Histochemistry (1986) 84:207–216

Histochemistry

Insect optic lobe neurons identifiable with monoclonal antibodies to GABA

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Accepted November 19, 1985

Summary. Five monoclonal antibodies against GABA were tested on glutaraldehyde fixed sections of optic lobes of three insect species, blowflies, houseflies and worker bees. The specificity of these antibodies was analyzed in several tests and compared with commercially available anti-GABA antiserum.

A very large number of GABA-like immunoreactive neurons innervate all the neuropil regions of these optic lobes. Immunoreactive processes are found in different layers of the neuropils. The immunoreactive neurons are amacrines and columnar or noncolumnar neurons connecting the optic lobe neuropils. In addition some large immunoreactive neurons connect the optic lobes with centers of the brain.

Some neuron types could be matched with neurons previously identified with other methods. The connections of a few of these neuron types are partly known from electron microscopy or electrophysiology and a possible role of GABA in certain neural circuits can be discussed.

Introduction

Gamma-aminobuturic acid (GABA) and the enzyme glutamic acid decarboxylase (GAD) involved in the synthesis of GABA have been demonstrated in the CNS of insects (Frontali 1964; Ray 1965; Osborne and Neuhoff 1974; Baxter and Torralba 1975; Klemm 1976; Breer and Heiligenberg 1985; Kingan and Hildebrand 1985). The quantity of GABA in e.g. the brain of bees and houseflies is relatively high, 1,091 μg/g.w.w. and 3.8 ng/mg protein, respectively (Frontali 1964; Clarke and Donnellan 1982). In the CNS of insects the action of GABA seems to be exclusively inhibitory as demonstrated by the response to the iontophoretical application of the substance (Gahery and Boistel 1965; Steiner and Pieri 1969; Pitman 1971). In the periphery, inhibitory motorneurons in crustaceans and insects are known to use GABA as a transmitter (e.g. Florey 1967; Usherwood 1969; Pitman 1971; Gerschenfeld 1973).

The neuronal localization of GABA in the insect CNS is poorly known. Earlier studies have relied on uptake of ^{3}H-GABA and demonstrated labelled terminals only in a few regions of the brain (Frontali and Pierantoni 1973) or showed mainly glial uptake (Campos-Ortega 1974). Apart from the fact that uptake experiments must be interpreted with caution, autoradiographs hardly ever provide information from which the morphology of entire single neurons or groups of neurons can be reconstructed.

On the other hand immunocytochemical mapping of neurons may be very specific, and permits resolution of entire nerve cells. Several neuroactive substances have been mapped immunocytochemically in the insect brain, e.g. serotonin (5-HT) (Bishop and O'Shea 1983; Nässel and Klemm 1983; Klemm et al. 1984; Tyrer et al. 1984); proctolin (Bishop and O'Shea 1982; Veenstra et al. 1985) FMRFamide (Veenstra and Schooneveld 1984) and several vertebrate type neuroactive peptides (Duve and Thorpe 1983a, b, El-Salhy et al. 1983; Veenstra and Schooneveld 1984; Veenstra et al. 1984). GABA-immunoreactive neurons have only been mapped in the antennal lobes of *Manduca sexta* (Hoskins et al. 1984).

In the present investigation, neurons reacting with different monoclonal antibodies to GABA are mapped in the insect optic lobe. The specificity of the antibodies, whose characteristics and reactivity in vertebrate nervous tissue are described elsewhere (Matute and Streit 1985; in prep.), was tested on insect brain tissue and compared to a commercially available anti-GABA antiserum. Since the optic lobes of insects have been studied anatomically and electrophysiologically in some detail (for review see Strausfeld and Nässel 1981; Laughlin 1981; Hausen 1984; Shaw 1984), the distribution of GABA-like immunoreactive processes in the optic lobes of flies and bees can be correlated with data on individual types of neuron and synaptic layers of the neuropils as well as on functional circuits. Certain types of neurons reacting with GABA-antibodies can be correlated with anatomically and physiologically identified neurons.

Materials and methods

Houseflies, *Musca domestica*, blowflies, *Calliphora erythrocephala*, and honey bee foragers, *Apis mellifera*, were caught in the wild in Zürich.

Five different monoclonal antibodies directed against GABA were tested (3A12, 18G10, 3D5, 12E5 and 7G3). The production and characteristics of these antibodies are described elsewhere (Matute and Streit 1985; in prep.). Briefly, mice were immunized with GABA coupled to bovine serum albumine with glutaraldehyde (GABA-GA-BSA) according to the procedure described by Storm-Mathisen et al. (1983), and hybridomas secreting antibodies reacting with GABA- but not with glutamate-BSA conjugates selected by enzyme linked immunoabsorbent assay (ELISA). Specificity of antibodies produced by selected cell lines was assessed by serial dilution of the antibodies in ELISA using BSA-conjugates including β-alanine-, glycine-, taurine-, glutamate- and glutamine-BSA, as well as glutaraldehyde-treated BSA and BSA. The antibody 3A12 showed the best specificity on ELISA and also gave the best immunostaining with brain tissue and has thus been used for mapping studies.

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For immunohistochemistry we adopted a postembedding procedure as follows. The brains were fixed for 2–4 h in ice cold 5% glutaraldehyde in 0.1 *M* phosphate buffer at pH 7.4. The tissue was dehydrated in 2,2 dimethoxypropane (Muller and Jacks 1975) and embedded in Epon 812. Sections were cut at 1 µm with glass knives and dried on microscope slides coated with a mixture of 0.3% gelatine and 0.05% chrome alum.

The Epon was removed from the sections by etching for 6 min in a mixture of methanol, propylene oxide and KOH (Maxwell 1978). After 5 min washes in methanol, methanol/phosphate buffered saline (PBS), and PBS, the sections were incubated at room temperature unless otherwise stated as follows: 5–15 min in 0.5% ovalbumin in PBS; overnight at 4° C in 1:1000 diluted antibody-containing ascitic fluid partially purified by ammoniumsulphate precipitation (protein concentration approximately 4 mg/ml); 1 h in goat anti-mouse IgG (H+L; Kirkegaard and Perry, Gaithersburg, Maryland) diluted 1:50; 1 h in mouse mono peroxidase-anti-peroxidase (PAP) complex (Sternberger-Meyer, Jarrettsville, Maryland) diluted 1:100. Three 10 min washes in PBS were done after incubation with antibodies. Before applying secondary and tertiary antibodies, sections were treated with 10% bovine serum (BS)/PBS for 20 min. All antibody solutions were prepared with 10% BS/PBS. The peroxidase reaction was run for 5–10 min in 0.05% diaminobenzidine and 0.01% H_2O_2 in 0.05 *M Tris* buffer (pH 7.6). The sections were dehydrated and coverslipped.

A few brains of bees and flies were fixed as above and cut with a vibratome. The vibratome sections (50 µm) were treated as floating sections. Furthermore, the reaction was run on cryostat sections (12 µm) of the bee and fly brains.

On cryostat sections the incubation in primary antibody was for 48–72 h, followed by the same procedure as with Epon sections. The immunoreactivity on cryostat sections was very low, even when using double incubations in secondary antiserum and PAP-complex. Hence, this method was abandoned.

For comparison, a commercially available anti-GABA antiserum (Immuno Nuclear Corporation) was tested on 1 µm Epon sections of one *Musca* and one *Apis* brain. The primary antiserum was used at 1:1500 (16 h at 4° C). The secondary antiserum was goat anti-rabbit IgG (H+L; 1:50; Kirkegaard and Perry) and the third was rabbit PAP complex (1:100; Sternberger-Meyer). The commercially available antiserum was compared on alternating 1 µm sections of bee and fly brains with the five different monoclonal antibodies.

Experiments were run on bee and fly brains to test the specificity of the reaction. *1)* Sections were incubated with BS/PBS instead of primary antiserum and then processed as above. *2)* Tissue was fixed in 4% paraformaldehyde instead of glutaraldehyde and then processed with primary antibodies, and the rest of the procedure was as usual. This was to test whether the antibody 3A12 reacted with antigenic sites on molecules fixed by formaldehyde. *3)* A staining test was run following incubation of the antibody 3A12 with the GABA-GA-BSA conjugate for 2 h at 4° C. The primary antibody (at 1:1000) was mixed with the conjugate at 6, 12, 30, 120, 300 µg/ml. The secondary and tertiary antibodies were diluted and processed as described above.

Results

The reactivity of the monoclonal antibodies

All of the five monoclonal antibodies tested, reacted with the same neurons as revealed in experiments using consecutive sections. There were, however, differences in the intensity of the immunoreactivity and in background staining. The antibody 3A12 was found to give optimal results and, hence, has been used throughout the mapping studies. Commercially available anti-GABA antiserum labelled identical structures (Fig. 1A, B), but there were differences in background staining. One exception in staining was seen in the optic lobes of *Musca*, where the commercial antiserum labelled almost the total compliment of large diameter neurons, whereas the monoclonal antibodies labelled only a specific subset of them (Fig. 1C, D).

When preabsorbing the monoclonal antibody (3A12) with antigen, the immunoreactivity diminished with increased concentration of antigen. At an antigen concentration of 30 µg/ml no reaction was found in brains of *Apis* or *Musca*. No reaction was seen in paraformaldehyde fixed tissue, nor in experiments in which the primary antibodies were excluded.

A few brain regions that were labelled in ^{3}H-GABA uptake experiments (Frontali and Pierantoni 1973) were checked for GABA-immunoreactivity. These regions which indeed showed immunoreactive neurons in bees and flies were the ellipsoid body of the central body complex, the antennal glomeruli and cell bodies in the pars intercerebralis (Fig. 2A–D). The antennal glomeruli and optic lobes in *Manduca* are furthermore known to be able to synthesize GABA (Maxwell et al. 1978). Altogether, many regions of the central neuropil contain GABA-like immunoreactive neurons; and a large number of reactive cell bodies can be resolved individually or in clusters. It should be noted that the GABA-like immunoreactivity can be found in cell bodies, axons, dendrites, and terminals.

GABA-like immunoreactive neurons in the fly optic lobes

The GABA-like immunoreactive neurons in the optic lobes of *Calliphora* and *Musca* are of several types: small-field columnar neurons, thin and thick axoned wide field neurons, and possibly anaxonal (amacrine) neurons. The neuropil regions, lamina, medulla, lobula and lobula plate contain immunoreactive processes in different layers.

In the lamina, all immunoreactive processes appear to be derived from one type of small-field columnar neuron, with cell bodies proximal to the medulla. The profiles seem identical to the centrifugal neurons, C2, which connect columns in the medulla to corresponding columns in the lamina (Strausfeld 1971). The immunoreactive neurons (as well as C2) form terminals in the distalmost part of the synaptic layer of the lamina (Fig. 3A). No immunoreactive cell bodies are found near the lamina.

The medulla contains four main layers of GABA-like immunoreactive processes (Fig. 3B). These layers are derived from processes of the columnar neurons in the lamina (C2), from processes of possibly columnar neurons possessing cell bodies distal to the medulla and axons ending in the lobula and lobula plate (Y-cells see Fig. 6A), and from at least one type of widefield neuron connecting the medulla and the midbrain. The latter types of neuron have their cell bodies anterior to the medulla, with axons running in an anterior tract. The slightly thicker tangential processes of these neurons run in three of the immunoreactive layers of the medulla.

The lobula (apart from the processes from the medulla) contains at least three types of GABA-like immunoreactive neurons (Fig. 3C, D). One type is columnar with processes in a distal and possibly a proximal layer, with axons connecting to the midbrain. The other two types have thicker processes and wider arborizations in the lobula proximally and distally, respectively. These neurons also connect to the midbrain. Immunoreactive cell bodies are found in clusters proximal to the lobula.

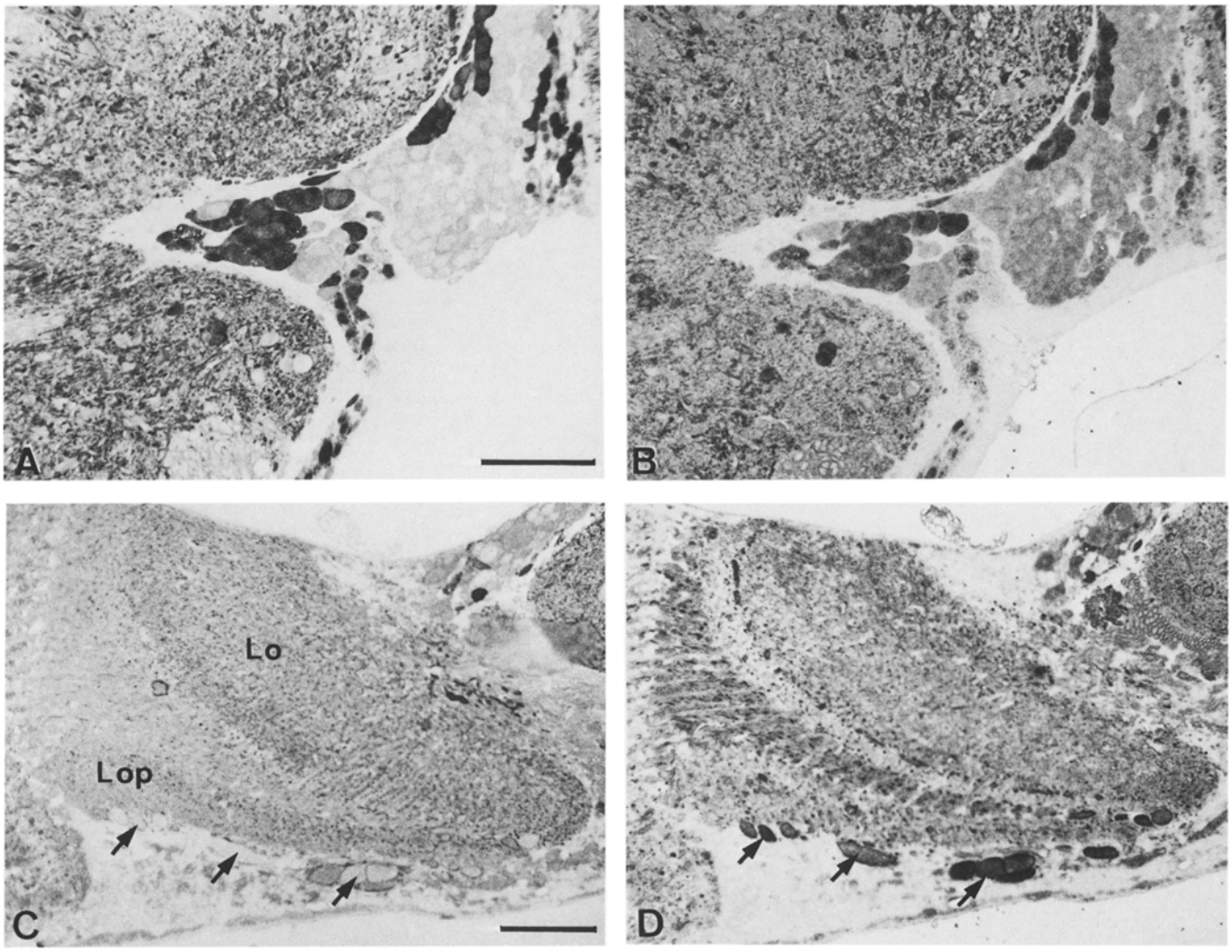


Fig. 1. Comparison between monoclonal antibody 3A12 (anti-GABA) and commercial anti-GABA antiserum. **A.** Ventro-lateral part of bee brain labelled with 3A12. **B.** Adjacent section labelled with commercial serum. **C.** Horizontal section through lobula (*Lo*) and lobula plate (*Lop*) of *Musca* labelled with 3A12. Arrows indicate profiles that were not immunoreactive with 3A12, but as shown in **D**, react with commercial serum. **D.** Adjacent section labelled with commercial serum. Scales 50 μm

In the lobula plate, immunoreactive terminals from the medulla and large wide field neurons can be resolved. At least four GABA-like immunoreactive large diameter axons enter each lobula plate. Reconstructions from 1 μm sections show the relation of these neurons to remaining large field neurons which were unlabelled, but could be identified in semithin sections (Pierantoni 1976; Hausen 1984). Two large GABA-like immunoreactive neurons have processes in the anterior-most part of the lobula plate (Fig. 4B), with their terminations anterior to those of the large HS-cells, and may hence correspond to the CH-cells (Hausen 1984). The other two have processes located posteriorly in the lobula plate (Fig. 4A). Their axons run posteriorly over the oesophageal foramen to the contralateral side. These cells may correspond to the V2 or V3 cells described by Hausen (1984). It should be noted that these correlations are tentative and mainly based on rather crude reconstructions of GABA-like immunoreactive neurons in 1 μm sections.

GABA-like immunoreactive neurons in the bee optic lobes

In the bee optic lobe, the anti-GABA antibodies react with a large number of cell bodies and extensive layers of terminals. A modest GABA-like immunoreactivity is found only in the lamina. In this neuropil, terminals are labelled in the most proximal layer-C of the external plexiform layer (epl-C) (Fig. 5A). These terminals are derived from cell bodies situated in clusters lateral to the lamina. Since no axons projecting to the medulla could be resolved, we assume that the lamina cells are amacrines.

The medulla has a complex pattern of layers with immunoreactive processes (Fig. 5A). At least nine thick or thin layers can be distinguished; four of these layers are especially densely packed with GABA-like immunoreactive terminals. At least three clusters of immunoreactive cell bodies can be related to medulla neurons. Two of them belong to columnar neurons. One type connects the medulla

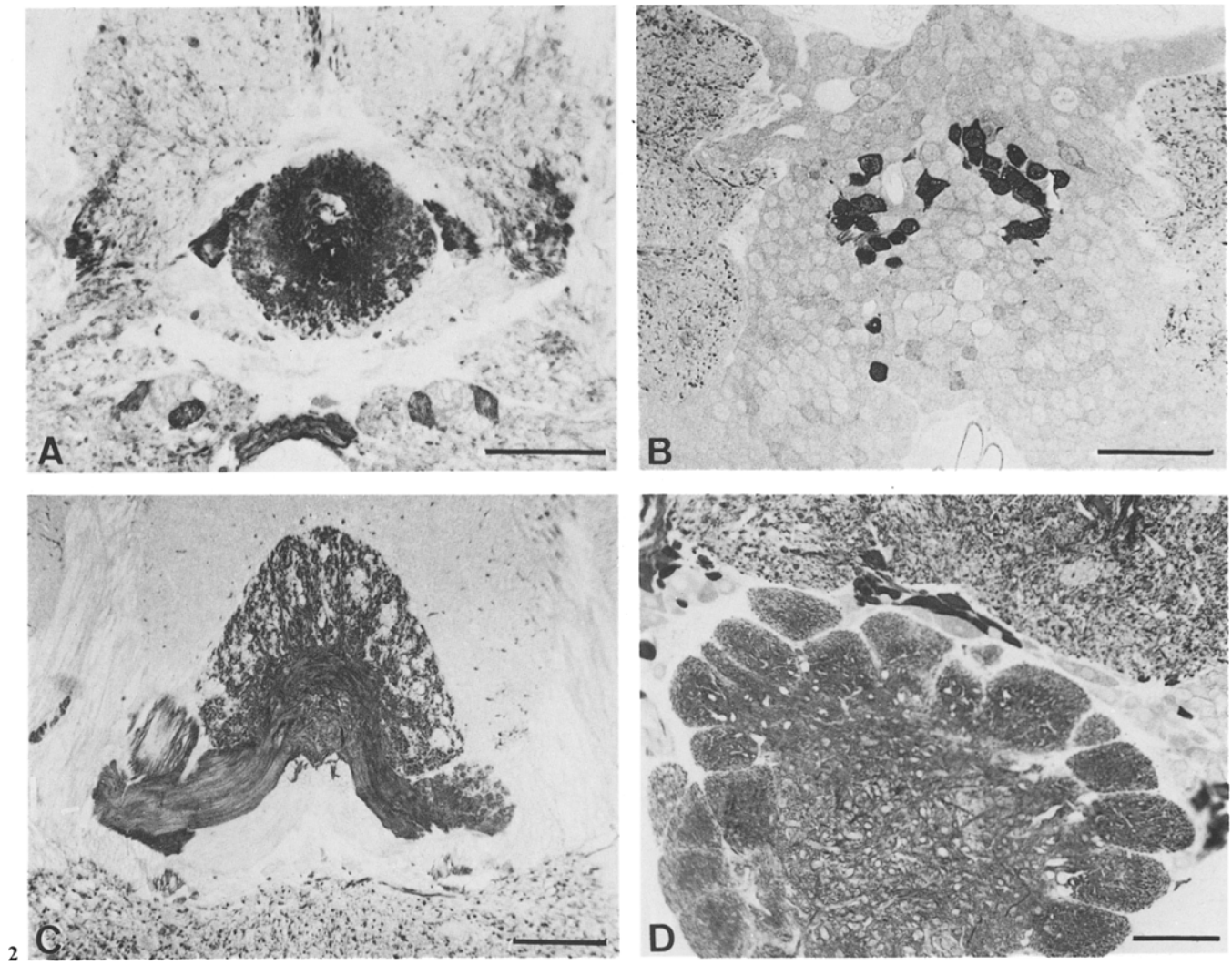


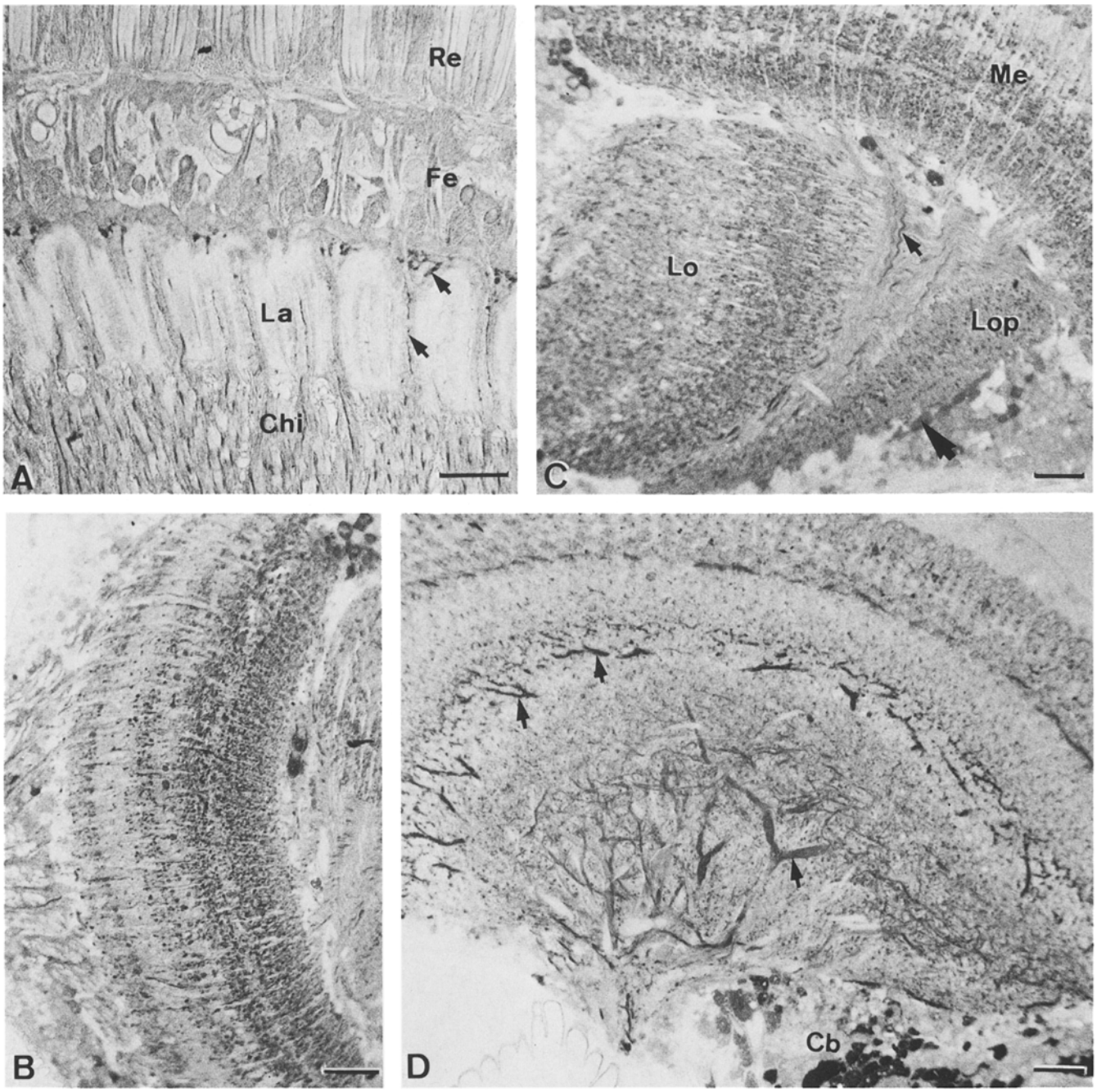
Fig. 2. Neurons reacting with monoclonal anti-GABA-antibodies (3A12) in fly and bee brains. The same neuron types were found to take up ^{3}H-GABA in cockroaches (Frontali and Pierantoni 1973). **A.** Ellipsoid body of central complex in *Musca*. Frontal section. **B.** Cell bodies of the pars intercerebralis in *Musca*. Horizontal section. **C.** Ellipsoid body in *Apis*. Frontal section. **D.** Antennal glomeruli in *Apis*. Horizontal section. Scales 50 μm

Fig. 3. Neurons reacting with anti-GABA antibodies (3A12) in optic lobes of *Musca*. **A.** Neurons probably corresponding to C2 neurons (one indicated by arrows) in the lamina (*La*). (*Re*) Receptor layer of retina; (*Fe*) fenestrated layer; (*Chi*) outer optic chiasma. **B.** Four layers of immunoreactive processes in the medulla. The lamina is to the left (not shown). **C.** Horizontal section of medulla (*Me*), lobula (*Lo*) and lobula plate (*Lop*). Note two main layers and the even distribution of immunoreactive terminals in the lobula plate. Thin arrow indicates immunoreactive fibres connecting medulla, lobula and lobula plate. Thick arrow points at large lobula plate processes. **D.** Frontal, (oblique) section of medulla showing layering and some thick tangential immunoreactive processes (*arrows*). Immunoreactive cell bodies (*Cb*). Scales 20 μm

Fig. 4. GABA-like immunoreactive neurons in the lobula plate of *Musca*. Frontal sections. **A.** Posterior portion of lobula plate. Immunoreactive processes of the two contralaterally projecting large neurons (*arrows*). The bundle of large unstained neurons are the vertical motion-sensitive neurons (*VS*). Immunoreactive cell bodies (*Cb*) **B.** More anterior section with the two large neurons (*arrows*) possibly corresponding to CH-neurons. The three large axons are the horizontal motion sensitive neurons (*HS*). Lobula (*Lo*); lobula plate (*Lop*). Scale 50 μm

to the lobula. The cell bodies of this type may be the ones located distally or proximally to the medulla. The other type could be columnar amacrines or possibly could also connect to the lobula. The third cluster of immunoreactive cell bodies are larger in size, and are situated anterior to the medulla. Their larger processes run parallel to the median layers of the medulla, and give rise to fine branches within the layers of the medulla. We assume that these neurons may be amacrines, since we have not been able to identify connections from the medulla to the midbrain with certainty.

The lobula also contains several layers of GABA-like immunoreactive terminals (Fig. 5B, C). These layers, which are of different densities, overlap and are difficult to distinguish clearly. Four denser layers can be resolved on a background of less densely packed terminals. The latter are derived from columnar neurons originating in the medulla and from at least four types of neurons connecting to the



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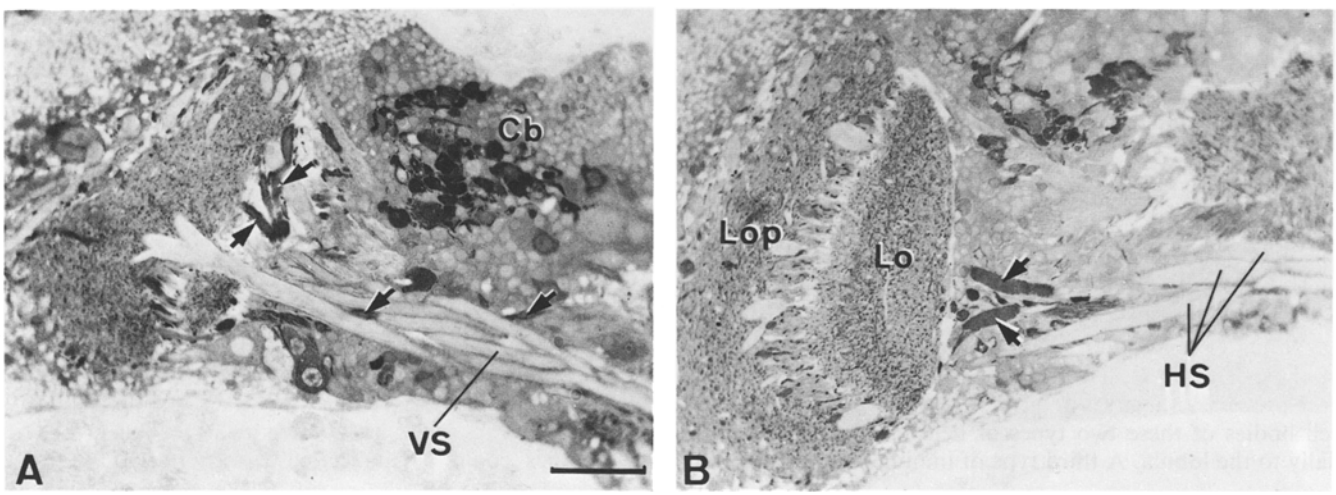

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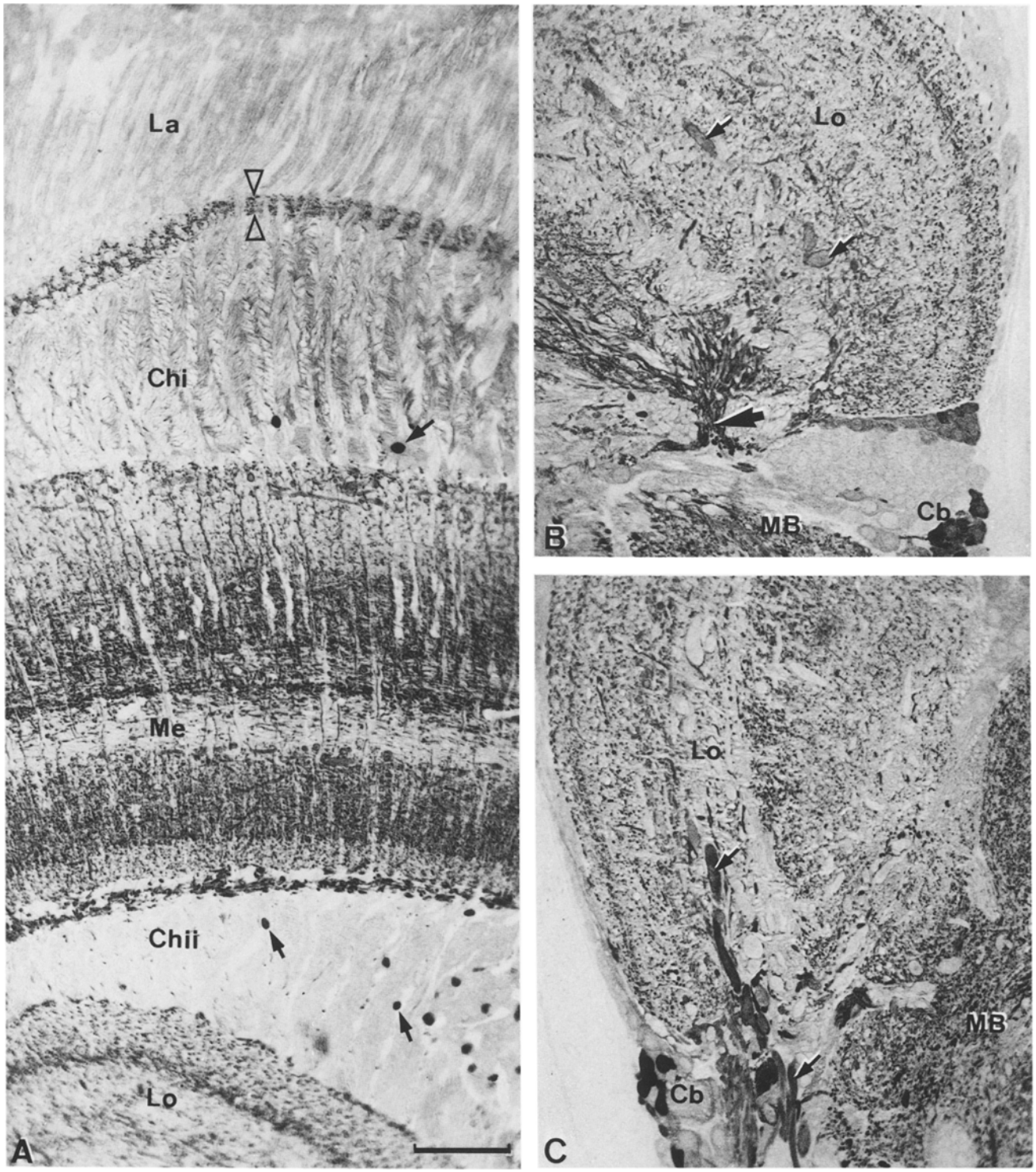


Fig. 5. GABA-like immunoreactive neurons in the optic lobes of the bee. **A.** The layers of immunoreactive processes in the lamina (*La*), medulla (*Me*) and part of the lobula (*Lo*). In the lamina, processes are restricted to the layer epl-C (between *open arrow heads*). Some immunoreactive cell bodies can be seen distal and proximal to the medulla (*arrows*). Inner (*Chii*) and outer (*Chi*) Chiasma. Frontal section. **B.** The lobula (*Lo*) with some large immunoreactive processes (*thin arrows*) and a bundle of thin axons (*thick arrow*) forming part of the layers. Mid brain (*MB*); cell bodies (*Cb*). **C.** Horizontal view of the lobula (*Lo*) with some large immunoreactive fibres (*arrows*) entering the midlayer of the neuropil. Mid brain (*MB*); cell bodies (*Cb*). Scale 50 μm

midbrain. One neuron type has numerous thinner processes in the posterior layers; the other type has thicker axons and processes in more anterior layers of the lobula. The cell bodies of these two types of neuron are situated caudally to the lobula. A third type of immunoreactive neuron consists of at least two large cells. Their huge arborizations extend over the entire projected retinal mosaic of the second and third posterior layers of the lobula. The finer ramifications of these neurons are found in the second layer. No cell bodies could be associated with certainity to these

fibres. The axons leave the lobula posteriorly within the posterior optic tract, and terminate in a ventral part of the protocerebrum. The fourth identifiable immunoreactive cell type in the lobula is a wide field neuron with branchings exclusively in the anterior two layers. This cell type could be traced from the lobula via the anterior optic tract and the intertubercle tract. Approximately six fibres can be counted belonging to this cell type. The cell bodies of these cells may be located frontally between medulla and protocerebrum. There are a few clusters of cell bodies near the lobula. Some are related to the lobula neurons, others innervate midbrain neuropil.

Discussion

In ELISA the five different antibodies showed a stronger immunoreactivity with GABA-BSA than with all the other BSA-conjugates tested. In the case of antibody 3A12, estimated crossreactivity with β-alanin-BSA was approximately 1:4000, being even lower, e.g., for glycine- and glutamate-BSA. In insect brain tissue fixed with formaldehyde, immunoreactivity with 3A12 was abolished showing that the antibody does not bind to residues on formaldehyde-fixed molecules. In addition, immunostaining also disappeared following application of antibodies previously incubated with GABA-GA-BSA conjugate. When tested on rat brain tissue reactivity was detected in neurons supposed to be GABAergic (Matute and Streit 1985; in prep.).

Except in antennal lobes of the sphinx moth *Manduca* (Hoskins et al. 1984) no GABA-containing neurons have been mapped in the insect brain with certainty using other methods. The ^{3}H-GABA uptake study of Frontali and Pierantoni (1973) is the only other investigation that can serve as a comparison with the present account. We could show (Fig. 2) that the cell bodies and terminals that take up ^{3}H-GABA also are immunoreactive. Furthermore, it was shown biochemically in *Manduca* that some regions of the brain are capable of synthesizing GABA: antennal lobes, optic lobes, protocerebrum and suboesophageal ganglia (Maxwell et al. 1978; Maxwell and Hildebrand 1981). The same regions contain large amounts of GABA-like immunoreactive cell bodies and terminals.

Distribution of GABA-like immunoreactive neurons in insect brains

The number of GABA-like immunoreactive cell bodies in the brains of flies and bees is high as compared to cell bodies that are immunoreactive with other antisera. For instance, we estimate that in the optic lobe of one hemisphere of *Musca* or *Calliphora* there are several thousand GABA-like immunoreactive cell bodies. In comparison, one optic lobe of *Calliphora* contains about 20 serotonin-immunoreactive cell bodies (Nässel et al. 1985), 40–60 cell bodies reacting with antibodies to dopamine-β-hydroxylase (Klemm et al. 1985) and a few gastrin-cholecystokinine- and noradrenaline immunoreactive cell bodies (Nässel et al. in prep.). The same applies to the remainder of the brain: GABA-like immunoreactive neurons are found in smaller and larger clusters in many regions of the brain and appear to outnumber all neurons that have so far been identified chemically (cf. Klemm 1976; Evans 1980; Duve and Thorpe 1983b; Veenstra et al. 1984).

In addition, the general abundance of GABA-like immunoreactive terminals is very impressive. Few neuropils (e.g. the protocerebral bridge) lack GABA-like immunoreactive terminals completely. This massive occurrance of GABA-like immunoreactive neurons correlates well with the high GABA content in the insect brain (Frontali 1964; Klemm 1976; Evans 1980; Clarke and Donnellan 1982; Kingan and Hildebrand 1985).

In the brains studied in the present account, several types of GABA-like immunoreactive neurons can be identified: local (amacrine type) neurons, and small and large projection neurons that connect different neuropils. The antibodies used by us labelled cell bodies, axon terminals and other processes. This allowed us to describe, at least occasionally, individual neurons in detail. Often, however, only a compound pattern produced by numerous neurons can be resolved. Within the optic lobes GABA-like immunoreactivity was not detected in neurons that had previously been found to be immunoreactive to other substances.

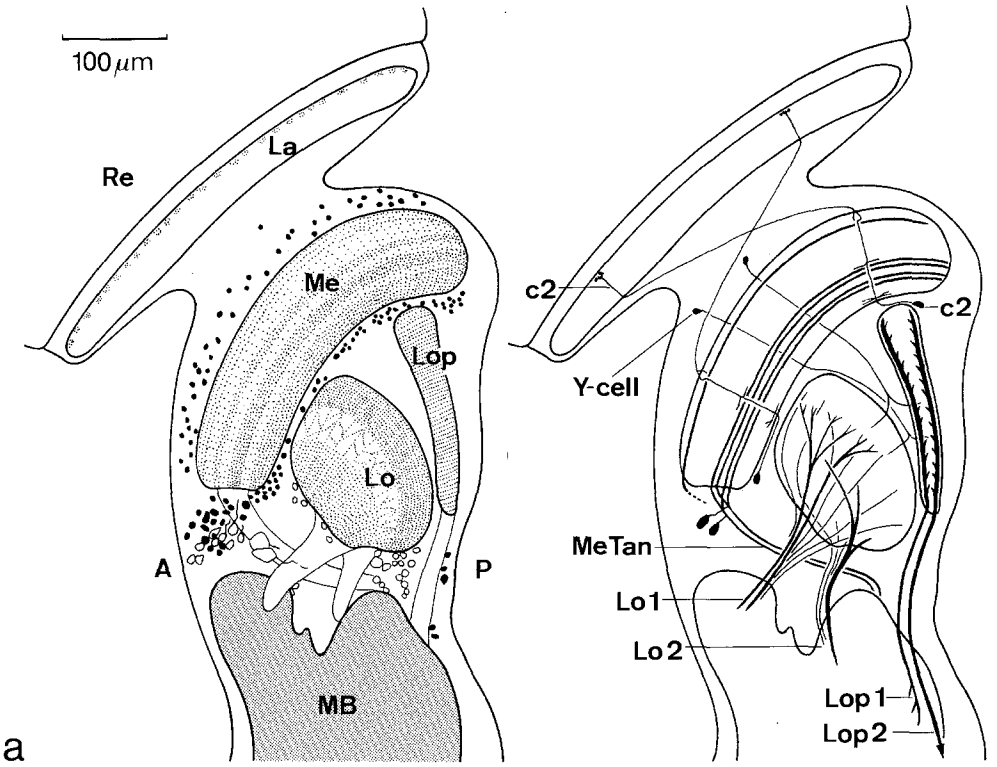
The optic lobes of flies

All regions of the optic lobe neuropil were found to contain GABA-like immunoreactive processes (Fig. 6A). Some of the immunoreactive neurons could be correlated with neurons described with other anatomical techniques (Strausfeld 1971; Hausen 1981, 1984).

One neuron type may correspond to the small field centrifugal neuron C2, which connects the medulla with the lamina. The C2 neurons are presynaptic to centripetal monopolar neurons (L1–L3) in the lamina (Strausfeld and Nässel 1981). Thus, these neurons may have inhibitory GABAergic synapses and form part of a recurrent pathway from the medulla. Circuits for lateral inhibition demonstrated in lamina monopolar neurons (Zettler and Järvilehto 1972) may utilize transmitters other than GABA. It should be noted that so far only neurons reacting with antibodies to GABA and serotonin have been detected in the lamina.

The other neurons that can be tentatively identified are six large neurons of the lobula plates (three on each side; of these, one within each lobula plate is bilateral). From the projection of their axons and the localization of their large tangential processes in the lobula plate, it can be proposed that these neurons correspond to the two CH-neurons of each lobula plate and the bilateral V2 or V3 neurons (Hausen 1984). For a more definite identification of these large field neurons, it would be necessary to analyse GABA-immunoreactivity in thicker sections. The CH-, V2- and V3-neurons are motion sensitive and are part of a large set of motion sensitive lobula plate units which interact with each other in complex ways (Hausen 1981, 1984). It has been shown that the CH-cells are inhibitory to some neural elements in the lobula plate (Hausen 1984).

The remaining cells of the fly optic lobes could not be identified individually, but their general appearance may provide us with some ideas about how they are connected. The medulla contains neurons that apparently connect to the lobula and lobula plate. These seem to be arranged in columns as the C2-neurons are. There are also wide field tangential neurons in the medulla (in three layers); some of these project to the midbrain. Hence, the immunoreactive layers in the medulla are the compound pattern of lateral processes from different cell types. It cannot be excluded



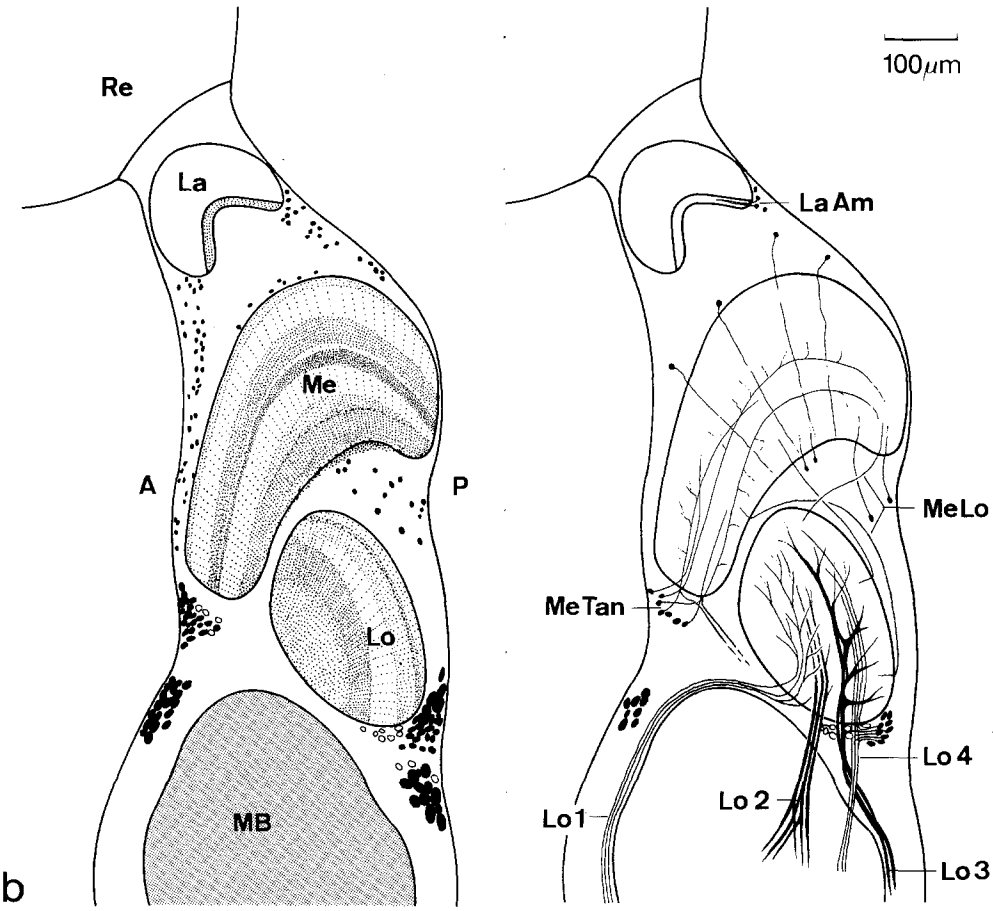


Fig. 6. Diagrammatic representation of GABA-like immunoreactive layers and distribution of cell bodies (*left*) and identified neurons in the optic lobes (*right*) of *Musca* and *Calliphora* (*A*) and *Apis* (*B*). Only a few representatives of each type of neuron are drawn. Filled cell bodies lie more dorsally, open ones more ventrally. (*A*) anterior; (*P*) posterior; (*Re*) retina; (*La*) lamina; (*Me*) medulla; (*Lo*) lobula; (*Lop*) lobula plate (in flies only); (*MB*) midbrain. Scales 100 μm.

A. (C2) narrow-field centrifugal cells. (Y-cells) columnar neurons connecting medulla to lobula and lobula plate. (*MeTan*) large tangential neurons of the medulla running to the midbrain. Tract (*Lo1*) containing thin columnar neurons and a smaller number of large lobula neurons with terminals located distally. Tract (*Lo2*) containing some large and numerous thinner neurons terminating in the inner layer of the lobula. Neurons in tract (*Lop1*) possibly correspond to CH-cells. Contralaterally projecting neurons of the lobula plate run in tract (*Lop2*).

B. Amacrines (*LaAm*) of the lamina layer epl-C. Columnar neurons connecting the medulla with the lobula (*MeLo*). Large tangential neurons of the medulla (*MeTan*) with possible connections to the midbrain. A few (approximately 6) thinner neurons (*Lo1*) with terminations in the inner layers of the lobula may have contralateral connections. Some thicker neurons (*Lo2*) of the inner lobula layers connect to the midbrain. At least two thick neurons (*Lo3*) and numerous thin neurons (*Lo4*) terminate in outer lobula layers connecting to the midbrain

that some medulla cells reacting with antibodies to GABA are amacrine neurons. In two of the four layers with GABA-like immunoreactive fibres, serotonin-immunoreactive processes were also found (Nässel and Klemm 1983). The serotonin-immunoreactive processes, however, are derived from widefield neurons whose morphology and cell-body location are different from the GABA-like immunoreactive cells (Nässel et al. 1985; Nässel and Byers in prep.).

The lobula receives GABA-like immunoreactive processes from the medulla and, in addition, contains neurons connecting to the midbrain. At least two such types were seen with thin or thick axons, respectively. The layering of the lobula is less pronounced, but a dense zone of GABA-like immunoreactive terminals corresponds to a synaptic layer with dendrites from several types of lobula output neurons (Strausfeld and Nässel 1981). This layer also contains serotonin-immunoreactive processes from large field neurons of a different type (Nässel and Klemm 1983; Nässel and Byers in prep.).

In addition to terminals from the medulla, and to the large possibly motion sensitive units, the lobula plate contains cells which have thin axons and may be columnar units. These cells project to the midbrain. The lobula plate is probably the best studied of the optic lobe neuropils, with respect to both anatomical and electrophysiological characterization of identified interneurons, and the large GABA-like immunoreactive neurons described above can thus be fitted into existing circuits analyzed in electrophysiological studies (e.g. Hausen 1984).

The optic lobes of bees

In the optic lobes of the bees, there are so far only a few types of neurons that have been identified outside the lamina (Ribi 1975; Ribi and Scheel 1981; DeVoe et al. 1982). Hence, our data on GABA-like immunoreactive neurons cannot be correlated reliably with types of neurons identified by other means.

In the lamina, the GABA-like immunoreactive processes in the epl-C appear to be derived from amacrine neurons. Each neuron has processes extending over more than one synaptic unit (cartridge). The epl-C layer was also found to contain processes of serotonin-immunoreactive neurons located more centrally (Schürmann and Klemm 1984; Nässel et al. 1985). Epl-C is a complex layer with numerous lateral processes, many of which extend over several cartridges (Cajal and Sanchez 1915; Ribi 1975). Lateral inhibition may take place in this layer. Although the involvement of GABA in such processes is by no means established, GABA-like immunoreactivity of amacrine neurons may indicate such a possibility.

The medulla and lobula are so rich in GABA-like immunoreactive terminals that only a few thin layers are devoid of such innervation (Fig. 6B). Some neurons are small field projection neurons, others wide field (tangential) projection neurons or amacrine neurons. Thus, several types of pathways exist which react with antibodies to GABA. Morphological data such as arborizations and projection patterns of axons, as well as the possibility of inhibitory action of the immunoreactive large field neurons in the lobula, make it tempting to compare them with some of the physiologically identified motion sensitive neurons (DeVoe et al. 1982). A certain similarity between the horizontal regressive (HR) motion sensitive cells and the most anterior immunoreactive cell type is obvious. However, as a complete reconstruction of immunolabelled cells is not possible on the basis of semithin sections, a definite answer is not yet possible. Part of the GABA-like immunoreactive layers overlap layers with serotonin-immunoreactive processes (Schürmann and Klemm 1984).

Comparison between bees and flies

One interesting finding is that some types of GABA-like immunoreactive neurons are analogous in the optic lobes of both flies and bees, whereas in other cases the neurons are of quite different types. For instance, in the lamina of flies the GABA-like immunoreactive C2-neurons connect the lamina with the medulla and are columnar small-field neurons. In bees, however, the immunoreactive lamina cells are wide field amacrine neurons. Most likely, these neurons are part of different circuits in flies and bees.

Other circuits may be analogous, such as the large neurons in the lobula complex or some medulla neurons. Since bees have a non-divided lobula, probably neurons analogous to lobula plate neurons in the fly are incorporated into the lobula. Therefore, the large-field, large diameter neurons reacting with GABA-antibodies in the lobula of the bee may serve functions similar to the motion sensitive neurons of flies. In conclusion, a very large number of neurons react with antibodies to GABA in the optic lobes of flies and bees. These neurons have processes in complex layers and interconnect different neuropil regions. Hence, if these neurons indeed contain GABA, this substance may play an important role in visual processing at all levels of the visual system.

Acknowledgements. We are grateful to Mr. H.J. Waldvogel for technical assistance, Mr. D. Rigoli for the graphics and Dr. R. Wehner and Mrs. V. Zwicky for reading the manuscript. The research was supported by grants from the Swiss National Science Foundation (grants 3.665-0.84; 3.228.82; 3.455.83 and 3.274.82), Dr. Eric Slack-Gyr-Foundation and the Swedish Natural Science Research Counsil. Carlos Matute was the recipient of a fellowship from the exchange program between the University of Zürich and the Consejo Superior de Investigationes Cientificas of Spain, and from IBRO (Swiss National Science Foundation; grant 83.161).

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