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GABA-immunoreactivity in processes presynaptic to the terminals of afferents from a locust leg proprioceptor

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Summary

Individually labelled sensory neurons from the femoral chordotonal organ, a proprioceptor at the femoro-tibial joint of a locust hindleg, were analysed by intracellular recording, and by electron microscopical immunocytochemistry to reveal the arrangement of their input and output synapses and to determine whether the input synapses were GABAergic. Intracellular recordings from these sensory neurons show spikes superimposed on a barrage of synaptic potentials during movements of the femoro-tibial joint. These synaptic inputs can be mimicked by GABA. Input synapses are made onto the vesicle-containing terminals of afferents and are often closely associated with the output synapses. By contrast, the axons of the afferents in the neuropil have no vesicles and neither make nor receive synapses. The input synapses to the afferent terminals are made from processes typically a few microns in diameter, whereas the output synapses are made onto much smaller processes of only 0.1–0.2 μm. Input synapses at which an afferent terminal is the only postsynaptic element are common. Where the synapse is dyadic the second postsynaptic element does not usually appear to be a chordotonal afferent. The output synapses from the afferent terminals are usually dyadic. At 78% of the input synapses, the presynaptic neurite showed immunoreactivity to a GABA antibody, supporting the physiological evidence that the presynaptic effects can be mediated by the release of GABA. The remaining (22%) immunonegative synapses are intermingled with those showing GABA immunoreactivity, but their putative transmitter is unknown. These morphological observations suggest that the presynaptic control of the chordotonal afferents is largely mediated by GABAergic neurons, but because other types of neuron also appear to be involved, presynaptic modulation may be more complex than has yet been revealed by the physiology.

Introduction

Synaptic inputs to the terminals of sensory neurons are common in both vertebrates and invertebrates and can modify the transmission of the sensory signals at the first integrative stage in the central processing (Schmidt, 1971; Rudomin, 1990). The most frequently documented form of presynaptic modification of sensory signals results from an inhibitory synaptic input to the terminals of the sensory neurons. In vertebrates, this is usually caused by the release of GABA from interneurons (Eccles *et al.*, 1963; Davidoff, 1972; Nicoll & Alger, 1979). GABA can also mimic the inhibitory synaptic input to the terminals of a particular crayfish limb receptor (El Manira & Clarac, 1991; Cattaert *et al.*, 1992). In insects, electron microscopy has shown that sensory neurons also receive synaptic inputs (Geisert & Altner, 1974; Altman *et al.*, 1980; Watson & Pflüger, 1984; Watson, 1992) and that many of the presynaptic processes show GABA-like immunoreactivity (Watson 1990; Watson *et al.*, 1991; Watson & England, 1991). For only a few insect sensory neurons, such as the filiform hairs on the cerci (Blagburn & Sattelle, 1987; Boyan, 1988), or the femoral chordotonal organ of the locust (Burrows & Laurent, 1993) is there physiological evidence for presynaptic effects.

The femoral chordotonal organ is a proprioceptor containing some 90 sensory neurons (Matheson & Field, 1990) that each respond to particular characteristics of the movements of the femoro-tibial joint (Usherwood *et al.*, 1968a; Hofman & Koch, 1985; Hofman *et al.*, 1985; Zill, 1985; Matheson, 1992a). The afferents synapse onto known elements of the local circuits that control leg movements (Burrows, 1987; Burrows *et al.*, 1988; Laurent & Burrows, 1988; Büschges, 1989) and mediate both intra- and interjoint reflexes (Usherwood *et al.*, 1968a; Field & Rind, 1981). Synaptic potentials are generated in the terminals of these afferents whenever the femoro-tibial joint of the

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hindleg moves (Burrows & Matheson, in preparation). These potentials are normally depolarizing but reverse a few mV above the resting membrane potential of an afferent, indicating that they are depolarizing IPSPs (Burrows & Laurent, 1993). The physiological & pharmacological evidence suggests that the synaptic potentials in the afferents are mediated by GABA (Burrows & Laurent, 1993). To obtain further evidence that these input synapses may use GABA, we have labelled individual chordotonal afferents with horseradish peroxidase to allow ultrastructural analysis, and then used an antibody raised against GABA to demonstrate immunoreactivity in the processes making synapses with them. We show that many of the presynaptic neurons are immunoreactive for GABA.

Materials and methods

Adult male and female locusts, *Schistocerca gregaria* (Forskål), from our crowded colony were used in all experiments.

For intracellular recording and staining of chordotonal afferents, the thorax was opened ventrally and the meso- and metathoracic ganglia were stabilised on a wax-coated silver platform. The ganglionic sheath was treated for approximately 1 min with a 1% (w/v) solution of protease (Sigma type XIV) in saline. For intracellular staining, the electrodes were filled with 4–8% horseradish peroxidase (HRP Sigma type VI) in 0.2 M Tris buffer (pH 7.2) containing 0.5 mM KCl. The apodeme of the chordotonal organ was exposed in the femur and grasped by forceps attached to a vibrator (Ling Dynamic Type 101). This allowed it to be moved in defined waveforms, under the control of a microcomputer, that simulated normal movements of the femoro-tibial joint (Field & Burrows, 1982). The summed spike discharge of the chordotonal afferents excited by these movements was recorded extracellularly from nerve 5B1 (N5B1) in the femur. These electrodes were also used to stimulate the afferents electrically. The axon of an individual chordotonal afferent was impaled in nerve 5 close to the point where it enters the metathoracic ganglion.

To label a physiologically characterised chordotonal afferent for electron microscopy, HRP was injected intracellularly for 15 min using 0.5 s pulses of 5–10 nA depolarizing current at 1 Hz. The ganglia were then left in saline for a further 20 min to allow the HRP to diffuse throughout the terminal branches before processing for electron-microscopy according to the method described by Watson & Burrows (1981). The ganglia were fixed initially for 10 min *in situ* in 5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.2) containing 0.2 M sucrose. The metathoracic ganglion was then removed from the locust and fixed for a further 1–2 h at 20–22° C in the same fixative. After several washes with phosphate and then Tris buffer, the ganglion was immersed in 0.5 M cobalt chloride in Tris buffer for 10 min. It was subsequently rinsed in Tris and phosphate buffer before being cut into 150 μm transverse slices using a vibratome. Each sliced ganglion was incubated in phosphate buffer containing diaminobenzidine (DAB) in which hydrogen peroxide was generated by the action of glucose oxidase (Sigma V) on β-D-glucose. The slices were checked under a binocular microscope to confirm that a chordotonal afferent had been labelled successfully. They were then treated with 1% osmium tetroxide for 30 min and stained *en bloc* with 2% aqueous uranyl acetate. The slices were subsequently dehydrated in graded ethanols and embedded in Araldite CY212 resin between sheets of cleared electron-microscope film. After curing at 57° C for 24 h, the film was peeled off and the arborizations of the afferent axons within each slice were photographed and drawn with the aid of a drawing tube. Sections were mounted on nickel slot grids coated with Pioloform.

Ultrathin sections were etched for 30 min in 2% periodic acid and, after washing in distilled water, for 30 min in sodium metaperiodate saturated at 50° C. This supersaturation of the sodium metaperiodate was necessary to obtain consistent immunostaining of Araldite CY212 sections. After further washing the grids were floated on droplets of 5% normal goat serum in Tris buffer (pH 7.0) for 30 min before being transferred to solutions of primary antibody for 2 h. The rabbit anti-GABA antiserum (Cambridge Research Biochemicals Ltd) used at a dilution of 1:100. After incubation in the primary antiserum, the grids were washed in Tris buffer (pH 7.0) and then transferred to Tris buffer (pH 8.3) containing 1% BSA for 5 min before incubation in 15 nm gold-labelled goat anti-rabbit antibody (Biocell Ltd) diluted 1:30, for 1 h. After further washing the grids were stained first with 2% uranyl acetate and then with lead citrate. Preabsorption of the GABA antiserum with a GABA-glutaraldehyde-BSA conjugate eliminated all staining.

Results

Identifying spikes and synaptic potentials in chordotonal afferents

Intracellular recordings from the axons of chordotonal sensory neurons as they enter the metathoracic ganglion show spikes and synaptic potentials during movements of the hindlegs. These synaptic potentials can also be evoked by experimentally imposed movements of the chordotonal apodeme that stimulate a movement of the femoro-tibial joint, or by an electrical stimulus to the sensory nerve (N5B1) carrying the sensory neurons of the chordotonal organ (Figs 1A and B). A chordotonal afferent can be identified by its responses to imposed movements of the chordotonal apodeme, by correlating spikes recorded intracellularly with those recorded extracellularly from N5B1 close to the organ, and by evoking a spike at the central recording site with an electrical stimulus to N5B1. The afferent spikes are generated at the chordotonal organ in the femur and propagate toward the metathoracic ganglion, whereas the synaptic potentials are generated in the terminals of the afferents within the ganglion, some 200–600 μm from the recording electrode. Pressure injection of GABA into the neuropil close to the terminal branches of the chordotonal afferents results in a depolarisation of an afferent and a decrease in the resistance of its membrane (Fig. 1C).

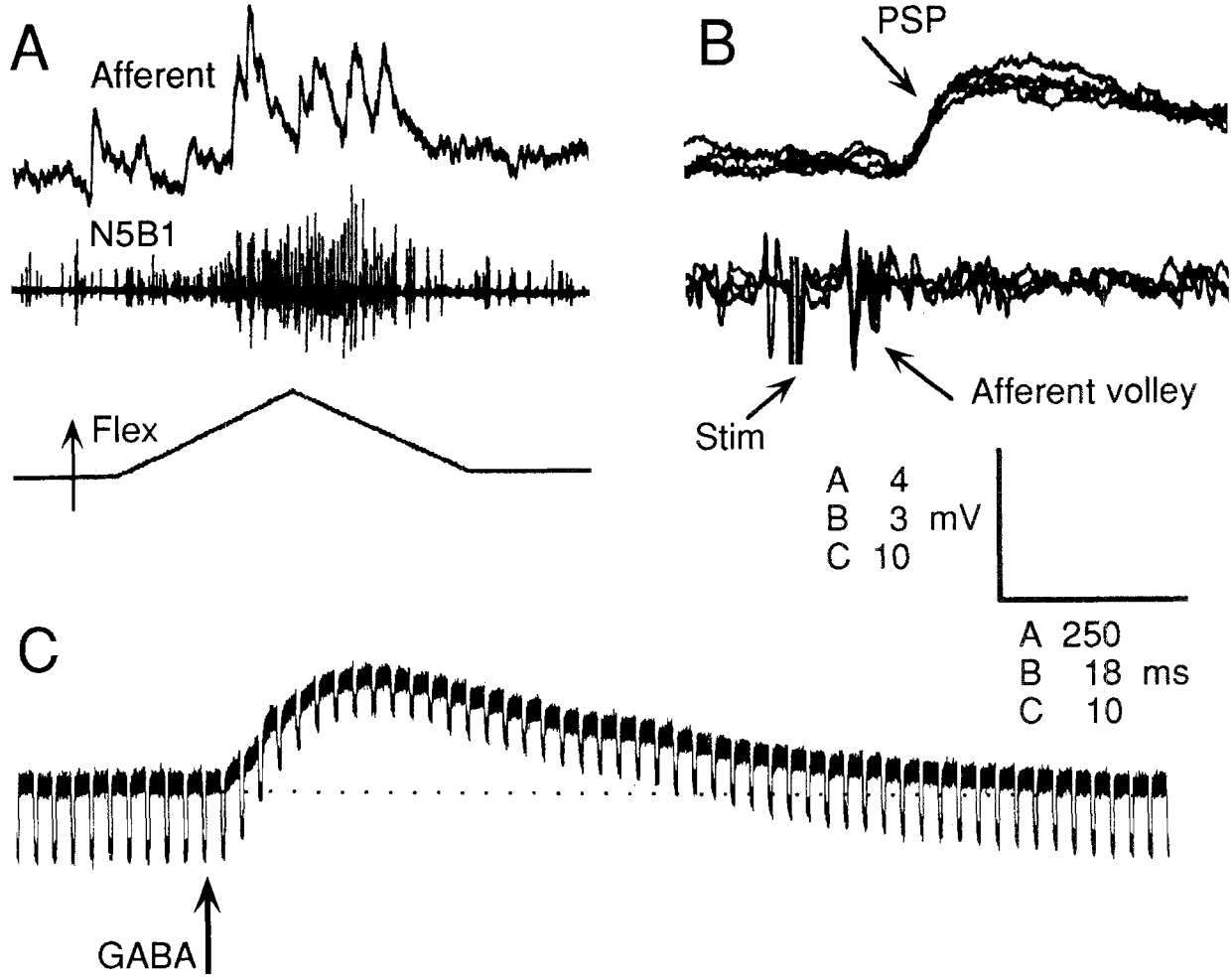
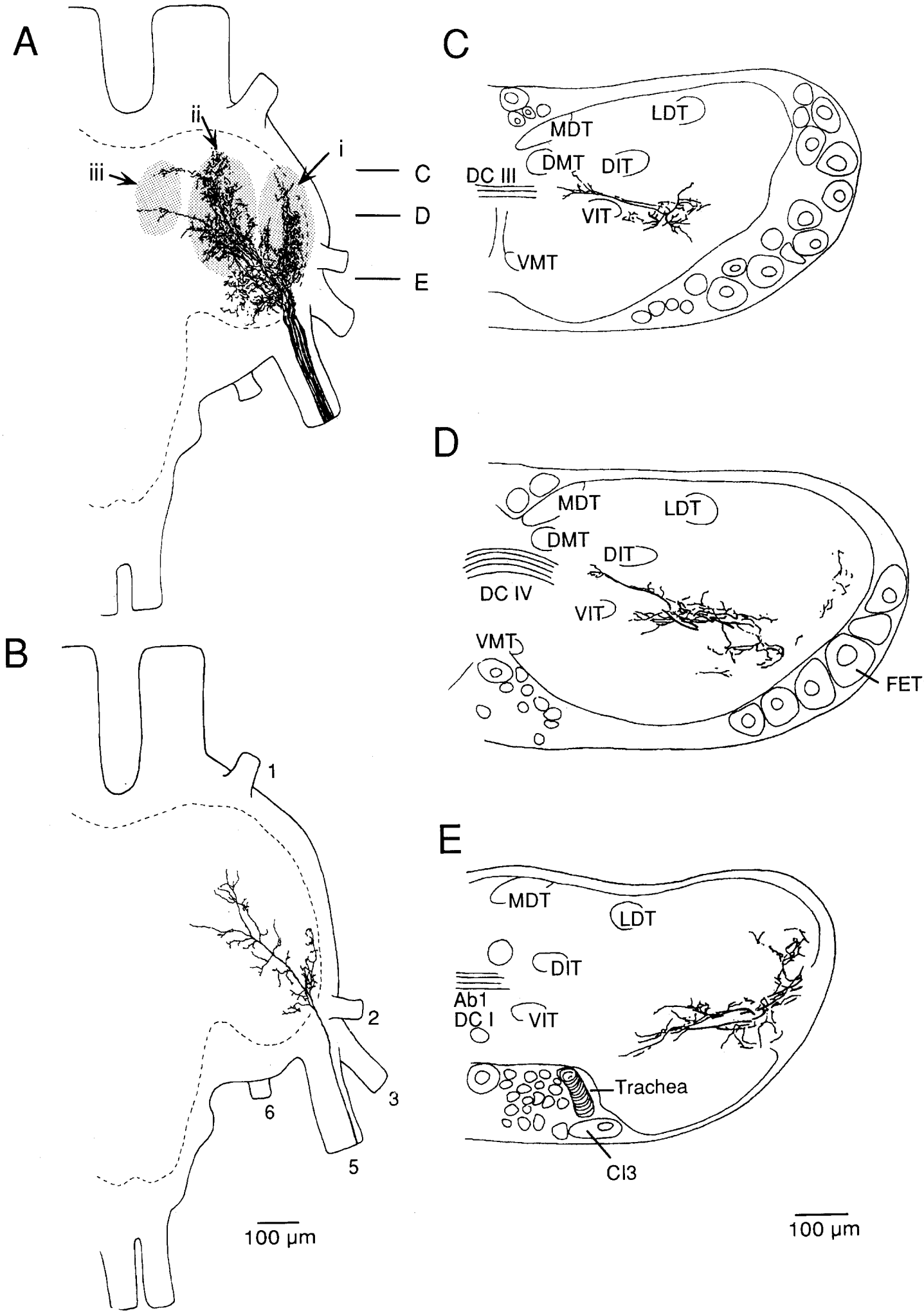


Fig. 1. Synaptic inputs to an afferent from the femoral chordotonal organ. (a) An imposed movement (lower trace) of the apodeme of the femoral chordotonal organ to simulate a movement of the femoro-tibial joint evokes spikes in many of its afferents (recorded extracellularly in N5B1, middle trace) and evokes depolarizing synaptic potentials in one afferent that is impaled with an intracellular electrode (upper trace). (b) Stimulation of the nerve supplying the chordotonal organ (N5B1) evokes an afferent volley in N5 as it enters the metathoracic ganglion (lower trace). The volley is followed by a synaptic potential in one of the chordotonal afferents (upper trace). Four traces are superimposed. (c) A pulse of 1 M GABA injected under pressure into neuropil containing the branches of chordotonal afferents evokes a depolarization of an afferent. The dotted line indicates the holding potential of the afferent. Pulses of hyperpolarizing currents show that the evoked depolarization is associated with an increase in the conductance of the membrane. The afferent was impaled in an isolated ganglion and current campled at −87 mV from its normal resting potential of −73 mV.

Detailed properties of these synaptic potentials are given in Burrows & Laurent (1993).

Morphology of the afferents

The morphology of afferents from the metathoracic femoral chordotonal organ has been described previously at the level of the light microscope following backfilling of N5B1 (Burrows, 1987) or, after physiological characterization, by intracellular injection of cobalt salts (Matheson, 1992b). The neurons which we identified here by their response to movements of the femoro-tibial joint and then injected with HRP all had morphologies consistent with these descriptions (Fig. 2). The terminal branches of these chordotonal afferents, many of which have a varicose appearance, project to three areas of neuropil (Fig. 2A). First (area (i) in Fig. 2A), the branches are in a lateral neuropil dorsal to the entry of the afferent axons (Figs 2A,B,D and E). Second (area (ii) in Fig. 2A), is a larger more medial area at the level of the ventral intermediate tract (VIT) that contains fine branches around the antero-medially running path of the afferent axons (Fig. 2A,B,D). Third (area (iii) in Fig. 2A) is a still more medial region that contains two bundles of afferent processes which run between VIT and the dorsal intermediate tract (DIT) to form terminal clusters of branches medial to these tracts (Fig. 2A–D). All three areas were examined in the electron microscope and were found to have a similar distribution of input synapses.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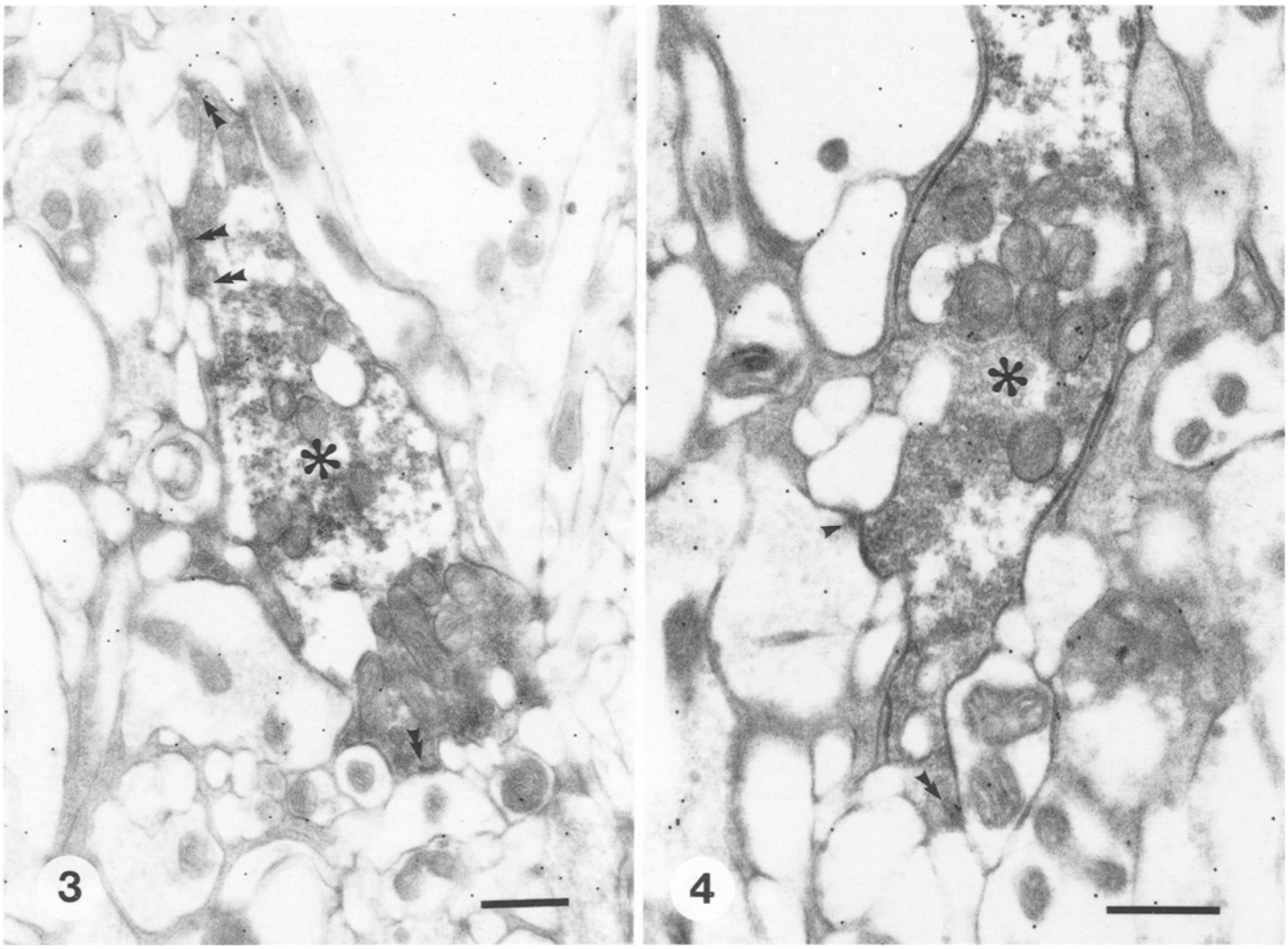


Fig. 3. A large terminal of an afferent that is labelled with HRP contains two regions where vesicles are clustered and makes several output synapses (double arrowheads) onto small diameter postsynaptic processes, immunonegative for GABA. Scale bar = 0.5 μm.

Fig. 4. An afferent terminal (asterisk) receives an input (arrowhead) from a process that does not show GABA-like immunoreactivity, close to an output synapse (double arrowhead). Scale bar = 0.5 μm.

Synaptic arrangements

The axons of the afferents, including those that project between DIT and VIT are devoid of vesicles, make no output synapses and receive no input synapses. In contrast, the fine terminal branches in the neuropil are filled with small, ovoid, electron-lucent vesicles, occasional large granular vesicles, and clusters of mitochondria. These branches not only make output synapses (Fig. 3), but also receive many input synapses (Fig. 4). The input and output synapses are

Fig. 2. Morphology of afferents from the femoral chordotonal organ drawn from light microscope images. (a) Drawing of a whole mount of the right half of the metathoracic ganglion in which chordotonal afferents were stained by backfilling nerve 5B1. The three areas of branching (i–iii) from which EM sections were taken are indicated. (b) A single afferent, impaled in its axon at the edge of the metathoracic ganglion, was stained intracellularly by the injection of cobalt ions following physiological characterization, and then drawn from a whole mount. The dashed line indicates the boundary of the neuropil, and lateral nerves 1–3, 5 and 6 are numbered. (c)–(e). Sections at the levels indicated in A of a ganglion in which a few chordotonal afferents were stained by the intracellular injection of HRP. These sections are of the material subsequently examined in the EM. The drawings show some of the major longitudinal tracts and commissures. Abbreviations: Ab1, first abdominal neuromere; CI3, common inhibitor 3 motor neuron; DC, dorsal commissure; DIT, dorsal intermediate tract; DMT, dorsal median tract; FETi, fast extensor tibiae motor neuron; LDT, lateral dorsal tract; MDT median dorsal tract; VIT, ventral intermediate tract; VMT, ventral median tract.

frequently seen in close proximity (Fig. 4). Output synapses are made onto processes which are often only 0.1–0.2 μm in diameter, that are devoid of vesicles and frequently clustered along one side of an afferent terminal (Fig. 3). A few of the postsynaptic processes are immunoreactive for GABA but the majority are not. The varicosities on these fine afferent branches, that often measure 2–3 μm in diameter, contain vesicles and mitochondria, and again have both input and output synapses.

Ultrastructural immunocytochemistry

The majority of the input synapses onto the terminals of the chordotonal afferents that were examined in all three regions of neuropil showed GABA-like immunoreactivity. Of 27 synapses observed in sections labelled with the GABA antibody, 21 (78%) were immunopositive (Figs. 5–9) while only 6 (22%) were immunonegative (Figs 4 and 9–11). Immunopositive processes making input synapses contain predominantly small agranular pleomorphic vesicles (Figs 5–8) but a few large granular vesicles are also occasionally present (Figs 5–6). Pleomorphic vesicles have been found at other known GABA immunoreactive synapses in crustaceans (Leitch *et al.*, 1990) and insects (Watson, 1988) and although their characteristic morphology is dependent on fixation (Tisdale & Nakajima, 1976), it is consistent with the distribution of GABA in these animals.

Input synapses to the afferents are generally made from processes that are 1 μm or more in diameter (Figs 6–8). Although many make synapses onto two postsynaptic processes (Figs 5–7, 9–11), input synapses in which the labelled chordotonal afferent is the only postsynaptic element are common (Fig. 8). Of 44 inputs synapses that were observed, 20 (45%) were of this type. As this seemed an unusually high proportion, results from other locust afferents that have been previously studied were re-examined for comparison. On the terminals of afferents from campaniform sensilla afferents 6% ($n = 78$) of the input synapses are one to one, from hair plates, 11% ($n = 104$), and from prosternal filiform hairs, 9% ($n = 78$) (data in Watson & England, 1991; Watson *et al.*, 1991; Watson & Pflüger, in preparation).

At immunopositive input synapses involving a postsynaptic dyad, the postsynaptic process that was not labelled with HRP was also not immunopositive for GABA (Figs 5–7,9). In some dyadic synapses, a rare arrangement was found in which the HRP-filled afferent received synaptic input from a GABA-immunoreactive and a non-immunoreactive process at the same site. For example, in Fig. 9 a GABA-immunoreactive process makes synaptic input onto the afferent and onto an unlabelled profile, which in turn makes a reciprocal synapse back onto the GABA-ergic process.

Discussion

Synaptic configurations

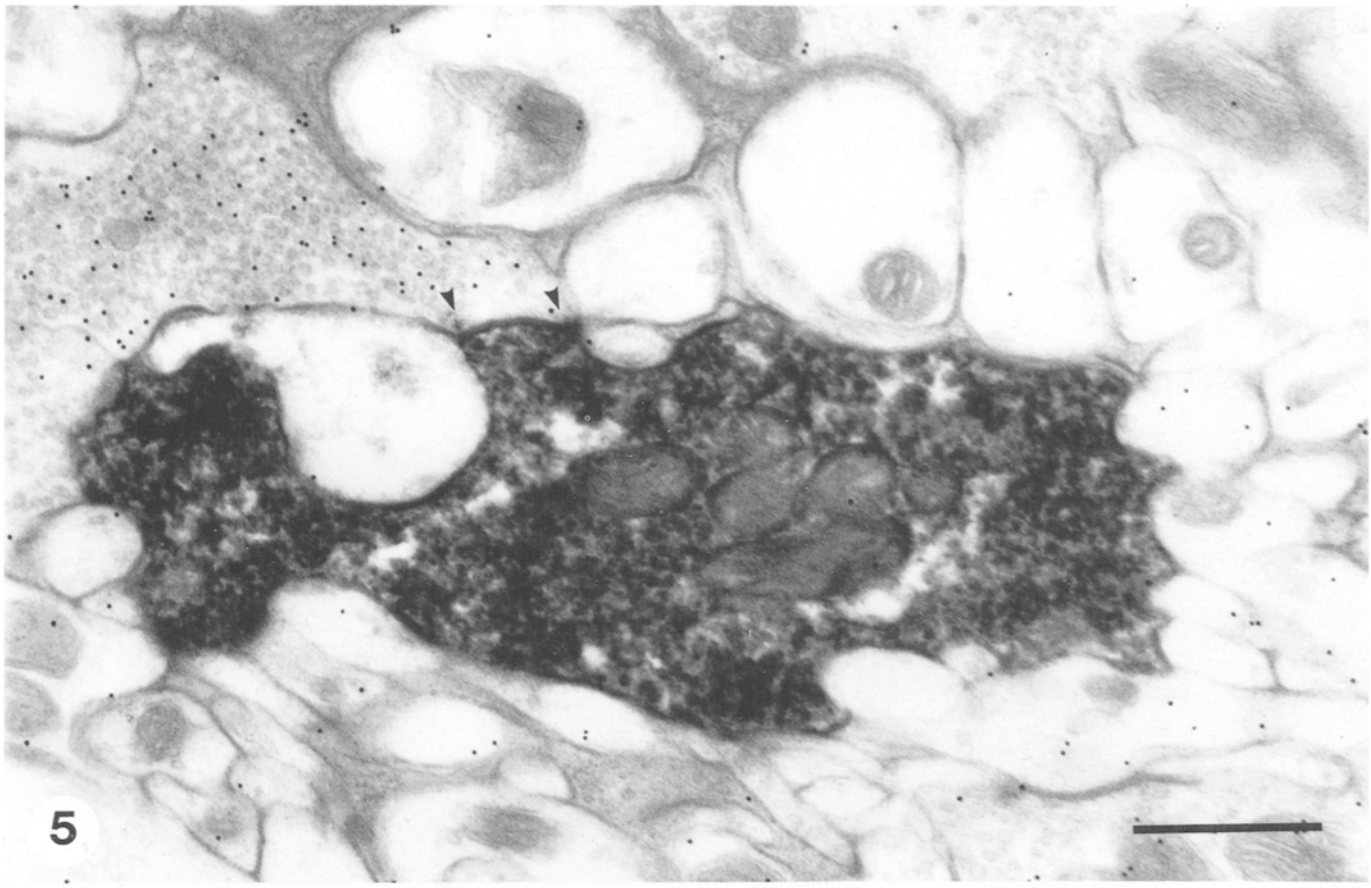
The close proximity of input and output synapses on the terminal branches of the chordotonal afferents is similar to that seen in other types of insect sensory neurons (locust hindwing stretch receptor, Altman *et al.*, 1980; prosternal filiform hairs, Watson & Pflüger, 1984; campaniform sensilla, Watson & England, 1991; hair plates, Watson *et al.*, 1991). By contrast, input synapses are absent from the main axons of these sensory neurons in the neuropil, and from terminal branches that have no output synapses. This close association of the input and output synapses suggests that the presynaptic control of transmitter release is precisely targeted. Furthermore, a single GABA-immunoreactive process may make several closely spaced input synapses on either chordotonal or other insect mechanosensory neurons (Watson & England, 1991; Watson *et al.*, 1991). This arrangement of multiple synaptic inputs, also occurs in other insect neurons (Watson & Burrows, 1982, 1983; Watson *et al.*, 1985; Burrows *et al.*, 1989). It would be expected here if a small number of neurons were responsible for the presynaptic modulation of a particular sensory neuron.

Synapses at which a chordotonal afferent is the only post-synaptic element are abundant, and even where the synapse is dyadic, the second postsynaptic element usually either lacks vesicles or contains many granular vesicles, features that are not characteristic of labelled chordotonal afferents. By contrast, a common synaptic configuration for locust hair plate afferents

Fig. 5. Process showing GABA-like immunoreactivity makes two input synapses (arrowheads) onto postsynaptic dyads comprising a labelled afferent and an unlabelled process. The presynaptic process contains small, agranular pleomorphic vesicles. The postsynaptic afferent process is large, whereas the second postsynaptic process is small. One of these contains a few agranular vesicles. The section is from neuropil region (ii). Scale bar = 0.5 μm.

Fig. 6. An immunpositive process makes input synapses (arrowhead) onto a small diameter afferent branch and a large unlabelled process which is devoid of vesicles. This section is from neuropil region (ii). Scale bar = 0.5 μm.

Fig. 7. An immunopositive process containing agranular pleomorphic vesicles makes an input synapse (arrowhead) onto a small diameter afferent branch and a large unlabelled process devoid of agranular vesicles. The section is from neuropil region (iii). Scale bar = 0.5 μm.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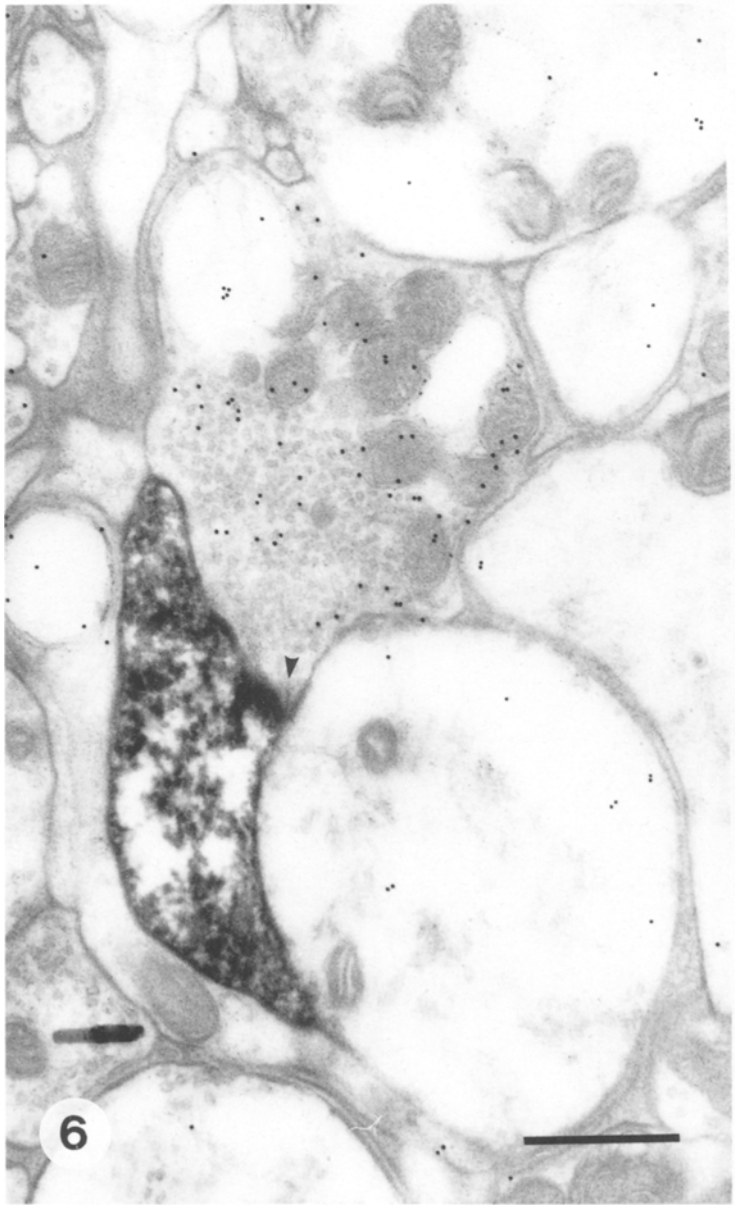
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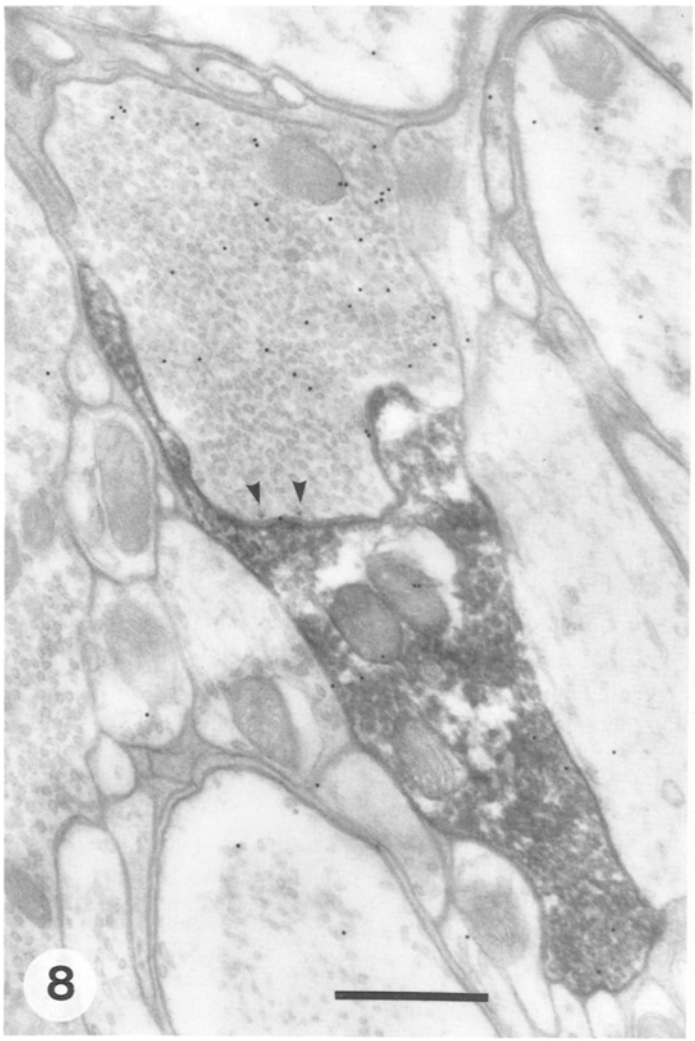
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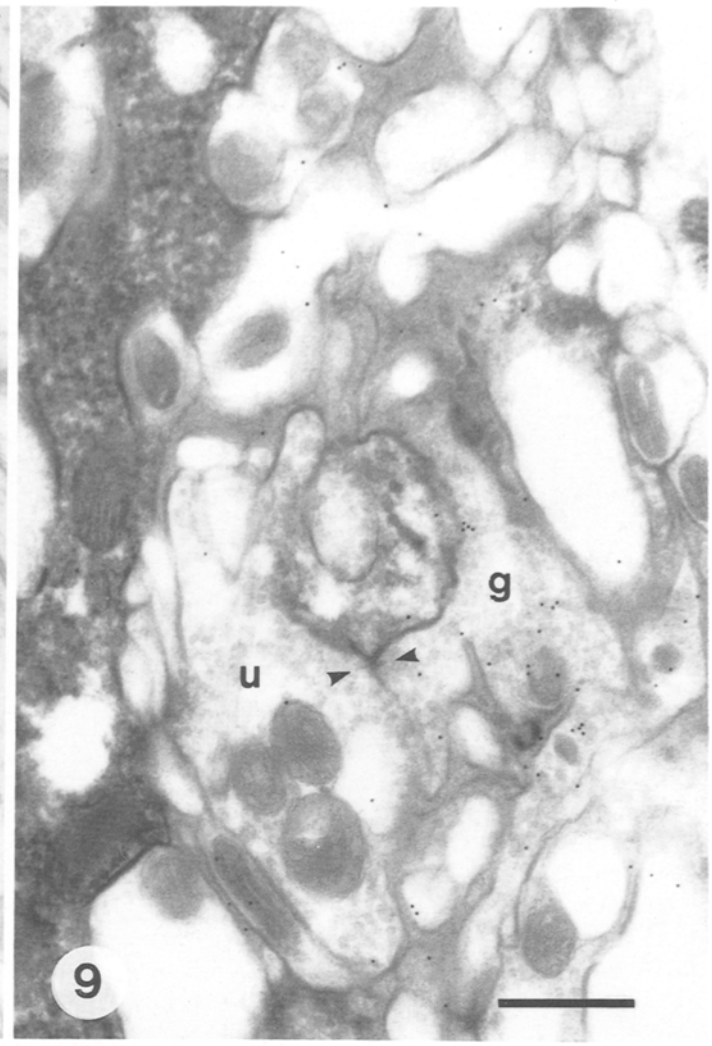
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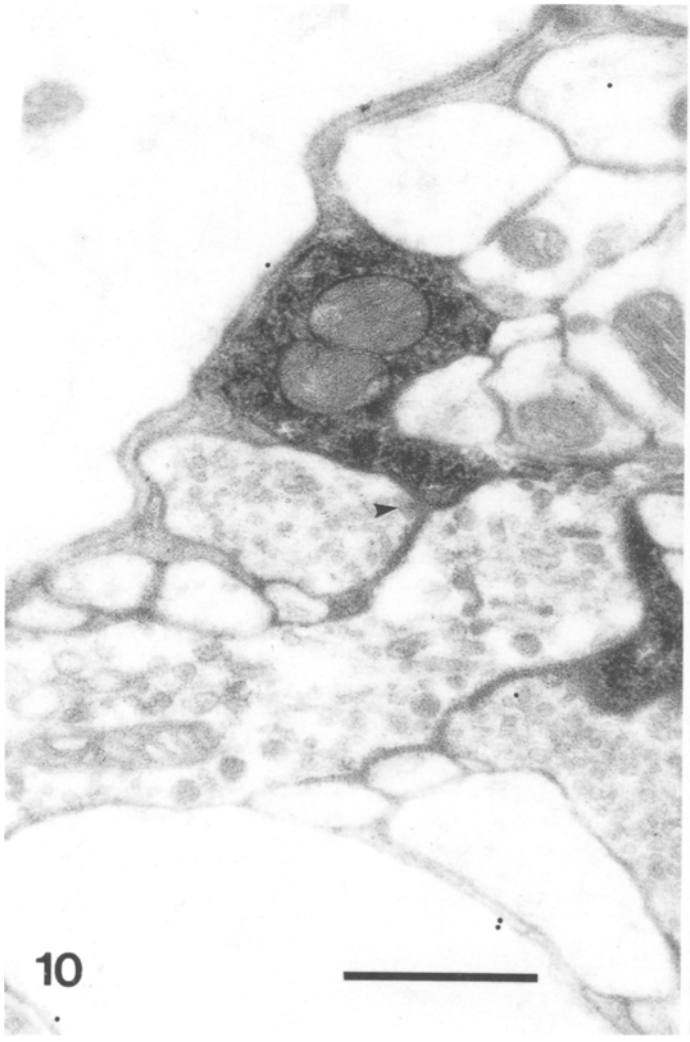
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consists of dyadic synapses where both postsynaptic processes are afferents (Watson *et al.*, 1991), indicating that one neuron is presynaptic to two sensory neurons at the same synapse. This arrangement was not seen for the chordotonal afferents. Part of the reason for the difference may lie in the way the two types of afferents were stained: many hair plate afferents were stained simultaneously by backfilling, whereas only a few chordotonal afferents were stained individually by intracellular injection. Another reason may lie in the nature of the populations of sensory neurons supplying each of these sense organs. The response properties of the hair plate afferents are qualitatively more homogeneous, with only two populations so far identified physiologically (Wong & Pearson, 1976), and therefore the role of presynaptic inhibition might be different. The chordotonal afferents show a much wider range of response properties and the presynaptic inhibition arises as a result of indirect interactions between them (Burrows & Laurent, 1993). Nevertheless, physiological experiments show that some chordotonal afferents receive common synaptic inputs though they do not indicate that they are controlled as a uniform population. Instead there seems to be differential control of sets of afferents with different response properties as a result of spikes in other chordotonal afferents (Burrows & Matheson, in preparation).

GABAergic presynaptic neurons

The role of GABA in presynaptic inhibition of sensory neurons is well established in both vertebrates and invertebrates (see review by Watson, 1992). Though not the only mechanism found in arthropods (Blagburn & Sattelle, 1987), a GABA mediated increase in chloride conductance is the one most commonly described (Hue & Callec, 1983; Boyan, 1988; El Manira & Clarac, 1991). For the chordotonal afferents analysed here, the physiological evidence also points to a dominant role for GABA in mediating the depolarizing inhibitory potentials recorded in their central terminals (Burrows & Laurent, 1993). GABA injected into the neuropil containing the processes of the afferents produces a depolarization in an afferent with the same reversal potential as the naturally occurring synaptic potentials, and can mimic the known effects of the synapses (Burrows & Laurent, 1993).

These synaptic inputs reduce the excitability of the afferent terminals (Burrows & Laurent, 1993), reduce the amplitude of the presynaptic afferent spikes and reduce the amplitude of the EPSPs that the afferents evoke in postsynaptic motor neurons (Burrows & Matheson, in preparation). The synaptic inputs to an afferent result from the spikes in other afferents from the same chordotonal organ but do not appear to involve direct interactions between the afferents themselves. Instead the pathway must involve at least one layer of interneurons. These interneurons, or other parallel sets of interneurons with similar properties, can also be activated by signals from other legs and by the networks that generate locomotory rhythms in isolated thoracic ganglia (Burrows & Matheson, in preparation). The consequence of these interactions is that the spikes of an afferent signalling a particular feature of the movement of the femoro-tibial joint will be superimposed on a synaptic input caused by the other afferents that are activated by this movement. The interactions may form a gain control mechanism that limits the effective output of the afferents on their target neurons in the local circuits that control posture and locomotion.

In the present study, ultrastructural immunocytochemistry shows that 78% of input synapses onto the chordotonal afferents are from processes showing GABA-like immunoreactivity. In other locust mechanosensory neurons the proportion of input synapses showing GABA-like immunoreactivity ranges from 51% for long prosternal filiform hairs (Watson & Pflüger, in preparation) through 72% for campaniform sensilla (Watson & England, 1991) to 93% for hair plates (Watson *et al.*, 1991). The chordotonal afferents are the only ones for which there is currently physiological evidence that GABA mimics the effects of their presynaptic inputs. The nature of the transmitter used by the presynaptic processes that show no immunoreactivity for GABA is unknown for the chordotonal afferents. For locust hair plate (Watson *et al.*, 1991) and prosternal hair afferents (Watson & Pflüger, in preparation) there is immuno-

Fig. 8. An afferent terminal receives two monodic input synapses (arrowheads) from a presynaptic process that is immunoreactive for the GABA antibody. Scale bar = 0.5 μm.

Fig. 9. Two posterior medial afferent branches are visible in this micrograph. The one more lightly stained with HRP receives two inputs (arrowheads) at the same synapse. One is made from a process immunoreactive for GABA (g), the other from one which is not (u). The section is from neuropil region (iii). Scale bar = 0.5 μm.

Fig. 10. A presynaptic process makes an immunonegative dyadic input synapse (arrowhead) onto a labelled afferent and onto an unlabelled process containing many large granular vesicles. Scale bar = 0.5 μm.

Fig. 11. An anterior medial branch of an afferent receives an input (arrowhead) from an immunonegative process. The section is from neuropil region (ii). Scale bar = 0.5 μm.

cytochemical evidence that some of their presynaptic neurons may be glutamatergic, but their effect is unknown. Glutamate is excitatory at insect neuromuscular junctions (Usherwood *et al.*, 1968b; Usherwood, 1981) and at the only central synapse so far described as glutamatergic (Sombati & Hoyle, 1981). Glutamate can, however, hyperpolarize some insect neurons (Usherwood *et al.*, 1980; Wafford & Sattelle, 1986; Dubas, 1990, 1991) though it is uncertain whether this effect in the central nervous system is mediated by synaptic or extrajunctional receptors. If glutamate is released by neurons presynaptic to chordotonal afferents then these neurons must either be activated by different pathways to those shown to cause presynaptic inhibition, or the synaptic potentials they evoke must have the same reversal potential as those evoked by GABA (Burrows & Laurent, 1993). The GABA-immunopositive and immunonegative input synapses are intermingled and in no regions of the arborizations is one type represented exclusively.

The electron microscopy reported in this paper therefore supports the conclusions of the physiological analysis of chordotonal afferents (Burrows & Laurent, 1993) that indicates a role for GABA presynaptic inputs. It also suggests that the control may be more intricate because other substances may be released from presynaptic processes. The action of these substances in modifying the afferent signals to the local circuits controlling movements of the legs must now be considered.

Acknowledgement

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References

ALTMAN, J. S., SHAW, M. K. & TYRER, N. M. (1980) Input synapses onto a sensory neurone revealed by cobalt-electron microscopy. *Brain Research* **189**, 245–50.

BLAGBURN, J. M. & SATTELLE, D. B. (1987) Presynaptic depolarization mediates presynaptic inhibition at a synapse between an identified mechanosensory neurone and giant interneurone 3 in the first instar cockroach, *Periplaneta americana*. *Journal of Experimental Biology* **127**, 135–57.

BOYAN, G. S. (1988) Presynaptic inhibition of identified wind-sensitive afferents in the cercal system of the locust. *Journal of Neuroscience* **8**, 2748–57.

BURROWS, M. (1987) Parallel processing of proprioceptive signals by spiking local interneurones and motor neurones in the locust. *Journal of Neuroscience* **7**, 1064–80.

BURROWS, M. & LAURENT, G. (1993) Synaptic potentials in the central terminals of locust proprioceptive afferents generated by other afferents from the same organ. *Journal of Neuroscience* **13**, 808–19.

BURROWS, M., LAURENT, G. J. & FIELD, L. H. (1988) Proprioceptive inputs to nonspiking local interneurones contribute to local reflexes of a locust hindleg. *Journal of Neuroscience* **8**, 3085–93.

BURROWS, M., WATSON, A. H. D. & BRUNN, D. E. (1989) Physiological and ultrastructural characterization of a central synaptic connection between identified motor neurones in the locust. *European Journal of Neuroscience* **1**, 111–26.

BUSCHGES, A. (1989) Processing of sensory input from the femoral chordotonal organ by spiking interneurones in the stick insect. *Journal of Experimental Biology* **144**, 81–111.

CATTAERT, D., EL MANIRA, A. & CLARAC, F. (1992) Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. *Journal of Neurophysiology* **67**, 610–23.

DAVIDOFF, R. A. (1972) GABA antagonism and presynaptic inhibition in the frog spinal cord. *Science* **175**, 331–3.

DUBAS, F. (1990) Inhibitory effect of L-glutamate on the neuropile arborizations of flight motoneurones in locusts. *Journal of Experimental Biology* **148**, 501–8.

DUBAS, F. (1991) Actions of putative amino acid neurotransmitters on the neuropile arborizations of locust flight motoneurones. *Journal of Experimental Biology* **155**, 337–56.

ECCLES, J. C., SCHMIDT, R. & WILLIS, W. D. (1963) Pharmacological studies on presynaptic inhibition. *Journal of Physiology* **168**, 500–30.

EL MANIRA, A. & CLARAC, F. (1991) GABA-mediated presynaptic inhibition in crayfish primary afferents by non-A, non-B GABA receptors. *European Journal of Neuroscience* **3**, 1208–18.

FIELD, L. H. & BURROWS, M. (1982) Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *Journal of Experimental Biology* **101**, 265–85.

FIELD, L. H. & RIND, F. C. (1981) A single insect chordotonal organ mediates inter- and intra-segmental leg reflexes. *Comparative Biochemistry and Physiology* **68A**, 99–102.

GEISERT, B. & ALTNER, H. (1974) Analysis of the sensory projection from the tarsal sensilla of the blowfly (*Phormia teranovae* Rob.-Desv., Diptera) *Cell and Tissue Research* **150**, 249–59.

HOFMANN, T. & KOCH, U. T. (1985) Acceleration receptors in the femoral chordotonal organ of the stick insect, *Cuniculina impigra*. *Journal of Experimental Biology* **114**, 225–37.

HOFMANN, T., KOCH, U. T. & BÄSSLER, U. (1985) Physiology of the femoral chordotonal organ in the stick insect, *Cuniculina impigra*. *Journal of Experimental Biology* **114**, 207–23.

HUE, B. & CALLEC, J. J. (1983) Presynaptic inhibition in the cercal-afferent giant-interneurone synapses of the cockroach, *Periplaneta americana* L. *Journal of Insect Physiology* **29**, 741–8.

LAURENT, G. & BURROWS, M. (1988) A population of ascending intersegmental interneurones in the locust with mechanosensory inputs from a hind leg. *Journal of Comparative Neurology* **275**, 1–12.

LEITCH, B., HEITLER, W. J., COBB, J. L. S. & PITMAN, R. M.

(1990) Anti-GABA antibodies label a subpopulation of chemical synapses which modulate an electrical synapse in crayfish. *Journal of Neurocytology* **19**, 929–36.

MATHESON, T. (1992a) Range fractionation in the locust metathoracic femoral chordotonal organ. *Journal of Comparative Physiology* **170**, 509–20.

MATHESON, T. (1992b) Morphology of the central projections of physiologically characterised neurones from the locust metathoracic femoral chordotonal organ. *Journal of Comparative Physiology* **170**, 101–20.

MATHESON, T. & FIELD, L. H. (1990) Innervation of the metathoracic femoral chordotonal organ of *Locusta migratoria*. *Cell and Tissue Research* **259**, 551–60.

NICOLL, R. A. & ALGER, B. E. (1979) Presynaptic inhibition, transmitter and ionic mechanisms. *International Review of Neurobiology* **21**, 217–58.

RUDOMIN, P. (1990) Presynaptic control of synaptic effectiveness of muscle spindle and tendon organ afferents in the mammalian spinal cord. In *The segmental motor system* (edited by BINDER, M. D. & MENDELL, L. M.) pp. 349–80. Oxford University Press, New York.

SCHMIDT, R. F. (1971) Presynaptic inhibition in the vertebrate central nervous system. *Ergebnisse der Physiologie* **63**, 20–101.

SOMBATI, S. & HOYLE, G. (1984) Glutamatergic central nervous transmission in locusts. *Journal of Neurobiology* **15**, 507–16.

TISDALE, A. D. & NAKAJIMA, Y. (1976) Fine structure of synaptic vesicles in two types of nerve terminals in crayfish stretch receptor organs, influence of fixation methods. *Journal of Comparative Neurology* **165**, 369–86.

USHERWOOD, P. N. R., RUNION, H. I. & CAMPBELL, J. I. (1968a) Structure and physiology of a chordotonal organ in the locust leg. *Journal of Experimental Biology* **48**, 305–23.

USHERWOOD, P. N. R., MACHILI, P. & LEAF, G. (1968b) L-glutamate at insect excitatory nerve-muscle synapses. *Nature* **219**, 1169–72.

USHERWOOD, P. N. R., GILES, D. & SUTER, C. (1980) Studies of the pharmacology of insect neurones in vitro. In *Insect Neurobiology and Pesticide Action (Neurotox 79)* pp. 115–28. Society of Chemical Industry. London.

USHERWOOD, P. N. R. (1981) Glutamate synapses and receptors on insect muscle. In *Glutamate as a neurotransmitter* (edited by DI CHIARA, G. & GESSA, L.) pp. 183–93. Raven Press, New York.

WAFFORD, K. A. & SATTELLE, D. B. (1986) Effects of amino acid neurotransmitter candidates on an identified insect motoneurone. *Neuroscience Letters* **63**, 135–40.

WATSON, A. H. D. (1988) Antibodies against GABA and glutamate label neurones with morphologically distinct synaptic vesicles in locust central nervous system. *Neuroscience* **26**, 33–44.

WATSON, A. H. D. (1990) Ultrastructural evidence for GABAergic inputs onto cercal afferents in the locust (*Locusta migratoria*). *Journal of Experimental Biology* **148**, 509–15.

WATSON, A. H. D. (1992) Presynaptic modulation of sensory afferents in the invertebrate and vertebrate nervous system. *Comparative Biochemistry and Physiology* **103A**, 227–39.

WATSON, A. H. D. & BURROWS, M. (1981) Input and output synapses on identified motor neurones of a locust revealed by the intracellular injection of Horseradish Peroxidase. *Cell and Tissue Research* **215**, 825–32.

WATSON, A. H. D. & BURROWS, M. (1982) The ultrastructure of identified locust motor neurones and their synaptic relationships. *Journal of Comparative Neurology* **205**, 383–97.

WATSON, A. H. D. & BURROWS, M. (1983) The morphology, ultrastructure and distribution of synapses on an intersegmental interneurone of the locust. *Journal of Comparative Neurology* **214**, 154–69.

WATSON, A. H. D. & PFLÜGER, H. J. (1984) The ultrastructure of prosternal sensory hair afferents within the locust central nervous system. *Neuroscience* **11**, 269–79.

WATSON, A. H. D., BURROWS, M. & HALE, J. P. (1985) The morphology and ultrastructure of common inhibitory motor neurones in the thorax of the locust. *Journal of Comparative Neurology* **239**, 341–59.

WATSON, A. H. D. & ENGLAND, R. C. D. (1991) The distribution of and interactions between GABA-immunoreactive and non-immunoreactive processes presynaptic to afferents from campaniform sensilla on the trochanter of the locust leg. *Cell and Tissue Research* **266**, 331–41.

WATSON, A. H. D., STORM-MATHISEN, J. & OTTERSEN, O. P. (1991) GABA and glutamate-like immunoreactivity in processes presynaptic to afferents from hair plates on the proximal joints of the locust leg. *Journal of Neurocytology* **20**, 796–809.

WONG, R. K. S. & PEARSON, K. G. (1976) Properties of the trochanteral hair plate and its function in the control of walking in the cockroach. *Journal of Experimental Biology* **64**, 233–49.

ZILL, S. N. (1985) Plasticity and proprioception in insects. I. Responses and cellular properties of individual receptors of the locust metathoracic femoral chordotonal organ. *Journal of Experimental Biology* **116**, 435–61.