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# Suboesophageal DUM Neurons Innervate the Principal Neuropiles of the Locust Brain

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# Suboesophageal DUM neurons innervate the principal neuropiles of the locust brain

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## SUMMARY

The morphology of the dorsal unpaired median (DUM) neurons of the suboesophageal ganglion (SOG) of the migratory locust, *Locusta migratoria*, were studied by using intracellular staining. The SOG lacks segmental DUM neurons with peripheral axons. All DUM neurons are either intersegmentally projecting (towards the brain or the thoracic nerve cord) or they are local. In addition to previously described DUM neurons with axons in peripheral nerves of the brain (Bräunig 1990), the SOG contains DUM neurons which, in the brain, innervate principal neuropile areas such as the antennal lobes, the pedunculi and calyces of the mushroom body, and the central complex. The number and location of DUM cell bodies stained with intracellular fills is compared with those obtained with either backfilling cervical or circumoesophageal connectives, or octopamine-immunocytochemistry. Additional experiments show that the locust brain, like the SOG, lacks both segmental DUM neurons with peripheral axons, and axons descending into the ventral nerve cord.

## 1. INTRODUCTION

In the insect central nervous system, the great majority of neurons occur as mirror image pairs. Exceptions are the so-called dorsal unpaired median (DUM) neurons (Crossman *et al.* 1971; Hoyle 1978) which have received much attention from insect neurobiologists because of their unique morphological, physiological and ontogenetic characteristics. DUM neurons are the progeny of unpaired embryonic precursor cells (Goodman & Spitzer 1979, 1981*a*, *b*; Bate *et al.* 1981; Goodman *et al.* 1981), they develop into bilaterally symmetrical neurons and they have spiking somata (Crossman *et al.* 1971; Heitler & Goodman 1978; Hoyle & Dagan 1978; Christensen & Carlson 1982; Lange & Orchard 1984; Brookes & Weevers 1988; Gras *et al.* 1990; Ferber & Pflüger 1990). A few identified DUM neurons have been shown to contain octopamine, a biogenic amine that acts as neurotransmitter, neuromodulator and neurohormone in insects (for review see Orchard (1982); Agricola *et al.* (1988)).

Most previous investigations have dealt with the DUM neuron of thoracic (Crossman *et al.* 1971; Evans & O'Shea 1978; Heitler & Goodman 1978; Hoyle & Dagan 1978; Watson 1984; Pollack *et al.* 1988; Gras *et al.* 1990) or abdominal ganglia (Christensen & Carlson 1982; Lange & Orchard 1984; Brookes 1988; Brookes & Weevers 1988; Pflüger & Watson 1988; Ferber & Pflüger 1990). Comparatively little is known about such neurons in the ganglia of the head, the suboesophageal ganglion (SOG) and the brain. In the locust embryo, the ganglionic primordia which later form the SOG contain DUM neuron precursor cells (Doe & Goodman 1985). A few cells with DUM neuron morphology have also been stained in the SOG of mature locusts (Boyan & Altman 1985; Kien *et al.* 1990).

We have recently shown that the segmental arrangement of DUM neurons as found in the thoracic and most abdominal ganglia is substituted in the head by intersegmentally projecting DUM neurons: the periphery of the SOG is exclusively supplied by a single prothoracic DUM neuron (Bräunig 1988), peripheral nerves of the brain receive axon collaterals from DUM neurons located within the SOG (Bräunig 1990; Bräunig *et al.* 1990). The brain itself appears to lack DUM neurons with axons in peripheral nerves.

In the course of these experiments it became clear that not all suboesophageal DUM neurons project into peripheral nerves of the brain. We occasionally encountered cells that, after staining, showed bilateral symmetry typical for DUM neurons, and axons ascending in both circumoesophageal connectives. Their cell bodies were smaller than those of DUM neurons projecting into the periphery, and their dendritic arborizations within the suboesophageal ganglion were different. Also, their soma spikes could not be correlated with spikes recorded from peripheral nerves of the brain. For one of these cells, we have shown that it innervates many of the principal neuropiles of the brain such as the mushroom bodies and the central complex (Hahnel & Bräunig 1989).

Because in locusts the circumoesophageal connectives are very long, it is difficult to get complete intracellular fills of these neurons showing all ramifications within the brain. The success rate, depending on the type of neuron ranged between 5–10% of all preparations. This applies even more to situations in which only the ganglion has been exposed for in-

tracellular recording during physiological experiments. One aim of the present study, therefore, was to prepare a catalogue of all suboesophageal DUM neuron types as a source of reference which allows identification of incompletely filled neurons by their branching pattern within the SOG. This was done in isolated, rigidly fixed ganglia that allowed stable penetrations for long iontophoresis periods. In addition, this paper gives an estimate of how many DUM neurons there are within the locust SOG by comparing the number of morphologically different cell types obtained by intracellularly staining individual cells with the numbers of cells obtained by either differentially staining the connectives or octopamine immunocytochemistry.

## 2. MATERIALS AND METHODS

### (*a*) *Insects*

Adult migratory locusts, *Locusta migratoria migratorioides* (R. & F.) of both sexes were used for all experiments. Before any manipulations they were anaesthetized by cooling in an ice box.

### (*b*) *Dissection*

The frontal part of the head (labrum, clypeus and frons) was removed by inserting a razor blade between the labrum and the anterior faces of the mandibles and making a vertical cut through the anterior tentorial arms towards the bases of the antennae. The insect was then decapitated and the head pinned frontal side up into a Sylgard-lined Petri dish with the mandibles and maxillae fixed in an opened position. After removal of pharynx, hypopharynx and numerous air sacs of the tracheal system, brain and suboesophageal ganglion were completely exposed and easy to dissect.

After dissection, the ganglia were transferred to a smaller dish on a small glass spoon. Care had to be taken not to bend the circumoesophageal connectives during this process. The ganglia were fixed in the dish dorsal side up with small insect pins. The suboesophageal ganglion was completely immobilized by using its peripheral nerves as 'tent ropes'. It was found necessary to keep the Ringer level in the dish as low as possible, to keep the preparation moist, but well aerated.

### (*c*) *Recording and staining*

It has been shown previously (Bräunig 1990) that the Nervus corporis cardiaci III (NCC III) of the brain receives axons of all suboesophageal DUM neurons so far studied. Accordingly, spikes were recorded from this nerve with small metal hook electrodes so that the neurons studied here could be tentatively identified as not being correlated with NCC III activity, i.e. neurons without axons in peripheral nerves of the brain.

The electrodes for intracellular recording and staining were filled with 0.1 mol $l^{-1}$ $[Co(NH_4)_6]Cl_2$ (Brogan & Pitman 1981). They had resistances between 40–80 MΩ. For staining, depolarising 5–10 nA current pulses of 200 ms duration were passed through the electrode at 2.5 Hz for 40–90 min. After filling, the preparations were stored in the refrigerator for 24–48 h to let the dye diffuse. In some cases, before filling the neuron, its activity was recorded on a FM-tape recorder (RACAL, Store 4 DS) and later transferred to a thermo-array digital chart recording device (SCHWARZER; Uniscript UD 210).

### (*d*) *Histology*

After precipitation of the $Co^{2+}$ with $(NH_4)_2S$, ganglia were fixed in alcoholic Bouin's for at least 2 h, washed in 70% isopropanol, rehydrated and intensified with silver (Bacon & Altman 1977). When necessary, silver precipitation on the ganglionic sheath was removed with Farmer's reducer (Pitman 1979). In some cases, the ganglia were cut into 100 µm horizontal or transverse (with respect to the neural axis) vibratome sections before intensification. In many preparations, the wholemount silver intensification method failed to reveal the neuronal arborizations deeper in the brain. Some of these preparations could be 'rescued' by cutting the brain into vibratome sections and intensifying those a second time. To study the branching patterns of cells in relation to prominent fibre tracts within the SOG, ganglia were embedded in soft plastic and serially sectioned at 10 µm. This was done because in 50 or 100 µm vibratome sections, tracts and commissures are comparatively hard to discern.

Neurons were drawn from wholemount ganglia (dorsal side up unless stated otherwise in the text) with a drawing tube attached to a compound microscope, or were reconstructed from serial 100 µm vibratome sections.

### (*e*) *Backfills*

To get an estimate of how many suboesophageal DUM neurons ascend towards the brain or descend towards the thoracic ganglia, circumoesophageal or cervical connectives were stained differentially with 2% $NiCl_2$ and 3% $CoCl_2$ (Quicke & Brace 1979; Sakai & Yamaguchi 1983). After precipitation with rubeanic acid, ganglia were fixed in Bouin's (both, alcoholic or aqueous solutions were found to yield good results), washed in several changes of 70% isopropanol to remove excess picric acid, dehydrated in isopropanol, cleared in methylsalicylate, and mounted in Canada Balsam (resin dissolved in methylsalicylate). This fixation and mounting procedure turned out to be superior to previously published ones (we observed considerable shrinkage of ganglia when the usual formaldehyde fixation was used, and fading of staining in ganglia embedded in commercial Canada Balsam which is dissolved in xylene) and was developed by C. Böhme in our laboratory.

Neuronal somata located along the dorsal midline of the SOG and which had acquired the red or purplish colour typical for double-labelled cells were counted from wholemount ganglia.




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### (*f*) *Immunocytochemistry*

The antiserum directed against octopamine (a kind gift of Dr H. G. B. Vullings, University of Utrecht) and the procedures used were essentially the same as those used by Konings *et al.* (1988). This reference also contains all relevant data concerning the specificity of this antiserum. Immunocytochemistry was done on 10 µm transverse paraplast sections of SOG fixed in GPA (glutaraldehyde–picric acid–acetic acid, fixative b of Konings *et al.* (1988)). Anti-octopamine staining of whole SOG was achieved by appropriate prolongation of incubation and washing times (incubation in the first antibody 3 or 4 days, overnight for the second antibody and peroxidase–antiperoxidase complex) and by the addition of 0.5 % Triton-X 100 to the antibody and washing media, except for the final rinse before peroxidase development with diaminobenzidine and $H_2O_2$.

### (*g*) *Nomenclature*

The naming scheme in use for thoracic DUM neurons (the capital letters 'DUM' followed by the Arabic numeral designating the peripheral nerve(s) into which a neuron sends axon collaterals) cannot be applied to the neurons studied here, because, for two reasons, it would lead to very complex designations. First, the peripheral nerves of the brain are usually referred to with names, not numerals. Second, suboesophageal DUM neurons may project into as many as four different peripheral nerves of the brain (Bräunig 1990). In addition, neurons that stay within the CNS could not be named in this fashion.

For these reasons, in the present study the nomenclature used by Boyan & Altman (1985) and Kien *et al.* (1990) is extended. Neurons are designated as 'DUM' followed by the letters 'S' for suboesophageal ganglion, 'A' for ascending, and/or 'D' for descending, followed by an arbitrary number. Neurons that send axons into peripheral nerves of the brain are further marked by a lower case 'p'. DUM SAp 1–6 would therefore designate the six DUM neurons previously described (Bräunig 1990), the neuron described by Hahnel & Bräunig (1989) becomes DUM SA 1.

The tracts and commissures of the CNS are labelled as in Strausfeld (1976) and Tyrer & Gregory (1982). All directions are given in relation to the neural axis of the CNS.

## 3. ABBREVIATIONS USED IN TEXT AND FIGURES

| | |
|---|---|
| CC | circumoesophageal connective |
| DIT | dorsal intermediate tract |
| DMT | dorsal median tract |
| DUM | dorsal, unpaired, median |
| FC | frontal connective |
| LDT | lateral dorsal tract |
| MDT | median dorsal tract |
| MVT | median ventral tract |
| NCC I, II, III | Nervus corporis cardiaci I, II, III |
| SOG | suboesophageal ganglion |
| T | main tracheal trunk of SOG |
| T1, T2 | pro- and mesothoracic ganglion |
| VIT | ventral intermediate tract |
| VMT | ventral median tract |

## 4. RESULTS

### (*a*) *Differential staining of circumoesophageal and cervical connectives*

Backfilling the connectives to double-label neurons with cobalt and nickel turned out to be a tricky procedure (see also Ferber & Pflüger 1990). One problem is that neurons with axons in both connectives might fail to take up one or the other dye, or might entirely fail to stain. A more serious problem is that staining of a nerve trunk as massive as a connective may cause artifacts due to leakage and transneuronal staining. However, when preparations with high background staining (indicative for leakage and unspecific diffusion) were discarded, the numbers of double-labelled cell bodies observed were fairly constant.

After filling the circumoesophageal connectives, maximally 14–17 (nine preparations) somata with the typical reddish colour formed a cluster along the dorsal

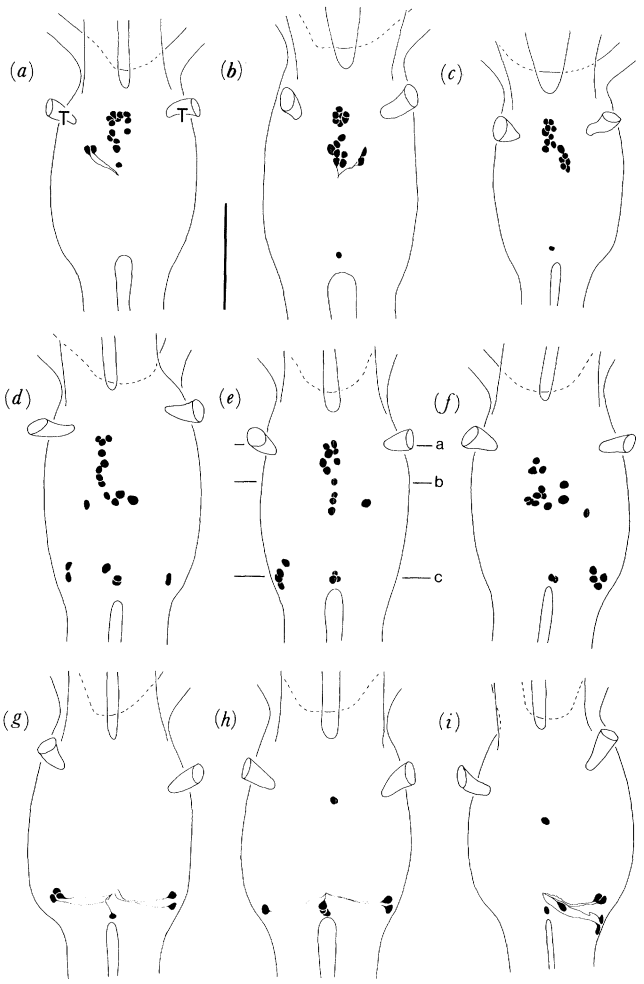


Figure 1. Comparison of number and location of dorsal cell bodies as revealed by differentially labelling the circumoesophageal (*a–c*) and the cervical connectives (*g–i*), or wholemount octopamine-immunocytochemistry (*d–f*). Scale: 500 µm.

longitudinal midline of the SOG (figure 1 *a–c*). In the following, these somata will be referred to as the *anterior cell body group*. The anterior border of this cluster is approximately determined by an imaginary line connecting the two large, funnel-shaped tracheal trunks that enter the SOG on each side dorsally and laterally (marked as T in all figures). The posterior margin of the cluster roughly corresponds to the borderline between maxillary and labial neuromeres of the ganglion. As a rule, a few cell bodies are displaced laterally, but their neurites can always be followed towards the midline (figure 1 *a*, *b*). The diameters of the somata in the anterior cell body group range between 30 and 40 μm. In a few preparations one additional, smaller (20–25 μm) cell body was located posteriorly in the ganglion, in the crotch between the cervical connectives (figure 1 *b*, *c*).

Via the cervical connectives, five or six somata in the posterior region of the SOG were stained (five preparations). These will be referred to as the *posterior cell body group*. The somata in this group are of the same size as those of the anterior group; their position, however, is variable. They are either located in the crotch between the connectives, or laterally, whereby the number of cell bodies in each location varies between preparations (figure 1 *g–i*). In a few cases, an additional seventh cell body located within the anterior cell body group was labelled (figure 1 *h*, *i*).

### (*b*) *Octopamine-immunocytochemistry*

Figure 1 *d–f* shows the position of octopamine-immunoreactive cell bodies in three different wholemount ganglia. The size, number and location of these cell bodies corresponds rather well to those obtained by filling the connectives. There are maximally 13–14 somata labelled in positions corresponding to those of the anterior cell body group, and maximally seven cell bodies observed in positions corresponding to those of the posterior cell body group. The number of immunoreactive cell bodies in anterior positions is slightly less than the number obtained in backfills of the circumoesophageal connectives. The number of posterior immunoreactive cell bodies would match the six neurons labelled via the cervical connectives plus the one labelled via the circumoesophageal connectives.

In wholemount SOG, immunolabelling of the somata was very weak. Much better results were obtained in sectioned material (figure 2). When counting the somata in sectioned ganglia (three ganglia) 13–15 cells were labelled in the anterior region of the ganglion, and seven cells in the posterior region. In addition to the dorsally located large cell bodies, two pairs of smaller cell bodies were stained ventrally in the SOG. Immunoreactive profiles within the neuropile were rarely observed, and if so, they were diffuse and weakly labelled. A further observation was that octopamine-immunocytochemistry completely failed in both wholemount and in sectioned SOG obtained from locusts shortly after imaginal ecdysis. All results presented here thus derive from mature locusts 4–6 weeks old.

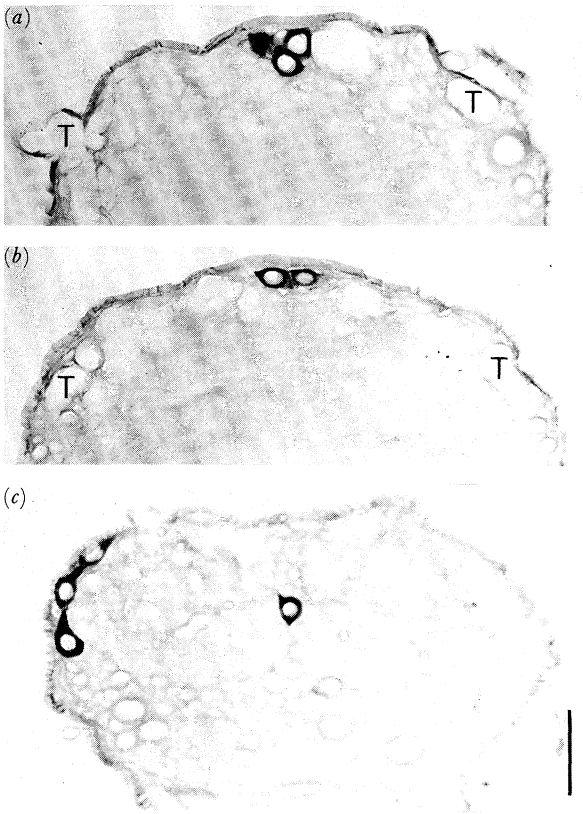


Figure 2. Octopamine-immunocytochemistry in sectioned SOG. (*a*) and (*b*) show the dorsal half of 10 μm transverse sections through the anterior cell body group (for definition see text); (*c*) shows a section through the posterior cell body group. The approximate planes of the sections *a–c* are indicated in figure 1 *e*. Scale: 100 μm.

### (*c*) *Intracellular staining of individual neurons*

*General observations*

The neurons DUM SA 1–DUM SA 5 described below have several features in common with the previously described DUM neurons (DUM SAp 1–6) with axons in peripheral nerves of the brain (Bräunig 1990; Bräunig *et al.* 1990). All these neurons have their somata in the anterior cell body group and their axons ascend towards the brain within the median dorsal tract (MDT). A further common feature is that they do not send any collaterals into the mandibular neuromere of the SOG.

The cells are either silent or spontaneously active at low frequencies. When active, they produce the typical large-amplitude soma spikes with pronounced undershoot. The neurons with peripheral axons appear to be the ones with the largest cell bodies, although one has to be careful with this interpretation, because the catalytic process of the intensification method might produce layers of silver deposits around the cell bodies of variable thickness, depending on the amount of cobalt present in the cell.

Even in the isolated ganglia used in the present and the previous studies, synaptic events can be observed. Excitatory potentials are particularly pronounced in case of DUM SA 1 (an example is shown in figure 3 *d*). In general, neurons that showed synaptic events and were spontaneously active, turned out after staining to




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be neurons without peripheral axons. Neurons with peripheral axons frequently spiked only upon current injection and showed fewer synaptic events. There were, however, exceptions to this rule.

*DUM SA 1*

This type of neuron was stained completely in nine preparations. Another two preparations showed only the branching pattern within the SOG. In four other preparations, the ramifications within the brain were incompletely filled, but sufficiently for identification.

Within the SOG, the branching pattern of this type of neuron is the least complex of all SOG DUM neurons stained so far. Arborizations are restricted to a dorsal area of the ganglion which, in a dorsal view, is roughly triangular in outline (figure 3*a*). Branches are found in the neuropile regions between the large dorsal longitudinal fibre tracts (MDT, DIT, VIT and LDT; figure 3*c*).

The ramifications of this type of neuron within the brain are very complex. The main axons proceed just below the dorsal surface of the brain through trito- and deutocerebrum, and about halfway into the protocerebrum, thereby converging medially. Here, they turn laterally and proceed towards the optic lobes. There are only a few branches in the dorsal proto-

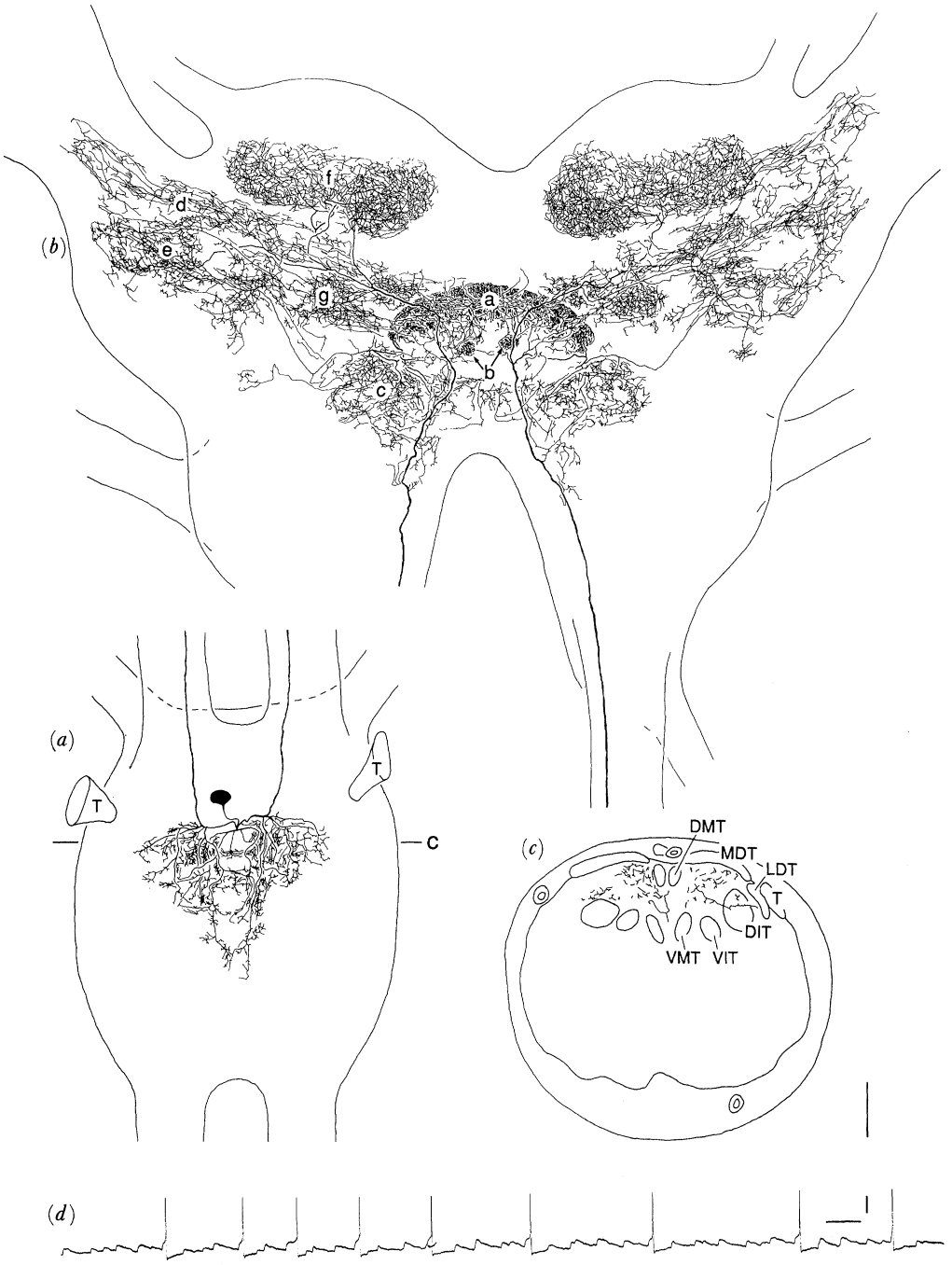


Figure 3. Branching pattern of DUM SA 1 in the SOG (*a*) and the brain (*b*) (anterior to the top). *a–g* in (*b*) indicate different neuropile areas described in the text. (*c*) shows a 10 μm transverse section of the SOG (dorsal to the top) in the region where the neuron has its maximal lateral extension (plane indicated in (*a*)) (scale for (*a–c*): 100 μm). (*d*) Spontaneous large-amplitude soma spikes and synaptic events recorded from the cell shown in (*a*) and (*b*) before filling. (Calibrations: horizontal, 100 ms; vertical, 10 mV.)

cerebrum, in some preparations also in the dorsomedial deutocerebrum.

Just before the main axons turn laterally, major collaterals emerge on both sides and supply the central body (area a in figure 3*b*) and the noduli (area b in figure 3*b*) of the central complex. In both neuropiles, the neuron terminates with a dense meshwork of ramifications (figures 3*b* and 8*a*, *b*). These first collaterals also supply area c (figure 3*b*), which consists of fibres and ramifications which wrap around the β-lobes of the mushroom bodies.

The main axons, on their way towards lateral regions of the protocerebrum, follow prominent fibre tracts which run just below the dorsal surface from the optic lobes towards the origin of the Nervus corporis cardiaci II (NCC II). These tracts probably correspond to the posterior optic tracts described in other insects (Strausfeld 1976). Numerous thin, parallel-running processes follow these tracts distally (area d in figure 3*b*), whereas the main axons proceed laterally to establish an area of ramifications in the lateroventral protocerebrum (area e in figure 3*b*).

Two to three anteriorly directed collaterals emerge from the main axons and establish a dense plexus of ramifications in the calyces of the mushroom bodies (area f in figure 3*b*, and figure 8*c*). Finally, a ventrally directed collateral supplies ramifications that wrap around the α-lobes of the mushroom bodies (area g in figure 3*b*).

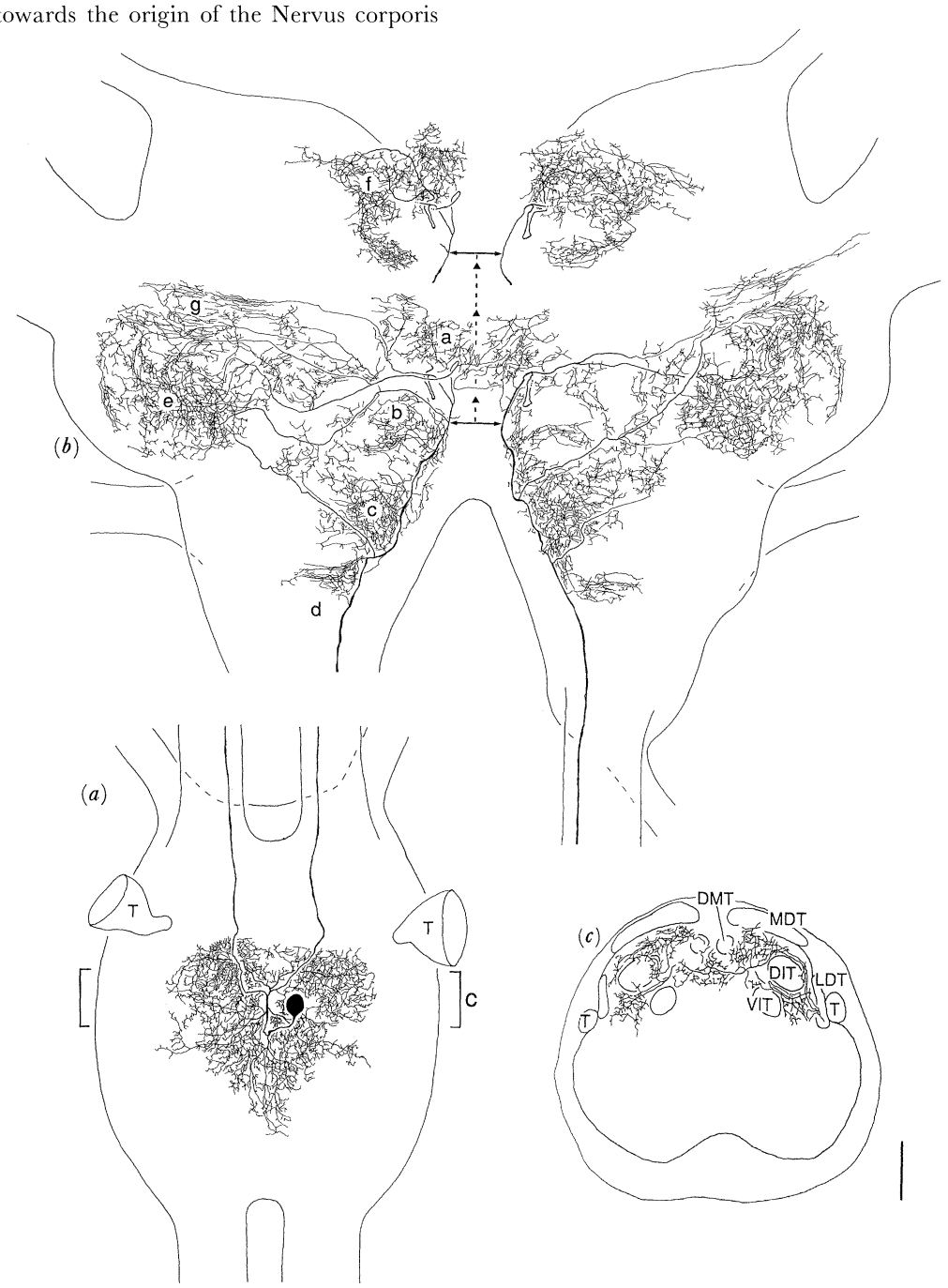


Figure 4. Branching pattern of DUM SA 2 in the SOG (*a*) and the brain (*b*) (anterior to the top). *a*–*g* in (*b*) indicate different neuropile areas described in the text. The ventral arborizations of area *f* are displaced towards the top as indicated by the broken line. (*c*) A 100 μm transverse section of the SOG (dorsal to the top) through the region indicated in (*a*). (Scale for (*a*–*c*): 100 μm.)

*DUM SA 2*

Most intracellular fills of this type of neuron (four preparations) were incomplete as far as ramifications in the brain are considered. In one of these, the ramifications in the brain were revealed after vibratome sectioning and re-intensification. The cell was stained completely in only one preparation.

Within the SOG, the branching pattern of DUM SA 2 differs from that of DUM SA 1 in only two respects. First, ramifications in the dorsal region of the SOG are much denser than those of DUM SA 1 (compare figures 3*a* and 4*a*). Second, DUM SA 2 has arborizations in the dorsolateral regions of the SOG, ramifications level with and below the lateral dorsal tracts (LDT) (figure 4*c*).

The course of the main axons of DUM SA 2 within the brain also resembles that of DUM SA 1. For this reason, it is difficult to distinguish both types of neurons in incomplete fills. However, when details of the branching patterns are compared, the two types are clearly different. DUM SA 2 not only has many more ramifications in the dorsal protocerebrum (areas a and b in figure 4*b*) than DUM SA 1, it also ramifies within the dorsal deutocerebrum (area c in figure 4*b*). Typically there are tufts of ramifications extending laterally at right angles to the main axon (d in figure 4*b*). These are very helpful in distinguishing DUM SA 2 from DUM SA 1 in incomplete fills.

DUM SA 2 also projects into the lateral protocerebrum, but whereas DUM SA 1 supplies the anteroventral regions, DUM SA 2 ramifications are mostly found in the dorsoposterior region (area e in figure 4*b*). Ventrally directed collaterals establish an area of dense ramifications just above the ventral surface of the brain in the vicinity of the α-lobes of the mushroom bodies (area f in figure 4*b*). A few branches also follow the posterior optic tracts (area g in figure 4*b*).

*DUM SA 3*

This type of neuron was stained in 10 preparations. The complete branching pattern in the brain could be seen in four of these. In one of the preparations, the brain branching pattern was revealed after vibratome sectioning and re-intensification. Five fills only revealed the branching pattern within the SOG.

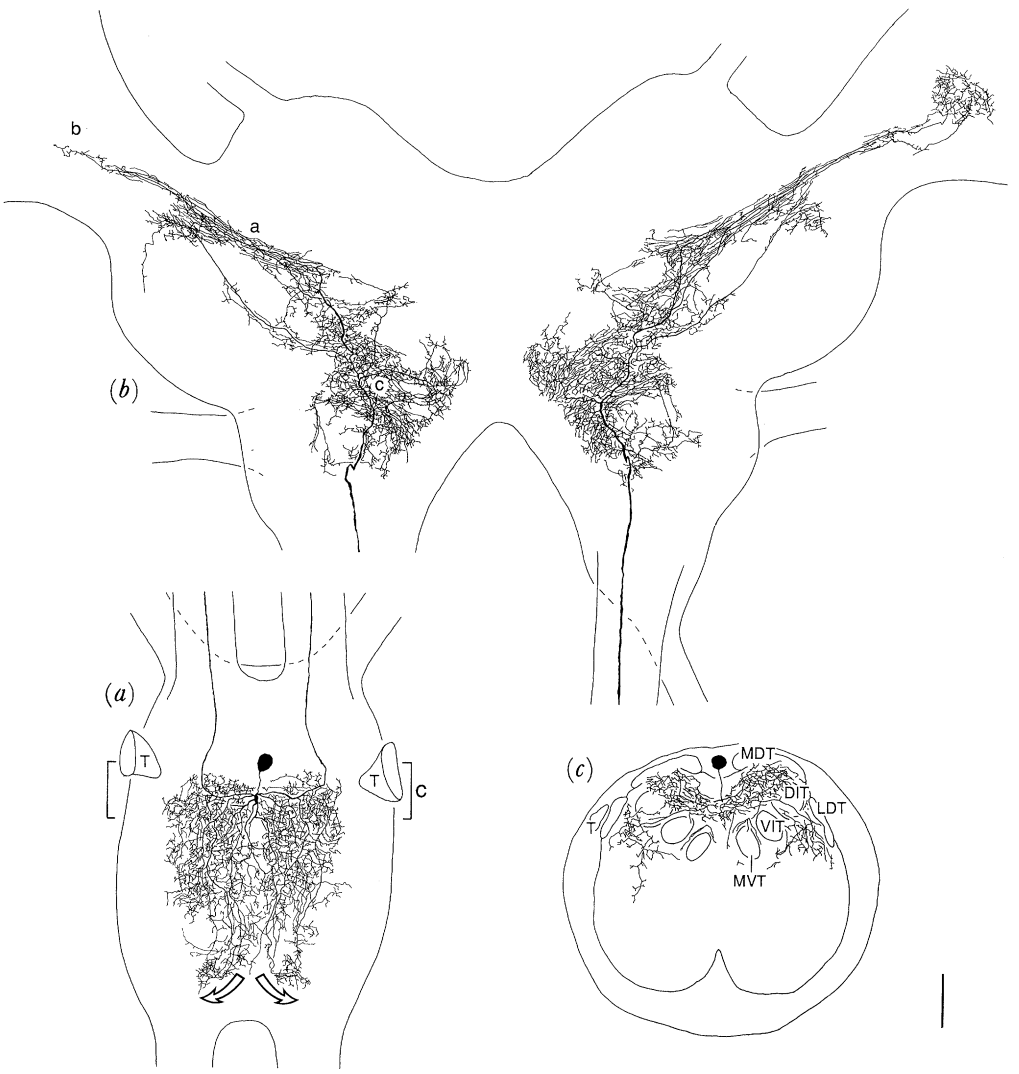


Figure 5. Branching pattern of DUM SA 3 in the SOG (*a*) and the brain (*b*) (anterior to the top). *a–c* in (*b*) indicate different neuropile areas described in the text. (*c*) A 100 μm transverse section of the SOG (dorsal to the top) through the region indicated in (*a*). (Scale for (*a–c*): 100 μm.)

Even within the SOG, DUM SA 3 establishes a typical branching pattern. The axons, all major dendritic arbors, and the thin neurite connecting to the soma emerge from a common origin. The ramifications, located in the dorsal region of the SOG, extend much further posterior than those of the two neuron types described so far. Typical for DUM SA 3 are the two hook-like dendritic tufts which emerge from medially running dendrites and curve in lateroposterior directions (indicated by curved arrows in figure 5*a*).

The ascending axons in the brain take a more lateral course than those of DUM SA 1 and 2. Also, the turns towards the optic lobes are less abrupt. One is tempted to call this type of neuron the 'optic DUM neuron', because of its branching pattern in the protocerebrum. Its projections into the posterior optic tract (a in figure 5*b*) are much more massive than those of DUM SA 1 and 2, and extend in some preparations into the lobula of the optic lobe (b in figure 5*b*). The third region of dense ramifications (c in figure 5*b*) corresponds to that region of the protocerebrum in which many large descending interneurons have their integrative segments and receive inputs from higher-order visual interneurons (O'Shea & Williams 1974; Griss & Rowell 1986; Hensler 1988; Rind 1990).

*DUM SA 4*

The branching pattern of this type of neuron in the brain was not visible in any wholemount preparation. The neuron is comparatively smaller than the ones described above and, for this reason, probably more difficult to stain completely. Five brains were sectioned and re-intensified. In two of these, the complete branching pattern was seen; in the other three at least the course of the main axon collaterals could be followed. In another three cases, cells were labelled only within the SOG, and showed the same type of branching pattern as DUM SA 4.

The differences in shape of DUM SA 4 and DUM SA 3 within the SOG are subtle (compare figures 5*a* and 6*a*). DUM SA 4 has a smaller cell body, its major dendritic arbors which proceed into posterior directions emerge from the main axons, and the hook-like tufts typical for DUM SA 3 are missing. The most posterior lateral projections are established by laterally running dendritic branches, not by medially running ones as in the case of DUM SA 3. In wholemounts, the density and the extent of the arborizations in anteroposterior and dorsoventral directions appears similar for both neuron types. Differences appear in sectioned material: although the arborizations of DUM SA 1–3 are mostly found between the dorsal longitudinal fibre tracts, those of DUM SA 4 are equally dense both within and between these tracts (compare figures 3*c*, 4*c*, 5*c* and 6*c*, *d*).

In the brain, the main axons of DUM SA 4 first takes routes similar to those of DUM SA 3. But while the main axons of DUM SA 3 proceed into the posterior optic tract, the axons of DUM SA 4 turn back medially and ventrally towards the pedunculi of the mushroom bodies. This 'pincer-shaped' course of the main axons provides a means for distinguishing the two neuron types even in preparations in which only the main axons are stained (figure 6*b*).

DUM SA 4 establishes four major areas of arborizations within the brain. First, it has many branches in the dorsal deutocerebrum (the antennal mechanosensory and motor centre; Rospars 1988; area a in figure 6*b*). Second, diffuse thin branches are found in the dorsal protocerebrum, just below the surface (area b in figure 6*b*). Third, the main axons approach the pedunculi and establish a meshwork of thin, parallel-running processes on their outer surface (area c in figure 6*b*; figure 8*d*). Some processes follow peduncular fibres towards the calyces, but, in contrast to projections of DUM SA 1, they do not project into the calyces. Finally, DUM SA 4 sends a few collaterals into the posterior optic tracts (d in figure 6*b*).

*DUM SA 5*

This type of neuron is also one of the smaller cells. It was first encountered in five preparations which showed the branching pattern in the SOG and the course of the main axons in the brain. One of these preparations showed a few weakly labelled profiles in the antennal lobes. With this information, the cell could be identified before filling by antidromic focal stimulation with a suction electrode placed on the surface of one antennal lobe. In this fashion, another three cells were identified and stained. The complete branching pattern within the brain, however, was only seen in sectioned material.

In contrast to the neurons described so far, there was no difficulty in unequivocally identifying DUM SA 5, even within the SOG. This is because arborizations of this type of neuron are not restricted to dorsal or dorsolateral neuropile areas between the most prominent dorsal longitudinal fibre tracts. Anteriorly in the SOG, between the mandibular and maxillary neuromeres, DUM SA 5 possesses ventrally directed processes that establish a vertical wall of projections (figure 7*d*). The ventralmost projections enter fibre tracts which so far have not been named. These tracts proceed on both sides in posterior directions (indicated by dots in figure 7*d*) and form a horseshoe- or omega-like structure. This tract is also used by some local DUM neurons (see below). How far DUM SA 5 collaterals follow these tracts posteriorly varies between preparations. Figure 7*a* shows the minimal, figure 7*c* the maximal extent.

The axons of DUM SA 5 in the brain first follow a course similar to those of DUM SA 3 and 4. In the dorsal deutocerebrum, however, they turn sharply, proceed laterally, and then descend more or less vertically into the antennal lobes. There are a few diffuse dendritic branches in the dorsal deutocerebrum and some of these extend into the region that corresponds to area b of DUM SA 4 (figure 6*b*). Primarily, however, DUM SA 5 innervates the antennal lobes. Within these lobes, it establishes extremely dense ramifications (figures 7*b* and 8*e*, *f*). A few thin processes also follow the antenno-glomerular tracts (marked with asterisks in figure 7*b*). It appears that the ramifications within the antennal lobes are as dense within olfactory glomeruli as they are between




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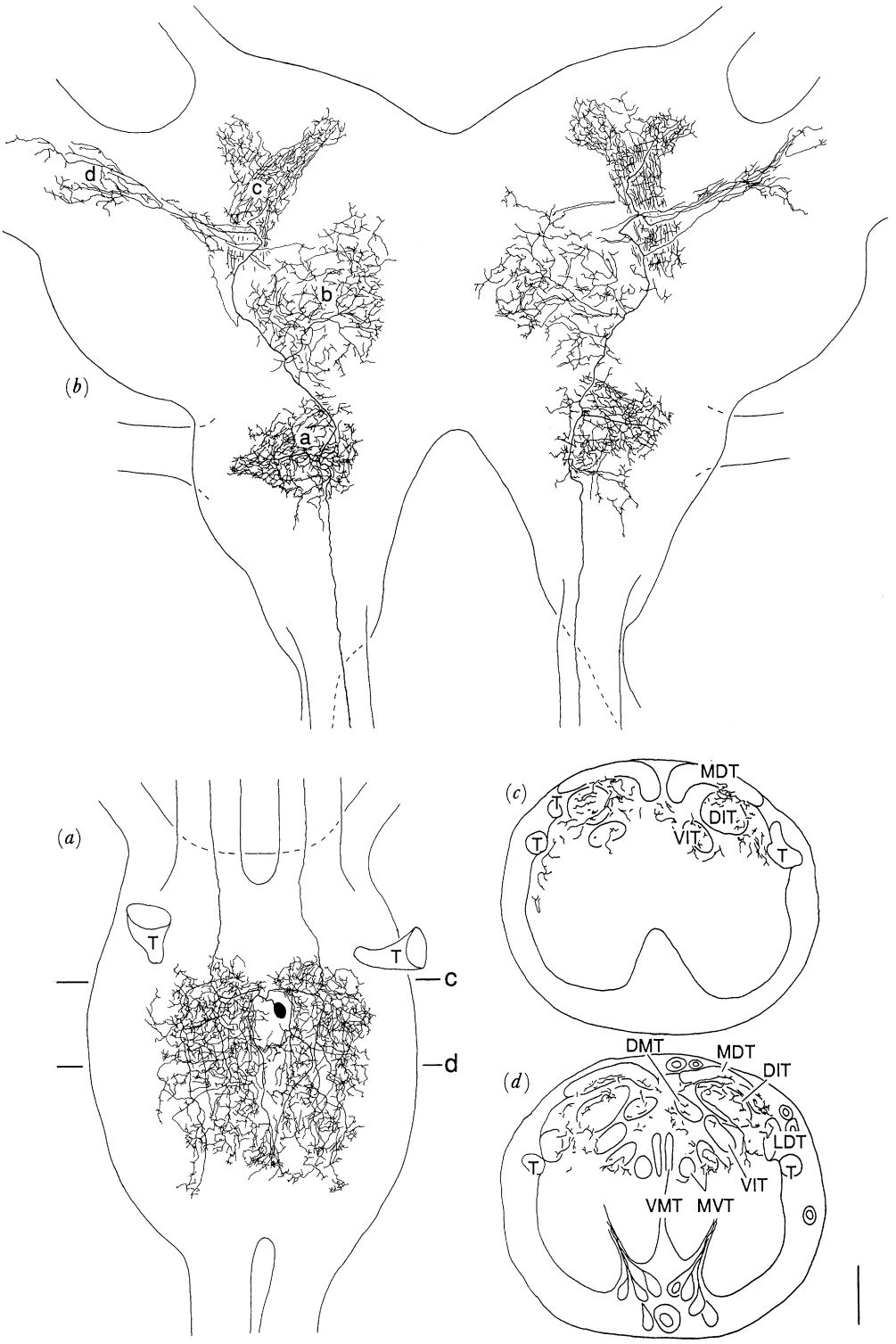


Figure 6. Branching pattern of DUM SA 4 in the SOG (*a*) and the brain (*b*) (anterior to the top). *a–d* in (*b*) indicate different neuropile areas described in the text. (*c*) and (*d*) 10 μm transverse sections of the SOG (dorsal to the top) through the regions indicated in (*a*). (Scale for (*a–d*): 100 μm.)

glomeruli. However, more such neurons have to be stained to provide material for fine-structural analysis, because the glomeruli could not be discerned very well in the thick sections used here.

*DUM SAD 1*

This type of neuron was stained in six preparations. Three of these showed the ramifications in the brain, in one of the other three the prothoracic ramifications were labelled.

DUM SAD 1 can already be distinguished from all other DUM neurons upon penetration. Typically, two spike sizes occur in recordings from the DUM SAD 1 cell body (figure 9*f*). The smaller spikes are attenuated spikes invading the soma. About 50% of these are topped by larger soma spikes with undershoot. As a rule, the soma spikes of DUM SAD 1 are smaller than

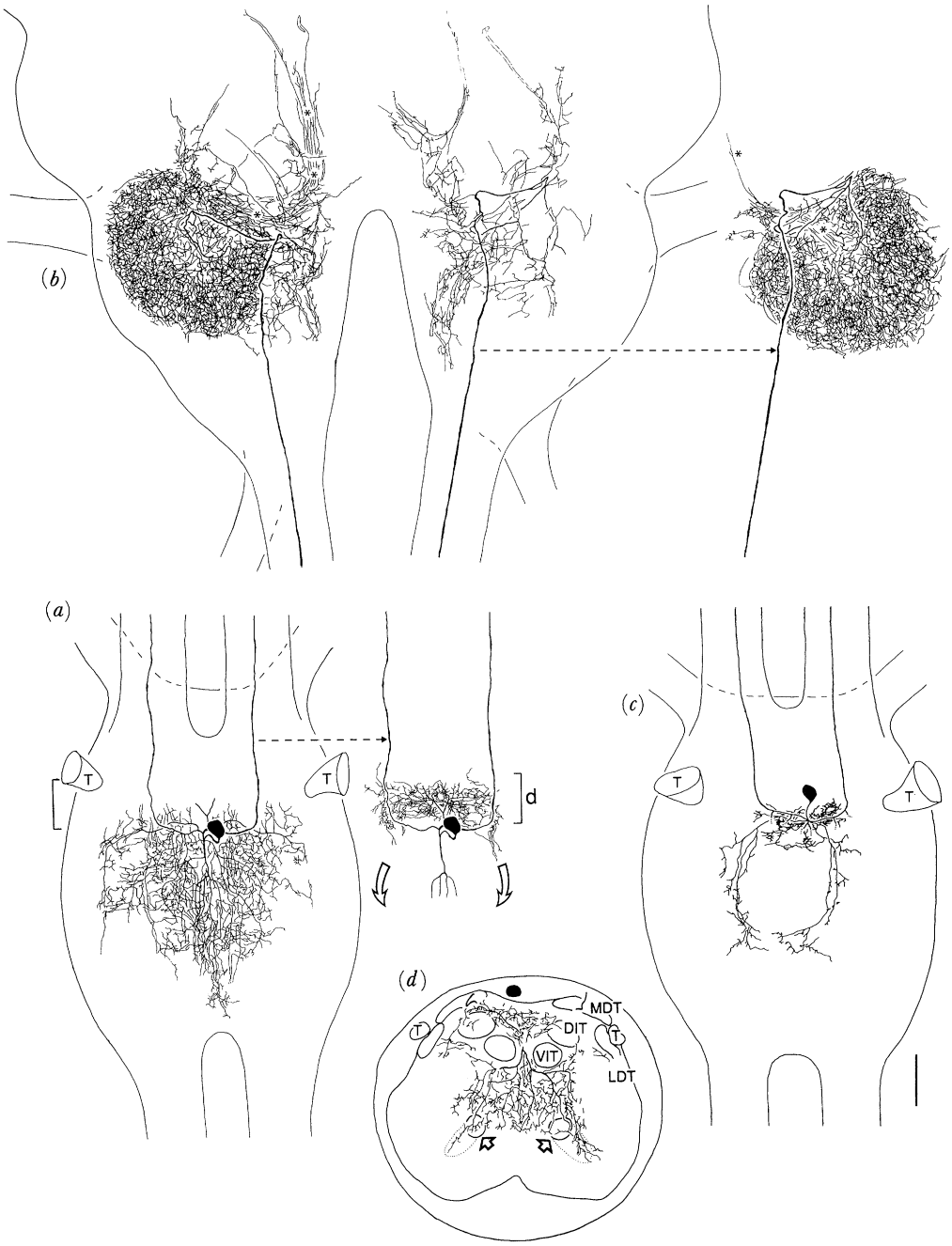


Figure 7. Morphology of DUM SA 5 in the SOG (*a*,*c*) and the brain (*b*) (anterior to the top). In (*a*) the dorsal branches of the neuron are shown on the left, the ventral projections are shown on the right as shown by the broken line. (*c*) The ventral projections of another preparation with fibres proceeding into the 'horseshoe tracts' (indicated by curved arrows in (*a*); see text for details). The 'anterior wall' of projections (see text) is shown in (*d*), a 100 μm transverse section (dorsal to the top) through the region indicated in (*a*). The 'horseshoe tracts' are shown by open arrows. The arborizations within the brain as reconstructed from 100 μm vibratome sections are shown on the left in (*b*). On the right, the dorsal projections and those in the antennal lobe are shown separately. The asterisks indicate fibres running within the antenno-glomerular tracts. Scale: 100 μm.

those recorded from the other DUM neurons. DUM SAD 1 ramifies profusely in the dorsal, dorsolateral and lateral neuropile of the maxillary and labial neuromeres of the SOG (figures 9*a*, *d* and 10*a*–*c*). Anteriorly directed processes also send numerous collaterals into the dorsal region of the mandibular neuropile (figures 9*a*, *c* and 10*b*, *c*).

DUM SAD 1 has both anteriorly directed and posteriorly directed axon pairs. Both pairs run in the lateral dorsal tract (LDT), not in the median dorsal tract (MDT) like the axons of all neurons described so far. The anterior axons are very thin and terminate in a few diffuse collaterals in the dorsal regions of trito- and deutocerebrum (figure 9*b*). The final destination of the posterior axons could not be determined. As figure 9*e* shows, the axons proceed beyond the prothoracic ganglion. Within this ganglion, processes follow the outer margin of the dorsal and dorsolateral neuropile. A few thin fibres also project for short distances into peripheral nerves (arrowheads in figure 9*e*).

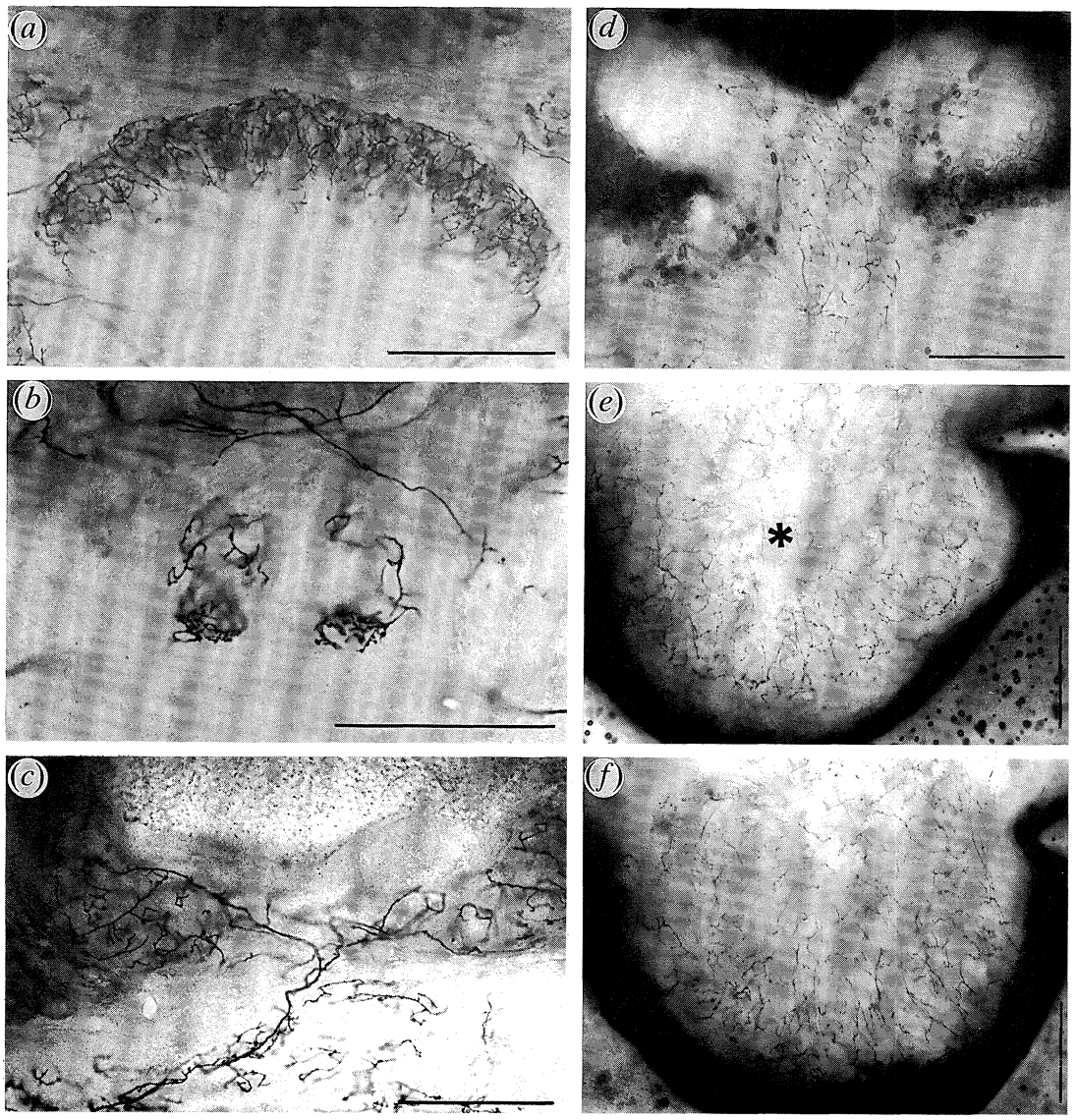


Figure 8. Photomicrographs of arborizations of SOG DUM neurons in principal neuropiles of the brain (100 μm vibratome sections; orientation as in previous figures; scales: 100 μm). (*a–c*) Projections of DUM SA 1 (comp. figure 3*b*) in the central body (*a*), the noduli (*b*), and the calyx of one mushroom body (*c*). (*d*) Meshwork of fine branches of DUM SA 4 around the pedunculus of the mushroom body (comp. figure 6*b*). (*e, f*) Two adjacent sections through the antennal lobe ((*f*) is ventral to (*e*)) after filling DUM SA 5 (figure 7*b*). Note that fibres are particularly dense in the periphery of the antennal lobe and less so near the centre, the origin of the antenno-glomerular tract (marked with an asterisk in (*e*)).

*DUM neurons of the posterior cell body group (DUM SD 1–6)*

Because the main interest of the present study lay on neurons projecting towards the brain, neurons of the posterior cell body group were not studied in as much detail as those of the anterior group. The results obtained by backfilling the cervical connectives suggest that there are six such neurons (figure 1 *g–i*). Only a few cells were stained individually to establish their DUM neuron character (soma spike, bilateral symmetry of arborizations). Figure 11 gives two examples of such neurons. It shows that the neurons have descending axons and that their arborizations exhibit bilateral symmetry. As already suggested by the results obtained with backfills of the posterior connectives and octopamine immunocytochemistry, the cell bodies of the posterior neurons may be located along the dorsal midline (figure 11*a*) or may be displaced laterally (figure 11*b*).

In this context it is interesting to note, that in our experiments on cricket SOG DUM neurons (Bräunig *et al.* 1990), we found that in crickets, also the cells of the anterior cell body group show much more variability in cell body position. It appears that in crickets, DUM neuron somata are dispersed over the entire dorsal and lateral surfaces of the SOG (P. Bräunig & C. Allgäuer, unpublished observations).

*Local DUM neurons*

When probing the dorsal surface of the SOG for DUM cell bodies, cells were occasionally penetrated which did not produce soma spikes. Instead, only attenuated spikes with rather small amplitudes could be recorded (figure 12*c*) both when the cells were

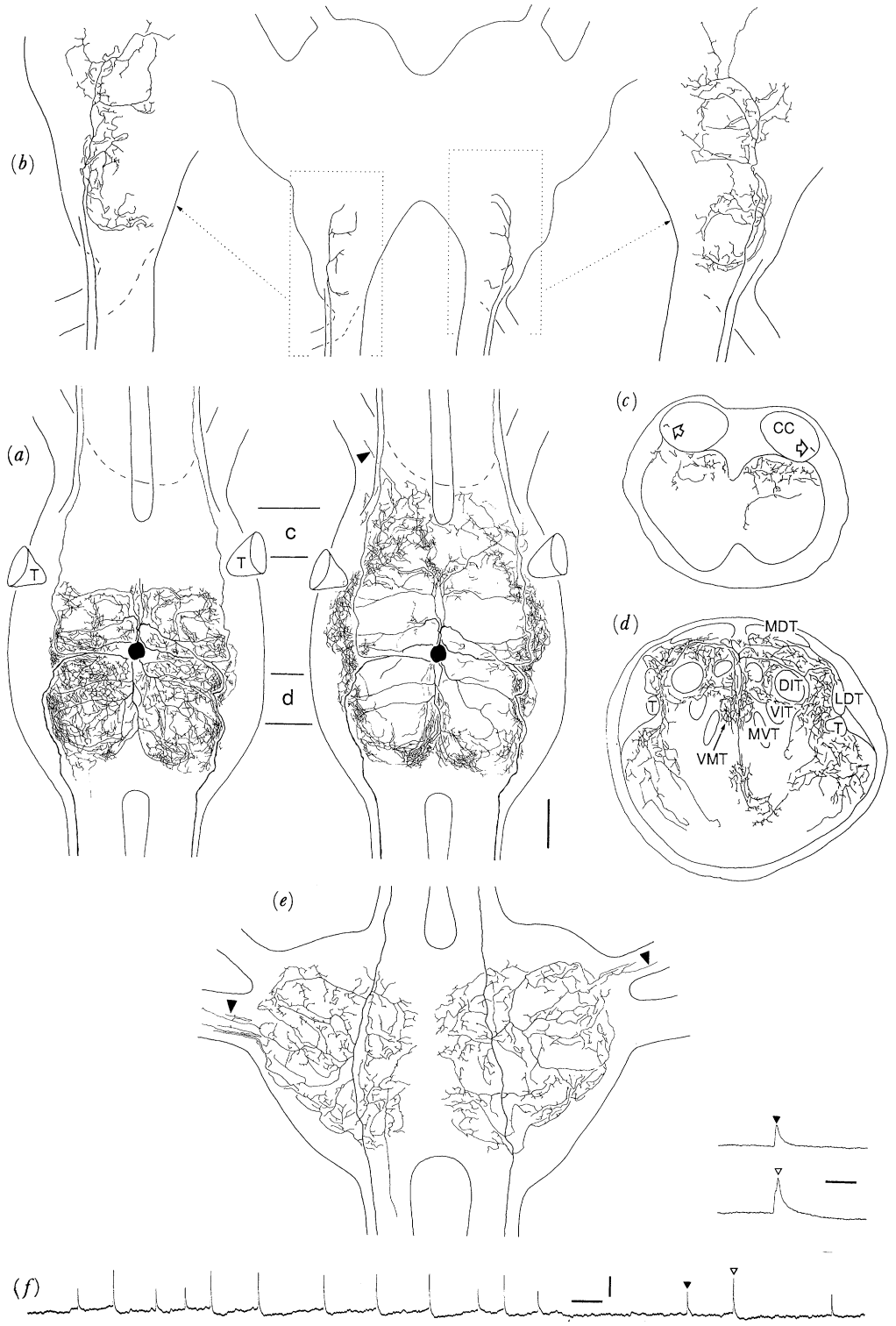


Figure 9. Morphology of DUM SAD 1 in the SOG (*a*), the brain (*b*), and the prothoracic ganglion (*e*) (anterior to the top). *a* and *e* are from the same preparation, *b* is from another preparation. *a* shows the dorsal and dorsolateral arborizations of the neuron within the SOG on the left, the ventrolateral and ventral arborizations on the right. (*c*) and (*d*) show two 100 μm transverse sections of the SOG (dorsal to the top). The planes of sectioning are indicated in (*a*). The open arrows in (*c*) indicate the anterior axons in the circumoesophageal connectives (CC). The arrowheads in *a* and *e* mark processes extending into peripheral nerves. Scale: 100 μm for (*a–e*), except for inset in (*b*). (*f*) shows a recording of the cell shown in *a* and *e* before filling (calibrations: horizontal, 100 ms; vertical, 10 mV). On the right, one attenuated spike (closed triangle) and one attenuated spike topped by a soma spike (open triangle) are shown with an expanded timescale (calibration: 10 ms).




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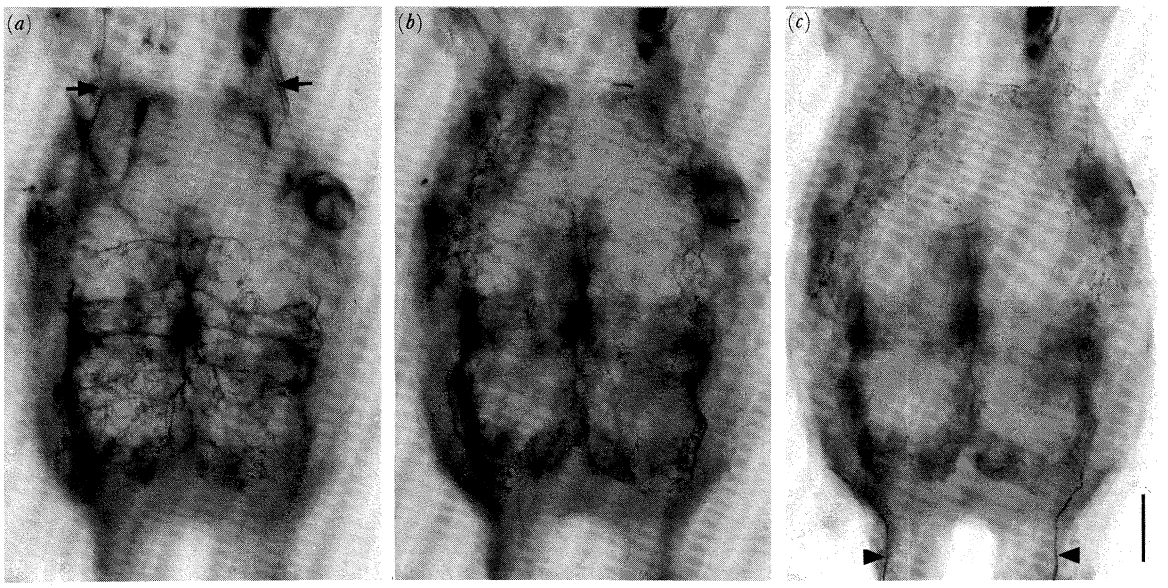


Figure 10. Photomicrographs of DUM SAD 1 arborizations within the SOG (same neuron as in figure 9*a*). (*a–c*) Three different focal planes (scale: 100 μm). The dorsal arborizations can be seen in (*a*), lateral and ventral arborizations in (*b*) and (*c*). The anterior axons (arrows) can be seen in *a* and *b*, the posterior axons (arrowheads) in (*c*).

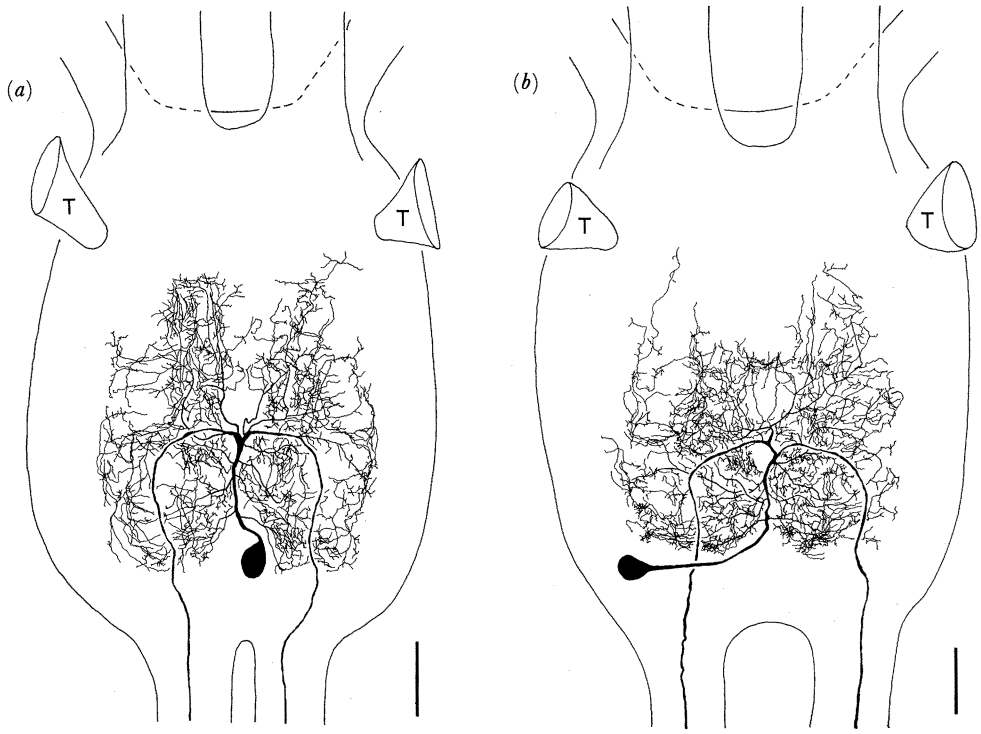


Figure 11. DUM neurons of the posterior cell body group (for definition see text). (*a*) A cell with a median cell body, (*b*) another cell with a laterally located cell body. Compare cell body size and location with that of cell bodies labelled by differentially staining cervical connectives (figure 1*g–i*), and cell bodies labelled with octopamine immunocytochemistry (figures 1*d–f*, and 2*c*). Scale: 100 μm.

spontaneously active, and when spikes were elicited by injecting depolarizing current pulses.

Staining revealed that such cells represent local neurons without any ascending or descending axons. Their cell bodies range in size between 15 and 20 μm, and, like the other DUM neurons, they have bilaterally symmetrical arborizations within the ganglion. Judged by the nine cells stained so far, the branching patterns of these local neurons are much more diverse than those of the large intersegmentally projecting ones. There are neurons that exhibit dorsal and dorsolateral projections (figure 12*a*), similar to DUM SA 4 and DUM SAD 1, and neurons that bear some resemblance to DUM SA 5 in that they project into the 'horseshoe tract' described earlier (figures 12*b* and 13*c*).

The most extreme type of local DUM neuron is shown in figure 13. This neuron has arborizations in the dorsal neuropile (figure 13*a*), the region cor-

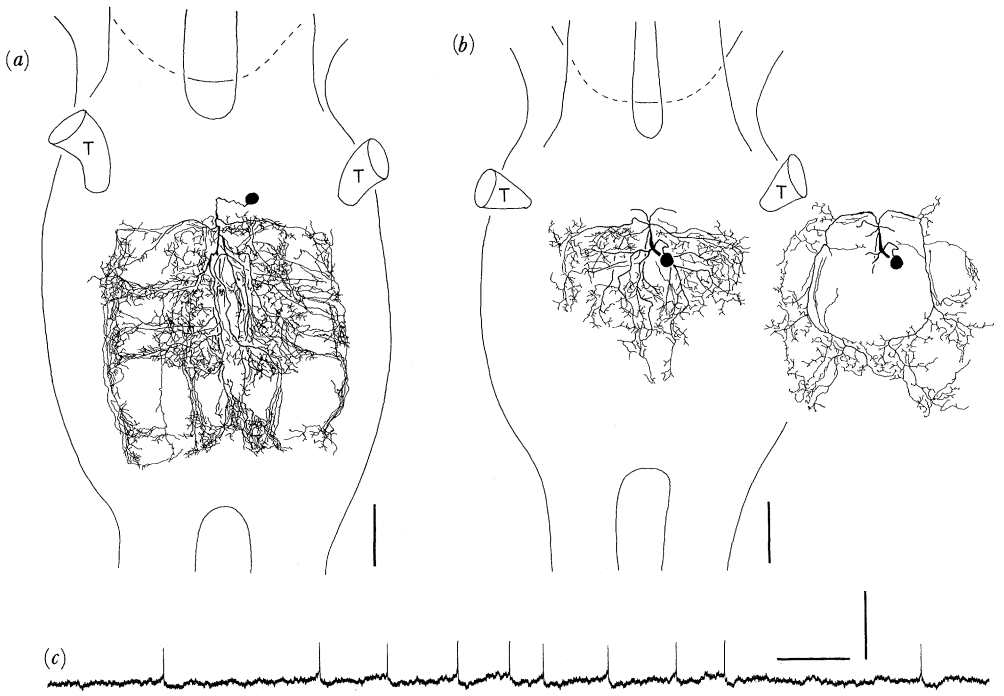


Figure 12. Two examples of local DUM neurons of the SOG (scales: 100 μm). The cell shown in *a* branches in the dorsal and dorso-lateral regions of the neuropile. The cell shown in *b* has dorsal (shown on the left) and ventral arborizations (shown on the right). The latter are supplied by collaterals running in the 'horseshoe tracts'. (*c*) A recording of spontaneous activity from the cell body of the cell shown in (*b*). (Calibrations: horizontal, 250 ms; vertical, 5 mV.)

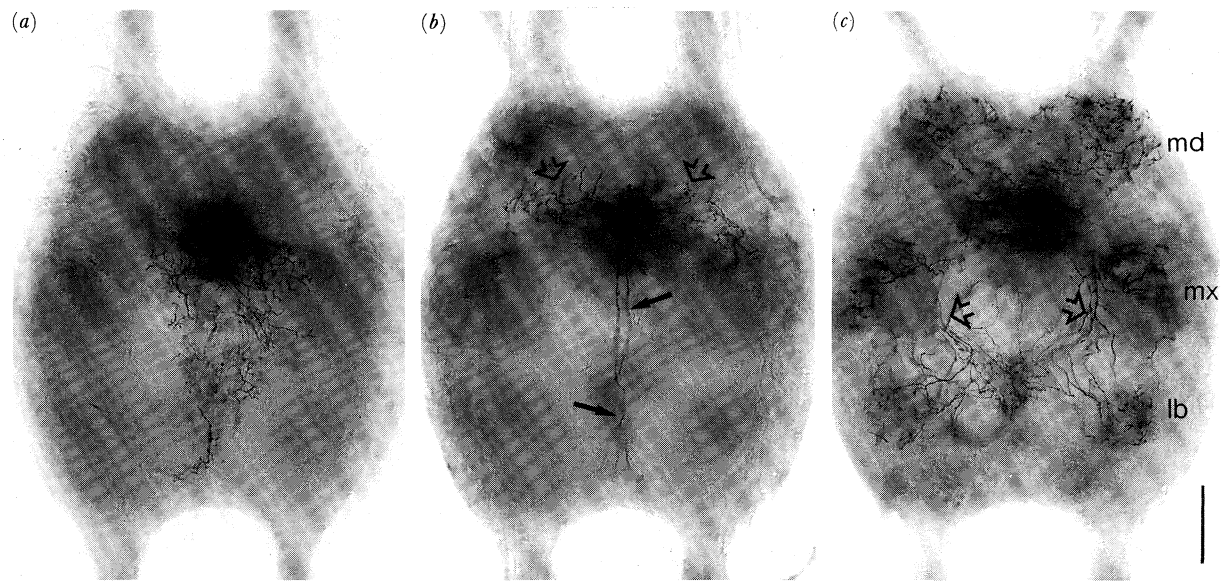


Figure 13. Photomicrographs of a third type of local DUM neuron (three different focal planes; scale: 100 μm). The cell has arborizations in the dorsal neuropile (*a*), the 'anterior wall' region (open arrows in (*b*); compare with DUM SA 5, figure 7*a*, *d*) and on the level of the median ventral tracts (*b*, arrows), and in the ventral neuropile areas of the mandibular (md), maxillary (mx), and labial (lb) neuromeres (*c*). The latter are supplied by collaterals running in the 'horseshoe tracts' (open arrows in *c*).

responding to the 'anterior wall' of DUM SA 5 (figure 7*a*, *d*; figure 13*b*, open arrows), and posteriorly directed collaterals on the level of the median ventral tracts (figure 13*b*, arrows). Particularly conspicuous are the arborizations in the ventral neuropiles of all three neuromeres of the SOG (figure 13*c*).

*Other DUM neurons*

In addition to the six cells described previously (DUM SAp 1–6; Bräunig 1990), only one neuron with axons in peripheral nerves of the brain was encountered during the present study. It was labelled DUM SAp 7 (figure 14). This neuron sends axons only into the frontal connectives. It resembles DUM SA 3 in that its

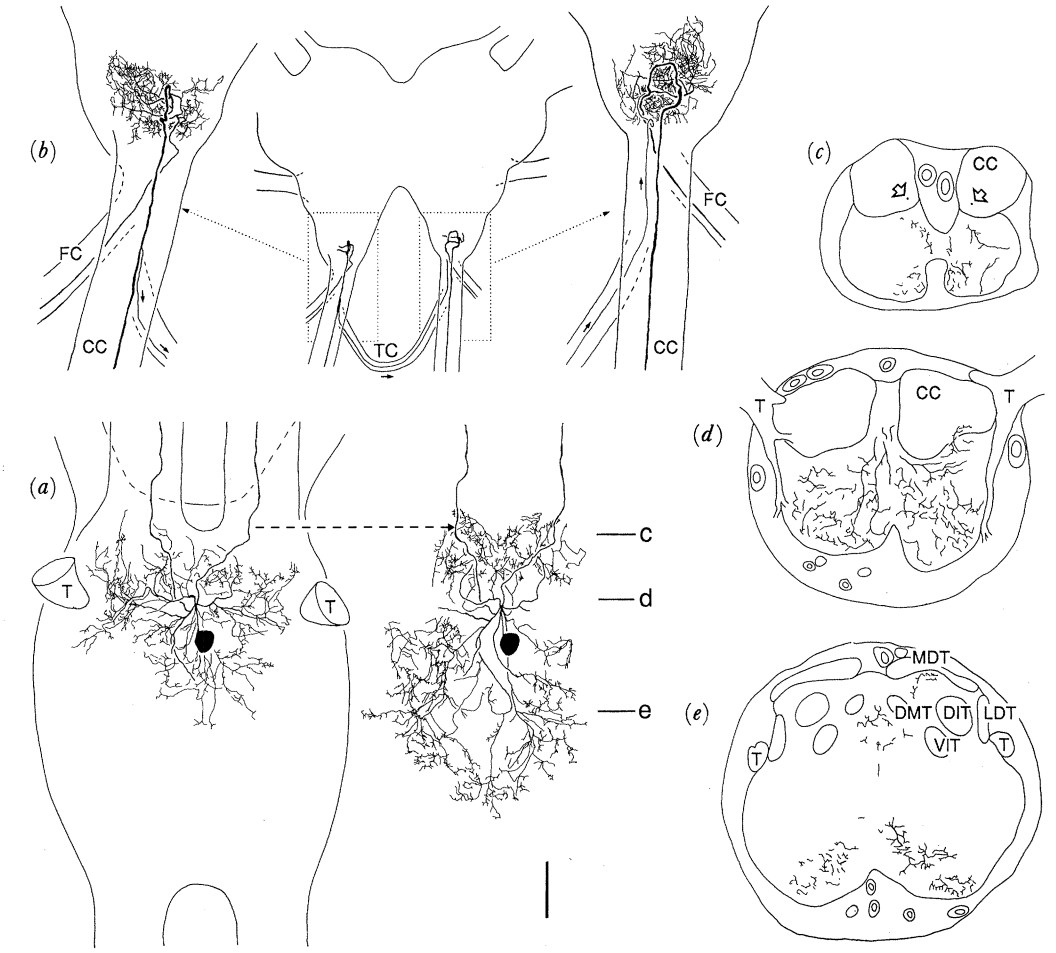


Figure 14. Morphology of DUM SAp 7 in the SOG (*a*) and the brain (*b*) (anterior to the top). In (*a*) the dorsal arborizations are shown on the left, the ventral arborizations on the right (indicated by broken line). (*b*) Projections in the tritocerebral lobes of the brain and the axons in the frontal connectives (FC). In this particular preparation a single collateral passes through the tritocerebral commissure (TC, arrows). (*c*, *d*, *e*) 10 μm transverse sections of the SOG (dorsal to the top, planes indicated in (*a*)). Note the ascending axons (open arrows in (*c*)) ventral in the circumoesophageal connective (CC). Scale: 100 μm (except for inset in (*b*)).

axons, major dendritic branches, and primary neurite have a common point of origin in the SOG. In other respects it differs very much from all other cells. In contrast to DUM SA 1–5 and DUM SAp 1–6, DUM SAp 7 has only a few branches in the dorsal neuropile of the SOG. Most of its arborizations are found ventrally, on a level with the ventral median and median ventral tracts (the VMT–MVT system), and in the ventral neuropiles of mandibular and maxillary neuromeres (figures 14*a*, *c–e*), but not the labial neuromere.

Another difference to other DUM neurons is that the ascending axons use a ventrally located longitudinal fibre tract, either the ventral median tract or the median ventral tract. Which tract could not be established with certainty, since the axons enter the root of the circumoesophageal connective anteriorly in the SOG, where all longitudinal tracts have already merged. In the brain, the cell establishes ramifications within the posterior, ventral tritocerebrum, close to the root of the frontal connective (figure 14*b*).

### (*d*) *Probing for DUM neurons in the locust brain*

When a particular peripheral nerve of the brain is electrically stimulated, suboesophageal DUM neuron spikes can be recorded from the corresponding contralateral nerve after a delay of 18–25 ms (Bräunig *et al.* 1990). When probing pairs of peripheral nerves of the brain in this fashion, such long-latency spikes can be recorded from antennal motor nerves (Bräunig *et al.* 1990), the frontal connective, the tritocerebral ventral nerve (TVN, described in Bräunig (1990)), the Nervus corporis cardiaci III (NCC III), but not from the Nervus corporis cardiaci I (NCC I). Moreover, no short-latency contralateral spikes, which would announce the presence of segmental, bilaterally projecting neurons, can be observed in these nerves.

Such short-latency spikes (2–4 ms) were, however, observed when stimulating the Nervus corporis cardiaci II (NCC II) on one side and recording from its contralateral counterpart. Staining both NCC II differentially with cobalt and nickel revealed two small red cell bodies in the Pars lateralis (figure 15*a*), but no large cell bodies along the dorsal midline of the brain. To find out whether these cell bodies belonged to




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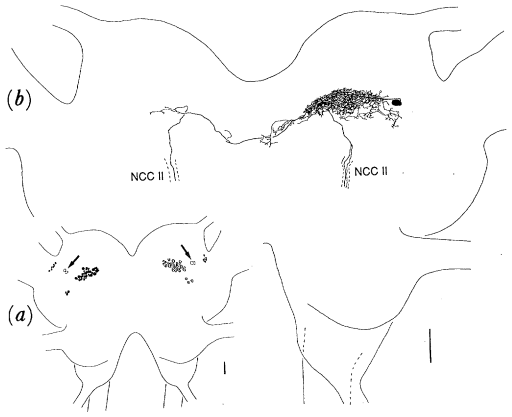


Figure 15. Morphology of neurons located in the Pars lateralis of the brain and projecting into both Nervi corporum cardiacorum II (NCC II) (ventral views; scales: 100 μm). (*a*) The location of cell bodies labelled with either nickel (black), cobalt (stippled), or nickel and cobalt (arrows) after differentially staining both NCC II. (*b*) An intracellular fill of one of the four bilaterally projecting neurons.

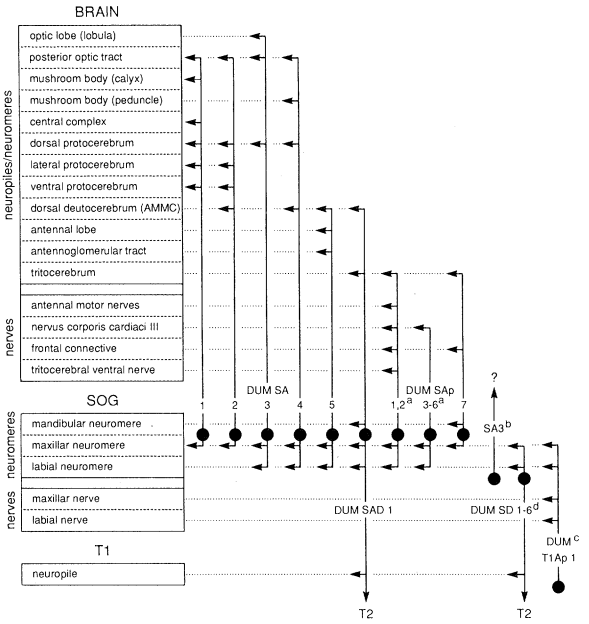


Figure 16. Summary diagram of DUM neurons of the head ganglia as described in this and previous publications (excluding local neurons). The somata are shown as dots, vertical lines represent ascending and/or descending axons. The arrows indicate into which neuropile or neuromere a neuron projects, or into which peripheral nerve(s) it sends axon collaterals. AMMC, antennal mechanosensory and motor centre (Rospars 1988); T1, T2, pro- and mesothoracic ganglion. (*a*) See Bräunig 1990; (*b*) see Boyan & Altman 1985; (*c*) see Bräunig 1988; (*d*) data acquired during revision of the manuscript show that DUM SD 1–6 (like DUM SAD 1) descend at least as far as T2 and project into the neuropile of T1.

DUM neurons with laterally displaced cell bodies (like the somata of the posterior cell body group in the SOG) such neurons were stained intracellularly. As figure 15*b* shows, their morphology is typical for Pars lateralis neurons and bears no resemblance to that of DUM neurons. In contrast to the great majority of Pars lateralis neurons, this type of neuron sends axons into both NCC II. Such neurons apparently went undetected in all previous studies of the locust NCC II (Mason 1973; Rademakers 1977; Koontz & Edwards 1980; Konings *et al.* 1989).

These results, taken together with the results obtained by octopamine-immunocytochemistry of the brain (Konings *et al.* 1988), strongly support the view that the brain lacks large DUM neurons that send axons into its peripheral nerves. This does not exclude the possibility that the brain contains unpaired cells with axons that descend into the ventral nerve cord, although we did not find any evidence for such neurons by differentially backfilling the circumoesophageal connectives towards the brain. Also, the brain might contain local DUM neurons.

## 5. DISCUSSION

### (*a*) *Classes of DUM neurons in the suboesophageal ganglion*

Unlike thoracic and abdominal ganglia, the SOG appears to lack segmental DUM neurons with peripheral axons. Evidence for this has been presented previously (Bräunig 1988), and all subsequent studies (Bräunig 1990; Bräunig *et al.* 1990; this present study) gave no indication for the presence of such neurons. All DUM neurons of the SOG are either intersegmentally projecting or local. They may be grouped into five different classes (figure 16 diagrammatically summarizes the results of the present and previous studies).

(i) Neurons which send axons into the brain, but not into its peripheral nerves. Morphologically, five different types can be distinguished (DUM SA 1–5; figures 3–8), which innervate principal neuropiles such as the antennal lobes, calyces and pedunculi of the mushroom bodies, the central complex, and the lobula of the optic lobe.

(ii) Neurons which project into the peripheral nerves of the brain (DUM SAp 1–7). Six such neurons have been described in a previous study (Bräunig 1990). All six project into NCC III, two of them send additional axons into the antennal motor nerves, the frontal connectives, and into a small tritocerebral ventral nerve (TVN; Bräunig 1990) that innervates pharyngeal dilator muscles. The present study adds to this list a seventh neuron, DUM SAp 7, which sends axons into the frontal connectives only (figure 14).

(iii) One neuron with axons in both anterior and posterior connectives (DUM SAD 1, figures 9 and 10), which resembles the H-cell identified in the thoracic and abdominal ganglia of grasshopper embryos (Bate *et al.* 1981; Goodman *et al.* 1981), and thoracic ganglia of mature cockroaches (Arikawa *et al.* 1984) and crickets (Gras *et al.* 1990).

(iv) Neurons with descending axons in the cervical connectives and cell bodies located either medially or laterally in the most posterior regions of the SOG (figure 11; see also Kien *et al.* 1990).

(v) Local neurons with bilaterally symmetrical arborizations and small cell bodies (figures 12, 13; Boyan & Altman 1985).

The neurons contained in classes (i–iii), with one exception, form the anterior cell body group, the




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neurons contained in class iv form the posterior group of cell bodies. A neuron with axons ascending towards the brain, but a posteriorly located cell body has been described by Boyan & Altman (1985, neuron SA3). One such neuron was also incompletely filled during the present study. The single, posterior cell body, double labelled after differential staining of the circumoesophageal connectives (figure 1*b*, *c*) probably belongs to this neuron.

Neurons with a cell body in the anterior cell body group and only descending axons as described by Kien *et al.* (1990, neuron SD33) were not stained in the present study. This neuron, however, might well correspond to DUM SAD 1. SD33 was stained with Lucifer Yellow and its small-diameter, anterior axons might well have been obscured by autofluorescent wrinkles of the ganglionic sheath frequently observed in such preparations. The single cell body found in the anterior cell body group after filling the cervical connectives (figure 1*h*, *i*) probably belongs to DUM SAD 1.

Finally, local neurons (class v) can be found within both anterior (present study) and posterior cell body groups (Boyan & Altman 1985).

### (*b*) *Are all suboesophageal DUM neurons unpaired?*

The question as to whether a neuron with a dorsal median cell body is a DUM neuron *sensu strictu*, that is an unpaired individual cell, can only be answered in the case of peripherally projecting cells. The number of such cells can be estimated by backfilling peripheral nerves or with electrophysiological methods and matched against the number of individual cell types obtained from intracellular fills. In the case of neurons that stay within the central nervous system, such as those presented here, the question is harder to answer. However, the results obtained with the various methods applied, at least for the intersegmentally projecting neurons with large somata, leave only a narrow margin of error, not only for the existence of cell pairs, but also of additional neurons that have not yet been identified.

Out of the anterior cell body group, seven SOG DUM neurons project into the peripheral nerves of the brain (DUM SAp 1–7), five cells project into principal neuropiles of the brain (DUM SA 1–5), and one cell projects both towards the brain and towards the thoracic nerve cord (DUM SAD 1). These cells add up to a total of 13. (One fill of a neuron within the SOG similar to but clearly distinct from DUM SAp 7 indicates that there might be a 14th type of DUM neuron.) This number corresponds well with the maximum number of cell bodies stained with the octopamine antibody (figure 1*d–f*), as well as with the numbers obtained by differentially labelling the circumoesophageal connectives (figure 1*a–c*).

Although the neurons of the posterior group were not studied in as much detail as the anterior ones, corresponding cell numbers are obtained with different methods for this group also. Six neurons were labelled by filling the cervical connectives (figure 1*g–i*), one neuron was occasionally labelled by filling the circumoesophageal connective (figure 1*b*, *c*). Octopamine immunocytochemistry labelled a maximum of seven cells posteriorly in the SOG (figure 1*d–f*). Both methods show that the cell bodies may be located near the midline of the ganglion or laterally, the numbers in each location being different in different preparations, but the total number not exceeding 7. The intracellular fills presented here and by Kien *et al.* (1990, neuron SD32) confirm the bilateral symmetry of the central arborizations of the posterior DUM neurons, regardless of their cell-body position (figure 11).

### (*c*) *Local DUM neurons*

The question as to whether the local cells (figures 12 and 13) are unpaired individuals or not cannot be answered at present. So far, only a few such cells have been stained in insect thoracic ganglia (Goodman *et al.* 1980; Pollack *et al.* 1988), although we know from an ontogenetic study (Goodman *et al.* 1980) that they probably exist in large numbers.

In the present study, five different types of local neuron have been stained (not all are shown). All had their cell bodies within the anterior cell body group. A posteriorly located, local DUM neuron has been previously described (Boyan & Altman 1985). The small local neurons of the SOG differ from the large ones in that they do not produce soma spikes (figure 12*c*). This corresponds to what is known about local DUM neurons of thoracic ganglia (Pollack *et al.* 1988; Thompson & Siegler 1989).

Local DUM neurons of thoracic ganglia are GABA-immunoreactive (Thompson & Siegler 1989). Small, dorsally located GABA-immunoreactive cell bodies also exist within the locust SOG (Watkins & Burrows 1989). These might correspond to the local DUM neurons. The octopamine antiserum, in contrast, did not label any small cell bodies in the dorsal region of the SOG (figures 1 and 2), nor any in the thoracic ganglia (Konings *et al.* 1988).

### (*d*) *Innervation of principal brain neuropiles*

Unfortunately, trials to provide direct evidence that the DUM neuron somata in the SOG are identical with the octopamine-immunoreactive somata by means of double-labelling have so far failed because of technical reasons. However, the good correspondence between number, size and location of DUM neuron somata labelled either intracellularly or by backfilling the connectives and the somata labelled with octopamine immunocytochemistry suggests that most, perhaps all, large intersegmentally projecting SOG DUM neurons are octopaminergic.

Synaptic events can be recorded from the cell bodies of the intersegmentally projecting neurons DUM SA 1–5 even in the isolated ganglia used in the present study. In all neurons studied, soma spikes were elicited by supra-threshold excitatory potentials or by slow depolarizations of the membrane potential. This suggests that at least parts of the arborizations of the neurons within the SOG represent the input regions, while the arborizations within the brain most likely

represent output regions. Supra-threshold input in the brain ought to trigger antidromic spikes, which, passively invading the cell bodies in the SOG, might trigger soma spikes or not. Such events were not observed when recording from DUM SA 1–5.

Except for the photomotor neurons of fireflies (Christensen & Carlson 1982), all DUM neurons with peripheral axons so far investigated have been shown to be neuromodulatory cells that affect neuromuscular transmission (for review see Orchard (1982); Agricola *et al.* (1988)). Consistent with the general assumption that DUM neurons also modulate synaptic transmission in the central nervous system, is the finding that focal injection of the DUM neuron transmitter octopamine into the neuropile of thoracic ganglia elicits different behaviours (Sombati & Hoyle 1984*a*, *b*; Stevenson & Kutsch 1988), and stimulation of individual DUM neurons may cause dishabituation and sensitization of central synapses (Sombati & Hoyle 1984*b*).

The suboesophageal DUM neurons project into the most prominent neuropiles of the brain such as the mushroom bodies, central complex, and antennal lobes. They could, therefore, modulate synaptic transmission in these neuropiles which, although still poorly understood, are generally regarded as 'the higher brain centres of insects'. The central body and the mushroom bodies represent areas of sensory convergence and are thought to feed information to descending neurons, mostly via polysynaptic pathways (Schürmann 1987), thus providing a formidable substratum for neuromodulation. This is particularly interesting in view of older reports that these neuropiles are involved in the control and generation of behaviour (Huber 1955, 1959, 1960).

Antennal lobes and mushroom bodies have been shown to be involved in the processing of olfactory information and olfactory learning and memory functions (for review see Boeckh & Ernst (1987); Erber *et al.* (1987); Heisenberg (1989)). Injection of octopamine into the protocerebrum affects evoked potentials in the honey bee (Mercer & Erber 1983) and focal injection of octopamine into the antennal lobe or the calyces of the mushroom bodies interferes with learning and memory retrieval in this insect (Bicker & Menzel 1989). It is important in this context that neurons with great overall similarity to the locust DUM SA 1–5 have recently also been found in the honeybee (M. Hammer, personal communication).

All these results permit the working hypothesis that suboesophageal DUM neurons affect integrative processes in the principal neuropiles of the insect brain, probably by octopamine-dependant, neuromodulatory effects. Even if this should turn out to be wrong, the present study shows that suboesophageal neurons participate in the innervation of the most prominent neuropile areas of the brain to a hitherto unknown extent. Furthermore it provides the neuroanatomical basis for future functional investigations at the level of identified neurons.

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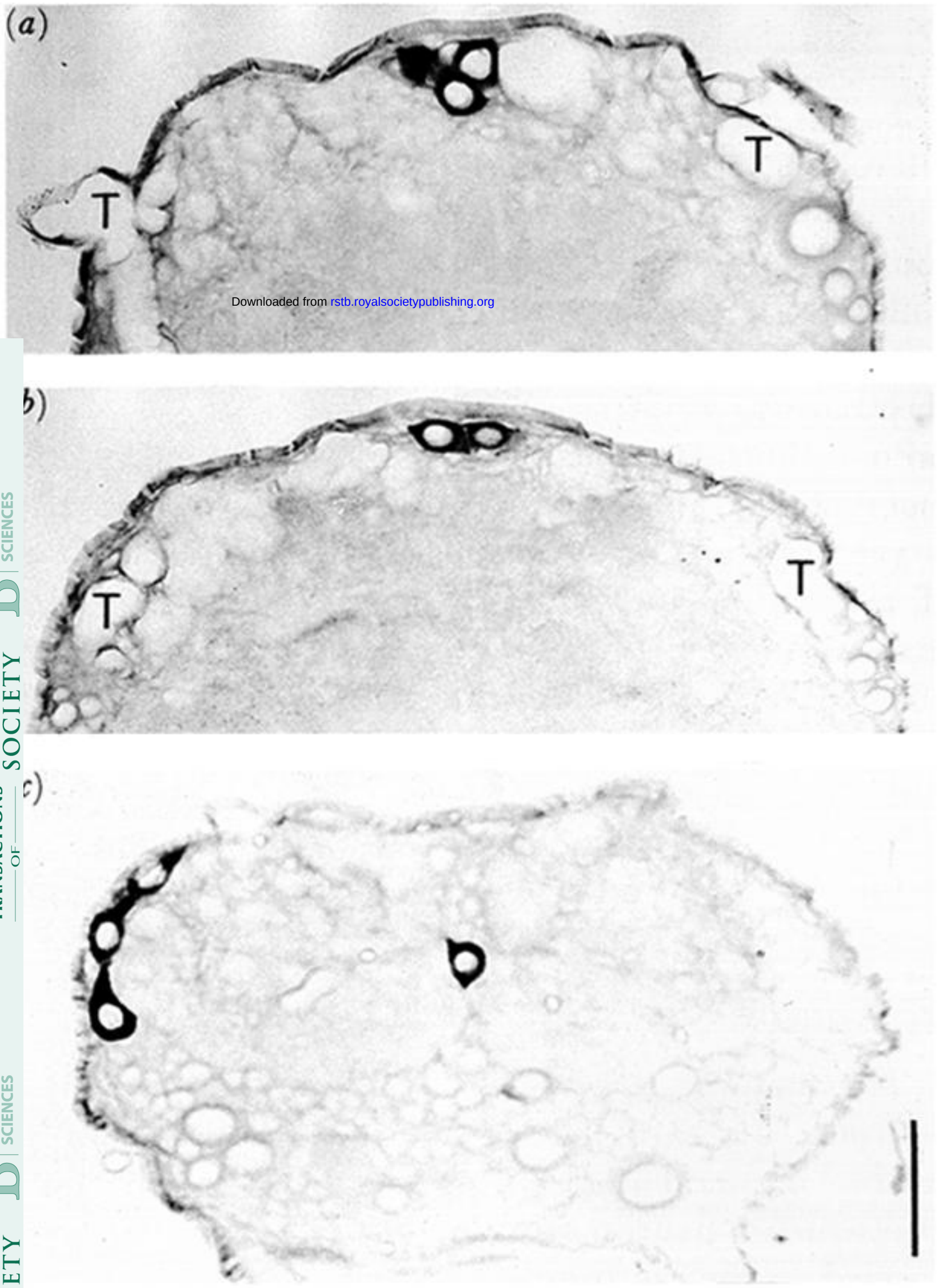




gure 2. Octopamine-immunocytochemistry in sectioned OG. (*a*) and (*b*) show the dorsal half of 10 µm transverse ctions through the anterior cell body group (for definition e text); (*c*) shows a section through the posterior cell body oup. The approximate planes of the sections *a*–*c* are dicated in figure 1*e*. Scale: 100 µm.

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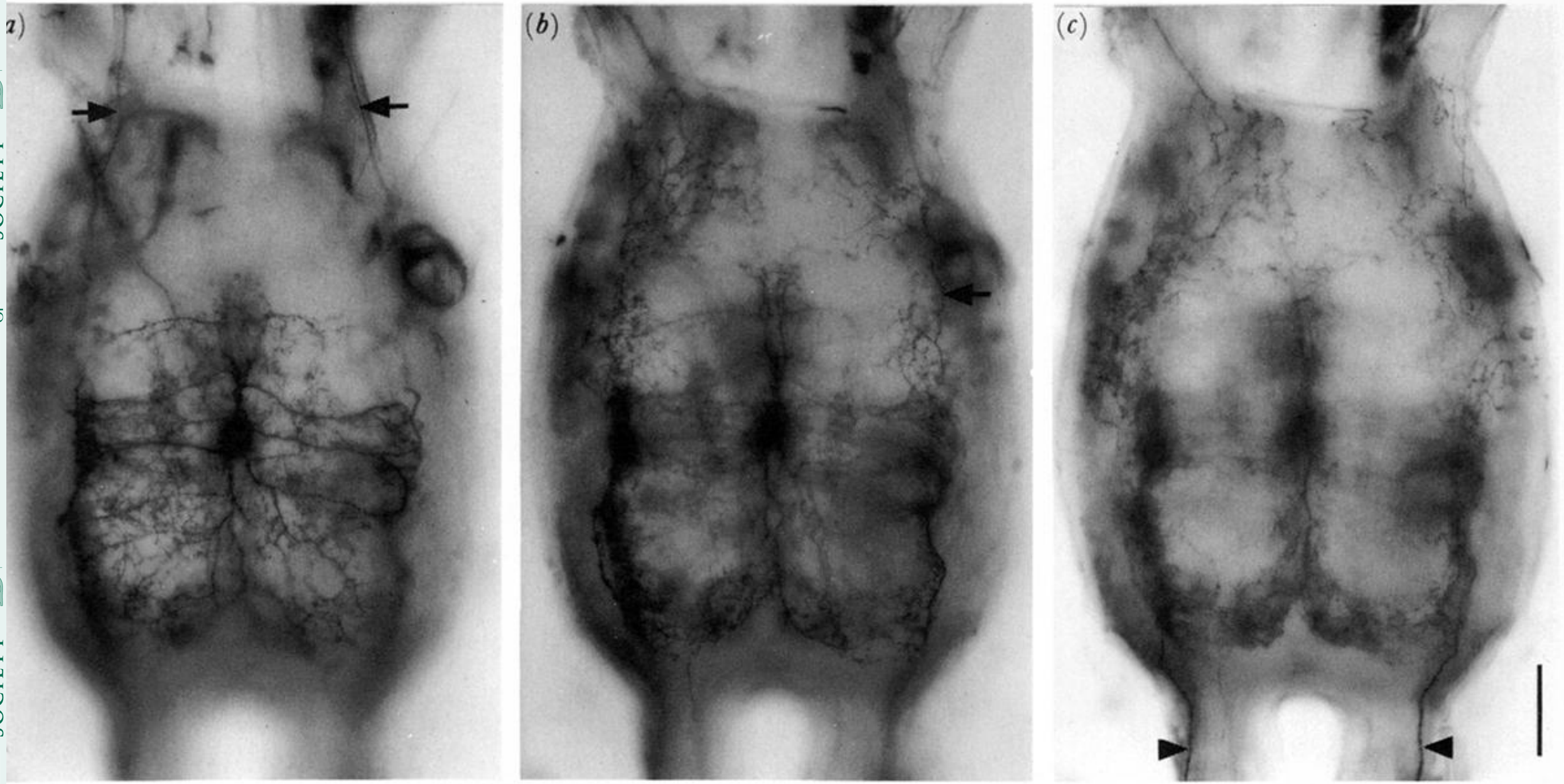
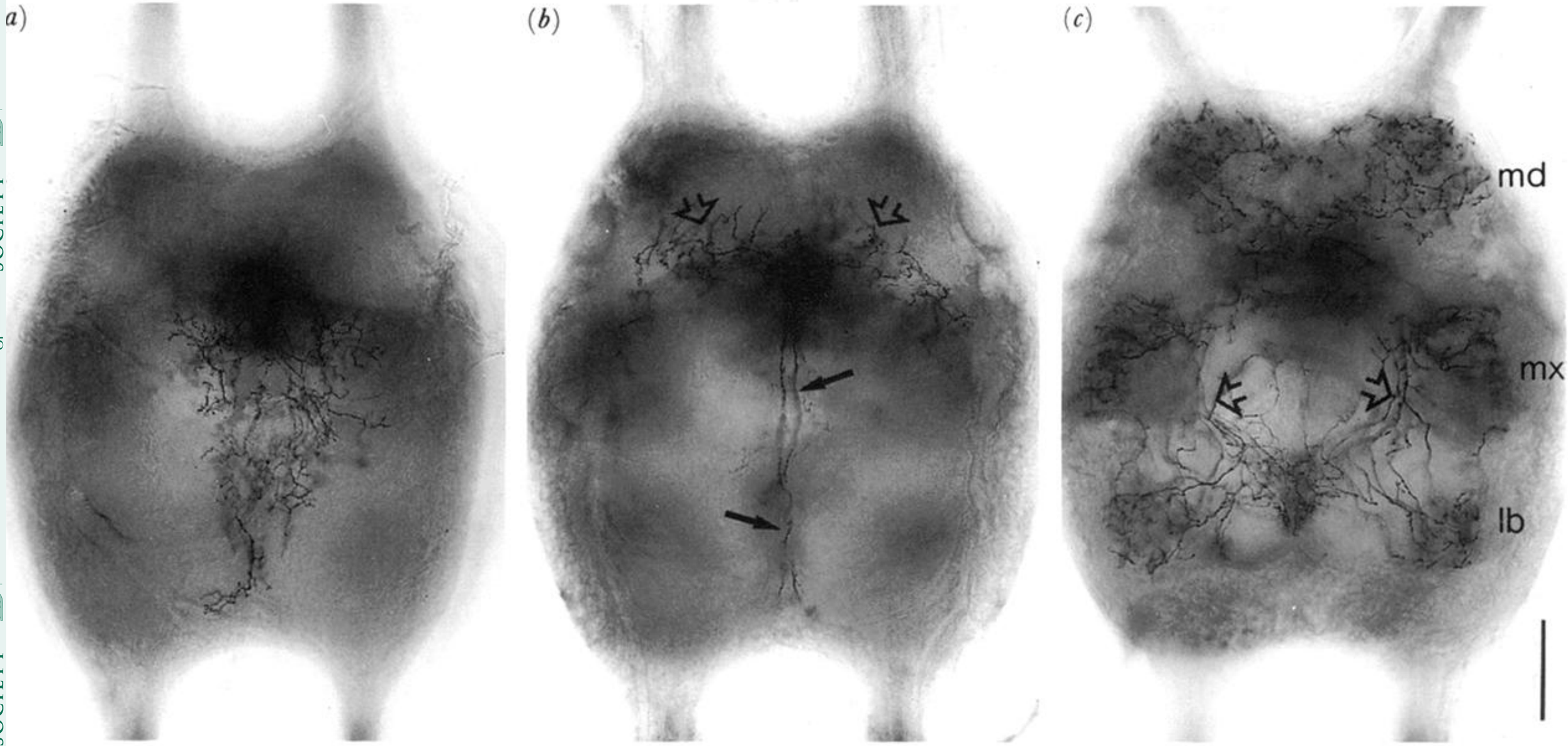
igure 8. Photomicrographs of arborizations of SOG DUM neurons in principal neuropiles of the brain (100 µm bratome sections; orientation as in previous figures; scales: 100 µm). (*a–c*) Projections of DUM SA 1 (comp. figure *b*) in the central body (*a*), the noduli (*b*), and the calyx of one mushroom body (*c*). (*d*) Meshwork of fine branches DUM SA 4 around the pedunculus of the mushroom body (comp. figure 6*b*). (*e*, *f*) Two adjacent sections through e antennal lobe ((*f*) is ventral to (*e*)) after filling DUM SA 5 (figure 7*b*). Note that fibres are particularly dense the periphery of the antennal lobe and less so near the centre, the origin of the antenno-glomerular tract (marked ith an asterisk in (*e*)).




gure 10. Photomicrographs of DUM SAD 1 arborizations within the SOG (same neuron as in figure 9*a*). (*a–c*) hree different focal planes (scale: 100 μm). The dorsal arborizations can be seen in (*a*), lateral and ventral borizations in (*b*) and (*c*). The anterior axons (arrows) can be seen in *a* and *b*, the posterior axons (arrowheads) (*c*).




igure 13. Photomicrographs of a third type of local DUM neuron (three different focal planes; scale: 100 μm). The ell has arborizations in the dorsal neuropile (*a*), the 'anterior wall' region (open arrows in (*b*); compare with DUM A 5, figure 7*a*, *d*) and on the level of the median ventral tracts (*b*, arrows), and in the ventral neuropile areas of the andibular (md), maxillary (mx), and labial (lb) neuromeres (*c*). The latter are supplied by collaterals running in e 'horseshoe tracts' (open arrows in *c*).