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Octopamine-immunoreactive neurons in the central nervous system of the cricket, *Gryllus bimaculatus*

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Summary. The distribution of octopamine-immunoreactive neurons is described using whole-mount preparations of all central ganglia of the cricket, *Gryllus bimaculatus*. Up to 160 octopamine-immunoreactive somata were mapped per animal. Medial unpaired octopamine-immunoreactive neurons occur in all but the cerebral ganglia and show segment-specific differences in number. The position and form of these cells are in accordance with well-known, segmentally-organized clusters of large dorsal and ventral unpaired medial neurons demonstrated by other techniques. In addition, bilaterally arranged groups of immunoreactive somata have been labelled in the cerebral, suboesophageal and terminal ganglia. A detailed histological description of octopamine-immunoreactive elements in the prothoracic ganglion is given. Octopamine-immunoreactive somata and axons correspond to the different dorsal unpaired medial cell types identified by intracellular single-cell staining. In the prothoracic ganglion, all efferent neurons whose primary neurites are found in the fibre bundle of dorsal unpaired cells are immunoreactive. Intersegmental octopamine-immunoreactive neurons are also present. Collaterals originating from dorsal intersegmental fibres terminate in different neuropils and fibre tracts. Fine varicose fibres have been located in several fibre tracts, motor and sensory neuropils. Peripheral varicose octopamine-immunoreactive fibres found on several nerves are discussed in terms of possible neurohemal releasing sites for octopamine.

Key words: Nervous system, central – Ganglia, invertebrate – Octopamine – Immunocytochemistry – DUM neurone – Neurosecretion – *Gryllus bimaculatus* (Insecta)

Octopamine (OA) acts as a neuroactive substance in several invertebrate groups (for review, see David and Coulon 1985; Evans 1985; Agricola et al. 1988). In the lobster, a change from aggressive towards evasive behaviour and vice versa can be induced by injection of OA or serotonin, respectively (Livingstone et al. 1980; Kravitz 1988). In the nervous system of insects, the presence of OA has been shown in several studies employing different techniques. Physiological and pharmacological experiments provide evidence that OA acts as a neurohormone (Goosey and Candy 1980, 1982; Davenport and Evans 1984), neuromodulator (Hoyle 1974, 1975, 1978; O'Shea and Evans 1979; Sombati and Hoyle 1984; Malamud et al. 1988; O'Gara and Drewes 1990) and neurotransmitter in insects (Christensen and Carlson 1981, 1982; Carlson and Jalenak 1986; Orchard et al. 1986).

Monoamine-containing cells have been stained by the vital dye Neutral Red in several invertebrates (see Adams et al. 1983). Although the chemical nature of this staining reaction is unclear, it has been considered as a selective but non-specific marker of putative octopaminergic neurons in insects (Evans 1980). Based on this staining, it has been suggested that large dorsal unpaired medial (DUM) neurons contain OA as a neuroactive substance (Goodman et al. 1980; Evans 1980). However, OA has been biochemically determined only in the isolated somata of some DUM cells (Hoyle and Barker 1975; Evans and O'Shea 1978; Dymond and Evans 1979; Orchard and Lange 1985).

DUM and ventral unpaired medial (VUM) neurons have been morphologically identified in the ventral ganglia of various insect species (Plotnikova 1969; Crossman et al. 1971; Evans and O'Shea 1978; Hoyle 1978; Watson 1984; Arikawa et al. 1984; Lange and Orchard 1986; Bräunig 1988; Pflüger and Watson 1988; Tanaka and Washio 1988; Ferber and Pflüger 1990; Hahnel and Bräunig 1989; Bräunig et al. 1990).

In a recent investigation, Konings et al. (1988) successfully employed an antibody against an OA-conjugate in the central nervous system (CNS) of an insect for the first time. They found OA-immunoreactivity in a number of unpaired and paired neurons in the metathoracic ganglion and in various neuropil areas in the brain of the locust.

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The present study deals with the immunocytochemical description of octopaminergic neurons in the entire CNS of the cricket, *Gryllus bimaculatus*. We show the number and distribution of OA-immunoreactive (OA-ir) neurons and provide some evidence for segment-specific variations of stained cells. For a detailed morphological study, we have selected the prothoracic ganglion, as different DUM cell types of only this ganglion have been thoroughly investigated by means of Lucifer-Yellow single-cell stains and electrophysiology during behaviour (Gras et al. 1990). The Lucifer-Yellow histology of the DUM cells thus serves as a comparison for the OA-ir medial cells of this ganglion.

Materials and methods

Immunocytochemistry

Female and male crickets (*Gryllus bimaculatus* de Geer), freshly moulted or up to 14 days after imaginal ecdysis, were collected from a breeding colony (28° C, 12 h–12 h light and dark cycle). Successful immunolabelling seemed to be dependent on the treatment of crickets prior to fixation. Enhanced motor activity of animals prior to immunostaining experiments was often correlated with a reduction of the intensity of immunolabelling. Therefore, collected animals were kept immobilized by cooling (4° C) 3–6 h prior to preparation. The animals were anaesthetized by cooling and then pinned down in a slightly stretched condition. During preparation, the ganglia were kept in special buffer (see Taghert and Goodman 1984) and then quickly immersed in fixative. In situ whole-mounts (for details, see Dircksen et al. 1991) were fixed during preparation. The immunostained ganglia of 65 animals were examined.

The following fixation procedures were used. (1) Glutaraldehyde fixative: 1.5% glutaraldehyde (v/v) in 0.1 M cacodylate buffer containing 1% sodium metabisulfite (SMB, $Na_2S_2O_5$, Merck, Darmstadt, FRG; w/v), pH 6.8 for 0.5–1 h and pH 4.0 overnight, or only pH 4.0 overnight, at 0° C. (2) Glutaraldehyde-fixative (see above) containing 0.25%–1% Triton X-100 (Sigma, Deisenhofen, FRG, w/v). (3) A mixture of glutaraldehyde and picric acid (GPA, Konings et al. 1988) containing 1% SMB (w/v), for 4–6 h or overnight, at 0° C. Fixation with the GPA fixative revealed only weak immunohistochemical staining.

After fixation, the ganglia were processed for whole-mounts or serial sectioning. Either Paraplast (thickness: 5–10 μm) or Vibratome sections (thickness: 40–70 μm) were used for immunolabelling. Horizontal, sagittal and frontal Vibratome sections were cut from agarose-embedded ganglia (agarose: Biomol, Hamburg, FRG; 5% v/w). Whole-mounts were rinsed in 0.05 M TRIS-HCl buffer (pH 7.2) containing 0.9% NaCl (w/v) and 0.25% Triton X-100 (Sigma; w/v), for 3–4 days. In addition, to facilitate antibody penetration, whole-mounts were subjected to a collagenase-dispase (Boehringer Mannheim, Mannheim, FRG) treatment prior to immunohistochemical staining (1 mg/ml buffer, for 0.5–1.5 h). Vibratome sections were treated with 0.05 M TRIS-HCl buffer (pH 7.2) containing 0.9% NaCl (w/v) and 0.25% Triton X-100 (w/v), for 2–12 h. In order to saturate double bonds (McRae-Degueurce and Geffard 1986), all preparations were washed in a 0.13 M sodium borohydride ($NaBH_4$, Sigma; 5–10 min) in TRIS-HCl buffer (pH 7.6). After subsequent washing in buffer, preparations were incubated with normal goat serum (10% v/v; Dakopatts, Hamburg, FRG; 1 h, 4° C) in phosphate buffer. Background labelling was reduced by diluting the antisera in 0.01 M phosphate buffer with a high sodium chloride concentration (Davis et al. 1989) and extended washes between successive antiserum incubations (1–2 h). The peroxidase-antiperoxidase (PAP) technique (Sternberger 1979) was used for sections and whole-mounts. Generally, we sequentially employed rabbit antiserum against OA (1:250–1:750 v/v in phosphate buffer containing 0.25% (w/v) Triton X-100, 12–24 h, 4° C), goat anti-rabbit antiserum (Sigma, 1:40 v/v, 12 h, 4° C), and rabbit PAP-complex (Dakopatts, 1:40–1:160 v/v, 12 h, 4° C). The staining process was monitored during treatment of the preparations with 0.02% 3.3-diaminobenzidine tetrachloride (DAB, Aldrich, Steinheim, FRG; w/v) in TRIS-HCl buffer (final pH 7.6) containing 0.005% H_2O_2 (v/v). After washing in TRIS-HCl buffer, the whole-mounts were dehydrated and embedded in Durcupan (Fluka, Neu-Ulm, FRG). Mounted Vibratome sections were post-fixed in osmium tetroxide (0.5%–1.5%, w/v, 1–5 min), rinsed in phosphate buffer, dehydrated and embedded.

Paraplast sections showed weak immunostaining or high background labelling together with poor neuropil preservation and were found to be of poor quality when compared with Vibratome sections. For comparative morphological studies, prothoracic ganglia were treated with osmium-ethylgallate staining modified after Wigglesworth (1957), embedded in Durcupan and cut in serial sections (thickness: 20 μm).

For comparison with unpaired medial OA-ir neurons, DUM neurons that had been intracellularly stained with Lucifer-Yellow (see Gras et al. 1990) were prepared as whole-mounts or sections. Our attempts to obtain double labelling of single DUM cells with Lucifer-Yellow and OA-immunocytology failed. This negative result is probably obtained because of the stress-induced release of OA during preparation and the membrane damage from intracellular recording.

With respect to classification, the largest diameters of somata were measured from photographs or camera lucida drawings, showing whole-mounts of cerebral ganglia in ventro-lateral view and the other ganglia in dorsal or ventral view.

Antiserum and specificity tests

The antiserum to OA was prepared by immunizing rabbits with OA coupled to thyroglobulin (Th) by glutaraldehyde. This antigen was obtained according to the method of Buijs et al. (1989) by mixing the following solutions: 0.8 ml Th (2.5 mg/ml), 0.1 ml OA (6.5 mg in 1 ml phosphate buffer, pH 7.4), and 0.1 ml glutaraldehyde (1% solution). After 30 min, the reaction was stopped by adding 5 mg $NaBH_4$. Thereafter, the immunogene was dialyzed against 0.2% $Na_2S_2O_5$-buffer (pH 6, 4° C). A rabbit was immunized with a mixture of 1 ml conjugate and 1 ml complete Freund's adjuvant administered in equal parts intramuscularly and subcutaneously. Blood was collected from the rabbit 6, 7 and 8 weeks after immunization; it was kept at 4° C for several hours, and centrifuged at 3000 rpm, for 10 min. The supernatant was collected and freeze-dried.

The specificity of the OA-antiserum was tested by determining the immunoreactivity of the antiserum with OA, noradrenaline, dopamine, tyramine and 5-hydroxytryptamine (serotonin). Known amounts of neuroactive substances (ranging from 0.6–1000 ng/μl per spot) were fixed by means of glutaraldehyde onto a nitrocellulose-gelatin matrix. The nitrocellulose was incubated according to Van der Sluis et al. (1988) and, after staining with DAB-nickel, the optical density of the spots was measured using an IBAS-Kontron image analyzing system.

As a control, ventral ganglia cut in sections were stained after omission of the first, second or third antibody, or with the primary antiserum preabsorbed with OA, tyramine, Th and the glutaraldehyde-coupled conjugates Th-OA and Th-tyramine.

Results

Antibody specificity

All specifity tests mentioned above demonstrated the very high affinity of the antiserum for the Th-OA conju-

Table 1. Results of control reactions with preabsorbed antiserum. The thyroglobulin(Th)-amine conjugates were prepared according to Geffard et al. (1984). +++, Regular staining (result as in controls without preabsorption); ++, moderate staining; +, weak staining; −, no staining

Octopamine antiserum at working dilution preabsorbed with:		Staining intensity
Octopamine	10^{-7} M	+++
	10^{-5} M	+
	10^{-3} M	±
Tyramine HCL	10^{-7} M	+++
	10^{-5} M	+++
	10^{-3} M	++
Tyramine CHR	10^{-7} M	+++
	10^{-5} M	+++
	10^{-3} M	+++
Th-Octopamine	2.5 µg/ml antiserum	+++
	25 µg/ml antiserum	±
	250 µg/ml antiserum	−
Th-Tyramine HCL	2.5 µg/ml antiserum	+++
Th-Tyramine HCL	25 µg/ml antiserum	+++
Th-Tyramine HCL	250 µg/ml antiserum	++
Th-Tyramine CHR	2.5 µg/ml antiserum	+++
Th-Tyramine CHR	25 µg/ml antiserum	+++
Th-Tyramine CHR	250 µg/ml antiserum	++
Thyroglobulin	250 µg/ml antiserum	+++

gate. The cross-reactivity with tyramine was greater than 1:20 (affinity of the OA-antiserum was 20 times greater for OA than for tyramine), with dopamine greater than 1:300, with noradrenaline and serotonin greater than 1:600. No immunoreactive structures were observed after the omission of the first, second or third antibody, and the control reactions with preabsorbed antiserum showed only a slight cross-reactivity with tyramine (Table 1). OA-ir immunostaining could not be blocked with OA itself or the OA-Th conjugate at concentrations of 10^{-7}, as has been shown for another antibody in a previous paper (Konings et al. 1988).

Immunolabelling

Whole-mount preparations were suitable for soma mapping but gave less information about the distribution of fibres. In Vibratome sections, axons, collaterals and fine varicose branches were labelled, in addition to the somata. The antiserum to OA consistently labelled distinct cell groups, but variations in the numbers of cells and intensity of labelling were observed. The partially incomplete and discontinuous staining of OA-ir neurons and the overlapping of fine fibres prevented single-cell description.

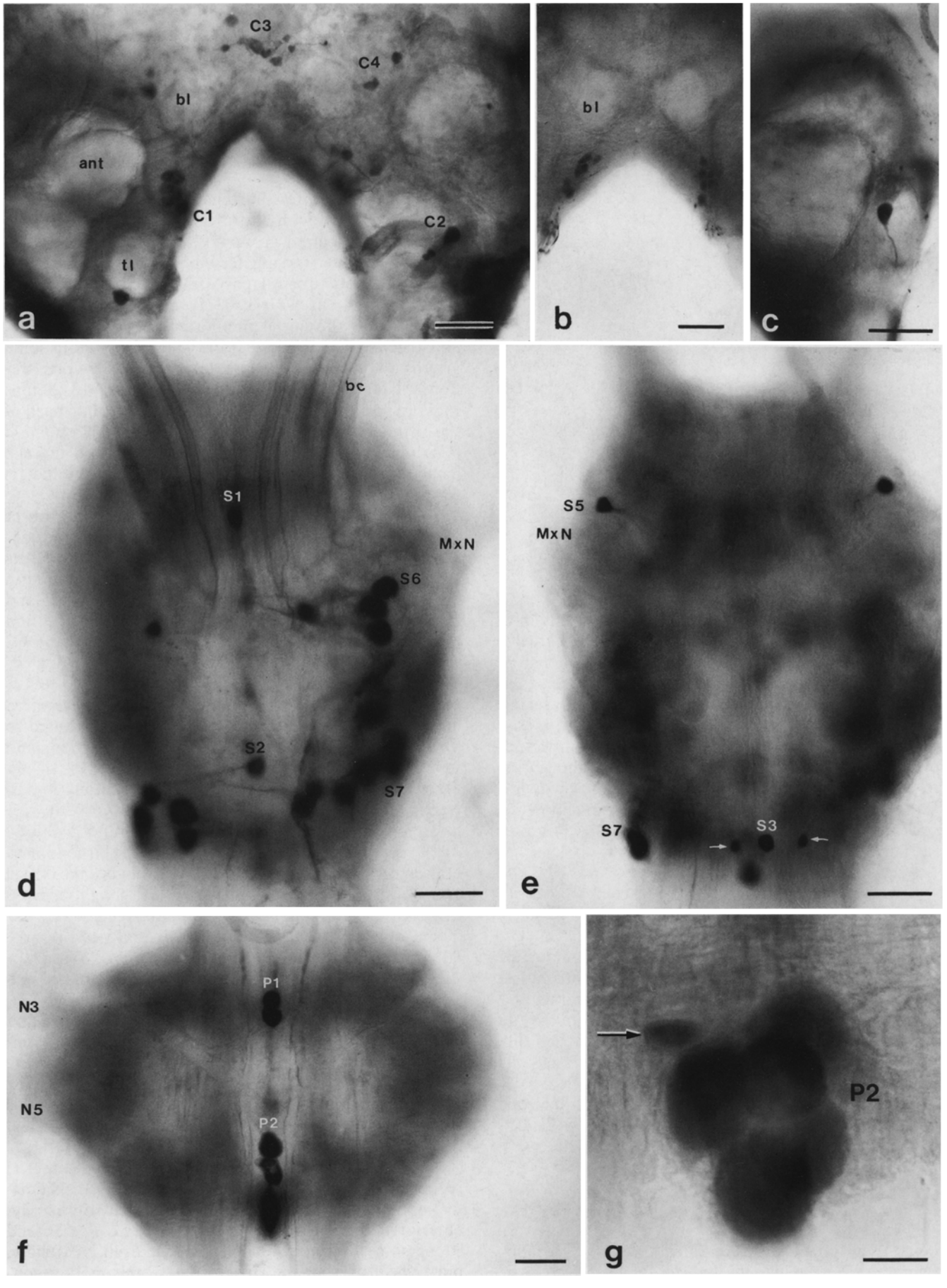
Distribution of OA-ir neurons in ganglia of the CNS

Whole-mount preparations revealed up to 160 OA-ir somata in the CNS; single cells and cell clusters were classified according to their location and soma size. Since whole-mounts gave only limited information about OA-ir fibre tracts and neuropils, we examined serial sections of the prothoracic ganglion. This specific approach allowed the identification of OA-ir neurons that corresponded with single-cell staining from DUM cells of the prothoracic ganglion obtained in electrophysiological experiments.

Cerebral ganglion. OA-ir somata ($n=11$–32) were primarily associated with rostral soma layers and were arranged in five paired groups (C1–5, Figs. 1a–c, 3a, 4). C1 somata ($n=6$–8, diameter: about 30 µm) were located in the ventro-rostral part of the cerebral ganglion (Fig. 1a, b). A pair of large OA-ir somata (C2; diameter: 30–50 µm) was found at the border between the deuto- and tritocerebrum (Fig. 1a, c). The other OA-ir soma groups (C3–5) could only be detected in a few preparations. Up to 10 somata (C3; diameter: 10–20 µm; Figs. 1a, 3a) were found in the rostro-medial part of the protocerebrum. Lateral to group C3, 3–5 somata (C4; diameter: about 20 µm) were distributed in each hemiganglion. Another pair of somata (C5; diameter: 30 µm) was found in the lateral brain area dorsal to the antennal nerves (Fig. 3a). Furthermore, a pair of weakly-labelled somata (diameter: about 30 µm) was observed at the lateral edge of the tritocerebrum. Some somata ($n=1$–3; diameter: about 10 µm) were found in the antero-lateral soma layer between the lamina and medulla of the optic lobes. OA-ir somata in the brain appeared to be labelled with varying intensity, the C2 somata usually being the most strongly stained.

Suboesophageal ganglion. Paired lateral and unpaired medial OA-ir somata ($n=17$–30) could be attributed to three neuromers of the suboesophageal ganglion, and were divided into 7 groups (S1–7, Figs. 1d, e, 3b, 4). Four groups of medial somata (diameter: about 30 µm) were present. Group S1 ($n=1$–3) and S2 ($n=0$–6) were located near the dorsal ganglion surface, and group S4 ($n=2$) near the ventral surface, whereas the large somata of group S3 ($n=2$–4) were distributed between the dorsal and ventral surface of the posterior ganglion parts (Fig. 1d, e). A pair of small somata (diameter: 15–20 µm) was always observed ventro-laterally to the large S3 somata. Each of the three dorsal medial OA-ir groups (S1–3) corresponded to one of the three neuromeres previously identified by serial homologous serotonin-ir neurons in *Gryllus* (Spörhase-Eichmann and Schürmann 1988) and *Locusta* (Tyrer et al. 1984). An additional ventral group (S4) was found in the second neuromere (Fig. 3b), whereas we only sporadically observed scattered ventro-medial somata in the first neuromere. The somata of group S4 were always less intensely labelled than the somata of the groups S1–3.

The soma groups S5–7 were located in the lateral region of the ganglion. One pair of somata (S5; diameter: about 20 µm) was usually located ventro-laterally, anteriorly to the maxillary nerve (Fig. 1e). The S6 and S7 somata (diameter: 30–50 µm) occurred bilaterally, but typically either the right or left hemiganglion con-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tained more somata; sometimes one side contained no labelled cells. In group S6, somata were more frequently absent in one hemiganglion than in group S7. Somata of S6 and S7, usually found in a dorso-lateral location, sometimes appeared shifted medially, projecting their axons towards the dorsal midline. The pear-shaped somata of group S6 ($n=0$–10 per ganglion, Fig. 1d) lay posteriorly to the margin of the maxillary nerve. Somata of group S7 ($n=2$–14 per ganglion, Fig. 1d, e) were located near the labial nerve roots. Up to 20 fibres in each brain connective and up to 6 OA-ir fibres in each neck connective were stained.

Prothoracic ganglion. In contrast to the head ganglia, exclusively medial unpaired OA-ir somata ($n=10$–15) forming an anterior (P1, $n=0$–2) and a posterior (P2, $n=8$–14) group were present in the prothoracic ganglion (Figs. 1f, g, 3c). One of the P1 somata (diameter: about 40 μm) was located medially between the ventral and dorsal surface; the other lay more posteriorly, close to the dorsal surface. The large medial P2 somata ($n=8$–11, diameter: 40–60 μm) were located close to either the posterior dorsal or ventral surface. In addition, up to 4 small OA-ir somata in group P2 (diameter: about 20 μm) were found near the large ventral medial OA-ir somata (Figs. 1g, 3c).

In each hemiganglion, up to 4 OA-ir fibres were marked at the level of the medial dorsal tract (MDT), further one at the lateral dorsal (LDT) and another near the lateral ventral (LVT) tract (tract terminology according to Wohlers and Huber 1985). A more detailed description is given below.

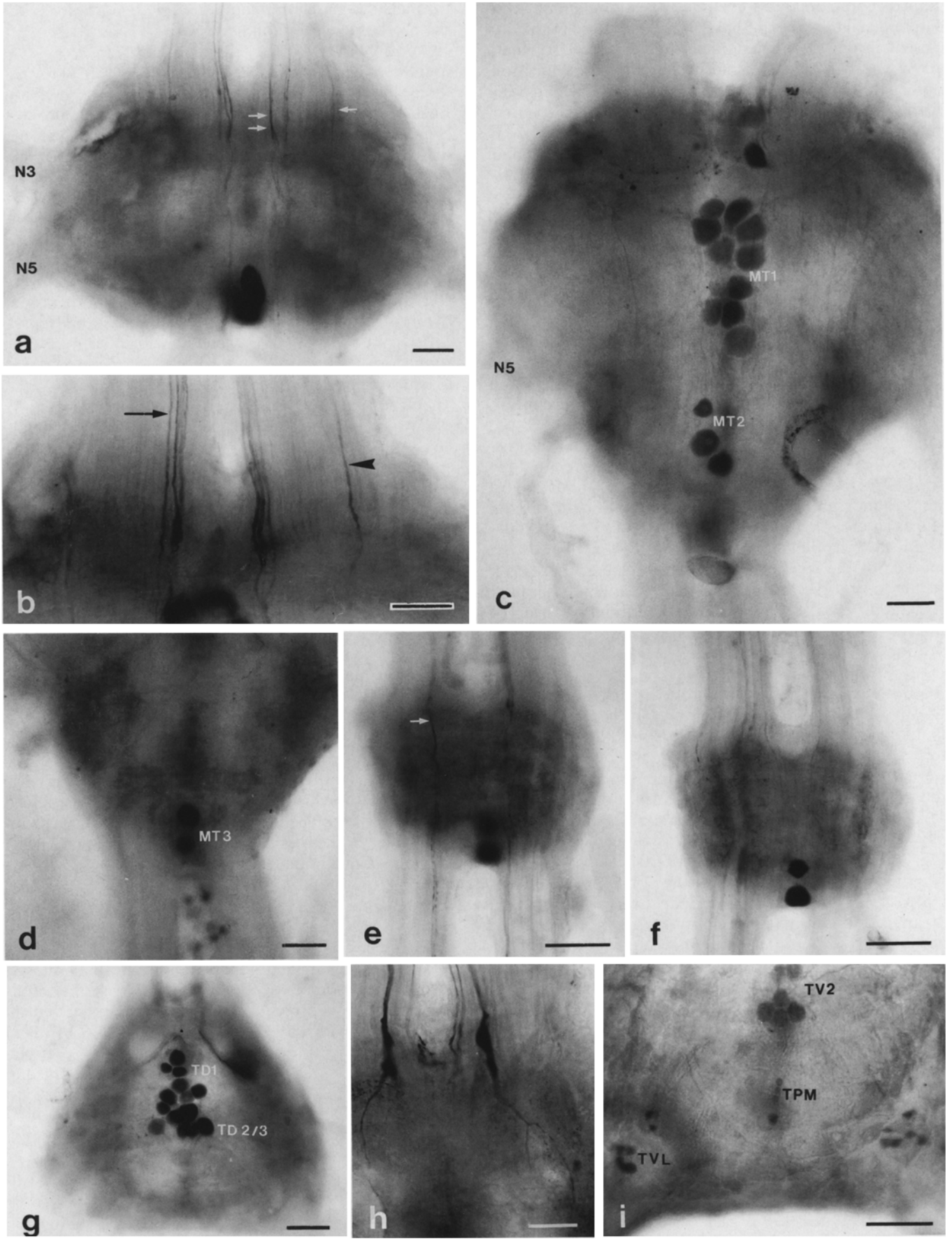
Mesothoracic ganglion. One group of unpaired medial OA-ir somata ($n=8$–12) was found in the posterior region of the mesothoracic ganglion (Figs. 2a, 3d, 4). Two to 3 somata were located dorsally, whereas the others were aligned near the posterior and ventral surface of the ganglion. As observed in the prothoracic ganglion, small somata ($n=1$–2, diameter: about 20 μm) lay laterally to the large ventral somata (diameter: 40–60 μm, Fig. 3d). Longitudinally running OA-ir fibres with collaterals appeared at the level of the dorsal fibre bundles. Up to 8 OA-ir fibres were traced in the dorsal half of an anterior connective and up to 6 in the posterior connective. In addition, a fibre in the LVT was traced through the ganglion, from anterior to posterior, as in the prothoracic ganglion.

Metathoracic ganglion. OA-ir somata ($n=16$–19) formed three clusters (MT1–3) along the midline of the ganglion. Each of them was located in one of the three neuromers (Figs. 2c–d, 3e, 4). In the metathoracic neuromere, medial OA-ir cell bodies (MT1; $n=11$–13; diameter: 40–60 μm) were aligned along the dorsal midline of the ganglion (Fig. 2c). In the first abdominal neuromere, 3 somata (MT2), viz. one small soma (diameter: about 30 μm) and two large ones (diameter: 40–50 μm), were located dorsally (Fig. 2c). In the second abdominal neuromere, two OA-ir cell bodies (MT3; diameter: 30–40 μm) had a ventral location (Fig. 2d); another soma (diameter: about 30 μm; Fig. 4) was occasionally present. Up to 8 OA-ir fibres were stained in each anterior connective (Fig. 2b). About 4 fibres were immunoreactive in each posterior connective.

Abdominal ganglia. Each of the unfused abdominal ganglia 1–4 contained a set of two medial somata of different sizes (diameters: about 30 μm and 40 μm, respectively) arranged remarkably regular in the posterior region of the ganglion (Figs. 2e, f, 3f). This set corresponded to the MT3 cells of the second abdominal neuromere of the metathoracic ganglion. Occasionally, we observed an additional cell in the fourth abdominal ganglion and the absence of one cell in the first abdominal ganglion (Fig. 4). In contrast to other ganglia, fine OA-ir fibres were stained in whole-mounts of these small abdominal ganglia. The dorsal fibre tracts (MDT, LDT) contained intersegmental OA-ir axons, that sent small collaterals into lateral neuropil areas. In some preparations, we traced one ventral and 4–6 dorsal intersegmental OA-ir fibres running from the anterior margin of the first abdominal ganglion through the abdominal connectives and ganglia posterior to the terminal ganglion. The number of stained intersegmental axon profiles agreed with results obtained in the thoracic connectives, whereas primary neurites of OA-ir somata were never immunostained in our preparations (Fig. 2e, f).

Terminal ganglion. Medial and lateral OA-ir somata (total number: $n=22$–50) were labelled in the terminal ganglion. A clear separation of large unpaired medial somata in the anterior ganglion region from small mostly lateral paired somata in the posterior part of the terminal ganglion was present. In contrast to all other ventral ganglia, large medial OA-ir somata (TD1–3, TV1–2) were not detected in each of the 5 neuromers described by Murphy (1981) in the terminal ganglion. These medial somata ($n=21$–24; diameter: 20–50 μm) belonged to three dorsal (TD1; $n=2$–3: TD2; $n=5$–9: TD3; $n=4$–5) and two ventral (TV1; $n=1$–5: TV2; $n=5$–11) soma clusters (Figs. 2g, i, 3g). In addition, a single large ventral cell body (diameter: about 50 μm) was labelled in some preparations (Fig. 3g). This soma was located laterally near the TV1 somata in the anterior ganglion region. Furthermore, up to 26 small cell bodies (diameter: about 10 μm) were stained in the posterior ganglion re-

Fig. 1a–g. OA-ir somata in whole-mount preparations of the cerebral, suboesophageal and prothoracic ganglion. **a–c** Cerebral ganglion, rostral views; *ant* antennal lobe of deuterocerebrum; *tl* tritocerebral lobe; *bl* β-lobe of mushroom bodies. **a** OA-ir somata of groups *C1–4*. ×115. **b** C1 somata. ×85. **c** Large C2 soma. ×125. **d, e** Suboesophageal ganglion, cell groups *S1–3* and *S5–S7*. **d** Dorsal view, OA-ir fibres in brain connectives (*bc*); *MxN* maxillary nerve root. ×120. **e** Medio-ventral aspect; small cell bodies of group *S3* (*arrows*) near the midline. ×125. **f, g** Prothoracic ganglion. **f** Anterior (*P1*) and posterior (*P2*) cell group; focus dorsal; *N3, N5* roots of lateral nerves 3 and 5. ×95. **g** Posterior cell group (*P2*), and small soma of *P2* (*arrow*). ×240. *Scale bars*: **a–f** 100 μm; **g** 50 μm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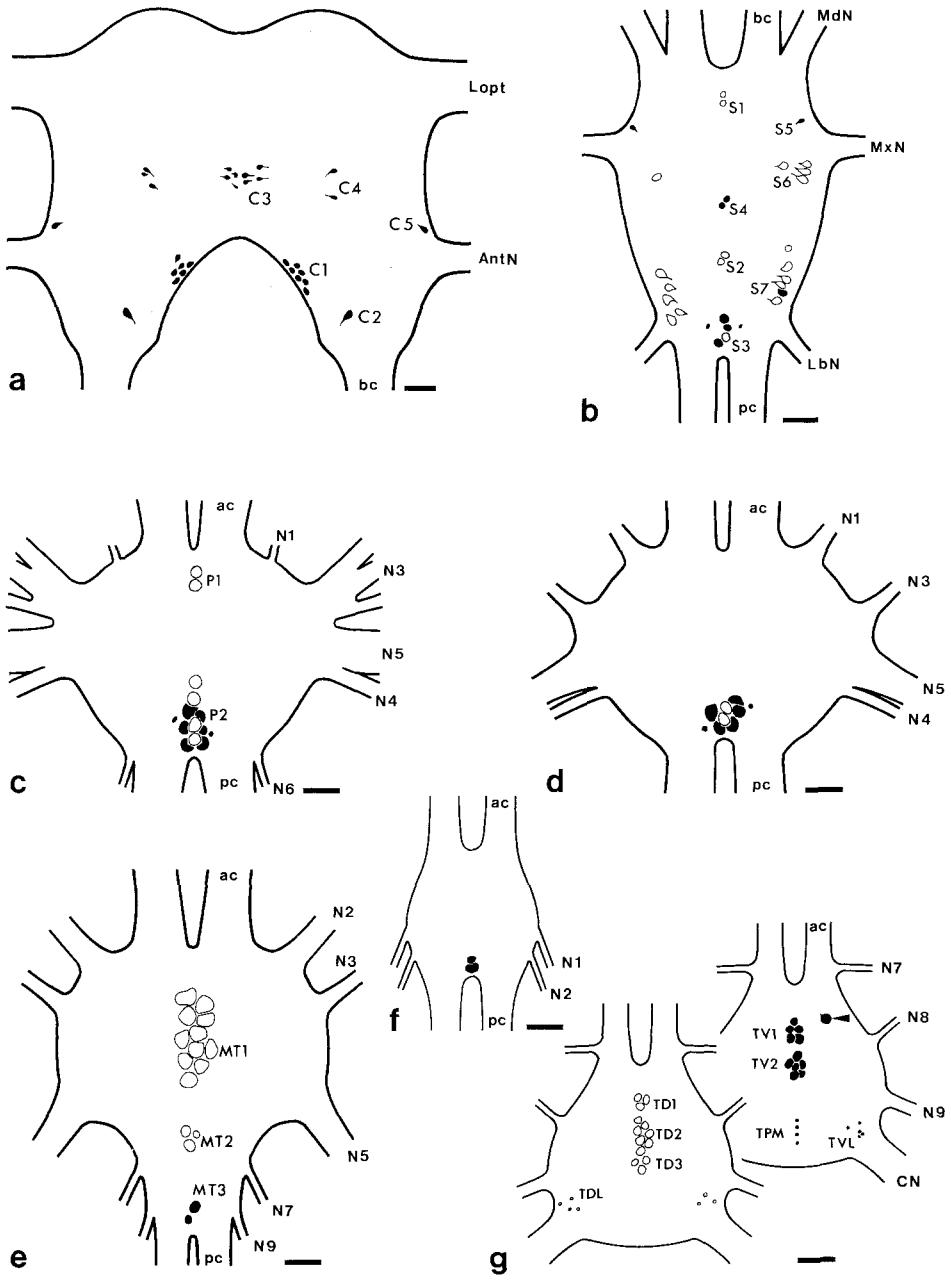


Fig. 3a–g. Schematic diagrams summarizing the general arrangement of OA-ir somata in the central ganglia of *Gryllus bimaculatus*. **a** Cerebral ganglion; *C1–5* OA-ir cell groups; frontal view. **b–g** *Black somata* are located in the ventral region of each ganglion; **b–f** dorsal views. **b** Suboesophageal ganglion; *S1–7* OA-ir cell groups. **c** Prothoracic ganglion; *P1–2* OA-ir cell groups. **d** Mesothoracic ganglion with posterior OA-ir cell cluster. **e** Metathoracic ganglion; *MT1–3* OA-ir cell groups. **f** Abdominal ganglion. **g** Terminal ganglion; dorsal aspect (*left*) with the OA-ir cell groups *TD1–3* and *TDL*; ventral aspect (*right*) with the OA-ir cell groups *TV1–2*, *TPM* and *TVL*. Note the large soma placed lateral to the *TV1* group (*arrowhead*). *ac* Anterior interganglionic connective; *AntN* antennal nerve; *bc* brain connective; *CN* cercal nerve; *Lopt* lobus opticus; *LbN* labial nerve; *MdN* mandibular nerve; *MxN* maxillary nerve; *N1–9* lateral nerves 1–9; *pc* posterior interganglionic connective. *Scale bars:* 100 μm

Fig. 2a–i. OA-ir somata in whole-mount preparations of the mesothoracic, metathoracic, abdominal and terminal ganglion. **a** Mesothoracic ganglion; OA-ir somata in the posterior ganglion region; OA-ir fibres at the level of the median dorsal tract (*double arrows*) and lateral dorsal fibre tract (*arrow*); dorsal view; *N3*, *N5* roots of lateral nerves 3 and 5. ×80. **b–d** Metathoracic ganglion. **b** OA-ir fibres in anterior connectives in the domain of the median dorsal (*arrow*) and lateral dorsal fibre tracts (*arrowhead*). ×110. **c** OA-ir cell groups *MT1* and *MT2*; *N5* root of lateral nerve 5. ×90. **d** Ventral OA-ir somata (*MT3*) in the second abdominal neuromere. ×90. **e, f** Third abdominal ganglion. **e** OA-ir intersegmental fibres (*arrow*); dorsal view. ×120. **f** OA-ir somata, same whole-mount as in **e**; ventral aspect. ×120. **g–i** Terminal ganglion. **g** Dorsal soma groups (*TD1–3*). ×80. **h** Branches of intersegmental OA-ir fibres in the anterior region of the ganglion. ×190. **i** Posteroventral aspect of the ganglion with medial somata *TV2*, small medial (*TPM*) and lateral somata (*TVL*). ×125. *Scale bars*: **a–g, i** 100 μm; **h** 50 μm

gion (Figs. 2i, 3g). These somata were arranged in 3 groups, one medial cluster (TPM) near the ventral surface of the ganglion and two bilateral clusters, positioned ventrally (TVL) and dorsally (TDL, Fig. 3g). In the anterior region of the ganglion, branches of intersegmental OA-ir fibres projected into different neuropil areas (Fig. 2h).

Segment-specific differences in number of OA-ir somata

Although the total number of immunostained cells varied to some extent between different animals, clear segment-specific properties existed in the composition of equivalent cell groups (Fig. 4). Whereas large numbers of segmentally organized large medial OA-ir cells were

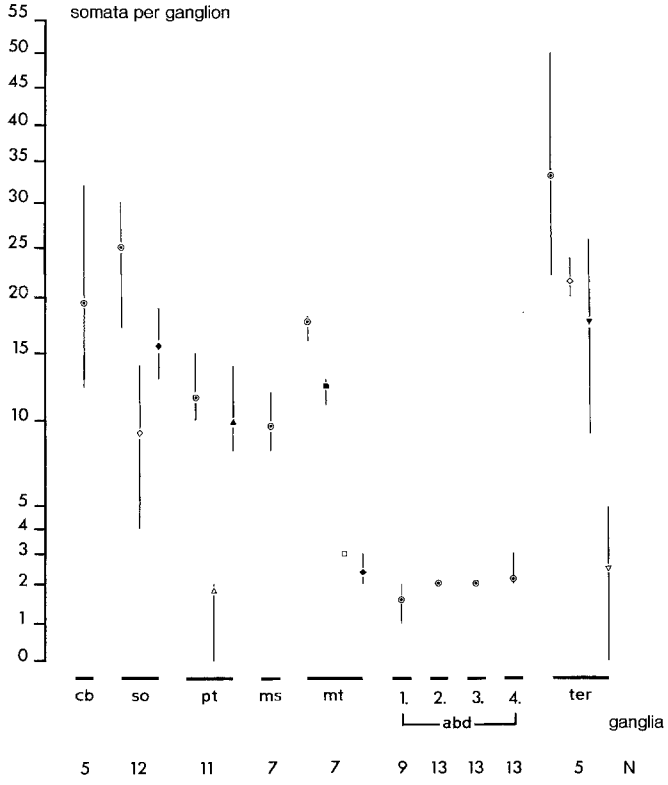


Fig. 4. Mean numbers of OA-ir somata (non-linear ordinate) in central ganglia of 31 animals. *Vertical bars* indicate the observed range (=max/min cell number) of interindividual variation for each ganglion and different cell groups. *abd* Abdominal; *cb* cerebral; *so* suboesophageal; *ms* mesothoracic; *mt* metathoracic; *pt* prothoracic; *ter* terminal ganglion; *N* number of preparations. Mean number of OA-ir somata (⊙) per ganglion; mean number of: large medial somata S1–4 (◇), P1 (△), P2 (▲), MT1 (■), MT2 (□), MT3 (◆), TD1–3 & TV1–2 (◇); large lateral somata S5–7 (◆), small somata TDL, TVL & TPM male (▽)/female (▼)

detected in the thoracic ganglia, abdominal neuromers contained only a few neurons of the given type. Generally, interindividual differences concerning the number and location of large medial OA-ir cells were small, especially in the second and third abdominal ganglia, where constant numbers were found throughout. In the suboesophageal ganglion, we found, in addition to large medial cells lateral groups of large cells in each of its three neuromeres. Interestingly, even within the neuromers of this fused ganglion, segment specific-variations in the number of cells were observed in both medial and lateral groups: the mean number of OA-ir cells assigned to the three neuromeres (group S1, S5; mean = 3.4: group S2, S4, S6; mean = 9.1: S3, S7; mean = 12.5) increased from anterior to posterior segments (ranges of values per neuromere: 2–5, 2–14, 7–18; $n = 12$). In the terminal ganglion, small somata (TDL, TVL, TPM) showed remarkable variations in number and between sexes (9–26 in females versus 0–5 in males), compared with medial somata.

Peripheral varicose OA-ir fibres

Fine varicose OA-ir fibres forming networks on nerves were revealed in known neurohemal areas of the median and transversal nerves, and on several lateral nerves. These OA-ir varicose fibre networks seemed to be restricted to abdominal parts of the ganglion chain and the metathoracic ganglion. They were found in nerve stumps of whole-mounts, and in situ preparations, but their somata could not be identified. In comparison with stainings of central somata and fibres, OA-ir fibre networks on nerves were less constant regarding labelling. Fine varicose OA-ir fibres were labelled reliably on the

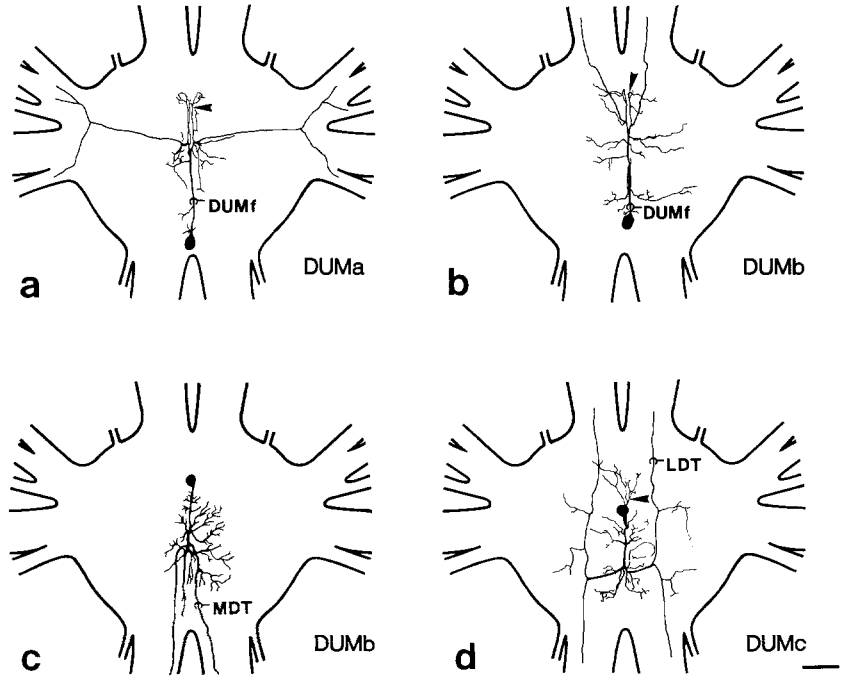


Fig. 5a–d. Prothoracic DUM cell types; camera lucida drawings of DUM neurons stained with Lucifer-Yellow. **a** DUMa neuron with lateral neurites in nerve 3, 4. **b, c** DUMb neurons. **b** Ascending DUMb neuron with posterior soma. **c** Descending DUMb neuron with anterior soma. **d** DUMc neuron with paired ascending and descending axons. *Arrowheads* indicate medial collaterals projecting into the MDT. *DUMf* fibre bundle of the DUM cell group; *LDT* lateral dorsal tract; *MDT* median dorsal tract. *Scale bar*: 100 μm

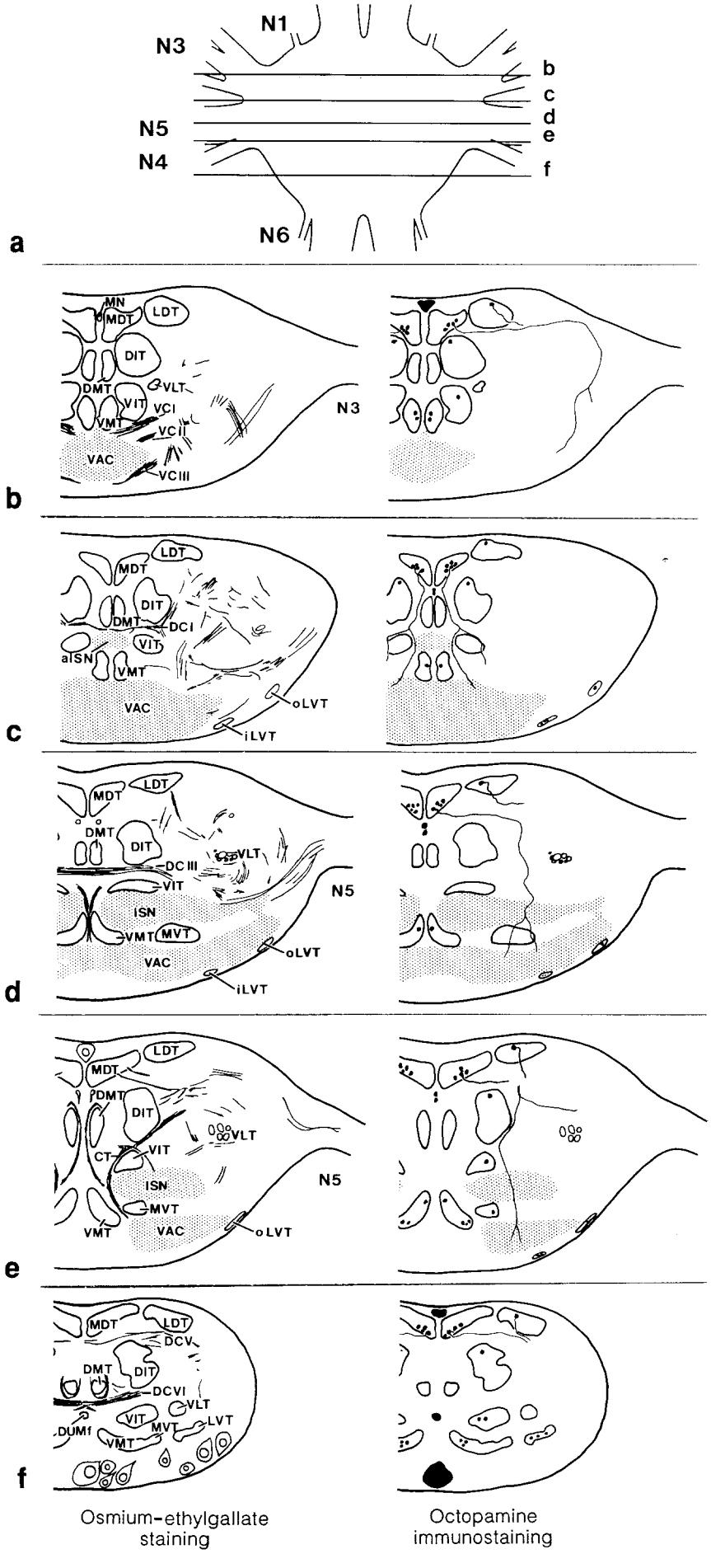


Fig. 6a–f. Schematic drawing showing the distribution and arrangement of prominent OA-ir somata and fibres in longitudinal fibre tracts and the course of prominent OA-ir collaterals compared with osmium-ethylgallate staining in the prothoracic ganglion. **a** Positions of selected frontal sections as shown in **b–f**. **b–f** OA-ir bilateral symmetrical collaterals originate in distinct regions of the ganglion. Each figure shows, on the *left*, a hemiganglion with prominent morphological structures as revealed from ethylgallate stainings, and on the *right*, OA-ir structures together with the margins of the longitudinal fibre tracts of the same region. OA-ir collaterals of the MDT run dorso-laterally and curve ventrally (**b, d–f**), or medio-ventrally (**c**). Collaterals in the LDT could only be traced over short distances (**b, d, f**). *alSN* anterior intermediate sensory neuropil (neuropil terminology according to Römer et al. 1988); *DCI-DCVI* dorsal commissures I–VI; *CT* C-tract; *DIT* dorsal intermediate tract; *DMT* dorsal median tract; *DUMf* fibre bundle of the DUM cell group; *ISN* intermediate sensory neuropil; *LDT* lateral dorsal tract; *LVT* lateral ventral tract; *iLVT* inner lateral ventral tract; *oLVT* outer lateral ventral tract; *MDT* median dorsal tract; *MN* median nerve; *MVT* median ventral tract; *N1–6* lateral nerves 1–6; *VAC* ventral association centre; *VCI–III* ventral commissures I–III; *VLT* ventral lateral tract; *VMT* ventral median tract

following nerves of the metathoracic ganglion: lateral branch of nerve 1, nerve 3, 5, 7, 8 and transverse nerves; unfused abdominal ganglia: nerve 1 and 2, all associated median and transverse nerves, and the connectives between the 4th abdominal and terminal ganglion (Fig. 7g–i); terminal ganglion: ventral and dorsal nerves of the abdominal neuromers 7–9. The OA-ir networks on nerves differed from each other with respect to fibre density and intensity of staining. Nerves 1 and 2 of all unfused abdominal ganglia showed an intense fibre staining and high fibre density. Typically, proximal regions of the peripheral nerves remained unstained, whereas varicose fibres were stained in their more distal parts; peripheral fibre staining was not observed.

OA-immunoreactivity in the prothoracic ganglion

Identified DUM cells. Three morphological types of DUM cells (terminology according to Gras et al. 1990) have so far been identified in the prothoracic ganglion (Fig. 5). For a comparison of medial OA-ir neurons with the identified DUM cell types, we give some morphological features of DUM cells that have not previously been described in detail (for tracts and neuropils see schemes in Figs. 5, 6).

DUMa neurons. These neurons with axons in the lateral nerves projected into the MDT, LDT, the dorsal median tract (DMT) and the ventral median tract (VMT, Fig. 5a). In addition, fine fibres of DUMa cells were predominantly observed in dorso-medial neuropils surrounding the fibre tracts and in some dorso-lateral neuropil areas, whereas fine branches were only rarely found in ventral neuropils. Collaterals of DUMb neurons with descending axons in the MDT projected mainly into the DMT, dorsal intermediate tract (DIT), ventral intermediate tract (VIT, Figs. 5c, 8f) and the dorso-medial neuropil. DUMc neurons with paired ascending and descending axons in the LDT projected predominantly into the DMT and DIT (Figs. 5d, 8e). Axon collaterals of DUMc neurons projected in dorso-medial and dorso-lateral neuropil regions, including more lateral parts not totally invaded by DUMa/b cells. Lucifer-Yellow-labelled terminals of intersegmental DUM cells were not observed in ventral neuropils.

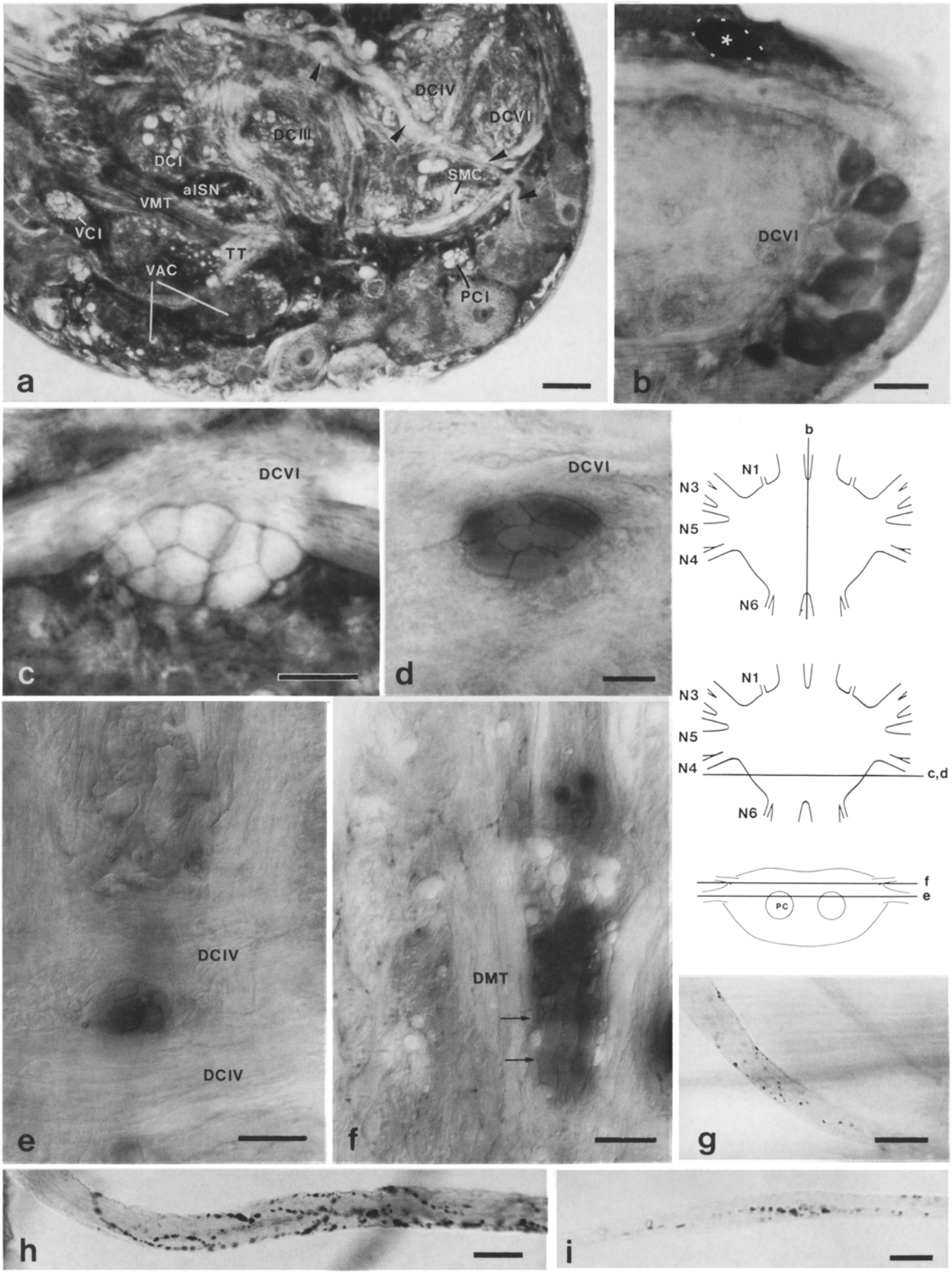
OA-ir somata. The OA-ir somata corresponded to the different types of DUM cells described above. The anterior P1 soma exactly matched the position of the descending DUMb neuron (for comparison, see Figs. 5c, 6b). The position of the P2 neurons correlated with those of DUMa and ascending DUMb cells. The most anterior soma of the P2 group had a similar or identical position of the DUMc cell.

Fibre bundle of DUM neurons. The primary neurites of DUM neurons located in the medio-posterior region of the ganglion, classified as DUMa and ascending DUMb neurons (Gras et al. 1990), formed a distinct bundle. This bundle ran ventrally to dorsal commissure VI (DCVI), then passed through DCIV, and projected further antero-dorsally (Figs. 5a, 7a), where the neurites bifurcated. In frontal sections, 12 fibre profiles (diameters: 4–8 μm) associated with some small fibres (diameter: 2 μm) formed a compact bundle (Fig. 7c).

In favourable preparations, a uniformly OA-ir labelled fibre bundle of DUM neurons could be traced antero-dorsad and clearly showed that Lucifer-Yellow-filled axons were identical with OA-ir fibres (Fig. 7d–f). In addition, some fine OA-ir fibres accompanying the bundle appeared similar to those seen in ethylgallate sections. In contrast to Lucifer-Yellow-staining, lateral DUMa neurites located within the ganglion or lateral nerves were never immunostained.

OA-ir fibres in tracts and neuropils. Longitudinal fibre tracts contained two types of OA-ir elements: straight longitudinally-running and fine twisted varicose fibres. Longitudinally oriented OA-ir fibres were traced along the whole antero-posterior axis of the prothoracic ganglion and were found in the following fibre tracts: the MDT contains 4–6 fibres (diameters: 4–5 μm; Fig. 8c), the LDT one fibre (diameter: 6–7 μm; Fig. 8d), the LVT 3 fibres (diameters: about 2 μm; Fig. 9g), and the VMT 2–3 fibres (diameters: 3–5 μm; Fig. 8h, i). One immunostained fibre was occasionally observed in the DIT and VIT (Fig. 8k). The majority of these fibres could be traced intersegmentally in the connectives (Fig. 8a). The position of some OA-ir fibres in the MDT corresponded well with fibres of the descending DUMb neuron revealed by Lucifer-Yellow-staining, and the OA-ir fibres in both LDTs could be correlated with the axons of a DUMc neuron (Fig. 8c–f). In addition, 2 or 3 longitudinal fibres were observed running medially between the MDT and DMT at the level of nerve roots 2–5; they probably represent fibre elements of descending DUMb

Fig. 7a–i. OA-immunoreactivity in the fibre bundle of DUM cells in the prothoracic ganglion and peripheral OA-ir fibres on abdominal nerves. **a** Sagittal section (thickness: 20 μm) of an osmium-ethylgallate stained ganglion with somata and axons (*arrowheads*) of DUM neurons. Note cross-sectioned commissures. ×180. **b–f** The position of each section is depicted in the insets. **b** Sagittal Vibratome section equivalent to **a** (thickness 40–50 μm) with OA-ir P2 somata along the postero-ventral and one near the dorsal (*asterisk*) ganglion border. ×200. **c** Frontal section (thickness: 20 μm) of an osmium-ethylgallate stained ganglion with the DUM fibre bundle. ×1500. **d** Frontal Vibratome section (thickness: 40 μm) of the same ganglion region as in **c** showing uniform labelling of the DUM cell fibre bundle. ×1000. **e** Horizontal Vibratome section (thickness: 40 μm) showing the OA-ir DUM fibre bundle passing through the *DCIV*. ×650. **f** Horizontal Vibratome section, OA-ir DUM fibre bundle (*arrows*) curving anteriorly. ×600. **g–i** Fine varicose OA-ir fibres covering nerves. **g** Transverse nerve of the third abdominal ganglion. ×100. **h** First lateral nerve of the third abdominal ganglion. ×190. **i** Median nerve located between the fourth abdominal and terminal ganglion. ×400. *alSN* anterior intermediate sensory neuropil; *DCI–VI* dorsal commissures I–VI; *DMT* dorsal median tract; *N1–6* lateral nerves 1–6; *PC* posterior interganglionic connective; *PCI* posterior ventral commissure; *SMC* supramedian commissure; *TT* T-tract; *VCI* ventral commissure I; *VAC* ventral association centre; *VMT* ventral median tract. *Scale bars*: **a**, **b**, **h** 50 μm; **c**, **d** 10 μm; **e**, **f**, **i** 20 μm; **g** 100 μm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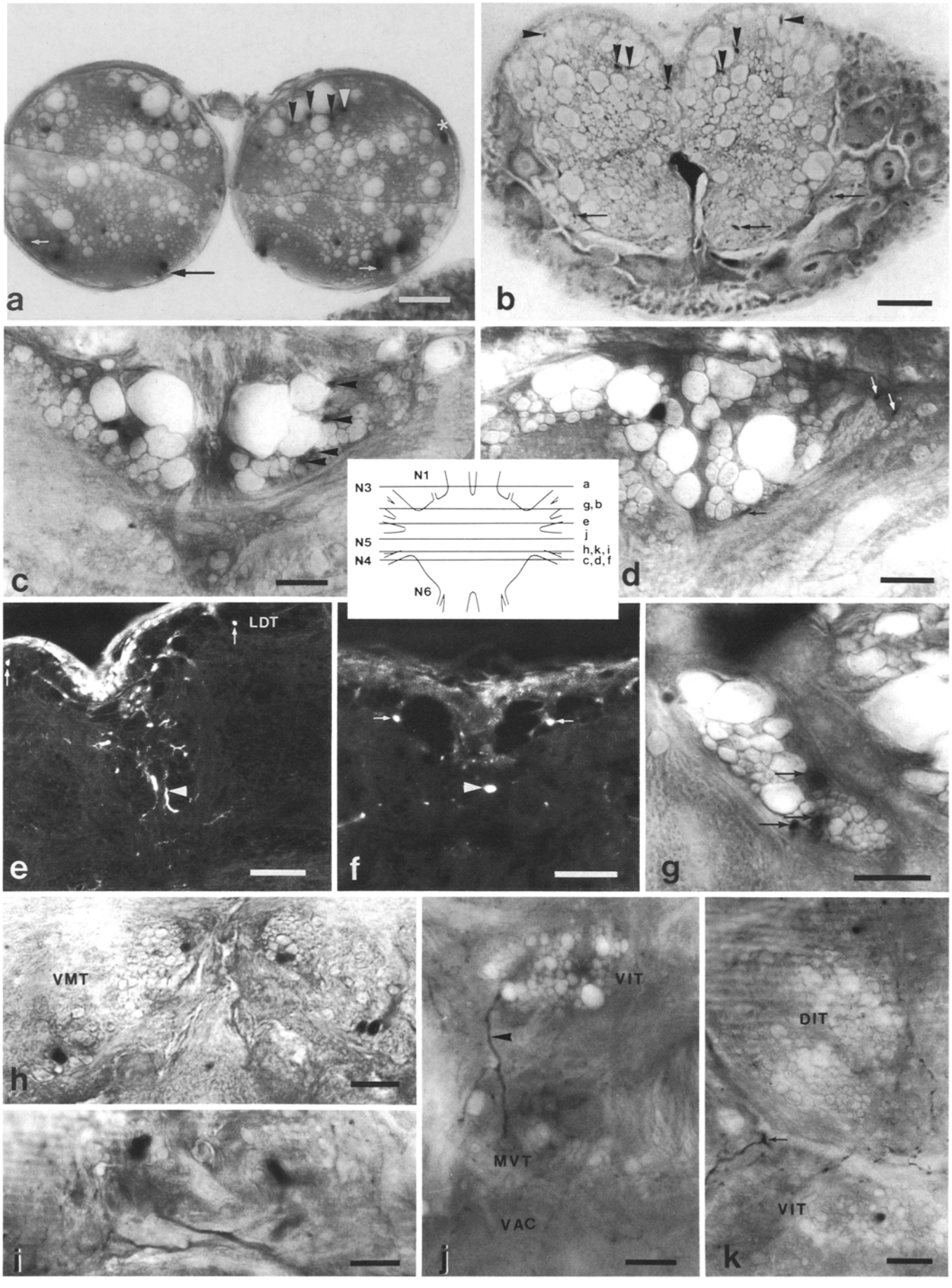
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neurons (Figs. 8f, 9f). No correspondence of intersegmental fibres of the ventral tracts (LVT, VMT) with neurites of intracellularly stained DUM cells of the prothoracic ganglion was found.

Fine varicose fibres, representing the second type of OA-ir fibres in the tracts, were predominantly found near the lateral margins of tracts. In the MDT, LDT, DIT and VIT, we observed both OA-ir and Lucifer-Yellow-stained terminals (Fig. 8j, k). However, prominent DUM cell arborizations in the DMT shown only by Lucifer-Yellow-filling could not be labelled with immunocytochemical methods, whereas in the median ventral tract (MVT), immunoreactive terminals were observed, although Lucifer-Yellow-stained branches were absent.

Varicose OA-ir fibre ramifications were observed in all neuropils. Generally, more OA-ir varicosities appeared in dorsal neuropils than in ventral neuropils. Typically, fine varicose OA-ir fibres were observed in the immediate vicinity of large fibres partly identified as elements of nerve roots and collaterals of intersegmental fibres (Fig. 9c). In some preparations, fibre branches (diameter: <1 µm) originated from collaterals of OA-ir fibres located in dorsal fibre tracts. These collaterals (diameters: 1–3 µm) arose in different regions of the ganglion (see Fig. 6). Unlike those of the LDT, collaterals in the MDT could be traced until they merged into synaptic neuropil areas. Collaterals originating from the MDT differed in their routes: some projected longitudinally (Fig. 9d), whereas others left the tract directly after arborization and showed a strict dorso-ventral orientation (Fig. 9b, f). The latter terminated symmetrically in different neuropils and ventral fibre tracts (MVT, VIT) in each hemiganglion. These collaterals of fibres in the MDT projected into functionally different compartments of the ganglion, as they were found in both neuropils and fibre tracts. Furthermore, functionally different neuropils (dorsal and ventral neuropils) contained branches of one and the same collateral. On their dorsolateral route, some collaterals were accompanied by fine varicose OA-ir arborizations and large unlabelled profiles originating from the MDT, and were part of the DCV (Fig. 9a, e, g, h).

Fig. 8a–k. Location of OA-ir elements in longitudinal tracts and various neuropil areas of the prothoracic ganglion. The position of each frontal section is depicted in the *inset*, dorsal being at the top. **a** Vibratome section (thickness: 70 µm); OA-ir fibres in the anterior connectives. Dorso-median fibres (*arrowheads*) are associated with the MDT, dorso-lateral fibres (*asterisk*) with the LDT, ventro-median fibres (*arrow*) with the VMT and ventro-lateral fibres (*white arrow*) with the LVT of the prothoracic ganglion. ×180. **b** Paraplast section (thickness: 10 µm); anterior P1 soma; some intersegmental OA-ir fibres (*arrowheads, arrows*) can be detected as in **a**. ×200. **c–d** Vibratome sections (thickness: 40–50 µm), OA-ir fibres in the dorsal fibre tracts. **c** OA-ir fibres (*arrowheads*) in the MDT. ×475. **d** OA-ir axon and fine fibres (*arrows*) in the LDT. ×500. **e, f** Intracellular Lucifer-Yellow-staining (thickness 10 µm). **e** DUMc neuron with paired axons (*arrows*) in the LDT and branches (*arrowhead*) in the DMT. ×215. **f** DUMb neuron with descending axons (*arrows*) in the MDT and a median collateral (*arrowhead*). ×230. **g–k** Vibratome sections (thickness: 50–60 µm). **g** OA-ir fibres (*arrows*) in the LVT. ×700. **h, i** OA-ir fibres in the VMT at different focus levels. ×450. **h** Longitudinal OA-ir fibres. **i** OA-ir commissural fibre branches. **j** Branch (*arrowhead*) of an OA-ir collateral originating from a MDT fibre and fine varicose fibres in the *VAC*. ×450. **k** OA-ir fibre in the dorsomedian region of the *DIT* and in the median region of the *VIT*; fine branches are associated with larger unlabelled elements (*arrow*). *DIT* Dorsal intermediate tract; *LDT* lateral dorsal tract; *MVT* median ventral tract; *N1–6* lateral nerves 1–6; *VAC* ventral association centre; *VIT* ventral intermediate tract; *VMT* ventral median tract. *Scale bars*: **a, b, e, f** 50 µm; **c, d, g–k** 20 µm

Discussion

OA-immunolabelling

We have demonstrated the presence of OA-ir cells in the CNS of *Gryllus bimaculatus* employing a well-characterized antiserum to an OA-conjugate. Stained cells, either grouped into ganglion-specific or serially homologous clusters, or singly arranged, belong to unpaired medial or lateral paired neurons. Variability in the number of stained somata of a given group has been detected mainly in the cerebral, suboesophageal and terminal ganglia. In such ganglia, insufficient penetration of the antibody may occur. Therefore, it is unclear whether the variability in the number of OA-ir cells is simply an experimental artefact or the result of real biological differences. In these ganglia, we find intensely stained cell groups with constant numbers of somata (cerebral ganglion: C1–2; suboesophageal ganglion: S1–3, S5; terminal ganglion: TD1–3, TV1–2) located close to less intensely immunoreactive groups with variable neuron number (cerebral ganglion: C3–5; suboesophageal ganglion: S4; terminal ganglion: TPM, TDL, TVL). Based on these findings, the differences concerning the cell number and intensity of immunostaining cannot merely be explained by the methods used. The variability in staining intensity may reflect differences in antigen distribution or expression of OA-titres in a given cell according to its physiological state.

Incomplete and variable staining intensity in OA-ir neurons is also detected at the level of nerve fibres. This can best be demonstrated by the segmental efferent DUM neurons previously stained with Lucifer-Yellow (DUMa cells, Gras et al. 1990). Their lateral neurites are not labelled by OA antibodies, whereas the bundle of their primary neurites is immunostained. In comparison, this bundle is less intensely stained than neurites of intersegmental OA-ir cells. Similar observations on the incomplete demonstration of neurons have been reported for immunolabelled serotoninergic neurons in snails (Murphy 1985) and insects (Orchard et al. 1989) and have been attributed to the inhomogeneous intracellular distribution of antigen.

Medial OA-ir and DUM neurons

Large medial OA-ir neurons grouped in serial homologous clusters exist in all ganglia of the ventral nerve

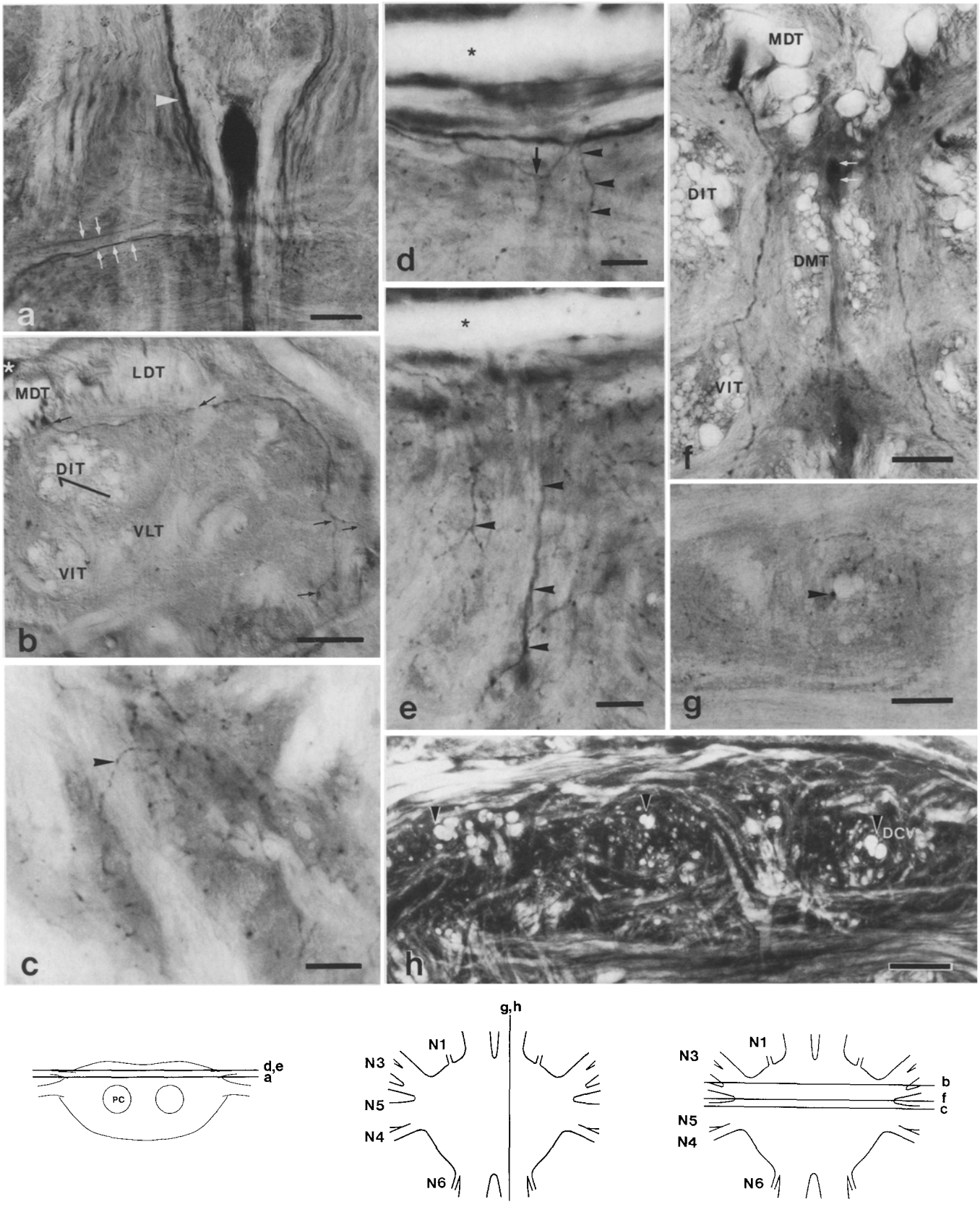

Fig. 9a–h. OA-ir collaterals originating from fibres of the median dorsal tract. **a–g** Immunostained Vibratome sections (thickness 40–50 µm); the location of each section is depicted separately in the *insets*. **a** Horizontal section; antero-median area of the ganglion with one P1 soma. OA-ir fibres in the MDT (*arrowhead*); latero-dorsal running collaterals (*arrows*). ×190. **b** Frontal section; collateral (*arrows*) of a MDT-axon; P1 soma (*asterisk*). ×240. **c** Frontal section; fine varicose OA-ir fibres (*arrowhead*) near the origin of nerve 5. ×500. **d, e** Horizontal sections; unlabelled large fibre of the MDT (*asterisk*). ×420. **d** OA-ir fibres in the MDT with lateral

cord in *Gryllus*. A direct interspecific comparison of OA-ir neurons in *Gryllus* can only be made for some of the selected ganglia so far investigated in *Locusta* (Konings et al. 1988; Stevenson et al. 1991) and *Periplaneta* (Rapus and Eckert 1990). OA-ir clusters C1 and C2 in the cricket brain correspond to similar cell groups in locusts (Konings et al. 1988). The metathoracic neuromere of the metathoracic ganglion contains OA-ir neurons in corresponding medial clusters (Konings et al. 1988; Stevenson et al. 1991). In contrast to locusts, paired OA-ir cells are not observed in the metathoracic ganglion of *Gryllus*, and the pattern in the fused abdominal neuromers differs. In the cockroach and cricket terminal ganglion, dorsal OA-ir clusters are considered as equivalently represented (Rapus and Eckert 1990). However, three ventral immunoreactive clusters that are stained in this ganglion of the cockroach using a different antibody against OA are absent in the cricket.

We demonstrate in detail that, in the prothoracic ganglion of *Gryllus*, the morphology of OA-ir cells corresponds to DUM cell types characterized by Lucifer-Yellow-staining and electrophysiology (Gras et al. 1990). In general, the position of medial OA-ir somata near the posterior border of each neuromere in the cricket corresponds to serial homologous clusters of DUM neurons shown for a number of insect species by ionophoretic and backfill staining (Crossman et al. 1971; Evans and O'Shea 1978; Hoyle 1978; Watson 1984; Arikawa et al. 1984; Pflüger and Watson 1988; Tanaka and Washio 1988; Thompson and Siegler 1991). As found regarding DUM neurons, the number of OA-ir neurons is large in thoracic neuromers in comparison to abdominal neuromers. In general, 2 OA-ir cells occur in abdominal ganglia not associated with genital organs. These OA-ir neurons correspond well to the two large efferent DUM neurons innervating the ventral and dorsal somatic muscles of the locust (Pflüger and Watson 1988). In *Gryllus*, the occurrence of additional DUM-type neurons can be expected, as shown for the locust (Ferber and Pflüger 1990), but they may contain neuroactive compounds other than OA. Such a discrepancy between the number of OA-ir neurons and DUM cells revealed by cobalt backfills (Yamaguchi et al. 1985) exists with respect to the terminal ganglion of the cricket. Recently, DUM neurons with a lateral soma position have been demonstrated in the suboesophageal ganglion of *Locusta* (Bräunig 1991). These neurons have also been suggested to contain OA and may correspond to the S6 and S7 neurons of *Gryllus*.

From a comparison of soma mapping of OA-ir cells in *Gryllus* with embryological studies (Bate 1976; Goodman et al. 1980; Goodman and Bate 1981), we can infer the neuronal precursor cells of the unpaired median neurons. (1) Serial homologous, medial immunoreactive neurons located posteriorly in each neuromere can be attributed to the first born progeny of the median neuroblast. (2) The anterior P1 neurons are assumed to stem from a different anterior neuroblast, exclusively described for the prothoracic ganglion of *Gryllus* (Miyamoto and Shimozawa 1983). (3) The OA-ir H-shaped DUMc neuron can be attributed to a third neuronal precursor cell (median precursor 3, Bate et al. 1981). The origin of the ventral medial cells and paired lateral OA-ir cells in the suboesophageal and terminal ganglion remains obscure. Positional variation of large lateral OA-ir neurons with a tendency to form asymmetric clusters in the suboesophageal ganglion can be interpreted as a lateral shift of medial cells during ontogenesis. This interpretation is also supported by the results of Bräunig (1991) in *Locusta*, described above. The progeny and functional role of unpaired medial neurons containing serotonin (Orchard et al. 1989), proctolin (Bishop and O'Shea 1982; Agricola et al. 1985; Breidbach and Dircksen 1989) or FMRF-amide (Ferber 1989) remain to be clarified.

Distribution of central OA-ir fibres

In the prothoracic ganglion, fine OA-ir varicosities are obviously distributed throughout the different synaptic neuropils and in most tracts. However, both OA-ir fibres and Lucifer-Yellow-stained branches of DUM cells appear more densely distributed in dorsal neuropils, where motoneurons predominantly have their dendritic domains (Honegger et al. 1984; Laurent and Richard 1986). Our immunohistochemical data and previous physiological studies (Sombati and Hoyle 1984; Gras et al. 1990) suggest a functional role of OA in motor neuropils.

Peripheral OA-ir fibres on nerves

In insects, OA is contained not only in the nervous system, but also in neurohemal organs and in the hemolymph (Evans 1978; David and Coulon 1979; Dymond and Evans 1979; Orchard et al. 1986). Hemolymph titres of OA correlate with physiological states and behavioural conditions (Goosey and Candy 1982; Baily et al. 1983; Davenport and Evans 1984; David et al. 1985; Woodring et al. 1988, 1989). Classical neurohemal organs (corpora cardiaca, perisympathetic organs) are generally considered to be the main release sites for secretory and neurosecretory products. In addition, the existence of neurohemal areas spread over peripheral nerves has been reported for some insect species (John-

(*arrowheads*) and longitudinal (*arrow*) running collaterals. **e** Laterally projecting collaterals (*arrowheads*); focus further dorsal than in **d**. Note fine varicose OA-ir fibres in the dorsal neuropil. **f** Frontal section, medio-ventral collateral of the *MDT*; immunoreactive fibres (*arrows*) run medially between the *DMT* and *MDT*. ×220. **g** Sagittal section; OA-ir collateral (*arrowhead*) associated with some large fibres of the DCV. ×550. **h** Sagittal section (thickness: 20 μm) of an osmium-ethylgallate stained prothoracic ganglion. *Arrowheads* indicate large fibres usually accompanied with OA-ir collaterals as seen in **g**. ×220. *DCV* Dorsal commissure V; *DIT* dorsal intermediate tract; *DMT* dorsal median tract; *MDT* median dorsal tract; *N1–6* lateral nerves 1–6; *LDT* lateral dorsal tract; *VIT* ventral intermediate tract; *VLT* ventral lateral tract. *Scale bars*: **a–e, f, h** 50 μm; **c, g** 20 μm

son 1966; Finlayson and Osborne 1968; Miller and Thomson 1968; Anwyl and Finlayson 1973; Fifield and Finlayson 1978). The OA-ir varicosities detected by our study in the cricket correspond to some areas of putative neurohemal release previously described by Raabe (1985), and are mainly associated with the surface of nerves. OA-ir fibre networks contribute to a distinct pattern of specialized neurosecretory nerves, and are often found to be co-distributed with other aminergic and peptidergic varicosities (Spörhase-Eichmann et al. 1991). Immunocytochemical studies on neuroactive compounds in fibre varicosities of nerve and ganglion sheaths of a number of insect species (Nässel and Elekes 1985; Bräunig 1987; Davis 1985, 1987; Duve et al. 1988; Spörhase-Eichmann and Schürmann 1988; Griss 1989; Orchard et al. 1989; Dircksen et al. 1991) complement the view of neurosecretory release of amines and peptides. A distinct pattern of putative neurosecretory release sites of amines and peptides emerges. Such a diversified system can be interpreted as one mode of supplying possible target organs by short diffusion pathways, not involving the large neurohemal organs.

Our immunohistochemical studies support the view that OA may serve multiple functions in the CNS of insects, including a role as neurotransmitter, neuromodulator and neurohormone (Agricola et al. 1988). In addition to the previously described peripheral OA actions via efferent DUM cell terminals at muscles, our results suggest the presence of central releasing sites and neurohemal areas spread over nerves. Our morphological findings indicate regions where the different roles of OA could be investigated in future.

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References

Adams ME, Bishop CA, O'Shea M (1983) Strategies for the identification of amine- and peptide-containing neurons. In: Strausfeld NJ (ed) Functional neuroanatomy. Springer, Berlin Heidelberg New York, pp 238–242

Agricola H, Eckert M, Ude J, Birkenbeil H, Penzlin H (1985) The distribution of a proctolin-like immunoreactive material in the terminal ganglion of the cockroach, *Periplaneta americana* L. Cell Tissue Res 239:203–209

Agricola H, Hertel W, Penzlin H (1988) Octopamine – neurotransmitter, neuromodulator, neurohormone. Zool Jb Physiol 92:1–45

Anwyl R, Finlayson LH (1973) The ultrastructure of neurons with both a motor and a neurosecretory function in the insect, *Rhodnius prolixus*. Z Zellforsch 146:367–374

Arikawa K, Washio H, Tanaka Y (1984) Dorsal unpaired median neurons of the cockroach metathoracic ganglion. J Neurobiol 15:531–536

Baily BA, Martin RJ, Downer GH (1983) Haemolymph octopamine levels during and following flight in the American cockroach, *Periplaneta americana* L. Can J Zool 62:19–22

Bate CM (1976) Embryogenesis of an insect nervous system. I. A map of the thoracic and abdominal neuroblast in *Locusta migratoria*. J Embryol Exp Morphol 35:107–123

Bate M, Goodman CS, Spitzer NC (1981) Embryonic development of identified neurons: segment-specific differences in the H cell homologues. J Neurosci 1:103–106

Bishop CA, O'Shea M (1982) Neuropeptide proctolin (H-Arg-Try-Leu-Pro-Thr-OH): immunocytochemical mapping of neurons in the central nervous system of the cockroach. J Comp Neurol 207:223–238

Bräunig P (1987) The satellite nervous system – an extensive neurohemal network in the locust. J Comp Physiol [A] 160:69–77

Bräunig P (1988) Identification of a single prothoracic 'dorsal unpaired median' (DUM) neuron supplying locust mouthpart nerves. J Comp Physiol [A] 163:835–840

Bräunig P (1991) Suboesophageal DUM neurons innervate the principal neuropiles of the locust brain. Philos Trans R Soc Lond (Biol) 332:221–240

Bräunig P, Allgäuer C, Honegger HW (1990) Suboesophageal DUM neurones are part of the antennal motor system of locusts and crickets. Experientia 46:259–261

Breidbach O, Dircksen H (1989) Proctolin-immunoreactive neurons persist during metamorphosis of an insect: a developmental study of the ventral nerve cord of *Tenebrio molitor* (Coleoptera). Cell Tissue Res 257:217–225

Buijs RM, Pool CW, Van Heerikhuize JJ, Sluiter AA, Van der Sluis PJ, Ramkema M, Van der Woude TP, Van der Beek E (1989) Antibodies to small transmitter molecules and peptides: production and application of antibodies to dopamine, serotonin, gaba, vasopressin, vasoactive intestinal peptide, neuropeptide Y, somatostatin and substance P. Biomed Res 10 [Suppl 3]:213–222

Carlson AD, Jalenak M (1986) Release of octopamine from photomotor neurones of the larval firefly lanterns. J Exp Biol 122:453–457

Christensen TA, Carlson AD (1981) Symmetrically organized dorsal unpaired median DUM neurones and flash control in the male firefly, *Photuris versicolor*. J Exp Biol 93:133–147

Christensen TA, Carlson AD (1982) The neurophysiology of larval firefly luminescence, direct activation through four bifurcating DUM neurons. J Comp Physiol 148:503–514

Crossman AR, Kerkut GA, Walker RJ (1971) Axon pathways of electrically excitable nerve cell bodies in the insect central nervous system. J Physiol (Lond) 218:55–56

Davenport AP, Evans PD (1984) Changes in haemolymph octopamine levels associated with food deprivation in the locust, *Schistocerca gregaria*. Physiol Entomol 9:269–274

David JC, Coulon JF (1979) Octopamine distribution in the *Locusta migratoria* nervous and non-nervous system. Comp Biochem Physiol 64C:161–164

David JC, Coulon JF (1985) Octopamine in invertebrates and vertebrates, a review. Prog Neurobiol 24:141–185

David JC, Coulon JF, Lafon-Cazal M (1985) Octopamine changes in nervous and non-nervous tissues of the locust, *Locusta migratoria* L., after different flight conditions. Comp Biochem Physiol 82C:427–432

Davis NT (1985) Serotonin-immunoreactive nerves and neurohemal system in the cockroach *Periplaneta americana* (L.). Cell Tissue Res 240:593–600

Davis NT (1987) Neurosecretory neurons and their projections to the serotonin neurohemal system of the cockroach *Periplaneta americana* (L.) and identification of mandibular and maxillary neurons associated with this system. J Comp Neurol 259:604–621

Davis NT, Velleman SG, Kingan TG, Keshishian H (1989) Identification and distribution of a proctolin-like neuropeptide in the nervous system of the gypsy moth, *Lymantria dispar*, and other Lepidoptera. J Comp Neurol 283:71–85

Dircksen H, Müller A, Keller R (1991) Crustacean cardioactive peptide in the nervous system of the locust, *Locusta migratoria*. An immunohistochemical study on the ventral nerve cord and peripheral innervation. Cell Tissue Res 263:439–457

Duve H, Thorpe A, Nässel DR (1988) Light- and electron-microscopic immunocytochemistry of peptidergic neurons innervat-

ing thoracico-abdominal neurohaemal areas in the blowfly. Cell Tissue Res 253:583–595
Dymond GR, Evans PD (1979) Biogenic amines in the nervous system of the cockroach, *Periplaneta americana*: association of octopamine with mushroom bodies and dorsal unpaired median (DUM) neurones. Insect Biochem 9:535–545
Evans P (1978) Octopamine distribution in the insect nervous system. J Neurochem 30:1009–1013
Evans P (1980) Biogenic amines in the insect nervous system. Adv Insect Physiol 15:317–474
Evans P (1985) Octopamine. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiol biochem and pharmacol, vol 11. Pergamon, Oxford, pp 499–531
Evans P, O'Shea M (1978) The identification of an octopaminergic neurone and the modulation of a myogenic rhythm in the locust. J Exp Biol 73:235–260
Ferber M (1989) Are all DUM-neurones octopaminergic? Evidence for peptidergic DUM-neurones in locust abdominal ganglia. In: Erber J, Menzel R, Pflüger HJ, Todt D (eds) Neural mechanism of behavior. Thieme, Stuttgart New York, p 249
Ferber M, Pflüger HJ (1990) Bilaterally projecting neurons in the pregenital abdominal ganglia of the locust: anatomy and peripheral targets. J Comp Neurol 302:447–460
Fifield SM, Finlayson LH (1978) Peripheral neurons and peripheral neurosecretion in the stick insect, *Carausius morosus*. Proc R Soc Lond (Biol) 200:63–85
Finlayson LH, Osborne MP (1968) Peripheral neurosecretory cells in the stick insect (*Carausius morosus*) and the blowfly larva (*Phormia terrae-novae*). J Insect Physiol 14:1793–1801
Geffard M, Buijs RM, Seguela P, Pool CW, LeMoal M (1984) First demonstration of higly specific and sensitive antibodies against dopamine. Brain Res 294:161–165
Goodman CS, Bate M (1981) Neuronal development in the grasshopper. Trends Neurosci 4:163–169
Goodman CS, Pearson KG, Spitzer NC (1980) Electrical excitability: a spectrum of properties in the progeny of a single embryonic neuroblast. Proc Natl Acad Sci USA 77:1676–1680
Goosey MW, Candy DJ (1980) The D-octopamine content of haemolymph of the locust *Schistocerca americana gregaria*. Insect Biochem 10:393–397
Goosey MW, Candy DJ (1982) The release and removal of octopamine by tissues of the locust *Schistocerca americana gregaria*. Insect Biochem 12:681–685
Gras H, Hörner M, Runge L, Schürmann FW (1990) Prothoracic DUM neurons of the cricket *Gryllus bimaculatus*-responses to natural stimuli and activity in walking behavior. J Comp Physiol 166:901–914
Griss C (1989) Serotonin-immunoreactive neurons in the suboesophageal ganglion of the caterpillar of the hawk moth *Manduca sexta*. Cell Tissue Res 258:101–109
Hahnel C, Bräunig P (1989) Dorsal, unpaired, median (DUM) neurones of the locust suboesophageal ganglion (SOG). In: Elsner N, Singer W (eds) Dynamics and plasticity in neuronal systems. Proceedings 17th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 54
Honegger HW, Altman JS, Kien J, Müller-Tautz R, Pollberg E (1984) A comparative study of neck muscle motor neurons in a cricket and a locust. J Comp Neurol 230:517–535
Hoyle G (1974) A function for neurones (DUM) neurosecretory on skeletal muscle of insects. J Exp Zool 189:401–406
Hoyle G (1975) Evidence that insect dorsal unpaired median (DUM) neurons are octopaminergic. J Exp Zool 193:425–431
Hoyle G (1978) The dorsal unpaired median neurons of the locust metathoracic ganglion. J Neurobiol 9:43–57
Hoyle G, Barker DL (1975) Synthesis of octopamine by dorsal median unpaired neurons. J Exp Zool 193:433–439
Johnson B (1966) Fine structure of the lateral cardiac nerves of the cockroach *Periplaneta americana* (L.). J Insect Physiol 12:645–653
Konings PNM, Vullings HGB, Geffard M, Buijs RM, Diederen JHB, Jansen WF (1988) Immunocytochemical demonstration of octopamine-immunoreactive cells in the nervous system of *Locusta migratoria* and *Schistocerca gregaria*. Cell Tissue Res 251:371–379
Kravitz AD (1988) Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science 241:1775–1781
Lange AB, Orchard I (1986) Ventral neurons in an abdominal ganglion of the locust, *Locusta migratoria*, with properties similar to dorsal unpaired median neurons. Can J Zool 64:264–267
Laurent G, Richard D (1986) The organization and role during locomotion of the proximal musculature of the cricket foreleg. I. Anatomy and innervation. J Exp Biol 123:255–283
Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce opposite postures in lobsters. Science 208:76–79
Malamud JG, Mizisin AP, Josephson RK (1988) The effects of octopamine on contraction kinetics and power output of a locust flight muscle. J Comp Physiol [A] 162:827–835
McRae-Degueurce A, Geffard M (1986) One perfusion mixture for immunocytochemical detection of noradrenaline, dopamine, serotonin and acetylcholine in the same rat brain. Brain Res 376:217–219
Miller T, Thomson WW (1968) Ultrastructure of cockroach cardiac innervation. J Insect Physiol 14:1099–1104
Miyamoto T, Shimozawa T (1983) Embryonic development of the central nervous system in the cricket, *Gryllus bimaculatus*. I. Segmental homologies in early neurogenesis. Zool Mag 92:317–331
Murphy RK (1981) The structure and development of a somatotopic map in crickets: the cercal afferent projection. Dev Biol 88:236–246
Murphy RK (1985) Sprouting and functional regeneration of an identified serotonergic neuron following axotomy. J Neurobiol 16:137–151
Nässel DR, Elekes K (1985) Serotonergic terminals in the neural sheath of the blowfly nervous system: electron microscopical immunocytochemistry and 5.7-dihydroxytryptamine labelling. Neuroscience 15:293–307
O'Gara BA, Drewes CD (1990) Modulation of tension production by octopamine in the metathoracic dorsal longitudinal muscle of the cricket *Teleogryllus oceanicus*. J Exp Biol 149:161–176
Orchard I, Lange AB (1985) Evidence for octopaminergic modulation of an insect visceral muscle. J Neurobiol 16:171–181
Orchard I, Martin RJ, Sloley BD, Downer RGH (1986) The association of 5-hydroxytryptamine, octopamine, and dopamine with the intrinsic (glandular) lobe of the corpus cardiacum of *Locusta migratoria*. Can J Zool 64:271–274
Orchard I, Lange AB, Cook H, Ramirez JM (1989) A subpopulation of dorsal unpaired median neurons in the blood-feeding insect *Rhodnius prolixus* displays serotonin-like immunoreactivity. J Comp Neurol 289:118–128
O'Shea M, Evans PD (1979) Potentiation of neuromuscular transmission by an octopaminergic neurone in the locust. J Exp Biol 79:169–190
Pflüger HJ, Watson AHD (1988) Structure and distribution of dorsal unpaired median (DUM) neurones in the abdominal nerve cord of male and female locusts. J Comp Neurol 268:329–345
Plotnikova SI (1969) Effector neurons with several axons in the ventral nerve cord of *Locusta migratoria*. J Exp Biochem Physiol 5:339–341
Raabe M (1985) Role of perisympathetic organs and other neurohemal organs in the neurosecretory system of insects. In: Gupta AP (ed) Neurohemal organs of arthropods. Thomas, Springfield Illinois, pp 552–580
Rapus J, Eckert M (1990) A new antiserum to octopamine for the demonstration of neurons with octopamine-like immunoreactivity in the American cockroach. In: Elsner N, Roth G (eds) Brain – perception – cognition. Proceedings 18th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 324
Römer H, Marquart V, Hardt M (1988) Organization of a sensory neuropil in the auditory pathway of two groups of Orthoptera. J Comp Neurol 275:201–215

Sombati S, Hoyle G (1984) Generation of behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. J Neurobiol 15:481–506

Spörhase-Eichmann U, Schürmann FW (1988) Serotonin-immunoreactivity in the central nervous system of the cricket *Gryllus bimaculatus*. In: Elsner N, Barth FG (eds) Sense organs: interfaces between environment and behavior. Proceedings 16th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 298

Spörhase-Eichmann U, Dircksen H, Hecht T, Helle J, Schürmann FW (1991) Neurohaemal-like fibre networks in an insect. In: Elsner N, Penzlin H (eds) Synapse – transmission – modulation. Proceedings 19th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 339

Sternberger LA (1979) Immunocytochemistry, 2nd edn. Wiley, New York

Stevenson PA, Pflüger HJ, Eckert M, Rapus J (1991) Octopamine immunoreactive neurons in the locust nerve cord. In: Elsner N, Penzlin H (eds) Synapse – transmission – modulation. Proceedings 19th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 1

Taghert PH, Goodman CS (1984) Cell determination and differentiation of identified serotonin-immunoreactive neurons in the grasshopper embryo. J Neurosci 4:989–1000

Tanaka Y, Washio H (1988) Morphological and physiological properties of the dorsal unpaired median neurons of the cockroach metathoracic ganglion. Comp Biochem Physiol 91A:37–41

Thompson KJ, Siegler MVS (1991) Anatomy and physiology of spiking intersegmental interneurons in the median neuroblast lineage of the grasshopper. J Comp Neurol 305:659–675

Tyrer MN, Turner JD, Altman JS (1984) Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. J Comp Neurol 227:313–330

Van der Sluis PJ, Pool CW, Sluiter AA (1988) Press-blotting on gelatin-coated nitrocellulose membranes after gel isoelectric focusing: application in the detection of peptides in the brain and the characterization of antiserum specificity. In: Van Leeuwen FW, Buijs RM, Pool CW, Pach O (eds) Molecular neuroanatomy. Elsevier, Amsterdam, pp 275–288

Watson AHD (1984) The dorsal unpaired neurons of the locust metathoracic ganglion: neuronal structure and diversity, and synapse distribution. J Neurocytol 13:303–327

Wigglesworth VB (1957) The use of osmium in the fixation of tissues. Proc R Soc Lond (Biol) 147:185–199

Wohlers DW, Huber F (1985) Topographical organization of the auditory pathway within the prothoracic ganglion of the cricket *Gryllus campestris* L. Cell Tissue Res 239:555–565

Woodring JP, Meier OW, Rose R (1988) Effect of development, photoperiod, and stress on octopamine levels in the house cricket, *Acheta domesticus*. J Insect Physiol 34:759–765

Woodring JP, McBride LA, Fields P (1989) The role of octopamine in handling and exercise-induced hyperglycaemia and hyperlipaemia in *Acheta domesticus*. J Insect Physiol 35:613–617

Yamaguchi T, Kushiro N, Waki T (1985) Sexual dimorphism of the terminal abdominal ganglion of the cricket. Naturwissenschaften 72:153–154