield final concentrations ranging between 10^{-7} and 10^{-3} mol l^{-1}.

Results

Innervation of strand receptors

The peripheral targets of two of the metathoracic DUM3,4,5 neurones have previously been described in detail (Bräunig, 1997). Like all other thoracic DUM neurones that have been studied, these two DUM3,4,5 neurones innervate skeletal muscles (Fig. 2A,C); in fact, they innervate all leg muscles except for the tibial extensor, the retractor unguis and the tarsal muscles. In addition, both neurones innervate strand receptors, but no other proprioceptive sense organs in the thoracic cavity or the leg. We consistently observed direct innervation of the tendons of the pleuro-trochantinal strand receptor located at the subcoxal joint (pltnSR, Fig. 2A) and the larger of the two strand receptors associated with the coxo-trochanteral joint (cxtrSR1; Fig. 2B). Innervation of the second coxo-trochanteral strand receptor (cxtrSR2; Fig. 2C) was obvious in only two of nine preparations, but this is probably a result of technical limitations. The DUM3,4,5 neurones consistently send axon collaterals into nerve $3B_5$a innervating cxtrSR1 and also into the distal part of nerve $3B_5$ (Figs 1, 2B), which innervates other proprioceptors including cxtrSR2 and the femoro-tibial strand receptor (fetiSR). Staining in these latter



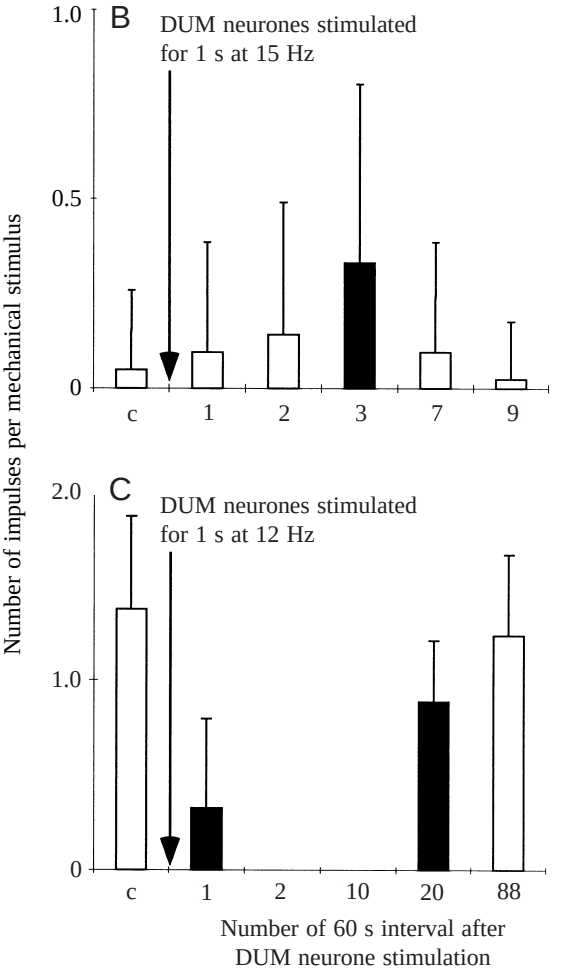


Fig. 3. Changes in the mechanoreceptive responses of strand receptor units after stimulation of DUM3,4,5 neurones. (A) DUM neurone activation causes the recruitment of a second unit during elongation (E↑) of the strand. The inset shows the first stimulation event on an expanded time scale. Strand receptor units may show an increase (B) or a decrease (C) in their response rates. Arrows indicate the 1 s interval during which the DUM3,4,5 neurones were activated. Filled columns represent values that differ significantly from controls (c; sign test, $P<0.01$). The unit shown in B became active after stimulus parameter adjustment using another unit, which is why it starts with far fewer than one impulse per stimulus. Values are means + 1S.D. ($N=42$).

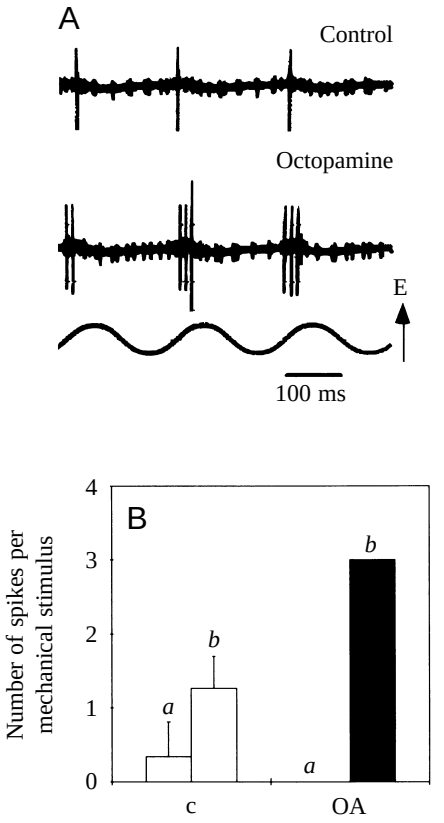


Fig. 4. Changes in the mechanoreceptive responses of strand receptor units after bath application of octopamine. (A) During sinusoidal elongation (E↑), adding 10^{-5} mol l^{-1} octopamine (OA) to the bath causes an increase in the response of one sensory unit and the recruitment of a second one. (B) Octopamine may have different effects on individual sensory units in the same receptor. In the experiment shown, 2 min after bath application of 10^{-7} mol l^{-1} octopamine, the response of unit *a* vanished while the response of unit *b* increased significantly (N=27 for each column; filled columns represent values that differ significantly from controls, c; sign test, P<0.01). Values are means + 1S.D.

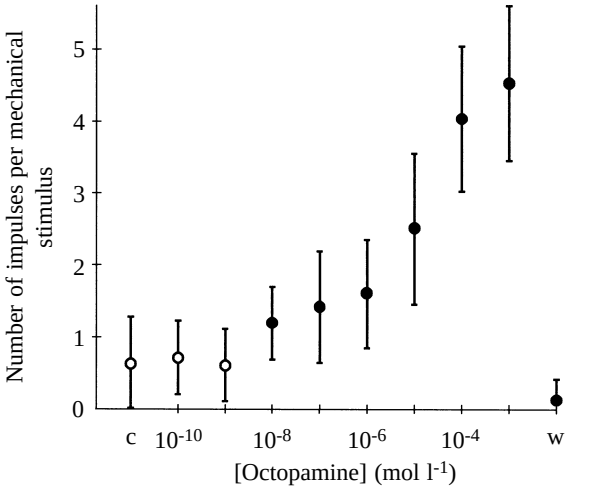


Fig. 5. Concentration-dependent increase in impulse frequency of a strand receptor unit during bath application of octopamine. The octopamine concentration in the bath was raised in a step-wise fashion, and the number of impulses per mechanical stimulus was measured for 54 cycles (60 s) starting 15 s after changing the solution. Values are means ± 1S.D. Filled circles represent values significantly different from the control (c, control; sign test, P<0.01). Washing (w) indicates that the effect is reversible, although the number of impulses was significantly lower than before octopamine application.

collaterals faded in more distal coxal regions in most preparations. For this reason, it remains an open question whether the two DUM cells studied also innervate fetiSR located in the distal femoral region.

Modulation of strand receptor responses by DUM neurones

In three out of seven experiments, stimulation of the DUM3,4,5 neurones changed the response of strand receptor units to mechanical stimuli (Fig. 3A–C). Stimulation of the DUM neurones caused a transiently increased response of single units and also the recruitment of additional units which had previously not responded to the same mechanical stimulus. The latency between the end of DUM neurone stimulation and the onset of effects varied between a few seconds (Fig. 3A) and several minutes (Fig. 3B). The effects lasted for several minutes before responses returned to baseline levels. These results are in accordance with previous observations on the behaviour of mechanoreceptive units of other insect proprioceptors after bath application of octopamine (Ramirez and Orchard, 1990; Ramirez *et al*. 1993; Matheson, 1997). Surprisingly, however, in one of our experiments, a single strand receptor unit behaved in just the opposite fashion: DUM cell stimulation caused its activity to cease (Fig. 3C).

Bath application of octopamine

Since the success rate of the experiments described above was rather low (for possible reasons, see Discussion), we also studied strand receptor responses during bath application of octopamine. In 6 out of 12 experiments, octopamine caused changes in the strand receptor unit responses to mechanical stimulation. Four experiments showed an increase in single-unit responses and recruitment of additional units (Fig. 4A). In one experiment, a unit decreased its response. Most interesting was an additional preparation which showed an increase in the response in one unit and a decrease in the response in a second unit in the same experiment (Fig. 4B). Octopamine effects could be reliably elicited with bath concentrations between 10^{-7} and 10^{-6} mol l^{-1}. A dose–response curve (Fig. 5) indicates threshold concentrations of 10^{-8} mol l^{-1} and no further increases of responses at concentrations higher than 10^{-3} mol l^{-1}.

Discussion

Our results are the first demonstration of a direct innervation of proprioceptive sense organs by neuromodulatory neurones

in insects. Since DUM3,4,5 neurones also innervate numerous leg muscles and form neurohaemal ramifications within a region of the peripheral nervous system (Bräunig, 1997), it is likely that these neurones represent a system performing several tasks simultaneously: modulation of neuromuscular transmission, modulation of a group of mechanoreceptors and release of octopamine into the haemolymph.

So far, there is no indication of a direct innervation of other proprioceptors such as stretch receptors and chordotonal organs by octopaminergic DUM neurones. For example, numerous chordotonal organs are innervated by nerve 2 (Bräunig *et al.* 1981), but there is no indication of any DUM cell projecting into this nerve. The response of the wing hinge stretch receptor is modulated by octopamine and by nonspecific activation of numerous DUM neurones (Ramirez and Orchard, 1990), but a recent study has clearly shown that neither DUM1 neurone, the only candidates for a direct innervation of this sense organ, projects into the stretch receptor nerve, which also innervates a chordotonal organ (Bräunig, 1997). Neither do these two DUM1 neurones project into the sensory nerve of the wing (nerve 1C), which also innervates chordotonal organs (Kutsch *et al.* 1980). It remains an open question whether other proprioceptors known to be modulated by octopamine, such as the femoral chordotonal organs (Ramirez *et al.* 1993; Matheson, 1997), are innervated by DUM cells. Recording from the nerve supplying the femoral chordotonal organ of the locust hindleg while its contralateral counterpart was being stimulated did not yield any indication of an innervation of the organ by DUM cells (P. Bräunig, unpublished results).

For these reasons, it is interesting to ask why the strand receptors might be the only proprioceptors directly innervated by DUM cells. In addition to the central location of the somata of their sensory cells, this direct innervation is another unusual feature of these sense organs. Both features hint at the possibility that phylogenetically this type of sense organ might derive from modified skeletal muscles. However, such speculation was not supported by further study of the innervation of the strand receptors (P. Bräunig, unpublished results). A feature common to all skeletal muscles of locust legs is their innervation by common inhibitory neurones ($CI_{1–3}$; Hale and Burrows, 1985). However, staining the peripheral branches of CI_1, the only CI neurone with axon collaterals in nerve 3B, which is the only nerve supplying strand receptors, clearly showed that the strand receptors are not innervated by this neurone.

We observed modulation of strand receptor unit responses in only approximately 50 % of our preparations. In experiments involving stimulation of the DUM neurones, possible explanations for this low success rate could be spike failures in the periphery at branching points of the neurones or conduction blocks caused by the recording electrode. Control experiments, however, made this explanation very unlikely: with an electrode attached to nerve $3B_5$ (recording site 3 in Fig. 1), we were able to record DUM neurone action potentials from the distal branch of this nerve (see also Fig. 1) which innervates sense organs located more distally (data not shown).

Spike failures and conduction blocks would also fail to explain the 50 % of experiments in which there was no response to octopamine during bath applications. We attribute such failures to the unknown pre-experimental history of our preparations. In approximately half the experiments, the strand receptors may have already been exposed to octopamine released by DUM3,4,5 neurones and/or octopamine circulating in the haemolymph prior to dissection. This might have caused saturation and/or inactivation of octopamine receptors prior to the experiments, causing unresponsiveness of sensory units. Such desensitization of responses has been observed previously in other insect systems. The wing hinge stretch receptor shows desensitization of its response during repeated injections of octopamine into the haemolymph (Ramirez and Orchard, 1990). Matheson (1997) observed that octopamine only transiently changed the responses of mechanosensory units of the femoral chordotonal organ of the locust hindleg. Desensitization of octopamine receptors has also been observed in haemocytes (Orr and Hollingworth, 1990). Octopamine receptors of *Drosophila melanogaster*, cloned and expressed in vertebrate cells, also desensitize during exposure to octopamine (Reale *et al.* 1997; Robb *et al.* 1994). Partial desensitization of sensory units might also be the cause of relatively long latencies in responses in some preparations (e.g. Fig. 3B).

Apart from these difficulties, both sets of experiments show that single units may either increase or decrease their response to mechanical stimulation after activation of octopaminergic neurones or bath application of octopamine. One experiment using bath application showed one unit increasing its response while the response of a second unit decreased (Fig. 4B). This experiment shows that a decrease in the response of some units is a genuine phenomenon and is not due to fatigue of the preparation. This is also illustrated by the return to baseline response levels of such units (Fig. 3C). It would be interesting to know whether it is always the same unit(s) that show(s) a decrease in response. In other insect proprioceptors, different physiological types of mechanoreceptive unit react differently to octopamine (Ramirez *et al.* 1993; Matheson, 1997). Unfortunately, the units of the strand receptor investigated here have similar physiological properties and also often similar amplitudes in extracellular recordings. This prevents the unequivocal identification of individual units in different preparations. This difficulty could, in principle, be overcome by investigating strand receptors that have only a single sensory neurone, such as the second coxo-trochanteral and the femoro-tibial strand receptors (Bräunig, 1982, 1985). Since the second coxal receptor is very small and difficult to record from, we are currently trying to determine whether the femoro-tibial strand receptor is also innervated by DUM neurones, since this might provide a preparation in which such tests would be possible.

The present study was supported by DFG grants Br 882/3-1 and Br 882/3-2. We are grateful to S. White for kindly correcting the English text, to B. Schmitz for sharing

equipment with our group and to G. F. Rast for constructive discussions. We also wish to thank two anonymous referees for their constructive criticism.

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