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Neuronal architecture of the antennal lobe in *Drosophila melanogaster*

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Summary. Computer reconstruction of the antennal lobe of *Drosophila melanogaster* has revealed a total of 35 glomeruli, of which 30 are located in the periphery of the lobe and 5 in its center. Several prominent glomeruli are recognizable by their location, size, and shape; others are identifiable only by their positions relative to prominent glomeruli. No obvious sexual dimorphism of the glomerular architecture was observed. Golgi impregnations revealed: (1) Five of the glomeruli are exclusive targets for ipsilateral antennal input, whereas all others receive afferents from both antennae. Unilateral amputation of the third antennal segment led to a loss of about 1000 fibers in the antennal commissure. Hence, about 5/6 of the approximately 1200 antennal afferents per side have a process that extends into the contralateral lobe. (2) Afferents from maxillary palps (most likely from basiconic sensilla) project into both ipsi- and contralateral antennal lobes, yet their target glomeruli are apparently not the same as those of antennal basiconic sensilla. (3) Afferents in the antennal lobe may also stem from pharyngeal sensilla. (4) The most prominent types of interneurons with arborizations in the antennal lobe are: (i) local interneurons ramifying in the entire lobe, (ii) unilateral relay interneurons that extend from single glomeruli into the calyx and the lateral protocerebrum (LPR), (iii) unilateral interneurons that connect several glomeruli with the LPR only, (iv) bilateral interneurons that link a small number of glomeruli in both antennal lobes with the calyx and LPR, (v) giant bilateral interneurons characterized by extensive ramifications in both antennal lobes and the posterior brain and a cell body situated in the midline of the suboesophageal ganglion, and (vi) a unilateral interneuron with extensive arborization in one antennal lobe and the posterior brain and a process that extends into the thorax. These structural results are discussed in the context of the available functional and behavioral data.

Key words: Antennal lobes – Antennal glomeruli – Antennal afferents – Maxillary afferents – Antennal interneurons – Computer reconstruction – Golgi impregnation – Backfilling – *Drosophila melanogaster* (Insecta)

The study of the antennal lobes of *Drosophila melanogaster* and their connections with other brain centers was pioneered more than 40 years ago by Power (1943, 1946). The first detailed information about the cellular elements of this system was provided by Strausfeld (1976) in *Musca*. Recently, the antennal system of *Drosophila* has been approached using neurogenetics. A number of mutants have been isolated that disrupt the olfactory system in different ways. For example, there exist mutants that affect olfactory function (Rodrigues and Siddiqi 1978; Siddiqi 1984, 1987; Helfand and Carlson 1989; McKenna et al. 1989; Woodard et al. 1989), the segmental identity of antennal afferents (Stocker et al. 1976; Stocker and Lawrence 1981), the pattern of antennal sensilla (Lienhard and Stocker 1987; Stocker and Gendre 1988), and the structure of central olfactory pathways (Heisenberg et al. 1985). Renewed interest in the anatomy of the antennal system has also been spurred by behavioral and functional studies. Behavioral assays on chemical orientation mechanisms and odor recognition by flies (Borst and Heisenberg 1982; Borst 1983) have led to new hypotheses about the evaluation of olfactory signals. Mapping of odor-induced activity by the 2-deoxyglucose method (Rodrigues and Buchner 1984; Rodrigues 1988) and mapping of choline uptake (Pinto et al. 1988) have supported the idea that antennal

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Abbreviations: *AC* antennal commissure; *AMMC* antennal mechanosensory and motor center; *iACT, mACT, oACT* inner/middle/outer antenno-cerebral tract; *bACTI, uACTI* bilateral/unilateral ACT relay interneuron; *AN* antennal nerve; *AST* antenno-suboesophageal tract; *FAI* fine arborization relay interneuron; *GSI* giant symmetric relay interneuron; *LI* local interneuron; *LPR* lateral protocerebrum; *SOG* suboesophageal ganglion; *TI* thoracic relay interneuron; *bVI* bilateral V-relay interneuron

lobes are subdivided into functionally specific subunits (Rodrigues and Pinto 1989).

To provide a better structural basis for the interpretation of these experimental data, more information about the anatomy of the antennal lobe of *Drosophila* is needed. As a first step towards this goal, Stocker et al. (1983) studied the glomerular organization of the lobes and the pattern of sensory projections they receive from the third antennal segment. Moreover, Singh and Nayak (1985) reported the presence of afferent input from the maxillary palps in the antennal lobe. Since both the third antennal segment and the maxillary palps possess sensilla of the basiconic type (Miller 1950; Nayak and Singh 1983; Venkatesh and Singh 1984), a comparison of their primary targets in the lobe might yield information about interactions between the two kinds of basiconic afferents.

The purpose of the present paper is to expand the knowledge of the anatomy of the system by adding data from two different approaches. Computer reconstruction of the antennal lobe should yield a more complete view of its glomerular organization (Stocker et al. 1983; Pinto et al. 1988). Golgi and transmission electron-microscopic (TEM) studies, on the other hand, should increase our knowledge about the neural elements involved in the antennal system. We have attempted to classify the different types of sensory inputs and interneurons that arborize in the lobe. The catalogue presented here is the most comprehensive description of the lobe anatomy in *Drosophila* to date. Yet, due to methodological limitations, a complete picture cannot be expected. A brief report of these data has been published in abstract form (Borst and Fischbach 1987). Our article belongs to a series of papers on the structure of the *Drosophila* brain, including the central complex (Hanesch et al. 1989) and the optic lobe (Fischbach and Dittrich 1989).

Materials and methods

Stocks

Drosophila melanogaster of the wild-type strains 'Sevelen', 'Berlin', and 'Kapelle' were used. Flies were kept on standard cornmeal medium at 21° C and 60%–65% relative humidity.

Golgi impregnation

Golgi impregnation was carried out according to the procedure of Colonnier (1964) as adapted to *Drosophila* by Fischbach and Götz (1981), or according to a modified 'Golgi rapid' method (Strausfeld 1980b) as follows: (1) fixation of heads (opened dorsally, proboscis removed) in a mixture of 2% paraformaldehyde and 0.5% glutaraldehyde in Millonig's phosphate buffer (2 h at 0° C); (2) 2 × 30 min rinse in Millonig's buffer at 0° C; (3) 2 × 30 min rinse in $K_2Cr_2O_7$ (2.5% in Holmes buffer) at 0° C; (4) storage in the dark for 7 days in a solution of 2.5% $K_2Cr_2O_7$ and 0.1% OsO_4; (5) 3 short rinses in Holmes buffer; (6) storage in the dark for 7 days in 0.75% $AgNO_3$ (in Holmes buffer); (7) repetition of steps 4–6; (8) rinse in Holmes buffer and H_2O at 0° C, 30 min each; (9) dehydration and embedding in Epon or Durcupan; (10) serial sectioning at 25 or 35 µm with a steel knife.

By removing particular appendages of the head before the fixation, the 'direction' taken by the Golgi reaction can be influenced. For example, amputation of antennae resulted in an enhancement of antennal afferents (see also Strausfeld 1980), though not of antennal interneurons. Antennal interneurons that send a process into higher brain centers could be demonstrated by removing the dorsalmost part of the head, which apparently produced small lesions in the dorsal brain.

Silver impregnation

For silver impregnation, heads were fixed in Carnoy's solution, embedded in Paraplast, and sectioned at 10 µm. After dissolving the Paraplast from the sections, specimens were impregnated using the Holmes-Blest technique (Blest and Davie 1980).

Orthograde filling of antennal afferents

Backfilling of afferents with $CoCl_2$ or with horseradish peroxidase (HRP) has been described by Stocker et al. (1983), and Schmid et al. (1986). For visualizing the glomeruli in the antennal lobes, the distal two-thirds of one antennal funiculus were cut off and a 5% aqueous solution of Lucifer Yellow was applied to the proximal stump for 2 h. Thereafter, the head was detached, opened dorsally and the proboscis was removed. Specimens were fixed in 4% paraformaldehyde for 4 h and embedded in Epon.

Electron microscopy

For semithin and ultrathin sectioning, heads were fixed in glutaraldehyde and OsO_4 and embedded in Epon (for details, see Technau 1984). Semithin sections were stained with AzurII/methylene blue.

Antennectomy

To induce degeneration of sensory fibers from the third antennal segment, either one or both funiculi were removed with forceps or iridectomy scissors from flies anaesthetized in CO_2 or N_2. Care was taken not to injure the proximal two segments. Successful surgery was indicated by non-degeneration of afferents from these two segments in the brain. After surgery flies were kept alive for 12 or 24 h before histological preparation.

Reconstruction of the antennal lobe

From 4- or 8-µm-thick serial Epon sections, micrographs were taken in a Zeiss epifluorescence microscope using an excitation filter BP 390–440 nm and a barrier filter LP 470 nm. Using a digitizing tablet (Summagraphics MM 1201), the outlines of the antennal lobes and glomeruli on the micrographs were registered for each section in a reconstruction program (Montage System; University of Pennsylvania). Internal head structures such as the oesophagus, the muscles running parallel to it, or the outline of the brain were used to establish by the 'best fit' method two arbitrary lines parallel to each other and perpendicular to the sections. These lines made it possible to align consecutive sections. Reconstructed antennal lobes could be viewed as stereo-plots or hidden-line plots. The plotted images of glomeruli were identified by visual comparison with the micrographs.

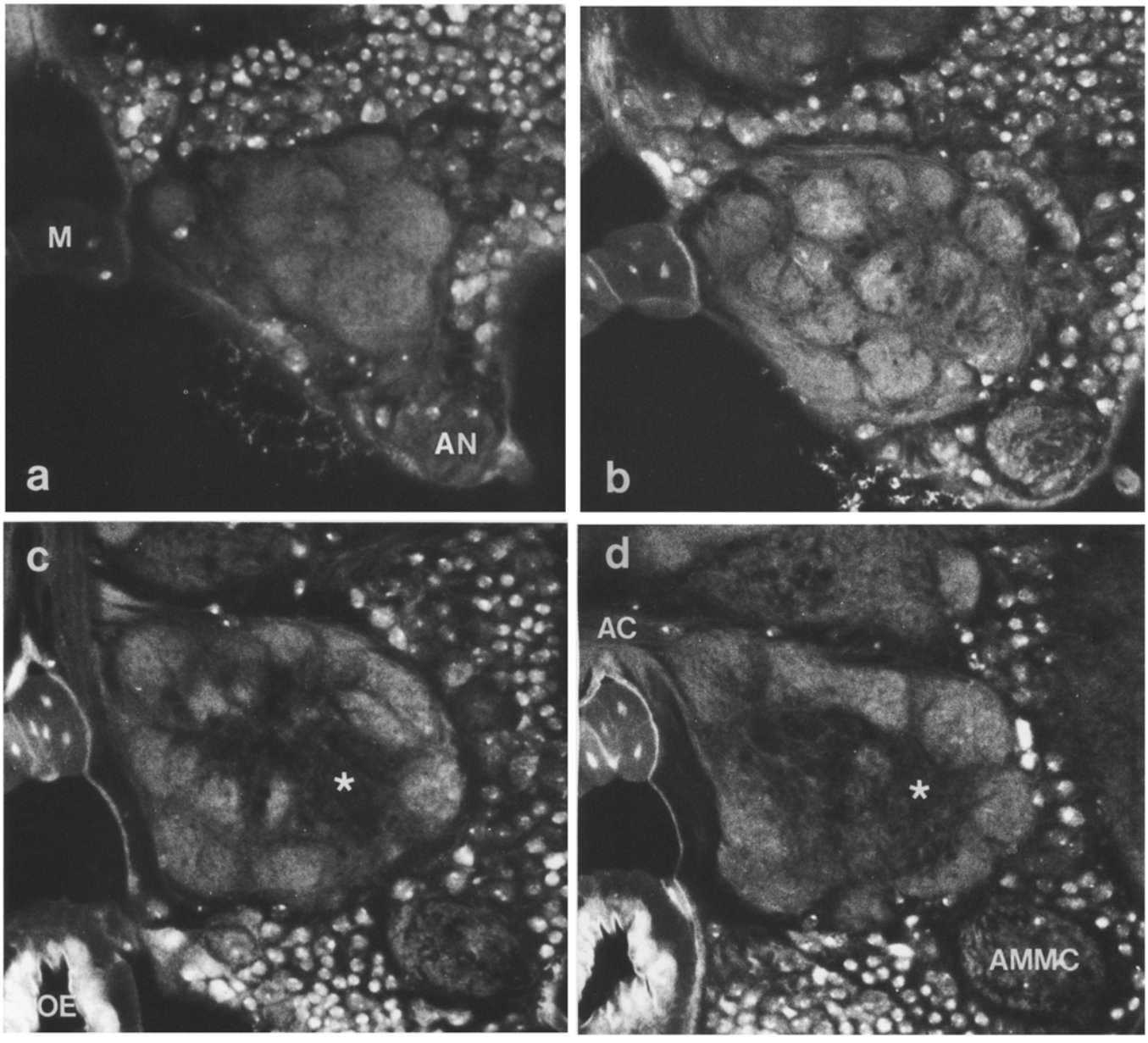


Fig.1 a–d. The glomeruli of the right antennal lobe of *D. melanogaster,* as visualized by orthograde filling of the afferents of the left funiculus with Lucifer Yellow. The four micrographs show representative 4-μm transverse sections from anterior (**a**) to posterior (**d**). In **c** and **d** the coarse non-glomerular central neuropil of the lobe is visible (*asterisks*). *M* muscle; *OE* oesophagus; *AN* antennal nerve; *AMMC* antennal mechanosensory and motor center. Dorsal is on *top*, lateral to the *right*. × 700

Results

The glomerular organization of the antennal lobe

The antennal lobes of *D. melanogaster* form a pair of protrusions situated in the anterior part of the brain, at the level of the oesophagus (Figs. 1, 4, 5a). They are connected to each other by the antennal commissure. The antennal lobes are the exclusive targets of receptor neurons from the third antennal segment (see below), and thus represent the primary olfactory association centers. Most of the center of the lobe consists of a coarse, large-fiber neuropil, whereas the periphery is occupied by a set of finely textured subunits, the so-called glomeruli. In *Manduca,* individual glomeruli are separated by a thin glial sheath (Oland and Tolbert 1987). Moreover, glial cells play a crucial role in the formation of the glomerular organization during metamorphosis (Oland and Tolbert 1989; Tolbert and Oland 1989). The glomeruli are the sites where sensory axons establish their terminal arborizations (Power 1946; Strausfeld 1976; Stocker et al. 1983). From studies in *Periplaneta* (Boeckh et al. 1970) and *Manduca* (Tolbert and Hildebrand 1981) it is known that synaptic contacts are essentially restricted to the glomerular neuropil. Numbers and arrangements of glomeruli are species specific (Rospars

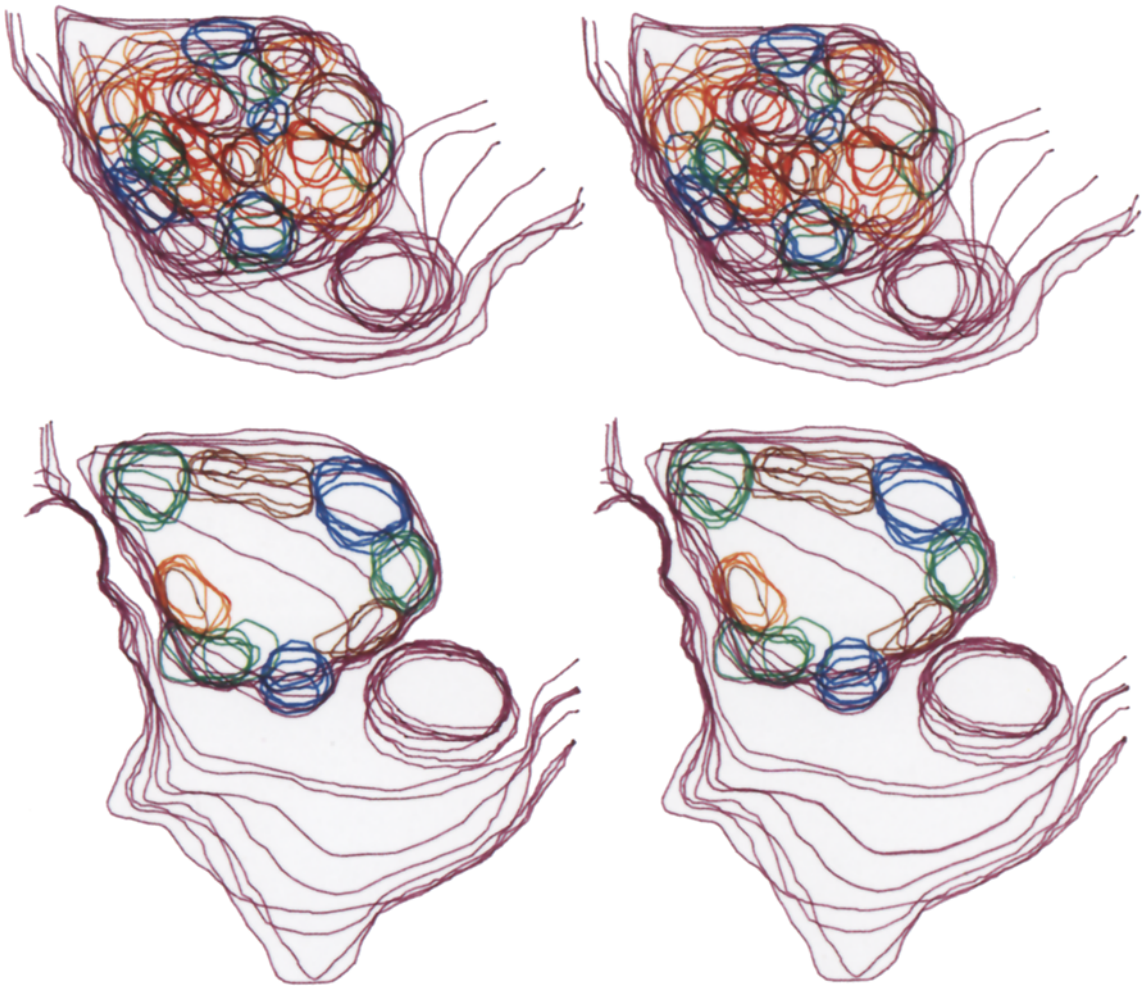


Fig. 2. Stereopairs of the right antennal lobe reconstructed from 4-μm transverse serial sections like those shown in Fig. 1. *Upper pair:* Glomeruli of the anterior half of the lobe. *Lower pair:* Glomeruli of the posterior half. In both pairs, the lobe is seen from anterior. Lateral is to the right, dorsal on top. The profile on the right side is the antennal nerve. For identification of glomeruli, refer to Fig. 3a, b

1988; Homberg et al. 1989; Rospars and Chambille 1989).

To visualize the glomerular architecture of the antennal lobe, we have applied various marking techniques, such as backfilling from the third antennal segment with Lucifer Yellow, $CoCl_2$, or HRP or using Golgi and silver impregnations of the brains. Combining the data from these methods allowed us to distinguish a total of 35 glomeruli, many more than described previously (Stocker et al. 1983; Pinto et al. 1988). Computer reconstruction of the lobe was performed with Lucifer Yellow preparations, which yielded optimal resolution of the glomeruli, in particular in the lobe contralateral to the filled antenna. The reconstruction was thus based on one 'contralateral' lobe sectioned transversely at 4 μm (Fig. 1). The results were then verified in the 'ipsilateral' lobe. Another transversely and two horizontally sectioned pairs of lobes, and four silver-impregnated antennal lobes (transverse sections) were used to confirm the reconstruction model. As a rule, individual glomeruli in *Drosophila* are not as clearly separated from one another as in other insects, probably because the enveloping glial sheath is less compact, or because the network of neuronal arborization within the glomeruli is less dense. Nevertheless, the resolution of Lucifer Yellow backfills allowed us to identify 31 glomeruli (Fig. 2), 16 of which had been described before. Their diameter range from 10 to almost 30 μm. Prominent landmark glomeruli like V, VA_{1-2}, VL_{1-2}, VM_1, VM_4, D, DA_{1-2}, DL_{1-2}, DM_{1-2}, or DP_1 are recognized in all preparations by combining the criteria of location, size, and shape, whereas the remaining glomeruli are identifiable only by their spatial relations with the prominent glomeruli. Of the newly detected glomeruli, VC_{1-4} and DC_1 lie clearly in the center of the lobe. Glomeruli VP_{1-3} and VM_3 are visible only in Golgi preparations and in antennal $CoCl_2$ backfills. Consequently, the glomerular organization of the antennal lobe, as shown in Fig. 3, relies on the combined data from the various techniques.

Our model is based on the reconstruction of female

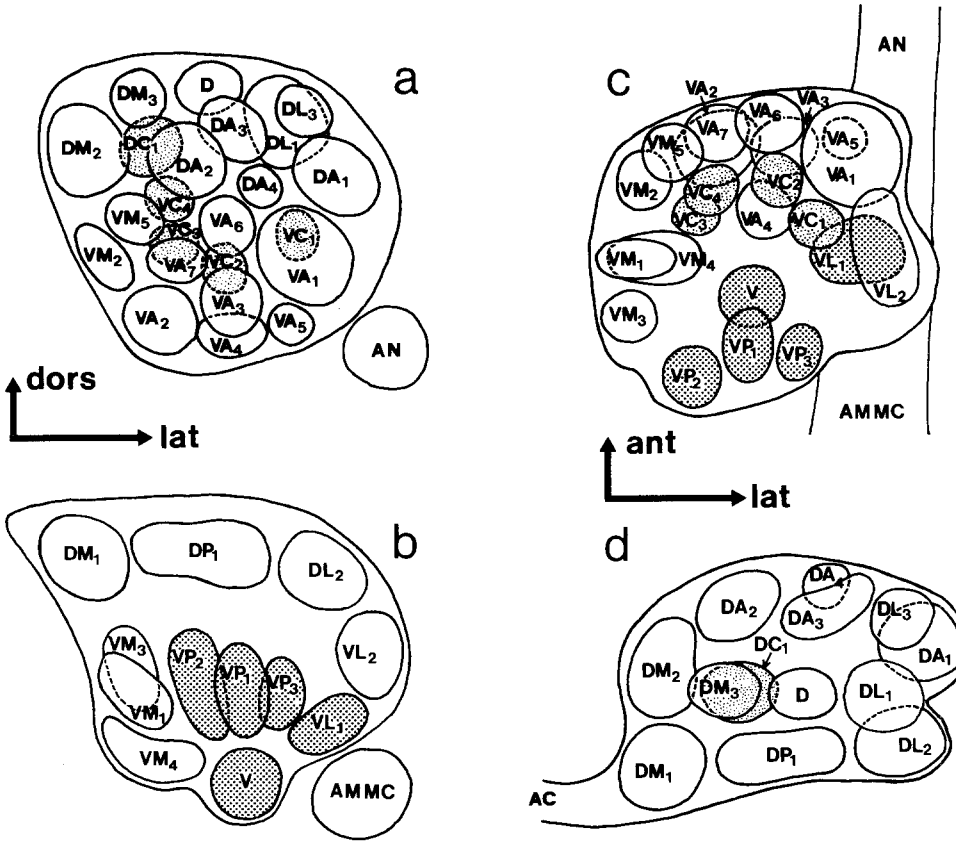


Fig. 3a–d. Schematic representation of the right antennal lobe in transverse (**a, b**) and horizontal view (**c, d**), showing the relative positions of 35 individual glomeruli. **a, b** Anterior and posterior halves, respectively, seen from anterior. **c, d** Ventral and dorsal halves, respectively, seen from dorsal. Heavily stippled glomeruli receive exclusively input from the ipsilateral antenna. Central glomeruli are lightly stippled. The designations of the glomeruli represent the relative positions (*A* anterior; *D* dorsal; *L* lateral; *M* medial; *P* posterior; *V* ventral; see also Stocker et al. 1983)

antennal lobes. However, a comparison with two preparations of male lobes revealed no obvious differences with respect to the number of glomeruli, their location, size, or shape.

Input and output tracts of the antennal lobe

The major input and output tracts of the antennal lobe have been described by Power (1946): these are the antennal nerve as a principal input tract, the antennal commissure as the connection of the two lobes, and two tracts extending into higher brain centers. Our Golgi preparations demonstrate additional tracts, some of which have also been reported from other insect species (Fig. 4). For the sake of uniformity, we adopt here the nomenclature used in recent anatomical work (Homberg et al. 1988, 1989; Rospars 1988).

The antennal nerve (AN) carries the majority of afferents to the antennal lobe (Figs. 4, 5a). Near its entrance into the brain, the AN consists of 1700–1800 axons (Stocker and Gendre 1988); approx. 1200 of them (essentially small diameter axons) are sensory fibers from the third antennal segment, the funiculus, (Stocker 1979; Venkatesh and Singh 1984). They are mainly olfactory and appear to project in toto into the antennal lobe, i.e., they enter the lobe ventrally at its antero-lateral corner (Fig. 5a). Twelve to 24 h after amputation of the funiculus, the periphery of the lobe is labelled by degeneration products, indicating where the funicular afferents terminate (Fig. 5b). The remaining 500–600 fibers in the AN (possessing larger diameters) stem mainly

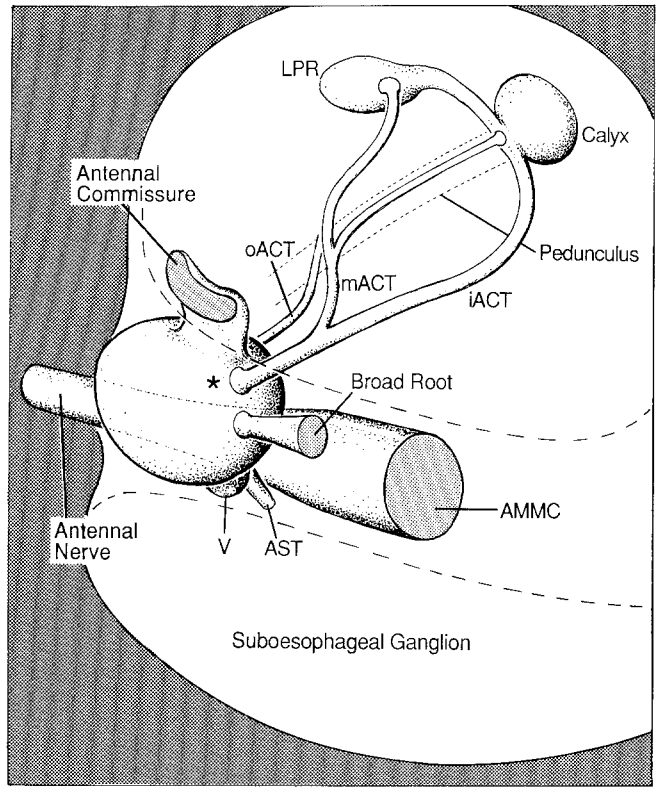


Fig. 4. Schematic diagram of the fiber tracts of the antennal lobe as seen from the midline and from slightly posterior. The oesophagus has been omitted. The AC is cut in the sagittal plane; the *dashed lines* indicate the outlines of supra- and suboesophageal ganglia in the sagittal plane. The area shown in white represents the brain. The *asterisk* marks the site of entry/exit of FAI. *V* glomerulus V

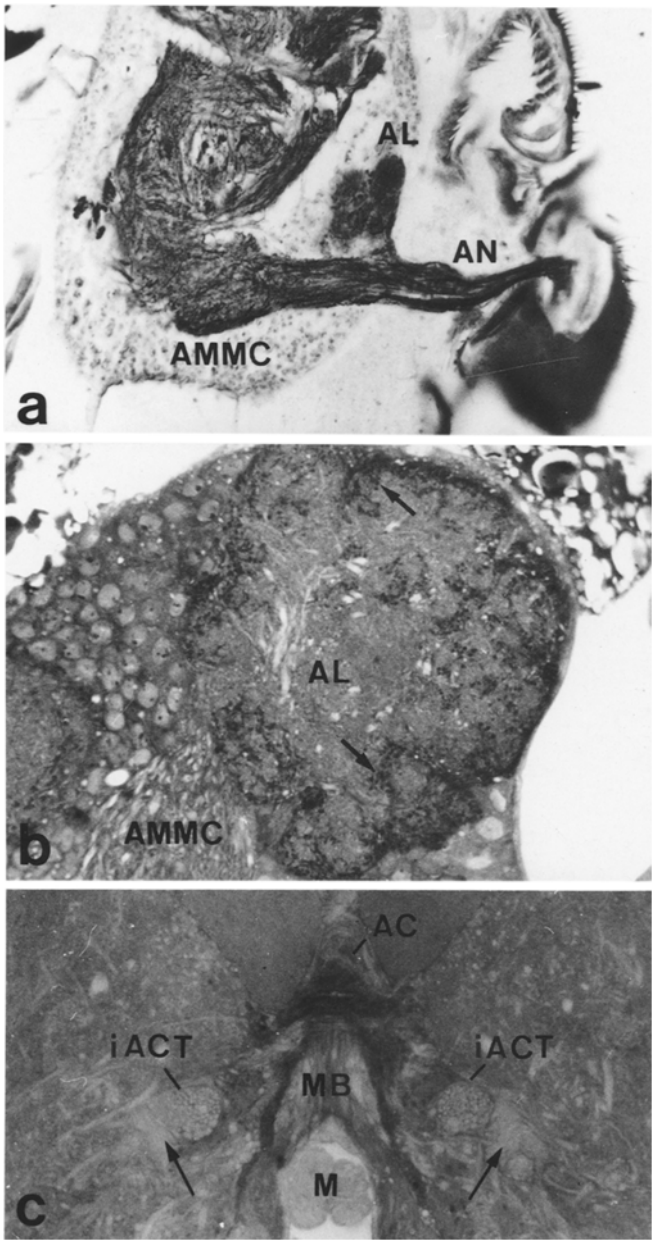


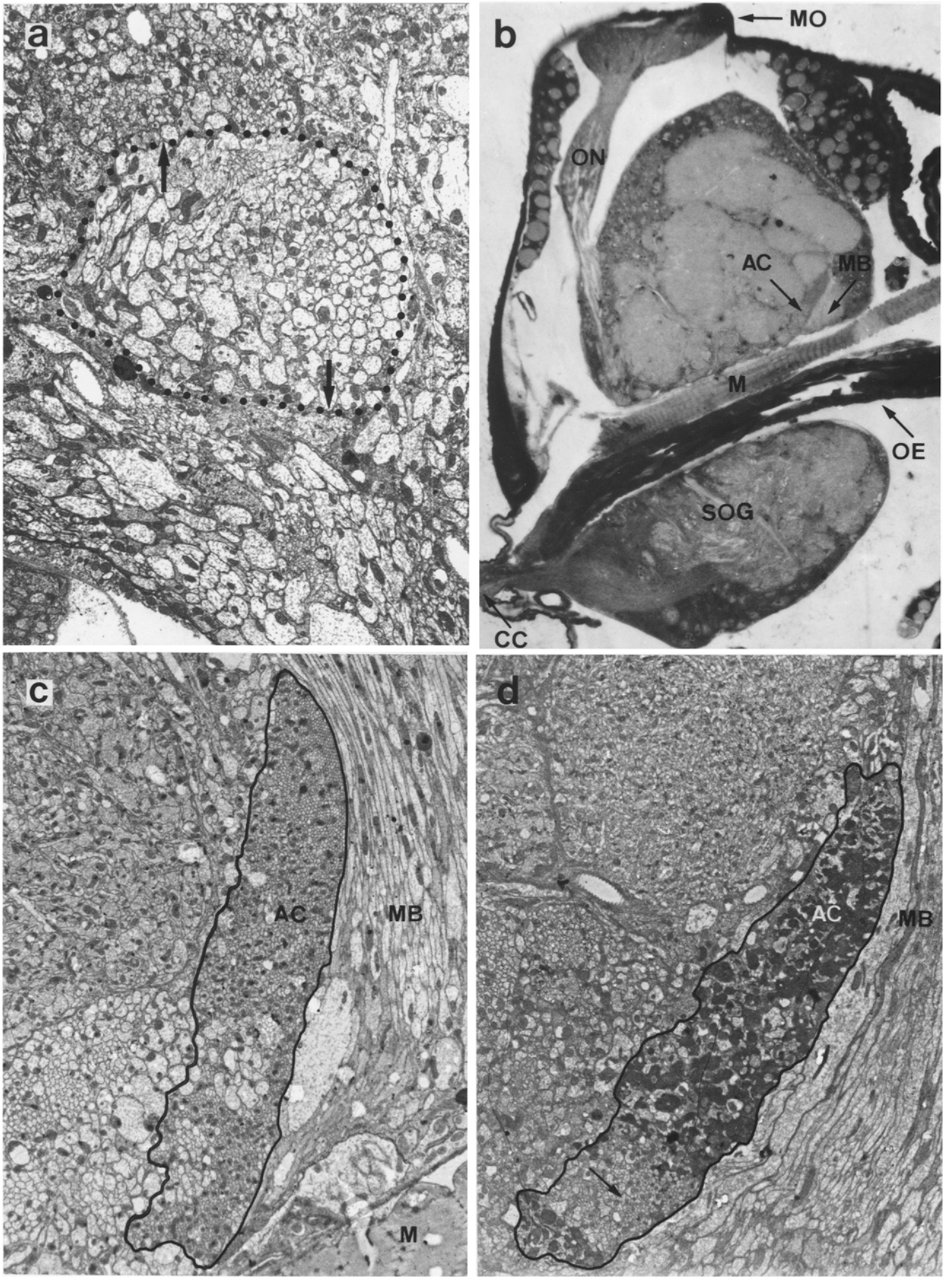
Fig. 5a–c. Parasagittal section through the brain (Holmes-Blest reduced silver impregnation). The AN carries small fibers from the smell receptors of the funiculus to the antennal lobe (*AL*), and thick mechanoreceptive axons from the Johnston's organ of the second antennal segment to the AMMC. ×300. **b** Transverse semithin section through the AL of a male fly with both funiculi removed 24 h before fixation. The degenerated antennal afferents are essentially localized in the periphery of the lobe and of individual glomeruli (*arrows*). No degeneration is visible in the AMMC. ×760. **c** More posterior section of same fly showing the degenerated AC and the cross-sectioned iACT, which is free of any degeneration. *Arrows* indicate fibers joining the iACT just behind the lobe. *M* muscle; *MB* median bundle. ×670

from mechanoreceptors in the second and first antennal segments, e.g., the Johnston's organ, and on the anterior head capsule. They bypass the lobe ventro-laterally and terminate in the lateral deutocerebrum (Fig. 5a; Power 1946; posterior antennal center: Stocker and Lawrence 1981; mechanosensory deutocerebrum: Strausfeld and Bacon 1983; antennal mechanosensory and motor center, AMMC: Rospars 1988; Homberg et al. 1988, 1989). In contrast to the lobe projections, these are purely ipsilateral. In *Calliphora,* specific afferents from the second segment extend into the suboesophageal ganglion (SOG) and even into thoracic ganglia (Nässel et al. 1984). The number of motor fibers in the AN (e.g., innervating antennal muscles; Miller 1950) is very small. In *Musca* their cell bodies and their dendritic arborizations are located in deeper levels of the AMMC (Strausfeld 1976).

The antennal commissure (AC) connects the two antennal lobes at their postero-dorsal corner (Figs. 4, 5c, 6b, c). In the 'Sevelen' strain, it consists of about 2500 fibers (female, adult 3 days: mean = 2528, $n = 2$, SE = 36). No significant sexual dimorphism or change in the axon number during the first 14 days of adult life have been observed (Stocker and Gendre 1988). Twelve to 24 h after amputation of a single funiculus, massive degeneration is present in the AC, although about 1500 fiber profiles survive (female 3 days: mean = 1539, $n = 4$, SE = 41). This suggests that almost 1000 of the estimated 1200 afferents of the funiculus have a process that extends via the AC into the contralateral lobe. If both funiculi are removed, degeneration in the AC is even more severe (Figs. 5c, 6d). Only a small number of axon profiles survive in the ventral part of the AC (Fig. 6d). Golgi impregnations suggest that most of these profiles represent contralateral projections of maxillary palp sensilla, whereas the minority may be processes of bilateral relay interneurons (see below).

In support of recent findings in bees (Mobbs 1982), moths (Homberg et al. 1988), and cockroaches (Kraus et al. 1988), our data demonstrate that three separate tracts are connecting the antennal lobe with higher protocerebral centers (Fig. 4). The major tract, called inner antenno-cerebral tract (iACT; synonyms: antenno-glomerular tract AGT, tractus olfactorio-globularis TOG), runs from the postero-dorsal end of the lobe straight up to the median dorso-posterior protocerebrum, then turns laterally towards the calyx of the mushroom bodies and extends further into the lateral protocerebrum (LPR; Figs. 4, 11e, 12a, b). We determined the number of axons running in the iACT by studying transverse brain sections, slightly tilted, so that they were perpen-

Fig. 6a–d. The structure of iACT and AC. **a** Electron micrograph of cross-sectioned iACT (dotted outline) at the level shown in Fig. 5c. Glial elements separate the iACT proper from other fibers adjoining it (*arrows*). ×4550. **b** Sagittal semithin section showing the location of the AC immediately behind the median bundle (*MB*) above the oesophagus (*OE*). ×275. **c, d** Sagittal TEM micrographs through AC (outlined) of intact (**c**) and operated male fly (**d**) with both funiculi removed 24 h before fixation. In antennectomized flies the vast majority of profiles has degenerated, demonstrating that they belong to bilateral receptor axons. Most of the persisting profiles (*arrow*) probably are maxillary palp afferents. **c, d** ×4000. *CC* cervical connective; *M* muscle; *MO* median ocellus; *ON* ocellar nerve; *SOG* suboesophageal ganglion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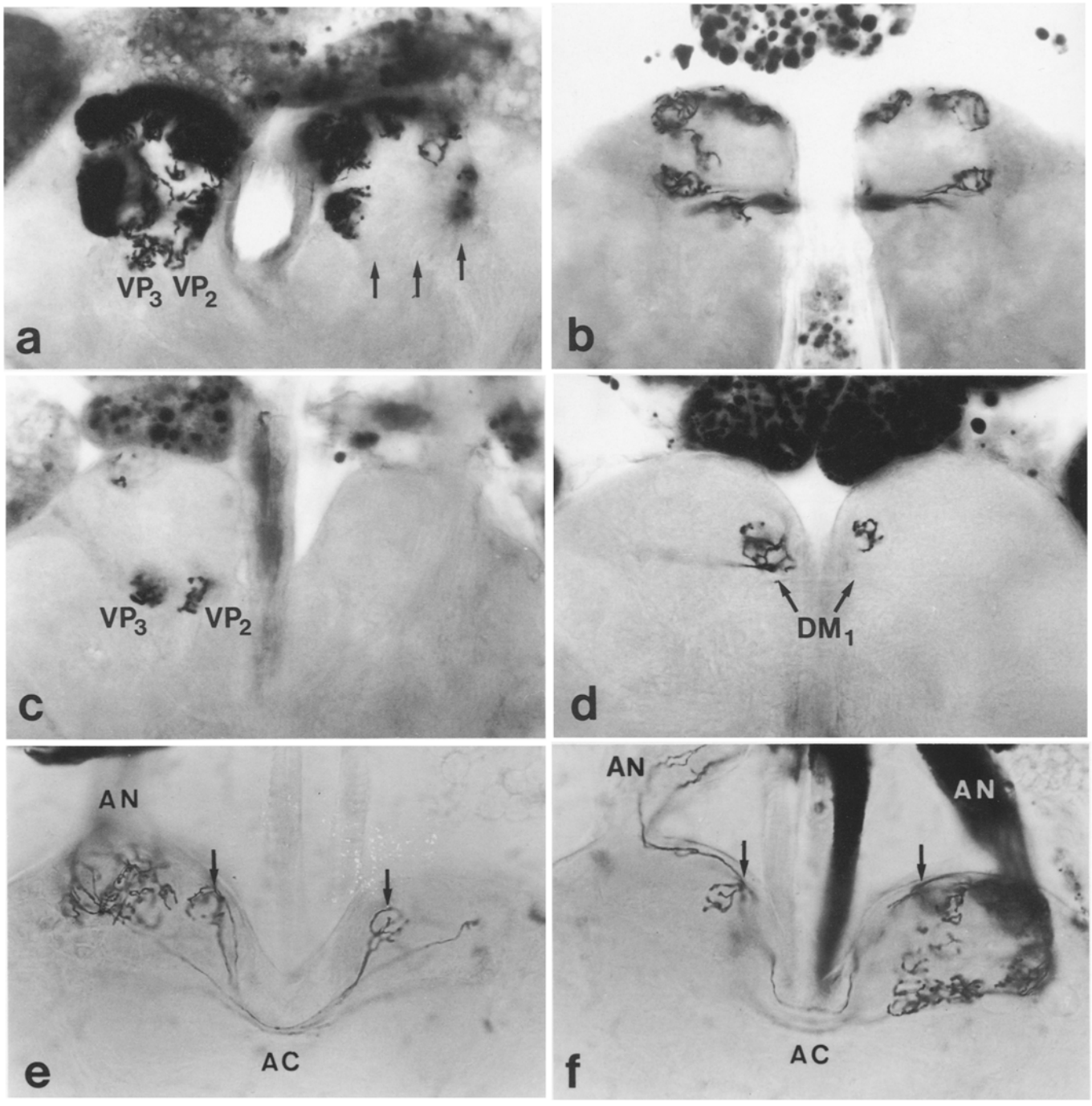


Fig. 7a–f. Golgi impregnations of antennal afferents in the antennal lobe (horizontal sections; anterior to top). **a** Massive impregnation of afferents from left antenna. In the left lobe, most of the glomeruli are heavily labelled (except VP_2 and VP_3, which are likely to receive only three afferent fibers each). In the right (contralateral) lobe, intense label in anterior and medial glomeruli demonstrates contralateral input. In contrast, the VP_{1-3}/VL_1-regions lack labelled terminals (*arrows*), indicating that they receive ipsilateral input only. **b** Dorsal section of same Golgi preparation. The symmetric projection pattern illustrates that dorsal glomeruli receive bilateral input. **c–f** 'Selective' Golgi impregnations reveal the projection pattern of individual funicular afferents. Each individual axon terminates only in one type of glomerulus. A few glomeruli get only ipsilateral input (**c**), but most of them receive afferents from both antennae (**d–f**). This leads to mirror-symmetric projection patterns (**e, f**, *arrows*). ×330

dicular to the iACT. Right behind the antennal lobe, the iACT is composed exclusively of fibers associated with the lobe (Technau 1984; Fig. 5c). At this level, about 200 medium to large axons can be counted on electron micrographs (Fig. 6a). After amputation of funiculi, no degeneration was observed in the iACT, indicating that funicular afferents do not extend into this tract. The iACT is probably the largest output tract of the antennal lobe, though it may also carry centrifugal fibers.

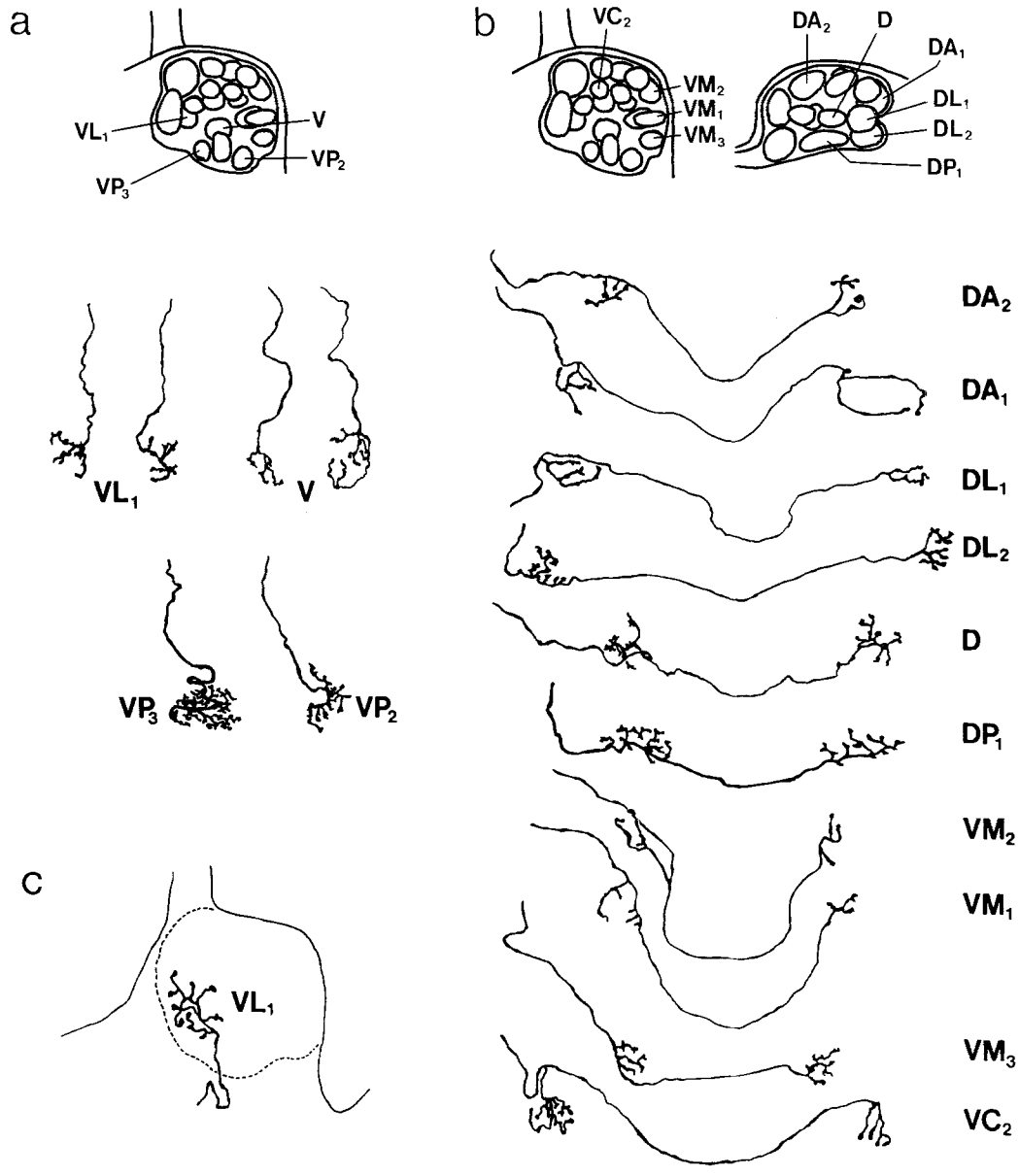


Fig. 8a–c. Camera lucida drawings of Golgi-impregnated receptor terminals from the antennal funiculus (**a, b**) and from a pharyngeal sensillum (**c**) (horizontal views). **a, c** Exclusive ipsilateral afferents. **b** Afferents with bilateral terminals in corresponding glomeruli. **c** The pharyngeal afferent enters the brain via the accessory pharyngeal nerve. Its terminals are in VL_1 (and probably adjacent glomeruli). The AL is shown by a d*ashed line*. ×430

The middle antenno-cerebral tract (mACT) branches off the iACT laterally some distance behind the antennal lobe and runs straight into the LPR (Figs. 4, 11f, 13b, d). Occasionally, fibers leave the mACT halfway and extend along the pedunculus of the mushroom bodies to the calyx (Fig. 13c). The outer antenno-cerebral tract (oACT) – revealed in a few Golgi preparations – emerges from the antennal lobe lateral to the iACT and extends into the LPR as well (Figs. 4, 12c).

The 'broad root' (Power 1946) leaves the lobe below the iACT as a rather ill-defined tract (Figs. 4, 14e, f). Fibers in the broad root pass into posterior brain areas, e.g., into the region of the great commissure. At the level of the iACT, just beside the oesophageal canal, there is another site of entrance/exit (Figs. 4, 14c, d, 15d).

Another important tract is the 'antenno-suboesophageal tract' (AST), which has also been reported from *Manduca* (Homberg et al. 1988). This tract connects the ventro-posterior corner of the lobe, slightly medial to the AMMC, to the anterior SOG (Fig. 4). The AST is used, for example, by afferents from maxillary palps (Fig. 9a), by processes of three different kinds of lobe interneurons (Figs. 14b, g, 16d, 17), and by afferents from homoeotically transformed antennae (Stocker and Lawrence 1981) or transformed labial palps (Stocker 1982).

Antennal afferents in the antennal lobe

The projections of afferents from the funiculus in the antennal lobes have been described on the basis of $CoCl_2$ backfills (Stocker et al. 1983). If suffices here to summarize the major features that become particularly obvious in Golgi impregnations. Funicular afferents can be

Table 1. Characterization of selected antennal glomeruli according to sensillar input and known relay interneurons

Glomerulus	Sensillar input	Relay interneurons				
		Monoglomerular uACTI	Oligoglomerular uACTI	bACTI (via AC)	bACTI (via SOG)	bVI
Type-a						
VP_2 [i]	aristal	+	+ (+VP_3)	+		
VP_3 [i]	aristal	+	+ (+VP_2)	+ [u]	+	
Type-b						
V [i]	basiconic	+				+
VM_2 [b]	basiconic	+	+ (+DM_2)			
D [b]	basiconic	+				
DL_1 [b]	basiconic	+				
DM_1 [b]	basiconic	+	+ (+VP_2/VP_3)			
VA_1 [b]	trichoid	+				
DA_1 [b]	trichoid	+				
VA_3 [b]	maxillary	+	+ (+DA_2/VA_2)			
VA_4 [b]	maxillary					
DM_2 [b]	maxillary	+	+ (+VM_2)			
Type-c						
VL_1 [i]	coeloconic/trichoid/pharyngeal	+				
VM_1 [b]	coeloconic/basiconic/[trichoid]	+				
DL_2 [b]	coeloconic/[basiconic]/[trichoid]	+				

Type-a and type-b glomeruli receive afferents from one sensillum type only, type-c glomeruli from more than one type (for a/b distinction, see text). Glomeruli with only ipsilateral or with bilateral afferent input are marked with [i] or [b], respectively. Minor antennal afferents are given in [ ]. In oligoglomerular uACTI, the shared glomeruli are shown in ( ). bACTI establish in certain glomeruli only unilateral arborizations [u]. Data about antennal afferents are from Stocker et al. (1983)

grouped into unilateral fibers that terminate in one of the five postro-lateral glomeruli V, VP_{1-3}, and VL_1 of the ipsilateral lobe (Figs. 7a, c, 8a), and bilateral fibers, which in addition to the ipsilateral branch send a collateral through the AC to the same glomerulus in the contralateral lobe (Figs. 7a, b, d–f, 8b). According to our data, bilateral fibers may terminate in any one of the remaining 30 glomeruli, i.e., VA_{1-7}, VC_{1-4}, VL_2, VM_{1-5}, D, DA_{1-4}, DC_1, DL_{1-3}, DM_{1-3}, and DP_1. The axons of both types extend along the periphery of the lobes until they are very close to the glomerulus in which they terminate; in massive impregnations this leads to an unlabelled center of the lobe (Fig. 7a, b). All the sensory fibers observed so far (uni- or bilateral) are essentially glomerulus specific, i.e., they terminate in a single type of glomerulus (Figs. 7c–f, 8a, b). However, terminal branches occasionally seem to pass beyond the glomerular border as defined by Lucifer Yellow fills, suggesting that adjacent glomeruli might often receive input as well. In most of the glomeruli, axon terminals have only 2–4 terminal branches (Fig. 8a, b). Yet, glomeruli VP_2 and VP_3 are characterized by multiarbor terminals that fill the entire glomerular volume (Figs. 7c, 8a). Bilateral fibers do not manifest conspicuous differences between ipsi- and contralateral arborizations (Fig. 8b).

Backfilling from specific areas of the funiculus and the analysis of an antennal sensillum mutant have elucidated some of the rules underlying funicular projections (Stocker et al. 1983; Stocker and Gendre 1988; Table 1). According to these data, glomeruli VP_2 and VP_3 appear to be unique targets of the ipsilateral aristal sensillum. Massive Golgi impregnations and $CoCl_2$ backfills demonstrate that VP_2 and VP_3 receive only few axons (Fig. 7a). This is in agreement with the small number of neurons present in the aristal sense organ (Foelix et al. 1989) and could explain why these glomeruli were not shown by the relatively faint Lucifer Yellow marker (see above). Glomerulus V is likely to be a specific target of basiconic sensilla from the ipsilateral antenna (Stocker and Gendre 1988). Other major targets of basiconic sensilla are VM_1 and DM_1, while minor targets are VM_2, D, and DL_1. Coeloconic sensilla, on the other hand, seem to project into VL_1, VM_1, and DL_2, whereas receptors from trichoid sensilla terminate predominantly in VL_1, VA_1, and DA_1 (Stocker et al. 1983).

The aforementioned descriptions are based on more than 400 antennal backfills and 70 selected Golgi preparations of male 'Sevelen' flies. In addition, we analyzed in detail 20 of 300 Golgi impregnations of male and female 'Berlin' and 'Kapelle' flies. Neither sexual dimorphism nor strain difference were observed.

Maxillary afferents in the antennal lobe

Afferents from maxillary palps were studied after removal of the palps both by the Golgi technique (eight preparations) and by orthograde filling with HRP (ten preparations). Although in the HRP preparations the axons

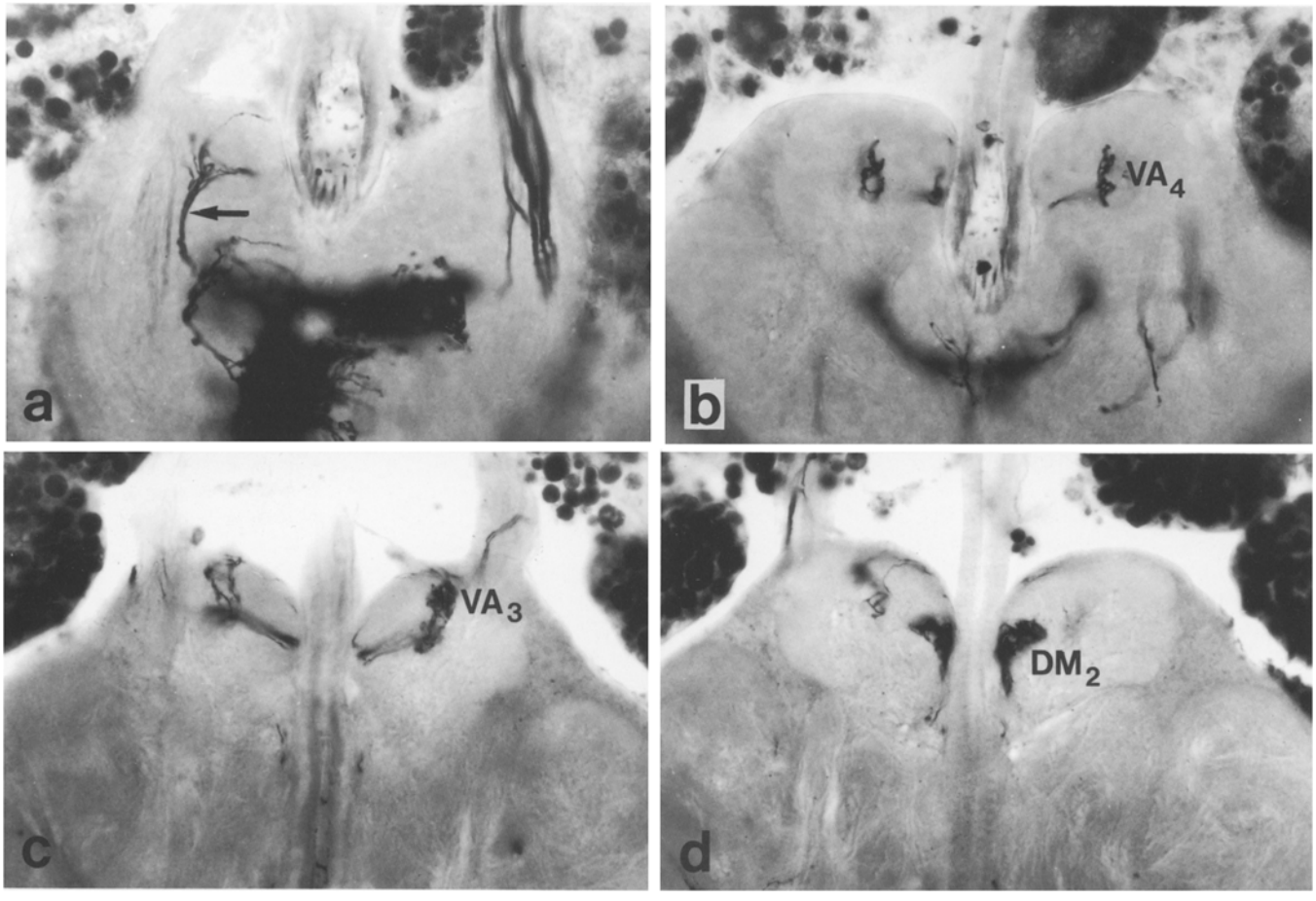


Fig. 9a–d. Projection patterns of maxillary palp sensilla in the antennal lobe (Golgi; horizontal sections). Maxillary afferents enter the lobe through the AST (**a** *arrow*). They exhibit bilateral arborizations in both glomeruli VA_4 (**b**), and in the glomeruli VA_3 (**c**, **d**) and DM_2 (**d**). Contralateral branches extend through the AC. In (**a**) and (**b**) non-maxillary elements are also impregnated. × 330

and their terminals had a dotted rather than continuous appearance, these precipitations occurred at the same sites as the uninterrupted projections seen in Golgi preparations. Hence, the HRP fills are a good argument in favor of the maxillary origin of the afferents described hereafter. Moreover, after filling from maxillary palps with $CoCl_2$, similar projection patterns have been obtained (Singh and Nayak 1985).

Maxillary palp afferents enter the suboesophageal ganglion (SOG) through the labial nerve. One group of fibers that we believe to be associated with maxillary bristles (see Discussion) terminates in the SOG; most of its arborizations remain ipsilateral, but a few are contralateral (Singh and Nayak 1985). A second group of axons, probably coming from basiconic sensilla, passes straight through the SOG to reach the ipsilateral antennal lobe via the AST, just beside the AMMC (Fig. 9a). The pattern of arborizations in the antennal lobe, which is almost identical in all of our preparations, is tripartite and mirror symmetric in both lobes (Table 1). A group of terminals is found in the VA_4 glomerulus (Fig. 9b) in an area that is devoid of antennal terminals. The other two regions are the glomeruli VA_3 (Fig. 9c) and DM_2 (Fig. 9d). Collaterals extend through the AC and terminate in the same glomeruli of the contralateral lobe. On their way to the AC, processes that enter VA_3 pass along the anterior edge of the lobe, whereas the remaining axons take a straight course through the lobe. As in antennal afferents (Stocker et al. 1983), every axon appears to terminate in the identical target glomerulus in each antennal lobe. Remarkably, these maxillary target glomeruli appear to be different from targets of any kind of antennal afferents, e.g., antennal basiconic sensilla.

Pharyngeal afferents in the antennal lobe

In one Golgi preparation a unilateral afferent projection into glomerulus VL_1 (and adjacent glomerular areas) was observed (Fig. 8c; Table 1). It was formed by an axon that entered the brain through the accessory pharyngeal nerve. This nerve has been shown to carry afferents from pharyngeal sensilla (Stocker and Schorderet 1981).

Interneurons of the antennal lobe

The cell bodies of antennal lobe interneurons are arranged essentially in three groups. Neuronal processes

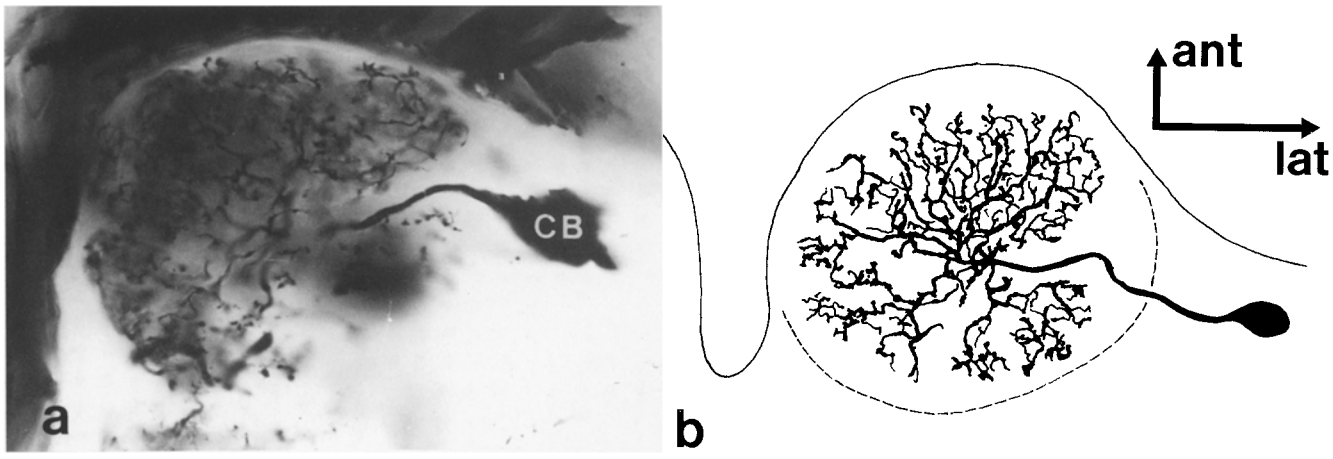


Fig. 10a, b. Local interneurons of the antennal lobe (LI). **a** Micrograph (CB cell body), **b** camera lucida drawing. both; ×600

of the most conspicuous dorso-lateral group enter the lobe in a common bundle, which creates a distinct dorso-lateral indentation in horizontal sections (Fig. 12d–i). A second cluster of cell bodies is located ventro-laterally, and a third group is scattered as a thin layer over the whole anterior surface of the lobe. The processes of the latter two groups extend into the lobe via individual pathways. No attempts have been made to estimate the numbers of these interneurons, mainly because not all of them may in fact be associated with the antennal lobe.

Local interneurons (LI). Local interneurons lack an axon and are intrinsic to one antennal lobe (Fig. 10a, b). In all the LI studied so far, a thick process extends from the laterally situated cell body into the center of the lobe. It exhibits extensive arborizations that ramify throughout the lobe and terminate in many, if not all, of the glomeruli. LI were found in 15 Golgi preparations of the 'Berlin' and in 1 of the 'Sevelen' strain.

Unilateral ACT relay interneurons (uACTI). Interneurons of this type connect the antennal lobe with the ipsilateral calyx or LPR, by an axonal process that extends through the iACT, mACT, or oACT. Most of the processes follow the iACT (Fig. 11e, 12a, b). They send one (or sometimes two or three) club-shaped side branches into the calyx of the mushroom body. The main branch continues in lateral direction and terminates in the LPR with profuse ramifications. Other uACTI fibers turn from the iACT into the mACT. They bypass the calyx and extend directly into the LPR (Figs. 11f, 13a, b, d–f). uACTI that use the oACT and terminate exclusively in the LPR have been observed only rarely (Fig. 12c). Unlike *Manduca* (Homberg et al. 1988, 1989) the various types of uACTI do not exhibit obviously distinct patterns of arborization in particular subregions of the LPR or the calyx.

Monoglomerular uACTI establish dense arborizations within a single glomerulus (Figs. 11, 12). Our data suggest that for most (if not all) of the known glomeruli, monoglomerular uACTI may exist (Table 1). Whereas, in general, only one such neuron was impregnated per glomerulus, a few multiple labellings in the same preparation suggest that glomeruli can possess more than one of these neurons. Their cell bodies are situated either dorso-laterally or in front of the lobe, although there is no close correlation between the cell body location and the site of the glomerular arborization (Fig. 12e, h). In the large majority of observations, monoglomerular uACTI send their process into both calyx and LPR through the iACT. Exceptionally, projections through the mACT or the oACT into the LPR have been observed; these neurons arborize mainly in glomeruli that receive only ipsilateral afferents, e.g., VL_1 or VP_3. In several insect species, monoglomerular uACTI have been shown to be output interneurons of the antennal lobe; glomerular arborizations appear thus to represent dendritic branching. We observed 92 interneurons of this kind in the 'Sevelen' strain, and 27 in 'Kapelle'.

Oligoglomerular uACTI arborize in a few antennal glomeruli (Fig. 13a–c; Table 1). Their branching pattern is less extensive than in the former type. Whenever cell bodies were visible, they were located in the ventro-lateral (or anterior) clusters (Fig. 13a). Virtually every neuron of this type observed projected into the target region via the mACT. In most cases, the target was the LPR (Fig. 13b), but in one case it was the calyx rather than the LPR (Fig. 13c). So far interneurons branching in the DA_2/VA_{2-3} region (Fig. 13a), in the VP_{1-3} region (Fig. 13b), in the VP_{1-3} and DM_1 region (Fig. 13c), and in the DM_2/VM_2 region have been found.

Polyglomerular uACTI arborize in a larger portion of the antennal lobe (Figs. 13d–f, 15a). Their sole target appears to be the LPR, which they reach via the mACT. The location of the soma could not be detected in our preparations. In some cases we observed branching in the lateral half (Fig. 13d), in the antero-lateral area (Fig. 13e), in the postero-medial area (Fig. 13f), in the whole dorsal half of the lobe plus in the VM_2/VA_2-region (Fig. 15a), or in certain posterior and anterior glomeruli (VP_3, DA_{1-3}; not shown).

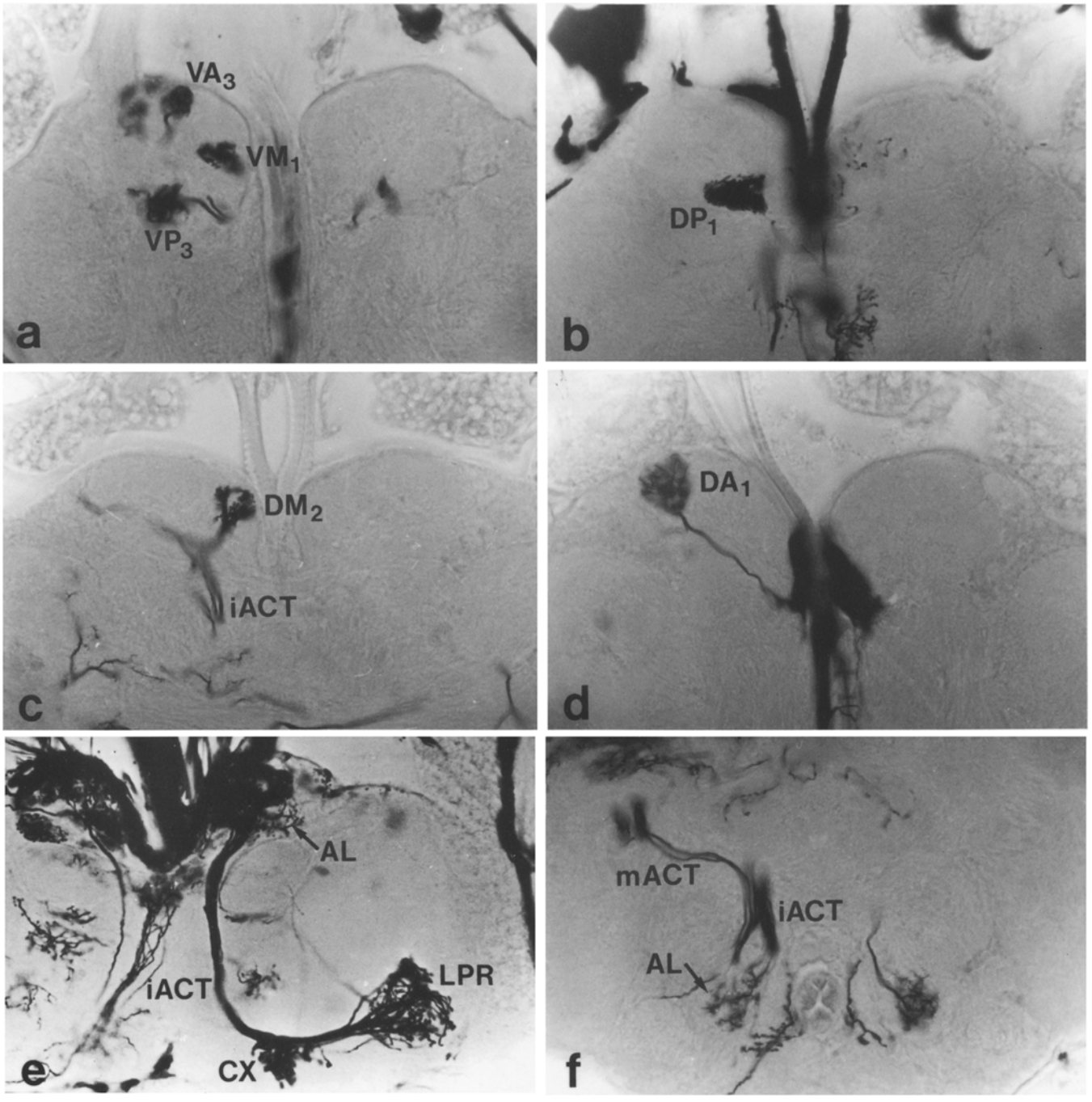


Fig. 11a–f. Golgi impregnations of unilateral, monoglomerular ACT interneurons (uACTI). They exhibit extensive arborizations in a single glomerulus. **a** Simultaneous labelling of three uACTI. **b–d** One neuron labelled in each. uACTI send a thick axon via the iACT into the calyx (*CX*) and the lateral protocerebrum (LPR) (**e**) or via the mACT directly into the LPR (**f**). **a–d** horizontal sections; **e** oblique section; **f** transverse section. ×330

Bilateral ACT relay interneurons (bACTI). Bilateral ACT interneurons connect one or a few particular glomeruli of *both* antennal lobes via the iACT to the calyx and the LPR.

Two bACTI were observed in which the contralateral antennal lobe is reached by a collateral through the AC (Fig. 14a). A relay process is established only in one of the two iACT. The location of the soma is not known. Neurons of this type arborize ipsilaterally in VP_2 and VP_3 and extend into the contralateral VP_2 (Table 1). Serotonin immunoreactivity is present in bACTI of somewhat similar shape (K. Störtkuhl, personal communication). Somata of this neuron type lie in the lateral group; they have a process in the ipsilateral iACT and a second one that extends via the AC into the contralateral iACT.

Other bACTI send a process into the contralateral lobe via the SOG (Fig. 14b). This process leaves the

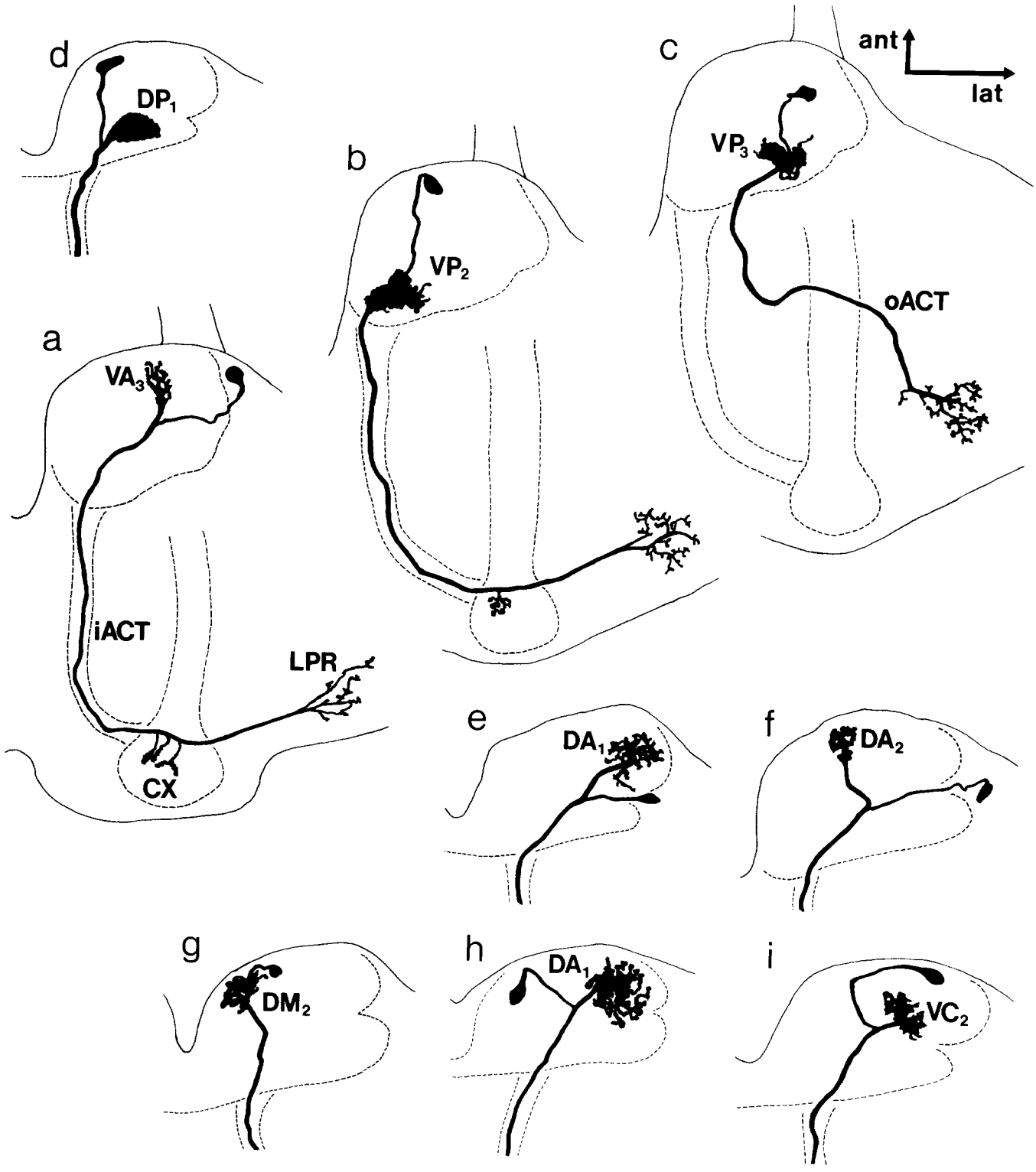


Fig. 12a–i. Monoglomerular uACTI with an output process in the iACT (**a, b**) and both calyx (CX) and LPR as targets, or directly extending via the oACT into the LPR (**c**). **a–c** Neurons arborizing in ventral glomeruli. **d–h** Neurons arborizing in dorsal glomeruli. **i** Arborization in central glomerulus. The site of the cell body is not related to the glomerular location (cf. **e** and **h**). ×375

lobe through the AST. The cell body is located in the lateral cortex of the SOG, slightly behind the AMMC. In contrast to the former type, processes are found in both iACT. The three neurons analyzed arborize bilaterally in the VP_3 region (Table 1).

Fine arborization relay interneurons (FAI). Interneurons of this type exhibit arborizations within a large portion of the antennal lobe (Figs. 14c, d, 15b–d). Most of these neurons cover more than half of the lobe, though they do not always invade the same glomeruli. A region that

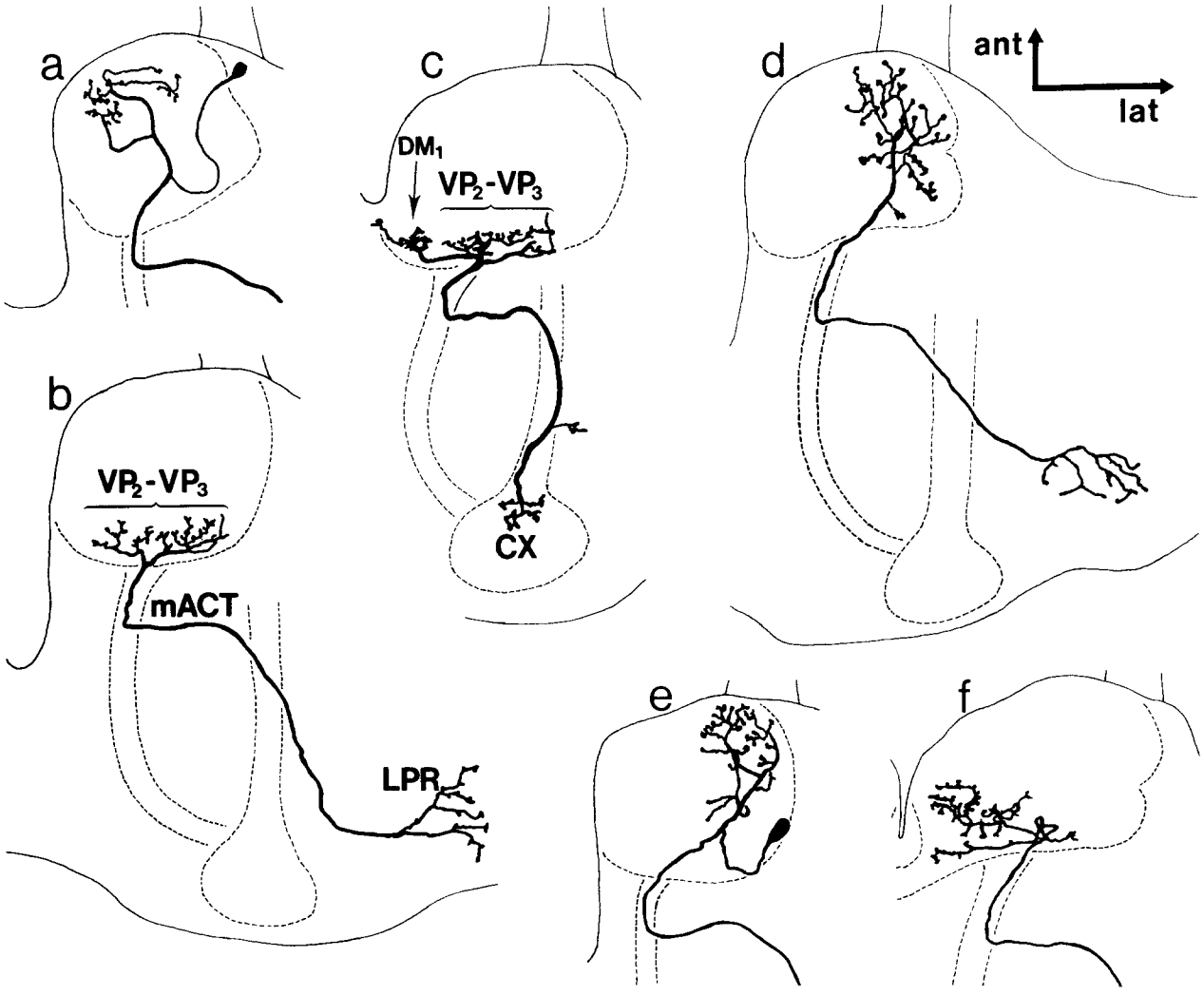
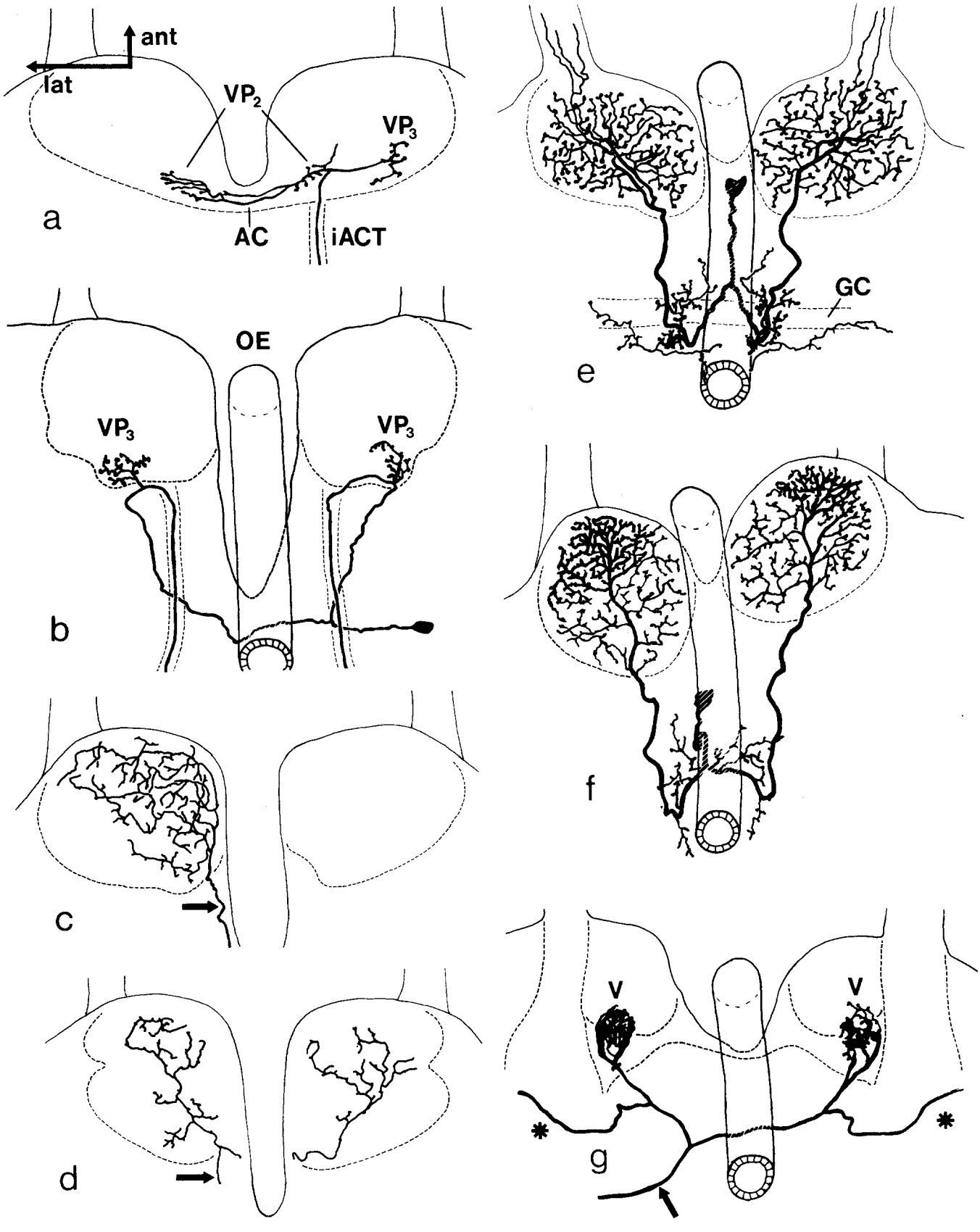


Fig. 13a–f. Oligo- and polyglomerular uACTI. **a–c** Oligoglomerular uACTI are restricted to a small number of glomeruli (e.g., **a** DA_2/VA_{2-3}). **d–f** Polyglomerular uACTI arborize in a larger number of glomeruli. Most of the processes extend via the mACT directly into the LPR (**b, d**) or into the calyx (**c**). The location of the cell body is often unknown. ×375

is spared in most cases is the glomerulus VA_1. Typically, all of the FAI processes are of small diameter, which makes the distinction between stem processes and terminal arborizations difficult. One or several fibers leave the lobe in posterior direction just adjacent to the oesophagus, at some distance from the iACT (Fig. 4, 14c, 15d). Due to staining of many other neurons, the projections of these fibers could not be elucidated. Likewise, the position of their cell body remains unknown. Eight of the FAI observed are unilateral (Figs. 14c, 15b). However, mirror symmetric patterns in both antennal lobes (two preparations; Figs. 14d, 15c), suggest that some of the FAI might be bilateral, although the connections between the two lobes remain obscure.

Giant symmetric relay interneurons (GSI). Giant symmetric relay interneurons, observed in two preparations, are very conspicuous because of their large size and their extensive symmetric arborization in both antennal lobes (Figs. 14e, f, 16a–c). The cell body of these neurons is located in the midline of the SOG, i.e., in its anteroventral cortex. A stout process extends dorsally from the cell body through the SOG neuropil and splits below the oesophagus into a bilateral pair of similarly large processes. Close to the branching point, the two processes give rise to a mirror-symmetric pattern of arborizations on both sides of the oesophagus. Some branches even extend into posterior brain regions behind the great commissure (Figs. 14e, 16a). The two main processes turn abruptly into the anterior direction to reach the antennal lobes through the broad root (Power 1946), just ventral to the iACT (Figs. 14e, f, 16b, c). They proceed into the center of the lobe and send off a large number of secondary and tertiary processes that extend into the periphery of the lobe. Virtually all of the glomeruli appear to be reached by these branches; in addition, terminal branches seem to extend some distance into the antennal nerve (Fig. 14e). With respect to the branching pattern, two subtypes of GSIs have been found. In the first one, branches are uniformly thin (Figs. 14e, 16a–c), whereas in the second one thick and thin branches are formed: the thick branches occupy


ant
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VP2
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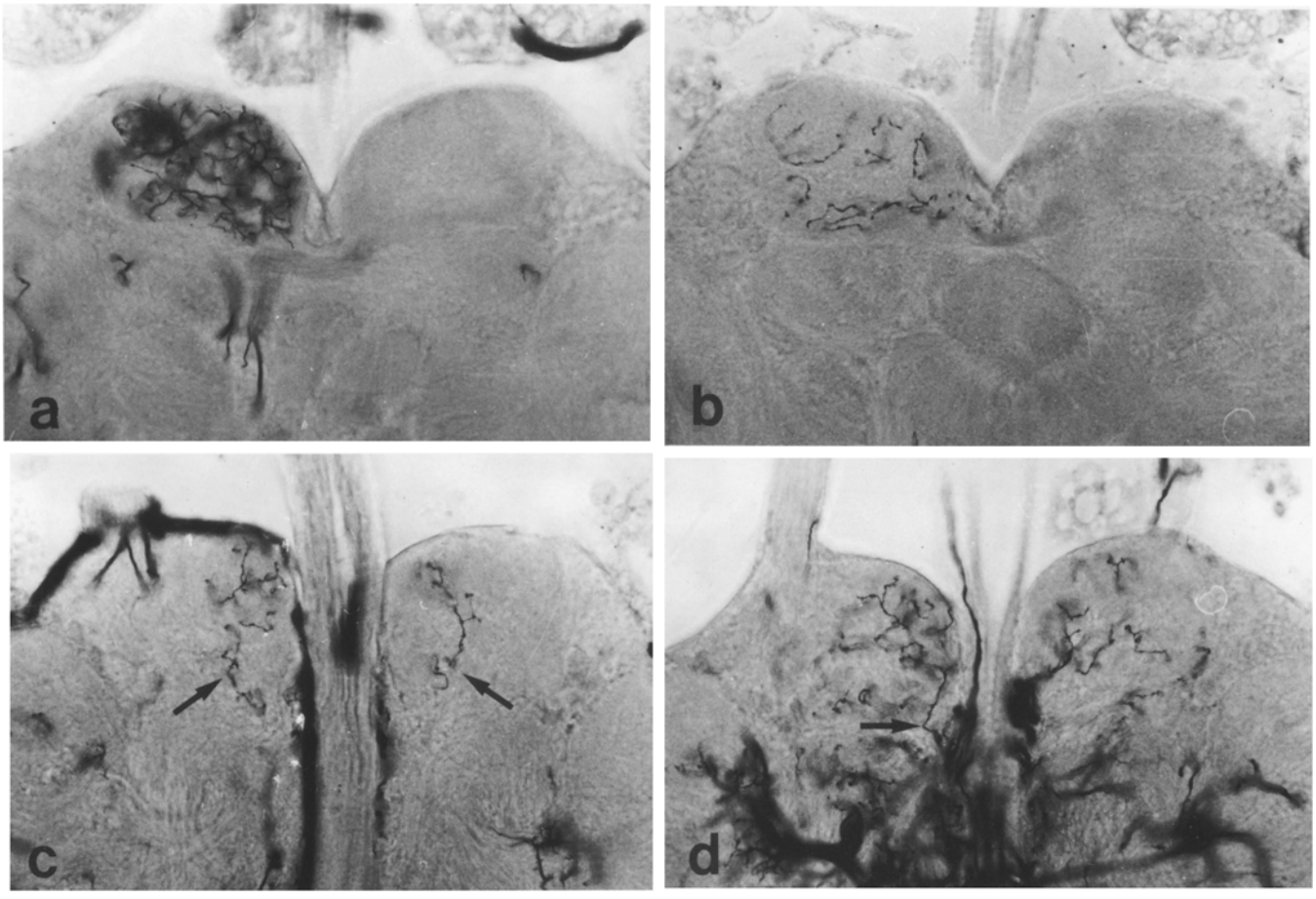

Fig. 15a–d. **a** Polyglomerular uACTI with extensive branching in the dorsal half of the left lobe. **b–d** Fine arborization relay interneurons (FAI) with unilateral **b** or mirror-symmetric bilateral pattern (**c** *arrows*). Processes leave the lobe lateral to the oesophagus (**d** *arrow*). ×330

Fig. 14a–g. Other types of antennal lobe interneurons. **a, b** Bilateral ACT relay interneurons (bACTI) arborize in one or a few glomeruli of both lobes and have a process in one (**a**) or both (**b**) iACT. Collaterals may reach the contralateral lobe via the AC (**a**) or the SOG (**b**). **c, d** Fine arborization relay interneurons (FAI) are characterized by small-diameter arborizations in a considerable portion of the lobe and have a process that leaves the lobe alongside the oesophagus (*arrows*). The pattern may be restricted to one lobe (**c**) or mirror-symmetric in both lobes (**d**). Neither the location of their cell bodies nor their target regions are known. **e, f** Giant symmetric relay interneurons (GSI) have their cell body in the ventral midline of the SOG. Its symmetric processes emanate from the bifurcation of the cell body fiber immediately below the oesophagus. They arborize in the posterior brain and extend into both antennal lobes, which they completely invade with extensive arborizations. One GSI subtype possesses only thin branches (**e**). In a second subtype thicker branches occupy the antero-lateral half of the lobe, whereas fine branches cover the rest (**f**). **g** Bilateral V-relay interneuron (bVI) with massive arborizations in both glomeruli V. Thick processes extend into the lateral brain (*asterisks*) and probably towards the cell body (*arrow*). *GC* great commissure; *OE* oesophagus. ×375

approximately the antero-lateral half of the lobe, whereas the fine branches cover the rest (Fig. 14f). Moreover, in the second type, the additional branching region in the posterior brain apparently does not reach the region of the great commissure.

Bilateral V relay interneuron (bVI). An asymmetric relay interneuron has been found that branches profusely in both left and right glomeruli V (Figs. 14g, 16d; Table 1). They are connected to each other via the SOG by a stout process. Slightly lateral to the midline, this process sends off a single asymmetric branch that extends into the lateral SOG, most likely towards the cell body. Furthermore, some distance behind each antennal lobe, two mirror-symmetric side branches arise from the stout process that connects the two lobes. These side branches extend straight into the ventro-lateral deutocerebrum; their final destination is not clear.

Thoracic relay interneuron (TI). A thoracic relay interneuron has been found that arborizes profusely in a

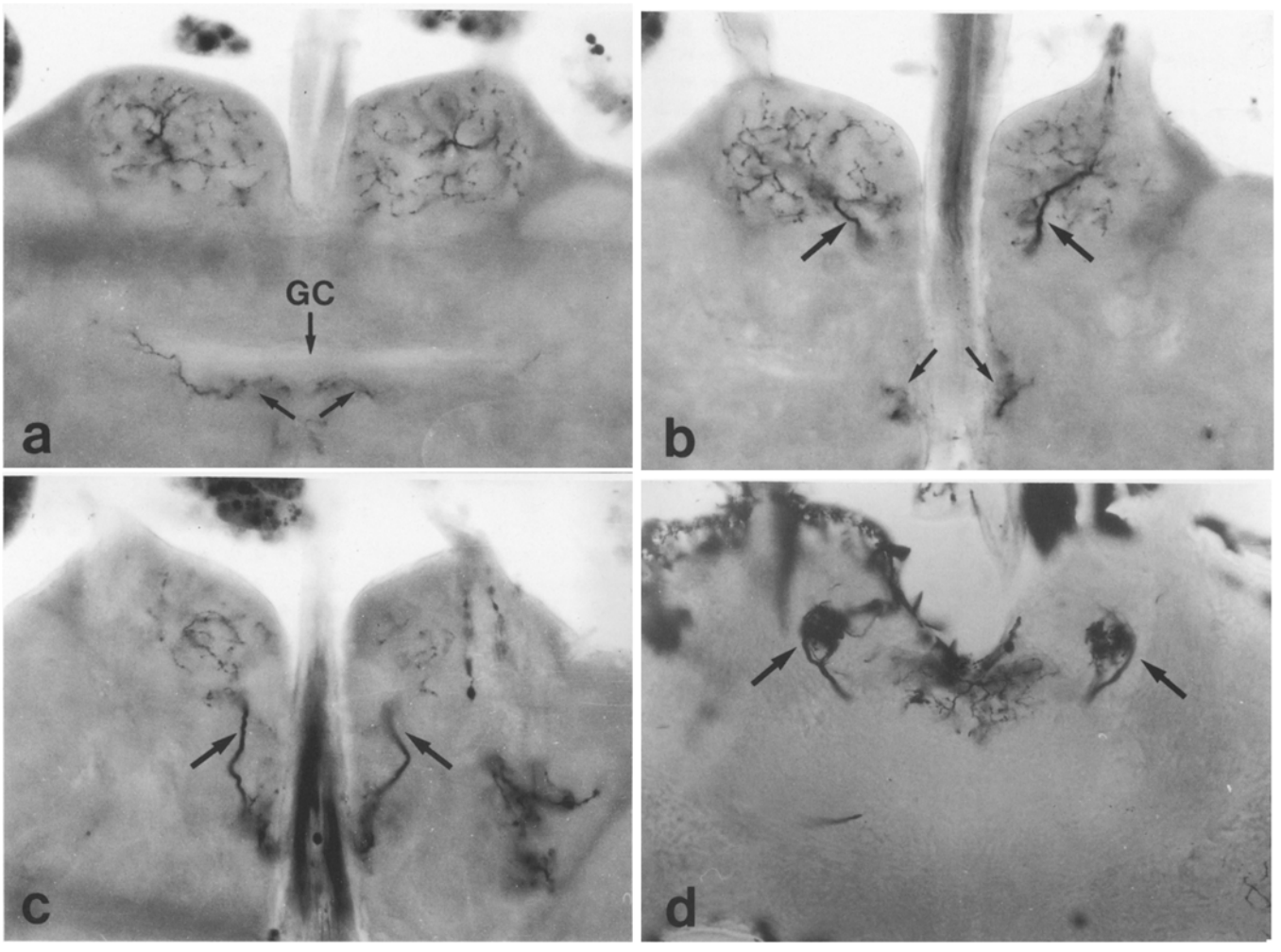

Fig. 16a–c. Dorsal (**a**), middle (**b**), and ventral (**c**) aspect of giant symmetric relay interneuron (GSI). Extensive symmetric arborizations are formed by the two main branches (*large arrows*) in both lobes and in the posterior brain (*small arrows*). GC great commissure. **d** Tight arborization of bilateral V-relay interneuron (bVI) in both glomeruli V (*arrows*). ×330

number of antennal glomeruli, i.e., in VA_3, DL_1/DA_3, and in the VP_{1-3} region (Fig. 17). These arborizations originate from a stout process that leaves the antennal lobe through the AST. Shortly behind the lobe, the process sends off a very long fiber towards the cell body, which is located in the antero-dorsal cortex of the lobe, near the midline. The main process divides once more. One branch extends into the posterior brain and establishes terminal arborizations just behind the great commissure; the other branch projects through the SOG and neck connective into the thoracic ganglion. Its thoracic targets are not known.

Discussion

The glomerular architecture of the antennal lobe

A three-dimensional (3D), computer-assisted mathematical reconstruction program has been used to identify glomeruli in the antennal lobe of a cockroach (Rospars and Chambille 1981), of a moth and of a butterfly (Rospars 1983). This method relied on a coordinate system, which was used as a reference for calculating relative positions of glomeruli in different individuals. Our technique is based on a computer drawing program in which serial sections are superimposed graphically. Whereas the mathematical method may be more accurate, the graphic method does not require the establishement of a sophisticated calculation program.

Our diagram of the antennal lobe reveals a total of 35 glomeruli (Fig. 3), 16 more than described previously (Stocker et al. 1983; VA_{4-7}, VC_{1-4}, VM_{3-5}, DA_4, DC_1, DL_3, DM_3, DP_1). Fifteen glomeruli are very prominent (V, VA_{1-2}, VL_{1-2}, VM_1, VM_4, D, DA_{1-2}, DL_{1-2}, DM_{1-2}, and DP_1) and can be used as landmarks for recognizing the smaller glomeruli. Some of the remaining glomeruli are clearly identifiable if neural elements are visible (e.g., VP_2, VP_3 or VM_3), but many of the smaller ones are identifiable only by comparing relative positions with the landmark glomeruli. Location, shape, and size of landmark glomeruli and of many

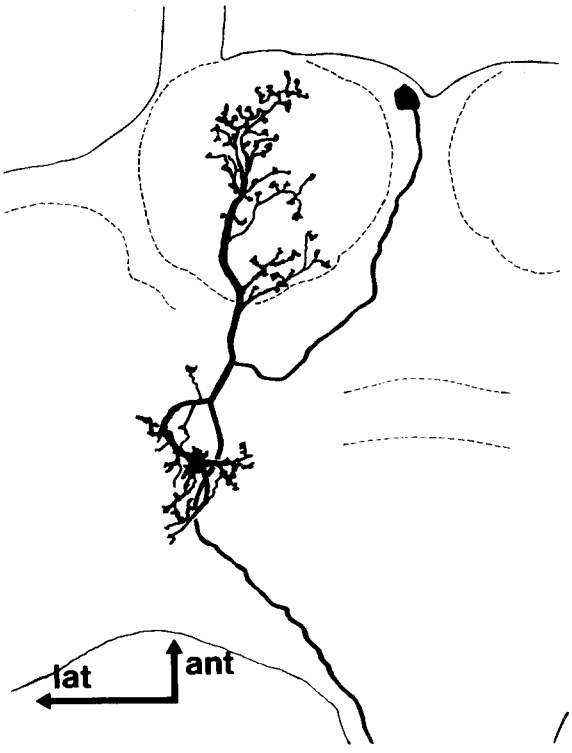

Fig. 17. A thoracic relay interneuron (TI) with extensive arborizations in the ventral half of the antennal lobe and the postero-lateral brain, as well as a process leading into the thoracic ganglion. The cell body is in the antero-dorsal cortex of the lobe. × 375

smaller glomeruli are invariant between different preparations. However, as the outlines of smaller glomeruli are not always evident, our model may still be incomplete. For example, in one preparation, we observed a small additional glomerulus between the triad DA_1/DA_3/DL_3, and in another preparation we found two glomeruli at the site of VM_4. Considering the relatively small number of specimens analyzed, we cannot distinguish whether such deviations are caused by interindividual variability or by poor resolution of some Lucifer Yellow backfills. Nevertheless, our data are consistent with a highly invariant glomerular organization of the insect antennal system (Rospars 1988; Rospars and Chambille 1989).

Three different lines of evidence suggest that certain glomeruli in *D. melanogaster* are located in the center of the antennal lobe. (1) Such glomeruli are clearly visible in Lucifer Yellow backfills; (2) antennal afferents and (3) uniglomerular iACTI were observed to establish distinct glomerulus-sized arbors inside the lobe. The presence of central glomeruli has been reported for a number of other insects, such as *Blaberus* (Chambille and Rospars 1981), *Mamestra* and *Pieris* (Rospars 1983). Due to the lack of functional studies, it remains unknown whether peripheral and central glomeruli differ from one another. Yet, their connectivity pattern does not manifest obvious differences.

The antennal lobe of Drosophila lacks an obvious sexual dimorphism

In moths or cockroaches, which attract their mate by long-range sex pheromones, sexual dimorphism of the antennal lobe has been described (for reviews, see Christensen and Hildebrand 1987; Rospars 1988; Rospars and Chambille 1989). One or several prominent glomeruli in the lobe of the male, termed macroglomerulus (or macroglomerular complex), seem to be specific sites of pheromone information processing. This dimorphism is always accompanied by a highly dimorphic pattern of antennal sensilla. In *Drosophila,* the male funiculus bears roughly 25% more trichoid and about 10% fewer basiconic sensilla than the female funiculus (Stocker and Gendre 1988). However, neither conventional staining of the antennal lobe (Pinto et al. 1988) nor Lucifer Yellow backfilling (this study) revealed an obvious sexual dimorphism in the antennal lobe. This is not surprising since in species like *Drosophila,* which possess less than 1500 olfactory sensilla, the dimorphism in sensillum number appears to be unrelated to the use of attractant sex pheromones (Chapman 1982). Studies of mutants have suggested that olfactory stimuli are less important in the courtship of *Drosophila* than visual or gustatory cues, at least in the sense that attractant pheromones are not effective over distances of more than 5 to 10 mm (reviewed by Tompkins 1984). In fact, several articles address the question of dimorphism in visual or gustatory systems. Whereas no obvious sexual dimorphism in the optic lobe of *Drosophila* was detected in a recent study (Fischbach and Dittrich 1989), sexually dimorphic visual neurons have been reported from *Calliphora* (Strausfeld 1980a) and *Musca* (Hardie 1983). In *Drosophila,* both the numbers of contact chemoreceptors on the foreleg (Nayak and Singh 1983; Possidente and Murphey 1989) as well as their central projections are sexually dimorphic (Possidente and Murphey 1989). Coating the male foreleg with dental wax results in considerably reduced courtship activity (Venard et al. 1989). Inhibitory compounds, on the other hand, may be mainly airborne. Evidence from maxillary-palp-deprived males that show higher than normal courtship rates towards mated females suggest that maxillary sensilla could be receptors for volatile antiaphrodisiacs (Stocker and Gendre 1989). Although this is not accompanied by obviously dimorphic numbers of maxillary sensilla (Singh and Nayak 1985), their central projection pattern should be examined for a possible dimorphism.

The neural elements of the antennal system

Several of the neuron types described here for *Drosophila* are known from the antennal system of other insects (for recent reviews, see Christensen and Hildebrand 1987; Rospars 1988; Homberg et al. 1989; Boeckh et al. 1990). For example, individual afferents from distal antennal segments have been shown to terminate in single glomeruli (Fig. 8a, b). This is also found in house flies

(Strausfeld 1976), moths (Matsumoto and Hildebrand 1981; Koontz and Schneider 1987; Homberg et al. 1988), honeybees (Mobbs 1982), and cockroaches (Schaller-Selzer 1984). Likewise, these studies indicate the presence of anaxonic local interneurons with processes throughout the lobe (Fig. 10), and interneurons that project from single glomeruli via the iACT to the calyx and the LPR (Figs. 11, 12). In addition to these frequent and widespread classes of neurons, we have found new types of antennal lobe neurons (Figs. 14, 17), most of which appear to be rare as judged by their low frequency of Golgi impregnation. Some of them are similar to known interneurons, but others have a completely different morphology. Whether these new neurons are peculiar to *Drosophila* or whether they are common elements of insect antennal systems will have to be shown by future studies.

Both Golgi and backfilling techniques are selective in a certain sense; the chances of successful impregnation or labelling are not the same for each type of neuron. Moreover, we have evidence that the Golgi technique often does not reveal neurons in their entirety. For example, the impregnation frequently stops in the cell body fiber. Therefore, our description should not be considered as a final list of the neural elements of the *Drosophila* antennal system. In fact, some of our preparations provide evidence of yet other types of interneurons (data not shown). Further progress in this field will probably rely on the application of antibodies against neurotransmitters (Nässel 1987; Rane et al. 1987) and other cell-specific antigens (Buchner et al. 1988), or the use of *Drosophila* strains expressing lacZ in selective classes of nerve cells (Ghysen and O'Kane 1989).

Antennal, maxillary, and pharyngeal afferents in the antennal lobe

A characteristic of the dipteran antennal system is the contralateral projection of most sensory fibers from the funiculus, as shown by Wallerian degeneration in *Calliphora* (Boeckh et al. 1970), by Golgi impregnation in *Musca* (Strausfeld 1976), and by cobalt backfilling in *Drosophila* (Stocker et al. 1983). Our antennectomy experiments reveal the fibers in the AC that are derived from primary sensory neurons in the funiculus. Removal of a single funiculus leads to a drop from about 2500 to 1500 fibers in the AC (Fig. 6c, d). Hence, roughly 1000 of the 1200 funicular afferents projecting into the lobe, i.e., 5/6, possess a contralateral axon. In our Golgi preparations, all of the newly detected glomeruli appear to receive bilateral input. Thus, only 5 of the 35 glomeruli – all of them clustered around the entrance of the antennal afferents into the lobe – are exclusive unilateral targets (Fig. 3). On average then, both bilaterally and unilaterally innervated glomeruli (with the exception of VP_2 and VP_3, see below) would receive 65–70 antennal fibers each. This is far below the average number of 10^3 to 10^4 given for cockroaches or moths (Boeckh et al. 1990).

Essentially two types of terminal branching of receptor axons can be distinguished. In most of the glomeruli, we observed a loose pattern formed by only a few branches (Fig. 8). This suggests that the axons terminate in the periphery of the glomerulus, which corresponds to the location of degeneration products after antennectomy (*Calliphora:* Boeckh et al. 1970; *Drosophila*: our data). In contrast, in the glomeruli VP_2 and VP_3, which are exclusive targets of the aristal sensillum, individual fibers establish numerous terminal branches, and there is an even higher density of terminals in VP_3 than in VP_2 (Fig. 8a; Lienhard and Stocker 1987). $CoCl_2$ backfills from the arista and massive Golgi impregnations reveal that VP_2 and VP_3 differ also from other glomeruli in receiving only a small number of afferent fibers. This agrees with the fine structural evidence that the aristal sense organ consists of only six neurons (Foelix et al. 1989). Hence, although VP_2 and VP_3 are as large as other glomeruli, they receive no more than six sensory neurons. This observation challenges the proposition that the size of individual glomeruli is controlled by the number of afferents (Rospars and Chambille 1989). Rather, we conclude that it is the overall extent of terminal arborizations that plays a crucial role in the control of glomerular size.

Golgi preparations confirm earlier results obtained by HRP backfilling (Singh and Nayak 1985) that antennal lobes also receive afferents from the maxillary palps (Fig. 9; Table 1). Two types of sensilla are present on these appendages, i.e., the basiconic and the bristle type. Based on ultrastructural criteria, basiconic sensilla are probably olfactory, whereas bristles appear to be mechanoreceptive (Singh and Nayak 1985). Our data do not allow us to associate directly the components of the projection patterns with each of these two sensillum types. However, the fact that afferents of the mechanosensory Johnston's organ and the bristles on the anterior head (entering the brain via the antennal nerve) bypass the antennal lobe, suggests that mechanosensory bristles do not project into the lobe. Hence, the maxillary terminals in the antennal lobe are likely to arise from basiconic sensilla, whereas the maxillary terminals seen in the SOG might originate from bristles. Consequently, both antennal and maxillary basiconic sensilla appear to project into the antennal lobe. Our Golgi and HRP data suggest that the target glomeruli of these two kinds of basiconic afferents are not the same. Single unit recordings of antennal basiconic sensilla have shown their role as receptors of a variety of odor components of food (Siddiqi 1984). In contrast, a courtship assay suggested that maxillary basiconic sensilla might represent pheromone receptors (Stocker and Gendre 1988). Hence, the non-overlap of antennal and maxillary projection regions might reflect the different odor specificities of the two kinds of basiconic sensilla. Alternatively, the projection pattern could also depend on the type of appendage, since the physical site of odor perception, i.e., antennae vs. maxillary palps, might be functionally important.

Non-antennal input into the antennal lobe also occurs in moths (Bogner et al. 1986; Kent et al. 1986) and in butterflies (Lee and Altner 1986). In both cases, sensil-

la located on the labial palp project bilaterally into a specific antennal glomerulus. Physiological evidence showed that these sensilla are sensitive to plant extracts (Lee et al. 1985) or to CO_2 (Bogner et al. 1986). Since the target glomerulus apparently does not receive antennal input, it was believed to possess a similarly specialized function as the macroglomerular complex (Christensen and Hildebrand 1987; Rospars 1988). Whether the target glomeruli of maxillary palp sensilla in *Drosophila*, i.e., VA_3, VA_4, and DM_2, are exclusive maxillary targets, has not been unequivocally demonstrated, although they apparently do not receive major antennal projections (Stocker et al. 1983).

In addition to maxillary input, our data show that antennal lobes may also receive afferents via the accessory pharyngeal nerve (Fig. 8c; Table 1). This nerve carries the processes of the labral sense organ, the ventral cibarial sense organ, and two bilateral rows of bristles within the pharyngeal lumen (Stocker and Schorderet 1981). Since the bristles appear to be mechanoreceptors (Nayak and Singh 1983), it is possible that the afferent observed in the antennal lobe stems either from the labral sensillum or from the ventral cibarial sensillum. By fine structural criteria, both sense organs are taste receptors (Nayak and Singh 1983). Thus, our observation is the first example of a gustatory afferent in the antennal lobe. Normal targets of pharyngeal sensilla are the tritocerebrum and the anterior SOG; the target of gustatory sensilla from labial palps is the SOG (Stocker and Schorderet 1981; Nayak and Singh 1985). Two additional points should be made: (1) In contrast to maxillary afferents or to the labial palp pit projection (Kent et al. 1986; Lee and Altner 1986), the new pharyngeal afferent is ipsilateral. (2) Its projection area, glomerulus VL_1, is also a main target of trichoid and coeloconic sensilla from the ipsilateral antenna (Stocker et al. 1983). These observations raise interesting ideas and conclusions. Obviously, glomerulus VL_1 receives exclusively unilateral input, regardless of the topographic site of the sensillum. Targets receiving unilateral input have been interpreted as being sensitive to directional information (Borst and Heisenberg 1982; Kent et al. 1986). However, the significance of directional information of pharyngeal input remains puzzling. Another intriguing point is the overlap of pharyngeal and antennal afferents in VL_1, a primary association region receiving multimodal input. This reflects an underlying association between odor perception and feeding. It also suggests that olfactory and gustatory information may converge onto common output neurons without the intervention of local interneurons.

Local interneurons (LI)

Local ('intrinsic') interneurons do not delineate single glomeruli but fill the entire (or almost the entire) antennal lobe (Fig. 10): they apparently connect the glomeruli with each other. It remains unclear, however, which regions of the neuron are postsynaptic and which are presynaptic. Hence, in *Drosophila*, no conclusion can be drawn about the information flow in these neurons. Also, Golgi impregnation did not allow us to estimate reliably their overall numbers. It has been proposed that LI may represent the neuronal substrate for concentration-invariant odor perception (Borst 1983). Alternatively, LI could be involved in a modulatory function. LI have been reported from *Periplaneta* (Selzer 1979) and *Manduca* (Matsumoto and Hildebrand 1981). In *Musca* the presence of both unilateral and bilateral LI has been claimed (Strausfeld 1976). In moths, at least some LI appear to receive excitatory and probably direct input from olfactory receptors (Matsumoto and Hildebrand 1981). Since such cells were found to be GABAergic (Hoskins et al. 1986; Distler 1989), it was hypothesized that LI might be involved in the synaptic inhibition observed between lobe interneurons (Christensen and Hildebrand 1987). LI are believed to possess multiple spike initiation sites, suggesting that different regions of such neurons might function as independent local units (Matsumoto and Hildebrand 1981).

ACT relay interneurons

The type of interneurons that we have called monoglomerular uACTI are cells that connect single glomeruli with the calyx and the LPR (Figs. 11, 12; Table 1). They occur in the antennal system of many insects, including house flies (Strausfeld 1976), locusts, cockroaches, (Ernst et al. 1977; Selzer 1979; Burrows et al. 1982), moths (Matsumoto and Hildebrand 1981; Olberg 1983; Kanzaki et al. 1989), honeybees (Mobbs 1982), and crickets (Schildberger 1983). The functional characterization proved clearly that monoglomerular uACTI represent output interneurons that respond either unimodally to olfactory, or multimodally to olfactory, mechanical and gustatory stimulation of the antenna (for review, see Light 1986; Christensen and Hildebrand 1987; Rospars 1988; Homberg et al. 1989). In some cases, even nonantennal input has been demonstrated in these interneurons (Homberg 1984; Schildberger 1984). It is assumed that normal glomeruli (aside from the macroglomerular complex) may be supplied by one or two monoglomerular uACTI in *Periplaneta* (Ernst and Boeckh 1983), and by five in *Manduca* (Homberg et al. 1988). This would lead to a convergence ratio between antennal afferents and uACTI of 1000:1 in *Periplaneta* (Boeckh et al. 1984) or 560:1 in *Manduca* (Homberg et al. 1988). In the cockroach, three times less monoglomerular uACTI than LI have been reported (Ernst and Boeckh 1983), whereas in the moth these two neuron types appear to occur in similar numbers (Homberg et al. 1988). Exact calculations in *Drosophila* are not possible because we could not count lobe interneurons. Assuming analogous numbers of monoglomerular uACTI as in the two species studied, an average convergence ratio would be only in the range of 30:1 to 20:1 (for both unilaterally and bilaterally innervated glomeruli).

Oligo- and polyglomerular uACTI (Fig. 13) have occasionally been found in the honeybee (Mobbs 1982), the cockroach (Ernst and Boeckh 1983), and in *Manduca* (Homberg et al. 1988; Kanzaki et al. 1989). Interesting-

ly, in the moth some of these neurons – most of them GABAergic (Hoskins et al. 1986) – project via the mACT and oACT, precisely as we have observed in *Drosophila*. Hence, it appears that in contrast to monoglomerular neurons, the uACTI receiving input from more than one glomerulus project directly into the LPR and bypass the mushroom bodies. Unlike *Manduca* (Homberg et al. 1988, 1989; Kanzaki et al. 1989) the different types of uACTI do not manifest distinct arborization patterns in subregions of the LPR or the calyx.

In addition to unilateral ACTI, we have evidence of ACTI with dendritic branching in *both* antennal lobes (bACTI: Fig. 14a, b). The connection between the two lobes may pass via the AC or the SOG. bACTI with a link through the SOG send a process into both iACT, whereas bACTI with an AC connection have apparently only one iACT process. However, the possibility of incomplete Golgi impregnation has to be considered. So far, only neurons supplying the VP_{2-3} region have been observed (Table 1). Hence, these neurons represent bilateral links of these purely unilaterally innervated glomeruli. Moreover, they seem to carry specific information from the aristal sensillum. Whether bACTI receiving input from other than aristal neurons exist, remains to be determined. A bilateral uniglomerular neuron with a SOG connection and a process passing into the calyx/LPR on one side has been recently reported from *Manduca* (Kanzaki et al. 1989).

Other types of relay interneurons

The FAI (Fig. 14c, d) is incompletely understood because we do not know its targets in the brain. Although its extensive arborizations bear some resemblance to the LI, its processes extending from the lobe differentiate it from the LI. FAIs could either be output neurons that carry information from many glomeruli to brain centers distinct from the calyx or LPR or they could represent centrifugal fibers that provide the lobe with central information.

A very conspicuous type of relay neuron is the GSI (Fig. 14e, f), which has not been observed in other insect antennal lobes. Its main characteristics are: (1) an extensive mirror-symmetric arborization in both antennal lobes, (2) a pair of giant processes leading towards a second arborization region in the posterior brain, and (3) a soma located in the ventral SOG midline. The GSI bears a superficial resemblance with a serotonin-immunoreactive neuron in the antennal lobe of *Manduca* (Kent et al. 1987), although this neuron is neither bilaterally symmetric, nor is its cell body situated in the midline. Furthermore, application of a polyclonal 5HT-antibody in *Drosophila* did not reveal neurons of GSI shape (K. Störtkuhl, personal communication). As in the case of the FAI, the direction of signal transfer is not known in the GSI. The secondary arborization in the region of the great commissure may overlap with contralateral branches of the cervical giant fiber neuron (Koto et al. 1981), which extends into the thoracic ganglion and is known to mediate jumping in response to visual or olfactory stimuli. Hence, GSI could be involved in mediating the smell-driven jump response. Alternatively, relay neurons with multiglomerular ramifications have often been interpreted to be centrifugal neurons (Homberg et al. 1989).

A peculiar type of bilateral interneuron branches exclusively in the V glomeruli (bVI: Fig. 14g; Table 1). bVIs not only interconnect the two V glomeruli, but also send a thick branch into the left and right ventrolateral deutocerebrum. Since glomeruli V appear to be a specific target of basiconic sensilla from the ipsilateral antenna (Stocker et al. 1983; Stocker and Gendre 1988), bVI might be part of a basiconic-specific pathway. However, because its target in the ventro-lateral deutocerebrum is not known, no convincing functional hypothesis can be proposed. In *Manduca,* an interneuron has been shown to link the macroglomerular complex of the antennal lobe with the origin of descending neurons in the lateral deutocerebrum (Strausfeld 1989b).

While descending interneurons link the AMMC with the thoracic ganglion (Strausfeld and Bacon 1983; Strausfeld et al. 1984), no direct connections have been reported so far between the antennal lobe and the thoracic central nervous system (CNS). The thoracic relay interneuron (TI) described here (Fig. 17) may be the first example of a neuron descending from the lobe directly into the thoracic ganglia. Its two branching regions in the lobe and in the posterior brain suggest that TI may carry multimodal information. Its function remains puzzling, mainly because of our ignorance of its thoracic targets.

A functional rather than topographic map of sensory inputs in the antennal lobe

Anatomical evidence from *Drosophila* supports the assumption of a functional rather than topographical map of sensory inputs in the antennal lobe. This is a striking difference from the retinotopic organization of the visual system (Fischbach and Dittrich 1989; for a comparison of the organization of visual and olfactory centers in insects, see Strausfeld 1989a). The evidence is as follows (Table 1): (1) Backfilling from selected funicular regions suggests that each of the three types of sensilla projects into a specific set of glomeruli, regardless of their location on the funiculus (Stocker et al. 1983). For example, the major targets of antennal basiconic sensilla are the ipsilateral glomerulus V, and bilateral glomeruli VM_1 and DM_1; minor targets include VM_2, D, and DL_1. According to these data, no other sensilla appear to project into glomeruli V, VM_2, D, DL_1, and DM_1. (2) *lozenge*[3] flies that lack antennal basiconic sensilla also lack glomerulus V (Stocker and Gendre 1988). (3) Glomeruli VP_2 and VP_3 are apparently reached only by afferents from the aristal sensillum (Stocker et al. 1983; Lienhard and Stocker 1987). This sensillum consists of only six neurons, three thermoreceptors and three receptors of unknown function (Foelix et al. 1989). (4) Backfilling from the funiculus also suggests that VA_1 and DA_1 are pure trichoid targets (Stocker et al. 1983). (5) Maxillary

basiconic sensilla project into glomeruli VA_3, VA_4, and DM_2, which do not appear to be significant targets of other sensilla (this study). (6) Functional mapping of the antennal lobe using [^{3}H]2-deoxyglucose autoradiography has shown that stimulation with different classes of organic odors (alcohols, acids, esters, aldehydes, ketones) results in labelling of specific but overlapping sets of antennal glomeruli (Rodrigues and Buchner 1984; Rodrigues 1988; Rodrigues and Pinto 1989). Out of the glomeruli that receive input from only one type of sensillum, seven were labelled, i.e., the basiconic glomeruli VM_2, DL_1, and DM_1, the trichoid glomeruli VA_1 and DA_1, and the maxillary glomeruli VA_3 and DM_2. Most of these glomeruli were activated by more than one class of chemical. Moreover, different glomerular targets of the same sensillum type showed different odor spectra. Accordingly, each sensillum type would be activated by a large variety of chemical odor classes. This conclusion is entirely consistent with recordings from single basiconic sensilla, which have revealed a surprisingly large odor perception spectrum (Siddiqi 1984).

These various lines of evidence suggest that the antennal lobe of *Drosophila* may be constructed of three types of glomeruli (Table 1): type-a glomeruli dealing exclusively with 'specialized' sensory input (VP_2, VP_3: aristal sensillum), type-b glomeruli receiving a more extensive spectrum of information, though only from one sensillum type (e.g., V, VM_2, D, DL_1, DM_1: basiconic sensilla), and type-c glomeruli that are targets of more than one type of sensillum (e.g., VL_1: coeloconic and trichoid) or even of both antennal and non-antennal sensilla (VL_1). Both type-b and -c glomeruli would be activated by a relatively broad spectrum of odors.

In conclusion, processing of 'specialized' sensory information may be performed in 'specialized' type-a glomeruli, whereas 'complex' odors may activate specific arrays of glomerular type-b and -c. Similar models of functional specification of antennal glomeruli have been proposed by other groups (Homberg et al. 1989; Rospars and Chambille 1989). A possible example of a type-a glomerulus is the male-specific macroglomerulus of moths (Boeckh and Boeckh 1979; Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1987; Koontz and Schneider 1987) and cockroaches (Ernst et al. 1977; Boeckh et al. 1984), which deals rather specifically with pheromonal information. The labial-pit-specific glomeruli of Lepidoptera, on the other hand (Bogner et al. 1986; Kent et al. 1986; Lee and Altner 1986), might represent type-b glomeruli.

Possible significance of bilateral afferents

The detection of many more glomeruli, each receiving input from both antennae, underscores the important role of bilateral antennal projections in *Drosophila*. Several attempts have been made to explain the functional significance of this peculiarity of the dipteran antennal system. If ipsi- and contralateral terminals were functionally identical, bilateral projections could enhance the signal-to-noise ratio by doubling the convergence ratio between afferents and target interneurons. However, this gain would be at the expense of lateral information. If, on the other hand, the information transferred at ipsi- and contralateral synapses were different (e.g., excitatory vs. inhibitory), ipsi- and contralateral input could be distinguished from one another. Golgi impregnations or cobalt backfills do not reveal striking differences between the ipsi- and contralateral branching patterns of bilateral afferents. However, this does not exclude possible differences in synaptic connectivity at the fine structural level. Unilateral antennal stimulation with attractants resulted in activity mapping predominantly in the ipsilateral antennal lobe, and only weak labelling of the corresponding contralateral glomeruli (Rodrigues 1988; Rodrigues and Pinto 1989). This was taken as an indication that the projections of the receptor neurons excite postsynaptic elements ipsilaterally but may inhibit contralateral targets. In contrast,unilateral stimulation with the repellent benzaldehyde has been shown to label corresponding glomeruli in both lobes, suggesting excitatory activity in both lobes (Rodrigues 1988; Rodrigues and Pinto 1989). These interpretations agree in general with the observation that attractive odors elicit positive osmotropotaxis, whereas repellents do not elicit negative osmotropotaxis (Borst and Heisenberg 1982).

Separate output channels formed by ACTI?

Assuming that all types of uACTI and bACTI are true output neurons of the lobe, they might belong to one of three functional categories, according to whether they arborize in type-a, -b, or -c glomeruli (Table 1). Intriguing examples of the first category are monoglomerular and oligoglomerular uACTI, as well as the two types of bACTI, all of which establish dendritic arbors in the two arista-specific glomeruli, VP_2 and VP_3. Hence, these interneurons may represent a specialized set that carries exclusively aristal information. Although the sensory input to type-b glomeruli may be less homogeneous than to type-a glomeruli, monoglomerular uACTI or the bVI arborizing in type-b glomeruli might also constitute separate output channels. According to these ideas, the concept of functional specification of antennal glomeruli could, to some extent, be pursued to the level of output neurons. We are aware, however, that this idea ignores the significance of interglomerular communication. As an interesting parallel, anatomical and physiological data in moths and cockroaches suggest that a group of ACTI may form a specific pathway for processing pheromonal information (Homberg et al. 1989).

As in all insect species studied so far, uACTI in *Drosophila* may project into two different protocerebral target regions, i.e., the calyx and the LPR (Strausfeld 1989a). Our data demonstrate that monoglomerular uACTI essentially terminate in both of these regions, whereas polyglomerular uACTI generally bypass the calyx region. The analysis of structural brain mutants with reduced mushroom bodies led to the hypothesis that qualitative and quantitative aspects of odor stimuli are evaluated in the LPR, whereas the calyces are involved

in olfactory learning (Fischbach and Heisenberg 1984; Heisenberg et al. 1985). Our data thus suggest that these two functions may be separated already at the level of uACTI. Accordingly, uACTI supplying more than one glomerulus might carry primary information from several glomeruli that would be selectively utilized for non-associative responses, whereas monoglomerular uACTI would be involved in both associative and non-associative responses. However, this model ignores the significance of LI in this network.

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