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Anatomical identification of glomeruli in the antennal lobes of the male sphinx moth *Manduca sexta*

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Summary. Computer-assisted neuroanatomical methods have been used to demonstrate unique identities of the glomeruli of the antennal lobes (ALs) in males of the sphinx moth *Manduca sexta*. The glomerular neuropil consists of the male-specific macroglomerular complex, which comprises two closely apposed bulky subunits, and 64 ± 1 "ordinary" glomeruli arrayed in a shell around a central region of coarse neuropil. Computer-generated maps show the exact locations of all glomeruli and adjacent groups of neuronal somata in a constant Cartesian coordinate system, such that these can be accurately identified in any individual. The glomeruli belong to three classes according to the number and type of identification criteria they satisfy. The larger class comprises glomeruli ($n=44$) identified only in the computer-generated maps on the basis of their relative positions. The other two classes include glomeruli that were also identified in sections, either directly from their proximity to readily identifiable structures and their shape and size ($n=10$, including the labial-palp–pit-organ (LPO) glomerulus), or indirectly from their positions relative to the former ($n=9$). Two very small glomeruli were present in only one AL, demonstrating the existence of anomalous glomeruli, whereas another glomerulus had no homologue in both ALs of one individual. The true number of ordinary glomeruli (per male AL) was thus estimated to be 64. The uncertainty in delineating some glomeruli might affect this number without implying modification of the homologies proposed. The locations of tracts and cell groups, both within and near the AL, are also invariant with respect to glomeruli, as shown in the computer maps. The methods employed are general and might be useful to researchers in related fields. The results obtained call for more attention to the precise geometry of neural structures.

Key words: Insect nervous system – Antennal lobe – Olfactory system – *Manduca sexta* (Insecta)

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Insects are favorable models for studies of neural mechanisms of olfaction because of their prominent olfactorily controlled behaviors, experimentally accessible sensory organs and corresponding cerebral centers, and relatively small number of neurons in comparison with vertebrates. Long-distance attraction by sex pheromones in insects offers one of the most striking examples of chemical signaling, especially for moths in which the pheromone is emitted by a female and detected by specific sensilla on the antennae of conspecific males. Neurons that respond to the pheromone have been studied in detail both in the antennae and at the first level of sensory integration in the antennal lobes (ALs) of the brain, and neurons responding to nonpheromonal olfactory stimuli have also been studied thoroughly, especially in cockroaches and to a lesser extent in moths (reviewed in Boeckh and Ernst 1987; Christensen and Hildebrand 1987b; Rospars 1988a; Homberg et al. 1989; Mustaparta and Masson 1990). Higher order processing of olfactory information relayed by AL output neurons has also emerged as a promising research theme (Olberg 1983; Fischbach and Heisenberg 1984; Heisenberg et al. 1985; Kanzaki et al. 1991a, b).

Evidence of identifiability of certain neurons in the ALs derives mainly from studies of the glomeruli, which are the sites of synaptic interactions among antennal receptor-cell axons and AL neurons, as well as studies of glomerular output neurons. First, the idea that glomeruli are individually identifiable units was initially proposed and tested in a cockroach (Chambille and Rospars 1981, 1985; Chambille et al. 1980; Rospars and Chambille 1981, 1986) and a moth (Rospars 1983). Confirmatory studies have been performed in other orders (e.g., flies: Stocker et al. 1983, 1990; Pinto et al. 1988; Rodrigues and Pinto 1989; honey bees: Arnold et al. 1985; Flanagan and Mercer 1988). Second, evidence from species of various orders supports the idea that the arborizations of the principal (output) neurons of the AL often are restricted to only one glomerulus (Goll 1967; Selzer 1979; Matsumoto and Hildebrand 1981; Mobbs 1982; Ernst and Boeckh 1983; Schildberger

1983; Homberg 1984; Boeckh and Ernst 1987; Christensen and Hildebrand 1987a; Homberg et al. 1988; Kanzaki et al. 1989). These two properties provide a basis for identification of the neurons associated with a given anatomically identified glomerulus. Such attempts have been reported (Boeckh and Ernst 1987; Flanagan and Mercer 1988; Stocker et al. 1990).

This study describes the specific criteria used to designate as unique each of the glomeruli in a formal three-dimensional (3D) map of the AL of male sphinx moths *Manduca sexta*. Subsidiary aims were to examine the spatial relationships of anatomically identified glomeruli with fiber tracts and cell groups and to describe anomalous glomeruli. The results are based on qualitative analyses of serial sections and computer-assisted reconstructions of the AL. Quantitative analyses of these data will be reported separately (J.P. Rospars, in preparation).

Materials and methods

Animals and histology

Manduca sexta (Lepidoptera: Sphingidae) were reared as described previously (Sanes and Hildebrand 1976; Tolbert et al. 1983). The animals used in this study were newly eclosed adult males. After dissection, brains were fixed in alcoholic Bouin's solution, embedded in Paraplast Plus (Monoject Scientific, St. Louis, Mo.), and sectioned at 8 μm in the horizontal plane. Sections were stained by Bodian's silver protargol technique (Gregory 1980).

Measurements

Photomicrographs of serial sections of two male brains were printed at 200×, and profiles of glomeruli, AL cell groups and tracts, and main protocerebral structures were traced from the photographs onto tracing paper. Unique designations were given to each profile of each glomerulus. Two axes (x and y) were drawn on each tracing by superimposing successive tracings as described previously (Rospars 1983).

The coordinates of profiles were acquired by a program, briefly described elsewhere (Rospars 1988b, c), with the use of a Benson 6440 digitizer, and the acquired and computed data were stored on the hard disk of a microcomputer. Two databases per AL were created, one for glomeruli and the other for tracts and cell groups. Each database included four files for storing the coordinates of points of profiles (file A), the area and center of each profile (file B, computed from file A), the volume and center of the set of profiles describing the same component (file C, computed from file B), and information about each section (file D). In the work described here, only data from file C were used. Instead of the volume of a component, the radius or diameter of the sphere having the same volume was used.

The acquired coordinate data (x, y, z) were corrected from the defects of orientation of the sectioning plane. Asymmetry angle α measured its deviation from the ideal plane, which is exactly perpendicular to the sagittal plane. Tilting angle γ measured the rotation of the sectioning plane around the X-axis (perpendicular to the sagittal plane) in different individuals (see Rospars and Chambille 1981). In the individual brains studied, angles α were about 2° and −1°, indicating a very good right-left orientation, and angle γ was about −12°. These corrections transformed (x, y, z) into (X, Y, Z). The center of the AL was taken as origin O of the axes. No other correction was performed.

Axes were named relative to the body axis of the animal according to Strausfield (1976), with X medio-lateral, Y postero-anterior, and Z ventro-dorsal.

Computer reconstructions

Computer reconstructions similar to those used previously (Rospars 1983; Chambille and Rospars 1985) were made from data (X, Y, Z, and radius) for each AL component. The AL components (glomeruli, cell groups, element of tracts) were schematized as spheres and drawn as disks, with the disks in the foreground (near the observer) hiding those in the background. Six different viewpoints were used, with the observer located successively on the six half-axes (X>0, X<0, etc.) and regarding each of these six views of the AL as one of the six sides of a cube. These options have several advantages: data are not numerous (four numbers per glomerulus); the computer programs are relatively simple and execute rapidly; graphics obtained can be used directly; they are complete, with all glomeruli visible; they do not favor any direction of observation; and their interpretation is relatively straightforward.

Two-dimensional graphics of ALs were obtained on-screen by means of an EGA card (Hercules and VGA cards were also used). The images were copied onto paper with a Hewlett-Packard LaserJet II printer. Programs used for computation, management of data, and graphical representations were written especially for this study.

Naming conventions

The glomeruli were named provisionally using a label peculiar to each glomerulus and referring to the side (R or L) and individual (Ms1 or Ms2). These names are objective and basic but cumbersome and relatively uninformative, because they are unrelated in different ALs. Thus glomerulus MS1-R-32 refers to a different glomerulus than Ms1-L-32 or Ms2-R-32. These names were used initially to match homologous glomeruli in different ALs. After matching (see Results), this naming system was replaced by a system in which the name of the glomerulus is intended to refer to the same glomerulus in the different ALs studied. These "definitive" glomerulus names are preceded by a G. In practice, glomeruli in Ms1 were ranked in decreasing order of their mean right/left Z-coordinate and numbered accordingly, low numbers referring to dorsal glomeruli and high numbers, to ventral glomeruli, except for two anomalous glomeruli (G65 and G66) and the macroglomerular complex (MGC-A and MGC-B).

Other structures, except certain tracts, were named according to the half-axes on which their centers project, i.e., the lateral (X>0, X+), median (X<0, X−), anterior (Y+), posterior (Y−), dorsal (Z+), and ventral (Z−) half-axes, in the order Z, Y, X. For example, the coordinates of the center of a structure named dorsoanterolateral (DAL) are all strictly positive. When a coordinate was 0 or close to 0, its name was omitted, e.g. a structure named dorsolateral (DL) is close to the XOZ plane (Y is ca. 0), and a structure named anterior (A) is close to Y+ (X=0 and Z=0). This convention was consistently followed only in naming a structure, not in describing its position; e.g., when a structure is dorsolateral, it may be in any frontal plane (i.e., parallel to XOZ).

Abbreviations: A, Anterior cell group of the AL; *AAT,* anterior antennal tract; *ACT,* antenno-cerebral tract; *AL,* antennal lobe; *AMMC,* antennal mechanosensory and motor center; *AMMT,* antennal mechanosensory and motor tract; *Ca,* calyces of the mushroom body; *DAAT,* dorsoanterior antennal tract; *DACT,* dorsal antenno-cerebral tract; *DM,* dorsomedian cell group of the AL; *DM2,* second dorsomedian cell group; *DMACT,* dorsomedial antenno-cerebral tract; *DMT,* tract of axons leaving the dorsomedian cell group of the AL; *DPAT,* dorsoposterior antennal tract; *Gn,*

ordinary glomerulus number n; *IACT*, inner antenno-cerebral tract; *IACT-DR*, dorsal root of the inner antenno-cerebral tract; *IACT-VR*, ventral root of the inner antenno-cerebral tract; *L*, left; *l*, lateral; *LPO*, labial-palp pit organ; *MACT*, middle antenno-cerebral tract; *MGC*, macroglomerular complex; *MGC-A*, subunit A of the macroglomerular complex ("cumulus"); *MGC-B*, subunit B of the macroglomerular complex ("toroid"); *Ms1*, individual *Manduca sexta* moth number 1; *Ms2*, individual *Manduca sexta* moth number 2; *OACT*, outer antenno-cerebral tract; *P*, posterior cell group of the protocerebrum; *p*, posterior; *PAT*, posterior antennal tract; *PM*, posteromedian cell group of the protocerebrum; *PVT1*, posteroventral tract 1; *PVT2*, posteroventral tract 2; *R*, right; *RP*, relative position (criterion for matching of glomeruli); *TA*, dorsal branch of the antenno-cerebral tract within the AL; *TB*, ventral branch of antenno-cerebral tract within the AL; *VL*, ventro-lateral cell group of the AL; *VLT*, tract of axons leaving the ventro-lateral cell group of the AL; *VPM*, ventroposteromedian cell group of the protocerebrum; *X*, mediolateral axis (medial view X−, lateral view X+); *Y*, posteroanterior axis (posterior view Y−, anterior view Y+); *Z*, ventrodorsal axis (ventral view Z−, dorsal view Z+).

Results

Organization of antennal lobes

The antennal lobes of *M. sexta* are similar to those of other moths, with a central core of relatively coarse neuropil surrounded by glomeruli and an outer cortex of neuronal cell bodies gathered in characteristic groups.

Glomeruli. Glomeruli are distinguished by their dense neuropil surrounded by a clearer area corresponding to a partial glial investment (Tolbert and Hildebrand 1981; Oland and Tolbert 1987). The silver staining used in this work did not permit the observation of the internal organization of glomeruli.

The male AL neuropil possesses two sets of glomeruli. The first is the male-specific macroglomerular complex (MGC) (Matsumoto and Hildebrand 1981; Hansson et al. 1991), located dorsolaterally near the entrance of the antennal nerve into the AL. The second set comprises an array of "ordinary" glomeruli, which are spheroidal and smaller (ca. 50–80 μm in diameter) than the MGC. The ordinary glomeruli surround the central neuropil, which is largely made up of principal neurites of AL neurons, and are all included in a spheroidal volume ventral to the MGC and anterior to the protocerebrum. The ordinary glomeruli are innervated by antennal fibers that surround and enter the glomerular array from its perimeter.

The cell groups, the bundles of neurites arising from them, and the tracts of afferent antennal fibers and of fibers connecting the ALs to other brain regions are useful landmarks for the identification of glomeruli.

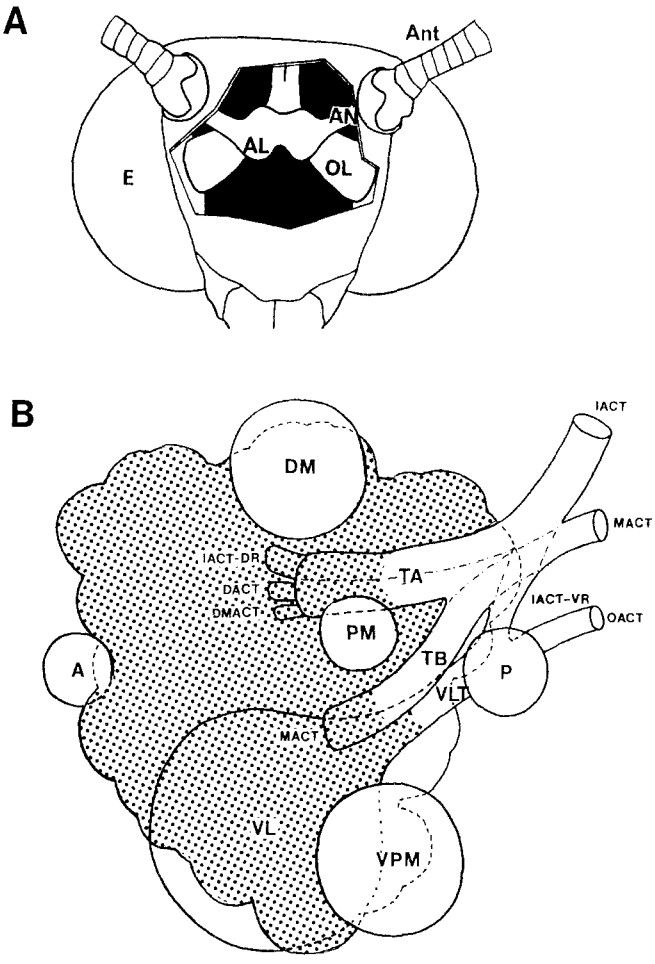


Fig. 1A, B. The antennal lobes of adult *Manduca sexta*. **A** Cutaway view of the brain in the head of the moth (dorsal aspect). *Ant* antenna; *Al* antennal lobe; *AN* antennal nerve; *E* compound eye; *OL* optic lobe (redrawn from Hildebrand et al. 1980). **B** Diagrammatic view of the right AL with cell groups and antenno-cerebral tracts as viewed from the left AL (see list of abbreviations). The *stippled area* represents the shell of ordinary glomeruli (the MGC of the male is on the far side and is not shown). Cell groups in AL (*A, DM, VL*) and in protocerebrum (*P, PM, VPM*) are shown. Inside and immediately outside the AL, three axonal tracts (*TA, TB, VLT*) connect the AL to the protocerebrum. These tracts are interpreted by comparison with the reconstruction given by Homberg et al. (1988; see also Hoskins et al. 1986). *TA*, the dorsal branch of the antenno-cerebral tract (ACT) within the AL, is the coalescence for a short distance of the dorsal root of the inner ACT (*IACT-DR*), the dorsal ACT (*DACT*), and the dorsomedial ACT (*DMACT*). *TB*, the ventral branch of antenno-cerebral tract within the AL, is probably the beginning of the middle ACT (*MACT*). Outside the AL, tracts TA, TB, and VLT give rise to the outer ACT (*OACT*), which continues the ventral VLT (Fig. 6A, D), the middle ACT (*MACT*, Fig. 5A, B, D), which projects directly to the ventral part of the calyces of the ipsilateral mushroom body in the protocerebrum, and the inner antenno-cerebral tract (*IACT*, Fig. 5B), which passes in front of the calyces dorsal to the pedunculus of the mushroom body and ends in the lateral protocerebrum

Cell groups. Three groups of neurons bordering the AL (Fig. 1) send axons into the central neuropil of the AL (Homberg et al. 1988, 1989): DM (dorsomedian, ca. 100 μm in diameter; Figs. 2B–D, 3A, B), VL (ventrolateral, 180 μm in diameter; Figs. 4B, D, 5, 6), and A (anterior, ca. 55 μm in diameter; Figs. 3D, 4C, E, F). Only the VL group interrupts the glomerular layer and abuts the central neuropil of the AL.

Three other cell groups are in contact with the glomeruli (Fig. 1) but do not send axons into the AL; they

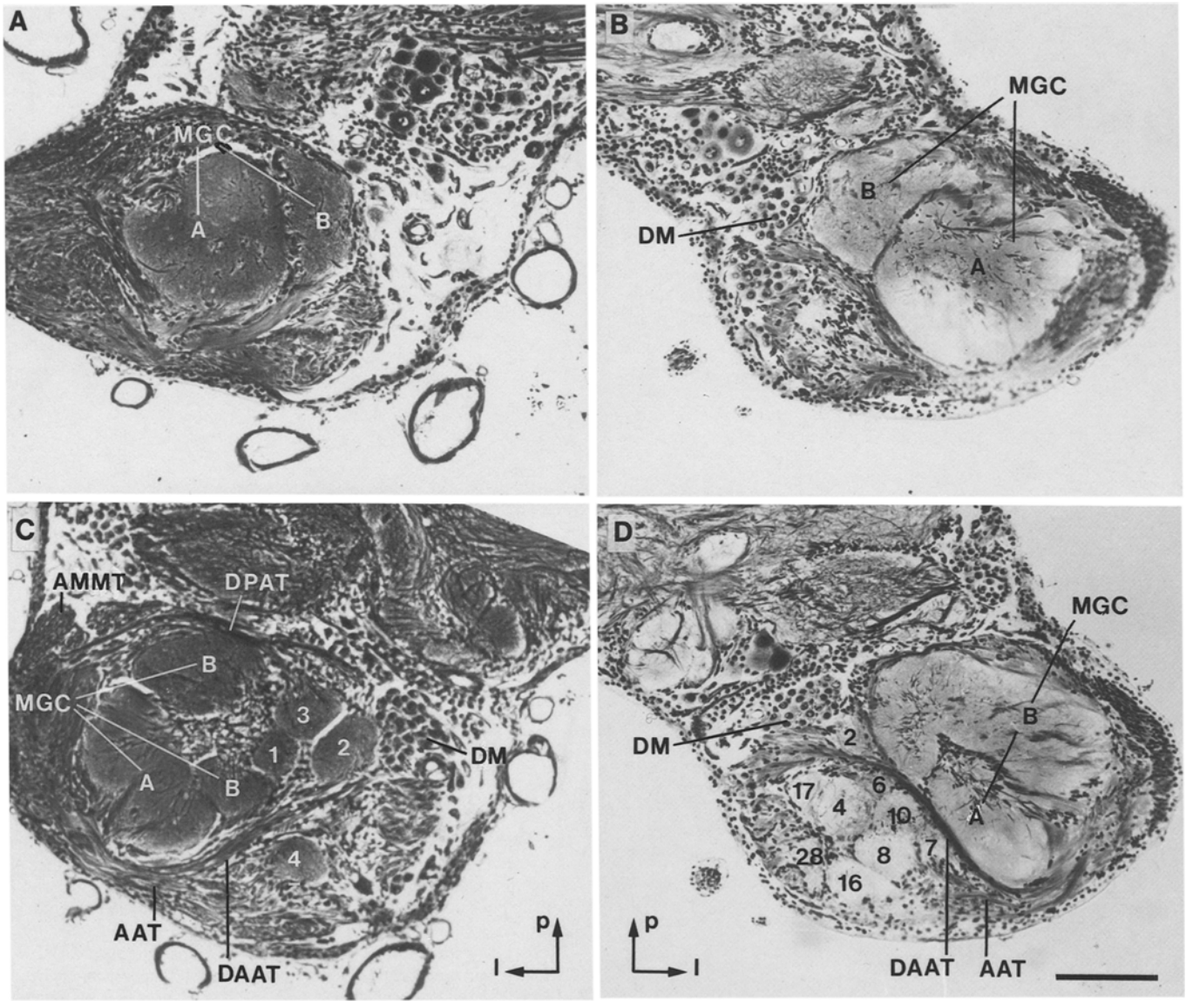


Fig. 2A–D. Comparison of dorsal aspects of ALs in two brains. Right AL of Ms1 (**A, C**) and left AL of Ms2 (**B, D**). At the plane of section shown in **C**, the cumulus (*MGC-A*) of the macroglomerular complex (*MGC*) is flanked by two parts of the toroid (*MGC-B*). Glomeruli identified from computer reconstructions. Horizontal sections **A** (no. 11) and **C** (no. 21) are 80 µm apart, **B** (no. 16) and **D** (no. 21) are 40 µm apart. Sections are numbered sequentially beginning at no. 1 at the dorsal pole of the AL. In Figs. 2–6 sections of the Ms1 right AL are on the left and in sequence from dorsal to ventral; sections of the Ms2 left AL are on the right and also in sequence, except in Fig. 4F. Because of tilting, if posterior parts of the ALs in the same row display the same areas, anterior parts do not. *AAT* Anterior antennal tract; *AMMT* antennal mechanosensory and motor tract; *DAAT* dorsoanterior antennal tract; *DPAT* dorsoposterior antennal tract; *numbers* mark individual glomeruli. Other abbreviations as in Fig. 1. Bodian-stained horizontal sections, *l* lateral, *p* posterior. *Bar:* 100 µm

may correspond to groups III, V, and VI described by Homberg et al. (1987) in the median protocerebrum. Group PM (posteromedian, 65 µm in diameter; Figs. 3D, 4A, B, D, F) with large somata (up to 20 µm in diameter), lies ventral to the DM group in the space delimited by the AL, the protocerebrum, and the esophageal foramen. Group P (posterior, 60 µm in diameter, Figs. 4B–E, 5B) is located in a wedge-shaped indentation of the protocerebral neuropil close to the posterior pole of the AL and separated from the glomerular layer by mediolateral fiber tracts that probably originate in the antennal nerve. Group VPM (ventroposteromedian, 100 µm in diameter; Figs. 5B, D, 6A–D) comprises large cell bodies (up to 35 µm in diameter), which probably belong to antennal motor neurons (Hildebrand et al. 1980). This group comes into contact with the glomerular layer and sends axons into an area posterior to the AL, probably the antennal mechanosensory and motor center (AMMC) of the deutocerebrum.

Tracts of antennal fibers. The antennal nerve enters the AL near its dorsal pole. A major subdivision of the nerve, the antennal mechanosensory and motor tract (AMMT, Figs. 2–6), is a thick, straight, posterolateral

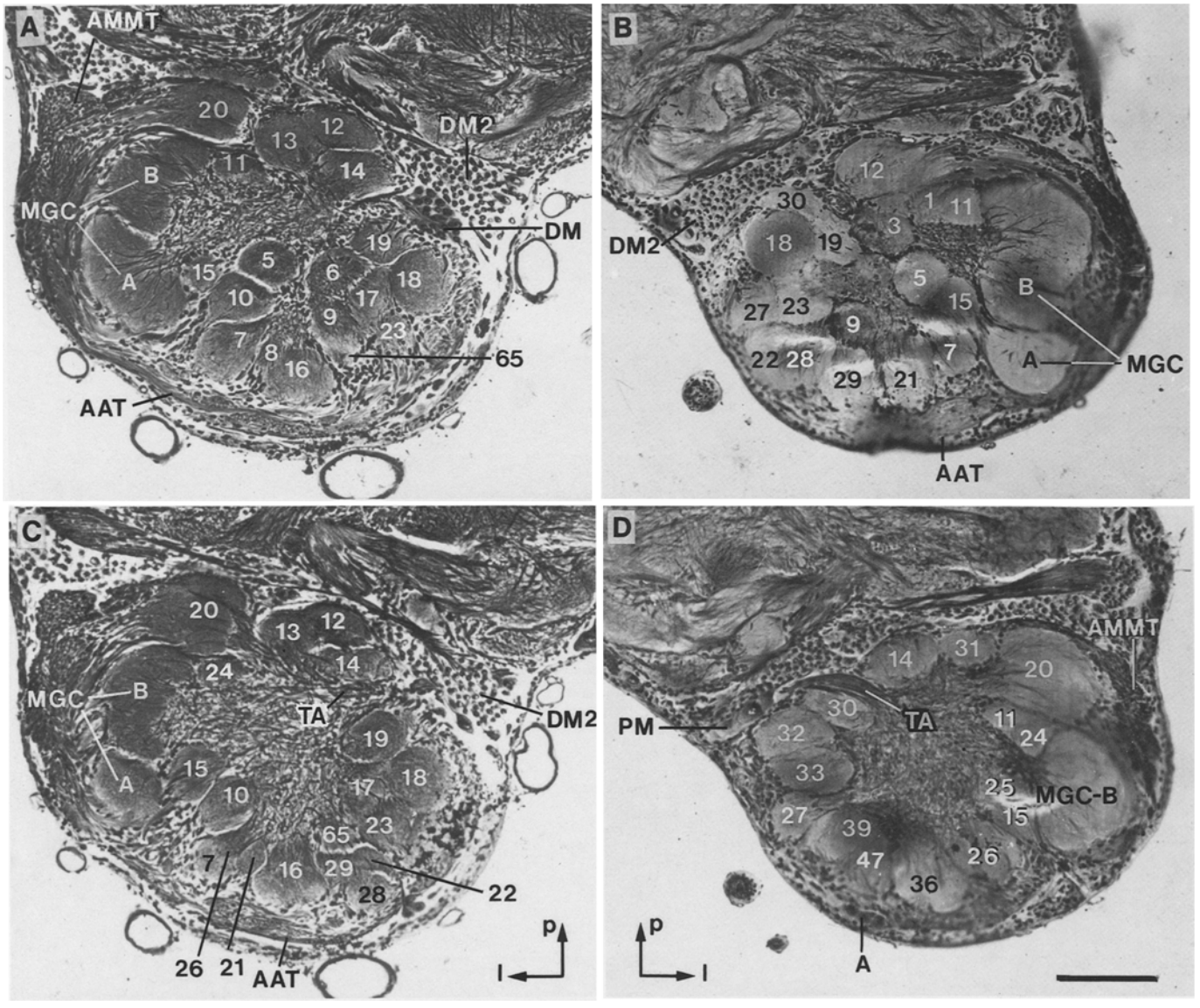


Fig. 3A–D. Middle part of the MGC and directly identified glomeruli of group I (G20 and G24). Other glomeruli identified from computer reconstructions. In **A**, fiber tract (adjacent to glomerulus 14) from the dorsomedian cell group (*DM*) enters the AL central neuropil. In **C** and **D**, glomerulus G20 shows diametrically opposite fibers coming from the antennal nerve and from the central neuropil. Other glomeruli also show entering fibers from the antenna (e.g., G10 in **A**, G22 in **B**, G31 in **C**) or AL (e.g., G27 in **D**). In **C** and **D** tract *TA* enters (or exits) the AL, and in **D** it exits the AL (see also Fig. 4A, B). Sections 29 (**A**) and 32 (**C**) in Ms1, 27 (**B**) and 34 (**D**) in Ms2. *DM2* Second dorsomedian cell group; other abbreviations as in Fig. 1. Sampled ALs, staining, and scale bar as in Fig. 2

bundle that projects dorsoventrally to the AMMC (Hildebrand et al. 1980; Hildebrand and Montague 1986). The AMMT projects past the MGC and the VL cell group between the protocerebrum and the AL.

The rest of the antennal nerve consists of fascicles of antennal receptor axons, which form tracts that innervate the glomerular neuropil of the AL. These sensory axons terminate in glomeruli (Hildebrand and Montague 1986). As in Dictyoptera (e.g., Chambille and Rospars 1981) but not Hymenoptera (e.g., Mobbs 1982), these tracts of receptor axons are relatively difficult to count. They are more readily recognized when sectioned lengthwise. Two input tracts are found near the dorsal pole of the AL. One runs posteriorly to the MGC and extends to the DM cell group (dorsoposterior antennal tract DPAT, Fig. 2C). The other runs around the anterior edge of the MGC from anterolateral to posteromedian and also approaches the DM group (dorsoanterior antennal tract DAAT, Fig. 2C, D); its ventral edge runs between the MGC and the most dorsal glomeruli. Two other tracts are located near the anterior and posterior poles of the AL. These tracts, the anterior (AAT; Figs. 2A, D, 3A–D, 4A, B, F) and posterior (PAT; Fig. 4C–E) antennal tracts, run in two horizontal planes close to each other and pass near the A and P cell groups, respectively. This list of antennal tracts is not exhaustive.

Antenno-cerebral tracts. The numerous individual tracts of the system that connects the AL to the protocerebrum (Homberg et al. 1988; see also Hoskins et al. 1986) can-

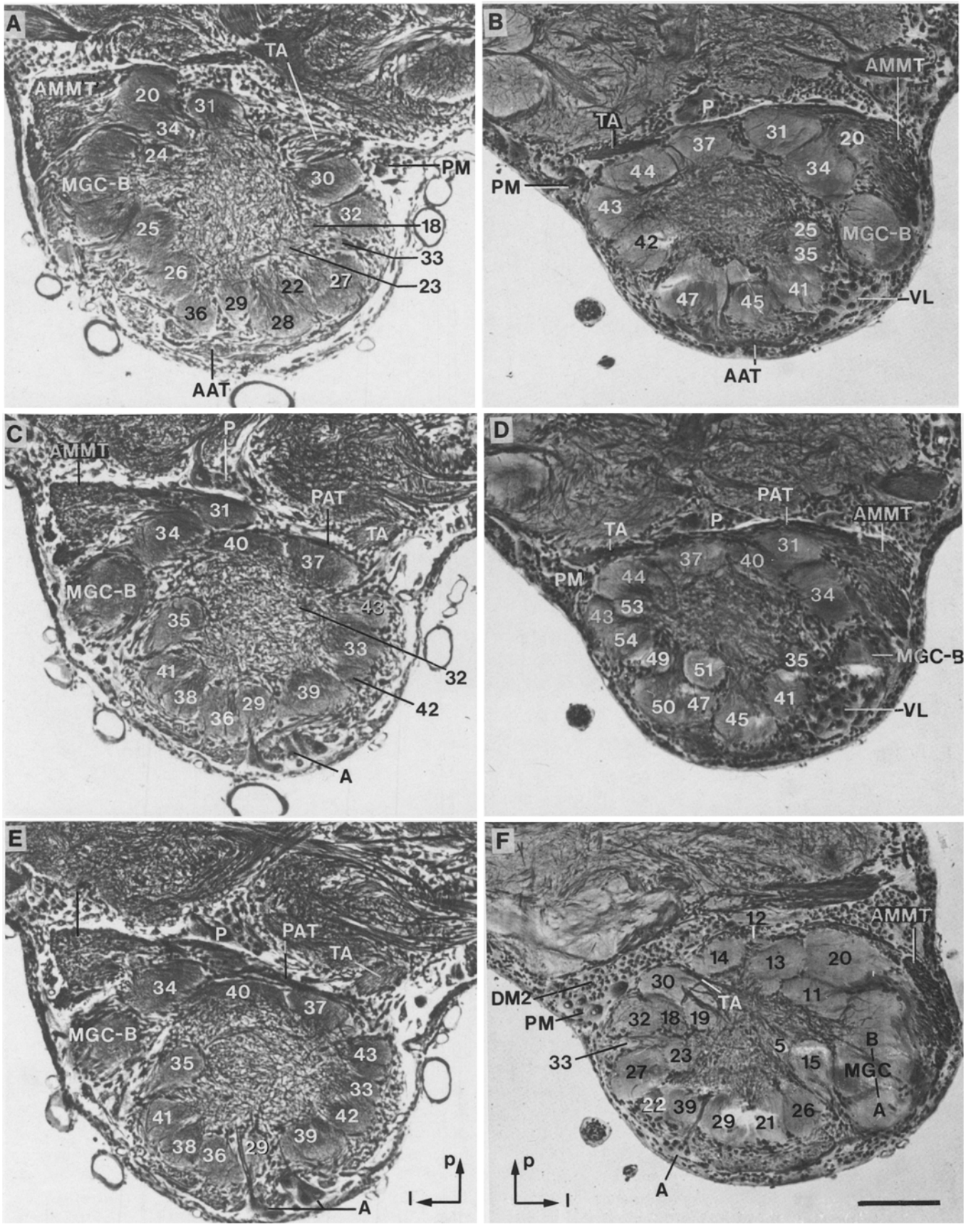


Fig. 4A–F. Ventral part of the MGC and directly identified glomeruli of groups II and III. Primary glomerulus G35 of group II (**B–E**) and secondary glomeruli G31 (**A–D**), G34 (**A–E**), G37 (**B–E**), and G40 (**C–E**) of group III. Glomeruli G31, G34, and MGC-B are aligned (**B, C, D**). In **E**, two tracts leave the anterior cell group *A* and enter the central neuropil on both sides of glomerulus G29. In **F**, only one tract leaving cell group **A** is seen. Note that section **F** is out of sequence; its anterior part is comparable with **A, C,** and **D** and shows that this AL area is more difficult to analyze in sections than is the posterior area. Sections 37 (**A**), 42 (**C**), and 43 (**D**) in Ms1, 38 (**B**), 41 (**D**), and 31 (**E**) in Ms2. *PAT* Posterior antennal tract; other abbreviations as in Fig. 1. Sampled ALs, staining, and scale bar as in Fig. 2

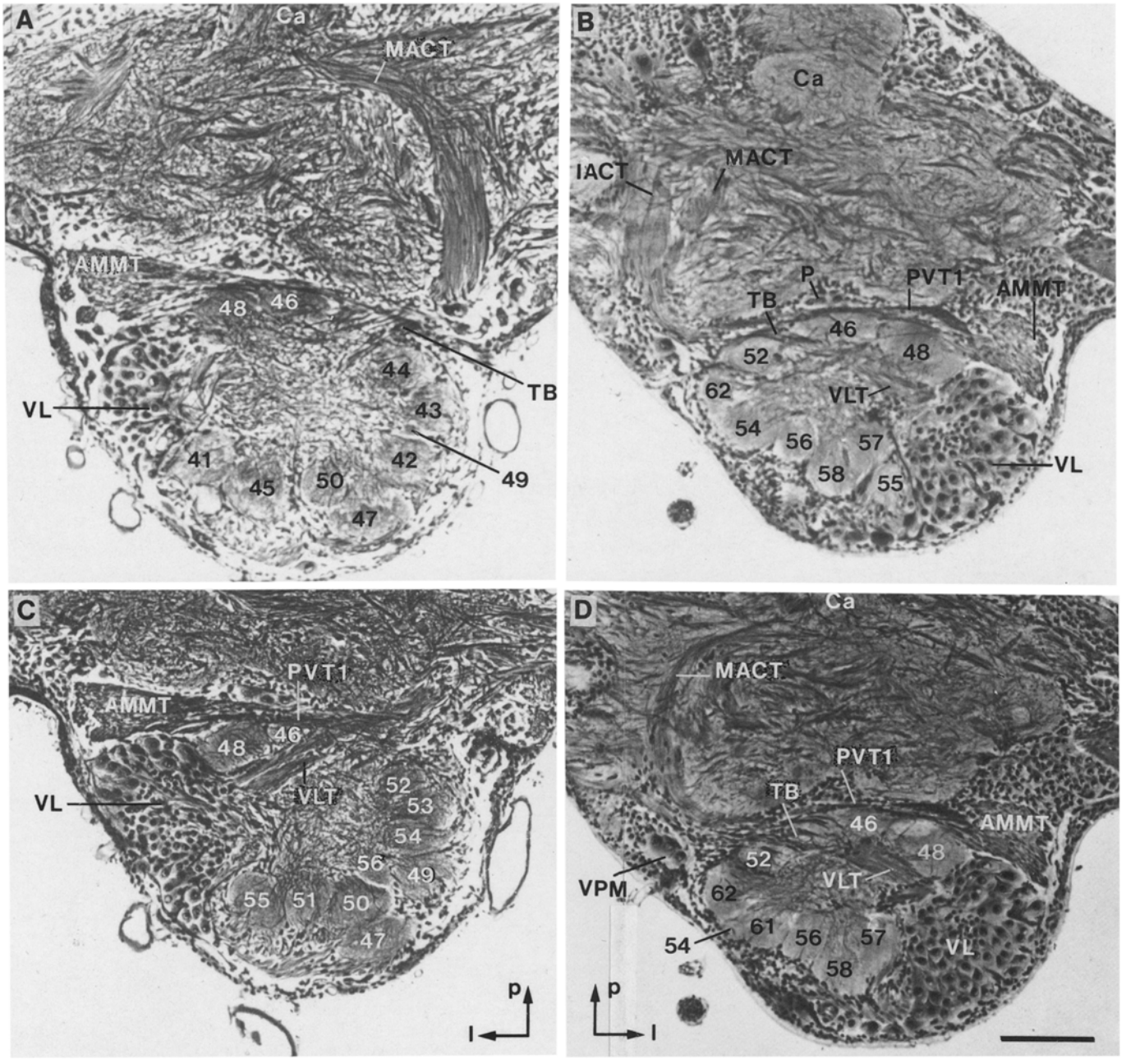


Fig. 5A–D. Directly identified glomeruli of group III–V and dorsal antenno-cerebral tracts. Primary glomeruli G46 and G48 of group III (**A–D**) are in characteristic locations with respect to tracts (**C, D**). Glomeruli G52 (**B–D**) and G44 (**A**, see also Fig. 4B, D) of group V. Tract *TB* enters/exits the AL (**A, B**). Tract *VLT* from the *VL* cell group joins the antenno-cerebral tract system (**C, D**). Dorsally this system shows two branches; the largest (**B**) is the inner ACT (*IACT*) that projects to the calyces of the ipsilateral mushroom body and the lateral protocerebrum (not shown); the smallest (**A, B, D**) is probably the middle ACT (*MACT*) that projects directly to the ventral side of the calyces (*Ca*). Sections 47 (**A**), 52 (**C**) in Ms1, 47 (**B**), and 48 (**D**) in Ms2. *PVT1* Postero-ventral tract 1; other abbreviations as in Fig. 1. Sampled ALs, staining, and scale bar as in Fig. 2

not be resolved in our Bodian-stained preparations. The more coarse-grain description obtained is informative, however. Only three main tracts – TA (Figs. 3C, D, 4A, F), TB (Fig. 5A, B, D), and VLT (Figs. 5, 6) – can be distinguished from dorsal to ventral inside and immediately outside the AL. Their interpretation with respect to previous studies is given in Fig. 1.

Glomeruli with distinctive features in sections

Some glomeruli exhibit distinctive features such as shape, size, proximity to recognizable structures, and singularity of location. These glomeruli were present in all four ALs studied and have been called "primary glomeruli." They may form distinctive patterns with neighboring glomeruli that permit those "secondary glomeruli" also to be recognized. We have accepted as candidates

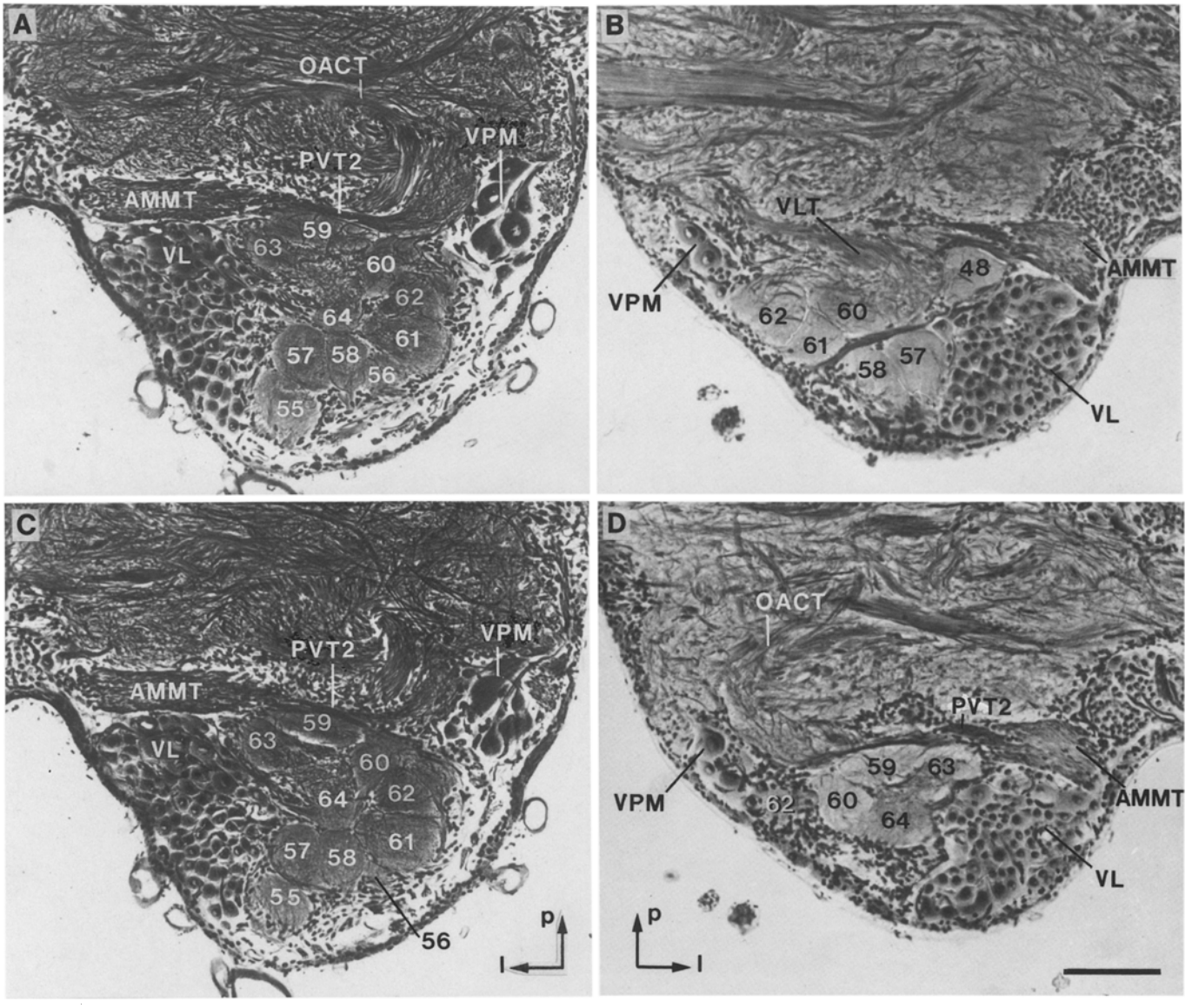


Fig. 6A–D. Ventral aspect of ALs with directly identified glomeruli of group VI and outer ACT (*OACT*). Primary glomeruli G59, G60, G63, and G64 (**C, D**) form a typical quadrangle. Secondary glomeruli G55 (**A, C**, see also Fig. 5C, B) G57 (**A–C**, also Fig. 5B, D), and G61 and G62 (**A, C**). The connection of the posteroventral tract 2 (*PVT2*) with the *OACT* has a characteristic appearance (partly visible in **A**): *PVT2* crosses the lateral edge of *OACT* while keeping anteriorly in contact with the glomerular layer, then turns posteriorly and enters the median edge of *OACT*. Sections 58 (**A**), 59 (**C**) in Ms1, 51 (**B**), and 54 (**D**) in Ms2. Other abbreviations as in Fig. 1. Sampled ALs, staining, and scale bar as in Fig. 2

for the latter class only glomeruli that could be found (1) directly in sections without reconstruction and (2) in at least three of the four ALs studied. Because such direct visual matching of glomeruli in different ALs can be achieved only on the same section or a few successive sections, those glomeruli appear in five distinct groups, in addition to the MGC. These glomeruli are listed in Table 1, and their locations in the ALs are given in Fig. 7.

Macroglomerular complex. The MGC comprises at least two subunits, of approximately equal volumes, which have been described and studied functionally (Hansson et al. 1991). Subunit MGC-A, the most dorsal, resembles a cumulus cloud and is located laterally (Fig. 2A, B). Subunit MGC-B is toroidal. Dorsally it is located medial to MGC-A, both subunits having a wide common edge oriented posteroanterioly (Fig. 2A, B). In a more ventral plane, MGC-B is posterior to MGC-A (Figs. 2C, D, 3A–C) and, finally, continues ventrally underneath MGC-A in a lateral position (Figs. 3D, 4A–E). The ventral subdivision is more clear-cut in Ms1 than in Ms2.

Group I. Identification of glomerulus G20 is easy and certain. It is the largest ordinary glomerulus (ca. 100 µm in diameter). G20 lies posterolaterally and is contacted on its posterior side by the protocerebral neuropil and, on its anterior side, by the MGC. A tract of antennal fibers enters the lateral side of G20, and another fiber tract is connected to its medial side (Fig. 3C, D).

Table 1. Glomeruli that appear to be identifiable in sections

Group	Glomerulus[a]	Features
I	*G20*	Largest ordinary glomerulus, posterior to MGC-A & B
	G24	Adjacent to MGC-B, anterior to G20
II	*G35*	Adjacent to ventral part of MGC-B, near cell group VL
III	*G46*	Between tracts PVT1 and VLT (medially)
	G48	Same as G46, laterally, near cell group VL
	G40	Dorsal to G48, ventral to G20, elongated
	G37	Adjacent to G40 (medially) near TA and PAT
	G31	Adjacent to G40 (posteriorly) near cell group P
	G34	Adjacent to G40 (laterally) between G31 and MGC-B
IV	*G52*	Adjacent to VLT and TB
	G44	Adjacent to G52 (dorsally)
V	*G64*	Most ventral glomerulus, adjacent to VL
	G63	Adjacent to G63 and VL (posteriorly)
	G59	Adjacent to G63 and PVT2 (posteriorly)
	G60	Adjacent to G59, G63, G64 (medially)
	G62	Adjacent to G60 (medially)
	G61	Adjacent to G62 (anteriorly)
	G57	Adjacent to G64 (anteriorly) and VL
	G55	Adjacent to G57 (anteriorly) and VL

[a] Glomeruli in *italics* are primary glomeruli with respect to which the other (secondary) glomeruli are identified

G24 (Figs. 3C, D, 4A, B) is contacted on its lateral side by the MGC and, on its posterodorsal edge, by G20 (3 ALs). Ventrally, on its posterior side, G24 contacts G34 of group III and, on its anterior side, G35 of group II (3 ALs).

Group II. The ventral end of G35 (Fig. 4B–E) is located in the horizontal plane in which the ventral end of the MGC meets the dorsal end of cell group VL. In this plane the glomerular sections are arranged in a "C" shape, the opening of which is occupied by the MGC and the VL group. G35 is located at the end of the anterolateral branch of the "C," and its ventroanterolateral edge contacts the dorsal pole of group VL. Dorsally G35 is in contact with or close to G24 of group I (3 ALs).

Group III. The identification of adjacent glomeruli G46 and G48 (Fig. 5A–D) is direct and certain because of their characteristic positions with respect to the surrounding fiber tracts. Ventrally, they lie between the tract leaving the VL cell group, which is anterior, and the PVT1 tract, which is posterior. G46 is located medially inside the angle formed by these tracts and G48 laterally, close to VL. Tract TB is close to or in contact with the medial side of G46.

G40 (Fig. 4D–E) is located dorsally with respect to G48 and has a characteristic, elongated shape. Its long axis is horizontal in one of the individuals studied and tilted in the other.

G37 (Fig. 4B–E) is located medially with respect to G40. Tract TA runs along the dorsal side of G37 and then along its posterior side and leaves the AL. At this level tract PAT runs along the posterior side of G37.

G31 (Fig. 4A–D) begins in the horizontal plane tangent to the ventral side of G20 (group I). At this level the anterior side of G31 contacts the medial fascicle of fibers entering G20. Ventrally, in the plane where G40 begins, G31 and G40 are adjacent, the former located posteriorly or posterolaterally with respect to the latter. Finally, cell group P is located on the ventral side of G31, either in contact with its posterior side (1 AL) or more medially. Tract PAT runs along its ventral side, and the antennal fibers that enter it run along the ventral side of G20.

G34 (Fig. 4A–E) is located ventromedially with respect to G20 and anterolaterally with respect to G31. More precisely, in the horizontal plane where G40 begins, G34 is located on the straight line joining G31, posterior and medial, to the ventral side of the MGC, anterior and lateral. These aligned profiles constitute a characteristic pattern. Also, G34 is innervated by antennal fibers that run along the posterior side of the MGC.

Group IV. G52 (Fig. 5B–D) ends ventrally in or close to the horizontal plane where the VL tract leaves the AL. At this level, G52 is located posteromedially with respect to tract VLT. The dorsal side of G52 is adjacent to tract TB, which runs dorsally (1 AL) or caudolaterally between this glomerulus and G46.

G44 is dorsal to G52. In one individual (Fig. 5A), tract TB runs on the lateral side of G44. In the other individual (Fig. 4D), however, this tract is less visible and passes more ventrally at the level of G52.

Group V. G64 is easily recognizable because it is bulky and the most ventral glomerulus. G59, G60, and G63 are located between its posterior side and the protocerebral neuropil. These four glomeruli make up a characteristic pattern (Fig. 6C, D). G59 is elliptical and elongated mediolaterally along the small tract PVT2. G63 is located posterolaterally to G59 and in contact with cell group VL. The lateral end of G63 is in contact with the AMMT. G60, the most medial, is located between the ventral branch of VLT and G64.

Three other glomeruli in the same area can be recognized with respect to the previous group in more dorsal planes. G62 (Fig. 6A–D) lies on the medial side of the AL and, on its lateral side, is adjacent to G60. G62 is located close to the small cell group VPM. G61 (Fig. 6A–C) is posterior to G62 and extends radially between the medial edge of the AL and G64.

Glomeruli G55 and G57 (Figs. 5B, C, 6A–C) can be recognized in the horizonal plane tangent to the dorsal side of G64. They are located posteriorly, in contact with the VL cell group. The ventro-posterior end of G57 is in contact with G64 except in one AL (left Ms1), where it is separated from G64 by an anomalous glomerulus (G66). G55 is more posterior and dorsal than G57.

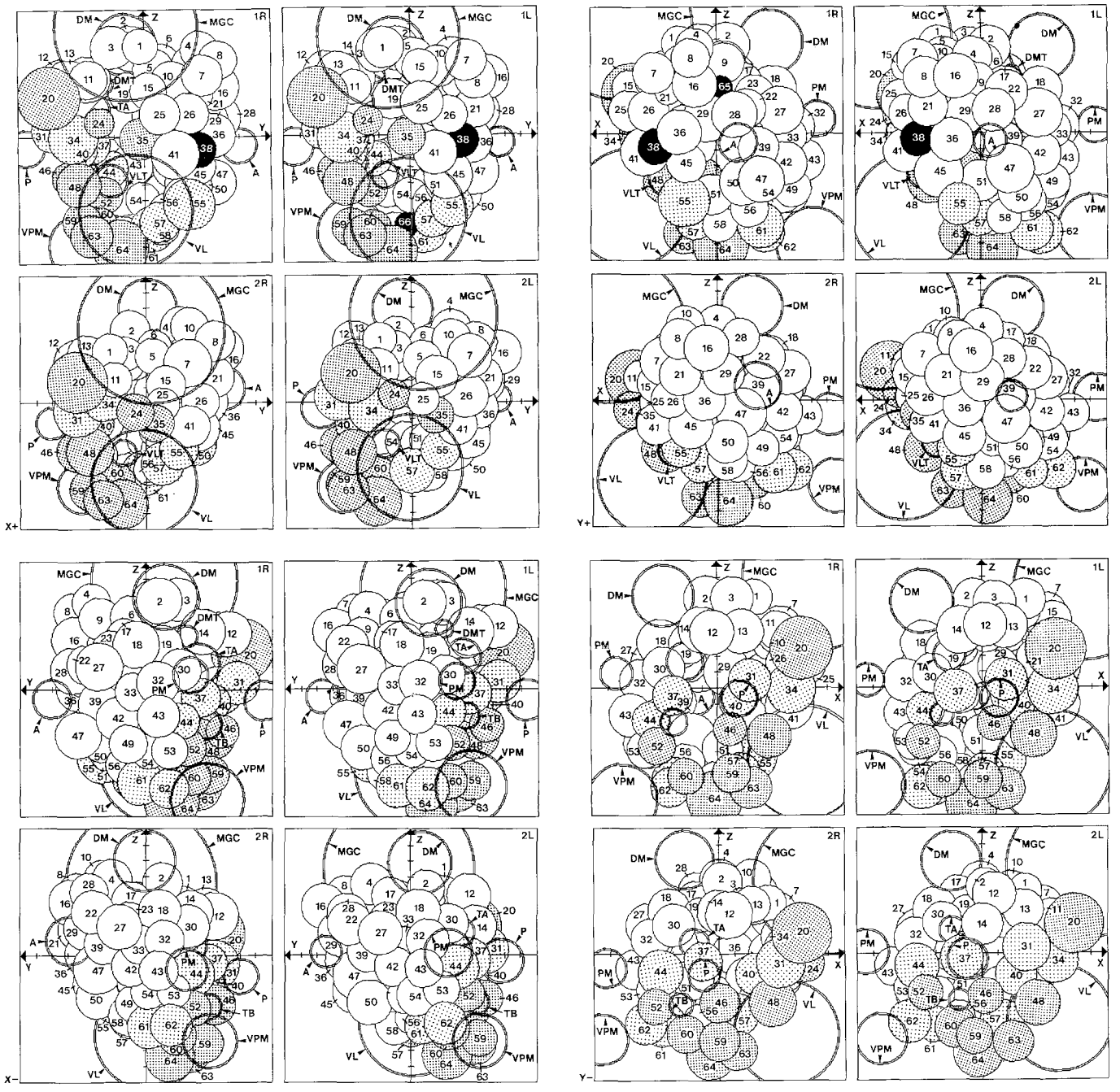
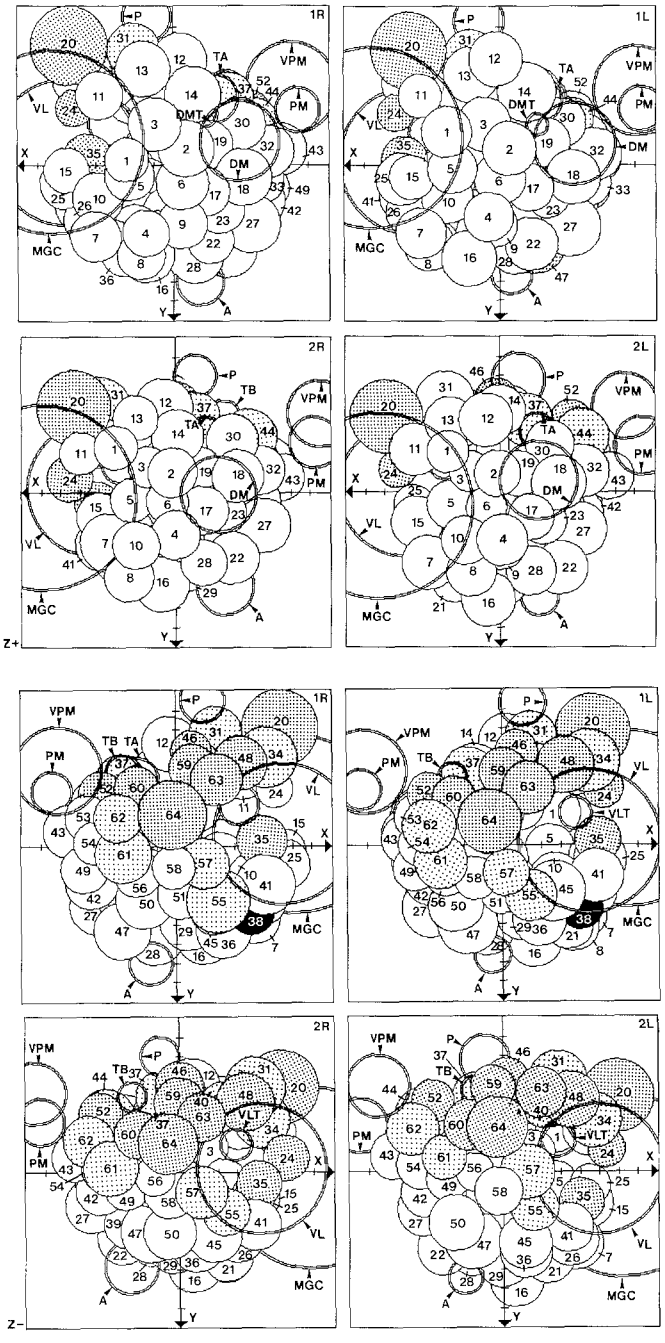


Fig. 7X–Z. Computer reconstructions of ALs in two adult male *M. sexta* brains. ALs are viewed from outside by an observer located successively on each of the six main axes. **X+** Lateral view; **X−** medial; **Y+** anterior; **Y−** posterior; **Z+** dorsal; **Z−** ventral. *Arrowheads* on axes within the individual views point in the positive direction (X+, Y+, Z+). For each point of view, the organization of right and left ALs of Ms1 (noted *1R* and *1L*) and Ms2 (*2R* and *2L*) can be compared intraindividually (in a row) and interindividually (between rows). Homologous glomeruli have the same numbers. Glomeruli that are candidates for direct identification are *dark gray* (primary) and *light gray* (secondary); anomalous glomeruli are *black*. MGC and nonglomerular structures (cell groups and tracts) are shown as *double rings*, as if transparent. Diameters and locations of circles give quantitatively accurate representations of the sizes and locations of corresponding structures in brains, except for tracts, in which case diameters of circles have no significance and centers indicate the location where the tracts cross the glomerular layer (*TA, TB*) or leave cell groups (*DMT, VLT*). Abbreviations as in Fig. 1. Length of square frame is 400 μm

Glomeruli with anomalously small sizes or uncertain outlines

Three glomeruli (observed only in Ms1) were represented by small profiles in five or fewer sections. The smallest ones are glomeruli Ms1-R-G65 (Fig. 3A, C; Fig. 7 Y+) and Ms1-L-G66 (Fig. 7 X+). Both appear in only four successive sections; at <40 μm in diameter, they are the smallest glomeruli observed in this study. They can be considered neither as erroneously delimited glomeruli,



in view of the excellent definition of their outlines, nor as homologous units, because they are not located in the same part of the ALs, G65 being dorsal and G66 ventral. Moreover, no glomerulus of such small size is present in Ms2.

Glomerulus Ms1-R-G21 is slightly larger and located in the same region as G65 (Fig. 3C, Fig. 7 Y+). Its definition is not as clear as that of G65 and G66, and it could be interpreted as an outgrowth of G7. No glomerulus of such small size is present in the same region in any of the other ALs. These observations suggest that these three glomeruli are anomalous, either because they are single and have no homologue in other ALs, or because their homologues are significantly larger. Other observations (see below) support the former hypothesis in the case of G65 and G66 and the latter in the case of G21.

About ten cases of uncertain boundaries between adjacent glomeruli, involving a total of 25 glomeruli in the four ALs studied, were encountered in this study. In seven of these cases, a satisfactory solution was found upon detailed examination, either by dividing or merging the initial glomerular masses. The other anomalies come from histological uncertainties that have not been solved completely. They involve the pairs of glomeruli Ms1-G22/G28 (Fig. 4A), Ms2-G15/G25 (Fig. 3D), and Ms2-G54/G61 (Fig. 5D), which are not clearly separated, especially in the right ALs. The histological data are compatible with these subdivisions but do not give decisive evidence for them.

Visual matching of glomeruli in computer reconstructions

Computer-graphic reconstructions of the four ALs were prepared, which unlike histological sections show the whole ALs and correct the misorientation of the sectioning planes. These reconstructions (Fig. 7) and their analysis (Figs. 8 and 9) are shown completely, allowing for an independent revision of this part of the work by anyone who wishes to pursue it. This analysis aims at matching the glomeruli in the different ALs in the course of three kinds of comparisons. The first kind involves the intraindividual (right-left) comparisons in Ms1 and Ms2; each can be done in only one step. Second, interindividual comparisons are performed on right ALs then on left ALs; they are more involved and need two steps. Finally, all ALs are compared simultaneously. These three kinds of comparison are summarized below. They permit the matching of almost all glomeruli. Throughout this section the numbering of glomeruli is a priori independent in the four ALs examined, e.g., Ms1-R-G48, Ms1-L-G48, Ms2-R-G48, and Ms2-L-G48 are considered as a priori unmatched glomeruli (even in the case in which they were shown above to have characteristic morphological features). The broader significance of the numbering system used is established only a posteriori.

Intraindividual comparisons (Fig. 7). Comparison of the right and left reconstructed ALs of MS1, as seen from a lateral point of view (see Fig. 7 X+ top row), shows that in both ALs, glomeruli form almost identical patterns. In the right AL, two relatively bulky glomeruli (G20 and G34) and a smaller one (G24) form a characteristic triangular pattern that can be found in the left AL in the same position and with the same orientation. Left glomeruli must therefore be matched to their right homologues and given the same name, as shown in Fig. 7. There is no acceptable alternative to these matchings, e.g. the matching of Ms1-R-G20 with Ms1-L-G24 or of Ms1-R-G20 with Ms1-L-G34 is clearly unacceptable because they break the spatial pattern. In this case, as in all others viewed similarly (with one exception, see below), there is one and only one solution that preserves in one AL the patterns observed in the other.

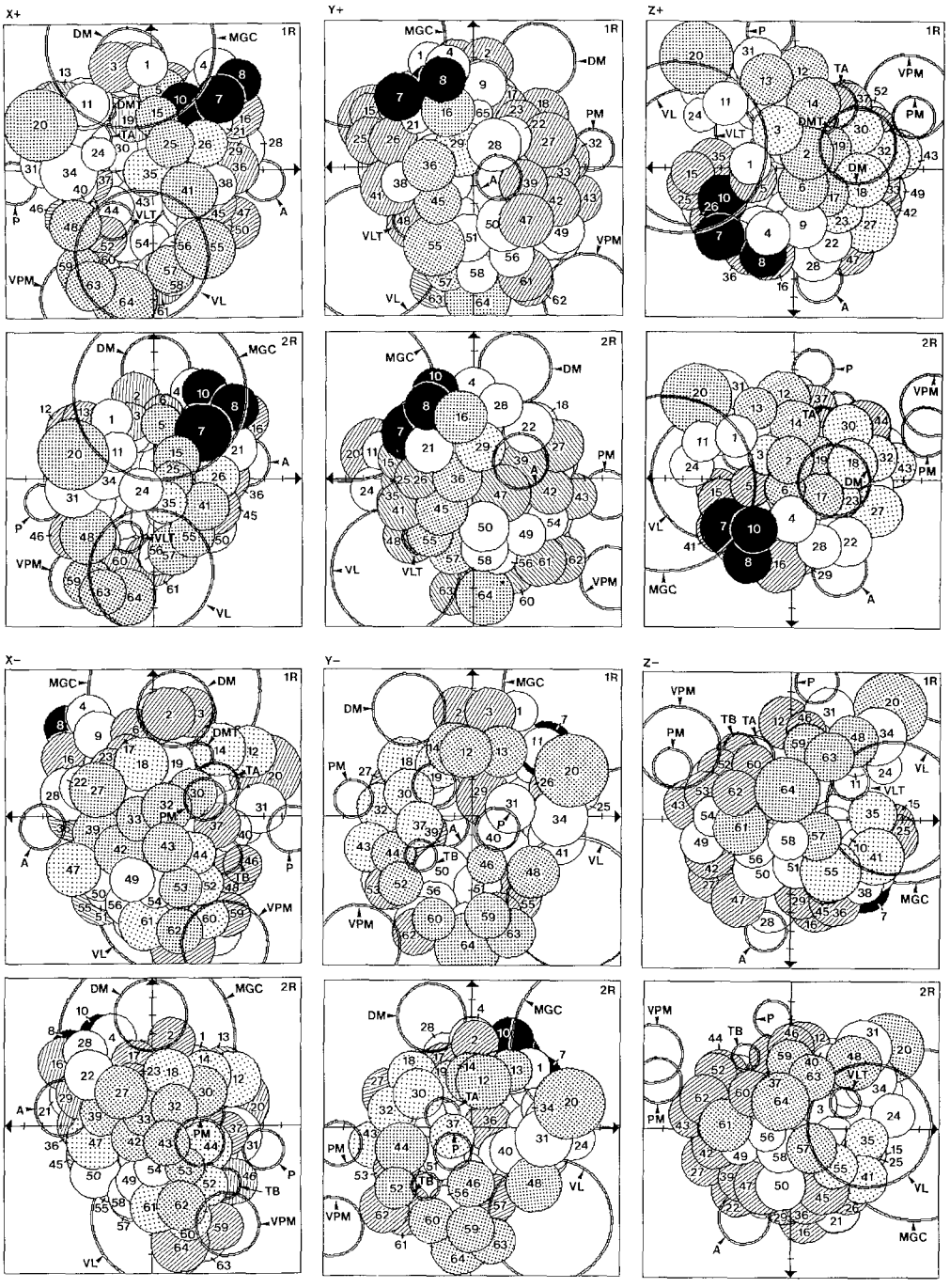


Fig. 8. Interindividual comparison of right ALs. Same computer reconstructions as in Fig. 7, arranged in pairs of comparable views of Ms1 (*1R, top*) and Ms2 (*2R, bottom*). Matchings of glomeruli done independently for each pair of views by the criterion of relative position (RP) and shown as found at the next-to-last step of the matching process. *Dark gray* glomeruli form the same patterns in the pair of views. *Light gray* glomeruli form slightly distorted but nonetheless clearly recognizable patterns. *Hatched* glomeruli are unmatched in this pair of views but matched (i.e., gray) in another one. *White* glomeruli are unmatched in all pairs. When reexamined at the last step of the matching process, most "white" glomeruli can also be matched unambiguously by the RP criterion, except for some adjacent glomeruli. Those are shown in *black* (see Table 4). Abbreviations as in Fig. 1

This example illustrates the principle of *visual matching by relative position (RP)* that has been used systematically in the present analysis. Because displacements and size differences as large as that shown by G24 with respect to G20 are rare in adjacent glomeruli, however, glomerular size was not used in practice. Therefore relative position prevails over relative size as a general and sensitive matching criterion in the present investigation.

In a few cases the RP criterion is not decisive when applied to one of the six pairs of views. Reexamination of these glomeruli shows that their patterns are generally unclear in only one pair of views and can be settled in the others. This is the case of Ms1-G8 and G16 in Z+, Ms1-G45 (hidden behind G55) in Z−, and Ms2-G25 and G35 in X+. The exceptions (Table 4) are Ms1-G7 and G8 (X+ and Y+), Ms2-G12 and G14 (X−,

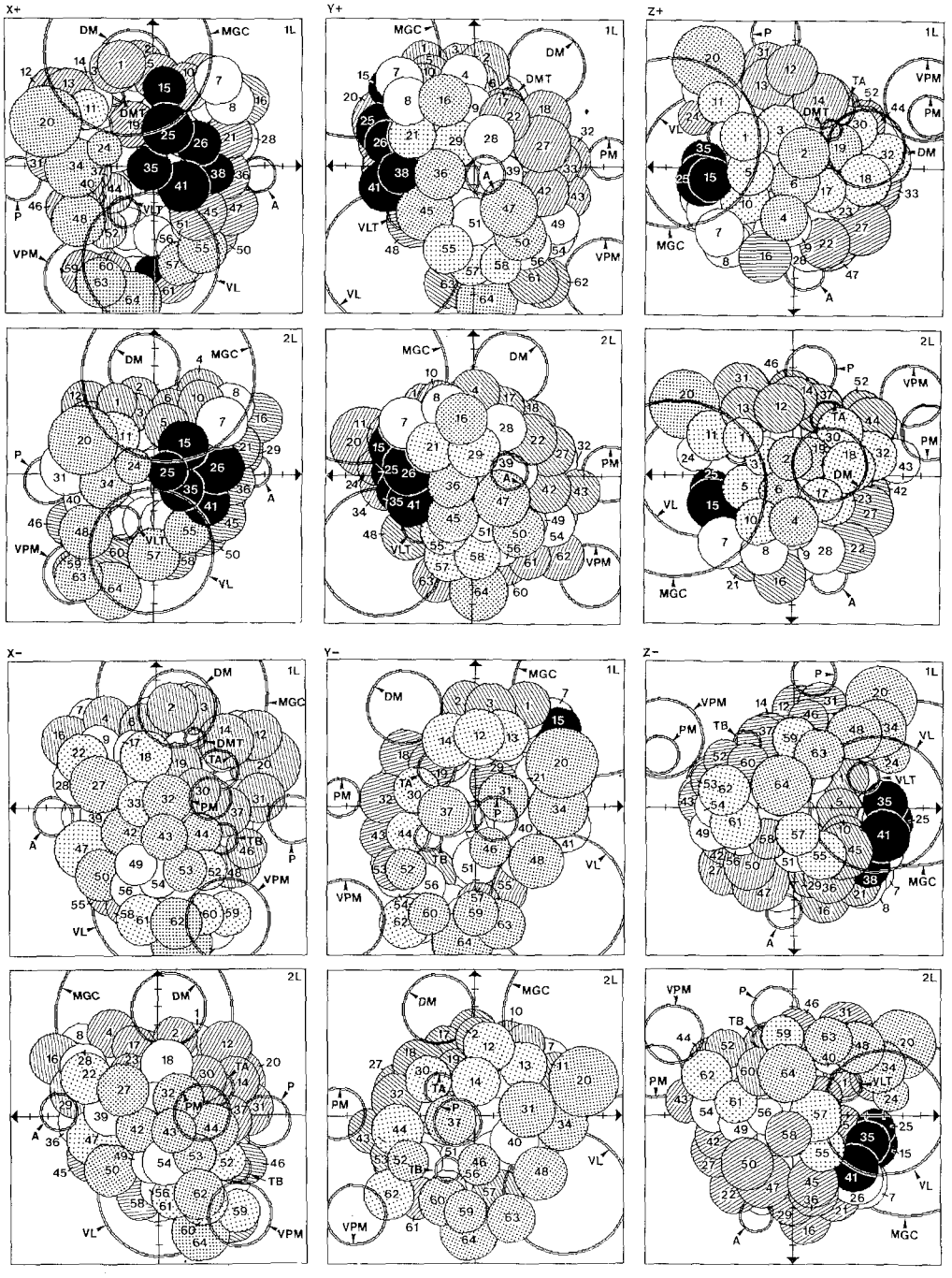


Fig. 9. Interindividual comparison of left ALs. Same legend as in Fig. 8

Y−, Z+), and to a lesser degree Ms1-G47 and G50 (X− and Y+). For all other glomeruli, visual RP matchings between right and left ALs were found to be unambiguous.

Interindividual comparisons (Figs. 8, 9). Comparison of ALs of different individuals, for example the right ALs, shows at once that interindividual variability in the positions of glomeruli is greater than intraindividual variability. The same method of matching, however, can be applied confidently to these ALs if performed step by step, i.e., without immediately attempting to match all glomeruli visible in any pair of views. The first step in the analysis aims at a careful distinction of the clear ("dark gray"), less clear ("light gray"), or unclear ("white") glomerular patterns in each pair of views. In the second step the "white" glomeruli are reexamined in isolation. Then, most of them can be matched, whereas the others are not matchable by the RP criterion alone ("black" glomeruli; Ms1-G7 and G8, Ms1-G47 and G50, and Ms2-G12 and G14, described above, are examples of intraindividual "black" glomeruli). The feasibility of this approach may be better appreciated in specific examples.

Step 1. Consider the pair of lateral views Ms1-R-X+ and Ms2-R-X+ (see Fig. 8 X+, first column). Two outstanding glomeruli in both ALs are G20 (on the left)

Table 2. Crossreference list of ordinary glomeruli

Glomeruli		Photomicrographs[a]		Computer reconstructions[b]						Comparisons[c]	
No.	Type[d]	Ms1-Right	Ms2-Left	X+	X−	Y+	Y−	Z+	Z−	1R/2R	1L/2L
1		2C	3B	*	−	−	*	*	−	.	g
2		2C	2D	−	*	−	*	*	−	d	d
3		2C	3B	*	−	−	+	*	−	g	g
4		2C	2D	−	*	*	−	*	−	.	d
5		3A	3B 4F	*	−	−	−	*	−	d	g
6		3A	2D	+	−	−	−	*	−	d	d
7		3A 3C	2D 3B	*	−	*	*	*	−	[b]	.
8		3A	2D	*	−	*	−	*	−	[b]	.
9		3A	2D 3B	−	−	−	−	+	−	.	.
10		3A 3C	3D	*	−	−	−	*	−	[b]	g
11		3A	3B 4F	*	−	−	−	*	−	.	g
12		3A 3C	3B 4F	*	*	−	*	*	+	d	g
13		3A 3C	4F	*	−	−	*	*	−	d	g
14		3A 3C	3D 4F	−	*	−	*	*	−	d	g
15		3A 3C	3B 3D 4F	*	−	*	−	*	+	d	[b]
16		3A	2D	*	*	*	−	*	−	d	d
17		3A 3C	2D	−	*	+	−	*	−	d	g
18		3A 3C 4A	3B 4F	−	*	*	*	*	−	g	g
19		3A 3C	3B 4F	−	−	−	*	*	−	d	d
20	P	32A 3C 4A	3D 4B 4F	*	*	*	*	*	*	d	d
21		3C	3B 4F	*	−	*	−	−	+	.	.
22		3C	3B 4F	−	*	*	−	*	−	.	g
23		3A 3C 4A	3B 4F	−	+	−	−	*	−	g	g
24	P	4A	3D	*	−	−	−	*	*	.	g
25		4A	3D 4B	*	−	*	−	+	*	d	[b]
26		3C 4A	3D 4F	*	−	*	−	−	−	g	[b]
27		4A	3B 3D 4F	−	*	*	*	*	−	d	d
28		3C 4A	2D 3B	−	*	*	−	*	*	.	.
29		3C 4A	3B 4F	−	−	*	−	−	*	g	g
30		4A	3B 3D 4F	−	*	−	*	*	−	d	g
31	S	4A 4C	3D 4B	*	*	−	*	*	−	.	d
32		4A	3D 4D 4F	−	*	*	*	*	−	d	d
33		4A	3D	−	*	−	−	−	−	d	g
34	S	4A 4C 4E	4B 4D	*	−	−	*	−	*	.	d
35	P	4C 4E	4B 4D	*	−	−	−	−	*	g	[b]
36		4A 4C 4E	3D	*	*	*	−	−	*	d	d
37	S	4C 4E	4B 4D	−	*	−	*	+	*	g	d
38	A	4C 4E	Absent	*	−	*	−	−	*	.	.
39		4C 4E	3D 4F	−	*	*	−	−	−	d	.
40	S	4C 4E	4D	*	−	−	*	−	−	.	.
41		4C 4E 5A	4B 4D	*	−	*	−	−	*	d	[b]
42		4E 5A	4B	−	*	*	−	−	*	d	d
43		4C 4E 5A	4B 4D	−	*	*	*	+	*	d	d
44	S	5A	4B 4D	−	*	−	*	*	−	d	d
45		5A	4B 4D	*	−	*	−	−	*	d	d
46	P	5A 5C	5B 5D	*	*	*	*	−	*	d	d
47		5A 5C	3D 4B 4D	−	*	*	−	−	*	g	d
48	P	5A 5C	5B 5D 6B	*	−	*	*	−	*	d	d
49		5A 5C	4D	−	*	*	−	−	*	.	.
50		5A 5C	4D	*	*	*	−	−	*	.	d
51		5C	4D	−	−	+	*	−	−	.	.
52	P	5C	5B 5D	−	*	−	*	−	*	d	d
53		5C	4D	−	*	−	*	−	−	d	d
54		5C	4D 5B	−	*	*	−	−	+	.	.
55	S	5C 6A 6C	5B 5D	*	+	*	−	−	*	d	d

[a] Crossreference to Figs. 2A–6D indicating on which sections a glomerulus is shown

[b] Crossreference to Fig. 7X+ to Z− indicating on which views a glomerulus is visible: * visible in four ALs, + visible in only three ALs, − visible in fewer than three ALs or invisible. This classification does not apply to anomalous glomeruli

[c] Crossreference to Figs. 8 (column 1R/2R) and 9 (column 1L/2L) indicating the type of matching in the right and left interindividual comparisons respectively: d "drak gray" (no distortion), g "light gray" (slight distortion), or [b] "black" (ambiguous relative positions). "White" glomeruli (.) are also unambiguous but could not be immediately classified "dark gray" or "light gray"

[d] Types are: P, primary; S, secondary (see Table 1); and A, anomalous. G38 is present in Ms1-R and Ms1-L, G65 in Ms1-R, and G66 in Ms1-L

Table 2 (continued)

Glomeruli		Photomicrographs[a]		Computer reconstructions[b]						Comparisons[c]	
No.	Type[d]	Ms1-Right	Ms2-Left	X+	X−	Y+	Y−	Z+	Z−	1R/2R	1L/2L
56		5C 6A 6C	5B 5D	+	+	*	*	−	*	.	.
57	S	6A 6C	5B 5D 6B	*	−	*	*	−	*	d	d
58		6A 6C	5B 5D 6B	−	+	*	−	−	*	.	g
59	P	6A 6C	6D	*	*	−	*	−	*	d	d
60	P	6A 6C	6B 6D	*	*	−	*	−	*	d	d
61	S	6A 6C	5D 6B	+	*	*	*	−	*	d	g
62	S	6A 6C	5B 5D 6B 6D	−	*	*	*	−	*	d	d
63	P	6A 6C	6D	*	*	*	*	−	*	d	d
64	P	6A 6C	6D	*	*	*	*	−	*	d	d
65	A	3C	Absent	−	−	*	−	−	−	−	−
66	A	Absent	Absent	*	−	−	−	−	−	−	−

and G64 (at the bottom). From G64, two main "lines" are recognizable. One line is directed toward G24 and includes G63, G59, and G48. The relative positions of these four glomeruli are identical in Ms1 and Ms2 and permit easy matching of these glomeruli. These are "dark gray" glomeruli (so represented in Fig. 8). Conversely, glomeruli located between G48 and G20, especially G31 and G34, show different relative positions that do not permit matching. These are "white" glomeruli (white in Fig. 8). The second main line ascends in the vertical plane XOZ and includes G57, G55, G41, G25, G15, and G5. These glomeruli resemble beads in a necklace, and there is only one way to match them according to the RP criterion ("dark gray" pairs). On both sides of this line are two glomeruli. G26 and G35, that form a diamond pattern with G41 and G25. Because they are matched in a second stage and their neighbors (e.g., Ms1-R-38 and Ms2-R-24) do not form clear patterns with them, they are considered as "light gray" pairs. All other readily visible glomeruli, such as G24, G11, G1, G7, G8, and G10, are not clearly matchable and thus left "white" (some of them are shown "hatched" or "black" on Fig. 8 for reasons explained below).

The same procedure applies to all pairs of views showing the right ALs (Fig. 8) and left ALs (Fig. 9) of Ms1 and Ms2. As a general rule glomeruli G20 and G64 stand out as clear landmarks in all ALs. Glomeruli near the center of the views are the most easily matched, while those at the margins, observed tangentially, cannot be matched.

At this stage, the glomeruli can be assigned to three classes: the "dark gray" class of glomeruli (each of which was a member of a "dark gray" pair), the "light gray" class that belonged at best to a "light gray" pair, and the "white" class of glomeruli that were never matched. This classification is detailed in Table 2. The numbers of glomeruli in these classes are 34, 9, and 21 in comparison Ms1-R/Ms2-R and 30, 18, and 16 in comparison Ms1-L/Ms2-L, i.e., 66% and 75% of glomeruli, respectively, were matched visually at this stage.

Glomeruli that were left unmatched (white) in some views but matched (dark and light gray) in others were coded "hatched" in Figs. 8 and 9 to signal this fact. The final "white" glomeruli are those that were not matched in any view. As could be expected, "hatched" glomeruli are located mostly at the margins of the ALs, whereas the "gray" glomeruli are in the center. Some "hatched" glomeruli, however, are clearly visible in some views and permit checking of their relative positions with respect to their matched neighbors (see for example G19, G18, G32, G43, and G53 in Fig. 9 Y−). Two displacements were spotted in this reexamination, G14 with respect to G30-G12 (X−) and G45 with respect to G55 (Z−), but no patent inconsistency. Thus, the various partial comparisons complete each other harmoniously. This checking is reconsidered on a broader basis below.

Step 2. The truly unmatched "white" glomeruli left at the end of the first step were analyzed separately in the right and left interindividual comparisons and gave other examples of harmonious completion of matchings. The unmatched glomeruli of the right (Ms1-R/Ms2-R) and left (Ms1-L/Ms2-L) comparisons are gathered into four distinct spatial groups (listed in Table 3) that correspond fairly well in both comparisons.

For example, the *right* anteroventral group G49, G50, G51, G54, G56, and G58 (Fig. 8 X−, Y+, Z−) contains the same number of glomeruli in both ALs. One of the reasons why this group remained unmatched is that Ms2-R-G51 is completely hidden behind other glomeruli in all views. This rare localization in a second glomerular layer in both ALs makes its matching certain. Although the subgroups G49 and G54 and G50, G56, and G58 exhibit internal displacements as well as displacements of one subgroup with respect to the other, their relative positions lead to only one possible matching. The *left* anteroventral group (Fig. 9 X−, Y+, Z−) involves the same glomeruli as its right counterpart except for G50 and 58. Their pattern in Fig. 9 Y+, where they are simultaneously visible, is compatible with only one matching, which is confirmed by the partial views (X−, Z+). Here again, the initial absence of matching is attributable not to any uncertainty about their relative positions but to partial masking in the computer reconstructions.

The same analysis applies to the three other groups

Table 3. Unmatched ("white") glomeruli in first step of interindividual comparisons

Unmatched glomeruli[a]		*n*	Figure	Comments
Anteroventral				
Right	G49, G50, G51, G54, G56, G58	6	8X−, Y+, Z−	Partial masking. G51 in a rare location (2nd glomerular layer) with Ms2-R-G51 completely hidden in all views
Left	G49, G51, G54, G56	4	9X−, Y+, Z−	Partial masking. Pattern in Y+ compatible with only one matching (confirmed by X− and Z+)
Posterolateral				
Right	G1, G11, G24, G31, G34, G40	6	8X+, Y−, Z+, Z−	G31, G34, G40 never simultaneously visible. Unusually large displacement of G1 and G24 (X+, Z+)
Left	G40	1	9Y−	Only unmatched glomerulus in both ALs behind quadrilateral G31, G34, G46, G48
Dorsoanteromedian				
Right	G22, G28, G9, G4	4–5	8X+, Y+, Z+	Anomalous (Ms1-R-G65), difficult histology (G22, G65, and G28), never simultaneously visible (G4 and G9, Ms2-R-G9 completely hidden)
Left	G28, G9, G39	3	9X−, Y+, Z+	Complete hiding of G9, partial obscuring of G39, slight displacement of G28
Dorsoanterolateral				
Right	*G38*, G21, *G7*, *G8*, *G10*	4–5	8X+, Y+, Z+	Ms1-R-G38 unmatchable; small Ms1-R-G21 matched to large Ms2-R-G21; subgroup G7, G8 and G10 not solvable by the RP criterion ("black" glomeruli)
Left	*G38*, G7, G8, *G15*, *G25*, *G26*, *G35*, *G41*	7–8	9X+, Y+, Z+, Z−	Difficult histology (Ms2-L-G15 and G25). Group not solvable by the RP criterion (except for G7 and G8). Extra glomerulus of Ms1 not identified at this stage

[a] Glomeruli listed are unmatched in Figs. 8 and 9 ("white"). Glomeruli in *italics* cannot be matched by the criterion of relative position ("black" glomeruli in Figs. 8 and 9)

and is summarized in Table 3. Once observed in isolation, the posterolateral and dorsoanteromedian groups are easily solved. The dorsoanterolateral group is the largest and most difficult to solve. It is impossible to propose a complete matching in this group because it includes one more glomerulus in Ms1 than in Ms2. In the *right* group the relative position suggests that Ms1-R-G38 should remain unmatched. Then the anomalously small Ms1-R-G21 must be matched to the larger Ms2-R-G21. The three remaining glomeruli, G7, G8 and G10, form a subgroup that is not solvable by the RP criterion. Indeed, each pair of views suggests different matchings (10:7, 7:8, 8:10 in X+; 7:7, 8:8, 10:10 or 10:8, 8:10 in Y+; 10:7, 7:8, 8:10 or 7:10, 8:8 in Z+). This does not come as a surprise, however, because G7 and G8 were already found to be difficult to match intraindividually in Ms1. Finally these three right glomeruli become "black" ones at this stage of analysis. The *left* dorsoanterolateral group could not be solved by the RP criterion, except for G7 and G8, which form a stable pattern in spite of their displacements. Consequently, the anomalous extra glomerulus could not be precisely identified, and the left subgroup G38, G15, G25, G26, G35, and G41 must also be labeled "black" at this stage of analysis.

Simultaneous comparison of the four ALs. Thirteen "black" glomeruli were left unmatched in one or more of the four (two intraindividual and two interindividual) comparisons carried out in this study (listed in Table 4). Except in the case of G7 and G8, these glomeruli can be matched, either logically from their known matchings (see Table 4) or equivalently by comparing the ALs not yet visually compared, i.e., Ms1-R with Ms2-L and Ms1-L with Ms2-R. This is equivalent to studying simulta-

Table 4. Glomeruli left unmatched ("black") by the criterion of relative position in at least one of the four comparisons performend

	Comparison				
	Intra	Inter	Intra	Inter	
Glom	Ms1-R	Ms1-L	Ms2-L	Ms2-R	Ms1-R
7	x− −?− −x	x	x	x −−?−−	x
8	x− −?− −x	x	x	x −−?−−	x
10	x	x	x	x −−−−−	x
12	x	x	x−−−−x		x
14	x	x	x−−−−x		x
15	x	x−−−−x	x	x	x
25	x	x−−−−x	x	x	x
26	x	x−−−−x	x	x	x
35	x	x−−−−x	x	x	x
38	x	x			
41	x	x−−−−x	x	x	x
47	x−−−−x	x	x	x	x
50	x−−−−x	x	x	x	x

x, Glomerulus found in the corresponding AL;
x——x, glomeruli matched in the corresponding comparison;
x−−−−x, glomerular matching deduced logically from the other three;
x− −?− −x, uncertain matching (ambiguous displacements of Ms1-R-G7 and G8)

neously all four AL reconstructions of each of the six points of view X+, X−, etc. This task can be conveniently done in Fig. 7, where all four ALs are shown side by side. Such a global examination can be attempted successfully at this late stage of analysis because overall glomerular organization is now well understood and attention can be focused on a small number of well-chosen glomeruli.

This simultaneous comparison of all ALs suggests that the unmatched glomeruli listed in Table 4 result not from unspecific spatial variations affecting all ALs but more probably from specific variation in positions of isolated or coupled glomeruli affecting only one AL at a time. This is the case of the intraindividually unmatched glomeruli of Ms1 and Ms2: unmatched G47 and G50 of Ms1 form very similar patterns in Ms1-L, Ms2-R and Ms2-L but are displaced in Ms1-R (see Fig. 7, Y+), and unmatched G12 and G14 of Ms2 form identical patterns except in Ms2-L (see Fig. 7 Y−). Similarly, most glomeruli in the interindividually problematic dorsoanterolateral group form a stable pattern except in Ms2-L. For example in Fig. 7 X+, G5, G15, G25, and G41 are lined up with G35 (posterior) and G26 (anterior) on both sides, except in Ms2-L where G25 moves away from the line and G15 comes closer to G41. Then the contralateral homologue of Ms1-R-G38, i.e., Ms1-L-G38, emerges as the anomalous supernumerary glomerulus of Ms1-L. Finally, the three unmatched glomeruli of this group (G7, G8, and G10) stand out as the only ones for which global examination gives no clear solution, because they are displaced in several ALs. In the tentative matching proposed in Figs. 7–9, G10 is slightly displaced in Ms1-R, whereas G7 and G8 are displaced in Ms1-L (see Fig. 7 X+, Z+).

Consistency of matchings

The glomerular matchings based on the RP criterion described above obey strong constraints of internal consistency. These tests of consistency show that the various matchings are consistent with one another and do lead to a unique solution.

Consistency of RP pairs for a given comparison. When all six pairs of views were compared independently, i.e., when stable patterns were found step-by-step all around the ALs of a pair, glomerular matchings were consistent because they fell into place in a kind of three-dimensional (3D) puzzle. This consistency was not a priori certain: matchings regarded as certain in two adjacent views could have been conflicting, either directly (a given glomerulus being paired differently in those views) or indirectly (because the same number of unmatched glomeruli would not have been present in the matched areas). A good example of the latter type is given by certain glomeruli initially left unmatched ("white") that finally happened to fill the holes of the puzzle because all of their neighbors were matched except themselves. A good case in point is the partially masked G40 in the left posterolateral group (see Table 3), which was found to be the only unmatched glomerulus in this region.

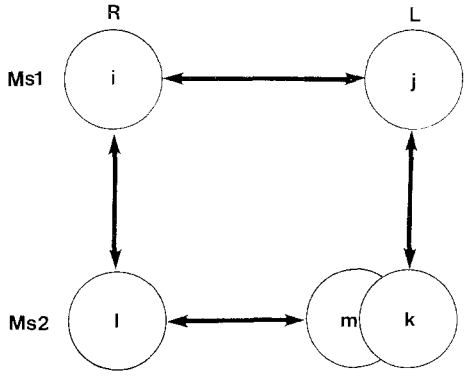


Fig. 10. Principle of the test of consistency of matchings between ALs. Four matchings of glomeruli by the RP criterion are performed directly within and between individuals (*arrows*). The matching not done directly between Ms1-R and Ms2-L (or equivalently Ms1-L and Ms2-R) can be derived logically in two different ways from the matchings already carried out. In the first way, Ms1-L is used as an intermediate; because glomerulus Ms1-R-Gi is intraindividually matched to Ms1-L-Gj and this Ms1-L-Gj is interindividually matched to Ms2-L-Gk, it follows that Ms1-R-Gi is logically matched to Ms2-L-Gk. In the second way, Ms2-R is used as an intermediate; Ms1-R-Gi is matched to Ms2-R-Gl, the latter is matched to Ms2-L-Gm, and then Ms1-R-Gi is logically matched to Ms2-L-Gm. The matchings are self-consistent if and only if Ms2-L-Gk and Ms2-L-Gm are the same glomerulus. This test applies only to the 51 glomeruli that were paired in all comparisons in this study, (i.e., all glomeruli except those mentioned in Table 4). In all cases, both logical paths end up in the same glomerulus. Therefore, the matchings achieved are self-consistent, which supports the idea that the RP criterion, when used cautiously (i.e., without attempting to impose a matching on unclear patterns), is objective and reliable

Consistency of RP matchings between comparisons. In two intraindividual and two interindividual comparisons, 89%–97% of the glomeruli were matched; moreover 80% of the glomeruli were matched simultaneously in all four comparisons. These four comparisons form a complete loop, which, starting from Ms2-L for example, goes through Ms1-L (inter left comparison), Ms1-R (intra Ms1), and Ms2-R (inter right) before coming back to Ms2-L (intra Ms2). If the loop would not close back on its starting point, the matchings done independently would contradict each other (see Fig. 10). Actually, no internal inconsistency of this type was found. Therefore the matching of the four ALs is unique and independent of the comparisons done for at least 80% of glomeruli.

Consistency of matchings in sections and in reconstructions. The matchings based on the RP criterion are identical to those based on morphological criteria in all cases in which the test can be applied, i.e., for the ten primary glomeruli. Most of these glomeruli are of the "dark gray" type in interindividual Ms1-R/Ms2-R and Ms1-L/Ms2-L comparisons, except G35 and G24. Similarly, as shown by the nine secondary glomeruli, the RP criterion gives the same matchings in sections and in computer reconstructions. This independent confirmation is valuable especially in the dorsoanterolateral group. This group (G38, G21, G7, G8, and G10 in comparison Ms1-R/Ms2-R, and G38, G15, G25, G26, G35, and G41 in

comparison MS1-L/Ms2-L) was labeled "black" in two interindividual comparisons of computer reconstructions because it exemplifies the main difficulties encountered in the analyses, i.e., a supernumerary glomerulus in Ms1, an intraindividual volumetric anomaly (Ms1-R-G21), and an ill-separated pair in Ms2 (G15/G25). The presence of one primary glomerulus in this group (G35) strengthens the homologies described previously and the interpretation of Ms1-G38 as supernumerary and Ms1-G21 as intraindividually anomalous in size.

Discussion

The hypothesis that the glomeruli in ALs are identifiable units was initially proposed for, and supported by extensive morphological and morphometrical analyses in, the cockroach *Blaberus craniifer* (Rospars and Chambille 1981) and the noctuid moth *Mamestra brassicae* (Rospars 1983). Other studies in the fly *Drosophila melanogaster* (Stocker et al. 1983, 1990; Singh and Nayak 1985; Pinto et al. 1988) and the honey bee *Apis mellifica* (Arnold et al. 1985; Flanagan and Mercer 1988), although based on less complete analyses, also gave evidence of glomerular identifiability. These earlier investigations supported the idea that the identifiability of AL glomeruli is a general property in insects (reviewed in Rospars 1988a; Rospars and Chambille 1989).

Computer-assisted reconstruction methods were first applied to the ALs of the sphinx moth *M. sexta* in preliminary studies aimed mainly at enumeration of glomeruli in male and female ALs (Schneiderman et al. 1983). Improved methods have been employed in the present, comprehensive investigation aimed at identifying the glomerular as well as nonglomerular components of ALs, based on computer-generated maps of ALs. We believe that maps such as that shown in Fig. 7 will lead to new hypotheses about the development and function of the ALs and prove to be important aids for experimental investigations in *M. sexta*, a species increasingly used for understanding the functional organization and physiology of the olfactory pathways in the central nervous system (for reviews see Christensen and Hildebrand 1987b; Homberg et al. 1989; Hildebrand et al. 1992).

The notion of proposing a unique map of AL glomeruli, and eventually AL neurons, is based on the demonstration that the glomerular maps of different individuals are almost identical, which means that the organization of glomeruli is invariant. This is shown in *M. sexta* by the direct identification in sections of some of the glomeruli on the basis of their own characteristics, and by the unique identification of all of the glomeruli on the basis of their constant relative positions in AL computer reconstructions. Four aspects of this invariance, which are summarized in the following statements, are examined in more detail below. (1) The identifiability of glomeruli is not all-or-none, because glomeruli meet a variable number of identification criteria. (2) The precision of 3D reconstructions is limited by uncertainties in delineating some glomeruli and finding the homologues of others. (3) Anomalous glomeruli exist and cannot be explained by these uncertainties. (4) Invariance applies to nonglomerular components as well as to glomeruli in the AL. (5) Specific conditions must be fulfilled and tests performed to demonstrate that the glomerular organization of a species is invariant.

Number and delineation of glomeruli

The normal number of glomeruli per male AL in *M. sexta*, not including the MGC, is 64 or very close to that number. A definite statement is hindered by the presence of anomalous glomeruli and of glomeruli that are difficult to delineate.

In this study, incorrect delineation of glomeruli in serial sections accounted for errors and delays in the complete reconstruction of an AL and determination of the homologies between ALs. The number of glomeruli concerned was 2–9 per AL, of which 2 in Ms1 and 4 in Ms2 were not conclusively solved from histological observations alone. The presence of 2 glomeruli can be certain, however, even if their boundaries are not unambiguous in all of their sections. The fact that the boundaries of some glomeruli are difficult to establish probably accounts for the variations in the number of glomeruli in ALs of male *M. sexta* reported by Schneiderman et al. (1983); 59, 59, and 61 for three ALs) and in this study (65, 65, 63, and 63). The differences (3–5 glomeruli) correspond to the normal uncertainty of counting in the absence of the supplementary information introduced by the identification of glomeruli. Actually, most delineation problems were raised and solved by intra- and interindividual comparisons. When a glomerular mass was clearly homologous to a pair of distinct glomeruli, in relative position and total volume, it was generally found that the mass comprised two closely juxtaposed glomeruli. If not, then the solution accounting for at least three ALs among four was selected, or the subdivision hypothesis was preferred because undifferentiation results from absence of evidence whereas subdivision can be clearly demonstrated. As a consequence, it is easier to demonstrate glomerular matchings than to establish the exact number of glomeruli, because the glomerular masses matched may be shown to be the same in the absence of knowledge about their delineation into one or two glomeruli. These two points are thus partially independent.

Three anomalous glomeruli were observed in one of the two individuals studied. A priori, several explanations may account for these anomalies: errors of delineation or matchings, partial separation of constant glomerular masses, or absence of homologues. The characteristics (good histological definition, unilaterality, very small size) of two of these glomeruli, G65 and G66, allow direct verification of their absence in the three other ALs and rejection of the idea that they result from errors of delineation or matching. These are the most fully verified exceptions to the invariance model and the only ones showing lack of symmetry between the ALs of the same individual, which was not observed previously in *M. brassicae* or *B. craniifer*. The small size

of these glomeruli suggests that they are supernumerary. A possible partial interpretation is that they are the sites of termination of antennal axons that normally terminate in neighboring glomeruli. The other anomaly (G38) is interindividual and therefore similar to those described previously for one male glomerulus and one female glomerulus in *M. brassicae* (Rospars 1983) and three glomeruli between nymphs and adult of *B. craniifer* (Chambille and Rospars 1985). The characteristics of G38 in Ms1 are not distinct enough to permit direct verification of its absence in serial sections of Ms2; one can show only that one glomerulus is lacking in the same area. The missing glomerulus of Ms2 was identified as Ms1-G38 in computer reconstructions. The status of Ms1-G38 remains to be established. Its normal size suggests that it is not supernumerary in Ms1 but is really missing in Ms2. If this interpretation is correct, then the actual number of ordinary glomeruli in male *M. sexta* would be 64 (G65 and G66 excluded).

This number is close to the 55–60 reported for *Bombyx mori* (Koontz and Schneider 1987). It is almost identical to the numbers determined under the same conditions, i.e., on the basis of a complete identification, in the noctuid moth *M. brassicae* (Rospars 1983), namely 64, 64, 65, and 65 in four male ALs (not including the MGC). This raises the question of the interspecific homology of glomeruli in phylogenetically related species, for which evidence was first obtained for the LPO glomerulus (see below) in several moths (Bogner et al. 1986; Kent et al. 1986) and a butterfly (Lee and Altner 1986).

Macroglomerular complex and LPO glomerulus

It has been shown in various species by anatomical and physiological methods that the MGC is the site of termination of axons of antennal neurons that respond specifically to the sex pheromone. The MGC of male *M. sexta* comprises two subunits, one of which (MGC-B) is toroidal and the other of which (MGC-A) resembles a cumulus cloud nestled above the toroid (see Hansson et al. 1991). This organization appears to be intermediate between previous descriptions in other moths. In *M. brassicae* (Rospars 1983), the MGC comprises two clearly distinct, egg-shaped glomeruli. In *B. mori, Lymantria dispar*, and *Antheraea polyphemus*, the MGC is made up of 3–4 subunits (Koontz and Schneider 1987). Koontz and Schneider reported that, in *B. mori*, "a central glomerulus (MGC-1) is partially surrounded by two large and one small ring-shaped glomeruli (MGC 2, 3, 4)." MGC-1 in this species might correspond to MGC-A in *M. sexta*, and MGC-2-4 to MGC-B.

The axons of the neuroreceptors in the sensilla of the labial-palp pit organ (LPO) project to an ordinary glomerulus in the ipsilateral AL called the LPO-glomerulus (Kent et al. 1986). It corresponds to the directly identifiable glomerulus G64 of this study. This conclusion is based on two criteria. (1) The LPO-glomerulus is located in a typical position in the posteroventral area of the AL (cf. Fig. 7 in Kent et al. 1986). Glomerulus G64 is in the same location, bulging near the ventral pole of the AL and close to the ventral end of the VL cell group (see Fig. 6 Z−). (2) In Ms1 a thick fiber tract of ventral origin that terminates in G64 is probably the end of the tract coming from the LPO via the first labial nerve and the subesophageal ganglion, described by Kent et al. (1986). Adjacent glomeruli, G62 medially and G63 laterally (see Fig. 6 Y−), with which G64 could possibly be confused, do not show any such innervation.

Nonglomerular invariance

To what extent do the nonglomerular elements, the cell groups and tracts, verify the invariance model? In *B. craniifer* (Chambille and Rospars 1981, 1985) and *M. brassicae* (Rospars 1983), fiber tracts can help in the identification of some glomeruli; this supports the idea of conservative mutual positioning. This point has been dealt with more fully in this paper.

The organization of the penetration sites of tracts TA and TB in the AL (see Fig. 1) appears different in sections and in computer reconstructions. Conservation of the relative positions of tracts and glomeruli is clear only in the latter. Consequently, in practice, tracts are excellent landmarks, but this does not imply the immediate identification of the adjacent glomeruli. Local reconstructions are still necessary to compensate for these variations. The same point applies to the tracts of neurites that leave the AL cell groups and enter the central neuropil of the AL. An interesting variation, however, is exhibited by the tract leaving group A, which in one AL is subdivided into two tracts by the interposition of a glomerulus. More generally, these cell groups always appear in constant relation to the adjacent glomeruli and can therefore be used as landmarks even for a group as bulky as VL.

Criteria of identification of glomeruli

According to a previous definition (Rospars and Chambille 1989), identification of a glomerulus requires fulfullment of the following two conditions: (1) it must be a stable unit, i.e., present in the corresponding (right or left) ALs of a given species, sex and stage of development, and (2) it must be recognized individually, i.e., similar to its homologues and distinct from all nonhomologues according to one or more criteria. The data gathered here from the four ALs of two male *M. sexta* moths permit consideration of the central problem of identification by comparison of ipsilateral ALs of different individuals, and of the related problem of bilateral symmetry. The criteria of recognition used in this work being only morphological, the question "are glomeruli in *M. sexta* identifiable?" becomes "are morphological criteria discriminative enough to demonstrate identifiability of glomeruli?" In this report the words "identification" and "identified" are used selectively to refer to glomeruli clearly meeting the conditions stated in the definition. Whenever these conditions have not been ful-

filled, the terms "recognition" and "matchings" have been preferred.

AL glomeruli may be assigned to three classes according to the number of their recognition criteria. (1) The most thoroughly identified glomeruli can be directly recognized in sections on the basis of their anatomical characteristics (location, shape, orientation, and volume). Ten such primary glomeruli (16%) were found (Table 1). Several (G20, G64, G46, and G48) have a strong individuality, which suggests that they could be recognized in sections of different orientation. The distinctive features of others probably depend more on the orientation of sections. (2) The second class includes glomeruli that also can be recognized directly in sections without reconstruction of the ALs. Their anatomical characteristics are not definite enough, however, and must be supplemented by their locations with respect to the primary glomeruli to ascertain their recognition. Nine secondary glomeruli were found (Table 1). (3) The minimal requirement for recognizing a glomerulus is that it be found repeatedly in the two-dimensional computer reconstructions of several ALs, i.e., that the supposedly homologous glomeruli are in approximately the same AL locations with the same positions relative to their neighbors. This individual recognition applies to all glomeruli, those of the first two classes included. In fact, 70% of them were recognized only in this way. This high proportion results from the number of glomeruli, their similar appearance, and the structural complexity of the ALs in *M. sexta*.

In all three classes the criterion of relative position (RP) is outstanding. It can be used for some glomeruli in sections and for all of them in computer reconstructions. The main criterion for recognition of a glomerulus in sections is its position with respect to fiber tracts and cell groups (first class) or to its neighbors (second class). Determination of relative positions in sections is hindered, however, by their defective orientation, the lack of a global view inherent in sectioning, and the fact that the third dimension, perpendicular to the sectioning plane, is inadequately assessed by the observer. For these reasons, most glomeruli recognized directly in sections are located in the posteroventral area of the ALs, in contact with the protocerebrum, where numerous landmarks are present. A similar observation was made in the cockroach *B. craniifer* (Chambille and Rospars 1981). This is unfortunate because these glomeruli are experimentally less accessible than those on the anterodorsal side of the AL. The relative size of this area is approximately the same in both species, which helps to explain why the proportion of primary and secondary glomeruli in sections is similar in *M. sexta* (30%) and *B. craniifer* (35%), although the number of glomeruli per AL is very different (107 in the latter species). Moreover, the patterns made by these adjacent glomeruli are characteristic of the sectioning plane used. The same glomeruli, viewed in histological sections with orthogonal sectioning planes, would form different patterns and, except for some primary glomeruli, would not be recognizable in those different sets of sections.

Computer reconstructions significantly aid the process of matching glomeruli by the RP criterion. A reconstruction is a set of six views corresponding to the six sides of an oriented cube that encloses the reconstructed AL. Each view shows an AL as seen from the outside by an observer located on the axis perpendicular to the side. Computer reconstructions correct for the three defects mentioned above and reflect only the experimental variability resulting from superimposition of sections and biological variability. Reconstructions show glomerular patterns on all sides, which is a major improvement over the limited view in sections, and display almost all glomeruli (which shows that glomeruli are arrayed in a single shell-like layer).

The feasibility of matching glomeruli by the RP criterion is established by comparing ALs two at a time. In given pairs of views, glomeruli are observed that define unambiguous local patterns. To be accepted a local pattern must either be devoid of apparent deformation for the direction of observation used (these are the pairs of "dark gray" glomeruli in Fig. 8), or have a deformation small enought to maintain the relative positions ("light gray" pairs) without any ambiguity. If there is the slightest uncertainty, either because glomeruli are not readily visible in the views, (e.g., glomeruli located at the margin of the AL) or because the glomerular pattern is not obvious, the glomeruli are left "white."

Glomeruli of the three classes do not have the same demonstrative value. The primary glomeruli meet clearly both conditions in the definition and can be regarded as identified. Because they do not otherwise differ from the other glomeruli, however, they support the identifiability of all glomeruli. For the glomeruli in the second and third classes to be regarded as "identified," the reliability of the RP criterion, which permits one to distinguish them, must be established first. Validation of the RP criterion (also called visual recognition) is based on the fact that if the RP criterion were not valid (or the glomerular patterns were poorly interpreted), then the various matchings would not be consistent with one another and would not lead to a unique solution. These tests of consistency show that the matching of ALs by the RP criterion in computer reconstructions is independent of the order in which glomeruli, views, and ALs (Fig. 10) are examined and identical with the matchings based on morphological criteria or on the RP criterion in sections. These properties support the conclusion that the RP matching is unique and make a good case for accepting relative position as an identification criterion and for regarding glomeruli matched in this way as identified.

Conditions of identification of glomeruli

What conditions must be fulfilled for the glomeruli in a species to be regarded as uniquely identified? The ALs considered here are supposed to be composed of glomeruli similar to each other and in sufficiently large number that special precautions must be taken to avoid confusing them. There are at least five such conditions, none of which can be relaxed without weakening the whole identification process.

1. At least two ALs from two different individuals must be compared. No claim to identification can be based on the reconstruction of only one AL.

2. The total number of glomeruli must be the same in the ALs investigated. Slightly different numbers, however, do not constitute proof of nonidentifiability because of the difficulty of precisely delineating some glomeruli and because of the presence of anomalous glomeruli. Conversely, equal numbers do not prove identifiability because the glomeruli are not necessarily the same. More specific criteria than the total number of glomeruli must be considered.

3. Glomeruli can be identified in sections if they possess distinctive morphological characteristics or are in constant relation with specific landmarks (tracts, other identified glomeruli, etc.). Identification is demonstrated only if the distinctive features are shown to be constant in at least two ALs (see first condition).

4. All glomeruli can be identified by the RP criterion in quantitative reconstructions of ALs, provided that orientational inaccuracies in serial sections (i.e., angles α and γ, see Materials and methods) are corrected. The identification of glomeruli is possible without correction in lateral (X+) and medial (X−) views if angles α are small, because the rotation of angle γ around X can be compensated easily by the observer. This compensation is not possible, however, for the other views along Y and Z; consequently, homologies are difficult to find and result in many uncertainties. Therefore, angles α and γ must be corrected when they exceed a few degrees.

5. Matchings based on the RP criterion must satisfy tests of internal consistency, both within and between comparisons, before being regarded as valid. Such tests can be made only if reconstructions are complete and all glomeruli are matched. Consequently, partial reconstructions or partial matchings do not provide a sound basis for identification.

6. The morphometric characteristics of glomeruli provide foundations for testing whether the homologous glomeruli have the same size and the same spatial coordinates. Up to now these tests have been applied completely only to *B. craniifer* and *M. brassicae*, with positive results. Similar and improved quantitative analyses are in progress in *M. sexta* (J.P. Rospars, in preparation).

Array of structurally identified glomeruli in M. sexta

The results obtained in this study may be summarized as follows. (1) Most glomeruli in the ALs of male *M. sexta* moths are arrayed in a patchy, shell-like monolayer. This array is not continuous but is interrupted by the MGC, cell groups, and fiber tracts, which occupy a large proportion of the "shell." (2) Each glomerulus maintains its location with respect to its neighbors in 3D space and is identifiable on the basis of this criterion alone, although the glomerulus may exhibit other specific features (e.g., shape and location with respect to nonglomerular elements such as tracts and cell groups). This 3D pattern is conservative between ipsilateral ALs and symmetrical between contralateral ALs and can be recognized consistently when viewed along orthogonal axes X, Y, and Z. (3) Among more than 250 glomeruli examined, only two (G7 and G8) have been found not to be soluble by the RP criterion because they are simultaneously anomalous in two ALs. The probability that fluctuations bring about the coordinated random displacement of two adjacent glomeruli, leading to the occupation of the same locus by two different glomeruli, seems to be <0.01. This suggests that the probability that a fluctuation will bring about an anomaly in relative positions of one glomerulus is $<(0.01)^{0.5}$, i.e. <0.1.

The usefulness and limits of this structural identification derive directly from the two conditions used for defining the notion of identification. The first condition restricts the fundamental definition to the comparison of ALs of different individuals of same species, sex, and age. The present results would not be affected by the observation of differences, however profound, between individuals of opposite sexes (sexual dimorphism), various stages of development (developmental variations), different species (interspecific variations), or between contralateral ALs in female *M. sexta* or in other species (bilateral dissymmetry). The second condition does not specify the criteria of identification that might also be morphometric, biochemical, physiological, developmental, etc. These extensions place the significance of the present results in a broad framework and raise the problem of a possible conflict of criteria. Thus, a glomerulus considered as identified from a morphological and morphometric point of view could possess a biochemical or physiological variability between ipsilateral ALs. Evidence of such a variability, affecting glomeruli identified morphologically (and morphometrically), would not weaken the value of that anatomical identification. It would merely invalidate the implicit generalization that two morphologically identified glomeruli are identical.

Consequently, the structural homologies are facts that must be taken into account and evaluated in their own right, independently of a more general theory of identifiable glomeruli that remains to be built. Indeed, only through the use of a set of well-defined morphological criteria will the generalization to other types of ALs and other criteria become profitable. In both cases, the initial morphological characterization will provide a sound basis for detection of similarities between sexes or correlations between criteria, and, with equal significance, possible physiological or biochemical variations, affecting anatomically homologous glomeruli.

The constancy of relative positions of glomeruli suggests consequences as to their microstructure, function, and development. Glomeruli appear not to be loosely connected structures but rather to be strongly connected ones ("invariance" hypothesis). This implies that the interactions among antennal neurons, AL neurons, and glial cells in the developing glomeruli involve not only a precise temporal sequence of events (Oland and Tolbert 1987, 1989) but also a precise use of positional information in ways that can be solved only through developmental studies.

The stability of the glomerular organization also opens the way to new and precise techniques of investi-

gation of the structure and function of the ALs. For example, stereotaxic applications could be considered because a microelectrode inserted at a particular set of Cartesian coordinates should encounter a given glomerulus with a definite probability. Conversely, the determination of the coordinates of a glomerulus marked in a preparation should permit the identification of this glomerulus. Such positive prospects encourage further analyses of the glomerular organization along the lines demonstrated in this paper. Indeed, stereotaxic and related applications call not only for precise 3D maps and thoroughly tested identification, but also for a better knowledge of the quantitative properties of the ALs. They also call for the development of specific mathematical tools and for new and fast computational procedures for use in an experimental context. Investigations are currently in progress to develop and test these new techniques. The hope is that such techniques will permit direct experimental approaches toward answering long-standing questions about the function of glomeruli.

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