

SYNAPTIC ACTIVATION OF EFFERENT NEUROMODULATORY NEURONES IN THE LOCUST *SCHISTOCERCA GREGARIA*

SYLVIE BAUDOUX* AND MALCOLM BURROWS

Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK

*e-mail: shb22@cus.cam.ac.uk

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Summary

The segmental ganglia of the locust contain efferent neuromodulatory neurones with cell bodies at the dorsal midline and axons that supply muscles and other tissue on both sides of the body. These are the dorsal unpaired median (DUM) neurones. Intracellular recordings were made from pairs of known metathoracic efferent DUM neurones in locusts in which all nerves were intact and in isolated metathoracic ganglia. The 19 metathoracic, efferent DUM neurones were identified according to the nerve roots through which their axons emerge from the ganglion. The synaptic potentials in these DUM neurones have been analysed to investigate how these neurones are activated and how their spikes are controlled.

The degree of correlation between the synaptic potentials in particular pairs of neurones was quantified using a correlation analysis. This allowed the population of DUM neurones to be divided into three subsets that also map onto an anatomical grouping based on the distribution of their axons in the lateral nerves: (i) DUM1 neurones (DUMDL and DUM1b); (ii) DUM3 and DUM3,4 neurones; and (iii) DUM3,4,5, DUM5b neurones and DUMETi. Individual neurones within each subset showed strong correlations between their synaptic potentials, in both intact locusts and

isolated ganglia, and tended to spike at the same time. Neurones in different subsets had few synaptic potentials in common and tended to spike independently. The persistence of common synaptic potentials in neurones of the three subsets in isolated ganglia indicates that they are derived from neurones within the metathoracic ganglion.

The DUM neurones that had many common synaptic potentials in a quiescent locust responded in similar ways to mechanosensory stimulation of different parts of the body. DUM3,4,5 and DUM5 neurones gave the clearest and most consistent responses to stimulation of mechanoreceptors on either hind leg. DUM3 and DUM3,4 neurones responded variably, but usually with a hyperpolarisation. DUM1 neurones were rarely excited by mechanosensory stimuli but, like the preceding group, their responses were dependent upon whether the locust was moving its legs.

These results lend further support to the idea that there is a subdivision of action amongst this population of DUM neurones, with those supplying the same targets being driven by the same presynaptic local neurones.

Key words: neuromodulation, motor programme, DUM neurone, octopamine, insect, locust, *Schistocerca gregaria*.

Introduction

The actions of neuromodulatory neurones can alter the output of the neural circuits that generate the many different patterns of movement used by animals during their normal behaviour. Their particular modulators can be released at specific sites within the central or peripheral nervous system, or into the circulatory system to exert a more widespread effect. The modulators can act on many components of the neural and motor machinery, including sensory neurones (Pasztor and Bush, 1989; Pasztor and Macmillan, 1990; Ramirez and Orchard, 1990), interneurones (Ramirez and Pearson, 1991a,b), central pattern-generating networks (Chiel *et al.* 1990; Katz and Harris-Warrick, 1990; Harris-Warrick and Marder, 1991; Harris-Warrick *et al.* 1992; Stevenson and Kutsch, 1988), motor neurones and muscle fibres (Evans and O'Shea, 1977, 1978; O'Shea and Evans, 1979). The

neuromodulatory neurones should be activated in such a way that they release their modulators at appropriate times during the performance of a particular motor pattern. Little is known about the pathways that drive neuromodulatory neurones in comparison with what is known about their targets and the effects of their modulators. To obtain some insight into these mechanisms, we have analysed the occurrence of synaptic potentials in a particular group of neuromodulatory neurones, the dorsal unpaired median (DUM) neurones in an insect.

The cell bodies of the DUM neurones are found at the dorsal midline of each segmental ganglion and were first described in locusts (Plotnikova, 1969) and later in other insects (Casaday and Camhi, 1976; Christensen and Carlson, 1982; Crossman *et al.* 1971). They are of three types: (1) efferent neurones with axons that supply targets on both sides of the body and which

are the subject of this paper; (2) local neurones with branches restricted to one ganglion (Thompson and Siegler, 1991); and (3) intersegmental neurones with axons projecting to other ganglia (Thompson and Siegler, 1991). The efferent neurones show octopamine-like immunoreactivity (Stevenson *et al.* 1992) and some are known to contain (Evans and O'Shea, 1978) and release (Morton and Evans, 1984) octopamine. They modulate neurotransmitter release from the terminals of motor neurones onto muscle fibres, and in skeletal muscles they reduce basal tension and increase the force of twitch contractions and relaxation rates (Evans and O'Shea, 1977, 1978; Evans and Siegler, 1982; Malamud *et al.* 1988; Whim and Evans, 1988). In visceral muscles, they inhibit both myogenic and neurogenic contractions (Kalogianni and Pflüger, 1992; Orchard and Lange, 1985). Octopamine also modulates the responses of leg proprioceptors in locusts (Matheson, 1997; Ramirez *et al.* 1993; Ramirez and Orchard, 1990), but it is not known whether these effects are mediated by the DUM neurones. Similarly, injection of octopamine into specific areas of the neuropile can produce the motor pattern for flying (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987, 1988). Although efferent DUM neurones have extensive branches within the central nervous system, few release sites have been demonstrated (Watson, 1984) so it is not known whether they mediate these central effects.

The metathoracic ganglion contains 19 efferent DUM neurones (Campbell *et al.* 1995; Stevenson and Spörhase-Eichmann, 1995) that were initially thought to act collectively as part of a general arousal mechanism. In the locust, they are active at the onset of flight (Ramirez and Orchard, 1990), during leg movements (Hoyle and Dagan, 1978) and during walking in crickets (Gras *et al.* 1990). Recent studies, however, have suggested that they should be regarded as individuals that are activated selectively during specific motor patterns. For example, a subset of these neurones spikes in repeatable patterns during the precise motor pattern that underlies jumping and kicking (Burrows and Pflüger, 1995). Similarly, the spikes of DUM neurones that supply muscles in the hind legs are coupled to a rhythmical motor pattern that is expressed in leg motor neurones of an isolated ganglion treated with pilocarpine (Baudoux *et al.* 1998) and that shows many characteristics of the normal walking motor pattern (Ryckebusch and Laurent, 1993, 1994). A more detailed analysis of their activity at the start of flight and during fictive flight (C. Duch and H. J. Pflüger, in preparation) points to further individual actions by showing that those supplying flight muscles are inhibited while the remaining neurones may be excited by the stimuli that induce and sustain flight. A first step in understanding how these neurones are recruited in specific functional groups is to identify those neurones that share common synaptic potentials, indicating that they are controlled by the same presynaptic neurones. We have therefore analysed the correlation between synaptic potentials in pairs of efferent DUM neurones within the metathoracic ganglion, and the correlation between the synaptic potentials or spikes evoked by sensory stimuli, in intact locusts and in

isolated ganglia. We show that different subgroups of DUM neurones have many synaptic potentials in common and are therefore excited or inhibited by the same presynaptic local interneurones. A parallel study by Duch *et al.* (1998) has analysed the intersegmental pathways that activate efferent DUM neurones in adjacent thoracic ganglia.

Materials and methods

Mature adult locusts, *Schistocerca gregaria* (Forskål), of either sex were taken from our crowded laboratory culture. Two types of preparation were used. First, an 'intact' preparation in which the locust was mounted dorsal surface uppermost in Plasticine with both hind legs rotated so that movements of the tibia and tarsi were unimpeded. The metathoracic ganglion was exposed by a longitudinal dorsal incision in the thorax followed by removal of the gut and overlying thoracic muscles. It was then stabilised on a wax-coated stainless-steel platform, and the thorax was bathed in a continuous flow of saline (Usherwood and Grundfest, 1965) at 20–24 °C. In the second 'isolated' preparation, the metathoracic ganglion was dissected from the locust with the longitudinal tracheae still attached. It was then pinned dorsal side uppermost in a Sylgard-lined chamber filled with the same locust saline. The tracheae were opened at the surface of the saline to maintain an air supply to neurones within the ganglion.

In both preparations, the sheath of the metathoracic ganglion was treated with protease (Sigma type XIV) to facilitate penetration of the somata of DUM neurones with glass microelectrodes filled with 2 mol l⁻¹ potassium acetate or 6 % hexamine cobaltic chloride (Brogan and Pitman, 1981). Intracellular recordings were made simultaneously from the somata of two DUM neurones so that the patterns of synaptic potentials or spike outputs in one neurone could be correlated with those in the other neurone. DUM neurones were initially recognised by the long duration, large amplitude and prominent after-hyperpolarisation of their spikes as recorded in their somata. They were characterised further according to the nerves in which their axons project bilaterally and by electrical stimulation of particular lateral nerves to evoke antidromic spikes. Characterisation was completed by intracellular injection of cobalt followed by silver intensification (Bacon and Altman, 1977) to reveal the dendritic branches and the pattern of axonal branches in particular lateral nerves. Three DUM neurones could be identified as individuals using morphological criteria alone: DUMDL (the DUM neurone supplying the dorsal longitudinal muscles in the thorax), DUM1b and DUMETi (the DUM neurone supplying the extensor tibiae muscles in both hind legs). The remaining neurones could only be named according to the branching patterns of their axons in different lateral nerves (see Table 1).

The analyses are based on recordings from 78 pairs of DUM neurones identified by physiological tests and subsequent staining. All recordings were stored on a Racal seven-channel

Table 1. *Efferent DUM neurones in the metathoracic ganglion*

Name	Number of neurones	Nerve	Known muscle and other targets
DUMDL	1	1	Dorsal longitudinal muscle (112) (Hoyle and Dagan, 1978; Whim and Evans, 1988)
DUM1b	1	1	Mesothoracic spiracle closer, thoracic ventral longitudinal muscles, salivary gland, median nerve neurohaemal organ (Bräunig <i>et al.</i> 1994)
DUM3	5	3	?
DUM3,4	6	3, 4	Muscles 113, 119, 129 (Kutsch and Schneider, 1987)
DUM3,4,5	3	3, 4, 5	Nerve 3: muscles 121, 125, 126, 130, 131, 133 Nerve 4: muscles 114, 115, 120, 122, 123, 124, 132 Nerve 5: muscles 133a,d, 136 (Bräunig, 1997; Stevenson and Meuser, 1997)
DUMETi	1	5	Extensor tibiae muscle (135) (Hoyle, 1978)
DUM5b	2	5	?

The number of DUM neurones is based on Campbell *et al.* (1995).
Muscle numbers are based on Snodgrass (1929).

FM tape recorder for later analysis with CED Spike2 software (Cambridge Electronic Design, Cambridge, UK) and printed on a laser printer.

Patterns of synaptic potentials and spike outputs were studied in both quiescent intact locusts and isolated metathoracic ganglia. Quiescent locusts were defined as those that were not moving their legs; active locusts were those that were moving one or more of their legs. In the intact locust, synaptic potentials were also evoked by sensory stimulation of specific parts of the body or by electrical stimulation of particular nerves. The sensory stimulation included tactile stimulation with a fine paintbrush of exteroceptors on the legs, head, antennae, wings and abdomen, imposed movements of the joints of either hind leg, and wind stimulation of hairs on the head and on the cerci. To localise the source of the synaptic inputs, particular nerves or connectives were cut.

To analyse the patterns of synaptic potentials in two DUM neurones and to quantify the potentials that might be common to both neurones, the following correlation technique was developed by P. C. Williams and implemented using 'Labview' software (National Instruments Corporation, Austin, Texas, USA). The technique was designed to be sensitive to correlations in synaptic potentials while remaining essentially unaffected by spikes, which can strongly bias more conventional correlation techniques. Direct current offsets were first subtracted to reduce transient effects in the subsequent filtering stages. Data records were cleaned of power mains artefacts using a line-synchronous 'bin and subtract' algorithm, which strongly attenuated the 50 Hz mains carrier frequency and its harmonics.

For each pair of neurones from the same locust, at least six separate sequences each 10 s long were digitised at 5 kHz and bandpass-filtered between 8 and 100 Hz. The recordings were then fed to the correlation routine in 200 μ s bins. If the resulting product of the two recordings at time '*i*' was greater than zero, then the two must share the same sign at that time, and the number +1 was assigned to a corresponding index '*i*'

of an array, indicating that the two recordings were instantaneously correlated. If the product at time '*i*' was negative, then the two recordings must have opposite signs, and -1 was written to a new array, indicating an anti-correlation. Finally, the cumulative sum over all previous bins (an integrated sign correlation) was plotted against the normalised duration of the sample. The slope of this plot was the correlation coefficient, *C*:

$$C(i) = \frac{n(i) - n'(i)}{n(i) + n'(i)},$$

where *n*(*i*) represents the number of points up to time '*i*' where both recordings were instantaneously correlated (same sign) and *n'*(*i*) represents the number of points where the recordings were instantaneously anti-correlated (opposite sign). The denominator *n*+*n'* is the total number of points (*N*) in the product, so that this plot had a slope equal to the 'fractional' instantaneous cross-correlation coefficient between the two recordings.

The following tests were performed to check this technique. First, if two recordings were identical, then *n*=*N* and *n'*=0, so the fractional correlation should be unity. This was checked by analysing recordings made with two electrodes in the soma of the same DUM neurone and by analysing synthetic data. Second, if two recordings were independent, then, on average, *n*=*n'*=*N*/2, so the fractional correlation should be [(*N*/2)-(*N*/2)]/*N* or zero. This check was made by analysing recordings obtained from identified DUM neurones in two different locusts and again by analysing synthetic data. Recordings in which signals are correlated, but with signals of opposite sign, will give a correlation of -1, implying maximum anti-correlation, whilst two fully independent recordings will yield a correlation of 0 (see Fig. 5 for an example of these analytical procedures). Third, one recording of a pair was shifted in time relative to the other by random amounts, a procedure that generated decreases in the correlation measures with increasing temporal shift. Fourth, to test for artefactual correlations from the mains line frequency,

one data set of a pair was shifted by 20 ms (1/50 Hz) relative to the other. No correlations synchronous with the mains frequency were unmasked by this test. Finally, to ensure that only correlations from an unmasked signal were analysed, correlated artefacts must be removed, because this technique specifically ignored correlations between the amplitudes of the signals in the two neurones and tested only the sign of their product. If artefacts are correlated in the two neurones and of a magnitude comparable with that of the synaptic potentials, the correlation will be more positive. Conversely, uncorrelated noise in the signal will bias the calculated correlation coefficient towards zero.

Results

Spontaneous synaptic activity

In quiescent intact locusts and in isolated metathoracic ganglia, efferent DUM neurones generated a continuous stream of synaptic potentials and spiked sporadically at low frequencies. At a particular time, individual DUM neurones differed in the frequency and pattern of their synaptic potentials, with either depolarising or hyperpolarising potentials predominating in some, whilst a mixture of the two was more prevalent in others. This pattern of synaptic potentials persisted for long periods, changing when the intact locust started to move or when a particular sensory stimulus was applied. In many of these neurones, depolarising potentials led to spikes, indicating that they were excitatory postsynaptic potentials (EPSPs), and hyperpolarising potentials reduced the frequency of tonic spiking, indicating that they were inhibitory postsynaptic potentials (IPSPs). In recordings from particular pairs of DUM neurones, there were differences in the correlation between their spontaneous synaptic potentials; in some pairs, the similarity of their potentials was obvious, but in others no correlation between their potentials was apparent.

Neurones with many common synaptic potentials

Particular pairs of DUM neurones had many synaptic potentials in common (Fig. 1A–C). This pattern of common synaptic driving always occurred in particular pairings of identified DUM neurones and was consistent from locust to locust. For example, in one locust, two DUM3,4 neurones (Fig. 1A) had very similar fluctuations in membrane potential in which both depolarising and hyperpolarising potentials were common to both. In a second locust, two DUM3 neurones showed prominent depolarising potentials of low frequency, and these were matched in both neurones (Fig. 1B). Occasionally one of these EPSPs led to a spike in one neurone but not in the other. Presumably variations in the spike thresholds in the two neurones, or the occurrence of synaptic potentials that were not shared, can explain why common synaptic potentials can sometimes lead to independent spiking in the two neurones. In other recordings from these neurones, common hyperpolarising potentials were also observed. In a third locust, two DUM3,4,5 neurones had a much higher frequency of common depolarising and hyperpolarising synaptic potentials (Fig. 1C). In a fourth

locust, a similar high frequency of depolarising and hyperpolarising potentials occurred in recordings from a DUM5b neurone and DUMETi (Fig. 1D).

When connectives anterior or posterior to the metathoracic ganglion or lateral nerves of the ganglion were cut, common synaptic potentials still occurred in pairs of neurones, suggesting that local interneurones were largely responsible for supplying these common inputs. A similar picture of synaptic potentials common to particular DUM neurones was observed in the isolated metathoracic ganglion (Fig. 2), indicating that the presynaptic neurones responsible were not sensory neurones but must therefore be interneurones within that ganglion. For example, two DUM3,4 neurones received a low frequency of common IPSPs (Fig. 2A) that, when they occurred at higher frequencies, could prolong the intervals between any spikes. Judged by the similarity of their shape, these IPSPs appeared to be derived from a small number of presynaptic local interneurones. None of the common depolarising potentials seen in an intact locust (Fig. 1A) were apparent in this particular recording. Similarly, a DUM3 and a DUM3,4 neurone had many common depolarising and hyperpolarising synaptic potentials (Fig. 2B). Typically, the synaptic potentials were of larger amplitude in DUM3,4 neurones than in DUM3 neurones. The two DUM neurones with axons in lateral nerve 1, DUMDL and DUM1b, also had many depolarising and hyperpolarising synaptic potentials in common. Occasionally, common EPSPs led to spikes in one neurone but not in the other (Fig. 2C).

The similarity in the waveforms recorded from these pairs of neurones made it essential to establish that the electrodes were not recording from the same soma. This was achieved in the following way. First, sporadic postsynaptic potentials occurred independently in all pairs of neurones, and spikes could occur at different times. Second, pulses of current injected through one electrode were not recorded in the other electrode. Third, dye injection revealed two neurones. These procedures were then extended to test whether two neurones were electrically coupled to each other or whether one neurone made a direct synaptic connection with the other, such that its spikes would cause a synaptic potential in the other neurone. For example, pulses of depolarising or hyperpolarising current injected into a DUM5b neurone did not alter the membrane potential of DUMETi (Fig. 3A), and when the current was injected into DUMETi there was no effect on the DUM5b neurone (Fig. 3B). Furthermore, spikes evoked in one neurone by depolarising current were not associated with a synaptic potential in the other neurone. Signal averaging from the evoked spikes failed to reveal any linked potential in the other neurone. The same tests were repeated for all pairs of neurones that showed many synaptic potentials in common.

Neurones with few common synaptic potentials

Many pairs of DUM neurones appeared to have only a few or even no synaptic potentials in common, either in the intact locust or in an isolated metathoracic ganglion (Fig. 4). A DUM3,4 neurone appeared to have few potentials in common with DUMETi in an intact locust (Fig. 4A) or with a

DUM3,4,5 neurone in an isolated ganglion (Fig. 4B). Similarly, a DUM3 neurone appeared to have few potentials in common with DUM1b in an intact locust, although a depolarisation in one sometimes corresponded to a hyperpolarisation in the other (Fig. 4C). The low frequency of synaptic potentials in a DUM3 neurone in an isolated ganglion also contrasted with the higher frequency of synaptic potentials in a DUM3,4,5 neurone (Fig. 4D), although on other occasions the contrast between the frequency of synaptic potentials in these neurones was less marked.

Quantifying the synaptic potentials in pairs of DUM neurones

The preceding examples illustrate the general occurrence of synaptic potentials in DUM neurones and show clearly that

some are driven by the same inputs, whereas others are not. To quantify the similarities and differences among the synaptic potentials in known neurones, a correlation coefficient was calculated for each of the 20 combinations of DUM neurones from which recordings were made. Two examples illustrate the analytical procedures (Fig. 5). When viewed by eye, a DUM3,4 neurone and DUMETi appear to have few synaptic potentials in common (Figs 4A, 5A). In the correlation procedure, the sign of the time series product showed fluctuations above and below zero, and a plot of the cumulative sum of the sign correlations over the duration of the recording gave a line with a slope of 0.03, representing the correlation coefficient. These two neurones therefore have few common synaptic potentials. In contrast, two DUM3,4 neurones appeared to have many

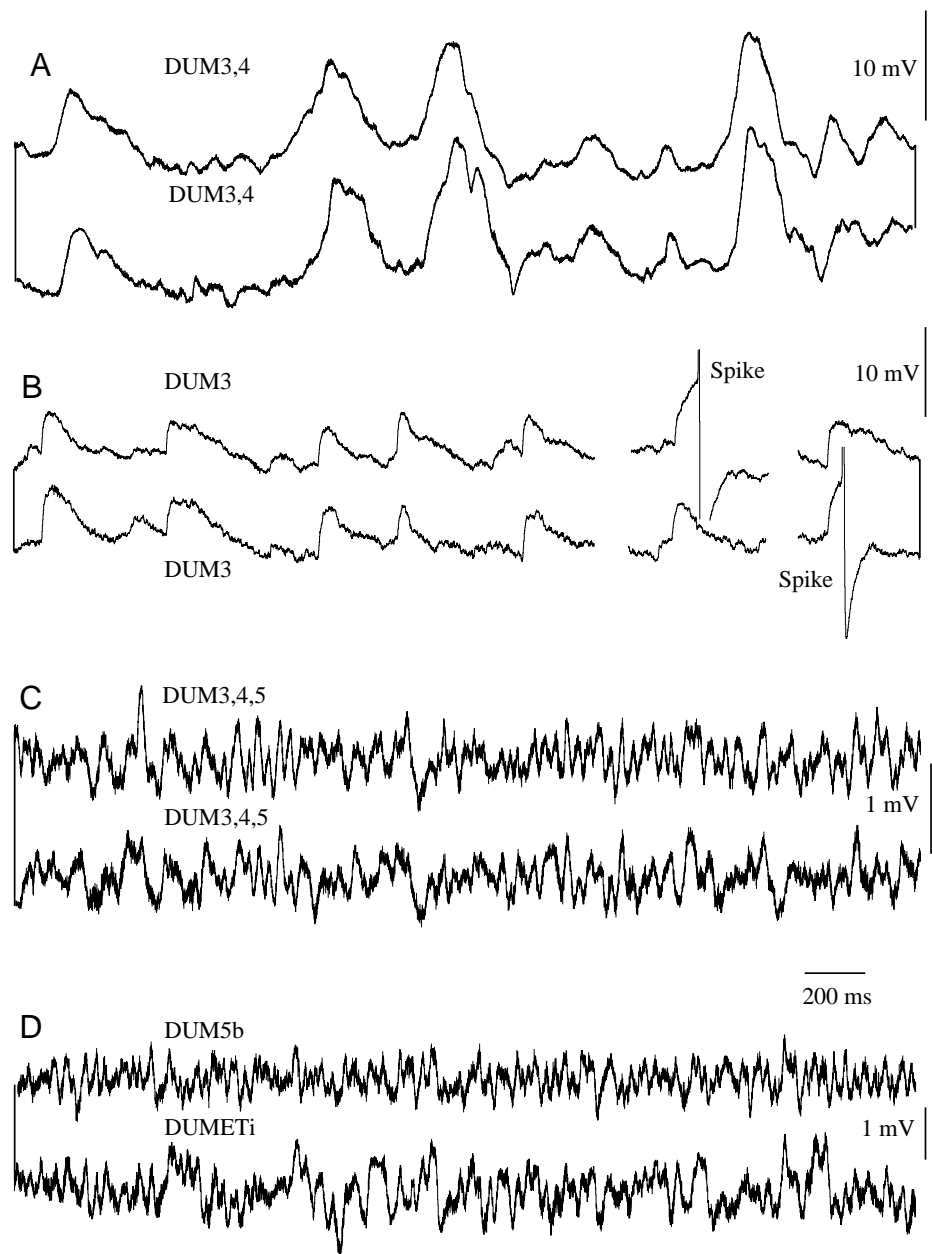


Fig. 1. Common synaptic potentials in particular pairs of dorsal unpaired median (DUM) neurones in a quiescent intact locust. (A) Recordings from two DUM3,4 neurones in which the waveforms of the depolarising and hyperpolarising synaptic potentials are closely matched. (B) Recordings from two DUM3 neurones in which depolarising synaptic potentials of low frequency are matched in both. Some common synaptic potentials can lead to a spike in one neurone but not in the other. The spikes have been clipped. (C) Recordings from two DUM3,4,5 neurones displayed at the same time scale as in A and B showing a high frequency of common synaptic potentials. Occasional potentials occur independently in the two neurones. (D) Recordings from a DUM5b neurone and DUMETi, showing many synaptic potentials in common.

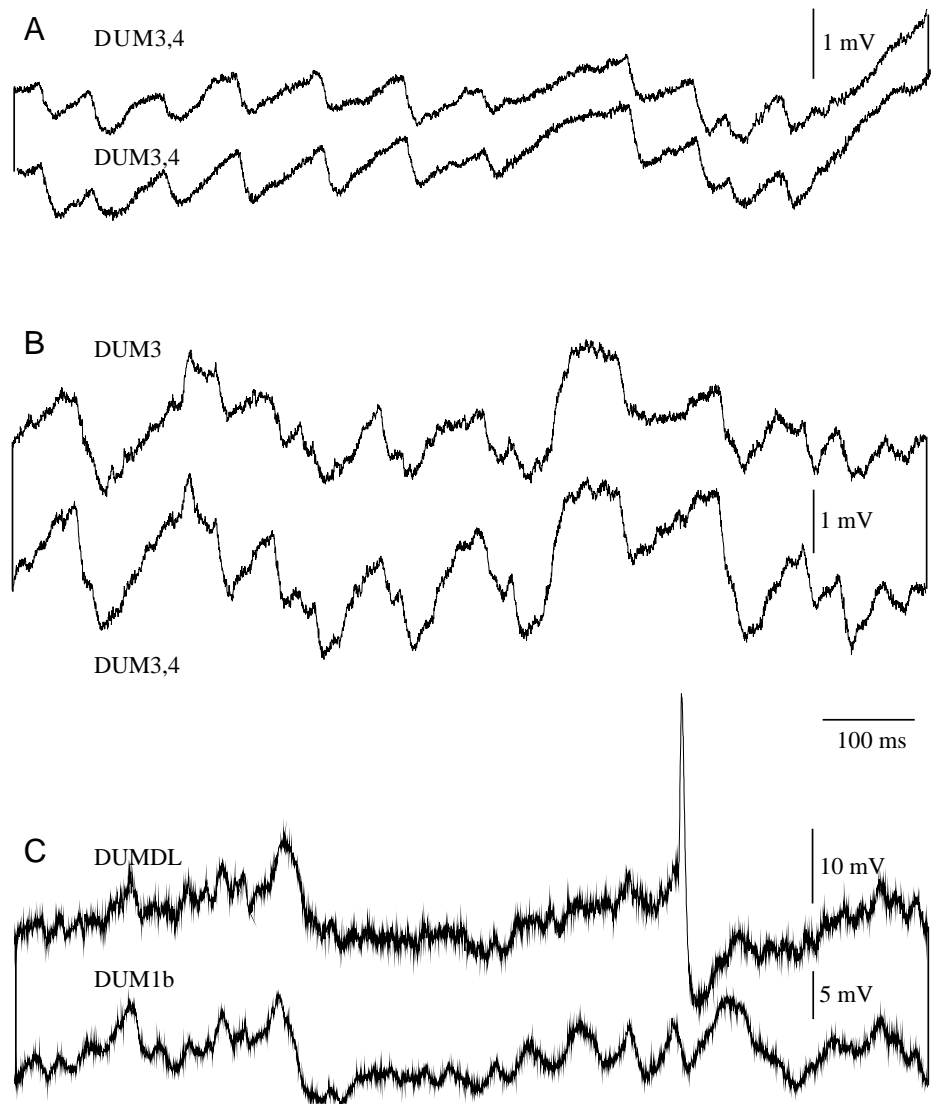


Fig. 2. Common synaptic potentials still occur in particular pairs of DUM neurones in an isolated metathoracic ganglion. (A) Recordings from two DUM3,4 neurones in which common hyperpolarising synaptic potentials predominate. (B) Recordings from a DUM3 and a DUM3,4 neurone showing closely matched waveforms of both depolarising and hyperpolarising synaptic potentials. (C) Recordings from DUMDL and DUM1b neurones sharing common depolarising and hyperpolarising synaptic potentials. A common synaptic potential can lead to a spike in one neurone but not in the other.

common synaptic potentials (Figs 1A, 2A, 5B). The sign of the time series product for these two neurones showed fluctuations predominantly above zero, and a plot of the cumulative sum of the sign correlations gave a line with a slope of 0.72, indicating that their synaptic potentials were strongly correlated.

Analyses of all the pairs of DUM neurones from which recordings were made produced a bimodal distribution of correlation coefficients; pairs of neurones either had a high correlation coefficient, indicating that their synaptic potentials were closely correlated, or they had a low correlation coefficient, indicating that they received few, if any, common synaptic potentials. Neurones whose synaptic potentials were most strongly correlated fell into three groups (Fig. 6) that also shared common anatomical features: (i) DUMDL and DUM1b; (ii) DUM3 and DUM3,4 neurones and (iii) DUM3,4,5, DUM5b and DUMETi.

Within each of these groups, the correlation coefficients were high, indicating that the majority of their synaptic potentials were common. In contrast, correlation coefficients

between neurones of the different groups were low. For example, the synaptic potentials in DUM3 and DUM3,4 neurones were not correlated with those in the DUM neurones, from which we recorded, that had axons in lateral nerve 5.

These groupings tentatively include all neurones that have the same distribution of axons where there are no adequate criteria that allow identification of individuals. The five DUM3 and the six DUM3,4 neurones within the metathoracic ganglion (Table 1) cannot yet be separated as individuals. Only one of the three DUM3,4,5 neurones can be distinguished in that it alone innervates a particular wing muscle (pleuroaxillary 114) (Stevenson and Meuser, 1997). Repeated paired recordings from these neurones and measurements of their correlation coefficients also suggest that there might be differences based on their synaptic potentials. For example, some paired recordings from a DUM5b and a DUM3,4,5 had a high correlation coefficient, but others had a much lower correlation (Fig. 6). Similarly, in different animals, paired recordings from two DUM3,4 neurones or from a DUM3 and

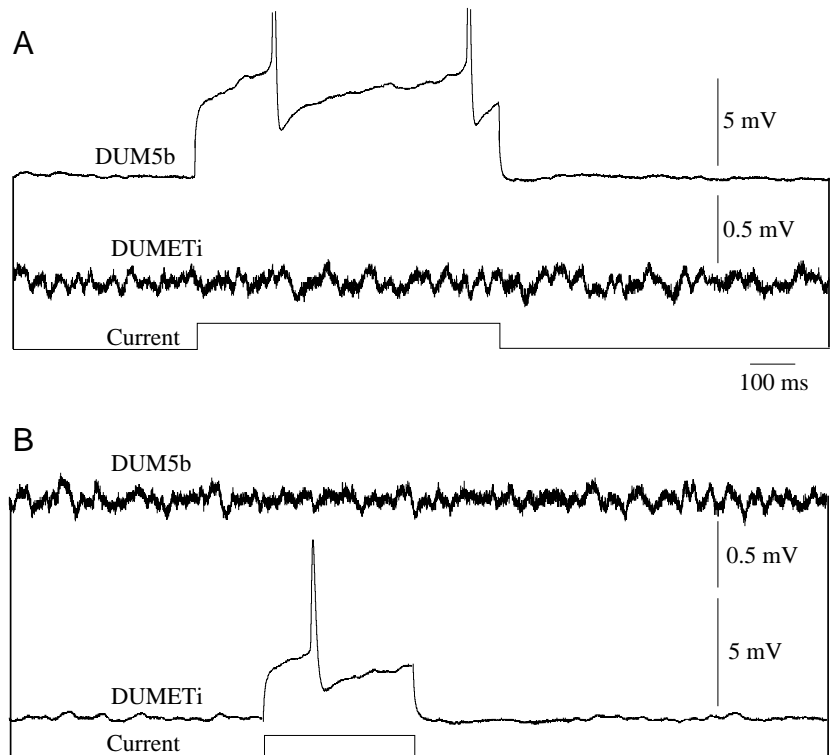


Fig. 3. DUM neurones that have many synaptic potentials in common are not coupled by electrical junctions or by direct synaptic connections mediated by their spikes. (A) Injection of depolarising current into a DUM5b neurone causes no change in the membrane potential of DUMETi, and spikes evoked by the current do not cause an obvious linked synaptic potential in DUMETi. The peaks of the spikes are clipped. (B) Current injected into DUMETi to cause a spike had no effect on the DUM5b neurone.

DUM3,4 neurone had different correlation coefficients, leading to an apparently large variation when the data were pooled (Fig. 6). Such differences are not seen in samples taken from long-term paired recordings from the same neurones in one locust, suggesting that the correlations result from individual differences amongst the neurones of a group. These observations might imply that there are differences in the synaptic drive to particular neurones within a subgroup.

Common synaptic potentials and spike outputs

In both intact locusts and isolated metathoracic ganglia, DUM neurones spiked irregularly and at low frequencies, usually less than 1 Hz. It might be expected that neurones with many synaptic potentials in common would show a tendency to spike at the same time, whereas those with no common synaptic potentials would spike with more independence. Visual inspection of the paired recordings suggested that this was what happened. Two DUM3,4 neurones in an isolated ganglion, whose synaptic potentials were strongly correlated, tended to spike at the same time (Fig. 7A). Similarly, two DUM3,4,5 neurones in an intact locust, whose synaptic potentials were also strongly correlated, often spiked at the same time, although spikes could occur independently (Fig. 7B).

To quantify the coupling of spike outputs, histograms were plotted of the occurrence of spikes in one neurone of a pair relative to those in the other neurone (Fig. 7C,D). In neurones showing a low correlation between their synaptic potentials, there was little correlation of spikes (Fig. 7C). In contrast, the histograms of neurones whose synaptic potentials were strongly correlated showed a pronounced peak, indicating that many spikes occurred at the same time in the two neurones (Fig. 7D).

Paired recordings were then analysed further to determine the number of spikes that occurred within 40 ms of each other in the two neurones. We tested intervals of between 20 and 100 ms without finding great differences in the correlations, probably because DUM neurones never spiked at the high frequencies seen in many other neurones within the same ganglion. The number of spikes that occurred within 40 ms of each other was expressed as a percentage and was compared with the percentage of spikes that might be expected to occur by chance in a 40 ms time window (Table 2). In those pairs of neurones whose synaptic potentials were highly correlated, the percentage of spikes occurring in a 40 ms time window ranged from 5.5 % in a DUM3,4,5 neurone and a DUM5b neurone, through 10.5 % in two DUM3,4 neurones, to 22.5 % in DUMDL and DUM1b. These percentages were higher than would be expected by chance. In pairs of neurones with few synaptic potentials in common, only 0–3.1 % of spikes occurred in a 40 ms time window; this value does not differ from the percentage expected by chance. The common synaptic driving of particular groups of neurones therefore biased their spiking so that they were more likely to spike at the same time.

Mechanosensory stimulation

When a mechanosensory stimulus was applied to specific parts of the body, particular DUM neurones responded with an increase in the frequency of synaptic potentials that often led to a spike or a short burst of spikes. To determine the source of the synaptic potentials in particular DUM neurones, stimuli were systematically applied to different regions of the hind legs and hind wings, and supplemented by stimuli to the other legs, to the abdomen and to the head (Figs 8–10). The response of

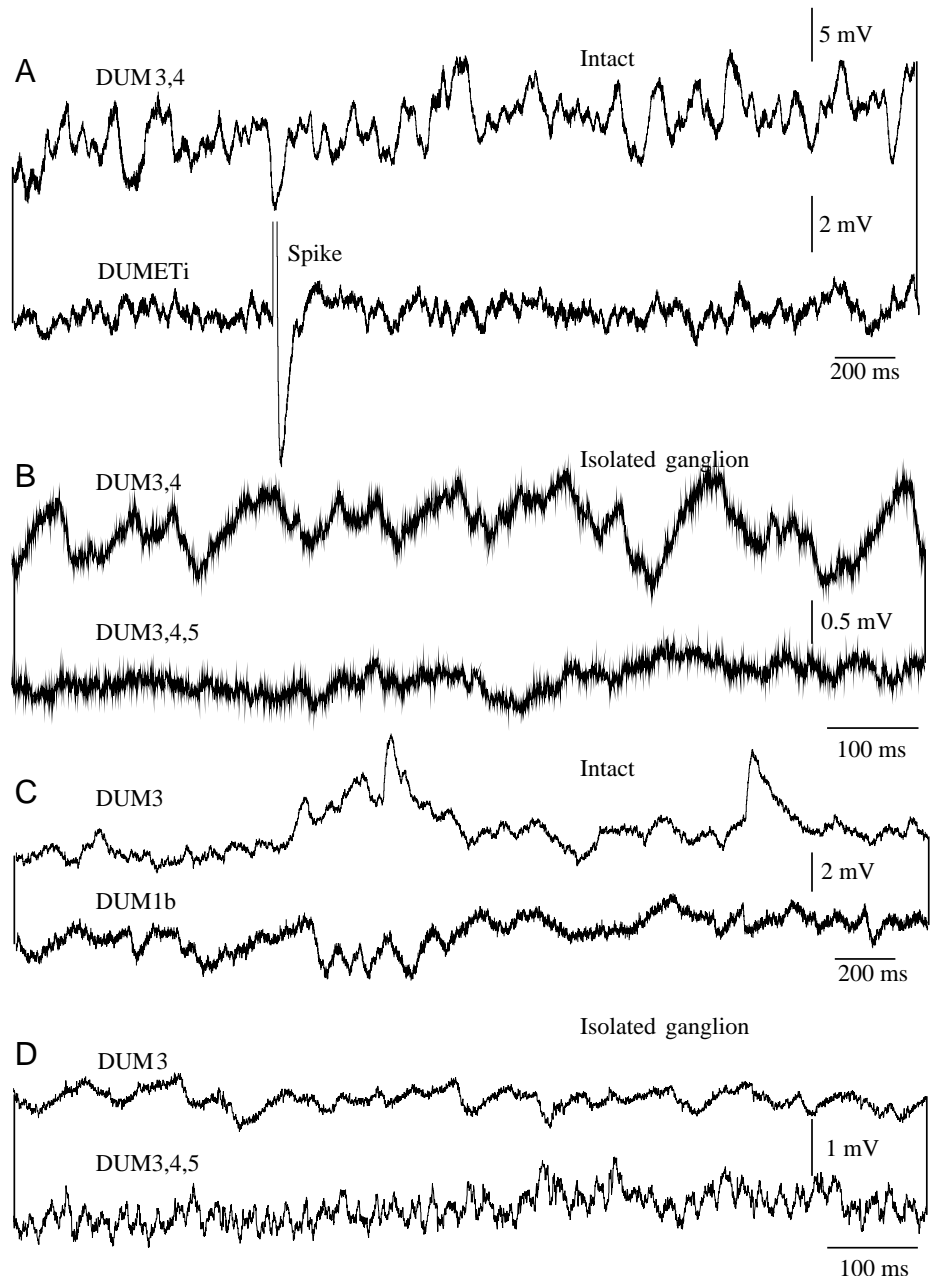


Fig. 4. DUM neurones that have few common synaptic potentials in either intact locusts (A,C) or isolated metathoracic ganglia (B,D). (A,B) DUM3,4 neurones have few synaptic potentials in common with either DUMETi or a DUM3,4,5 neurone. The spike in DUMETi is clipped. (C,D) DUM3 neurones similarly have few synaptic potentials in common with either DUM1b or with those that occur at a higher frequency in a DUM3,4,5 neurone.

all the DUM neurones to a particular stimulus occurred with a delay of 150–200 ms, suggesting that the sensory neurones did not make direct connections with the DUM neurones. Furthermore, the response to the same stimulus was often variable and waned substantially if stimuli were repeated more frequently than at intervals of a few seconds.

DUM3,4,5 and DUM5 neurones

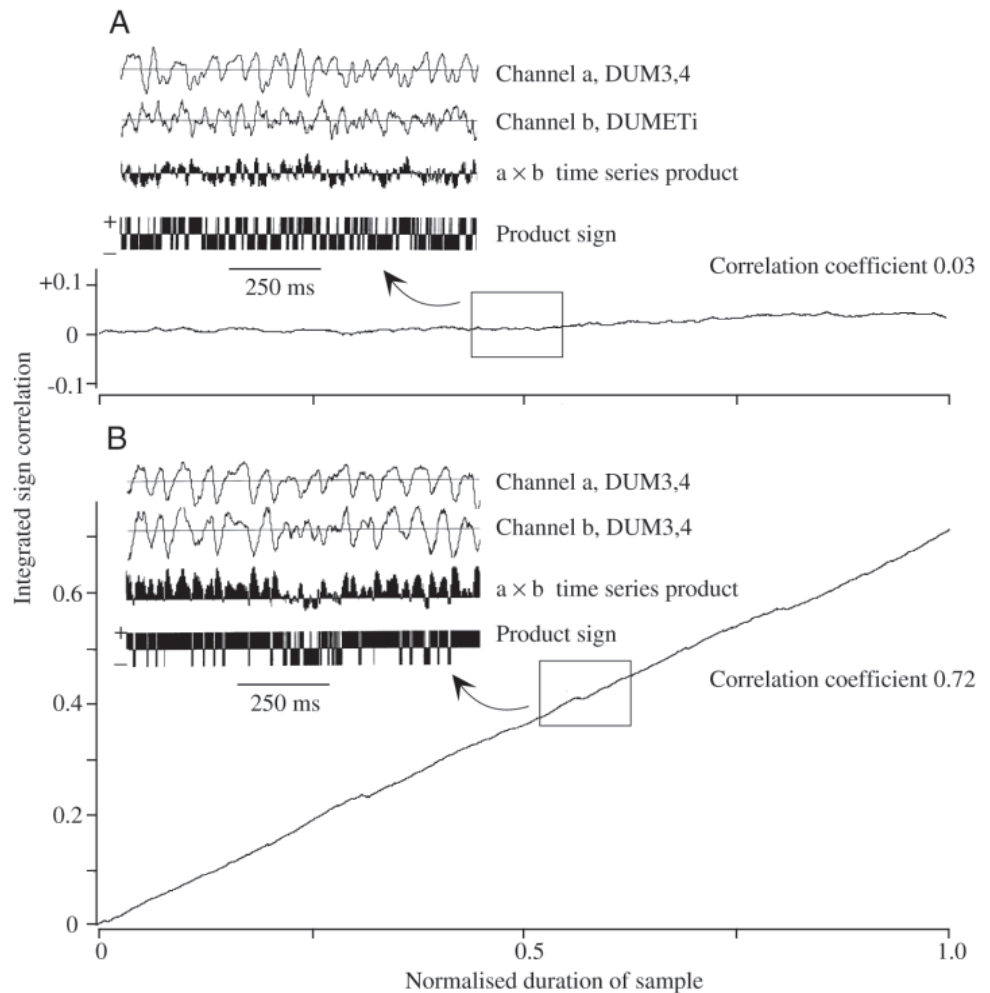
DUM3,4,5 and DUM5 neurones showed similar responses when mechanosensory stimuli were delivered to different parts of either hind leg, or the tibia was moved forcibly about the femur. Touching exteroceptors around the femoro-tibial joint, or on the tarsus, and forcibly moving the tibia evoked a depolarisation and spikes in a DUM5b neurone and in DUMETi (Fig. 8A–C). Stimuli to either hind leg were effective. Touching

the abdomen (Fig. 8D) or the cerci led to a depolarisation of both neurones with some spikes. Wind stimuli to the head and mechanical stimulation of exteroceptors on the head or antennae (Fig. 8E) also elicited spikes in these neurones. Similar responses were seen when a DUM3,4,5 neurone was recorded with either DUMETi or a DUM5b neurone. These neurones were the most responsive to mechanosensory stimuli and gave the most reliable responses. Their responses to stimulation of either hind leg persisted when the anterior and posterior connectives of the metathoracic ganglion were cut.

DUM3 and DUM3,4 neurones

DUM3 and DUM3,4 neurones showed variable responses to mechanosensory stimuli (Fig. 9) and none responded with the same intensity or reliability as shown by the neurones discussed

Fig. 5. Quantification of the correlation between the synaptic potentials in pairs of DUM neurones. (A) DUM3,4 and DUMETi. The inset shows a sample from a 10 s recording from the two neurones. Beneath are shown the time series product (channel a \times channel b) and the product sign (+1 or -1). Deflections above zero indicate a sign correlation between the two recordings, and deflections below zero indicate that the recordings were anti-correlated. The graph plots the cumulative, integrated sign correlation against the normalised duration of the sample recording, and the slope of the line (0.03) indicates no correlation between the synaptic potentials in these neurones. The box indicates the region from which the inset recordings were taken. (B) Two DUM3,4 neurones. The time series product shows mostly positive deflections, and the slope of the plot (0.72) indicates a strong correlation between the synaptic potentials in these neurones. Full details of the procedures used are given in Materials and methods.



in the preceding section. Touching the metathoracic or mesothoracic tarsus often led to a depolarisation of a DUM3 and a DUM3,4 neurone, which could sometimes evoke one or more spikes when the locust was active (Fig. 9A). When the locust was quiescent, the same stimuli could sometimes evoke no response or could lead to a movement of a leg and a

hyperpolarisation of both neurones. Imposed movements of either hind tibia were generally without effect on these neurones. A dependency between the response and the state of activity of the locust was also seen for stimuli applied elsewhere on the body. Touching the tarsus of a prothoracic leg could lead to parallel excitatory synaptic potentials in both neurones,

Table 2. Percentage of spikes in pairs of DUM neurones during a 40 ms time window

	DUMDL	DUM1b	DUM3	DUM3,4	DUM3,4,5	DUMETi	DUM5b
DUMDL	—	22.5 (1.1)		2.0 (1.1)			
DUM1b		—	3.1 (1.9)	0 (0.3)			0 (2.6)
DUM3			—	5.5 (0.7)	0 (1.0)	0 (0.5)	2.2 (0.5)
DUM3,4				10.5 (1.5)	2.2 (1.1)	2.3 (0.6)	2.5 (1.1)
DUM3,4,5					9.8 (3.0)	14.1 (0.7)	5.5 (0.7)
DUMETi						—	5.9 (4.3)
DUM5b							—

The numbers show the percentage of spikes that occurred within a 40 ms time window of each other; the numbers in parentheses show the percentage of spikes that would be expected to occur by chance within the same time window. The estimation of this expected value involved three steps. First, the probability of a spike in one neurone occurring in any 40 ms interval was calculated by dividing the total number of spikes by the total number of intervals of the recording. Second, these probabilities for each channel were multiplied to yield the probability that both neurones spiked in any of the intervals. Third, this probability was multiplied by the total number of spikes in both neurones to yield the expected number of spike pairs occurring by chance.

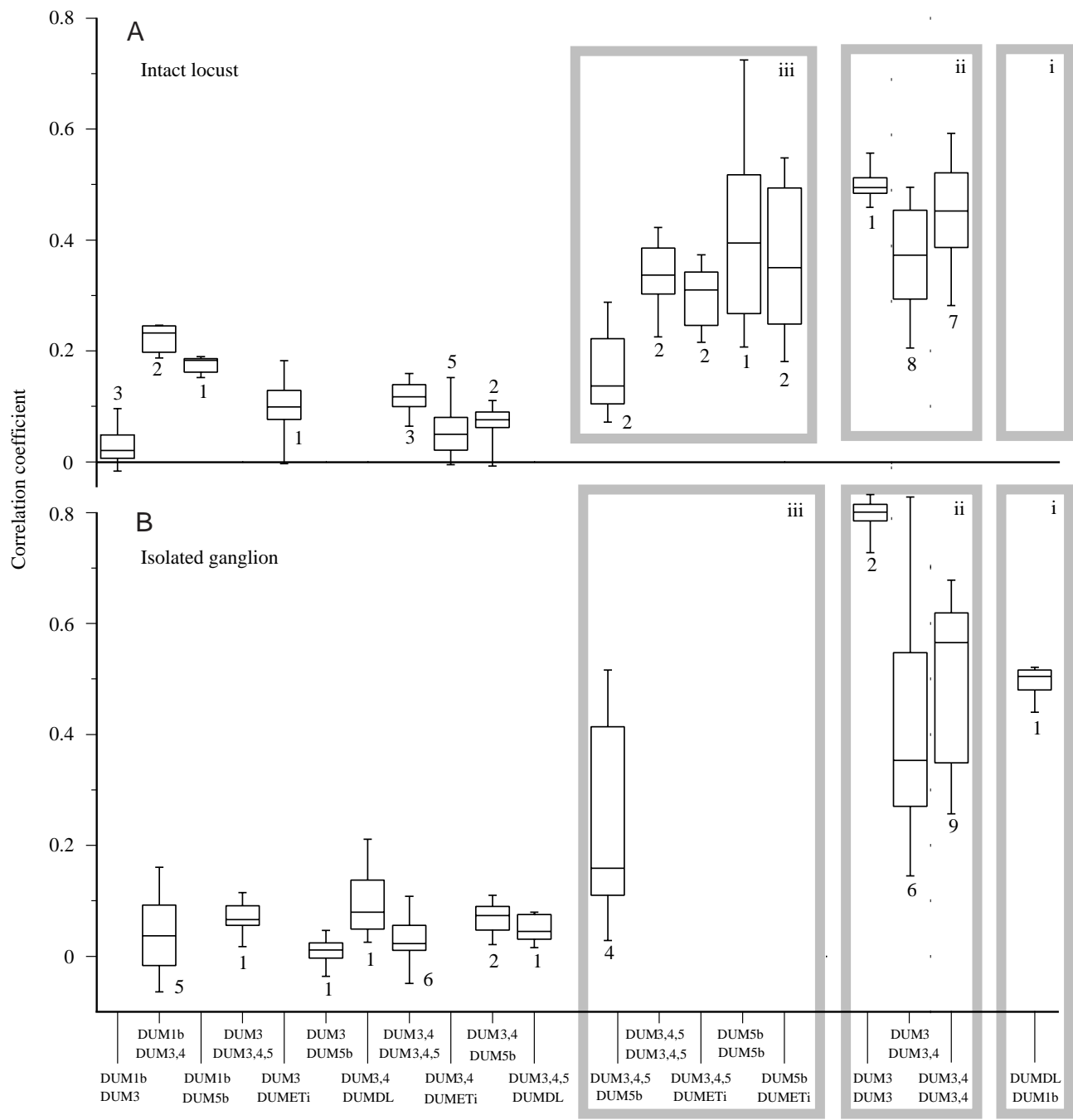


Fig. 6. Summary of the quantitative correlation analyses for 20 combinations of different DUM neurones in intact locusts (A) and in isolated metathoracic ganglia (B). The correlation coefficient for each pair, derived as in Fig. 5 from at least six sample periods, is displayed as a 'box and whisker' plot. The numbers beside each box indicate the number of locusts from which the recordings were made. The boxes show the twenty-fifth to seventy-fifth percentile with the median value inside, and the whiskers show the fifth to ninety-fifth percentile. The large grey boxes divide the DUM neurones into three subsets that share the strongest correlations between their synaptic potentials: (i) DUMDL and DUM1b; (ii) DUM3 and DUM3,4; and (iii) DUM3,4,5, DUM5b and DUMETi.

evoking a spike in one neurone in active locusts, whereas on other occasions the same stimulus led to a mixed depolarisation and hyperpolarisation of both neurones (Fig. 9B). Similarly, touching head hairs in an active locust led to a depolarisation and spikes in an active locust, but the same stimulus in a quiescent locust led only to a small hyperpolarisation (Fig. 9C).

Thus, none of the stimuli we used elicited responses that were reliable diagnostic criteria for identifying these neurones.

DUM1b and DUMDL neurones

As in the case of DUM3 and DUM3,4 neurones, both DUM1b and DUMDL showed labile responses to

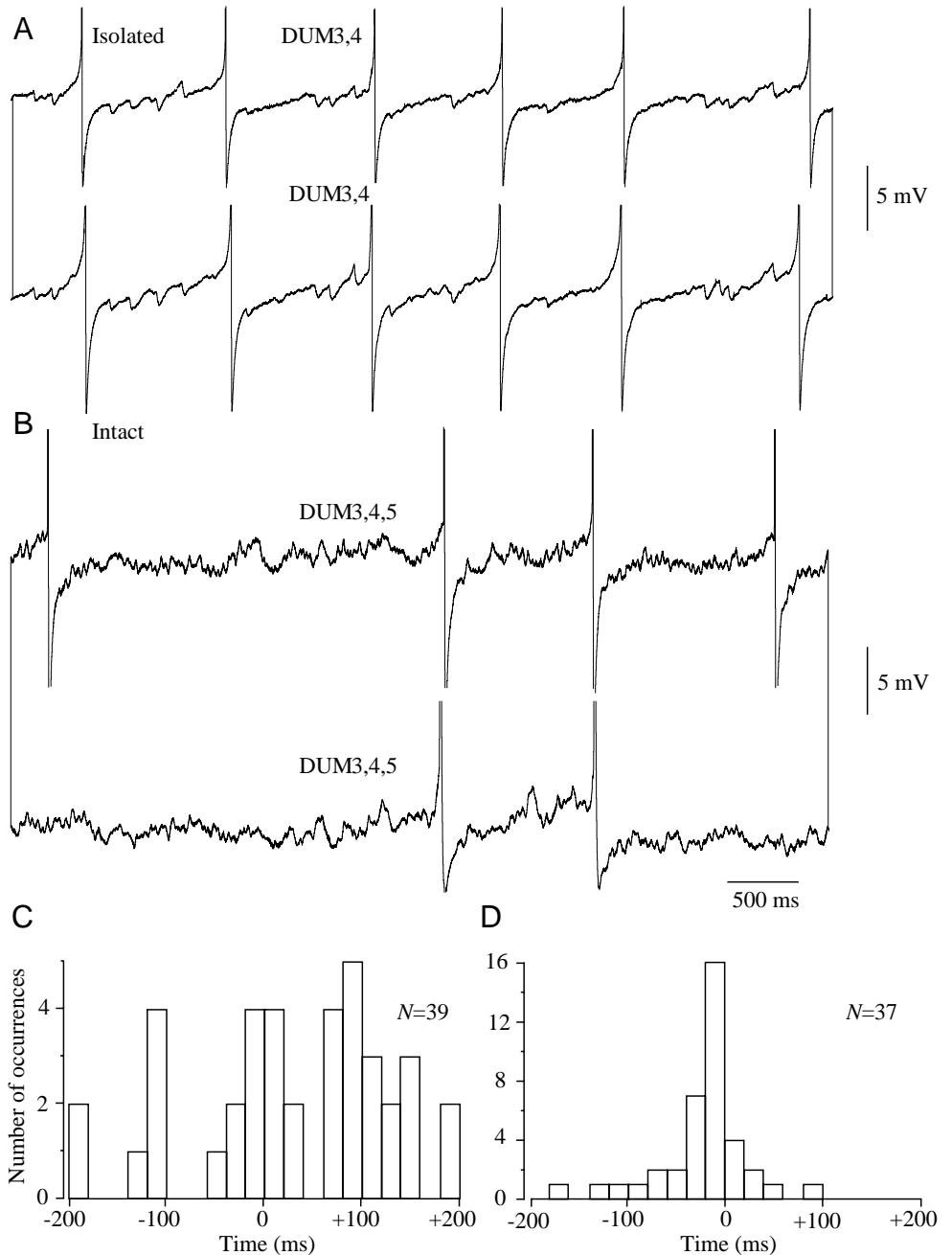


Fig. 7. DUM neurones that have many common synaptic potentials may spike at the same time. (A) Two DUM3,4 neurones in an isolated metathoracic ganglion have many synaptic potentials in common that generate spikes at the same time in both neurones. (B) Two DUM3,4,5 neurones in a quiescent, intact locust tend to spike at the same time, although spikes also occur independently. Spikes have been clipped. (C,D) Histograms to show correlations between spikes in neurones that have few synaptic potentials in common (C) and those in neurones whose synaptic potentials are strongly correlated (D). N =the total number of correlations. The 400 ms sampling period was triggered by a spike in one neurone, and the histograms plot the time when spikes in the other neurone occurred spontaneously relative to this spike. The paired recordings each lasted for 15 min. In C, 174 spikes occurred in the neurone used as the trigger and 283 in the other neurone, indicating that most spikes occurred at intervals of more than 200 ms relative to one another. In D, the numbers of spikes were 54 and 92 respectively.

mechanosensory stimuli. Touching either hind leg had no consistent effect on either DUMDL or DUM1b neurones. Touching either hind wing led to a depolarisation and an occasional spike in DUMDL, but had no effect on DUM1b. Of the stimuli tested, the most effective for these neurones was wind directed at the head. Touching the abdomen sometimes led to response in these neurones. These responses were, however, always smaller than those in a DUM5 or DUM3,4,5 neurone. Air blown over the head caused an initial hyperpolarisation of DUM1b followed by a depolarisation and a low frequency of spikes if there was an active movement of the locust (Fig. 10A). DUMDL was initially depolarised and spiked when there was no active movement, but the frequency

of evoked spikes was much lower than that in a DUM3,4,5 neurone recorded simultaneously (Fig. 10B). Touching the abdomen led to a hyperpolarisation in both neurones, but simultaneously evoked a burst of spikes in a DUM5b (Fig. 10C) or a DUM3,4,5 (Fig. 10D) neurone. The response to all sensory stimuli tested was very labile in DUM1b. In 60% of the preparations this neurone did not change its spike activity.

Discussion

Intracellular recordings from different pairs of efferent DUM neurones in the metathoracic ganglion of a locust have shown that the background synaptic potentials in particular pairs are

strongly correlated whereas those in other pairs are not. Similar correlations were seen both in intact locusts and in isolated metathoracic ganglia, indicating that many of the neurones responsible for the common synaptic potentials are contained within the metathoracic ganglion. In some pairs of neurones, the synaptic potentials were so closely matched that it was necessary to establish that the electrodes were not recording from a single neurone. Even in such pairs, however, independent synaptic potentials did occur and the neurones sometimes spiked individually. Pairs of DUM neurones with many common synaptic potentials were more likely to spike spontaneously than were neurones with few synaptic potentials in common. They were also activated by the same mechanosensory stimulation.

Subsets of DUM neurones

Quantitative correlations of the common synaptic potentials in different pairs of DUM neurones provided a diagnostic feature that enabled the metathoracic population of 19 efferent DUM neurones to be divided into three subsets. Within a subset the synaptic potentials were strongly correlated, but between the subsets the low correlations indicated that few synaptic potentials were common. This grouping based on physiology also maps onto an anatomical grouping based on the axonal projections of the different neurones in particular lateral nerves (Campbell *et al.* 1995; Stevenson and Spörhase-Eichmann, 1995). Thus, the two neurones with axons in nerve

1 (DUMDL and DUM1b) form one subset because their synaptic potentials are strongly correlated with each other but are not correlated with synaptic potentials in other DUM neurones. The five neurones with axons in nerve 3 (DUM3 neurones) and the six neurones with axons in both nerves 3 and 4 (DUM3,4 neurones) have strongly correlated synaptic potentials and form a second subset. A third subset is formed by neurones that have an axon in nerve 5; the three DUM3,4,5 neurones, the two DUM5b neurones and DUMETi have strongly correlated synaptic potentials. Only a few of the efferent DUM neurones can be unequivocally identified as individuals, so that the others, including the five DUM3 neurones, the six DUM3,4 neurones, the three DUM3,4,5 neurones and the two DUM5b neurones, can only be treated as members of anatomically defined groups. Controlling DUM neurones with common synaptic drives may be a widespread mechanism because recordings in other insect species have also revealed some common synaptic potentials in the blood-feeding insect *Rhodnius prolixus* (Orchard *et al.* 1989), in the moth *Manduca sexta* (Pflüger *et al.* 1993) and in the silk moth *Antheraea pernyi* (Brookes and Weevers, 1988).

In our analyses of the correlations between synaptic potentials, we have pooled data on neurones from the same anatomical group obtained in different animals that could not be identified as individuals. In recordings from neurones of the same anatomical group in different locusts, there were,

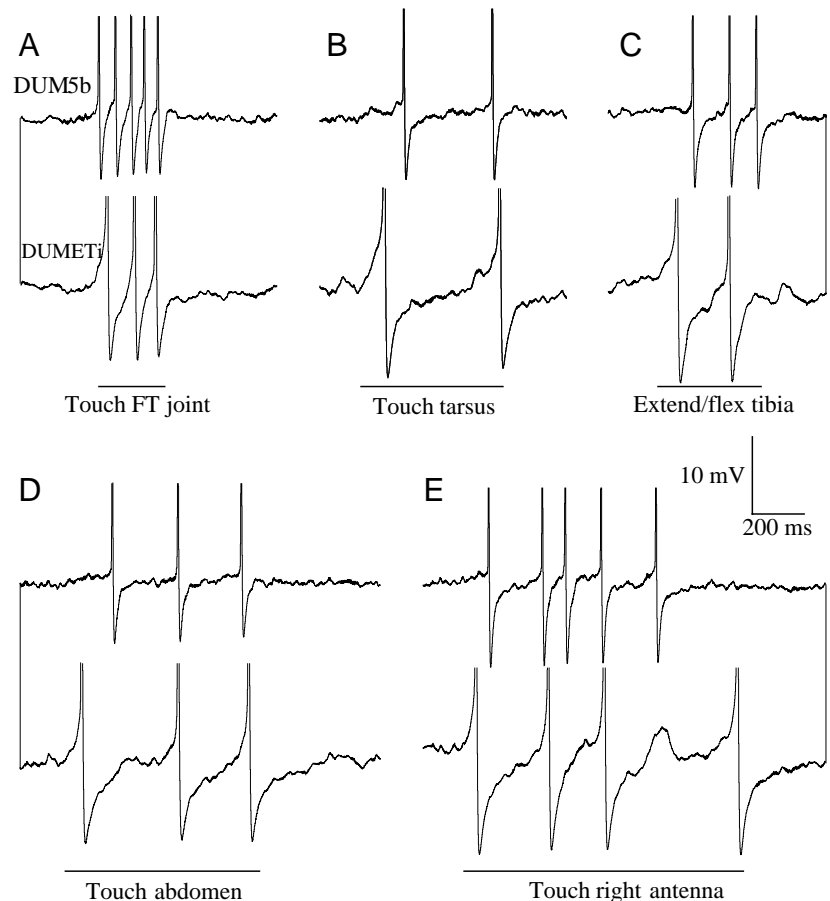


Fig. 8. DUMETi and a DUM5b neurone respond in a similar way to mechanosensory stimulation of various parts of the body. (A–C) Stimulation of the right hind leg leads to a burst of spikes in both neurones. (D,E) Touching the abdomen (D) or the right antenna (E) also leads to a burst of spikes. Spikes have been clipped. FT, femoro-tibial joint.

however, variations in the degree of coupling between their synaptic potentials. Do all members of a particular anatomical group therefore share the same pattern of synaptic potentials (see, for example, the DUM5b and DUM3,4 neurones in Fig. 6)? Some of these differences could have resulted from variations in the patterns of synaptic potentials that occurred at different times in the same pair of neurones in one locust. Nevertheless, the overriding pattern in a particular pair of neurones when analysed from sample periods taken at different times from long recordings was of strongly correlated synaptic potentials. An alternative explanation is that there are differences in the synaptic drive to different members of the

same anatomical group. Our pooling of data would inevitably have obscured such differences. On this basis, therefore, it may be appropriate to assume that all the DUM neurones are potentially identifiable as individuals and that, within an anatomical group, there may be neurones with differing synaptic drives and thus potentially different behavioural roles. This conclusion is supported by the observation that the three DUM3,4,5 neurones can be distinguished anatomically; two have primary neurites in the superficial DUM tract of the metathoracic ganglion, whereas the third has its primary neurite in the deep DUM tract (Campbell *et al.* 1995). Only one of the three neurones innervates a small wing muscle

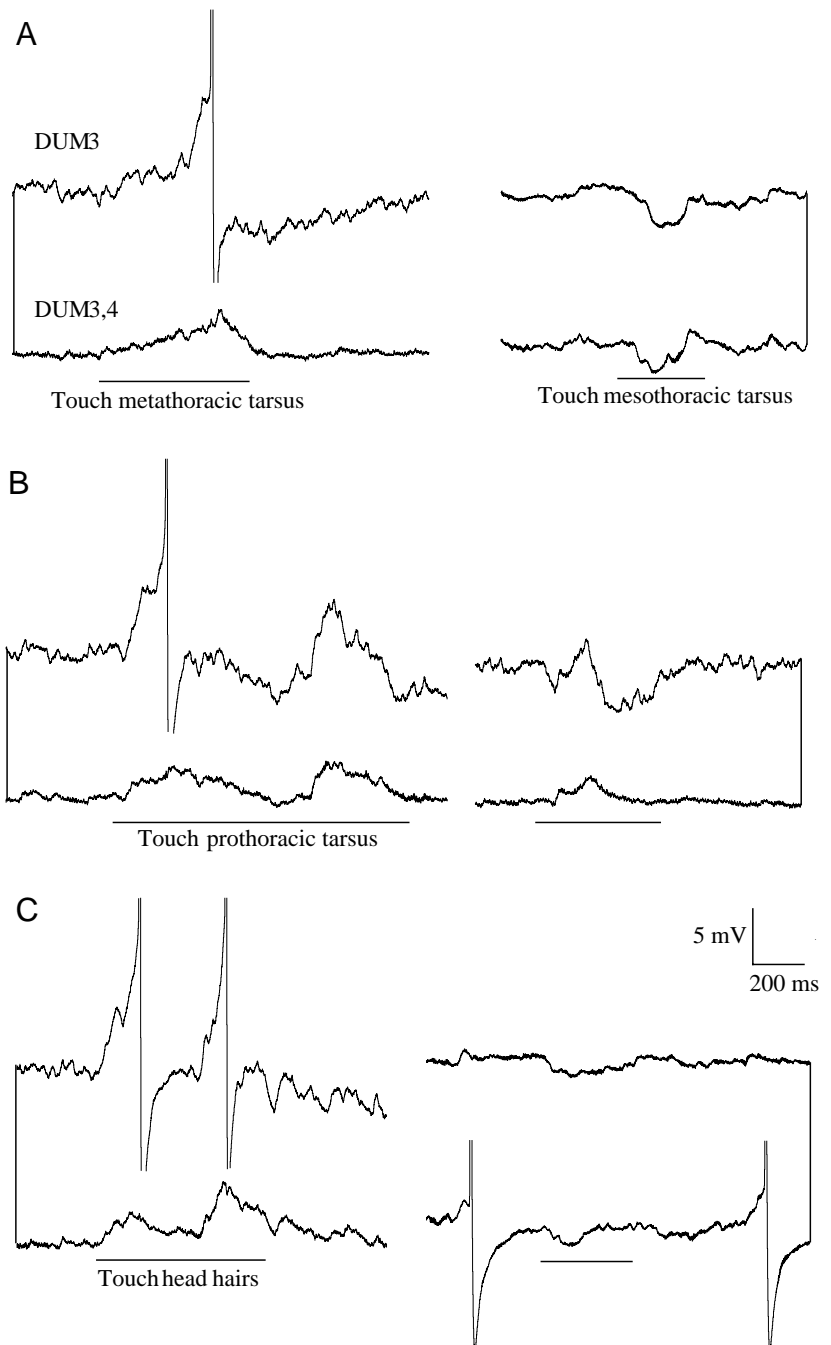


Fig. 9. DUM3 and DUM3,4 neurones have a variable response to mechanosensory stimuli. (A) Touching a metathoracic tarsus evokes a depolarisation and spikes in a quiescent locust (recordings on left), but touching a mesothoracic tarsus leads to a hyperpolarisation (right) if there is an active movement of a hind leg. (B) Touching a prothoracic tarsus evokes a depolarisation and spikes in an active locust that is moving all its legs (left), but a small depolarisation followed by a hyperpolarisation when a hind leg alone is moved (right). (C) Touching head hairs evokes a depolarisation and spikes in an active locust that is moving all its legs (left), but the same stimuli elicit a hyperpolarisation when a leg is moving actively (right). Spikes have been clipped.

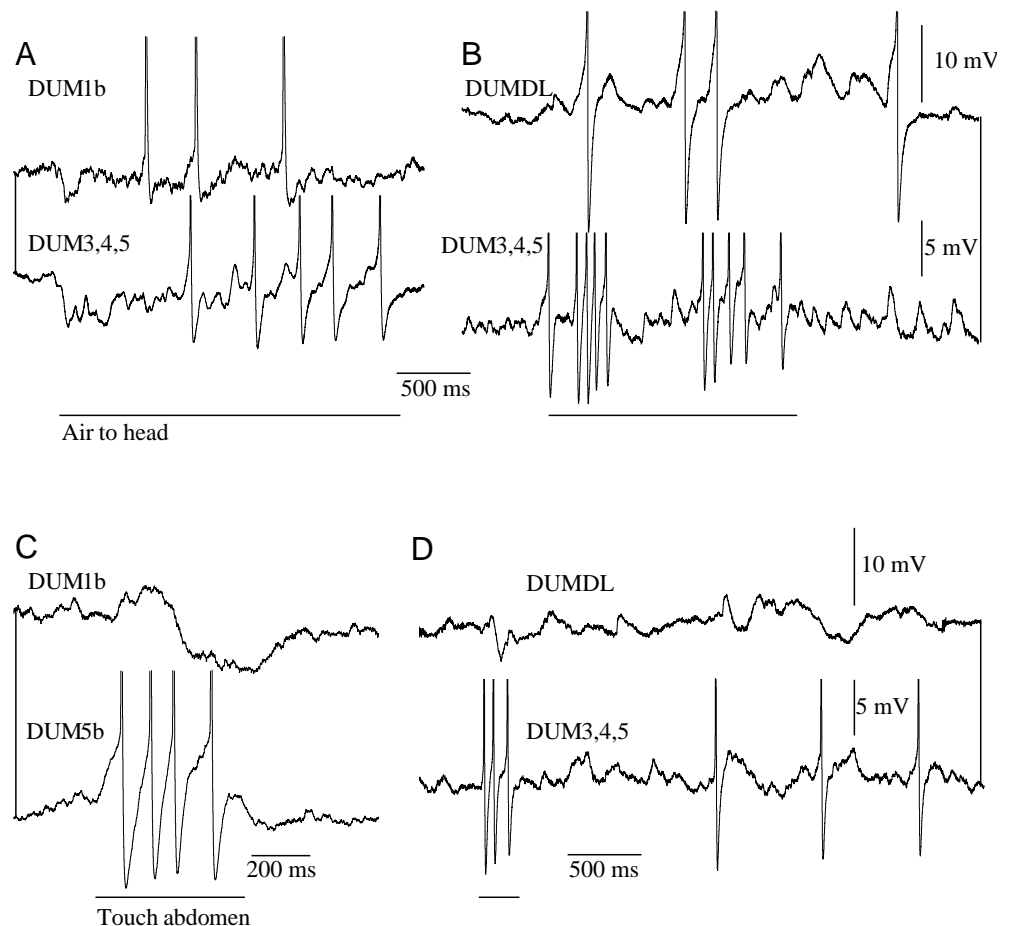


Fig. 10. Responses of DUM1b and DUMDL to mechanosensory stimulation. (A) Blowing air at the head evokes an initial hyperpolarisation followed by spikes in DUM1b and in a DUM3,4,5 neurone, when an active movement occurs. (B) A similar stimulus evokes depolarising synaptic potentials and a few spikes at low frequency in DUMDL, but a higher frequency of spikes in a DUM3,4,5 neurone. (C,D) Touching the abdomen evokes a hyperpolarisation in DUM1b (C) and DUMDL (D) when the locust moves, but evokes bursts of spikes in DUM5b or DUM3,4,5 neurones. Spikes have been clipped.

(pleuroaxillary 114, which twists a hind wing during flight) and its primary neurite is in the superficial DUM tract (Stevenson and Meuser, 1997). In *Locusta migratoria*, two of the three DUM3,4,5 neurones establish a small neurohaemal area near muscle 115 in the thorax (Bräunig, 1997).

Source of the synaptic drive

The occurrence of common synaptic potentials in particular DUM neurones indicates that these neurones are controlled by the same networks of presynaptic neurones. The simplest assumption is that a sustained sequence of common synaptic potentials of a particular amplitude and form is caused by spikes (or graded signals in non-spiking neurones) in the same presynaptic neurone. Alternatively, the neurones directly presynaptic to the DUM neurones could be independent but themselves be driven by common presynaptic neurones. More complex patterns of connections between the presynaptic neurones could also give rise to apparently common synaptic potentials. These latter arrangements would, however, not lead to the tight coupling between synaptic potentials seen, for example, in DUM3 (Fig. 1B) or DUM3,4 (Figs 2A, 7A) neurones, because the neurones directly presynaptic to the DUM neurones would not always spike simultaneously.

Cutting the connectives anterior and posterior to the

metathoracic ganglion reduced the synaptic drive to the DUM neurones, but correlated synaptic potentials still occurred. Moreover, when the metathoracic ganglion was completely isolated, the synaptic potentials in neurones of the three subsets remained strongly correlated and must therefore be derived from neurones within the metathoracic ganglion. Some of these could be local interneurones restricted to the metathoracic ganglion, but others could be intersegmental interneurones with their input and output branches within this ganglion.

In locusts that are quiescent, DUM3,4,5 and DUM5 neurones in the metathoracic ganglion have many synaptic potentials in common with the equivalent neurones in the mesothoracic ganglion, whereas the remaining different types of DUM neurones in the two ganglia share few synaptic potentials (Duch *et al.* 1998). During active movements, DUM3 and DUM3,4 neurones in the two ganglia also receive a common hyperpolarising synaptic drive. The common potentials must be caused by intersegmental interneurones, but the location and identity of these neurones have not been established. Similarly, in the moth *Manduca sexta*, abdominal unpaired median neurones in different ganglia have common synaptic potentials that are caused by intersegmental interneurones, apparently originating in the suboesophageal ganglion (Pflüger *et al.* 1993). As for the local drive from neurones within the metathoracic ganglion, the common intersegmental synaptic

drive biases particular groups of DUM neurones in the different ganglia to spike at same time (Duch *et al.* 1998).

Activation of DUM neurones

DUM neurones that belong to the same subsets, as defined by the strong correlations between their synaptic potentials and by their anatomy, tend also to respond with similar patterns of synaptic potentials and spikes to mechanosensory stimulation of the hind legs, hind wings or other parts of the body. Responses to mechanosensory stimuli were labile, depended on whether the locust was moving its legs, and occurred with a long latency. These observations and the lack of anatomical overlap between the neuropilar branches of DUM neurones (Watson, 1984) and the central projections of most exteroceptive mechanosensory neurones (Newland, 1991) indicate that unidentified interneurones must be involved in the sensory pathways. The subset of neurones formed by DUMETi, DUM5b and DUM3,4,5 neurones showed the strongest and most consistent response to mechanosensory stimulation, receiving a common synaptic drive and often producing a spike or burst of spikes. The same stimuli that excited neurones of this subset led, however, to a hyperpolarisation of other DUM neurones such as DUM1b, again emphasising differential control. No mechanosensory stimuli consistently led to spikes in DUM3,4 neurones, but instead their usual response to a stimulus on the hind legs or other parts of the body was a hyperpolarisation, particularly if there was an active movement of a leg. They, along with DUM3 neurones and DUMDL, also receive inhibitory input from the tegula receptors associated with the wings (C. Duch and H. J. Pflüger, in preparation). It is therefore not known what stimuli can make these particular DUM neurones spike and thus release octopamine at their targets within the thorax.

Behavioural implications

The different local and intersegmental synaptic drives to particular efferent DUM neurones and the way that these neurones are activated by different mechanosensory stimulation is further evidence that they are not a homogeneous population with a role only in controlling non-specific arousal. This adds to the accumulating evidence that particular DUM neurones are activated only during specific movements, an idea originally proposed by Sombati and Hoyle (1984). For example, DUMETi and DUM3,4,5 neurones, which supply muscles in the hind legs, spike in particular ways and at particular times during the motor pattern that generates kicking movements of the hind legs (Burrows and Pflüger, 1995). The same neurones also spike rhythmically during a fictive motor rhythm that resembles walking, whereas other DUM neurones do not (Baudoux *et al.* 1998). At the onset of flight and during fictive flight motor patterns, DUMDL, DUM3 and DUM3,4 neurones, which supply flight muscles, are hyperpolarised, whereas DUM neurones that supply leg muscles are depolarised and may spike during each cycle of the pattern (C. Duch and H. J. Pflüger, in preparation). Clearly, these neurones must be seen as integral parts of the neural machinery that generates and controls specific motor patterns and movements. Only specific DUM

neurones are activated during a particular movement through the local pathways characterised in the present paper and by intersegmental pathways (Duch *et al.* 1998; Pflüger *et al.* 1993). Efferent DUM neurones are therefore linked by their particular patterns of synaptic drive to the behaviour of the locust to fulfil specific modulatory functions. The task ahead is to identify the interneurones responsible for both the local and intersegmental drive of these neuromodulatory neurones.

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