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Synaptic relationships between GABA-immunoreactive neurons and an identified uniglomerular projection neuron in the antennal lobe of *Periplaneta americana*: a double-labeling electron microscopic study

D. Malun

Institut für Zoologie, Universität Regensburg, Universitätsstrasse 31, W-8400 Regensburg, Federal Republic of Germany

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Summary. Two types of central neurons in the antennal lobe of the American cockroach *Periplaneta americana* were labeled with a combination of two specific markers. Their synaptic contacts were characterized and their distribution on the neurons examined. A uniglomerular pheromone-sensitive projection neuron with dendritic arbor in the male-specific macroglomerulus (attractant neuron) was characterized physiologically by intracellular recording and then filled with biocytin, which was converted to a marker for this individual neuron by a preembedding procedure. In a postembedding procedure local, multiglomerular interneurons were marked by immunogold labeling of GABA. Two kinds of synaptic contacts were found on the attractant neuron. (i) Input synapses from GABA-immunoreactive profiles. There were many of these, which (together with results of previous studies) suggests that local interneurons mediate polysynaptic transmission from antennal receptor fibers to the projection neuron. (ii) Output synapses onto GABA-immunoreactive profiles and onto non-identified neurons. These contacts indicate that signals generated by the projection neurons in a given glomerulus are passed back to multiglomerular interneurons and hence are also transmitted to other glomeruli.

Introduction

The glomeruli of the antennal lobe constitute the first central relay station in the insect olfactory pathway (for review see Boeckh and Ernst 1987; Homberg et al. 1989; Masson and Mustaparta 1990). The antennal receptor fibers terminate in these glomeruli, forming synapses with central neurons (Boeckh et al. 1970; Schürmann and Wechsler 1970; Tolbert and Hildebrand 1981). Two types of neurons involved in the processing of olfactory signals have been known for some time, and their morphology and physiology have been thoroughly investigated in a variety of insect species (Matsumoto and Hildebrand 1981; Burrows et al. 1982; Ernst and Boeckh 1983; Boeckh et al. 1984; Christensen and Hildebrand 1987; Kanzaki et al. 1989). One type comprises the local interneurons, with processes restricted to the antennal lobe; each of these innervates several glomeruli (Fig. 1a). Neurons of the second type are called projection neurons, because they send an axon into projection regions in the protocerebrum. Within the antennal lobe, as a rule, their dendrites arborize in a single glomerulus. The axons of the uniglomerular projection neurons that have been most closely examined in cockroaches pass through olfactory-globular tract I and terminate in the calyces of the mushroom bodies and in the lateral lobe of the protocerebrum. Their somata are located in the ventrolateral cell group of the antennal lobe (Fig. 1b; Ernst and Boeckh 1983). Information about the connections of these neurons in the glomerular neuropil was first obtained by electron-microscopic examination of preparations in which interneurons had been stained with cobalt and the axons of antennal receptors had been marked by experimentally induced degeneration (Boeckh et al. 1989); synaptic contacts between the receptor fibers and associated local interneurons were described. In both *Manduca sexta* and *Periplaneta americana*, a large population of multiglomerular interneurons were found to be immunoreactive for a GABA antiserum (Hoskins et al. 1986; Distler 1989), which made it possible to identify such neurons in the electron microscope. When the immunolabeling method was combined with the marking of antennal receptor fibers, synaptic contacts between the latter and GABA-immunoreactive interneurons were demonstrated, confirming the earlier results (Distler 1990a, b). Contacts between receptor fibers and projection neurons have very rarely been observed and appear to be the exception (Malun 1989; Boeckh et al. 1990). It seemed likely, therefore, that the receptors were connected indirectly, by way of interneurons, to the neurons projecting to higher centers. The projection neurons have also been individually stained and the distribution of their synapses within the glomeruli of the antennal lobe examined (Malun 1991). The many input synapses observed in this study corrobo-

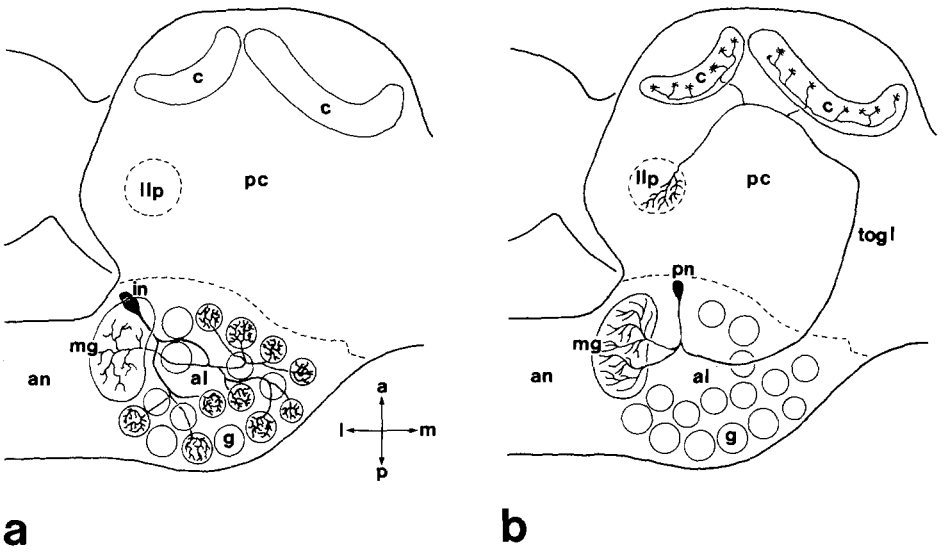


Fig. 1a, b. Schematic drawing of the right half of the brain of the cockroach in frontohorizontal section (modified from Ernst and Boeckh 1983). **a** Local interneuron (*in*) that innervates several glomeruli and has processes restricted to the antennal lobe (*al*). *an*, antennal nerve; *g*, glomerulus; *mg*, macroglomerulus. The orientation of the diagram is indicated by the axes: *a*, anterior; *p*, posterior; *m*, medial; *l*, lateral. **b** Projection neuron (*pn*) with dendritic arbor in the macroglomerulus. Its axon passes through olfactory-globular tract I (*tog l*) into the protocerebrum (*pc*) and ends in the calyces of the mushroom body (*c*) and in the lateral lobe (*llp*)

rated the supposition that the main input region of the projection neurons is in the antennal lobe. This interpretation is consistent with the relay function ascribed to these neurons – to transmit information received in the antennal lobe to the protocerebrum (Ernst et al. 1977). However, a large number of output synapses were also found, indicating that the projection neurons also participate in the neuronal circuitry within the antennal lobe.

In the present study the glomerular fine structure was examined further with respect to the synaptic connections between multiglomerular interneurons and projection neurons. Therefore, a projection neuron was first characterized physiologically and then marked individually by intracellular dye injection, while the multiglomerular local interneurons were marked by exploiting their GABA-like immunoreactivity. With this method, the contacts between a single projection neuron and such local interneurons were visualized.

Material and methods

Recording and olfactory stimulation

Adult male cockroaches of the species *Periplaneta americana* were taken from the institute's culture and prepared for recording by standard methods (Waldow 1975). During the recording and staining procedures the exposed brain was covered with the ringer solution of Rees (1974). For intracellular recording glass capillaries were filled with biocytin (5% in 50 µl *Tris*-buffer containing 0.5 *M* KCl, pH 7.4; Horikawa and Armstrong 1988; King et al. 1989). The electrode resistance was ca. 40 MOhm. The electrodes were inserted into single somata within the ventrolateral group of cells in the antennal lobe. A syringe olfactometer (as designed by Sass 1976) was used to present odor stimuli known to excite projection neurons (Waldow 1977; Burrows et al. 1982; Ernst and Boeckh 1983; see also Malun 1991). The female attractants periplanone A and periplanone B were also used as odor stimuli (periplanone A in the concentrations of 10^{-10} and 10^{-12} g/ml; periplanone B in the concentrations 10^{-10}, 10^{-11} and 10^{-12} g/ml). These stimuli were presented by charging a piece of filter paper with 50 µl of solution and placing the paper in a syringe. As controls, syringes were left empty or contained clean pieces of filter paper. For mechanical stimulation a small brush was moved along the antenna.

To mark the attractant neuron for fine-structural examination after the physiological data had been collected, a hyperpolarizing current of 4–10 nA was applied for 5 min to inject biocytin. 20 min were allowed to elapse between dye injection and further preparation, during which the biocytin diffused with no applied current.

Preparation for microscopy

The brain was dissected out, fixed for 2 h at 4° C in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer with 5% sucrose, and then washed overnight in 0.1 *M* phosphate buffer with 5% sucrose. After it had been embedded in gelatine, the brain was cut into 40-µm-thick sections in the frontohorizontal plane (as described by Ernst et al. 1977) with a vibratome (Microcut H 1200, Bio Rad, München, FRG). The sections were collected in 10 m*M* phosphate buffer containing 0.14 *M* NaCl (phosphate buffered saline = PBS, pH 7.4) and incubated free-floating for 3 h in avidin peroxidase (Sigma, St. Louis, Mo., USA; dilution 1:800). Finally, the neuron was made visible by means of the DAB (diaminobenzidine) reaction. The sections with the marked neuron were examined in a light microscope (Zeiss, Oberkochen, FRG; Axioplan) and photographed, after which they were embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, FRG) in Eppendorf cups at low temperatures.

Protocol for embedding in Lowicryl K4M: 30% ethanol 0° C 30 min; 50% ethanol −20° C 30 min; 70% ethanol −30° C 30 min; 95% ethanol −30° C 15 min; 2x 100% ethanol −30° C 15 min; 100% ethanol/Lowicryl K4M 1:1 −30° C overnight; 100% ethanol/Lowicryl K4M 1:2 −30° C 1 h; 2x Lowicryl K4M −30° C 1 h; Lowicryl K4M −30° C 1 day polymerization under UV light; 2 days polymerization at room temperature under UV light.

Series of thin sections were prepared with a Reichert Ultracut E with diamond knife and caught on nickel grids covered with pioloform foils.

Immunogold electron microscopy

Selected grids were successively preincubated in (i) 0.01% Tween 20 (Sigma) in PBS at pH 7.3 for 5 min, (ii) 50 m*M* NH_4Cl in PBS for 5 min, to block free aldehyde groups of the fixative solution,

(iii) PBS for 5 min, (iv) a block buffer consisting of 0.1% ovalbumin (Sigma) and 0.5% fish gelatine (Sigma) in PBS for 10 min, to block nonspecific protein-binding sites. The sections were then incubated in GABA antiserum for ca. 15 h at 4° C in a humid chamber. The GABA-KLH antiserum (polyclonal rabbit antiserum against GABA conjugates to keyhole-limpet hemocyanin = KLH; produced and characterized by Dr. T. Kingan; Hoskins et al. 1986) and the GABA-GA-BSA antiserum (polyclonal rabbit antiserum against GABA conjugates to bovine serum albumin = BSA and glutaraldehyde = GA; produced and characterized by Dr. Wulle and Dr. Schnitzer; Wässle and Chun 1989) were added to the block buffer to give final dilutions of 1:800 to 1:10000. In control experiments block buffer or normal rabbit serum were used instead of the primary antiserum. These controls were always negative. To remove unbound primary antibody, the grids were washed twice, for 5 min each time, in PBS with 0.01% Tween 20, and twice for 5 min each in 0.01 *M* phosphate buffer pH 7.3 with 0.01% Tween 20, 0.01% ovalbumin, 0.5% fish gelatine and 0.5 *M* NaCl. The grids were then incubated for 1 h in goat anti-rabbit serum coupled with 10-nm colloidal gold (Auro Probe EM GAR 10, Amersham, Braunschweig, FRG), diluted 1:75 in the latter buffer. Finally, after the grids had been washed several times in PBS and distilled water, the thin sections were stained with 2% ethanolic uranyl acetate and lead citrate (Reynolds 1963) and examined in a Zeiss transmission electron microscope 109.

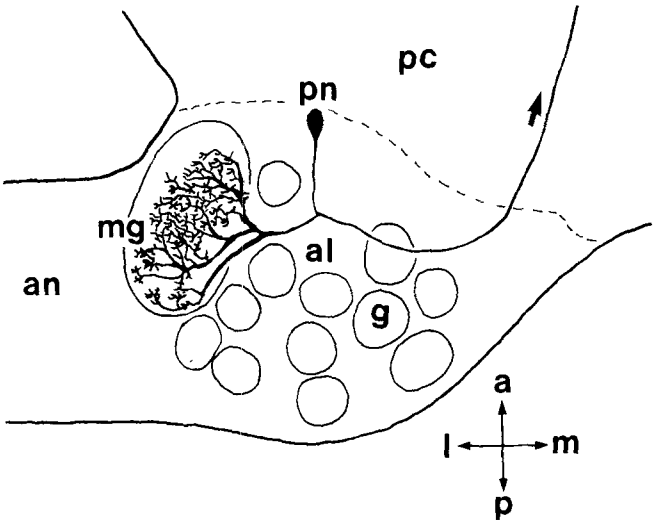


Fig. 2. Schematic drawing of the right half of the brain in frontohorizontal section, with a reconstruction of the attractant neuron studied here (*pn*). Its dendritic arbor is confined to the macroglomerulus (*mg*), and its axon (*arrow*) runs into the protocerebrum (*pc*). *al*, antennal lobe; *an*, antennal nerve; *g*, glomerulus. The orientation of the diagram is indicated by the axes: *a*, anterior; *p*, posterior; *m*, medial; *l*, lateral

Results

On the basis of physiological properties and morphology (Fig. 2), the neuron studied electrophysiologically here is a typical representative of a category well known in the cockroach, the Type B pheromone-sensitive projection neurons (attractant neuron of Type B: Burrows et al. 1982; Boeckh and Selsam 1984; Hösl 1990a).

When recording from a neuron had been completed, the intracellular injection of biocytin (with subsequent conversion by avidin peroxidase and DAB reaction) served to mark the neuron for both light-microscopic and electron-microscopic analysis. The soma of the neuron is situated in the ventrolateral cell group of the antennal lobe, and its dendritic arbor is entirely restricted to the macroglomerulus. The axon passes through olfactory-globular tract I into the protocerebrum. Its projection regions there, in the calyces of the mushroom body and in the lateral lobe of the protocerebrum, were incompletely stained due to the brief time allowed (ca. 5 min).

Fine structure of the attractant neuron

The neuron that had been filled with biocytin after electrophysiological recording appeared in the thin sections of the antennal lobe as profiles marked with DAB reaction product. For examination of fine structure, parts of the vibratome sections were chosen in which the DAB precipitate only slightly masked the intracellular structures of the neuron.

The fine structure of the attractant neuron studied here corresponded to that described previously for projection neurons (Malun 1991). At their entry into the glomerulus, the thick, sparsely branched fibers are enclosed in the processes of glia cells. Within the glomerulus the fibers ramify, their diameter is reduced and they are free of glia. The fibers terminate as fine processes, 0.3–0.5 μm in diameter.

Postembedding labeling and fine structure of GABA-immunoreactive neurons

The immunogold precipitate was regarded as a specific marker and indicator of GABA-like immunoreactivity only when three criteria were met, as follows. (i) Only individual profiles were so labeled, whereas neighboring profiles in the neuropil were unlabeled, (ii) the labeled profiles could be followed through a series of thin sections, and (iii) control preparations showed no labeling. An additional demonstration of the specifity of the marking derives from the fact that positive immunogold labeling was not found in any profile of the stained attractant neuron (Figs. 3–8).

The fine-structural characteristics of the GABA-immunoreactive profiles in this study corresponded to those reported by Distler (1990a, b). The immunogold-labeled profiles can exhibit not only clear vesicles, with a diameter of 25 nm, but also dense-core vesicles ca. 60 nm in diameter (Fig. 6).

In the sections examined, none of the intracellular structures were covered by precipitate. Therefore, synaptic contacts could reliably be identified. The variation in density of labeling in comparison with the background results from the differences in treatment from a given preparation (two different GABA antisera were used in different concentrations; see 'Material and methods').

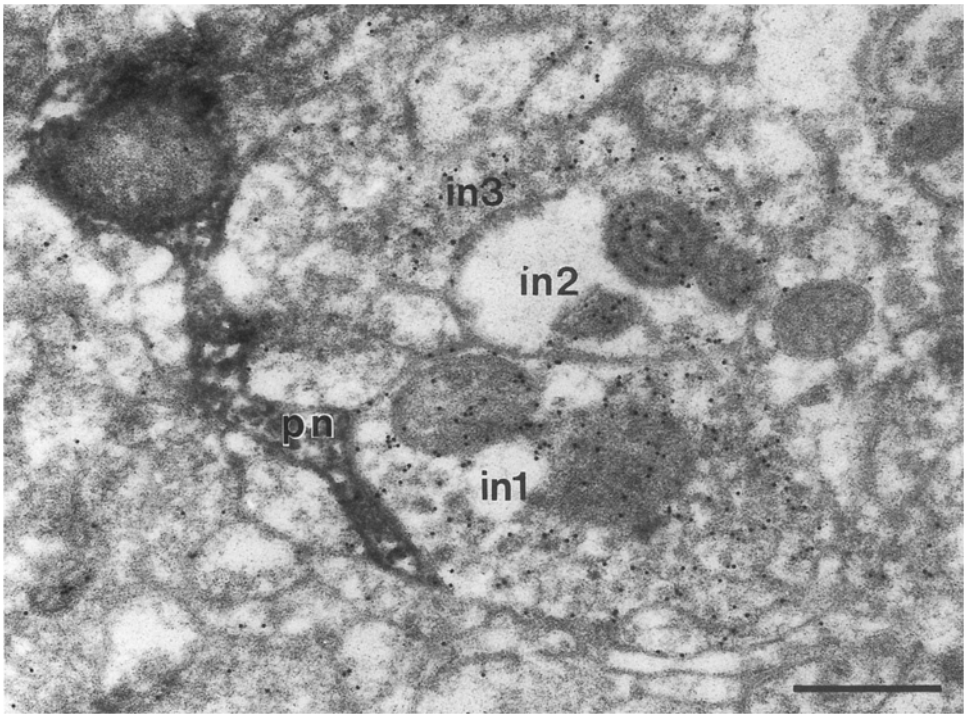


Fig. 3. Electron micrograph of part of the neuropil in the macroglomerulus. A fiber terminal of the attractant neuron (*pn*) filled with DAB reaction product is apposed to a GABA-immunogold-labeled profile (*in 1*). Two other profiles (*in 2* and *in 3*) also exhibit GABA-immunoreactivity. *Scale*: 0.5 μm

Synaptic contacts

Synaptic contacts are identifiable by a number of characteristic structural features. These are (i) the active, presynaptic zone, consisting of an electron-dense bar and many synaptic vesicles, (ii) a distinctly expanded intercellular space, the synaptic cleft, and (iii) the dense material that commonly appears just inside the postsynaptic membranes. These characteristics (iv) were observable in several consecutive ultrathin sections.

In the profiles marked with DAB precipitate, the synaptic vesicles of output synapses were often not clearly discernible. These contacts were nevertheless regarded as synapses if all the other criteria given above were met.

Furthermore, in interpreting the fine structure in these sections allowance was made for the fact that the cell membranes in tissue that have not been fixed in osmium appear considerably less electron-dense than those in osmium-fixed tissues.

In all thin sections, through the neuropil of the macroglomerulus, profiles of the stained attractant neurons were apposed to GABA-immunoreactive profiles (Fig. 3), and in many cases synaptic contacts between them were observed. Most of these exhibited a divergent, dyadic arrangement; that is, a single presynaptic element made contact with two postsynaptic elements. Monadic arrangements, with only presynaptic and one postsynaptic element (cf. Goodman et al. 1977), were considerably less common.

Input synapses on profiles of the attractant neuron. A total of 20 input synapses from GABA-immunolabeled fibers onto attractant-neuron profiles of small diameter (0.3–0.5 μm) were observed. In one case an input synapse from a GABA-immunoreactive neuron was found on a larger-diameter fiber of the attractant neuron (see Fig. 8c).

Input synapses on the attractant neuron ocurred in various configurations, as follows.

(i) Fig. 4 shows a GABA-immunoreactive profile that forms two output synapses on three postsynaptic profiles. One of the postsynaptic elements is a profile of the attractant neuron, and the other two are unlabeled. One of the latter participates in both synapses, so that it receives two inputs from the same GABA-immunoreactive neuron.

(ii) Profiles of the attractant neuron can also receive two input synapses from one GABA-immunoreactive profile (Fig. 5).

In both of the above cases, the input synapses on fibers of the attractant neuron are dyadic. One of the two postsynaptic elements is a process of the attractant neuron, whereas the other profile is unlabeled. This situation was by far the most frequently observed (in 14 cases).

(iii) In another synaptic arrangement, a dyadic output synapse of a GABA-immunolabeled profile has as postsynaptic elements an attractant-neuron profile and another GABA-immunoreactive profile (Fig. 6). Four examples of this kind of connectivity were found.

(iv) Finally, two monadic contacts were identified in which an attractant neuron receives an input synapse from a GABA-immunoreactive profile (Fig. 7).

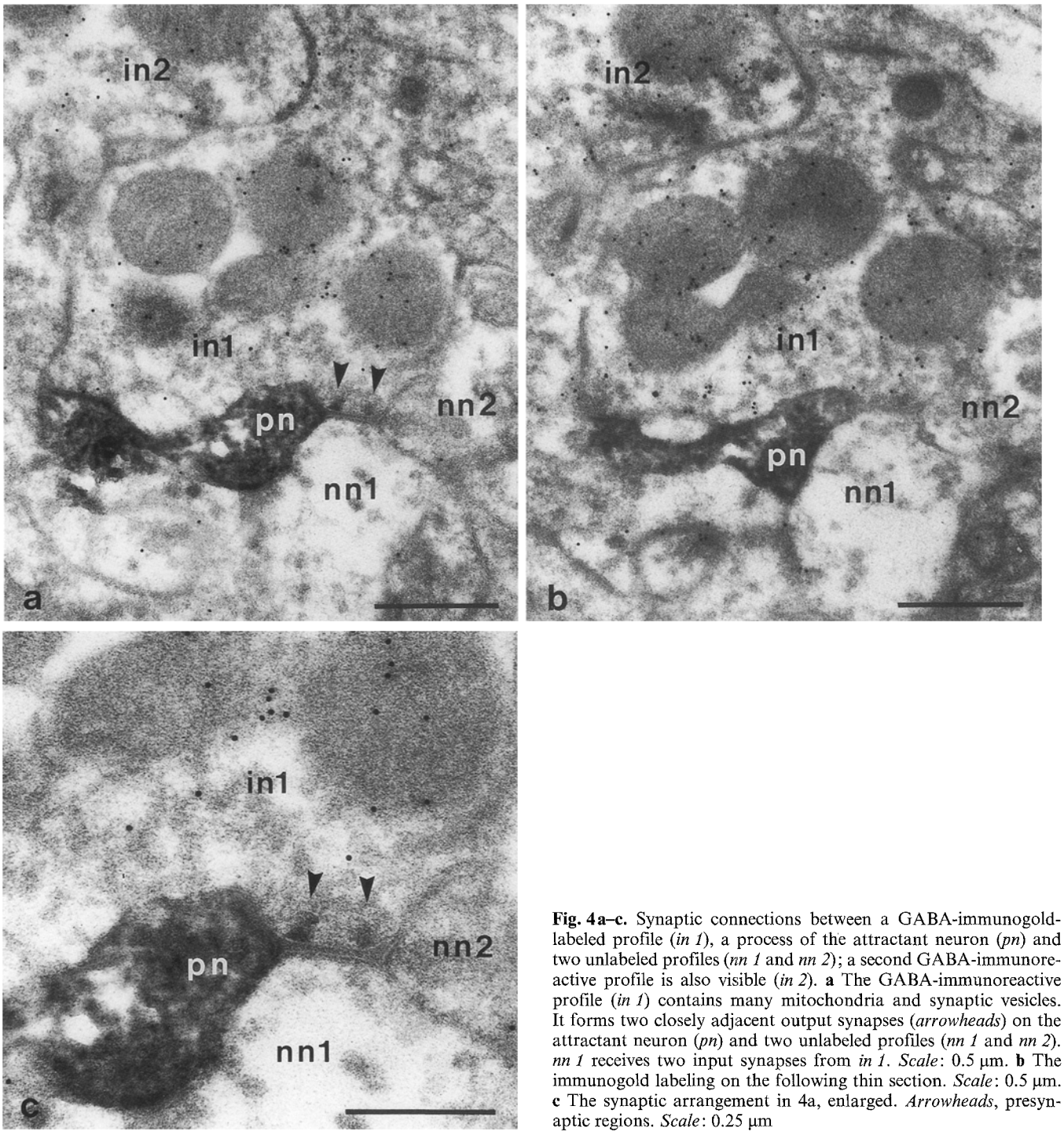
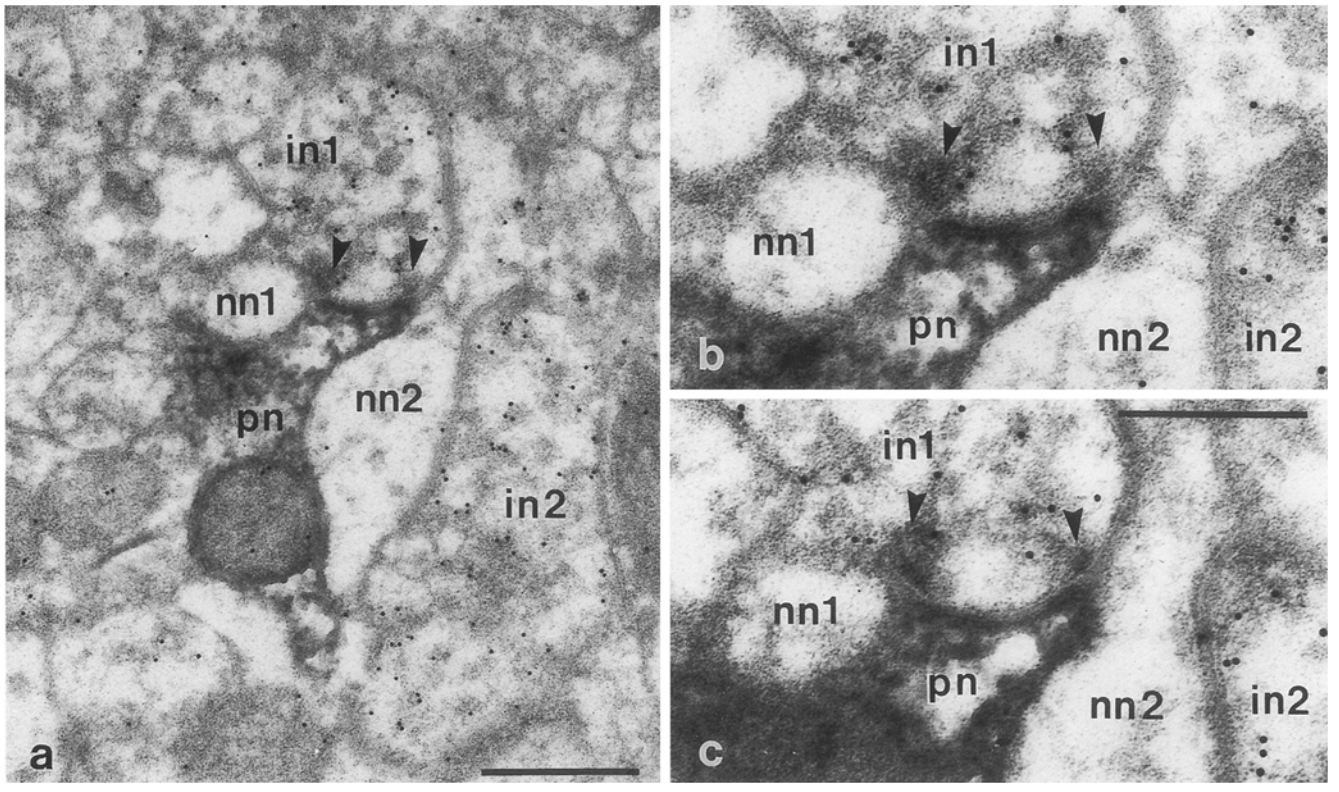


Fig. 4a–c. Synaptic connections between a GABA-immunogold-labeled profile (*in 1*), a process of the attractant neuron (*pn*) and two unlabeled profiles (*nn 1* and *nn 2*); a second GABA-immunoreactive profile is also visible (*in 2*). **a** The GABA-immunoreactive profile (*in 1*) contains many mitochondria and synaptic vesicles. It forms two closely adjacent output synapses (*arrowheads*) on the attractant neuron (*pn*) and two unlabeled profiles (*nn 1* and *nn 2*). *nn 1* receives two input synapses from *in 1*. *Scale*: 0.5 μm. **b** The immunogold labeling on the following thin section. *Scale*: 0.5 μm. **c** The synaptic arrangement in 4a, enlarged. *Arrowheads*, presynaptic regions. *Scale*: 0.25 μm

Output synapses of the attractant neuron. As had been expected at the outset, output synapses – from profiles of the projection neuron onto postsynaptic neurons – were less reliably identifiable than input synapses, because of the DAB reaction product in the projection neuron. Hence, although the arrangement of the contacts that were observed will be described here, nothing can be said about the number of these synapses.

Projection-neuron profiles exhibiting output synapses, as a rule, are larger in diameter (ca. 1 μm or larger) than those with input synapses (Fig. 8). Again, these synapses are predominantly dyadic. Projection-neuron processes were found that were presynaptic to (i) two GABA-immunoreactive profiles (Fig. 8a), or (ii) a GABA-immunoreactive fiber and an unlabeled profile (Fig. 8b). In other cases (iii) both postsynaptic profiles were unlabeled (Fig. 8c). One output synapse onto two unlabeled profiles was situated on a spine-like process



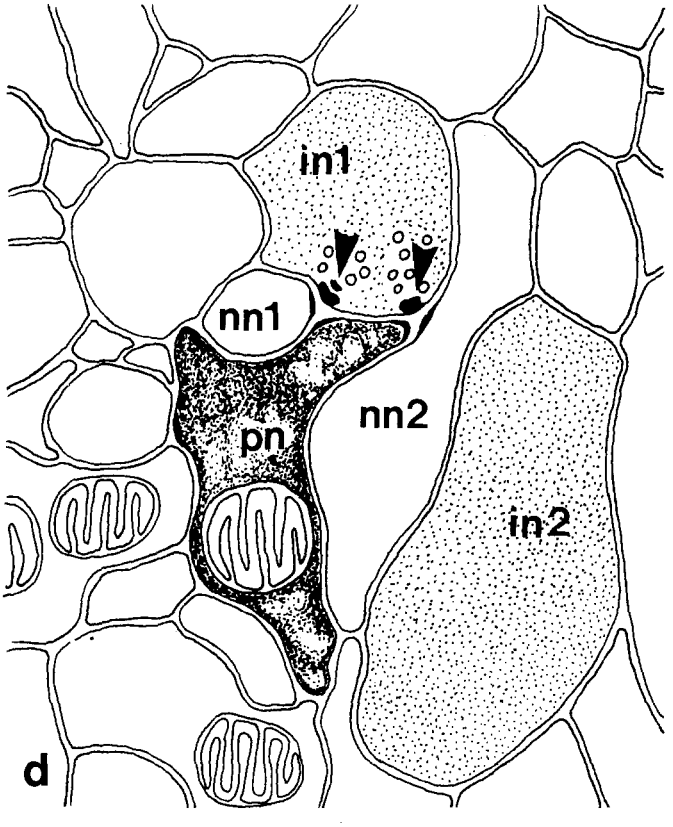


Fig. 5a–d. Synaptic connections between a GABA-immunogold-labeled profile (*in 1*), a process of the attractant neuron (*pn*) and two unlabeled profiles (*nn 1* and *nn 2*). **a** The attractant neuron (*pn*) receives two inputs (*arrowheads*) from a single GABA-immunoreactive profile (*in 1*); *in 2* is another GABA-immunoreactive profile. *Scale*: 0.5 µm. **b** The synaptic arrangement at higher magnification and **c** the synapses on the following thin section. *Scale*: 0.25 µm. **d** Schematic drawing of the synaptic arrangement in 5a

of a projection-neuron fiber about 1 µm in diameter. In the immediate vicinity of this synapse, the projection neuron received an input synapse from a GABA-immunoreactive profile (Fig. 8c).

Discussion

Methodological aspects. The synaptic connections between antennal receptor fibers and GABA-immunoreactive neurons having been clarified by Distler (1990a, b), in this study a double-labeling method has been used to examine the connections of the GABA-immunolabeled neurons with another type of antennal-lobe element, the projection neurons. One of the latter was made visible in both the light and the electron microscope as individually identifiable neuron, by the intracellular injection of biocytin and its subsequent conversion to an electron-dense DAB reaction product in a preembedding procedure. At the same time, the choice of fixation technique and the embedding in Lowicryl K4M made possible a postembedding immunogold staining for GABA. Although the degree of contrast in the osmium-free tissue is far less than in osmium-treated material, structural details such as synaptic contacts, vesicles and organelles can be identified.

With two types of neurons labeled in this way, it is possible to examine their synaptic contacts with one another and with unlabeled neurons. A similar combina-

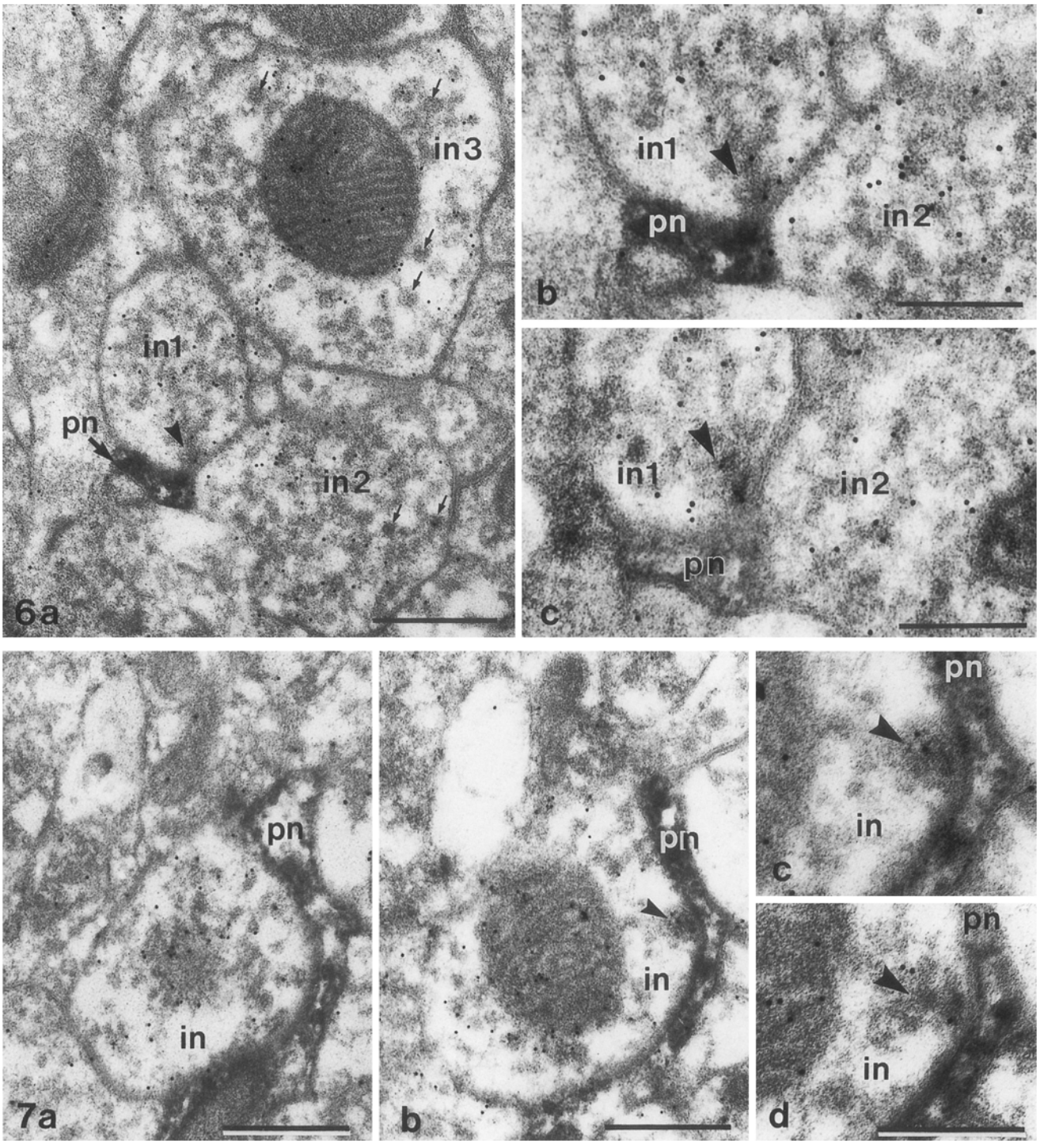


Fig. 6a–c. Input synapse (*arrowhead*) on a small process of an attractant neuron (*pn*), from a GABA-immunogold-labeled profile (*in 1*); the dyadic synapse includes another GABA-immunoreactive profile (*in 2*). (*in 3*) a third GABA-immunoreactive profile. Two of the GABA-immunoreactive profiles contain dense-core vesicles (*small arrows*) as well as clear vesicles. *Scale*: 0.5 μm. **b** Enlarged view of the input synapse. **c** The same synapse, in the following thin section. *Scale*: 0.25 μm

Fig. 7a–d. Monadic input synapse on an attractant neuron (*pn*), from a GABA-immunogold-labeled profile (*in*). **a** A GABA-immunoreactive profile (*in*) is apposed to a thin process of the attractant neuron (*pn*). **b** In the following thin section the monadic input synapse (*arrowhead*) from the GABA-immunoreactive profile onto the attractant neuron (*pn*) is visible. *Scale*: 0.5 μm. **c** Enlarged view of the synapse. **d** The synapse in the section following that in (b). *Scale*: 0.25 μm

tion of recording and staining of identified neurons with subsequent postembedding labeling for GABA has been employed by Freund et al. (1989) in vertebrates, Watson and Laurent (1990) in *Locusta* and by other authors.

The gold particles that bind to GABA-immunoreactive sites are relatively uniformly distributed over vesicles, cytoplasm and mitochondria, and there was no indication that gold particles are localized to a particular

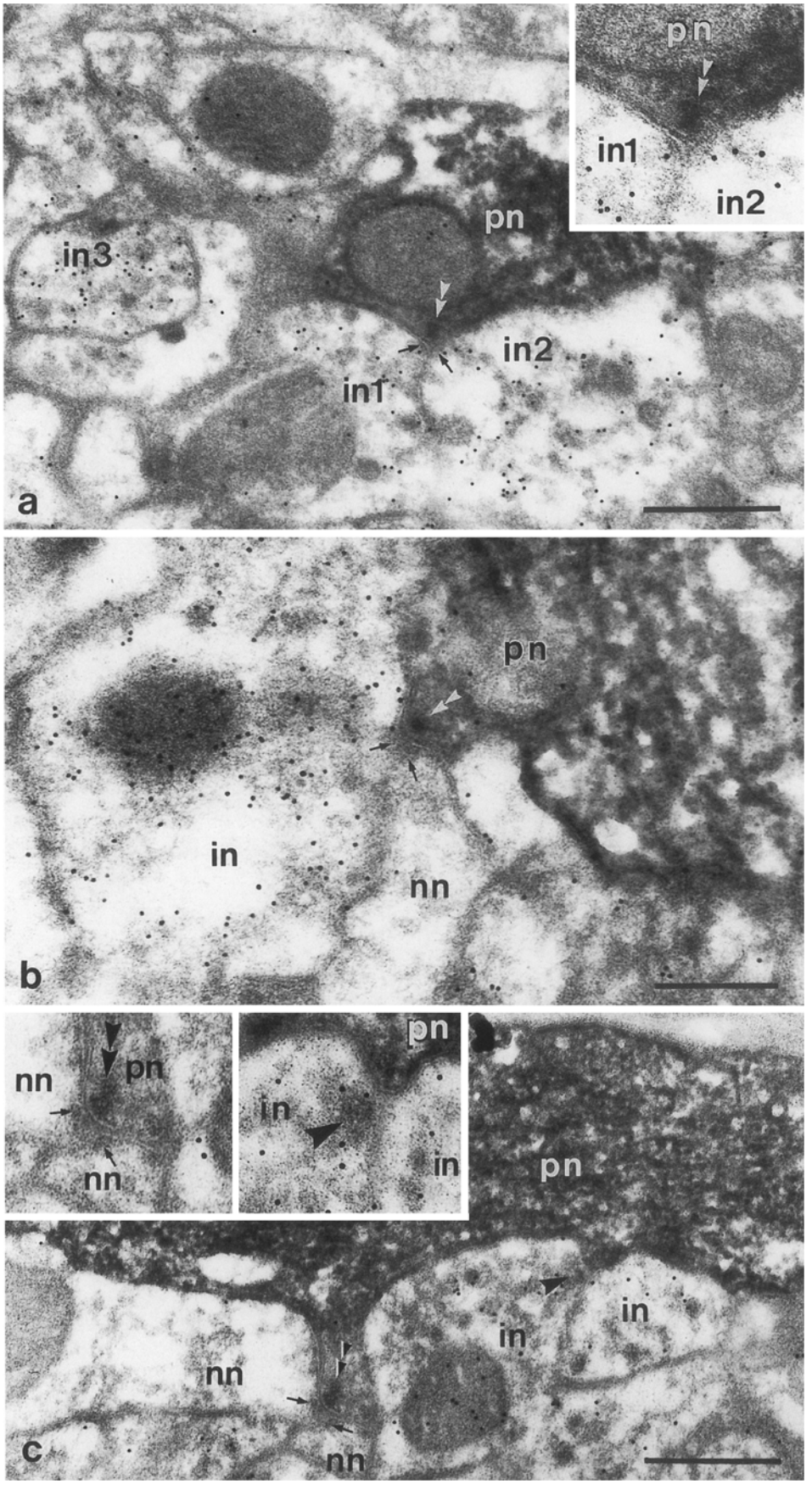


Fig. 8a–c. Output synapses of the attractant neuron (*pn*). **a** Output synapse (*double arrowheads*) onto two GABA-immunoreactive profiles (*in 1* and *in 2*); (*in 3*) another GABA-immunoreactive profile. *Small arrows*: postsynaptic densities. *Scale*: 0.5 µm. *Inset*: Enlarged view of the synapse. **b** Output synapse (*double arrowheads*) of a process of the attractant neuron (*pn*) onto a large-diameter GABA-immunoreactive profile (*in*) and onto a smaller, unlabeled profile (*nn*). *Small arrows*: postsynaptic densities. *Scale*: 0.25 µm. **c** On a spinelike process of a ca. 1-µm-thick fiber of the attractant neuron (*pn*) there is an output synapse (*double arrowheads*) onto two unlabeled profiles (*nn*). *Small arrows*: postsynaptic densities. The same attractant neuron receives an input synapse (*arrowhead*) from a GABA-immunoreactive profile (*in*). *Scale*: 0.5 µm. *Left inset*: Enlargement of the output synapse. *Right inset*: Enlargement of the input synapse, from the following thin section

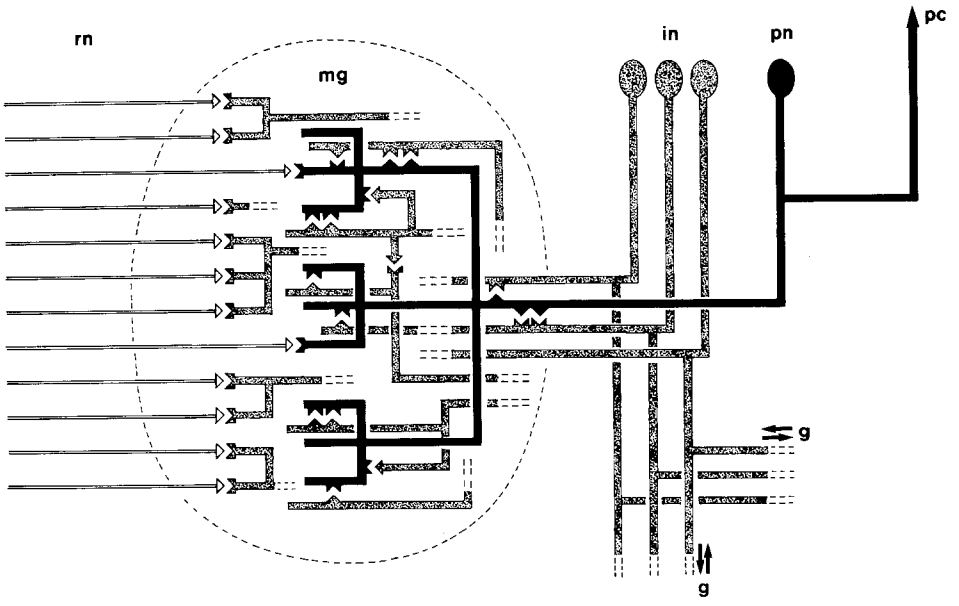


Fig. 9. Schematic representation of synaptic contacts between receptor fibers (*rn*), GABA-immunoreactive local interneurons (*in*), and a projection neuron (*pn*) in the macroglomerulus (*mg*) of male *Periplaneta americana*. Individual interneurons might possibly not exhibit all types of synaptic contacts shown. Non-GABA-immunoreactive interneurons are not shown in the diagram. (*pc*) protocerebrum; (*g*) glomerulus of normal size

type of vesicle. Only in rare cases was the density of the labeling found to be higher in the presynaptic region of a synapse. The reasons might lie in the existence of a cytoplasmic GABA component (Somogyi and Hodgson 1985) or in the pretreatment of the tissue (Distler 1990b; Decavel and van den Pol 1990).

Synaptic contacts

Synaptic contacts of the labeled projection neuron. The present observations of the fine structure of the attractant neuron support certain general conclusions about the inventory and distribution of its synapses, because (i) a large number of thin sections of labeled fibers from (ii) various regions of the macroglomerulus were examined, and (iii) the results are consistent with those for projection neurons studied previously (Malun 1991). A prominent feature of the distribution is the extensive segregation of output and input synapses. Output synapses are found in glia-free regions of fibers of large diameter (1 μm or more), whereas the terminals of the projection-neuron fibers bear only input synapses. In the intervening transitional regions, outputs predominate and only a few inputs are found. The fiber terminals, then, constitute the main input region of the projection neurons in the glomerulus.

Fine structure and synaptic contacts of GABA-immunoreactive fibers. Both the fine-structural details and the arrangement of the synapses observed in this study correspond to the findings of Distler (1990b), as follows. (i) In addition to the small, clear vesicles GABA-immunoreactive profiles contain large dense-core vesicles. (ii) Both input and output synapses were found on GABA-immunoreactive profiles. (iii) Only in a few cases were synaptic contacts between GABA-immunolabeled profiles observed.

Synaptic connections between GABA-immunoreactive neurons, the attractant neuron and unlabeled neurons. The large number of synapses from GABA-immunoreactive profiles onto small-diameter fibers or terminal branches of the attractant neuron indicates that the main input the projection neuron receives is chiefly GABA-mediated and hence inhibitory. This finding provides additional evidence that the transfer of information from the receptor fibers that enter the glomerulus to the projection neurons involves inhibition. Previously such a pathway had been suggested by physiological studies of *Manduca*, in which inhibitory postsynaptic activity was recorded in some projection neurons during their responses to electrical and olfactory stimulation of the antenna, and by neuropharmacological evidence in the same insect (Christensen and Hildebrand 1987; Waldrop et al. 1987; Waldrop and Hildebrand 1989). GABA was proposed as the transmitter mediating the inhibtion (Waldrop et al. 1987). It was shown to be synthesized in the antennal lobe of *Manduca* (Kingan and Hildebrand 1985), and in immunohistochemical tests it was found mainly in amacrine local interneurons (Hoskins et al. 1986). Taken together, the results gave rise to the hypothesis that these interneurons are excited by antennal receptor fibers and, in turn, inhibit the projection neurons (for review see Homberg et al. 1989).

The findings in *Manduca* were confirmed and extended by physiological, morphological and immunohistochemical studies of *Periplaneta americana* (Distler 1989, 1990a, b; Hösl 1990b). Distler (1989) found that the GABA-like immunoreactivity in the antennal lobe of the cockroach resides primarily in the elements known as local, multiglomerular interneurons (Ernst and Boeckh 1983). Although the possibility that other neurons in the antennal lobe are also GABA-immunoreactive cannot be ruled out (cf. Distler 1989), such interneurons most probably account for the majority of the labeled profiles. It would follow that the synapses found

on GABA-immunoreactive profiles are representative of these interneurons. The uniglomerular projection neurons, with somata in the ventrolateral cell group of the antennal lobe and with axons that project into the protocerebrum in olfactory-globular tract I, exhibit no GABA-like immunoreactivity (Distler 1989).

Boeckh et al. (1989), in a study of the glomerular fine structure, described input synapses on identified local, multiglomerular interneurons in which antennal receptor fibers were the presynaptic elements. Distler (1990a, b) found synaptic contacts between three neurons in series, from antennal receptor fibers to GABA-immunoreactive neurons to unidentified neurons that formed the third link in the chain. In the present study, many output synapses of GABA-immunoreactive profiles onto processes of a projection neuron were found. Given the evidence of connectivity discussed above, at least some of the unidentified neurons in Distler's study would be expected to be projection neurons. In an earlier study, synaptic contacts between antennal receptor fibers and projection neurons were observed only very rarely (Malun 1989; Boeckh et al. 1990). Accordingly, polysynaptic connections between the input to the glomerulus and its output are likely to predominate (see also Boeckh and Ernst 1987; Boeckh et al. 1989). The GABA-immunoreactive local interneurons are proposed as the intermediate element (Distler 1990; Fig. 9).

So far, most of what is known about synaptic connectivity in the insect olfactory neuropil relates to the inhibitory pathway just described. The high level of excitation in the responses of the projection neurons to adequate stimulation (Burrows et al. 1982; Boeckh and Selsam 1984; Kanzaki et al. 1989; Hösl 1990a) implies that there is a second, excitatory route. This pathway is also thought to be polysynaptic (Waldrop et al. 1987), but the neurons involved are still unknown.

The output synapses of GABA-immunoreactive profiles are mainly dyadic (Distler 1990b and the present study) – that is, with two postsynaptic elements. Whereas one of the postsynaptic profiles is often a fiber of the labeled attractant neuron, in all but a few cases the second element is unlabeled (one of the exceptions is shown in Fig. 6, in which the second postsynaptic profile exhibits GABA-immunoreactivity). These unlabeled neurons might be other non-GABA-immunoreactive interneurons, serotonin-immunoreactive fibers (Salecker and Distler 1990), antennal receptor fibers (Distler 1990b) or other not yet identified types of neurons. In the case of the macroglomerulus, which contains the dendritic arbors of ca. 15 attractant neurons (Ernst and Boeckh 1983), these might also be postsynaptic partners of the GABA-immunoreactive neurons.

The many input synapses on the projection neurons (Malun 1991 and this paper) reflect the high degree of convergence in the antennal lobe, the first central relay station in the olfactory pathway of insects. In view of their morphology, it was suggested that the projection neurons transmit information to higher centers of the brain (Ernst et al. 1977). This idea was corroborated when output synapses were found to predominate in the regions of which these neurons project, the calyces of the mushroom bodies and the lateral lobe of the protocerebrum (Ernst and Boeckh 1983; Kraus et al. 1988). On the other hand, the large number of output synapses found on these neurons within the glomeruli implies that they are part of neuronal circuits inside their own glomeruli (Malun 1991). According to Distler (1989) most of the GABA-immunoreactive neurons in the antennal lobes of the cockroach are multiglomerular interneurons. On the assumption that at least part of the output synapses between the projection neuron and GABA-immunoreactive neurons, demonstrated in this study, represent outputs to multiglomerular interneurons, it is conceivable that this information is not confined to one glomerulus but is also transmitted to other glomeruli.

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