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Supplemental Information

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A Systematic Nomenclature for the Insect Brain

(Supplemental Informartion)

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Introduction

Here we provide a nomenclature system for the adult insect brain using that of *Drosophila melanogaster* as a framework. The nomenclature system is based on discussions amongst the Insect Brain Name Working Group, a team of invertebrate neurobiologists working towards this end point since 2007. This effort was triggered by a broad realization that existing nomenclatures used for arthropod brains suffered from several problems: (1) comparable brain regions have been given different names depending on the species and the researcher; (2) the same words have been used to refer to different structures; (3) boundaries of many brain regions have not been defined clearly; (4) various brain structures have no established names and thus no defined boundaries. Such a "Tower of Babel" has caused confusion in comparing results described in different studies. As molecular, physiological and behavioral analyses of diverse insect species proliferate, taxonomic comparisons of brains assume greater relevance. Put simply, modern neuroscience urgently requires a systematic and consistent naming scheme for the entire insect brain.

The necessity to address this issue was acknowledged at the Insect Neuroanatomy Meeting held in 2007 on the HHMI Janelia Farm Research Campus and the meeting sponsored by the NIH Neuroscience Blueprint for Neuroscience Research, and a working group of neurobiologists studying the brains of diverse insects and other arthropods was formed. The group subsequently discussed possible suggestions via an email-based online forum and a number of face-to-face workshops. Preliminary versions of the proposed system were reported and discussed at various meetings and symposia, which allowed us to gather opinions from a wider community. After considering that input we here report the final coordinated nomenclature system of the insect brain.

Attempts to consolidate nomenclature for corresponding neural structures across insect species began in the 19th century (Flögel, 1876) and several recent studies have continued this tradition, either by providing 3D renditions of the brains of specific insect species or by resolving the organization of neuronal subsets by chemical neuroanatomy (see, for example, Nässel, 2002; Nässel and Homberg, 2006; Homberg, 2002; Brandt et al., 2005; Kurylas et al., 2008; Dreyer et al., 2010). However, because the fly *Drosophila melanogaster* is currently the most commonly used species in insect neurobiology and the attempts to comprehensively map all of the neurons in an insect brain are focused on this organism, we chose the brain of *Drosophila melanogaster* as a framework.

Many of the brain regions identified in *Drosophila* have obvious counterparts in other insect species (for example, grasshoppers, cockroaches, honey bees, moths). By including colleagues working on those other species in the Insect Brain Name working group, we are confident that the nomenclature system presented here is extendable across insect taxa and also to crustaceans. Amongst segmented animals, insect species are the most abundant and diverse. Nevertheless, despite 400 million years of divergent evolution, their different parts correspond across taxa. The same holds for their brains: neuropils likely correspond across species; however, their volumes and shapes may differ drastically. They would thus invite terms different from those used here. In such cases, reference should additionally be made to the term used for the homologous neuropil in the fly. The present nomenclature system is therefore meant to serve as a framework for a better understanding of the fly brain as well as a point of reference for the study of the brains of other insects and their arthropod sister group, the crustaceans.

In addition to this document, we provide (1) supplemental movies through frontal, horizontal, and sagittal serial sections that show synaptic labeling and the map of neuropils ("Movie_S1_Neuropils_frontal.mov", "Movie_S2_Neuropils_horizontal.mov", and "Movie_S3_Neuropils_sagittal.mov"), (2) supplemental movie showing synaptic labeling and the map of fiber bundles ("Movie_S4_Fiber_Bundles_frontal.mov"), and (3) interactive, three-dimensional clickable maps of neuropils and fiber bundles ("Movie_S5_Interactive_Map_40x.pdf" and "Movie_S6_Interactive_Map_20x.wrl"). Raw confocal image files and image files of the neuropils required for three-dimensional volume registration are provided via Flybase (http://www.flybase.org).

I. Overview of nomenclature

I-1. Names for major brain parts

Historically, there have been several ways to divide the insect brain into subdivisions. To refer to unambiguous subdivisions, we suggest the following terms (Fig. S1). The term *brain* refers to the fused assemblage of the central nervous system within the head capsule, penetrated by the esophagus (Fig. S1A). When the *optic lobes* are separated from this structure, the rest of the brain will be referred to as the *central brain* (Fig. S1B). We do not recommend *midbrain* to refer to the *central brain*, because the former is in longstanding use in vertebrate neuroanatomy for the most rostral portion of the brainstem.

The insect brain has also been parceled into the *supraesophageal* and *subesophageal ganglia* (*SPG* and *SEG*). However, these terms have caused confusion, because there have been two contradicting ways to define these terms. To resolve this, we employ two sets of terms to clearly distinguish the two definitions. When we refer to the segmental neuromeres of the brain, we use the terms *cerebral ganglia* (*CRG*) and *gnathal ganglia* (*GNG*) for the rostral three and the subsequent three neuromeres, respectively (Fig. S1C). When we refer to those parts of the brain above and below the esophagus, irrespective of their neuromeric origins, we use the terms *supraesophageal zone* (*SPZ*) and the *subesophageal zone* (*SEZ*) (Fig. S1D).

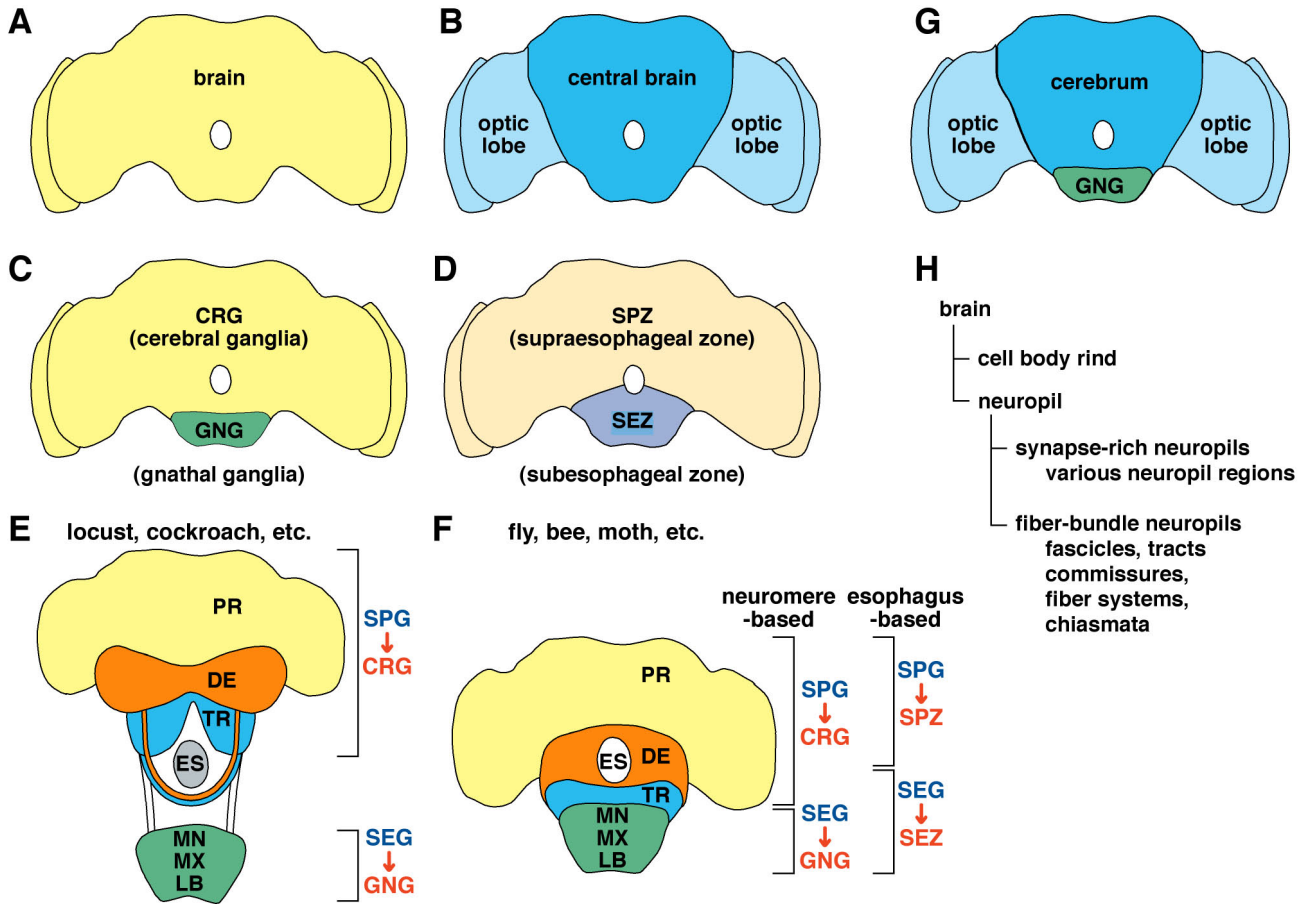


Figure S1. Names for brain parts (using the adult *Drosophila melanogaster* brain as the generic representative)

The rationale for this change is as follows. Developmentally and evolutionarily, the insect (as well as malacostracan and chelicerate) brain comprises six neuromeres (Scholtz and Edgecombe, 2006; see also section III-2, p. 17). The first three neuromeres: the *protocerebrum* (*PR*), *deutocerebrum* (*DE*), and *tritocerebrum* (*TR*) have been termed *supraesophageal*, whereas the subsequent three neuromeres: the *mandibular* (*MN*), *maxillary* (*MX*) and *labial* (*LB*) *ganglia* have been termed *subesophageal.* However, contrary to what these names imply, developmental studies show that the esophagus penetrates the *supraesophageal ganglia* at the level of the *deutocerebrum* (Boyan et al., 2003). In many Hemimetabola, such as locusts and cockroaches, neuropils of the *supraesophageal* neuromeres below the level of the esophagus are reduced to thin commissures in the adult, allowing a clear distinction of three neuromeres above and three below the esophagus (Fig. S1E). In many Holometabola, however, such as flies, bees and moths, the fused neuropils of the *deutocerebrum* and *tritocerebrum* lie around and below the level of the esophageal foramen, thus imparting contradictory definitions of the *supra-* and *subesophageal ganglia* (Fig. S1F). The terms employed here – the *cerebral* and *gnathal ganglia* (*CRG/GNG*) – have been used historically and are independent from the location of the esophagus (Haeckel, 1896; Snodgrass, 1956).

The existence of the *deutocerebral* and *tritocerebral* components below the esophageal foramen is often overlooked, and therefore the term *SEG* has often been used to refer to the entire volume of the brain tissue below the level of the esophagus. Such volume should not be called ganglia, because the division does not match with the neuromeres. However, we recognize the usefulness of such definition of the volume, because the esophagus is a prominent positional landmark in the insect brain. To denote this condition we have introduced the neuromere-free term *zone* (*SPZ/SEZ*).

There has been no clear term to refer to the subdivision that corresponds to the *central brain without GNG* (or *CRG without the optic lobe*). We suggest the term *cerebrum* to refer to this subdivision (Fig. S1G). The *cerebrum* includes the *protocerebrum* without the *optic lobes* as well as the entire *deutocerebrum* and *tritocerebrum*.

Unlike those of vertebrates, neurons in the insect brain have few, if any, synaptic sites on their cell bodies (somata). Usually, a single neurite extends from the soma and projects to either nearby or distant targets where it provides a highly branched system of synaptic arborizations that may give rise to one or more axon-like extensions and their terminals (Strausfeld, 1976). All the neuronal cell bodies are distributed near the surface of the brain, forming the *cell body rind* (*CBR*), which also contains certain types of glial cells (surface-associated glia and cell body glia). Because there are few synapses in the cell body rind, we use the term *rind* rather than the often-employed term *cortex* to avoid unintended analogy with the cortex of the vertebrate brain or to imply that this covering plays any substantial role in computation.

The *neuropil* comprises the axons, dendrites, terminals and their synaptic elements, as well as neuropil-associated glial cells. The neuropil can be categorized into two types: *Synapse-rich neuropil* (often called just *neuropil*) is composed of neuronal processes that possess synapses, whereas *fiber-bundle neuropil* (often called just *fiber bundle*) refers to the bundles of fibers – axons and cell body fibers – that are largely devoid of synapses (Fig. S1H). *Fiber bundles* include *fascicles*, *tracts*, or *bundles* that connect two different regions ipsilaterally; *commissures* are *fiber bundles* that connect two regions contralaterally; *fiber systems* are regions where *fiber bundles* from various directions converge and segregate; and *chiasmata* are arrays of axons that cross over each other in an orderly fashion to remap one level of *synapse-rich neuropil* onto another, maintaining lateral order.

Synaptic markers such as anti-Bruchpilot (mAb nc82) or anti-Synapsin antibodies, and pan-neuronal expression of synapse-targeted reporters such as n-syb-GFP (neuronal Synaptobrevin-GFP fusion) or syt-HA (hemaglutinin-tagged Synaptotagmin) can be used to visualize *synapse-rich neuropils* (e.g., Rein et al., 2002; Brandt et al., 2005; Kurylas et al., 2008; Kvello et al., 2009; Dreyer et al., 2010; Heinze and Reppert, 2012). When such reagents are used, *fiber-bundle neuropils* appear as dark, mostly unlabeled regions. (Note, however, that *fiber bundles* may feature a few synapses along them.) Volumes occupied by thick *fiber-bundle neuropils* are clearly segregated from *synapse-rich neuropils* and are therefore excluded from the mass of *neuropils* listed in the next section. Thinner *fiber-bundle neuropils* tend to run within *synapse-rich neuropils* and are not distinguished within those *neuropils*. Although the *synapse-rich neuropils* and *fiber-bundle neuropils* are also distinguishable in preparations stained by conventional histology such as Bodian silver and hematoxylin-eosin, the difference is less pronounced because neuronal fibers with and without synapses are visualized equally well.

I-2. Hierarchical tables of synapse-rich neuropils

Different types of studies require different levels of spatial resolution. To satisfy diverse needs, we defined the nomenclature of brain structures in a hierarchical manner (Levels 1, 2 and 3, see Tables S1-3). It should be noted that levels refer to the degree of detail rather than to any suggested functional/developmental distinction that may or may not exist. See Figs. S2, S7-S13 for the arrangements of the defined neuropils. The *body axis* is used primarily to describe neuropil positions (see Section III-1, p. 16). The categorization and names of the neuropils were then determined according to the following policy:

- Retain classic terminology whenever possible.
- When multiple names have been used to refer to an identical structure, choose one that seems most appropriate.
- When a single name has been used to refer to different structures in different insect species, or by different researchers, a new term is devised to avoid confusion with any previous term.
- For regions whose precise boundaries have not previously been unambiguously resolved, new boundaries have been proposed based upon known projection patterns of neurons, distribution of glial processes, and developmental aspects.
- For the regions with no established names, both names and boundaries are newly defined. For the sake of brevity, convenient electronic text searching, and minimizing acronyms, simple unique names are proposed that are suggestive of shapes or relative positions. This follows the convention of giving descriptive names to genes or mutants, and the classic style of naming neuropils (e.g. mushroom, olive, Ammon's horn). Names associated with neural functions and neuromeres are avoided, because future studies may reveal yet-unknown functions and because precise neuromere boundaries in the neuropils remain unresolved. Acknowledging that names based on relative positions within the brain may be useful in some cases, alternative position-based synonyms are also provided (see Section VII-1, p. 59).
- In all cases where a name has been changed, we track all previous names and alternatives as synonyms.

The working group felt it was useful to establish a systematic abbreviation of nomenclature. We used the following rationale:

- Retain conventional abbreviations when possible.
- If often-used abbreviations are ambiguous (e.g. a single abbreviation is historically used to refer to more than one structure), devise alternative abbreviations.
- If the definitions or boundaries of the neuropils are modified significantly from historical descriptions, devise alternative abbreviations to distinguish old and new terms.
- As with gene names, unique combinations of a few characters are preferred in most cases.
- Uppercase letters are preferred in most cases although nomenclature and abbreviations are case insensitive.
- For the last character of the neuropil names, the letters C, T, and F are usually avoided as they are used for Commissures, Tracts and Fascicles. This makes it easier to distinguish the names of *synapse-rich neuropils* from *fiber bundles*.

I-2a: Synapse-rich neuropil supercategories (Level 1)

These comprise a set of large neuropil blocks that together divide the entire brain comprehensively in a non-overlapping manner (Table S1; Fig. S2). Boundaries between supercategories are mainly defined by the mass of neuronal somata, glial processes, and streams of fiber bundles, which appear dark and unlabeled with the synaptic markers nc82 or anti-Synapsin antibodies as well as pan-neuronal ectopic expression of presynapse-targeted reporter genes such as n-syb-GFP or syt-HA. In some cases in which the boundaries appear rather contiguous, easily identifiable nearby structures are used as landmarks to define boundaries. Note that, as mentioned above, the neuropils in the *CRG* are categorized independently from the segmental neuromeres, because precise segmental identity is not known in all cases; for example, many neuropils in the ventromedial neuropils (*VMNP*) and periesophageal neuropils (*PENP*) (see Section III-2, p. 17).

Table S1. Neuropil supercategories (level 1)

Cerebral ganglia (CRG)				Gnathal ganglia (GNG)	
OL	optic lobe	SNP	superior neuropils	GNG	gnathal ganglia
MB	mushroom body	INP	inferior neuropils		
CX	central complex	AL	antennal lobe		
LX	lateral complex	VMNP	ventromedial neuropils		
VLNP	ventrolateral neuropils	PENP	periesophageal neuropils		
LH	lateral horn				

I-2b: Systematic list of synapse-rich neuropils (Level 2)

The synapse-rich neuropils within each supercategory (Table S2) comprise a set of discernible neuropil regions whose boundaries divide the entire supercategory comprehensively in a non-overlapping manner (see Figs. S2 and S7-S13). Boundaries between neuropils are identifiable with neuropil markers, glial processes, and fiber bundles, as described above.

Table S2. Neuropil names (level 2) (**Red bold letters = Level-1 supercategories**)

(Structures that might not exist in *Drosophila* are indicated by §.)

abbreviation	neuropil name	abbreviation	neuropil name
OL	**optic lobe**	**LH**	**lateral horn**
LA	- lamina	**SNP**	**superior neuropils**
ALA§	- accessory lamina§	SLP	- superior lateral protocerebrum
ME	- medulla	SIP	- superior intermediate protocerebrum
AME	- accessory medulla	SMP	- superior medial protocerebrum
LOX	- lobula complex	**INP**	**inferior neuropils**
LO	– lobula	CRE	- crepine
LOP	– lobula plate	CL	- clamp
MB	**mushroom body**	SCL	– superior clamp
CA	- calyx	ICL	– inferior clamp
ACA	- accessory calyx	IB	- inferior bridge
PED	- pedunculus	ATL	- antler
SPU	- spur	**AL**	**antennal lobe**
VL	- vertical lobe	**VMNP**	**ventromedial neuropils**
ML	- medial lobe	VX	- ventral complex
YT§	- Y tract§	VES	– vest
YL§	- Y lobe§	EPA	– epaulette
CX	**central complex**	GOR	– gorget
CB	- central body	PS	- posterior slope
FB	– fan-shaped body *or upper division of CB (CBU)*	SPS	– superior posterior slope
		IPS	– inferior posterior slope
EB	– ellipsoid body *or lower division of CB (CBL)*	**PENP**	**periesophageal neuropils**
		SAD	- saddle
PB	- protocerebral bridge	AMMC	– antennal mechanosensory and motor center
NO	- noduli	FLA	- flange
LX	**lateral complex**	CAN	- cantle
BU	- bulb	PRW	- prow
LAL	- lateral accessory lobe	**GNG**	**gnathal ganglia**
VLNP	**ventrolateral neuropils**		
AOTU	- anterior optic tubercle		
VLP	- ventrolateral protocerebrum		
AVLP	– anterior VLP		
PVLP	– posterior VLP		
PLP	- posteriorlateral protocerebrum		
WED	- wedge		
POTU§	- posterior optic tubercle§		

I-2c: Finer subregions of synapse-rich neuropils (Level 3)

Some neuropils can be further subdivided into distinct subregions. In several cases only some parts of the level-2 neuropil are identified as named subregions. Some are hard to identify with nc82 and require other markers for labeling. Note that some of the level-3 subregions have so far been identified only in the brain of *Drosophila melanogaster*.

Table S3. Finer neuropil subregions (level 3)

(Red bold = Level-1 supercategories, Black bold = Level-2 neuropil names, Blue = Level-3 subregions)

(Structures that might not exist in *Drosophila* are indicated by §.)

OL optic lobe
- **LA** - **lamina**
  - LADRA lamina dorsal rim area
  - PLLA plexiform lamina
- **ALA** § - **accessory lamina** § (e.g. in Orthoptera)
- **ME** - **medulla**
  - MEDRA medulla dorsal rim area
  - PLME plexiform medulla
  - OME outer medulla (layer 1-6 in flies)
  - SPL serpentine layer (layer 7 in flies)
  - IME inner medulla (layer 8-10 in flies)
- **AME** - **accessory medulla**
- **LOX** - **lobula complex**
  - **LO** – lobula (layer 1-6 in flies)
  - **LOP** – lobula plate (layer 1-4 in flies)

MB mushroom body
- **CA** - **calyx**
  - MCA medial calyx
  - LCA lateral calyx
- **ACA** - **accessory calyx**
- **PED** - **pedunculus**
  - PEDN pedunculus neck
  - PEDD pedunculus divide
- **SPU** - **spur**
- **VL** - **vertical lobe**
  - α'L α' lobe (α' division in some species)
  - αL α lobe (α division in some species)
  - α_pL α_p lobe (or vertical lobelet)
  - VγL § vertical γ lobe § (e.g. in Hymenoptera)
- **ML** - **medial lobe**
  - γL γ lobe (γ division in some species)
  - β'L β' lobe (β' division in some species)
  - βL β lobe (β division in some species)
  - β_pL β_p lobe (or medial lobelet)
  - TRA § trauben § (only in Ephemeroptera and Zygentoman insects)
- **YT** § - **Y tract** § (e.g. in Lepidoptera)
- **YL** § - **Y lobe** § (e.g. in Lepidoptera)

CX central complex
- **CB** - **central body**
  - **FB** – **fan-shaped body** or **upper division of CB (CBU)** (layers 1-8 and slices 1-8 in flies)
  - **EB** – **ellipsoid body** or **lower division of CB (CBL)** (layers 1-4 and slices 1-8 in flies)
- **PB** - **protocerebral bridge** (slices 1-8 per side in flies)
- **NO** - **noduli** (subunits I-IV in flies)

LX lateral complex
- **BU** - **bulb**
  flies: superior bulb (SBU), inferior bulb (IBU), anterior bulb (ABU)
  locust: medial bulb (MBU), lateral bulb (LBU)
- **LAL** - **lateral accessory lobe**
  - ULAL upper LAL
  - LLAL lower LAL
  - GA gall

VLNP ventrolateral neuropils
- **AOTU** - **anterior optic tubercle**
  - UU upper unit
  - LU § lower unit § (not prominent in flies)
- **VLP** - **ventrolateral protocerebrum**
  - **AVLP** – **anterior VLP**
  - **PVLP** – **posterior VLP**
    optic glomeruli (OG)
- **PLP** - **posteriorlateral protocerebrum**
  optic glomeruli (OG)
- **WED** - **wedge**
- **POTU** § - **posterior optic tubercle** § (not prominent in flies)

LH lateral horn

SNP superior neuropils
- **SLP** - **superior lateral protocerebrum**
  anterior SLP, posterior SLP
- **SIP** - **superior intermediate protocerebrum**
  ring neuropil
- **SMP** - **superior medial protocerebrum**
  anterior SMP, posterior SMP

INP inferior neuropils
- **CRE** - **crepine**
  - RUB rubus
- **CL** - **clamp**
  - **SCL** – **superior clamp**
  - **ICL** – **inferior clamp**
- **IB** - **inferior bridge**
- **ATL** - **antler**

AL antennal lobe
- GL - AL glomeruli
  - MGC § macroglomerular complex § (in Lepidoptera)
- ALH - AL hub

VMNP ventromedial neuropils
- **VX** - **ventral complex**
  - **VES** – **vest**
  - **EPA** – **epaulette**
  - **GOR** – **gorget**
- **PS** - **posterior slope**
  - **SPS** – **superior posterior slope**
  - **IPS** – **inferior posterior slope**

PENP periesophageal neuropils
- **SAD** - **saddle**
  - **AMMC** – **antennal mechanosensory and motor center** (zones 1-5 in flies)
- **FLA** - **flange**
- **CAN** - **cantle**
- **PRW** - **prow**
  - SPhS superior pharyngeal sensory center

GNG gnathal ganglia
- IPhS inferior pharyngeal sensory center
- AMS anterior maxillary sensory center
- PMS posterior maxillary sensory center
- LS labial sensory center

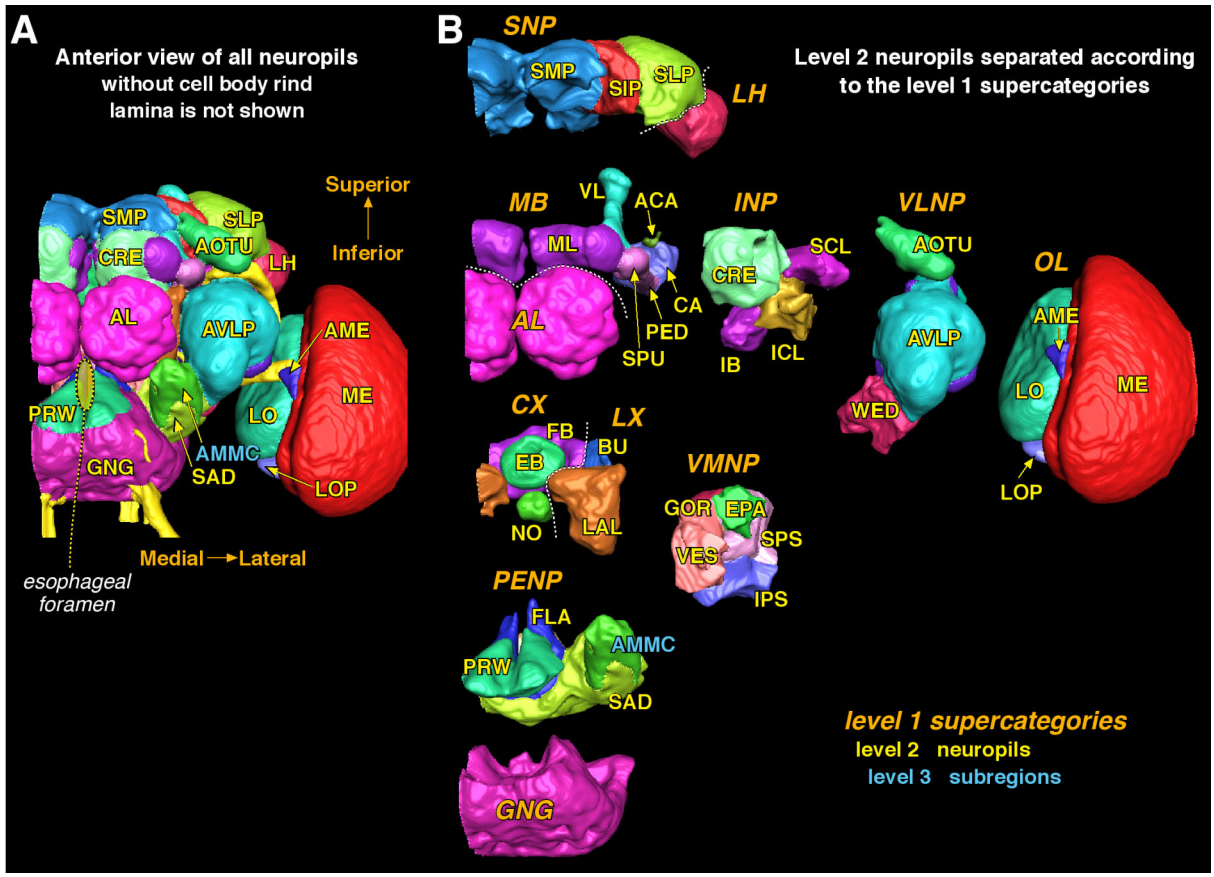


Figure S2. Summary of the level-1 and level-2 neuropils

I-3. Brief description of synapse-rich neuropils

See Figs. S7-S15 (pp. 23-30) for their spatial arrangement in the *Drosophila* brain and Section V (pp. 31-53) for detailed description.

* Asterisks indicate the neuropils whose boundaries and/or names have not been determined clearly in the previous literature and are thus newly defined here, based primarily on the structures in the adult *Drosophila melanogaster* brain.

§ indicate the structures that might not exist in *Drosophila*.

- Directions are indicated according to body axes. *Blue italic characters* indicate the direction according to the neuraxis (e.g. ventral according to the neuraxis is written *n-ventral*). (See Section III-1, p. 16.)
- For brevity, terms “above/below A” were used to indicate “superior to/inferior to A” and “between A and B” were used to indicate the arrangement in the horizontal direction of the body axis (“posterior to A and anterior to B” or “lateral to A and medial to B”).
- For the sake of clarity, names of neuropils and fiber bundles are written in *italics* in this document. However, unlike the names given to genes, they do not have to be italicized when used in regular papers or presentations.

optic lobe (OL) : Nested neuropils in the lateral part of the brain, lying beneath the compound eye.

- lamina (LA) : The distalmost neuropil, lying just beneath the compound eye.
- accessory lamina (ALA) § : Discrete posterior part of the *lamina* having a distinctive architecture, supplied by modified photoreceptor axons. (§possibly absent in flies)
- medulla (ME) : The second and (in many cases including flies) largest neuropil of the *OL*.
- accessory medulla (AME) : Discrete part of the anterior *medulla* near the entry of the *posterior optic commissure*.
- lobula complex (LOX) : The medialmost region of the *optic lobe*; either a single neuropil or two neuropils with opposing retinotopic maps in, e.g. flies, or with a few satellite volumes as in, e.g. locusts.
  - lobula (LO) : Lying anteriorly, it houses many columnar neurons.
  - lobula plate (LOP) : In a *lobula complex* composed of two distinct neuropils, the *lobula plate* lies posteriorly and is the shallower neuropil.

mushroom body (MB) : Lobed neuropils, in many species having cap- or cup-like neuropils in the superior-posterior (*n-anterior*) brain and lobes in the anterior (*n-ventral*) brain.

- calyx (CA) : The region of bulbous or cup-like structures just adjacent to the cluster of Kenyon cell somata, at the dorsoposterior end (*n-anterior end*) of the *MB*.
- accessory calyx (ACA) : The region protruding from the bulbous *calyx*, housing dendrites of a subset of Kenyon cells.

- pedunculus (PED) : The stem-like part of the *mushroom body* containing densely bundled projections of Kenyon cells (equipped with synapses) extending from the *calyces* into the *lobes*.
- spur (SPU) : A small protrusion lateral to the anterior (*n-ventral*) end of the *pedunculus*, identifiable in adult flies.
- vertical lobe (VL) : One of the *lobes* comprising the branches of Kenyon cell fibers that originate from the anterior (*n-ventral*) end of the *pedunculus*, projecting approximately vertically (upwards in flies, forwards, e.g. in honey bees, or recurved dorso-posteriorly e.g. in cockroaches).
- medial lobe (ML) : The second major *lobe* comprising the fibers of Kenyon cells projecting directly or obliquely towards the midline of the brain from its origin from the *pedunculus divide*.
- Y tract (YT) [§] : A separate bundle of Kenyon cell processes identified in moths/butterflies, which takes a more medial trajectory than the *pedunculus*.
- Y lobe (YL) [§] : Swollen anterior end of the *Y tract*, observed in moths/butterflies. It lies near the branch point of the vertical and medial lobes.

central complex (CX) : A system of interconnected neuropils lying at, or about, the midline of the *protocerebrum*.

- central body (CB) : The most prominent set of neuropils of the *central complex*.
  - fan-shaped body (FB)/upper division of CB (CBU) : Modular neuropil having various forms: fan-like (e.g. in flies, locusts, honey bees) or rectangular/bar-like shape depending on the species.
  - ellipsoid body (EB)/lower division of CB (CBL) : Modular neuropil having various forms: ellipsoid or toroidal (e.g. in Diptera), shallow crescent or a bar-like (e.g. in locusts) neuropil. In flies, the *FB* lies posterior (*n-dorsal*) to the *EB*, whereas in locusts, the *CBU* lies superior (*n-anterior*) to the *CBL*. Their arrangements are therefore different by about 90 degrees along the transverse axis. Taxon-specific shapes and modularity can characterize species.
- protocerebral bridge (PB) : Bridge-like neuropil lying posterior (*n-dorsal*) to the *FB*.
- noduli (NO) : Glomerular structures composed of a few stacked discs or swellings lying ventral (*n-posterior*) to the *FB*.

lateral complex (LX) : Neuropils lying anterior-lateral (*n-ventral-lateral*) to *CX* and closely associated with it.

- bulb (BU) : Neuropils on both sides of the *EB* (between the *EB* and *MB pedunculus*), comprising many discrete bulbous domains.
- lateral accessory lobe (LAL) : Pyramidal neuropil inferior-lateral (*n-posterior-lateral*) to the *EB*, behind the *AL*.

ventrolateral neuropils (VLNP) : Neuropils at the lateralmost extent of the *cerebrum* ventrolaterally (*n-posterior-laterally*).

- anterior optic tubercle (AOTU) : The most anterior-superior (*n-ventral-anterior*) optic glomerulus of the brain; in flies appearing slightly separated from the *VLP*. It receives inputs from the *ME* and *LO* via the *anterior optic tract* (*AOT*).
- ventrolateral protocerebrum (VLP) : A large mass of neuropils in the anterior (*n-ventral*) *VLNP*, between the *AL* and *OL*.
  - anterior VLP* (AVLP) : Non-glomerular region of the *VLP* protruding in the anterior (*n-ventral*) *VLNP*.
  - posterior VLP* (PVLP) : A region with many glomeruli, in front of (*n-ventral to*) the *great commissure* (*GC*). It appears contiguous with *PLP* that lies behind it (*n-dorsal to it*). The *PVLP* and *PLP* house many *optic glomeruli* formed by the terminals of axon bundles arising from the *OL*.
- posteriorlateral protocerebrum (PLP) : A region with many glomeruli in the posterior (*n-dorsal*) *VLNP,* between the *PS* and *OL*.
- wedge* (WED) : Non-glomerular region lying inferiorly (*n-posteriorly*) to the *VLP*, extending down to the level of the *GNG*.
- posterior optic tubercle (POTU) [§] : The most posterior (*n-dorsal*) optic glomerulus of the brain; it has a distinct and prominent structure in e.g. locusts, crickets, cockroaches, and monarch butterfly ([§] not prominent in *Drosophila*).

lateral horn (LH) : A neuropil flanking the superior-posterior (*n-dorsal-anterior*) *protocerebrum*. It receives terminals of various uni- and multiglomerular projection (output) neurons from the *AL*.

superior neuropils (SNP) : The most superior (*n-anterior*) neuropils of the central brain.

- superior lateral protocerebrum (SLP) : Lateral part of the *SNP*. Its lateral region covers the medial part of the *LH*.
- superior intermediate protocerebrum* (SIP) : A relatively small region around and posterior (*n-dorsal*) to the *MB*'s *vertical lobe*.
- superior medial protocerebrum (SMP) : Medial part of *SNP*, which lies roughly above the region flanked by the *MB*'s *lobes*, *pedunculus*, and *calyx*(*-ces*). (Note: *SMP* and *MB pedunculus* are separated by *SCL*, see below.)

inferior neuropils* (INP) : Neuropils below (*n-posterior to*) the *SNP*, around the level of the *MB medial lobe* and *pedunculus*.

- crepine* (CRE) : The region encircling the *MB medial lobe*.
- clamp* (CL) : The region between *FB*/*PB* and *MB pedunculus*, including the region above and below the *pedunculus*. (It is distinct from *CRE*, being separated by *LX* that lies between them.)

– superior clamp* (SCL) : Superior (*n-anterior*) part above the superior surface of the *MB pedunculus*.
– inferior clamp* (ICL) : Inferior (*n-ventral*) part medial and inferior-medial to the *MB pedunculus*.
- inferior bridge (IB) : Posteriormost (*n-dorsalmost*) midline region behind the *FB* and below the *PB*
- antler* (ATL) : Thin elongated structure connecting the *IB* and the *SLP*.

antennal lobe (AL) : Glomerular neuropil in the anteriormost (*n-ventralmost*) brain, below the *MB medial lobe*, with many glomeruli that receive axons from olfactory sensory neurons.

ventromedial neuropils* (VMNP) : Region just above (*n-anterior*) or lateral to the esophagus. It lies below the *CX* and *INP*, behind the *LAL*, and medial to the *VLNP*.

- ventral complex* (VX) : A group of neuropils between the *LAL* and *great commissure*.
– vest* (VES) : The medial and major part of the *VX*, lying either side of the esophagus and situated above the *saddle*.
– epaulette* (EPA) : A small lateral part lying below the *ICL*. It is separated from the *VES* by the *inferior fiber system* (*IFS*) in *Drosophila*.
– gorget* (GOR) : A small thin region below the *FB* and above the *great commissure*.
- posterior slope (PS) : Neuropils behind the *VX* and the *great commissure*.
– superior posterior slope (SPS) : Superior component, above the plane of the *great commissure* and *posterior optic commissure*.
– inferior posterior slope (IPS) : Inferior component, below the level described above.

periesophageal neuropils* (PENP) : Region below (*n-posterior to*) or lateral to the esophagus but above the *GNG*.

- saddle* (SAD) : Region covering the superior surface of the *GNG*, housing the *AMMC*.
– antennal mechanosensory and motor center (AMMC) : Region comprising terminals from the mechanosensory neurons of the antennae and dendrites of the motor neurons to the antennal muscles.
- flange* (FLA) : A small triangular neuropil protruding from the anterior end of the *saddle*, flanking the anteriormost esophageal foramen.
- cantle* (CAN) : A small triangular neuropil protruding from the posterior end of the *saddle*, flanking the middle esophageal foramen.
- prow* (PRW) : Anterior region below the opening of the esophageal foramen. It lies above the anteriormost region of the *GNG* and houses part of the peripheral axon terminals from the pharyngeal nerve.

gnathal ganglia (GNG) : Fused post-oral neuropils of the most inferior (*n-posterior*) part of the brain. It houses parts of the axon terminals from the *pharyngeal* and *maxillary-labial nerves* as well as terminals from the *thoracico-abdominal ganglia*.

Note: differences between classic and new terminology systems

The curved boundaries of the neuropils described in this terminology system are not the same as the more or less straight boundaries in the terminology system published previously for the fly brain (Strausfeld, 1976; Otsuna and Ito, 2006). Various terminologies are revised. Lookup Tables providing the equivalent classic and new terminologies and the relevant explanations of boundary distinctions are provided in Section VII-2 (p. 60).

Note: fine subregions identifiable with other markers

Each neuropil may further be divided into finer subregions. Some of such subregions, e.g., glomeruli in the *antennal lobe*, are identifiable using synaptic markers like nc82. Other subregions can be identified by the projection patterns of specific types of neurons. Cell-specific antibodies and gene expression drivers, as well as single-cell labeling of neurons with Golgi staining, intracellular tracer injections, or molecular genetic techniques, are useful for revealing such subregions.

Note: variability between insect species

Previous studies showed that the structures of well-investigated neuropils have variability between different species. For example, the *lobula complex* is a layered neuropil in some insects but separated into two regions lying opposite each other in others (Strausfeld, 2005). Numbers and arrangements of the subdivisions in the *MB calyx* and *lobes*, and the morphology and arrangement of the *FB*/*CBL* and *EB*/*CBU*, are different depending on species (Farris, 2005; Strausfeld et al., 2009). Similarly, the shape and relative positions of some of the newly defined neuropils are based primarily on the arrangement in the *Drosophila* brain, especially those in level-2 and level-3 categories (black or blue characters in Tables 2, 3) and may well look different in other insects. We suggest using the same terms if topological, structural or developmental similarity is observed. In other cases a description with level-1 supercategories (red characters) would be useful, as it would most likely be compatible across taxa.

II. Conflicting terms and their solutions

II-1. List of solutions

We tried to resolve controversial or conflicting terminologies and abbreviations. Detailed arguments follow below Table S4.

Table S4. Controversial terminologies and solutions

Controversial terminologies	Solutions
Names of brain parts	
supraesophageal ganglia (SPG) and **subesophageal ganglia (SEG)**?	➜ **CRG** and **GNG for neuromere masses** **SPZ** and **SEZ for general zones of brain tissues**
brain, **cerebrum,** or **supraesophageal ganglia**?	➜ **brain = entire central nervous system in the head** **cerebral ganglia (CRG) = including the optic lobe** **cerebrum = CRG without the optic lobe**
midbrain or **central brain**?	➜ **central brain**
cerebrum or **protocerebral lobe**?	➜ **cerebrum** (for the regions including deuto- and trito-cerebral neuromeres)
P or **pr** for **protocerebrum**?	➜ **P for the neuropils, but PR for the neuromere**
Neuropil names and abbreviations	
MB: **CA** or **CX** for **calyx**?	➜ **CA** (and CAL for corpora allata)
MB: **vertical lobe** or **dorsal lobe**?	➜ **vertical lobe**
MB: **horizontal lobe** or **medial lobe**?	➜ **medial lobe**
MB: **heel**, **knee**, **lobe junction** or **pedunculus divide**?	➜ **pedunculus divide**
MB: **spur**, **heel**, or **knee**?	➜ **spur**
MB: **SP** or **SPU** for **spur**?	➜ **SPU**
lateral horn or **lateral protocerebrum**?	➜ **lateral horn**
central complex or **central body**?	➜ **central body = FB + EB** **central complex = FB + EB + PB + NO**
CC or **CX** for central complex?	➜ **CX** (and CCA for corpora cardiaca)
Segments, **columns**, **modules**, or **slices** for the transverse divisions of **central complex** and **MB lobes**?	➜ **slices**
numbering of the central-complex slices	➜ **from medial to lateral**
lateral triangle, **median olive**, or **bulb**?	➜ **bulb**
ventral body or **lateral accessory lobe**?	➜ **lateral accessory lobe**
larval AL or **LAL**?	➜ **larval AL**
ventrolateral protocerebrum or **anterior optic foci**?	➜ **ventrolateral protocerebrum**
wedge or **ventral protocerebrum (VPC)**?	➜ **wedge**
optic tubercle (OTU) or **anterior optic tubercle (AOTU)**?	➜ **anterior optic tubercle (AOTU)**
olfactory receptor neuron (ORN) or **olfactory sensory neuron (OSN)**?	➜ **olfactory sensory neuron (OSN)**
Cell body rind names	
cortex, rind, or **cell body layer**?	➜ **cell body rind** (and cell body glia for the cortex glia)
Fiber bundle names	
iACT, mACT, oACT or **mACT, mlACT, lACT**?	➜ **mALT, mlALT, lALT**
tracts or **fascicles**?	➜ **fascicles** (with some reservation)
cerebro-cervical fascicle or **ascending/descending fiber bundles**?	➜ **cerebro-cervical fascicle**
axon, **neurite**, **primary neurite**, or **cell body fiber**?	➜ **neurite = entire neuronal fibers** **axon = neurite connecting synaptic arborizations** **cell body fiber = neurite between cell body and axons / synaptic arborizations**
CC or **CV** for **cervical connective**?	➜ **CV**
Spelling	
oesophagus or **esophagus**?	➜ **esophagus**
OES or **ES** for **esophagus**?	➜ **ES**
SOG or **SEG** for **subesophageal ganglia**?	➜ **GNG or SEZ depending on definitions**
labellar or **labial** for the nerve and neuromere names?	➜ **labial**
neuropile or **neuropil**?	➜ **neuropil**
deutocerebrum or **deuterocerebrum**?	➜ **deutocerebrum**
pedunculus–pedunculi or **peduncle–peduncles**?	➜ **pedunculus–pedunculi**
maxillary **palpus–palpi** or **palp–palps**?	➜ **palp–palps**

II-2. Detailed reasoning

Names of brain parts

supraesophageal ganglia (SPG) and **subesophageal ganglia (SEG)** ➜

cerebral ganglia (CRG) and **gnathal ganglia (GNG) : for ganglia as masses of neuromeres**
supraesophageal zone (SPZ) and **subesophageal zone (SEZ) : for general zones of brain tissues**

As discussed in Section I-1 (P. 3), the terms *supra-* and *subesophageal ganglia* have been used in two ways to refer either to the masses of first or second three sets of neuromeres or to the general brain tissues above or below the level of the esophagus. In holometabolous insects like flies, significant parts of the putative *deutocerebrum* and *tritocerebrum* lie around and beneath the esophagus (e.g., *prow, saddle,* and *AMMC*), but these regions have often been referred to as parts of the *subesophageal ganglia*. To avoid ambiguity, we employ two sets of terms to distinguish two definitions clearly. The terms *CRG/GNG* should be used when discussing neuromeres, and the terms *SPZ/SEZ* should be used when referring to general regions above or below the esophagus, especially when the boundary of the *GNG* is not clear for the user (Fig. S1C, D). Descriptions using the conventional terms *SPG/SEG* should be interpreted with care, as the treatment of the regions around and beneath the esophagus may vary depending on studies.

brain, **cerebrum, supraesophageal ganglia** or **cerebral ganglia** ? ➜

brain = for the entire central nervous system in the head
cerebral ganglia (CRG) = for the regions including the optic lobe
cerebrum = for the CRG without the optic lobe = the central brain without the GNG

The *cerebral ganglia* (former *supraesophageal ganglia*) have sometimes been called just the brain. To avoid confusion, we define the *brain* as the "entire central nervous system in the head" (Fig. S1A). The *cerebral ganglia* include the *optic lobe* (Fig. S1C). The *cerebrum* refers to the "*cerebral ganglia* without the *optic lobe*," which also corresponds to the "*central brain* without the *gnathal ganglia*" (Fig. S1G). The term *supraesophageal ganglia* is no longer used in this terminology system in favor of less ambiguous *cerebral ganglia* (neuromere-based) and *supraesophageal zone* (location-based).

midbrain or **central brain**? ➜ **central brain**

The term *midbrain* is also used to refer to the brain without the *optic lobe*. However, this term is commonly used in vertebrate neuroanatomy as the name of the segmental neuromere in the developing brain (forebrain, midbrain, and hindbrain). Therefore, *midbrain* should be avoided in insect neuroanatomy to avoid misleading segmental implication.

cerebrum or **protocerebral lobe**? ➜ **cerebrum** (in the context of this terminology system)

The term *protocerebral lobe* is used to refer to the central part of the *protocerebrum* (without the *optic lobe*) in honey bees. This term is useful to refer to the superior region of the central *protocerebrum.* However, the extent of the *protocerebrum* in the inferior part of the *central brain* remains still ambiguous. Therefore, to refer to the entire neuropil mass of the central part of the *CRG*, which also includes the *deutocerebrum* and *tritocerebrum*, we here used the more generic neuromere-free term *cerebrum.* Also, because the term *protocerebral lobe* contains the word "lobe," it is sometimes misused to refer specifically to the lobe-like protruded region of the *protocerebrum,* such as the region around the *MB vertical lobe*, rather than to the entire protocerebral neuropils of the *central brain*.

P or **pr** for **protocerebrum**? ➜ **P for the neuropils, but PR for the neuromere**

The *protocerebrum* has often been abbreviated as *pr* (e.g. *superior lateral protocerebrum* → *slpr*). Here we use the abbreviation *P* for two reasons. First, it would make the abbreviations shorter. Second, given that the classic boundaries of some protocerebral neuropils are modified in the new terminology system (see Section VII-2, p. 60), using a different abbreviation should clearly indicate which terminology system the authors have applied. Using upper case letters also clarifies the difference. If authors write *slpr*, they follow the classic definition, and if they write *SLP*, they are using the new definition. Using all uppercase effectively makes the terminology case-insensitive for computational ease of use.

To abbreviate *protocerebrum* as a single-word term of the segmental neuromere, using a single character "P" looks too short and can be fused with the term *posterior*, which is often abbreviated as "P" in the figures. It also demands the usage of the same single-character acronyms for other neuromeres like *deutocerebrum* and *tritocerebrum* of the *CRG* and *mandibular*, *maxillary*, and *labial neuromeres* of the *GNG*. To avoid this, we suggest using *PR* when referring alone to the segmental neuromere (it is important to note that the boundaries between segmental neuromeres are not yet identified unambiguously).

Neuropil names and abbreviations

MB **CA** or **CX** for **calyx**? ➜ **CA** (and CAL for corpora allata)

The abbreviation *CX* is sometimes used for referring to the *calyx*. This is not recommended, as it conflicts with the abbreviation of the *central complex*. To avoid ambiguity we also suggest abbreviating *corpora allata* as *CAL* instead of *CA*.

MB **vertical lobe** or **dorsal lobe**? ➜ **vertical lobe**

Several pioneering studies identified subdivisions within the lobe-like neuropils of the *Drosophila mushroom body* (Yang et al., 1995; Crittenden et al., 1998). Here we have consolidated and standardized terminology for the lobes and their parallel divisions. The *vertical lobe* of the *MB* is sometimes called the *dorsal lobe*. We recommend the former term, because in some insect species this lobe is so oriented towards anterior or posterior that it cannot be regarded as "dorsal."

In some cases the term α *lobe* is used to refer to the entire *vertical lobe*. The generic term *vertical lobe* is preferable because, as in flies, the *vertical lobe* may consist of multiple subdivisions of which the α *lobe* is just one component.

MB **horizontal lobe** or **medial lobe**? ➜ **medial lobe**

The *medial lobe* of the *MB* is sometimes called the *horizontal lobe*. We recommend the former term, because in some species this lobe does not project horizontally but obliquely. Even in such species, it always projects medially (Fig. S3). In some cases the term β *lobe* is used to refer to the entire *medial lobe*. The generic term *medial lobe* is preferable because, as in flies, it may consist of multiple subdivisions of which the β *lobe* is just one component.

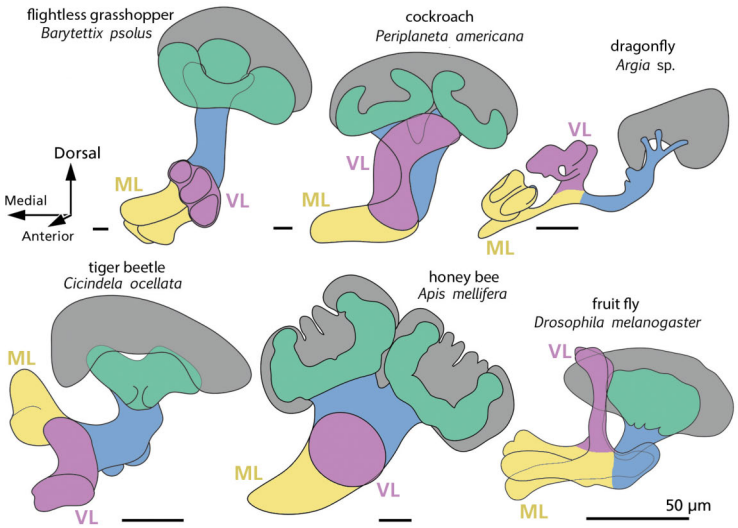


Figure S3. Mushroom body lobes of various insects. Colors denote the regions of the *ML, VL,* and *pedunculus* (Strausfeld et al., 1998; Strausfeld, 2012. See also: Farris and Sinakevitch, 2003).

Note: These suggestions lead to an apparently odd combination of the two lobe names – *vertical / medial* instead of *vertical / horizontal* or *dorsal / medial*. Between the latter two, *vertical/horizontal* would be better because the *vertical lobe* in some insects extends not dorsally at all but almost anteriorly (e.g. in honey bees), whereas the *medial lobe* never projects vertically. However, considering that the two lobes happen to be arranged perpendicularly in flies but not in many other species (Fig. S3), and that research on *mushroom body* function is performed extensively on many other species, cross-species compatibility is more important than using terms that in just a few species reflect lobe orientations.

MB **heel**, **knee**, **lobe junction** or **pedunculus divide**? ➜ **pedunculus divide**

The anterior (*n-ventral*), or distal, end of the *pedunculus*, where the Kenyon cell fibers bifurcate to project to the two lobes, is sometimes called the *heel, knee,* or *junction of the lobes/lobe junction*. Considering that at this point the bundle of Kenyon cell fibers projecting from the *calyx* diverges into multiple lobes rather than converges, the term *junction* is somewhat misleading and the term *pedunculus divide* is more appropriate. We also recommend avoiding *heel* and *knee* to prevent ambiguity, because they have also been used for referring to the more lateral protruded region that is here called the *spur.*

MB **heel**, **knee**, or **spur**? ➜ **spur**

Terms *heel, knee,* and *spur* have been used for referring to the region of the *MB* that protrudes laterally from the *pedunculus divide* (root of the *vertical* and *medial lobes*). We recommend avoiding *heel* and *knee*, because these terms (which imply the bending point of neuronal fibers) have also been used for referring to the *pedunculus divide* and therefore cause more ambiguity. The term *spur* (which means a thing that projects or branches off from a main body), on the other hand, refers specifically to the lateral protrusion from the *pedunculus divide* and not the *divide* itself. (See Section V-2-4, p. 34 for the detail of this region.)

SP or **SPU** for **spur**? ➜ **SPU**

Spur has often been abbreviated as *SP*, but we recommend abbreviating it as *SPU*, because *SP* may be confused with the *superior protocerebrum*.

lateral horn or **lateral protocerebrum**? ➜ **lateral horn**

The *lateral horn* is also called the *lateral protocerebrum*. However, the latter may also include other regions of the lateral neuropils such as the *ventrolateral protocerebrum* and *posterior lateral protocerebrum*. To refer to the terminal region of the antennal lobe projection neurons specifically, we recommend using *lateral horn* rather than *lateral protocerebrum*.

central complex or **central body**? ➜ **central body (CB) = FB + EB**
central complex (CX) = FB + EB + PB + NO

The terms *central complex* and *central body* have been used somewhat interchangeably. We examined the context in which these terms appear, and recommend using *central body* to refer specifically to the combination of the *fan-shaped body* and

ellipsoid body (*FB* and *EB*), and using *central complex* to refer to the combination of all the four components including the *protocerebral bridge* (*PB*) and *noduli* (*NO*).

CC or **CX** for **central complex**? ➜ **CX** (and CCA for corpora cardiaca)

The abbreviations *CC* and *CX* have been used to refer to the *central complex*. *CC* should best be avoided, because it has also been used for both the *cervical connective* and *corpora cardiaca*. Although *CX* has sometimes been used to refer to the *calyx,* we assigned *CA* to abbreviate that term. Thus, *CX* can be used specifically to refer to the *central complex*. To avoid ambiguity, we also suggest abbreviating *corpora cardiaca* as *CCA* instead of *CC*.

Segments or **columns** for the transverse divisions of the **central complex** and **MB lobes**? ➜ **slices**

In many insects the *EB* and *FB* have two types of subdivisions, one along their longitudinal (left to right) axes and the other perpendicular to it (transverse divisions). The *PB* also has transverse divisions. The *MB lobes* also have longitudinal and transverse divisions. Whereas the longitudinal divisions are called *layers*, the transverse divisions have often been called *segments*, or *columns*. Both terms, however, cause potential confusions.

Because the word "segment" is used for the rostro-caudal segmentation of the body, and because the segmental origin of the *central complex* or the *MB* is itself a focus of developmental analysis, using the term for other purpose than referring to the rostro-caudal segmentation may cause confusion/misinterpretation among readers. The word "column" infers a structure that has elongated, cylinder-like morphology. The transverse divisions especially in the *PB* and *MB lobes* do not have such columnar shape. After long discussion, we concluded to suggest using another word "slice" for these transverse divisions. This word does not cause confusion with other developmental or functional organization of the insect brain. It is also suitable for describing the transverse divisions of cylinder-like structure such as the *PB* and *MB lobes*.

Neurons that extend through such slices have been called *columnar neurons*. We do not find necessity to change this term, because such neurons do have elongated, cylinder-like projections.

Numbering of the central complex slices? ➜ **from medial to lateral**

The slices in the *FB*, *EB*, and *PB* were numbered from lateral to medial ("12345678-midline-87654321") in the locust brain by Williams (1975) but from medial to lateral ("87654321-midline-12345678") in the fly brain by Hanesch et al. (1989). Both schemes are being used in current literature. After careful consideration, we propose using the latter, medial-to-lateral numbering because it follows a widely used convention of numbering elements from the center to the periphery (e.g., in ship and airplane construction) and might thus be more intuitive to general audiences.

lateral triangle, **median olive**, or **bulb**? ➜ **bulb**

The terms *lateral triangle*, *median olive*, and *isthmus* have been used somewhat confusingly to refer to the neuropil regions lateral to the *ellipsoid body* (or *lower division of central body* in locusts). In *Drosophila*, the entire region has been called the *lateral triangle* (Hanesch et al., 1989). In locusts, the term *lateral triangle* refers only to the lateral part of this structure, and the term *median olive* was used to refer to its more medial part (Müller et al., 1997). Thus, the same term has been used to refer to different structures.

To avoid ambiguity and confusion, we recommend using the new term *bulb* to refer to the entire structure, named after the bulbous glomerular structure characteristic of this neuropil. The fly's *lateral triangle* is now called the *bulb*, and the locust *lateral triangle* and *median olive* are now called the *lateral bulb* and *medial bulb*, respectively.

ventral body or **lateral accessory lobe**? ➜ **lateral accessory lobe (LAL)**

Though the term *ventral body* was used originally to refer to this region in houseflies, the term *lateral accessory lobe* is used more often for descriptions of many other insect species, particularly for studies that ascribe possible functions. The latter name is therefore more appropriate to refer to this structure.

larval AL or **LAL**? ➜ **larval AL**

The antennal lobe (*AL*) in larvae has sometimes been abbreviated as *LAL*. This should be avoided, because it would be confused with the *lateral accessory lobe* (see above), which lies just posterior to the *AL*. (It is not yet known whether a functional *LAL* exists in larvae.)

ventrolateral protocerebrum (VLP) or **anterior optic foci**? ➜ **ventrolateral protocerebrum**

VLP has also been called the *anterior optic foci*, but this is not a suitable name to refer to the entire *VLP*. An *optic focus* is another name for *optic glomerulus*, which refers to the glomerular terminals of the visual projection neurons arising from the *optic lobe* to terminate in various regions of the *central brain*. However, within *VLP* there are also regions that do not belong to any *optic glomeruli* (and hence are not *optic foci*).

wedge or **ventral protocerebrum (VPC)**? ➜ **wedge**

The neuropil newly termed the *wedge* anatomically appears similar to the region called the *ventral protocerebrum* (*VPC*) in moth and locust. We avoided this name because (1) the generic term *ventral protocerebrum* may infer a much larger brain region including *VLP*, *PLP*, *posterior slope*, etc., and because (2) it is not yet confirmed whether this region indeed belongs to

the *protocerebral neuromere*, as the term *ventral protocerebrum* would suggest.

optic tubercle (OTU) or **anterior optic tubercle (AOTU)**? ➜ **anterior optic tubercle (AOTU)**

The *anterior optic tubercle* is sometimes called simply the *optic tubercle*. In the brain of locusts, crickets, monarch butterflies and other species, there is another prominent tubercle in the posterior brain called the *posterior optic tubercle* (Homberg et al., 1991; Heinze et al., 2013). To distinguish these, we suggest using *anterior optic tubercle*.

olfactory receptor neuron (ORN) or **olfactory sensory neuron (OSN)**?➜ **olfactory sensory neuron (OSN)**

Both terms are being used almost equally to refer to the sensory neurons that detect odorants. Neurons in the peripheral sensory system are more often called *sensory neurons* than *receptor neurons*. The word "receptor" is often used to refer to cell-surface molecules that bind to ligands. To distinguish clearly the sensory neurons themselves from receptor molecules within them, we suggest using the term *olfactory sensory neurons.* This aligns well with the use of "sensory neurons" of other modalities, such as gustatory sensory neurons, auditory sensory neurons, and somatosensory neurons.

Cell body rind names

rind, cortex, or **cell body layer**? ➜ **cell body rind** (and cell body glia for the cortex glia)

The *cell body rind* (*CBR*) is often called the *cortex*. We suggest avoiding the term *cortex*, because researchers who are familiar with vertebrate neurology may assume that it should be a site of synaptic connections and neuronal computation, whereas in insects this region has few synapses, if any, and therefore is not a site of computation. The term *cell body layer* (*CBL*) has also been used. However, because the *CBR* does not have layered structures in most cases, we suggest avoiding the term *layer*. We also suggest that for clarity it is preferable to call the region *cell body rind* rather than just *rind*.

The shift from *cortex* to *rind* affects the name of what has been called the *cortex-associated glial cells* (or *cortex glia*). We suggest calling them the *cell body glial cells*, which embraces entire subgroup of this glial category.

Fiber bundle names

iACT, mACT, oACT or **mACT, mlACT, lACT**? ➜ **mALT, mlALT, lALT**

In flies, moths and some other insects, the terms *inner*, *middle*, and *outer antennocerebral tract* (*i, m, oACT*) have been used to describe fiber bundles that extend from the *antennal lobe* to various regions of the *protocerebrum* (Homberg et al., 1989; Stocker et al., 1990; Schachtner et al., 2005). In honey bees, the terms *medial*, *mediolateral*, and *lateral antennocerebral tract* (*m, ml, lACT*) have been used (Brandt et al., 2005; Kirschner et al., 2006). The term *mACT* therefore meant the most medial tract in bees but the middle tract in flies. Because medial/lateral are used throughout the nervous system to refer to entities arranged in a mediolateral manner, and because inner/outer are often used for referring entities that are arranged concentrically (which is not the case in the *i, m, oACT*) we chose the combination of *m, ml, and l*.

The term *ACT* (*antennocerebral tract*) is also changed to *ALT* (*antennal lobe tract*) to avoid confusion between the classic *mACT* (either medialmost of middle tract) and the new *mALT* (always the medialmost tract). *Antennal lobe tract* is more appropriate to describe these tracts, because they connect the *antennal lobe* (which is also within the *cerebrum*) and various neuropils of the *protocerebrum*. (See Section VI-1-1, p. 54, for the detailed structure of these tracts.)

Note that there might be several tracts between the *mALT* and *lALT*. In honey bees three such tracts have been identified (Galizia and Rössler, 2010) and are named *ml1ALT*, *ml2ALT,* and *ml3ALT.* Flies also seem to have more than one such tract, but only one of them is prominent and has been called the *mACT* = *mlALT*. To avoid confusion, we suggest calling other minor *ALTs* of the fly brain *transverse ALTs* (*tALTs*; Tanaka et al., 2012a, b).

Note: APT (antennal lobe-protocerebral tract):

During the course of our discussion, a tentative term "APT" was proposed and, in anticipation of the employment of this new term, it has already been used in some recent literature (e.g., Galizia and Rössler, 2010). However, the term is too long and some of the neuronal fibers running via this tract might actually terminate in the brain regions other than the *protocerebrum*. Because of this, the working group opted to change the name to the simpler origin-based *ALT*.

fascicles or **tracts**? ➜ **fascicles** (with some reservation)

Both *fascicles* and *tracts* have been used to name fiber bundles that run ipsilaterally in the neuropils. They might be classified according to (1) whether the fiber bundles are spread loosely or bound tightly, or (2) whether the fibers fasciculate from (or spread to) multiple neuropil regions or connect single neuropil regions at both ends. Because of the difficulty in clearly classifying each fiber bundle according to one of these criteria, and especially because neuronal fibers in a single bundle tend to arise from/project to more than one brain region before/after they pass through the particular fiber bundle, we chose the term *fascicle* as the generic name to denote ipsilateral fiber bundles. The term *tract* is used in those instances when existing names that have been used extensively in the literature are still retained.

cerebro-cervical fascicle or **ascending or descending fiber bundles**? ➜ **cerebro-cervical fascicle**

The dorsal and ventral parts of the *cervical connective* predominantly, but not exclusively, contain descending and ascending

axon. Considering that ascending and descending fibers are not exclusively separated from each other and that they are mixed in fascicles that diverge from, or converge to, the *cervical connective*, we collectively call these fascicles the *cerebro-cervical fascicles* (*CCFs*). This avoids any assumptions from neuroanatomy alone, about the directions of information flow. (See Section VI-1-3, p. 55, for the detailed structure of these fascicles.)

axon, **neurite**, **primary neurite**, or **cell body fiber? ➜**

neurite = **entire part of the neuronal fibers**
axon = **neurite that connects between synaptic arborizations**
cell body fiber = **neurite between cell body and axons/synaptic arborizations**

The terms *axon, neurite, primary neurite,* and *cell body fiber* have often been used confusingly to refer to either all or some part of a neuron's arborizations. Here we define the generic term *neurite* to refer to any process of a neuron. Thus, in cases where it is not possible to securely determine if a process is dendritic, axonal, or between these and neuronal cell bodies, the term *neurite* suffices in all cases. The term *axon* should be used to refer to any elongated process that conveys encoded information between two distinct arborizations in synapse-rich neuropils. A *cell body fiber* refers to the slender process that connects the neuronal cell body (also called *soma*/*somata* or *perikaryon*/*perikarya*) situated in the cell body rind to the integrative part of the neuron in neuropil. Although the *cell body fiber* is sometimes called the *primary neurite*, this term should be avoided because, when used for describing vertebrate nervous system, it is used to refer to the most prominent fiber of a multipolar neuron.

CC or **CV** for **cervical connective**? ➜ **CV**

The abbreviation *CC* has often been used for the *cervical connective*. Considering that this acronym is also popular not only for the *central complex* but also for the *corpora cardiaca*, we suggest using *CV* to abbreviate the *cervical connective*. As stated above, the suggested abbreviations for *central complex* and *corpora cardiaca* are *CX* and *CCA*, respectively.

Spelling

oesophagus or **esophagus**? **oesophageal** or **esophageal**?➜ **esophagus, esophageal**
OES or **ES**? ➜ **ES** (for *esophagus*)
SOG or **SEG**? ➜ **SEZ** (for general brain tissue below esophagus) or GNG (for gnathal ganglia)

Because US English often simplifies "oe" as "e", the original term *oesophagus* in UK English is often modified to *esophagus*. Because of this, the *suboesophageal ganglion* (*SOG*) is often written and abbreviated as *subesophageal ganglion* (*SEG*), These linguistic differences have led to inconsistencies in abbreviating this brain structure. Many journals have switched to US English spelling. The tendency is to alter spelling of *oesophagus* to *esophagus* during the editorial process. Coordinated ontology projects in other fields of anatomy also favor US English. The present nomenclature accords with this trend and thus uses the US spelling of *esophagus* (thus abbreviated to *SEG*) even though *oesophagus* and *SOG* are still in common use. It follows that the abbreviation *ES* rather than *OES* denotes the *esophagus*. Note that the term *SEG* is no longer used in this terminology system in favor of less ambiguous sets of terms *SEZ* and *GNG*.

labellar or **labial** for the nerve and neuromere names? ➜ **labial**

The *labial nerve* and *labial neuromere* are also called *labellar nerve* and *labellar neuropil*. The *labellum* refers to the flat tip of the *labium* (mouth part) of the licking insects, such as many species of flies. The *labium* of sucking or biting insects does not feature a *labellum*. We therefore suggest using *labial* for the general names of the nerve and neuromere.

neuropile or **neuropil**? ➜ **neuropil**

Literature surveys reveal that both forms of spelling are used in roughly the same frequency. However, the term introduced originally by Wilhelm His in the 19th century was *neuropil* (or *neuropilem*), whose ending derives from the Greek word "pilema", the material known as felt. The term is therefore not a combination of "neuro-" and "pile". Thus the original spelling *neuropil* has been used for the present nomenclature.

deutocerebrum or **deuterocerebrum**? ➜ **deutocerebrum**

Though the original Greek form of the word for "the second' is "deuteros", we suggest using the *deutocerebrum* rather than *deuterocerebrum* because a survey of the literature shows it is used much more often. Furthermore, *deutocerebrum* aligns better phonetically with *proto-* and *tritocerebra*.

pedunculus–pedunculi or **peduncle–peduncles**? ➜ **pedunculus–pedunculi**

We recommend using the Latin spelling, because (1) it is currently used much more often in the literature, and because (2) *peduncle* is used in many other contexts of neuroanatomy, resulting in false hits using electronic literature searches.

maxillary **palpus–palpi** or **palp–palps**? ➜ **palp–palps**

Though not consistent with above, we here recommend English spelling, because (1) it is currently used much more often in the literature, and because (2) the word *palp* is not used very frequently in other contexts, thus simplifying searches.

III. Other terminology issues

III-1. Body axis and neuraxis

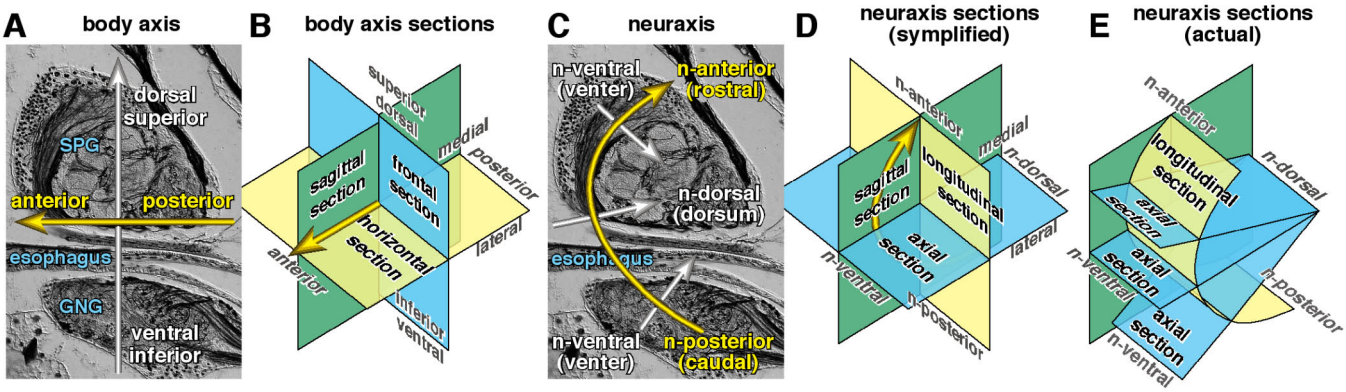


Figure S4. Names of the axis and sections

Directions in the central nervous system (CNS) are usually described as *anterior*/*posterior* (A/P), *dorsal*/*ventral* (D/V), and *medial*/*lateral* (M/L). However, there are two ways to define the A-P axis in the arthropod brain: The *body axis* (BA) sets the direction parallel to the longitudinal axis of the body (Fig. S4A). The *neuraxis* (NA) sets the direction according to the way segmental neuromeres of the central nervous system (CNS) are sequentially aligned (Fig. S4C). Thus, reference to the neuraxis is most useful for cross-taxonomic descriptions.

The two axes are identical for that part of the CNS that includes the *thoracico-abdominal ganglia* and, in some but not all species, posterior parts of the *gnathal ganglia*. However, because the anterior portion of the brain is bent upward or even reflected backwards, the orientations of the two axes differ with regard to the *cerebral ganglia* (Fig. S4A, C). Confusion has often occurred, because the same directional terms (A–P and D–V) have been used for defining both the body axis and neuraxis. Many authors do not even define the axis system in their manuscripts, and when they do the declaration is so vague as to be useless. This is particularly problematic for computer-aided searching for references to brain structures.

Table S5. Names of the axis and planes

body axis	**neuraxis**
anterior/**posterior** (A/P) (b-anterior/b-posterior to distinguish explicitly from the neuraxis)	**n-anterior**/**n-posterior** (NA/NP) rostral*/caudal
dorsal/**ventral** (D/V) (b-dorsal/b-ventral to distinguish explicitly from neuraxis) superior/inferior (S/I)	**n-dorsal**/**n-ventral** (ND/NV) dorsum/venter**
medial/lateral (M/L)	medial/lateral (M/L)
body axis plane	**neuraxis plane**
frontal plane	longitudinal plane
horizontal plane	axial plane
sagittal plane	sagittal plane

Example of the combined terms
anterior-ventral ➜ n-anterior-ventral
lateral-dorsal ➜ lateral-n-dorsal

* Though the direction of *caudal* is unambiguous, that of *rostral* might be confusing because the base segment of the proboscis, which lies below the *GNG* (caudalmost part of the brain), is called the rostrum.

** Dorsum/venter have been used in some old literature and could be used as specific terms for neuraxis.

To minimize confusion, it would be helpful to use dedicated directional terms for the body axes and neuraxes. However, the search for simple terms that can be used for these was unsuccessful. Instead of *dorsal*/*ventral*, terms like *superior*/*inferior* have often been used to specify body axes. However, *dorsal*/*ventral* cannot simply be abandoned to refer to body axes, because many published neurons and brain regions have already been given names using *dorsal*/*ventral* according to body axes. The terms *rostral*/*caudal* may be used to substitute *anterior*/*posterior* according to the neuraxis, but the term *rostrum* has also been used to refer to the basal part of the proboscis, which is situated below the caudal part of the brain (*GNG*). Thus, it is inevitable to use *anterior*/*posterior* and *dorsal*/*ventral* for both body axis and neuraxis. To distinguish them clearly, we therefore propose using adding a suffix "n-" to indicate the terms that are used in neuraxis. Suffixes are not required when the terms are used to refer to a body axis, but when both body axis- and neuraxis-terms appear in the same literature, "b-" may be added to the former so as to avoid confusion.

Terms describing orientation of planes (as in planes of sectioning, and those aspect of viewing) have also caused much confusion: for example, the directions of *frontal* and *horizontal* planes do not refer to the neuraxis. The supposed neuromere axis is actually not straight but is curved (Fig. S4C, E). The plane parallel to the neuraxis should therefore be curved, and those planes that are perpendicular to the neuraxis should be aligned radially depending on their position along the neuraxis. (Note that it is impossible to cut microscopy sections according to such curved or radial planes.) Thus, we propose to use the terms *frontal* and *horizontal* strictly in terms of the body axis (Fig. S4B). To indicate the planes that are parallel or perpendicular to the neuraxis, we propose to use *longitudinal* and *axial*, respectively (Fig. S4D, E). For describing the direction of microscopy sections, body axis should better be used.

III-2. Segmental neuromeres of the brain and thoracico-abdominal ganglia

Developmental and evolutionary data suggest that there are three segmental neuromeres in the *CRG* (*protocerebrum*, *deutocerebrum*, and *tritocerebrum*), three in the *GNG* (*mandibular*, *maxillary*, and *labial neuromeres*), three contributing to the thoracic ganglia (*pro, meso,* and *meta*; *T1-T3*), seven contributing to the abdominal ganglia *A1-A7*, and four that compose the (so-called) terminal abdominal "ganglion" A8. It should, in principle, be possible to divide neuropils in the *brain* and *thoracico-abdominal ganglia* (*TAG*) of any insect into these neuromeres.

Table S6. Names and abbreviations of neuromeres

cerebral ganglia (CRG)

PR	protocerebrum
DE	deutocerebrum
TR	tritocerebrum

gnathal ganglia (GNG)

MN	mandibular neuromere
MX	maxillary neuromere
LB	labial neuromere

thoracic ganglia (TG)

T1	prothoracic neuromere
T2	mesothoracic neuromere
T3	metathoracic neuromere

abdominal ganglia (AG)

A1-A7	1st–7th abdominal neuromere
A8	fusion of the 8th-11th abdominal neuromere

***thoracico-abdominal ganglia (TAG)**
as a general term for the TG and AG combined (whether or not they are fused)

Because neuronal fibers originating from one neuromere can extensively project to other neuromeres, there is no clear neuromere boundary in the mature adult brain. The same is true for the thoracico-abdominal ganglia. Though some data have been presented to suggest neuromere boundaries, further studies are required to unambiguously resolve these. In order to keep the neuropil nomenclature independent from ongoing studies that are attempting to identify neuromere boundaries, we have classified the neuropils of the brain not according to neuromeres but according to identifiable landmarks (except for the regions where it is very obvious, e.g. in the superior part of the *superior protocerebrum*). For example, terms such as the *ventral neuropils* were employed instead of *ventral protocerebrum* so that the term would not be affected even if future studies might identify a neuromere boundary within this general volume. The term *protocerebral lobe*, which is used in some species to refer to the central neuropil mass without the *optic lobe*, was not used and the neuromere-free term *cerebrum* was used instead, for the same reason.

Nevertheless, having an established naming scheme for neuromeres and their abbreviations is useful for minimizing incompatibility between studies, and therefore we here suggest the already defined names and abbreviations for neuromeres: *protocerebrum*, *deutocerebrum* and *tritocerebrum* of the *CRG*, and *mandibular*, *maxillary* and *labial neuromeres* of the *GNG*.

Some or all the ganglia in the thorax and abdomen are fused to form distinct masses (or a mass) depending on species. Because of the high variability in the way they are fused, we have not here attempted to define names for fused ganglia.

III-3. Names for the landmark fiber bundles

Because there are many fiber bundles in the brain, we define only those that are most prominent and form useful landmarks for determining neuropil boundaries (Table S7, Fig. S5). Detailed explanations are provided in Section VI, pp. 54-59.

Terms like *fascicles*, *tracts, bundles,* and *commissures* are used to refer to systems of fiber bundles. The present systematic terminology defines *fascicles, tracts* and *bundles* as connecting two different brain regions ipsilaterally and *commissures* as connecting two regions contralaterally.

Fascicles, tracts, and bundles

These terms refer to the fiber bundles that run between two different regions of the ipsilateral brain. In principle, fascicles and tracts might be classified according to (1) whether the fiber bundles are spread loosely or bound tightly, or (2) whether the fibers fasciculate from (or spread to) multiple neuropil regions or connect single regions at both ends. As a matter of practice,

it is difficult to unambiguously classify each fiber bundle according to one of these criteria. In addition, because unambiguous distinctions of axons, cell body fibers, or mixtures of both are sometimes difficult, we adopt the term *fascicle* as the generic name of ipsilateral fiber bundles. The terms *tract* and *bundle* are used only when retaining existing names that have already been used extensively in the literature. (See Section VI-1, pp. 54-56.)

Commissures

Commissures refer to fiber bundles that cross the midline thereby connecting both hemispheres. Certain fiber bundles that have previously been called *tracts* in the literature are here renamed *commissures* when these connect neuropils in both sides of the brain hemispheres (e.g. *posterior optic tract* → *posterior optic commissure*). (See Section VI-3, pp. 56-57.)

Fiber systems

"Fiber systems" refer to a few specific regions of the brain where *fascicles* and *commissures* from various directions meet and merge. They appear as large hollow regions (immunonegative) in sections treated with synaptic labels, such as nc82, and as extensive arrays of fibers in silver (Bodian) staining. (See Section VI-2, p. 56.)

Chiasmata

A *chiasma* is a system of crossing fibers that remaps or systematically permutates the linear order of neurons between two neuropils. (See Section VI-4, p. 58.)

Nerves and connectives

Nerves are the bundles of neuronal axons connecting with the peripheral nervous system. *Connectives* are the bundle of fibers connecting segmental neuromeres. (See Section VI-5, p. 58.)

Table S7. Names of landmark fiber bundles

antennal lobe-associated tracts

mALT	medial antennal lobe tract
mlALT	mediolateral antennal lobe tract
lALT	lateral antennal lobe tract
AST	antenno-subesophageal tract

fiber bundles in the cerebrum

AOT	anterior optic tract
PYF	pyriform fascicle
PLF	posterior lateral fascicle
aSLPF	anterior SLP fascicle
hVLPF	horizontal VLP fascicle
vVLPF	vertical VLP fascicle
MBDL	median bundle
MEF	medial equatorial fascicle
LEF	lateral equatorial fascicle

cerebro-cervical fascicles

pCCF	posterior cerebro-cervical fascicle
mCCF	medial cerebro-cervical fascicle
aCCF	anterior cerebro-cervical fascicle
lCCF	lateral cerebro-cervical fascicle

fiber systems

SFS	superior fiber system
IFS	inferior fiber system

chiasmata

OCH1	first optic chiasma of the OL
OCH2	second optic chiasma of the OL
ACH	anterior chiasma of the CX
PCH	posterior chiasma of the CX

commissures

sALC	superior AL commissure
iALC	inferior AL commissure
LALC	LAL commissure
SEC	superior ellipsoid commissure
SAC	superior arch commissure
sPLPC	superior PLP commissure
pPLPC	posterior PLP commissure
POC	posterior optic commissure
GC	great commissure
sAMMCC	superior AMMC commissure
WEDC	wedge commissure

nerves and connective

AN	antennal nerve
TgN	tegumentary nerve
LbrN	labral nerve
FrN	frontal nerve
* LbrFrN	labro-frontal nerve
RcN	recurrent nerve
NCC I, II, III	corpora cardiaca nerve I, II, III
PhN	pharyngeal nerve
APhN	accessory pharyngeal nerve
MnN	mandibular nerve
MxN	maxillary nerve
LbN	labial nerve
* MxLbN	maxillary-labial nerve
OCN	ocellar nerve
CV	cervical connective

* indicates the fused nerve observed in some insects including *Drosophila*.

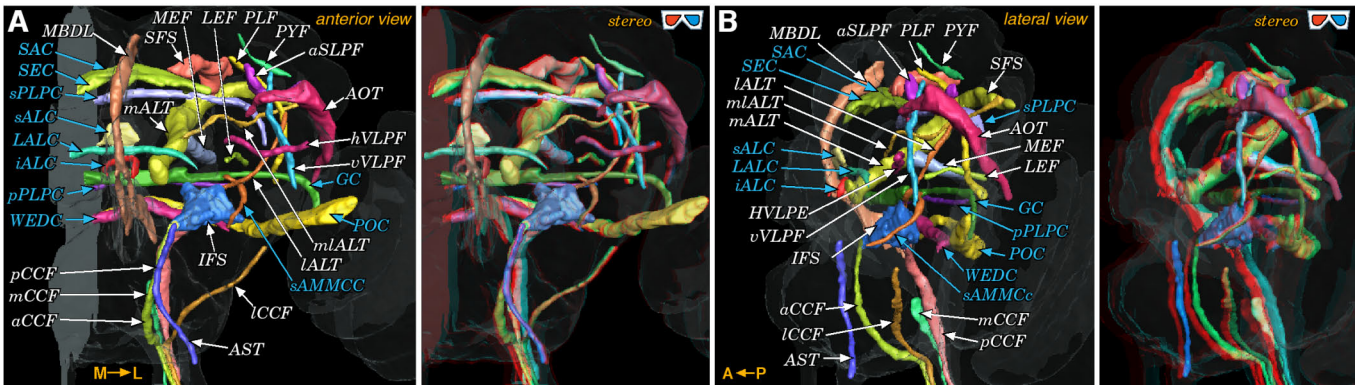


Figure S5. Landmark fiber bundles

(Spectacle icons indicate red-cyan 3D color stereograms viewable with a red-cyan stereogram filter.)

As with neuropil names, the names of landmark fiber bundles were determined according to the following policy.

- Retain classic terminology whenever possible.
- When multiple names have been used to refer to an identical structure, choose one that seems least confusing.
- When a single name has been used to refer to different structures depending on different insect species or researchers, devise a new term to avoid confusion. (Do not use the same term to refer to different structures.)
- Names of the commissures that were given the suffix "... tract" are changed to "... commissure".
- Names of commissures such as "inter-XXX commissure" or "commissure of XXX" are all changed to "XXX-commissure" to keep consistency of the naming scheme.
- Fascicles and commissures with no established names are given names according to the neuropils they connect or project through.

The abbreviations of the fiber bundles were suggested using the following rationale:

- Retain conventional abbreviations whenever possible.
- Uppercase letters are used for the core part of the tract and commissure names (e.g. *ALT*, *PCCF*, *ALC,* etc.). If multiple fibers exist within a particular category, the prefixes for distinguishing them are abbreviated with lower-case letters to increase readability (e.g. *m, ml* and *lALT*). In spite of this, abbreviations are chosen so that they are all distinguishable using a case-insensitive search.

Note: the target of the fiber bundles and the target of each single axon

The target of a fiber bundle is determined as the region in which the prominent bundle disperses. This does not necessarily mean that all the neurons of the fiber bundle terminate in that region. Neuronal fibers may spread out, or subsets of fibers may extend further to other neuropils.

III-4. Names for the cell body rind

In the insect nervous system, except for between the base of the retina and the neuropil of the *lamina*, neuronal cell bodies are all located in the *cell body rind* (CBR) near or at the surface of the brain. The locality of any area or cluster of neuronal cell bodies can most simply be described with reference to the name of the next nearest (often adjacent) neuropil (Fig. S6). We suggest the following scheme to name the cell body rind subdivisions.

- Use the name of the adjacent neuropils and add prefix "r" to distinguish the neuropil name and the cell body rind name.
- Some neuropils have multiple surfaces, e.g. anterior, superior, and posterior surfaces of the SMP and posterior, superior, and lateral surfaces of the LH. If it is necessary to distinguish the location of cell bodies with respect to any surface, the addition of a, p, d, v, etc., (according to the body axis) will indicate the particular surface.

Three regions of the cell body rind have been given special names.

Pars intercerebralis (PI) (Latin word for "the body that is between-the-brain")

The *PI* can be defined as the region of the rind on the midline of the superiormost brain (*n-anteriormost brain*). Neurons and neurosecretory cells with large cell bodies form clusters, many of which send axons that project to the median bundle. It corresponds to the *rSMPm* (ma, md and mp) in the list below.

Pars lateralis (PL) (Latin word for "the body that is lateral")

The *PL* refers to the region of the rind that contains the cluster of neurosecretory cells that lies laterally. Because these neurosecretory cells are scattered in some cases, an unambiguous delineation of the *PL* is not always possible. In *Droso-*

phila it roughly corresponds to the *rSLPl* in the list below.

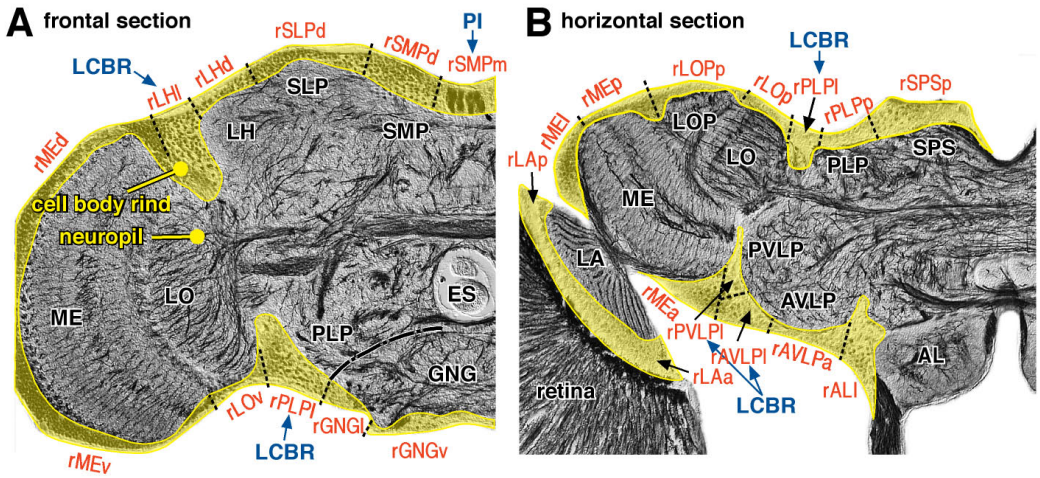
Lateral cell body region (LCBR)

The *LCBR* is a broad region of the cell body rind between the *central brain* and the *optic lobe*. It corresponds to the entire regions of the *lateral cell body rind* in the list below.

Table S8. Names of the cell body rind

- In the following table, possible occurrences in the *Drosophila* brain are listed. In certain insect species, these may vary depending on the specific arrangements of the neuropils and cell body rind.

optic lobe cell body rind (OLBR)		
rLAl	lateral to LA surface	
rLAm	medial to LA medial surface (if any)	
rLAa	anterior to LA rim	
rLAp	posterior to LA rim	
rLAd	dorsal to LA rim	
rLAv	ventral to LA rim	
rMEl	lateral to ME surface	
rMEa	anterior to ME rim	
rMEp	posterior to ME rim	
rMEd	dorsal to ME rim	
rMEv	ventral to ME rim	
rLOa	anterior to LO rim	
rLOp	posterior to LO rim	
rLOd	dorsal to LO rim	
rLOv	ventral to LO rim	
rLOPp	posterior to LOP surface	
rLOPd	dorsal to LOP rim	
rLOPv	ventral to LOP rim	
anterior cell body rind (ACBR)		
rSMPa	anterior to SMP	
rSIPa	anterior to SIP	
rSLPa	anterior to SLP	
rAOTUa	anterior to AOTU	
rAOTUv	ventral to AOTU (between AVLP and AOTU)	
rAVLPa	anterior to AVLP	
rCREa	anterior to CRE	
rALad	anterodorsal to AL (between AL and CRE)	
rALld	laterodorsal to AL (between AL, ML and AVLP)	
rALl	lateral to AL (between AL and AVLP)	
rALv	ventral to AL (between AL and PRW)	
rSADmv	medioventral to SAD (between SAD and GNG)	
superior cell body rind (SCBR)		
rSMPd	dorsal to SMP	
rSIPd	dorsal to SIP	
rSLPd	dorsal to SLP	
rLHd	dorsal to LH	
posterior cell body rind (PCBR)		
rSMPp	posterior to SMP	
rSLPp	posterior to SLP	
rLHp	posterior to LH	
rCAp	posterior to CA	
rPBp	posterior to PB	
rIBp	posterior to IB	
rSPSp	posterior to SPS	
rPLPp	posterior to PLP	
lateral cell body rind (LCBR)		
rAOTUl	lateral to AOTU	
rSLPl	lateral to SLP	(= PL)
rLHl	lateral to LH	
rAVLPl	lateral to AVLP	
rPVLPl	lateral to PVLP	
rWEDl	lateral to WED	
rPLPl	lateral to PLP	
rSADl	lateral to SAD	
rIPSl	lateral to IPS	
cell body rind along the midline (MCBR)		
rSMPma	medioanterior to SMP	(= PI)
rSMPmd	mediodorsal to SMP	(= PI)
rSMPmp	medioposterior to SMP	(= PI)
rCREm	medial to CRE	
rATLm	medial to ATL	
cell body rind around the GNG (GCBR)		
rGNGla	lateroanterior to GNG	
rGNGlm	lateral to GNG (middle region)	
rGNGlp	lateroposterior to GNG	
rGNGva	ventroanterior to GNG	
rGNGvm	ventral to GNG (middle region)	
rGNGvp	ventroposterior to GNG	



black: neuropil name, red: cell body rind name based on adjascent neuropil, blue: unique rind name

Figure S6. Names of the cell body rind

III-5. Colloquial names of insects and their representative taxonomic names

Colloquial names have been used to refer to those insects that are often used for neuroanatomical studies. The following Table lists their Latin names, the order to which they belong, and in some cases their superfamily. The list also provides alternative common names for certain genera.

Table S9. Colloquial names of insects and their representative taxonomic names

Name mentioned in the text	include	Latin name of representative species	Order (and superfamily)
Fly	Fruit fly, Vinegar fly	*Drosophila melanogaster*	Diptera (Ephydroidea)
	Blowfly	*Calliphora erythrocephala*	Diptera (Muscoidea)
	House fly	*Musca domestica*	Diptera (Oestroidea)
	Flesh fly	*Sarcophaga carnaria*	Diptera (Oestroidea)
	Bluebottle	*Phaenicia sericata*	Diptera (Oestroidea)
Mosquito	Malaria mosquito	*Anopheles gambiae*	Diptera (Culicoidea)
	Yellow fever mosquito	*Aedes aegypti*	Diptera (Culicoidea)
Locust	Desert locust	*Schistocerca gregaria*	Orthoptera (Acridoidea)
	American grasshopper	*Schistocerca americana*	Orthoptera (Acridoidea)
	Bow-winged grasshopper	*Chorthippus biguttulus*	Orthoptera (Acridoidea)
Cricket	House cricket	*Acheta domesticus*	Orthoptera (Grylloidea)
Moth	Tobacco hawkmoth	*Manduca sexta*	Lepidoptera
	Silk moth	*Bombyx mori*	Lepidoptera
Butterfly	Cabbage butterfly	*Pieris brassicae*	Lepidoptera
	Monarch butterfly	*Danaus plexippus*	Lepidoptera
Bee	Honey bee	*Apis mellifera*	Hymenoptera
	Bumble bee	*Bombus impatiens*	Hymenoptera
Wasp	Paper wasp	*Polistes* sp.	Hymenoptera
	Solitary wasp	*Pepsis thisbe*	Hymenoptera
Ant	Carpenter ant	*Camponotus floridanus*	Hymenoptera
	Leaf cutting ant	*Atta vollenweideri*	Hymenoptera
		Apterostigma mayri	Hymenoptera
Beetle	Scarab beetle	*Maladera castanea*	Coleoptera
	Red flour beetle	*Tribolium castaneum*	Coleoptera
	Meal worm	*Tenebrio molitor*	Coleoptera
	Whirligig beetle	*Dineutus sublineatus*	Coleoptera
	Tiger beetle	*Cicindela ocellata*	Coleoptera
Cockroach	American cockroach	*Periplaneta americana*	Dictyoptera
	Madera cockroach	*Rhyparobia maderae*	Dictyoptera
		Leucophaea maderae	Dictyoptera
Dragonfly	Hawker	*Aeshna* sp.	Odonata
	Damselfly	*Sympetrum* sp.	Odonata
Bristletails	Firebrat	*Thermobia domestica*	Zygentoma
	Silverfish	*Lepisma saccharina*	Zygentoma

IV. Figures of neuropils and selected annotated sections

In the following pages, Fig. S7 shows a three-dimensional reconstruction model of the entire synapse-rich neuropils and major fiber bundles of the adult female *Drosophila melanogaster* brain (5-10 days old), and Figs. S8-S10 show cross-sections of the same brain at different levels along three body axes. Figs. S11-S13 present representative confocal serial sections. Frontal sections (Fig. S11) are labeled with *elav*-GAL4 > UAS-n-syb-GFP labeling (which provides images similar to the nc-82 antibody-labeled brains) and with "tricolor" labeling (see below) that overlies neuropil boundaries. For horizontal and sagittal sections (Figs. S12, S13), only tricolor labeling with boundary overlays are shown. See Tables S1-S3 and detailed explanation from p. 31 for abbreviations.

Figs. S14 and S15 present examples of identified clones with respect to identified neuropils (Ito et al., 2013). Clonal analyses demonstrate that the progeny of single neuroblasts (called a *clonal unit*) provide processes (neurites) that form arborizations occupying specific volumes of the brain. The extents of such arborizations tend to match landmarks identifiable by antibody markers. These are taken into account when determining neuropil identities and limits.

Synapse-rich neuropil boundaries and landmark fiber bundles were identified by comparing the data obtained from the following labeling procedures (Shinomiya et al., 2011) and the projection and arborization patterns of known neurons and clones (see also p. 67).

Silver stain

Used to visualize neuronal fibers using a reduced silver stain following the Holmes-Blest protocol (Blest, 1961).

nc82 (anti Bruchpilot) antibody

Used to visualize synapse-rich neuropils according to the density of an active synaptic zone-specific protein Bruchpilot. Regions occupied by the fiber bundles and glial processes are left unlabeled. nc82 is thus convenient for estimating the regions of synapse-rich neuropils. Anti-Synapsin antibody provides similar labeling patterns. Anti-Bruchpilot (nc82), available from Developmental Studies Hybridoma Bank (University of Iowa) (Wagh et al., 2006).

anti-DLG (discs large) antibody

Used to label membranes of the neuronal cell bodies, neuronal fibers, and synapses, by detecting DLG proteins required for septate junction structure. Unlike nc82, anti-DLG also labels fiber bundles and fiber structures within neuropils. – Anti-Discs large (4F3), available from Developmental Studies Hybridoma Bank (University of Iowa) (Parnas et al., 2001).

anti-β-Tubulin antibody

Used to visualize fibrous structures of both neurons and glial cells. – Anti-β Tubulin (E7), available from Developmental Studies Hybridoma Bank (University of Iowa) (Chu and Klymkowsky, 1989; Popodi et al., 2005).

repo-GAL4 > UAS-GFP

Used to visualize glial cells and their processes, which form bounding sheaths surrounding many (but not all) neuropils (Lai and Lee, 2006).

elav-GAL4 > UAS-GFP

Used to visualize fibers of whole neurons (Lin and Goodman, 1994). Neurons are labeled with different intensities because of the differences in *elav* expression levels. Using cytoplasmic GFP or DsRed reporters, thick fibers are labeled more intensely than thin fibers. Regions occupied by the glial processes are left unlabeled.

"Tricolor" labeling (*elav*-GAL4 > UAS-DsRed, UAS-n-syb-GFP, UAS-Rdl-HA)

Used to visualize various aspects of neuropils and fiber bundles. Cytoplasmic DsRed (red; Verkhusha et al., 2001) visualizes synaptic and non-synaptic fibers. Synaptic vesicle-targeted n-syb-GFP (green; Estes et al., 2001) visualizes the distribution of the presynaptic sites, similar (but not completely equal) to that obtained by nc82 and anti-Synapsin antibodies. GABA receptor-targeted Rdl-HA signal (blue; Sánchez-Soriano et al., 2005) indicates postsynaptic sites and cell bodies.

Figures of the neuropils yellow: neuropil names, *white italic*: fiber bundles, white normal: other structures

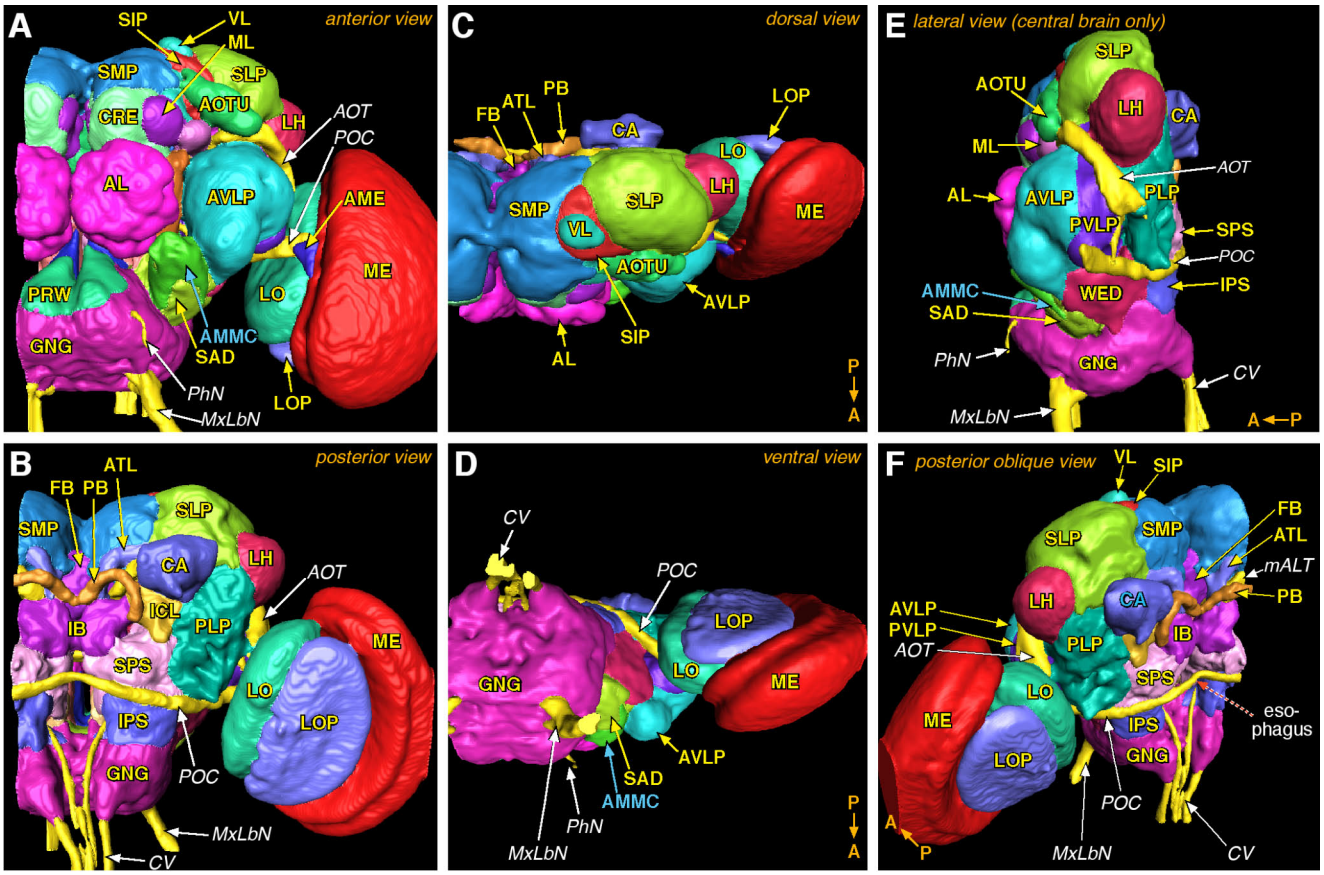


Figure S7. General view

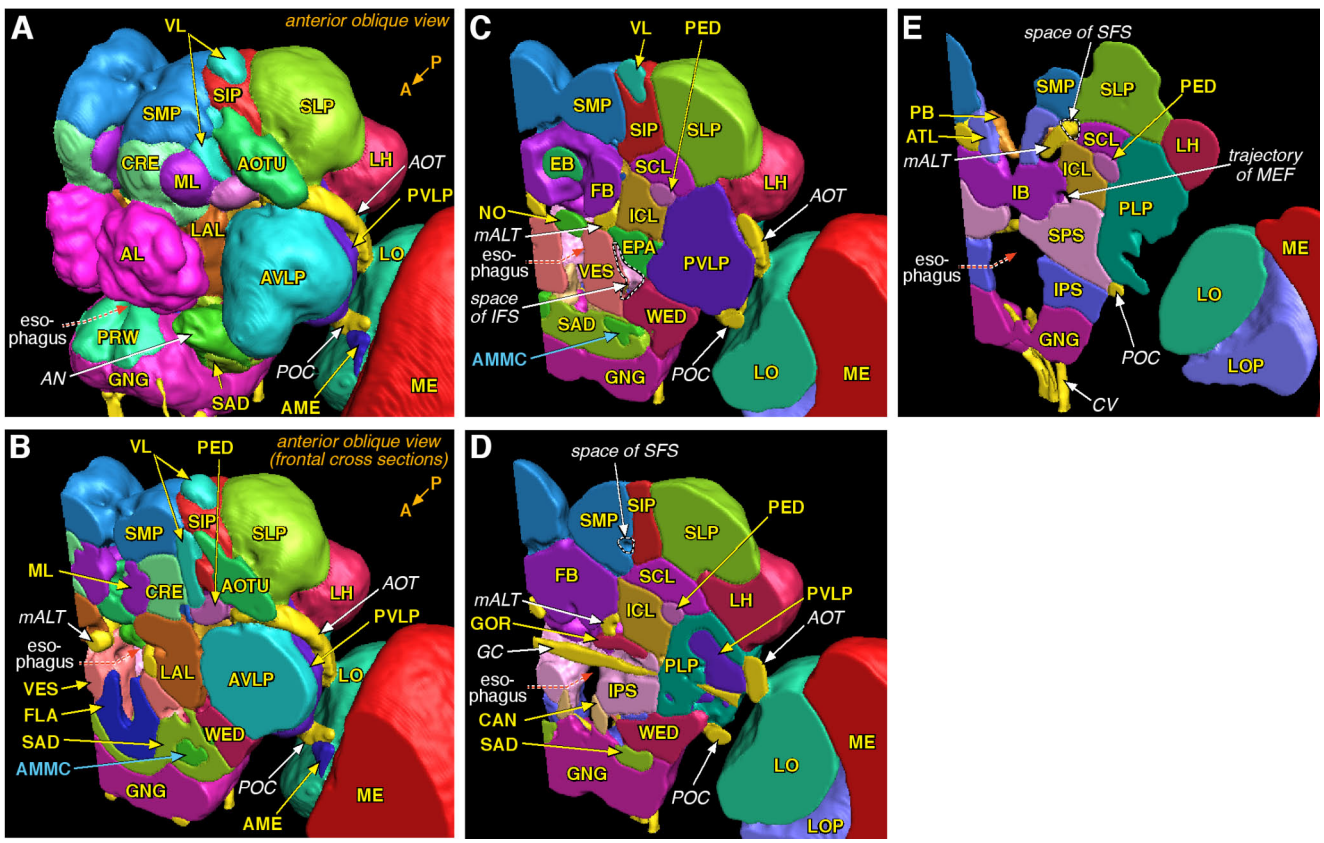


Figure S8. Frontal cross sections (anterior to the left bottom)

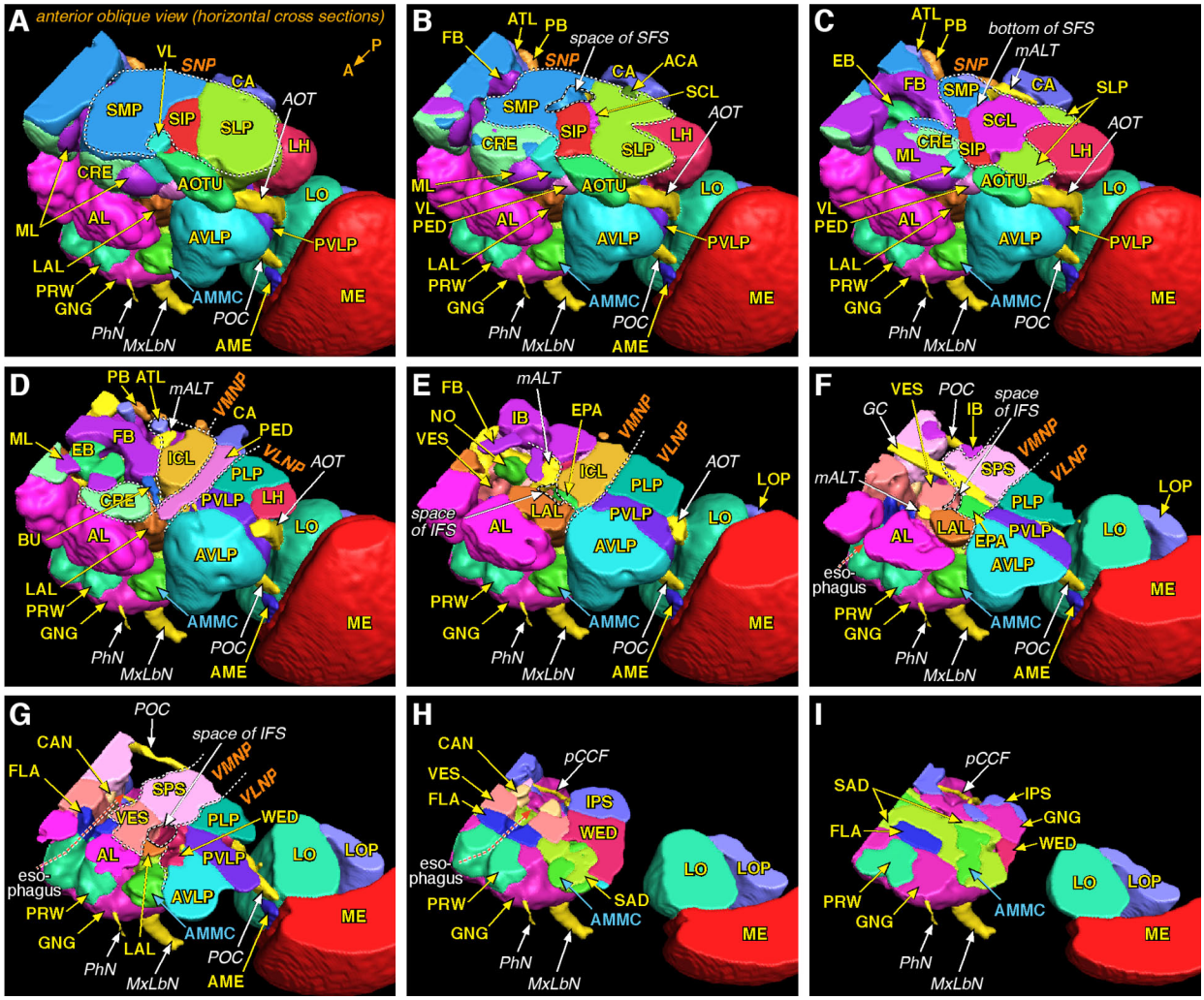


Figure S9. Horizontal cross sections (anterior to the left bottom)

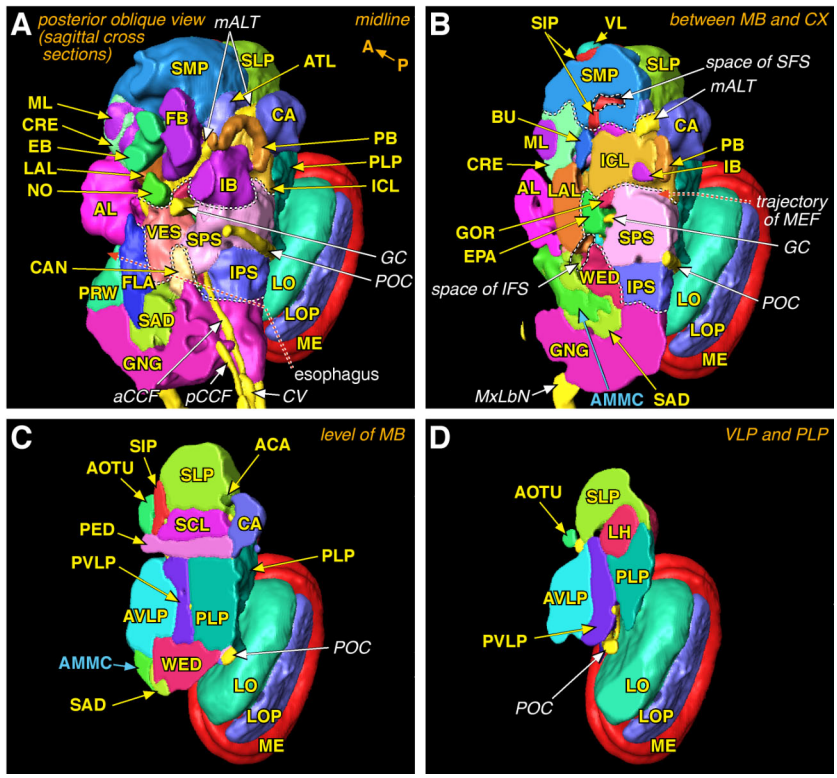


Figure S10. Sagittal cross sections (anterior to the left top)

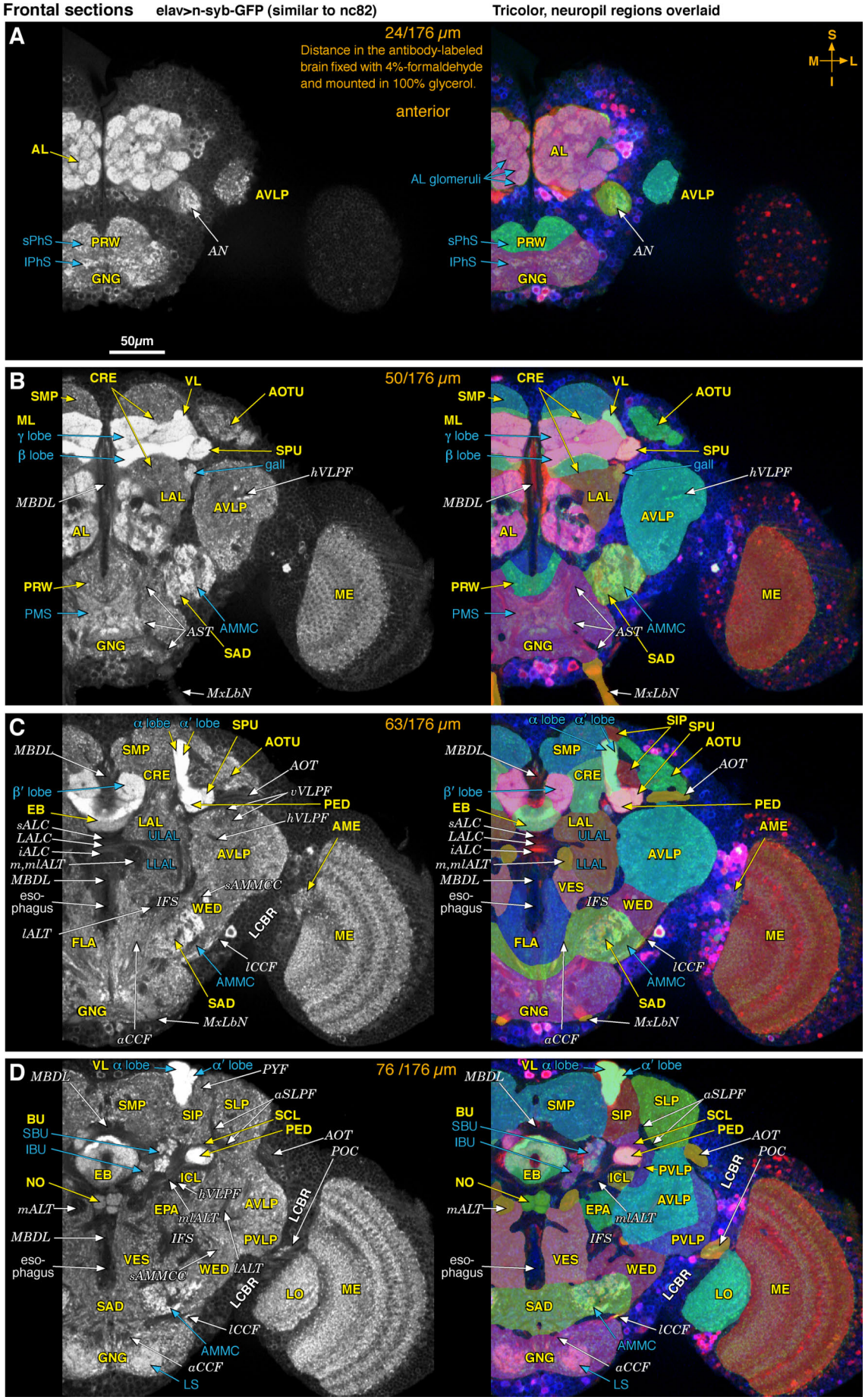


Figure S11. Annotated sections (frontal, part 1) yellow: neuropils (level 2), blue: subregions (level 3), *white italic*: fiber bundles

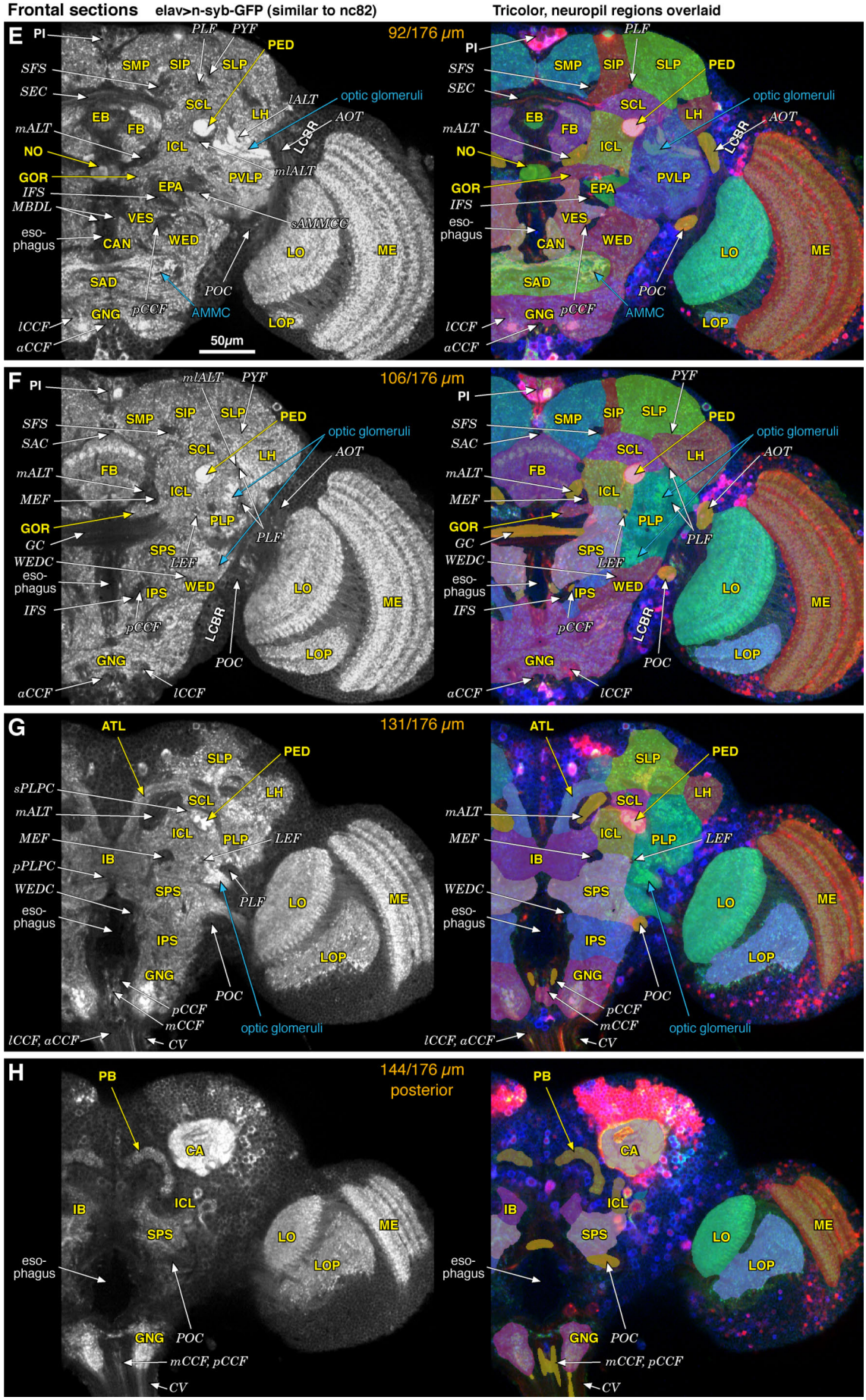


Figure S11. Annotated sections (frontal, part 2) yellow: neuropils (level 2), blue: subregions (level 3), *white italic*: fiber bundles

Horizontal sections (Tricolor, neuropil regions overlaid)

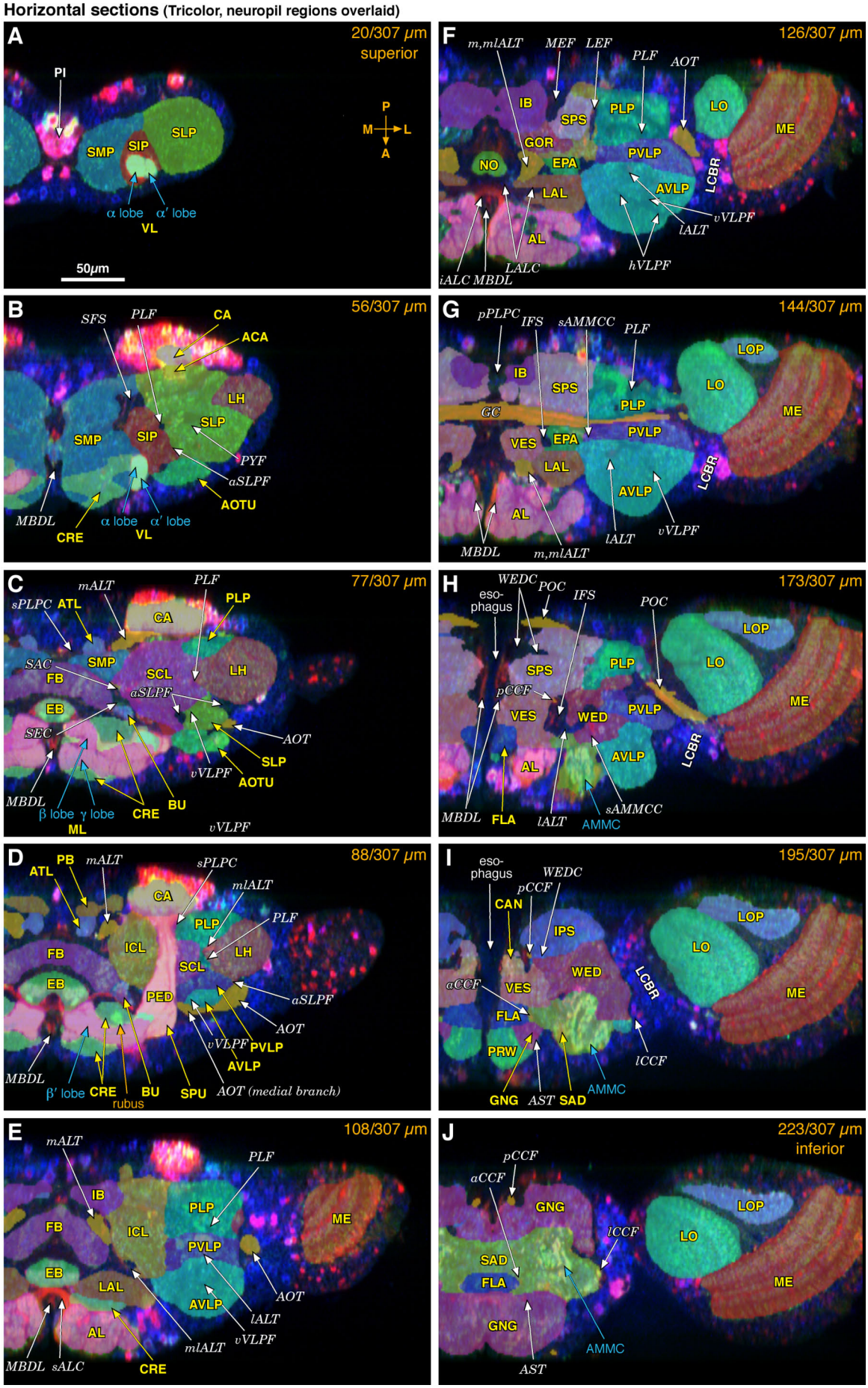


Figure S12. Annotated sections (horizontal) yellow: neuropils (level 2), blue: subregions (level 3), *white italic*: fiber bundles

Sagittal sections (Tricolor, neuropil regions overlaid)

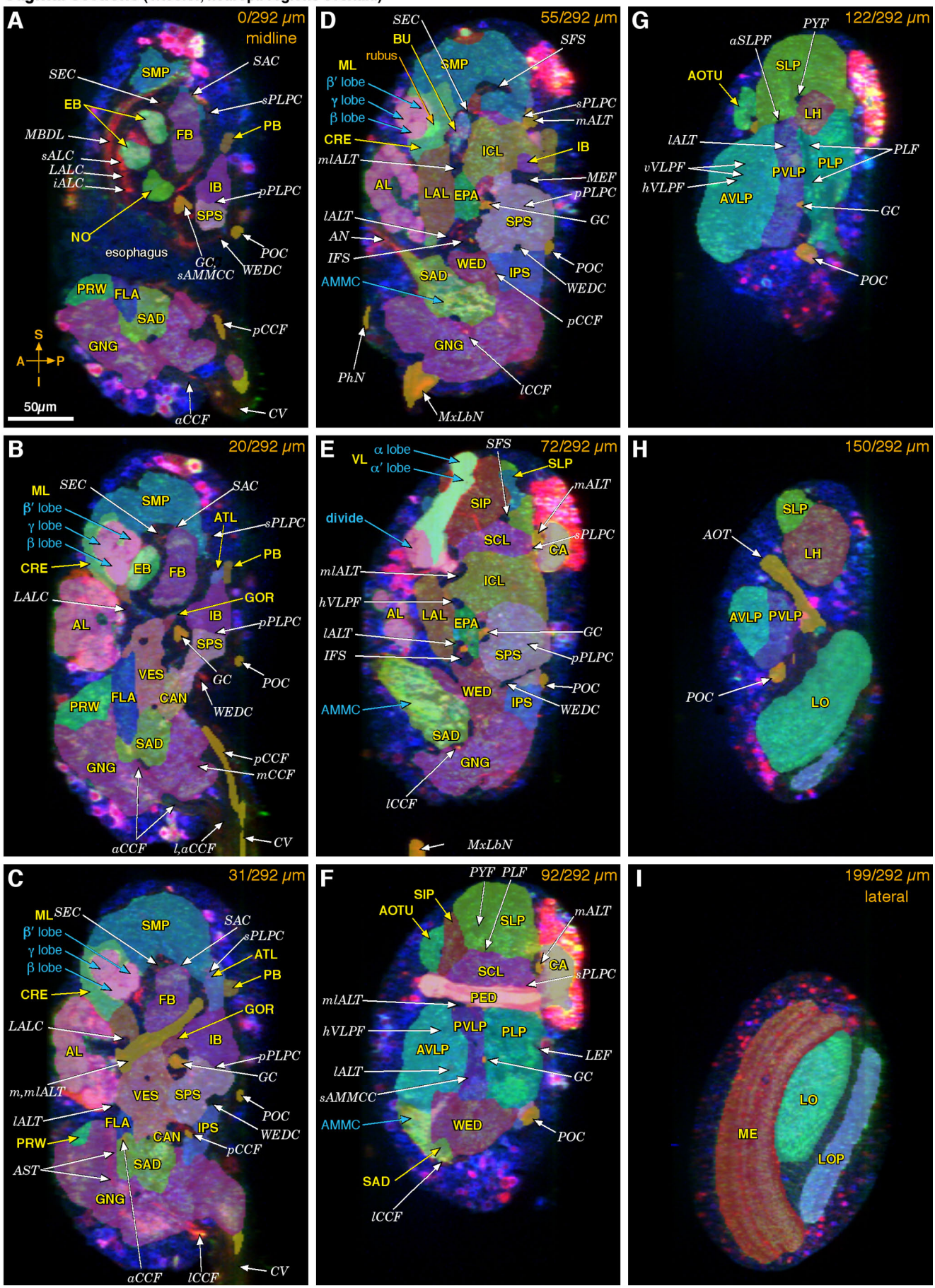


Figure S13. Annotated sections (sagittal) yellow: neuropils (level 2), blue: subregions (level 3), *white italic*: fiber bundles

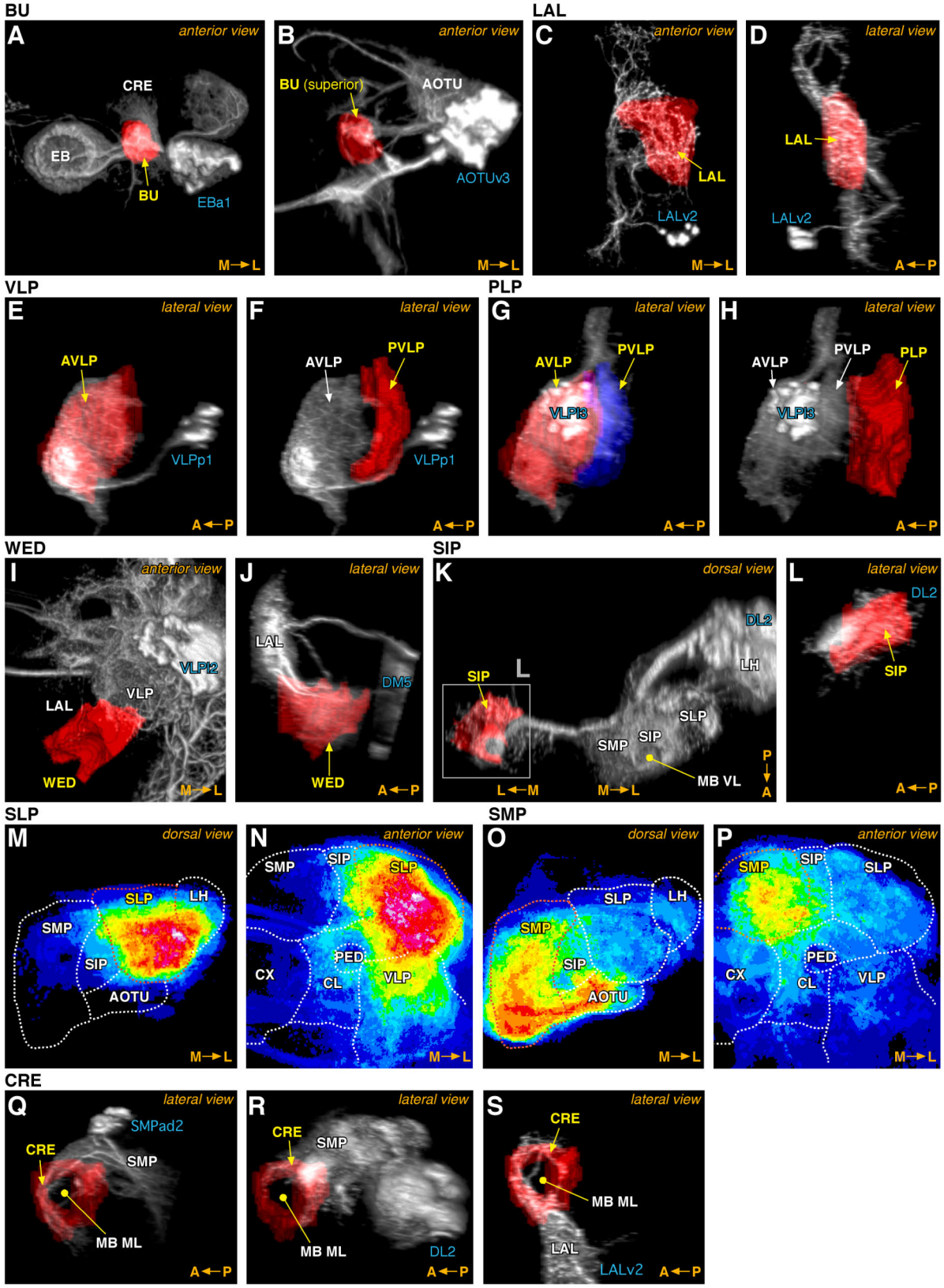


Figure S14. Neuropils and arborizations of clonally related neuron groups (clonal units) (part 1)

Red or blue volumes indicate the extent of discrete neuropils. Structures shown in white and gray reveal the arborizations of a group of clonally related neurons generated by a single neuroblast. All structures are 3D reconstructions. Blue letters indicate the clone name (Ito et al., 2013). **(A)** A clone innervating the *EB* has extensive arborization in the *BU* (and sparse arborization in the *CRE*). **(B)** Clonal projections from the *AOTU* terminate in the superior part of the *BU*. **(C, D)** A clone arborizing broadly in the *LAL*. **(E, F)** A clone arborizing broadly in the *AVLP* but not in the *PVLP*. **(G, H)** A clone arborizing broadly in both *AVLP* and *PVLP* (G) but not in the *PLP* (H). There is no

known clone that arborizes broadly in the *PLP*. **(I)** A clone arborizing broadly in the *VLP* but not in the underlying *WED*. **(J)** Projection from a clone arborizing broadly in the *WED*. **(K, L)** Projections from a clone arborizing in the ipsilateral *SIP* and terminating in the contralateral *SIP*. Though the ipsilateral arborization spread in the neighboring *SLP* and *SMP*, the contralateral one is confined in the *SIP*. **(M, N, O, P)** Clones in the *SLP, SIP* and *SMP* form rather contiguous arborizations, making it difficult to clearly demarcate neuropil boundaries. However, when arborizations of multiple clones that innervate preferentially in the *SLP* (M, N) and *SMP* (O, P) are overlaid (color coded from blue to yellow to red), consensus regions demarcate the boundaries between them as well as with the underlying neuropils such as the *VLP* and *CL* (N, P). **(Q, R, S)** Neuropil of the *CRE* contributed by the clones surrounding the *MB medial lobe*.

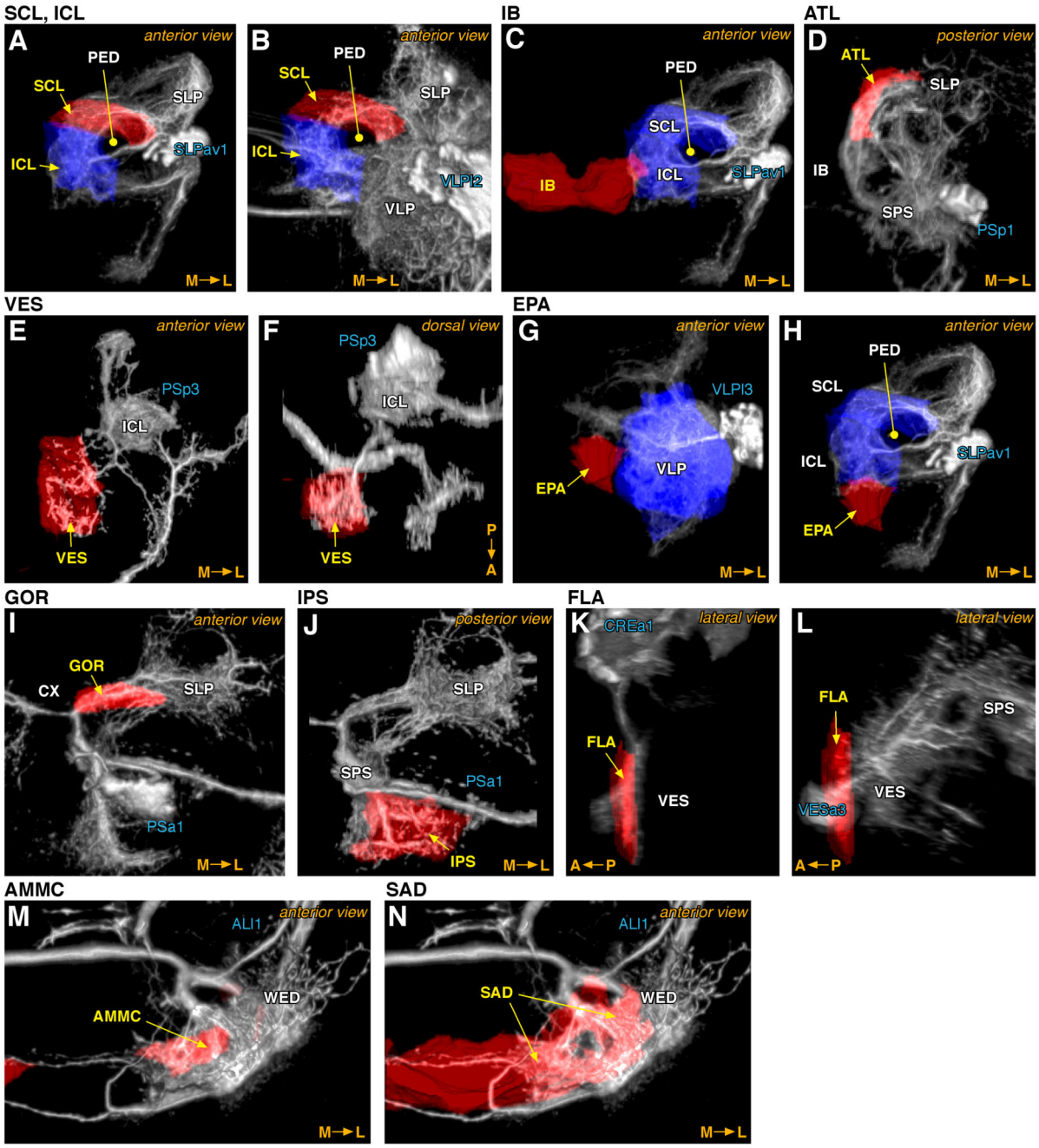


Figure S15. Neuropils and arborizations of clonally related neuron groups (clonal units) (part 2)

(A, B) Projections of clones surrounding the *MB pedunculus*. Their arborizations in regions other than the *SLP* and *VLP* match the *clamp* neuropil (*CL*). **(C)** A clone arborizing in the *CL* but not in the *IB* that lies medially. **(D)** Projection of a clone that arborizes through the elongated neuropil of *ATL*. **(E, F)** A clone, one of whose branches forms confined arborizations in the *VES*. **(G, H)** Clones that arborize in the *VLP* (G) and *CL* (H) but not in the neighboring *EPA*. **(I)** A clone projecting from the *SPS* through the region below the *central complex* (*CX*), forming arborizations in the *GOR*. **(J)** Projection of a clone that arborizes broadly in the *IPS*. **(K)** A clone arborizing in the thin neuropil of *FLA*. **(L)** A clone that projects through the *FLA* but arborizes mainly in the neighboring *VES*. **(M, N)** A clone arborizing not only in the *AMMC* (M) but also in the surrounding *SAD* (N).

V. Guide to synapse-rich neuropils

This section describes the positions, shapes, and arrangements of each synapse-rich neuropil – thus, a more detailed version of Section I-3. To make documentation self-consistent, some parts of the text necessarily repeat parts of previous sections. The present descriptions are based on the adult brain of female *Drosophila melanogaster*. Colors of the section titles (red, black and blue) correspond to the three levels of hierarchical categories described in Table S3 (p. 6). Because likely homologous structures may not appear to have the same shape and organization in other insect species, interspecific comparisons are mentioned when and where sufficiently understood.

For the sake of clarity, the names of neuropils and fiber bundles are written in *italics.* However, unlike the names given to genes, they do not have to be italicized when used in regular papers or presentations. Directions are indicated according to the body axis. Those according to the neuraxis are supplemented where necessary with the suffix "n-".

Detailed definition of the neuropil boundaries are provided in the appendix at the end of this document.

Cerebral ganglia (CRG)

The *cerebral ganglia* (*CRG*) comprise the volume of the brain above, around, and partially below the esophagus (Fig. S1A). The *CRG* developmentally consists of three neuromeres: the *protocerebrum, deutocerebrum,* and *tritocerebrum*. In insects, the *deutocerebrum* and *tritocerebrum* both extend commissures beneath the esophagus, because the esophagus penetrates the middle of the *deutocerebrum* during development (Boyan et al., 1993).

Because neurons that developmentally originate in one particular neuromere can send extensive processes to other neuromeres (Ito et al., 2013), these three neuromeres of the brain appear almost completely fused: there are no clear boundaries between them.

- *Cerebral ganglia* are sometimes called the *brain, cerebrum*, or *supraesophageal ganglia*, but we suggest calling it the *cerebral ganglia* (*CRG*, see p. 3 and p. 11).

V-1. Optic lobe (OL)

The *optic lobe* (*OL*) is a substantial lateral outgrowth of the *protocerebrum* usually comprising several nested neuropils (Fig. S16). These reconstruct visual information from patterns of light intensity and wavelengths detected by visual sensory neurons (photoreceptors) of the compound eyes. In most insects, each *OL* consists of three distinct neuropils, the *lamina* (*LA*), *medulla* (*ME*), and the *lobula complex* (*LOX*) (Strausfeld, 2005). The *medulla* is divided into an outer and inner part, deriving from separate embryonic components (Meinertzhagen and Hanson, 1993). The l*obula complex* is divided into the *lobula* (*LO*) and the *lobula plate* (*LOP*). In many insect orders, the *lobula plate* is integrated into the *lobula*, occupying a deep layer rather than a separate neuropil opposite the *lobula* (Strausfeld, 2005).

The *lamina, medulla, lobula,* and *lobula plate* are all easy to identify, because they are separated clearly by the cell body rind of the *optic lobe* (Fig. S12F-J) as well as by fibers of the *first* and *second optic chiasmata* (Fig. S17).

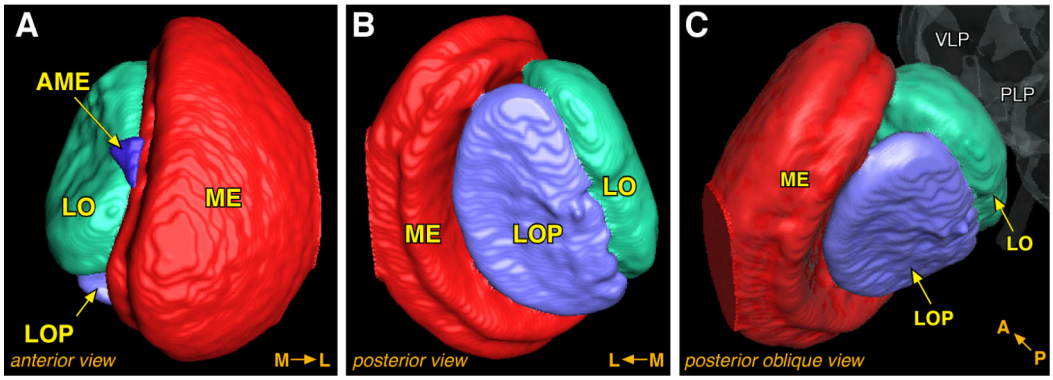


Figure S16. Optic lobe (lamina is not shown)

V-1-1 Lamina (LA)

The *LA* is the most distal neuropil, lying beneath the compound eye (in flies, the *LA* lies just beneath the retina above, whereas in some other insects it is more distant from the retina). The principle organization of the *LA* is that of a palisade of repeating columnar units, each of which receives axons from those photoreceptors that share the same optic alignment. In many species, such optically coherent photoreceptors reside in the same ommatidium. In flies and related Diptera, which have neural superposition eyes, identically aligned photoreceptors are distributed amongst seven neighboring ommatidia (Braitenberg, 1967; Kirschfeld, 1967).

According to the parts of the retina from where their axons derive, the lamina can be divided into two parts:

lamina dorsal rim area (LADRA): In many insects, several horizontal rows of columns in the dorsal *lamina* receive axon terminals from ommatidia in the *dorsal rim* of the compound eye (Strausfeld and Wunderer, 1985; Blum and Labhart, 2000; Homberg and Paech, 2002). The photoreceptor rhabdoms in the ommatidia of the *dorsal rim* are specialized for the detection of the E-vector of polarized light (Homberg and Paech, 2002).

plexiform lamina (PLLA): The *PLLA* refers to the bulk of the *lamin*a that does not contain the *lamina dorsal rim area*.

Note: retina of insects and vertebrates

- Here, the term “retina” is not synonymous with the term “retina” used for vertebrates. In vertebrates, the retina consists of the photoreceptor layer (rods and cones) and the several strata of neurons and connections comprising the inner and outer plexiform layers. In insects, and other arthropods with compound eyes, the retina refers exclusively to the layer of photoreceptor neurons and their non-neuronal accessory cells.

V-1-2 Accessory lamina (ALA)

In certain species (e.g. cockroaches, caddisflies) there is a secondary neuropil that has been called *accessory lamina* (Hagberg, 1986; Loesel and Homberg, 2001). The *ALA* is not visible (presumed absent) in *Drosophila melanogaster*.

V-1-3 Medulla (ME)

The *ME* is the second and (in many species, including *Drosophila melanogaster*) largest neuropil of the *optic lobe*. Like the *lamina*, it consists of a *medulla dorsal rim area* and the rest (*plexiform medulla*).

medulla dorsal rim area (MEDRA): The dorsal region of the *medulla* receiving the terminals of long visual fibers of some retinal photoreceptors as well as terminals of *lamina* relay interneurons from the corresponding columns in the *lamina dorsal rim area, which* represent the dorsal rim of the *retina* (Homberg and Paech, 2002; Pfeiffer and Kinoshita, 2012).

plexiform medulla (PLME): The *PLME* refers to the bulk of the *medulla* excluding the *medulla dorsal rim area*.

The organization of tangential neurons and layer-specific branches of columnar neurons divide the *medulla* (both *MEDRA* and *PLME*) into three successive layers, which can further be divided into discrete strata (10 in the *PLME* of *Drosophila*; Fischbach and Dittrich, 1989). The sequence, from distal (= lateral; closer to the *lamina*) to proximal (= medial; further from the *lamina*), is as follows:

outer medulla (OME): Tangential neurons, amacrine cells, local interneurons, and lateral processes of columnar neurons contribute to several plexiform strata through the *outer medulla*.

serpentine layer (SPL): This layer, which was originally referred to as *Cuccati's bundle*, contains extensive tangential axons of the neurons that project to or from the *medulla* via the *posterior optic commissure*. Some of these neurons connect the two *medullae* heterolaterally, whereas others terminate ipsi- or contralaterally in the *central brain*.

inner medulla (IME): A layer comprising about 1/3rd of the *medulla*’s depth, lying medial to the *serpentine layer*. Amacrine cells, local interneurons, and the lateral processes of the retinotopic neurons contribute to three plexiform strata through the *inner medulla*.

layers 1-10: The *medulla* of *Drosophila* is divided into about 10 strata, of which 6 (layer 1-6) are in the *outer medulla* and 3 in the *inner medulla* (layer 8-10), separated by the *serpentine layer* (layer 7) (Fischbach and Dittrich, 1989).

V-1-4 Accessory medulla (AME)

The *AME* is a small, separated region of neuropil located at the anterior-medial edge of the *medulla*, close to the outgoing fibers from the *serpentine layer* to the *posterior optic commissure*. Unlike the *accessory lamina*, the *accessory medulla* exists in flies (reviewed by Helfrich-Förster, 2004).

V-1-5 Lobula complex (LOX)

The medialmost region of the *optic lobe*. It exhibits various organizations depending on species (Cajal and Sánchez, 1915). In wasps/honeybees/ants and cockroaches, the *lobula complex* consists of a single neuropil called the *lobula* (*LO*), the lower part of which is characterized by large-field tangential cells and small neurons that typify the *lobula plate* in species with a *lobula complex* comprising a *lobula* and *lobula plate*. In cockroaches/mantis, the *lobula* is associated with several satellite lobe-like neuropils. In locusts/crickets/grasshoppers, as many as five subdivisions have been recognized, which are called the *outer lobe, dorsal lobe, anterior lobe,* and *inner dorsal* and *inner ventral lobes*. Future studies will have to determine if some of these are laterally displaced *optic glomeruli* from the *central brain*. In flies/mosquitoes, moths/butterflies, beetles, and dragonflies/damselflies, the *lobula complex* is divided into two separate neuropils: the *lobula* (*LO*) and *lobula plate* (*LOP*).

V-1-5a Lobula (LO)

In flies the *lobula* is located in the anterior medial region of the *lobula complex* and houses many ensembles of columnar neurons. Their axons segregate, according to type, to *optic glomeruli*.

layers 1-6: The *Drosophila lobula* is divided into 6 layers (Fischbach and Dittrich, 1989). Layers 1-6 from posterior medial to anterior lateral. Layer 1 is topologically closest to the *medulla*, and layer 6 is the furthest from it.

V-1-5b Lobula plate (LOP)

In Diptera, the *lobula plate* lies in the posterior lateral region of the *lobula complex* and contains many large tangential neurons (Fischbach and Dittrich, 1989).

layers 1-4: The *Drosophila lobula plate* is divided into 4 layers (Fischbach and Dittrich, 1989). Layers 1-4 from anterior to posterior. Layer 1 is topologically closest to the *medulla*, and layer 4 is the furthest from it. Layer 1 of the *lobula* faces layer 1 of the *lobula plate*.

Note: first optic chiasma and second optic chiasma

- The system of fiber bundles between the lamina and medulla is called the first optic chiasma (OCH1). Axons reverse the linear order of horizontal rows of columns between the two neuropils.
- The system of axons between the *medulla* and *lobula complex* is termed the *second optic chiasma* (*OCH2*). In those taxa with a single *lobula*, the axons from the *medulla* reverse in the *lobula complex* the linear order of columns along the horizontal axis (Fig. S17A). However, the situation is different in those taxa with a *lobula plate* (Fig. S17B). The *lobula* lies opposite to the *lobula plate*. Both regions are columnar, with the columns representing anterior in the visual field being closest to the *medulla*, and those representing posterior in the visual field being furthest from the *medulla*. For the linear order of columns to be the same in the *lobula* and *lobula plate* requires that axons from the *medulla* to the *lobula* form a chiasma, whereas axons from the *medulla* to the *lobula plate* do not (Strausfeld, 2005). Neither do axons connecting the *lobula* and *lobula plate*.

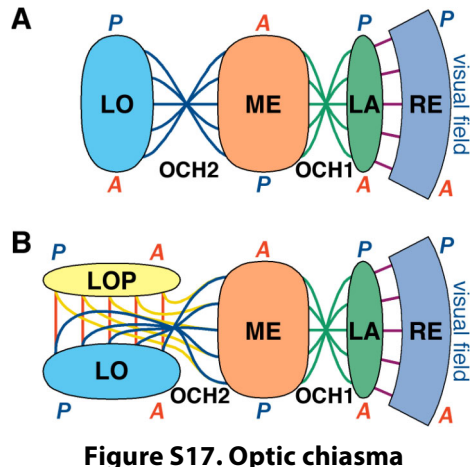


Figure S17. Optic chiasma

V-2. Mushroom body (MB)

The *mushroom bodies* are paired mushroom-shaped neuropils in the *protocerebrum* (Farris, 2005; Strausfeld et al., 1998, 2009). They are characterized by extensive and confined arborizations of intrinsic neurons called Kenyon cells. The *MB* consists of four major parts (*calyx, pedunculus, vertical lobe,* and *medial lobe*). In some species, there are also small satellite neuropils (*accessory calyx*, *spur, Y tract*, and *Y lobe*; Pearson, 1971; Weiss, 1981; Homberg et al., 1988; Tanaka et al., 2008; Heinze and Reppert, 2012). Except for the *accessory calyx,* most of the *MB* neuropils are separated from the surrounding neuropils by glial sheaths.

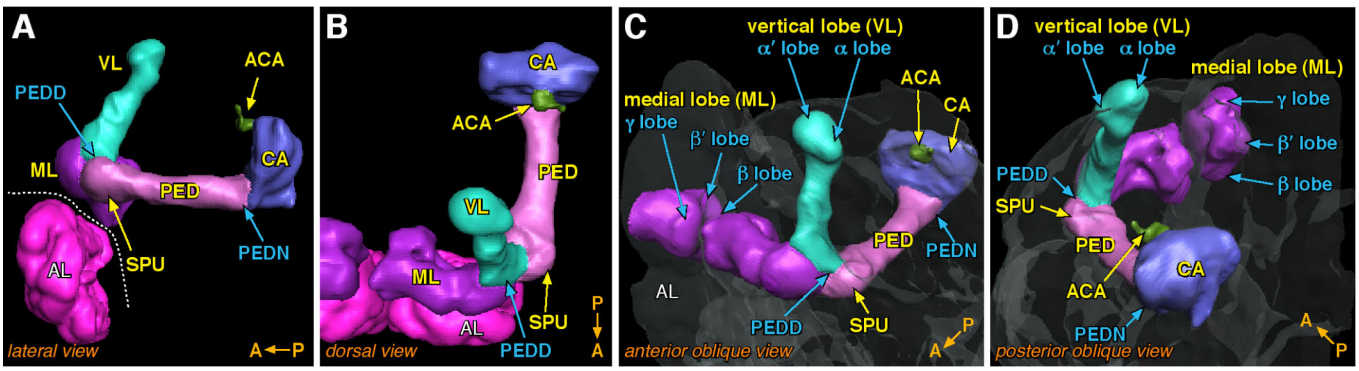


Figure S18. Mushroom body

V-2-1 Calyx (CA)

The region of bulbous or cup-like structures immediately beneath the cluster of Kenyon cell bodies is the *calyx*. In most insects, the *calyx* receives extensive inputs from *antennal lobe* projection neurons. In dragonflies and many aquatic insects (adults), that have very few, if any, olfactory sensory neurons in their antennae, both *antennal lobe* projection neurons and *calyx* are essentially missing as are the *antennal lobes* (Strausfeld et al., 2009).

- *Calyx* is sometimes abbreviated as *CX*, but we suggest the abbreviation *CA* (see p. 11).

Lateral calyx (LCA), medial calyx (MCA)

In some insects, such as honey bees and ants, the *calyx* forms twin cup-like structures (Kenyon, 1896; Ehmer and Gronenberg, 2004). Even in flies, moths, and butterflies, which apparently have a single bulbous *calyx*, the *calyx* comprises two fused entities. For example, the *Drosophila MB* is made by four clonally associated groups of Kenyon cells, but the lateral two groups are more closely associated with each other as are the medial two groups. Fiber bundles arising from the lateral and medial pairs of Kenyon cell groups fuse at the proximal (posterior) part of the *pedunculus*, but the resulting two bundle structures merge only at the distal (anterior) end of the *pedunculus* (Ito et al., 1997).

Note: concentric structure of the calyx (lip, collar, basal ring)

- In honey bees, wasps, ants, and many other Hymenoptera, the *lateral* and *medial calyces* consist of three major concentric zones: the *lip, collar,* and *basal ring*. The *lip* primarily receives olfactory information, whereas the *collar* primarily receives visual information (Mobbs, 1984; Ehmer and Gronenberg, 2002). These zones can be further subdivided on the basis of additional inputs of other sensory modalities. Such clear concentric organization and massive visual input to the *calyces* are observed in some other orders such as Coleoptera (beetles) (Larsson et al., 2004).

V-2-2 Accessory calyx (ACA)

The *ACA* is a small region protruding from the *calyx* into the *SLP* (*superior lateral protocerebrum*). A subset of Kenyon cells arborizes in this region. So far the *ACA* has been reported in *Drosophila*, moths/butterflies, cockroaches, crickets/locusts, firebrat/silverfish, and termites (Weiss, 1981; Homberg et al., 1988; Tanaka et al., 2008; Farris, 2008; Heinze and Reppert, 2012). Unlike the *calyx,* the *ACA* does not appear to receive input from *antennal lobe* projection neurons. The boundary between *ACA* and the surrounding *SLP* is not visible with nc82 synaptic label; there is no glial sheath separating them. The location of *ACA* varies among species. It is located on the anterior side of the *calyx* in *Drosophila*, but posterior of it in moths/butterflies as well as in locusts/crickets. Though they all share the same characteristics described above, whether they indeed refer to a single, homologue structure remains to be investigated.

V-2-3 Pedunculus (PED)

The mass of Kenyon cell fibers connecting the *calyx* and *lobes* forms the *pedunculus*. However, this is not a fascicle, rather a synapse-rich neuropil because it features many synapses within it (Schürmann, 1970). It runs almost horizontally in flies, but in honey bees it is almost upright (relative to the body axis). Thus, the *pedunculus* appears as a cross section in frontal sections of the fly brain, whereas in honey bees it appears as a parallel stream of fibers.

- *Pedunculus* is sometimes spelled *peduncle,* but we suggest calling it *pedunculus* (see p. 15).

Pedunculus neck (PEDN)

The proximal (posterior or posterior superior) point of the *pedunculus*, where Kenyon cell fibers converge beneath the *calyx* to form the *pedunculus*.

Pedunculus divide (PEDD)

The distal (anterior or anterior inferior) point of the *pedunculus*, where Kenyon cell fibers bifurcate to extend into the *vertical* and *medial lobes*.

- *Pedunculus divide* is sometimes called *heel*, *knee*, or *junction of the lobes*, but we suggest the term *pedunculus divide* (see p. 12).

V-2-4 Spur (SPU)

A small protrusion lateral to the *pedunculus divide*, identifiable in some flies and dragonflies (Tanaka et al., 2008; Strausfeld et al., 2009). It is formed either by the specific set of Kenyon cells (γ neurons) that run through the lateral part of the *pedunculus divide* and project to the *γ lobe* (in flies), or the collaterals (third branches) of Kenyon cells arising from the *pedunculus divide* (in dragonflies).

- *Spur* is sometimes called *heel* or *knee*, but we suggest calling it *spur*. It is sometimes abbreviated as *SP*, but we suggest the abbreviation *SPU* (see p. 12).

Note: Is SPU a part of the pedunculus or lobes?

- In *Drosophila,* the *spur* is formed almost entirely by the γ neuron fibers that circumvent the lateral part of the *pedunculus divide* and project to the *medial lobe* (Tanaka et al., 2008). Many extrinsic neurons arborize in this region and form synapses with γ neurons. In this respect, the *spur* in *Drosophila* can be regarded as the most proximal part of the *medial lobe*, even though it lies lateral to – not medial to – the *pedunculus* and appears as a part of it. In other insects, neurons projecting to the *vertical lobe* may also contribute to the *spur*. Because of these multiple identities, we classified *spur* as a separate entity rather than a part of the *pedunculus*, *vertical lobe*, or *medial lobe*.

V-2-5 Vertical lobe (VL) and medial lobe (ML)

Kenyon cell fibers bifurcate at the *pedunculus divide* to provide two lobes, called the *vertical* and *medial lobes*. The *vertical lobe* extends from inferior to superior in flies, but approximately from posterior to anterior in honey bees. Thus, in frontal sections the fly *vertical lobe* appears as a stream of parallel fibers whereas the corresponding structure in the honey bee is seen in cross section.

- The *vertical lobe* is sometimes called the *dorsal lobe*, but we suggest calling it the *vertical lobe*. The *medial lobe* is sometimes called the *horizontal lobe,* but we suggest calling it the *medial lobe* (see p. 12).

The *vertical lobe* consists of three components:

vertical γ lobe (VγL) or γ division of the vertical lobe (not in adult flies)
α prime lobe (α'L) or α' division of the vertical lobe
α lobe (αL) or α division of the vertical lobe

The *medial lobe* also consists of three components:

γ lobe (γL) or γ division of the medial lobe
β prime lobe (β'L) or β' division of the medial lobe
β lobe (βL) or β division of the medial lobe

In some insects, such as Diptera, these substructures form distinct lobes. In other insects (e.g. honey bees and cockroaches) they are not discrete but occupy different slabs within the *vertical/medial lobes*. In such case these structures are called *divisions* (Strausfeld, 2002, Sinakevitch et al., 2001)

The α/β neurons of the Kenyon cells bifurcate to project to the *α lobe* and *β lob*e. The α'/β' neurons contribute to the *α' lobe* and *β' lobe*. In most insects, γ neurons contribute to the *γ lobe* and the *vertical γ lobe* (or *γ divisions* of the *medial* and *vertical lobes*; Sinakevitch et al., 2001; Strausfeld, 2002), but in flies, γ neurons project only to the *γ lobe* (and therefore, a *vertical γ lobe* does not exist in flies) (Lee et al., 1999). In honey bees γ neurons enter only a little way into the *medial lobe* (Strausfeld, 2002). In paper wasps (Polistinae), where there is a complete absence of the *medial lobe*, γ neurons enter the *vertical lobe* only (Farris et al., 2004).

Layers and slices

Layers in the lobes

Each lobe may have either a concentric or laminar layered structure. These finer layers are given letters or numbers depending on species. In *Drosophila*, e.g., (Tanaka et al., 2008)

α' lobe: α' anterior (αa), α' middle (αm), and α' posterior (αp) layers
α lobe : α posterior (αp), α surface (αs), α core outer (αco), and α core inner (αci) layers
β' lobe: β' anterior (βa), β' middle (βm), and β' posterior (βp) layers
β lobe : β posterior (βp), β surface (βs), β core outer (βco), and β core inner (βci) layers

αp layer/vertical lobelet and βp layer/medial lobelet

Kenyon cell fibers deriving from the *accessory calyx* form a specific layer (called the *α accessory calyx layer* and *β accessory calyx layer*) in the *vertical* and *medial lobes* and form small protrusions called the *vertical* and *medial lobelet* (Farris and Strausfeld, 2003). In *Drosophila* the *α* and *β accessory calyx layers* are called the *αp layer* and *βp layer* shown in the above list, as they occupy the *p*osterior part of the *α* and *β lobes* and are formed by the neurons that are generated *p*rior to other α/β neurons (Tanaka et al., 2008).

slices by the MB extrinsic neurons

Lobe-associated extrinsic neurons, connecting the *MB lobes* with neighboring neuropils, tend to arborize in distinct transverse divisions of the lobes, dividing the lobes into several slices. In flies, e.g., (Tanaka et al., 2008)

α' and α lobes: 1 (base), 2 (shaft), and 3 (tip) of the lobes
β' and β lobes: 1 (proximal = lateral) and 2 (distal = medial) parts of the lobes
γ lobe: 1-5 from proximal (lateral) to distal (medial)

and in cockroaches (Li and Strausfeld, 1999),

medial lobe: 1-6 from proximal (lateral) to distal (medial)
vertical lobe: precise number unknown yet

Trauben (TRA)

In the *MB* of firebrats and silverfish (Zygentoma) and mayflies (Ephemeroptera), swollen protrusions called the *trauben* are observed in the *medial lobe*. (Böttger, 1910; Strausfeld et al., 1998). A similar organization of the *medial lobe* into numerous ovoid swellings has also been observed in some Heteroptera (Farris, 2004; Strausfeld et al., 2009).

Accessory lobe (ACL)

In some species, elongated processes originating from the *accessory calyx* often extend separately from the pedunculus. These fibers enwrap the *vertical lobe* to form what is called the *accessory lobe* (e.g., Dictyoptera).

V-2-6 Y tract (YT)

The *Y tract*, or *secondary pedunculus*, is a separate bundle of Kenyon cells identified in moths/butterflies (Pearson, 1971). It emerges from a more dorsal region of the *calyx* than the *pedunculus neck* and takes a more medial trajectory than the *pedunculus* to reach the region dorsal to the *medial lobe* and medial to the *vertical lobe*. There it forms a distinct swollen lobe called the *Y lobe*. (The *Y tract* is presumed absent in *Drosophila*.)

V-2-7 Y lobe (YL)

The *Y lobe* is observed in moths/butterflies at the anterior end of the *Y tract* (Pearson, 1971). It lies near the branch point of the *vertical* and *medial lobes* but is structurally isolated from them. Two lobelets, called the dorsal lobelet and the ventral lobelet, exist here (Sjöholm et al., 2005; Heinze and Reppert, 2012). (The *Y lobe* is presumed absent in *Drosophila*.)

V-3. Central complex (CX)

The *central complex* comprises a group of neuropils lying on the midline at the center of the brain (Strausfeld, 1976; Hanesch et al., 1989; Young and Armstrong, 2010a, 2010b). There are four major components: *fan-shaped body* (*FB*), *ellipsoid body* (*EB*), *protocerebral bridge* (*PB*), and *noduli* (*NO*). In flies, the *FB*, *EB* and *NO* are covered entirely by extensive glial sheaths, making them easy to distinguish from the surrounding neuropils. The *PB* is extensively connected with the *FB*, but its position is somewhat detached from the rest of the neuropils, embedded in the cell body rind in the superior posterior brain.

- *Central complex* is sometimes called *central body*, but we suggest using the term *central body* specifically for the combination of the *FB* and *EB* and *central complex* for the combination of all the four components. *Central complex* is sometimes abbreviated as *CC*, but we suggest the abbreviation *CX* (see pp. 12-13).

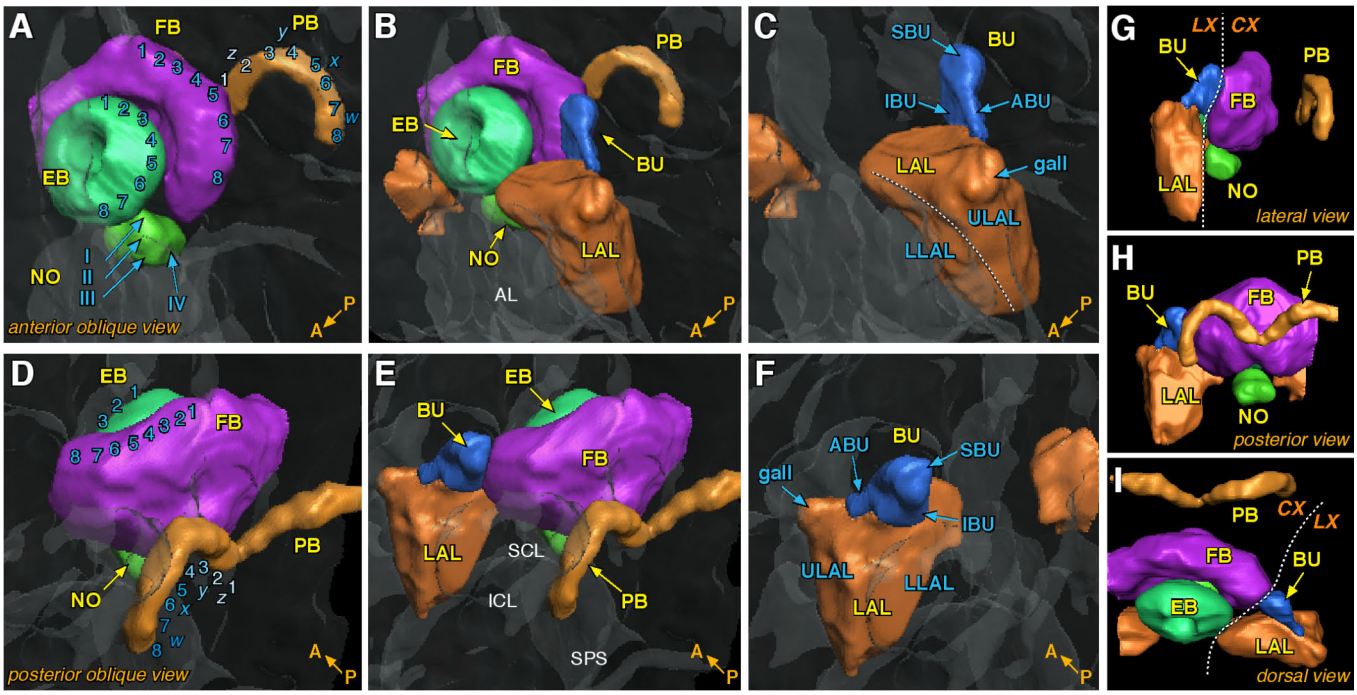


Figure S19. Central complex and lateral complex

V-3-1 Central body (CB)

The *central body* is here determined as the combination of the *fan-shaped body* and *ellipsoid body*.

V-3-1a Fan-shaped body (FB) or Upper division of the central body (CBU)

The *fan-shaped body* consists of a matrix of columnar (small-field) and large-field neurons. In flies the columnar components form a radiating fan-like organization, and thus the entire *fan-shaped body* has a fan-like shape (Hanesch et al., 1989). In other insects, the shape of the *fan-shaped body* may be arch-like, bar-like, or kidney-like (Homberg, 1987, 2008; Loesel et al., 2002; Strausfeld, 2012). It is greatly elongated in decapod crustaceans (Utting et al., 2000; Strausfeld, 2012). The structure can still be called the *fan-shaped body* because of the homology with the fly counterpart, but it can also be called the *upper division of the central body* (*CBU*, e.g. in locust).

Slices

In many species, including *Drosophila* and *Schistocerca*, *fan-shaped body* is usually separated into 8 discrete modules, here termed *slices* (*slices* 1-8 from medial to lateral), on each side of the midline (i.e. a total of 16) that reflect the columnar arrangement of neurons projected through the *FB* neuropil. In some taxa there may be more slices (e.g. *Mantis religiosa*, where there are nine; Strausfeld and Hirth, 2013) or fewer (e.g. in aquatic Hemiptera; Strausfeld, 2012). In *Drosophila*, two neighboring slices, i.e., 1-2, 3-4, 5-6, and 7-8, are associated more closely because they receive small-field columnar neurons generated by the same stem cells, forming 4 groups on each side of the midline (Ito and Awasaki, 2008; Boyan and Williams, 2011).

- The slices are sometimes counted from lateral to medial, but we suggest counting them from medial to lateral.
  The slices have often been called segments or columns, but we suggest calling them slices (see p. 13).

Layers

Processes of various neurons divide the *fan-shaped body* into several layers. In *flies* the *fan-shaped body* is divided into 6-8 layers depending on the specific labeling method. These layers have been numbered from top to bottom in Hanesch et al. (1989), but recent studies number the layers from bottom to top (Young and Armstrong, 2010a; Phillips-Portillo and Strausfeld, 2012) following the direction of projections of prominent fan-shaped neurons. We therefore recommend counting the layers in the fly *fan-shaped body* from bottom to top (from inferior to superior).

Between three and four layers have been identified in the *fan-shaped body* (*CBU*) of locusts and its homologue in flightless grasshoppers (e.g. *Barytettix psolus*), monach butterflies, and the tobacco hornworm *Manduca sexta*. In locusts and monarchs, these layers have been numbered from top to bottom (Müller et al., 1997; Heinze et al., 2013). Generally, it has been difficult to compare layers in the *FB*/*CBU* amongst different taxa because in many of them the central body has a taxon-specific shape and orientation (see Note in the next page), and it is not possible to reconcile such taxon-specific features with a numbering system devised for *Drosophila* and certain other Diptera.

Elements

Because of the matrix arrangement of layers and slices, the *fan-shaped body* of *Drosophila* can be separated into up to 64 (8 x 8) elements on each side. The number of these elements is subject to change if future studies identify finer divisions.

Note: Superior arch and lateral protuberance

- In *Drosophila*, the most superior layer (layer 8) of the *fan-shaped body* is sometimes called the *superior arch* (Strausfeld, 1976). In addition, in some Diptera (e.g. *Musca domestica*) the lateralmost modules (numbers 7, 8) protrude anteriorly, providing a substructure sometimes referred to as the *lateral protuberance* (Strausfeld, 1976). They are both parts of the *fan-shaped body*, however.

Note: Left-right asymmetry of the fan-shaped body

- In *Drosophila* and in blowflies, a pair of neuropil domains in layer 1 of the *fan-shaped body* shows an anatomical left-right asymmetry, where one of these *asymmetric bodies* is larger than the other (Pascual et al., 2004; Phillips-Portillo and Strausfeld, 2012).

V-3-1b Ellipsoid body (EB) or Lower division of the central body (CBL)

The *ellipsoid body* also consists of a matrix of vertical (or radial) and tangential fibers (Strausfeld, 1976; Hanesch et al., 1989). In flies, the structure is so curved that the lateral edges of the *ellipsoid body* fuse at the inferior midline, forming a torus. In many other insects, the structure is much less curved, ranging from a prominent inverted U to a shallow arch (Homberg, 1987, 2008; Loesel et al., 2002; Strausfeld, 2012). Those differently shaped neuropils can still be called the *ellipsoid body* because of their correspondence with the fly counterpart. They can also be called the *lower division of the central body* (*CBL*, e.g. in locust).

Slices

In flies, as in other insects, the *ellipsoid body* is separated into 8 slices each side of the midline (in total 16). In the bar-shaped *ellipsoid body* (*CBL*) of various insects, these slices should be counted from medial to lateral as in the *fan-shaped body*. Because the fly *ellipsoid body* is toroidal, its slices are arranged 1-8 from superior medial (topologically medial) to inferior medial (topologically lateral).

- The slices are sometimes counted from lateral to medial, but we suggest counting them from medial to lateral.
  The slices have often been called segments or sectors, but we suggest calling them slices (see p. 13).

Layers

The bar-like *ellipsoid body*/*CBL* in most insects is organized into horizontal layers, which are often counted from top to bottom (Müller et al., 1997; Heinze et al., 2013). Because of its toroidal shape, the fly *ellipsoid body* has a concentric arrange-

ment of layers. Tney are counted from the center to the periphery (layers 1-4: Hanesch et al., 1989; Renn et al., 1999) that topologically corresponds to the order from bottom to top. As in the layers of the *fan-shaped body*/*CBU*, we decided not to resolve the possible discrepancy in the counting order of these layers because of the inconsistent layer boundary identities and difference in the orientation of the *ellipsoid body*/*CBL*.

Elements

Because of the matrix arrangement of layers and slices, the *ellipsoid body* can be separated into 32 (8 x 4) elements on each side in case of *Drosophila*. The number of these elements may be subject to change if future studies identify finer divisions.

Note: orientation of the central body

- In flies the *fan-shaped body* and *ellipsoid body* are arranged horizontally so that the former lies posterior to the latter. In locust, the *CBU* and *CBL* are arranged vertically (according to the body axis) so that the former lies above the latter (el Jundi et al., 2010). Thus, the orientation of the *central body* is rotated along the transverse axis by about 90 degrees. The relative arrangement of the *FB*/*CBU* and *EB*/*CBL* varies considerably and in a taxon-specific manner in other insects (Strausfeld, 2012), and their relative orientations cannot be precisely matched to the orientation of other landmark structures such as the *MB pedunculus*, whose direction also shows variability across taxa. Thus, in many insects the upper and lower divisions of the *CB* are not necessarily aligned vertically as in the locust. This being the case, position-independent terms like *FB* and *EB* might be more widely applicable.

V-3-2 Protocerebral bridge (PB)

The *protocerebral bridge* usually has the form of an arched or handle bar-shaped profile just posterior to the *fan-shaped body* (Strausfeld, 1976). In many taxa it is unbroken across the midline and is half-embedded in the cell body rind of the superior posterior brain. In some species, especially lepidopteran, its neuropil comprises two volumes each side of the midline that are linked by fiber bundles (Heinze et al., 2013). In flies, the lateral edge of the *PB* ends at the posterior end of the *inferior clamp* (*ICL*) near the *medial equatorial fascicle* (*MEF*).

Slices

Like the *fan-shaped body*, in *Drosophila* the *protocerebral bridge* is separated into 8 slices each side of the midline (in total 16, *slices 1-8* from medial to lateral) by the dendrites of the *fan-shaped body* columnar neurons projecting through the *protocerebral bridge*. Neighboring slices in the *protocerebral bridge*, i.e., 1-2, 3-4, 5-6, and 7-8, each form a more closely associated unit in the *fan-shaped body*, thereby suggesting four subunits each side of the *fan-shaped body*'s midline. There is no layer organization in the *protocerebral bridge*.

w, x, y, and z bundles

The fibers projecting from the cell body rind via the *protocerebral bridge* to the *fan-shaped body* form four bundles on each side. Each of them is formed by clonally associated neurons generated by the same stem cell (Ito and Awasaki, 2008; Boyan et al., 2008; Boyan and Williams, 2011). These four bundles are called *w, x, y,* and *z* from lateral to medial (Williams, 1975).

Though we defined the slices in the *protocerebral bridge*, *fan-shaped body,* and *ellipsoid body* counting from medial to lateral, we did not change the counting direction of these bundles because, unlike the slice numbers, these alphabets for the bundles (w-z) have been used unanimously in this order.

Posterior chiasma and anterior chiasma of the central complex

Between the *protocerebral bridge* and *fan-shaped body*, about half of the fibers cross the midline to project to the contralateral *fan-shaped body* (Williams, 1975; Boyan et al., 2008), forming the *posterior chiasma* (*PCH*) of the *central complex*.

Similarly, in the region anterior to the *fan-shaped body*, some fibers arising from the *fan-shaped body* cross the midline to innervate the contralateral *lateral accessory lobe* (*LAL*). They form the *anterior chiasma* (*ACH*) of the *central complex*.

V-3-3 Noduli (NO)

Noduli are the structures lying beneath the inferior tip of the *fan-shaped body* and *ellipsoid body* and are connected to both of them (Hanesch et al., 1989; Müller et al., 1997; Heinze and Homberg, 2008; Heinze et al., 2013). Unlike the other three components of the *central complex*, the *noduli* are paired, one *nodulus* on each side of the midline.

Subunits

Each *nodulus* consists of several subunits that are separated by glial processes. Four subunits can be identified in *Drosophila*. There are 3 subunits (*I*, *II* and *III*) lying on top of each other (Young and Armstrong, 2010a; Kashia et al., 2012), in front of a fourth subunit (*IV*) in the posterior (Fig. S19A). In monarch butterflies, the *noduli* are subdivided into four layers; in locusts an upper unit (consisting of three layers) is distinguished from a lower unit (without further substructure) (el Jundi et al., 2010). These components have been called *layers*, but because of their highly variable appearance (layered, lobular, etc.), they are better termed *subunits*.

Note: left-right asymmetry of the noduli

- At least in *Drosophila*, the *noduli* show slight anatomical left-right asymmetry. The *nodulus* of one side of the brain almost always lies slightly more anteriorly compared to that on the other side.

V-4. Lateral complex (LX)

Two structures (the *bulb* and the *lateral accessory lobe*) lie anterior laterally (*n-ventral laterally*) to the *central complex*. They are closely associated with the *central complex* but are distinguished from it in that they are not clearly separated from surrounding neuropils by extensive glial sheaths. They are also slightly detached from the *central complex* itself. We introduced the new term *lateral complex* to refer to these regions.

V-4-1 Bulb (BU)

The *bulb* is the structure lying lateral to the *ellipsoid body* and anterior lateral to the *fan-shaped body* (i.e. between anterior parts of the *central complex* and *MB pedunculus*). It is formed mainly by the collateral arborizations of neuronal fibers that project to the *ellipsoid body* and by the terminals of fibers projecting from the *anterior optic tubercle* and other neuropils (Fig. S14A, B). The *bulb* may lie just adjacent to the *central body* (e.g. in flies) or in the region a bit distant from it (e.g. in locusts). The terms *lateral triangle* and *median olive* have been used in the past to refer to some parts of the *bulb* (Hanesch et al., 1989; Müller et al., 1997; Heinze and Homberg, 2008). To avoid ambiguity, we suggest the new term *bulb*, because it contains many bulbous glomerular structures in it (Fig. S11D).

Subregions of the bulb (in *Drosophila*)

superior bulb (SBU), inferior bulb (IBU), anterior bulb (ABU)

In *Drosophila*, the *bulb* forms an essentially fused single structure. The classic terminology *lateral triangle* referred to all the regions of the *bulb* (Hanesch et al., 1989). Examination of sections showed that three subregions can be identified (Fig. S19C, F). The *SBU* and *IBU* lie in the region between *ellipsoid body* and *MB pedunculus*. They are characterized by many microglomerulus-like structures. The lateral part of the *SBU* extends antero-laterally and inferiorly to form the *ABU*, which lies beneath the *MB pedunculus*. The *ABU* is closer to the somata of the *ellipsoid body* neurons than the *SBU* and *IBU*.

In monarch butterflies, the *bulb* is slightly more detached from the *ellipsoid body* than in *Drosophila* but forms a single piece as in *Drosophila*. It consists of at least two sub-compartments (Heinze et al., 2013).

Subregions of the bulb (in locusts)

medial bulb (MBU), lateral bulb (LBU)

In desert locusts, the *bulb* is slightly more detached from the *ellipsoid body*/*CBL* than in *Drosophila*. Unike *Drosophila,* the locust *bulb* consists of two separated pieces.

MBU corresponds to *median olive*, or *olive*, in the previous locust terminology. It is embedded in the *lateral accessory lobe* (*LAL*), around the boundary between the upper and lower *LAL* (Müller et al., 1997; Heinze and Homberg, 2008, el Jundi et al., 2009).

LBU corresponds to the locust *lateral triangle* in the previous locust terminology. It lies near the lateralmost region of the *LAL*. *LBU* is closer to the somata of the *ellipsoid body*/*CBL* neurons than *MBU.*

Yet to be resolved are the parts of the *SBU*, *IBU* and *ABU* in flies that correspond to the locust *LBU* and *MBU*.

Note: Isthmus (IST)

The term *isthmus* is also used sometimes to refer to a part of the *bulb* in flies (Strausfeld, 1976). It was originally defined as a bridge of neuropil between the *fan-shaped body* (*CBU*) and the *LAL*/*bulbs* in locusts (Williams, 1975). Fiber fascicles called the *isthmus tract* pass through it (Homberg, 1987). Because of its original meaning, we suggest defining *isthmus* as a distinct synapse-rich neuropil and the *isthmus tract* as the fiber bundle between the *bulb* and the *fan-shaped body*. In flies, fibers that correspond to the *isthmus tract* exist, but a distinct *isthmus* neuropil has not been identified and is presumed not to exist.

V-4-2 Lateral accessory lobe (LAL)

The *lateral accessory lobe* has a roughly pyramidal shape and lies inferior lateral (*n-posterior lateral*) to the *ellipsoid body* (Fig. S19B). Its anterior (flanked by the *antennal lobe, AL*) and lateral (flanked by the *ventrolateral protocerebrum, VLP*) sides are clearly demarcated by thick glial processes (Figs. S11C, S12F). It is flanked by the *bulb, inferior clamp* and *epaulette* posteriorly and by the *vest* inferiorly. The *bulb*'s boundary is clearly demarcated by its glomerular structure. Boundaries with other neuropils appear to be less distinct because of extensive connections between them. We determined the extent of the *LAL* by defining its boundaries with neighboring neuropils as well as by the projections of clonally-associated

neurons (i.e., the clonal units) that arborize broadly in the *LAL* (Fig. S14C, D). Massive fiber streams of the *medial antennal lobe tract* (*mALT*) and the *inferior fiber system* (*IFS*) lie in its medial and lateral sides (Fig. S11C).

- *LAL* was originally called the *ventral body* (Strausfeld, 1976) but we have adopted the term *LAL* (see p. 13).

Upper LAL (ULAL) and Lower LAL (LLAL)

The *LALs* of both hemispheres are connected by many fibers that run via the *LAL commissure* (*LALC*). Within the *LAL* the fibers of this commissure run through the center of the *LAL*, dividing it into *upper LAL* and *lower LAL*. This upper/lower division exists in the *LAL* of many species including *Drosophila*, *Manduca*, monarch butterfly, and locust (el Jundi et al., 2010; Iwano et al., 2010; Heinze and Reppert, 2012).

Gall (GA)

The *gall* is a newly defined small volume of the *LAL.* It is a small protruding region identifiable in the superior-lateral tip of the fly *LAL*, just medial to the *VLP* and beneath the *spur* of the *MB*. It is densely labeled with synaptic markers, and sparsely surrounded by glia (Fig. S11B). The name *gall* (= a pathological outgrowth from a plant) comes from its protruding shape. It receives specific output terminals of fibers projecting from the *central complex* (Ito et al., 2013). A similar structure is identified also in the monarch butterfly and called the *anterior lobelet of the LAL* (aLobl) (Heinze and Reppert, 2012). Whether the *gall* also exists in other insect species is yet to be determined.

V-5. Ventrolateral neuropils (VLNP)

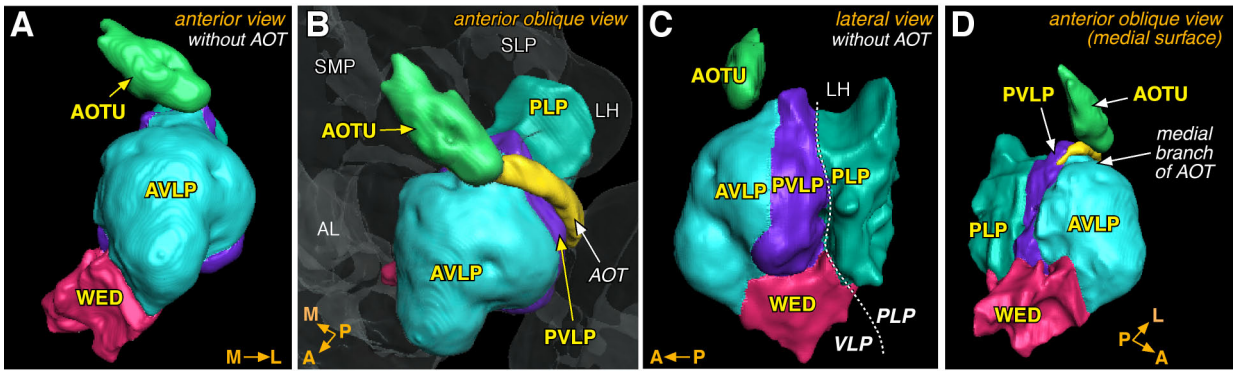


Figure S20. Ventrolateral neuropils

Compared to the *optic lobe, mushroom body, central complex, lateral complex, and antennal lobe,* the boundaries in the remaining neuropils are far less distinct. Glial processes do not form continuous boundaries that unambiguously delineate the neuropils. Arborizations of identified neurons tend to overlap extensively with each other and often cross the proposed neuropil boundaries. Nevertheless, with these limitations in mind, discrete regions can be recognized and categorized into named neuropils based on the organization of glial processes, the arrangement of neuronal processes, and easily identifiable landmarks such as fiber bundles. These regions are first divided according to the positions in the brain (*ventrolateral*, *superior*, *inferior*, *ventromedial*, and *periesophageal*, level 1) and then into smaller entities (level 2 and level 3). Furthermore, there are corresponding divisions of protocerebral neuropils identified in *Drosophila* in other dipterous species (e.g. Phillips-Portillo and Strausfeld, 2012).

The *ventrolateral neuropils* occupy the ventrolateral region of the *central brain* (*n-posteriorlateral in neuraxis*). It consists of the *anterior optic tubercle* (*AOTU*), *ventrolateral protocerebrum* (*VLP*), *posterior lateral protocerebrum* (*PLP*) and *posterior optic tubercle* (*POTU*; not prominent in some species including flies). Many (but not all) neuropils in this region are associated with visual information pathways from the *optic lobe*.

Note: Optic glomeruli (OGL)

- Within the *VLNP* there are many discrete regions where the synaptic density is higher than the surrounding areas. Many (but maybe not all) of them are associated with the efferent axons projecting from the *optic lobe* and are supposed to be the primary sites of visual information pathways in the *central brain*. Those that are associated with the visual system are called the *optic glomeruli* (Strausfeld et al., 2007). The *AOTU* and *POTU* are themselves discrete *optic glomeruli*. There are also many *optic glomeruli* in the volumes of the *posterior VLP* and *PLP*. The functions of other glomeruli in the *posterior VLP* and *PLP* without visual connections are currently unknown.

V-5-1 Anterior optic tubercle (AOTU)

The *anterior optic tubercle* is determined by the terminals of neurons projecting from the *optic lobe* via the *anterior optic tract* (*AOT*) (Strausfeld, 1976; Strausfeld and Okamura, 2007). It is covered by glial processes and is discernible by the thick density of synapses of the neurons projected from the *optic lobe*. It lies in the anteriormost (*n-ventralmost*) region of the lat-

eral *cerebrum* (anterior to the *SLP*/*SIP* and superior to the *AVLP*) and is the most prominent *optic glomerulus*. It is slightly detached from the rest of the *ventrolateral neuropils*. The structure protrudes anteriorly from the contour of the adjacent synapse-rich neuropils, thus giving its name.

In locusts, bees, moths and monarch butterflies, the *anterior optic tubercle* is divided into two major parts: the *upper* and *lower units* (Homberg et al., 2003; el Jundi and Homberg, 2012; Mota et al., 2011; Pfeiffer and Kinoshita, 2012; Heinze and Reppert, 2012).

upper unit — A large main part lying more dorsally, called *major unit* in honey bees. It receives massive bundles of fibers via the *anterior optic tract.*

lower unit — A smaller part that lies more ventrally, called *ventrolateral unit* in honey bees. It receives a branch of fiber bundles from the *anterior optic tract* and also houses dendrites of the neurons projecting towards the *bulb*.

There are also species-specific additional parts reported in various insects.

nodular unit — Identified in moth and butterflies (Heinze and Reppert, 2012; el Jundi et al., 2009). It is a small part in the ventralmost region and consists of several compartments.

strap — Identified in butterflies (Heinze and Reppert, 2012), an elongated, rather irregular structure between the *lower unit* and *nodular unit*.

lateral unit — Identified in honey bees (Mota et al., 2011), lying in the lateral part between the *dorsal* and *ventral lobe* of the *upper* (*major*) *unit* of the *anterior optic tubercle*.

In *Drosophila* and blowflies (*Phaenicia*), the *anterior optic tubercle* is not clearly divided into subunits and appears as a single structure. Within it there are three zones (Otsuna and Ito, 2006; Strausfeld and Okamura, 2007). Considering that the *lateral zone* appears to receive separate fibers from the *anterior optic tract* and that it contains fibers projecting towards the *bulb*, it might correspond to the *lower unit* in non-dipteran insects.

medial zone — The largest region with relatively sparser labeling of fibers and synapses.

intermediate zone — The narrowest region, with weaker synaptic labeling.

lateral zone — The region with dense labeling of fibers and synapses.

V-5-2 Ventrolateral protocerebrum (VLP)

The *ventrolateral protocerebrum* (*VLP*) is a large mass of neuropil in the anterior (*n-ventral*) brain between the *antennal lobe* and the *optic lobe* (Strausfeld, 1976). In *Drosophila*, the anterior part of the *VLP* is segregated clearly by a thick glial wall from the more medial neuropils (*antennal lobe* and *LAL*). The posterior (*n-dorsal*) part appears more contiguous with the neighboring neuropil lying posteriorly (*PLP*).

In *Drosophila*, the *VLP* can be separated into two parts, the *anterior* and *posterior VLP* (*AVLP* and *PVLP*, respectively). The *PVLP,* together with the *PLP* (*posterior protocerebrum*) behind it, contains many discrete neuropil volumes characterized by high synaptic densities (i.e., extensive labeling with nc82), many of which are *optic glomeruli*. The *AVLP* is non-glomerular and lacks such architecture.

Several clonal units arborize broadly across the *VLP*. There are also clonal units that arborize specifically across the *AVLP*. On the contrary, so far there are no known clonal units that arborize specifically and broadly in the *PVLP* (Fig. S14E-H, Ito et al., 2013).

- *VLP* is sometimes called the *anterior optic foci*, but we suggest the term *VLP* (see p. 13).
- *VLP* is expected to have glomerular and non-glomerular components also in other insects, but the relative arrangements of these divisions may not exactly be the same across taxa.

V-5-2a Anterior VLP (AVLP)

The *AVLP* is determined as the volume of *VLP* that is devoid of glomerular structures with high synaptic density (i.e., extensive labeling with nc82). It protrudes in the anterior (*n-ventral*) neuropil surface between the *antennal lobe* and *optic lobe*. In *Drosophila* it lies in front of the *PVLP*, below the *anterior optic tubercle* and *SLP,* above the *saddle* and *wedge*, and lateral to the *AL* and *LAL*.

Whereas most of the visual input from the *optic lobe* is sent to the *optic glomeruli* in the *PVLP* and *PLP*, the lateral region of the *AVLP* receives visual input from the visual neurons that form loose terminal arborization in this region (Otsuna and Ito., 2006). The *AVLP* also receives terminals of the *antennal lobe* projection neurons via the *lateral antennal lobe tract* (*lALT*; Tanaka et al., 2012a, b).

V-5-2b Posterior VLP (PVLP)

The *PVLP* is determined as the volume of *VLP* that contains glomerular structures with high synaptic density. Many, if not all, of these glomeruli correspond to *optic glomeruli* that receive terminals of projection neurons from the *optic lobe*. Note that, because these glomeruli are scattered across the neuropil, the regions between them are also included in, and defined as, *PVLP* neuropil. The extent of the neuropil was determined by enveloping the contours of these glomeruli. *PVLP* lies behind the *AVLP*, in front of the *PLP*, above the *wedge*, below the *lateral horn*, *SLP* and *superior clamp*, and lateral to the *MB pedunculus, inferior clamp,* and *epaulette.*

At its mid-level, *PVLP* and *PLP* are separated by the *great commissure*. Both above and below this level, the two neuropils appear contiguous. The *PVLP* mainly contains *optic glomeruli* supplied by axons from the *lobula*, whereas the *PLP optic glomeruli* are supplied by axons of neurons from the *lobula plate* or neurons shared by the *lobula* and *lobula plate* (in *Drosophila* and in larger dipterous species). The boundary between *PVLP* and *PLP* was determined by the contours based on these *lobula*-supplied and *lobula plate*-supplied glomeruli. There are also several clonal units that arborize broadly in the *VLP* but not in the *PLP* (Fig. S14G, H).

V-5-3 Posteriorlateral protocerebrum (PLP)

The *PLP* is a neuropil similar to the *PVLP* in that it receives many terminals of the visual projection neurons, which form *optic glomeruli*. Unlike the *PVLP*, most of these projections derive from the *lobula plate*. The extent of the neuropil was determined by enveloping the contours of these glomeruli.

The *PLP* lies behind the *PVLP*, above the *wedge*, below the *lateral horn*, *SLP,* and *superior clamp*, and lateral to the *MB pedunculus, inferior clamp,* and *superior posterior slope*. The contour of the *PVLP* is not delineated by a clear glial boundary. There are no known clonal units that arborize specifically and broadly across the *PLP*.

Note: association between the clamp and superior medial PVLP/PLP

- We defined the boundary of the *PVLP* and *PLP* using the extent of the *optic glomeruli* as landmarks. On the other hand, the superior medial part of the *PVLP* and *PLP* is rather contiguous with the *superior clamp* and *inferior clamp* (above and below the *MB pedunculus*, respectively; Fig. S11D-G), and various neuronal fibers arborize around the *MB pedunculus* to connect the superior medial *PVLP*/*PLP* extensively with the *superior* and *inferior clamps* (Fig. S15B). The superior medial *PVLP*/*PLP* therefore have dual associations with the visual pathways and the *clamp*.

Note: difference between the PLP and posterior slope

- In some cases the *PLP* has been regarded as a part of the *posterior slope*, which also receives projections from the *optic lobe* (Strausfeld, 1976). Here the *PLP* is defined as the region that features *optic glomeruli*, whereas the *posterior slope* is defined as a region that has no glomeruli.

V-5-4 Wedge (WED)

The *wedge* is the inferiormost region of the *ventrolateral neuropils* (*VLNP*), lying between the *AVLP*/*PVLP*/*PLP* and the *saddle*/*GNG*. It lies lateral to the *inferior fiber system* and *vest.* It extends much more medially compared to the *AVLP*/*PVLP* lying above it. The *wedge* is non-glomerular like the *AVLP*. Unlike the *PVLP* and *PLP*, the *wedge* has essentially no connection with the *optic lobe* but receives neurons connecting with the underlying *saddle* (and *AMMC* within it). The *wedge* receives direct input from some of the antennal mechanosensory neurons via the underlying *AMMC*, as well as secondary neurons deriving from the *AMMC* (Kamikouchi et al., 2006, 2009).

The superior extent of the *wedge* houses sensory arborizations from the antennal mechanosensory neurons. This level matches that of the inferior limits of the *VLP* as determined by the specific broad arborizations of clonal units (Fig. S14I). The medial, inferior, and posterior extents were determined by the boundaries of the *vest*, *saddle,* and *SPS*, respectively.

Note: affiliation of the wedge in respect to the VLP

- The *wedge* has previously been regarded as a part of the *VLP* and called the *inferior VLP* (*IVLP*) or *caudal VLP* (*CVLP*), because many fibers connect the *wedge* and *VLP* almost contiguously (Kamikouchi et al., 2009; Chiang et al., 2011). However, as described above, the *wedge* receives direct input from antennal mechanosensory neurons, which are regarded as deutocerebral. Also, it lies at a level as low as the *vest,* which is likely to be a part of the *deutocerebrum*. These observations suggest that the *wedge* might not be a part of the *protocerebrum*, as the term *VLP* would have suggested. To avoid inferring segmental association, and to leave that question for further study, we propose using a segment-free term. *Wedge* is an architectural term that refers to a stone block in an arch. The trapezoidal shape of this neuropil and its organization that fills the space between the overlying *VLP* and underlying *saddle* is best described by this word.
  - A part of the wedge has been called the *ventral protocerebrum* (*VPC*) in moths, but here we avoided the term *ventral protocerebrum* because it is too vague and infers unproven segmental identity (see p. 13).

V-5-5 Posterior optic tubercle (POTU)

The *posterior optic tubercle* is the posteriormost optic glomerulus of the lateral brain, behind the *PLP* and ventral to the *MB calyx.* It has a prominent discrete structure in many locusts, crickets and cockroaches (Homberg, 1991; Homberg et al., 1991) as well as in monarch butterflies (Heinze and Reppert, 2012), but appears to be just one of several similar *optic glomeruli* of the *PLP* in *Drosophila.*

V-6. Lateral horn (LH)

The *lateral horn* is a horn-shaped lateral neuropil in the superior posterior brain (*n-dorsal-anterior*). It is determined as the volume that receives terminals of the *antennal lobe* projection (output) neurons projecting via several *antennal lobe tracts* (*ALTs*). It is therefore one of the second-order olfactory centers (Stocker et al., 1990). It also receives inputs from the centers serving other modalities, such as from the optic glomerular complex in the blowfly *Phaenicia* (Strausfeld et al., 2007). The *lateral horn* protrudes laterally from the superior posterior surface of the neuropils in flies as well as in locust, honey bee, cockroach and moth. In other insects it may be embedded more deeply in the overall neuropils, without forming particular horn-like protrusion. In *Drosophila* it lies behind the *SLP* and *PVLP*, above the *PVLP* and *PLP*, below the *SLP*, and lateral to the *superior clamp.*

In *Drosophila*, the medial part of the *lateral horn* has a cone-like shape formed by the radial terminal arborization of the *antennal lobe* projection neurons via the *mALT* pathway (Fig. S26C). This region is sandwiched between the *SLP* and *superior clamp*. The anterior inferior-medial part also protrudes slightly to accommodate the projections via the *mlALT* pathway. The *posterior lateral fascicle* (*PLF*) intersects the *mlALT* at this point (Tanaka et al., 2012a).

Unlike the *MB calyx*, the other second-order olfactory center, the *lateral horn* is not clearly separated from surrounding neuropils by glial processes. The region is best visualized by labeling a large population of the *antennal lobe* projection neurons (Fig. S26C). Labeling with *elav*-GAL4 > UAS-n-syb-GFP shows slightly higher synaptic density within the *lateral horn* (Fig. S11F). The nc82 antibody does not, however, clearly visualize its boundary.

Note: classification of the lateral horn – superior neuropil or lateral neuropil?

- The *lateral horn* has sometimes been called the *lateral protocerebrum*, and thus regarded as a part of the *lateral neuropils*. Indeed, *antennal lobe* projection neurons via the *lALT* and some other fascicles connect the *lateral horn* with the *AVLP*. The *lateral horn* is connected with the *PVLP* and *PLP* less extensively, though. Nevertheless, the *lateral horn* has extensive connections with various regions of the *superior protocerebrum* (Ito et al., 2013) and developmentally it seems to arise together with these neuropils (Pereanu et al., 2010). Because of this dual association, we classified the *lateral horn* as a single entity rather than a part of either the *superior neuropils* or *lateral neuropils*. To make it clear that the lateral neuropils do not contain parts of the *lateral horn*, we call these the *ventrolateral neuropils.*

V-7. Superior neuropils (SNP)

The *superior neuropils* comprise neuropils occupying the superiormost region of the brain. In *Drosophila*, this region is rather large and lies over other neuropils. In contrast, in the honey bee the collective regions appear to be compressed into a relatively small volume between the massive *MB calyces* and the *MB lobes* (Mobbs, 1982).

A major landmark in this region (at least in *Drosophila*) is the system of fiber bundles called the *superior fiber system* (*SFS*; newly termed here). In *Drosophila*, this lies in the region superior-medial to the *MB pedunculus*. Unlike the surrounding neuropils, there are few synapses in the *SFS*, and it therefore appears dark when using general synaptic labels (Figs. S11E, F, S12B). The inferior surface of the *SFS* and the fiber bundles that lie at similar levels (e.g. *pyriform fascicle* and *posterior lateral fascicle*), demarcate the boundary between the *superior neuropils* and underlying *inferior neuropils*.

The *superior neuropils* are obviously contiguous. As a matter of practice they have previously been separated into two parts: the *superior lateral protocerebrum* (*slpr*) and *superior medial protocerebrum* (*smpr;* Strausfeld, 1976; Otsuna and Ito, 2006). Closer examination of neuronal fiber arrangements and the preferential arborizations of clonal units (Fig. S14M-P) in *Drosophila* led us to suggest defining the boundary between the two regions obliquely rather than parallel to the body axis (see Section VII-2, p. 60), and defining a new intermediate small region around the *MB vertical lobe,* which we named the *superior intermediate protocerebrum* (*SIP*). The abbreviations of the other two neuropils are also changed to *SLP* and *SMP*, respectively, to distinguish the classic (*slpr, smpr*) and new (*SLP, SMP*) terminologies.

- These three regions (i.e. *SLP*= the lateral region, *SIP*= the region around the *MB vertical lobe*, and *SMP*= the medial region) are likely to be found in the brains of other insects, but their precise arrangements and boundary landmarks may vary between species (Phillips-Portillo and Strausfeld, 2012).

Note: deletion of the inferior protocerebrum

- Previous classification set the regions called the *inferior lateral protocerebrum* (*ilpr*) and *inferior medial protocerebrum* (*impr*) between the overlying *slpr*/*smpr* and underlying *central complex* and *VLP*/*PLP* (Fig. S30C; Strausfeld, 1976;

Otsuna and Ito, 2006). Developmental studies suggest that the entire region above the level of the *central complex* and *VLP/PLP* would arise in a similar manner, which is distinct from the developmental pattern of the more inferior region that is now called the *clamp* (Pereanu et al., 2010). Clonal units projecting in the superior neuropil tend to arborize broadly above this level (Fig. S14N, P). We therefore lowered the inferior boundaries of the superior *protocerebrum* so that it is now flanked directly by the *central complex* and *VLP/PLP* (see also Section VII-2, p. 60).

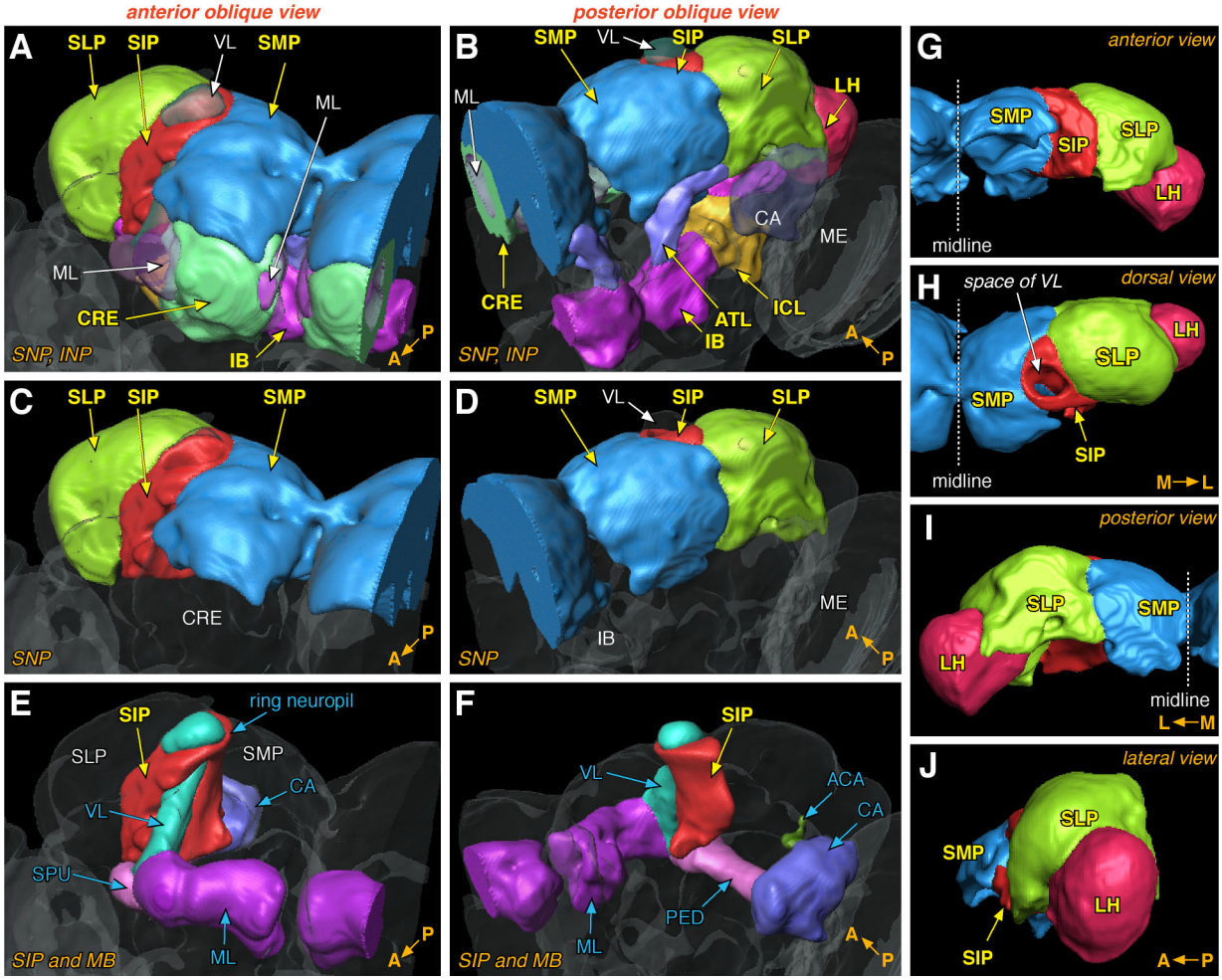


Figure S21. Superior neuropils and lateral horn

V-7-1 Superior lateral protocerebrum (SLP)

The *SLP* occupies the lateral part of the *superior neuropils*. It lies behind the *anterior optic tubercle,* in front of the *MB calyx*, and above the *superior clamp, superior fiber system, AVLP, PVLP*, and *lateral horn*. Its medial boundary runs obliquely from anterior lateral to posterior medial, flanked by the *SIP* (anteriorly) and *SMP* (posteriorly). This oblique boundary reflects the major direction of neuronal fibers in the *SLP* and the averaged extent of arborizations of clonal units in this region (Fig. S14M). Externally it is also demarcated as an indentation on the superior neuropil surface.

The boundary with the *anterior optic tubercle, MB calyx*, and *lateral horn* are determined by the specific projection patterns of the visual projection neurons and *antennal lobe* projection neurons in these regions. Boundaries with the *superior clamp, AVLP* and *PVLP* are determined based on the characteristic patterns of clonal projections in these regions and defined with clearly distinguishable fiber bundles as practical landmarks (see document S4_Neuropil_Boundary.pdf).

Anterior SLP and posterior SLP

Various neurons arborize only in the anterior or posterior part of the *SLP*. However, there is no prominent natural boundary that could clearly separate the *SLP* into smaller subregions. A frontal plane extrapolated from the boundary between the *PVLP* and *PLP*, which corresponds to the anteroposterior level of the *great commissure*, can be used as a practical boundary between the *anterior SLP* and the *posterior SLP* (*ASLP* and *PSLP*). This boundary corresponds to the boundary between classic *middle slpr* and *posterior slpr* (*mslpr* and *pslpr*; see Section VII-2, p. 60).

V-7-2 Superior intermediate protocerebrum (SIP)

SIP is a relatively small region around and posterior to the *MB vertical lobe.* It lies between the *SMP* and *SLP*, in front of the

superior fiber system, and above the *superior clamp*. Because the lateral boundary with the *SLP* is rather oblique, in the horizontal view the *SIP* has a prism-like shape. Unlike the *SLP* and *SMP*, the *SIP* extends through only about one half of the longitudinal extent of the brain, bounded by the large *superior fiber system* just behind it. In its anterior part, *SIP* effectively surrounds the *MB vertical lobe*; in this region the *SIP* is characterized with an empty territory through which the *MB vertical lobe* penetrates.

The *SIP* contains arborizations of many neurons that connect with the *MB vertical lobe* (Fig. S14K, L). It is also equipped richly with loose fibers that project from the *anterior optic tubercle* in a medial-posterior direction. This stream of fibers, which characterizes the *SIP*, in contrast to the *SLP*, is observed in horizontal sections of *elav*-GAL4 > UAS-GFP, etc. (Fig. S12B). These fibers, however, may not terminate in the *SIP* itself.

- The *SIP* is a newly determined neuropil in the *Drosophila* brain, but because of its close association with the *MB vertical lobe*, other insects may also have a corresponding structure around their *MB vertical lobes*.
- The region defined newly here as *SIP* has previously been regarded as the lateral part of the *anterior* and *middle smpr* (*asmpr* and *msmpr*; see Section VII-2).

Ring neuropil

The *ring neuropil* is identified in honey bees as the terminals of the *mlALT* neurons (Abel et al., 2001). The axon branches wrap around the distal tip region of the *MB vertical lobe.* The corresponding structure is identifiable also in flies. Here the *ring neuropil* is regarded as a component of the *SIP* in its anterior superior region.

V-7-3 Superior medial protocerebrum (SMP)

The *SMP* is the most medial region of the *superior neuropils*. It roughly occupies the neuropil region above that flanked by the *MB lobes*, *pedunculus*, and *calyx*. The *SMP* is flanked directly by the *MB vertical lobe* and *calyx,* but not by the *medial lobe* and *pedunculus*. The *crepine* lies between the *medial lobe* and *SMP,* and the *superior clamp* insulates the *pedunculus* and *SMP*. The inferior boundary of the *SMP* is determined by the extent of clonal units that arborize preferentially in this neuropil (Fig. S14P).

- As explained above, the lateral region of what has been called the *smpr* is now regarded as the *SIP* (anterior half) and the medial part of the *SLP* (posterior half). Thus, the *SMP* is narrower than the previous *smpr* (see Section VII-2).

Anterior SMP and posterior SMP

Because the *fan-shaped body* protrudes deeply into the *SMP*, the *SMP* is effectively separated into anterior and posterior parts. Various neurons arborize only in the anterior or posterior part of the *SMP*. The superior apex of the *FB* can therefore be used for a practical boundary landmark between the *anterior* and *posterior SMP* (*ASMP* and *PSMP*). This plane is essentially in line with the practical boundary between the *anterior* and *posterior SLP* (*ASLP* and *PSLP*) mentioned above. This boundary corresponds to the boundary between classic *middle* and *posterior smpr* (*msmpr* and *psmpr*; see Section VII-2).

V-8. Inferior neuropils (INP)

The *inferior neuropils* occupy the region below the *superior neuropils*, around the level of the *MB medial lobe* and *pedunculus.* They also surround the superior half of the *central complex*. In *Drosophila*, the *lateral complex* (comprising the *bulb* and the *LAL*), lying on both sides of the *central body*, is so large that it effectively separates the *inferior neuropils* into anterior and posterior parts. The anterior part wraps around the *MB medial lobe* and is newly termed here as the *crepine* (*CRE*). The posterior part consists of two major parts and one small region. First, the region between the *central body* and the *MB pedunculus* (including the region superior to the *pedunculus*) is termed the *clamp* (*CL*), which is further divided practically into superior and inferior parts (*SCL* and *ICL*). Second, the region posterior to the *central body* is termed the *inferior bridge* (*IB*). Finally, a thin elongated region extending from the *inferior bridge* to the *SLP* is called the *antler* (*ATL*).

- The regions that correspond to the fly's *crepine, clamp* and *inferior bridge* are also likely to exist in other insects. Existence of the *antler* depends on whether there are fiber connections between the *inferior bridge* and *SLP*. Because the shapes and relative arrangements of the *MB* and *central complex* vary greatly among species, the neuropil arrangements of the *inferior neuropils* may also show corresponding variability.

Note: impr and crepine/clamp/inferior bridge/antler

- The region of the *inferior neuropils* is to some extent similar to the region that has previously been called the *inferior protocerebrum*, which was further separated into *inferior medial protocerebrum* (*impr*) and *inferior lateral protocerebrum* (*ilpr*). For the following reasons, the boundaries of these regions are modified so drastically that we decided not to use these terms so as to avoid confusion (see also Section VII-2, p. 60).

1. As explained in the previous section, the boundary between the *superior neuropils* and *inferior neuropils* is lowered significantly. The lateral half of the *ilpr* is now regarded as a part of the *SLP,* and the medial half of the *impr* is now regarded as a part of the *SMP*.

2. There was no unambiguous inferior boundary of the *impr*/*ilpr*. The inferior surface of the *MB pedunculus* has previously been used as a practical landmark to demarcate a boundary. However, in the current terminology system we distinguished the *inferior neuropils* and *ventromedial neuropils* (*VNP*) lying below it as the regions that are relatively free from and rich in, respectively, the arborizations from the ascending/descending neurons associated with the *cervical connective*. According to this definition, the inferior boundary of the *inferior neuropils* is lowered significantly downwards, close to the level of the superior surface of the *great commissure*. Because of this change, the *inferior neuropils* is now taken to include the superior part of what has been called the *ventromedial protocerebrum* (*vmpr*).
3. The *clamp*, which includes the medial part of *ilpr* and lateral part of *impr*, is subdivided into two parts reflecting the stream of fiber bundles running above the *MB pedunculus* and *central complex*. The boundary plane is therefore almost horizontal, making it unnecessary to call the two parts “lateral” and “medial.”

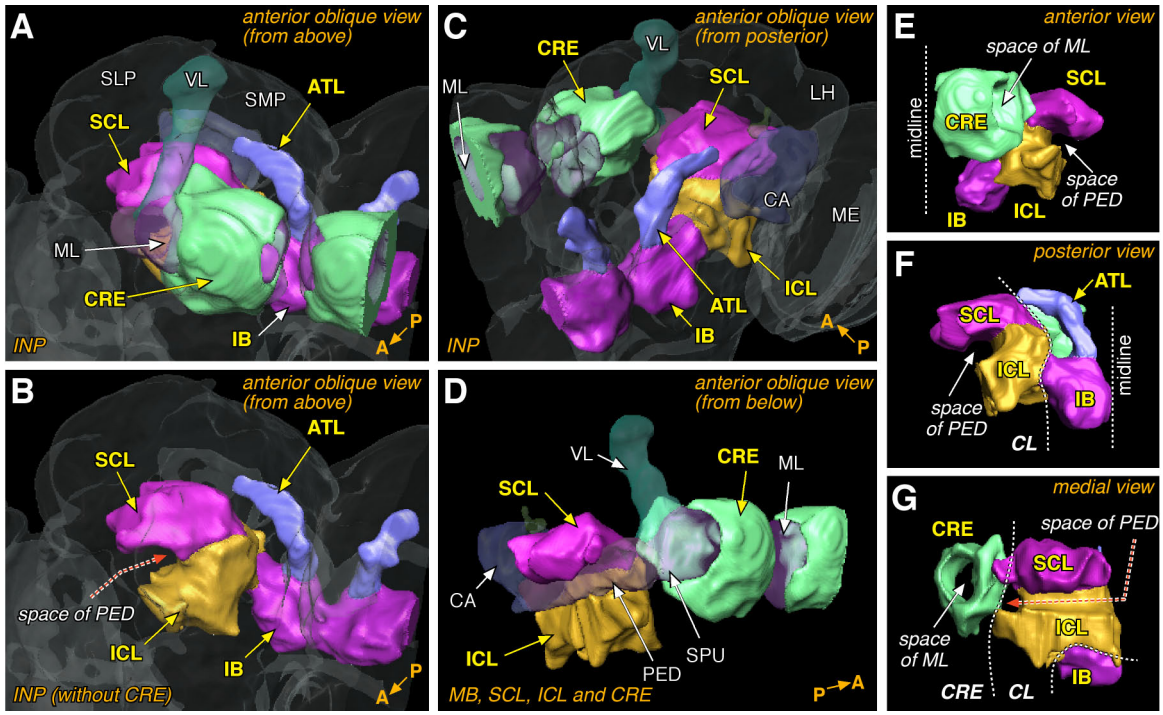


Figure S22. Inferior neuropils

V-8-1 Crepine (CRE)

The *crepine* is a thin neuropil that surrounds the shaft of the *MB medial lobe.* The name *crepine* (meaning a caul, or cloak) is from the French (and other) culinary disciplines, and is a term used to denote bacon or a sliver of another meat that is wrapped around certain delicacies, such as a mushroom. The *crepine* lies in front of the *SMP* and *bulb,* below the *SMP*, above the *AL*, *LAL* and *bulb*, and medial to the *MB vertical lobe* and the *SIP*. The region roughly corresponds to the *anterior inferior medial protocerebrum* (*aimpr*) in the previous terminology. Many neurons in this region extend around the *MB medial lobe* and enter the lobe to form extensive connections with Kenyon cell fibers (Tanaka et al., 2008). There are also many other neurons that project around the *medial lobe* without entering it (Fig. S14Q-S).

- *Crepine* is a newly determined neuropil in the *Drosophila* brain, but because of its close association with the *MB medial lobe*, other insects are likely to have a comparable structure around their *MB medial lobes*.

Rubus (RUB)

The *rubus* is a newly defined small subregion that is embedded in the *crepine,* with a diameter of about 10 microns, lying just behind the *MB medial lobe* (Fig. S12D). There is no glial boundary around it. It consists of a small rough-surfaced roundish structure that looks like a Raspberry; we took the name *rubus*, the Latin for this fruit. It is very rich in small fibers and presynaptic sites, and is prominently visible with elav > n-syb-GFP and elav > GFP but not with nc82 or anti-DLG. Fibers from the *central complex* form specific output terminals in this subregion, and project further towards the *gall* of the *LAL* (Ito et al., 2013). Whether the *rubus* also exists in other insects is yet to be determined.

V-8-2 Clamp (CL)

Clamp is a newly defined term to refer to the region between the *central body* and the *MB pedunculus*, including the region above and below the *pedunculus*. The space occupied by the *pedunculus* forms a deep tunnel-like recess in its inferior-lateral side. The name was taken from its shape, which looks like a clamp holding the *pedunculus*. Whereas neuropils

surrounding the *MB vertical lobe* and *medial lobe* (i.e. *SIP* and *crepine*, respectively) receive many *MB* extrinsic neurons connecting the *MB lobes* with these neuropils, few neurons in the *clamp* seem to penetrate into the *pedunculus.*

- Because of its spatial correlation with the *central body* and *MB pedunculus*, other insects should also have a neuropil volume comparable to the *clamp.*

The *clamp* has a generally uniform appearance; many neurons arborize broadly in it without forming clear subdivisions (Fig. S15A, B). However, a thin sheet-like stream of neuronal fibers deriving from the *superior ellipsoid commissure* (*SEC*) and *superior arch commissure* (*SAC*) project through the middle level of the *clamp,* spanning the superior surface of the *central complex* and the *MB pedunculus* (Fig. S11E). These fiber streams can be used as a landmark to separate the *clamp* into superior and inferior parts.

V-8-2a Superior clamp (SCL)

The superior part of the *clamp* (*SCL*) lies above the streams of *SEC* and *SAC*. It lies behind the *SLP and SIP,* in front of the *MB calyx* and superior part of the *mALT,* above the *MB pedunculus* and *inferior clamp,* beneath the *SIP*, *SLP* and *SMP,* lateral to the *central complex*, medial to the *lateral horn,* and superior medial to the *PVLP* and *PLP*.

V-8-2b Inferior clamp (ICL)

The inferior part of the *clamp* (*ICL*) lies below the streams of *SEC* and *SAC*, between the *central complex* and *MB pedunculus.* It lies behind the *LAL, bulb*, and *AVLP*, above the *epaulette* and *gorget* of the *ventral complex* as well as the *superior posterior slope*, below the *superior clamp,* lateral to the *central body*, and medial to the *pedunculus, PVLP* and *PLP*. It extends to the posterior surface of the brain, where the lateral end of the *protocerebral bridge* is located near the root of the *medial equatorial fascicle*.

Anterior SCI/ICL and posterior SCL/ICL

Though the *SCL* and *ICL* are relatively long neuropils, there is no prominent natural boundary that could clearly separate them into anterior and posterior subregions. A frontal plane extrapolated from the boundary between *VLP* and *PLP* (the level of the *great commissure*) can be used as a practical boundary between the *anterior* and *posterior SCL/ICL* (i.e., *ASCL/PSCL* and *AICL/PICL*).

Note: association between the clamp and superior medial PVLP/PLP

- The *clamp* covers the superior and medial surface of the *MB pedunculus.* The inferior lateral surface of the *pedunculus* is flanked almost directly by the medialmost *optic glomeruli* of the superior medial *PVLP* and *PLP* (Fig. S11D-G). However, some fibers of the *clamp* extend in this part of the *PVLP/PLP* and continue around the *pedunculus* (Fig. S15A, B), forming characteristic cylindrical architectures around the *pedunculus*. Thus, the superior medial part of the *PVLP/PLP* has dual association with the *optic glomeruli* and the *clamp*.

V-8-3 Inferior bridge (IB)

The *inferior bridge* is a neuropil in the posterior brain, behind the *central body, gorget* and *great commissure,* below the *protocerebral bridge*, above the *superior posterior slope*, and medial to the posterior end of the *inferior clamp.* Other than the *central complex*, it is the only “fused” neuropil structure contiguous across the midline of the *protocerebrum*, where there is no separation between hemispheres.

The anterior and superior sides of the *inferior bridge* are clearly demarcated by fiber bundles and glial processes of the *fan-shaped body* and the *mALT*. The boundary is rather contiguous in its lateral and inferior sides. Its lateral extent was determined by the medial boundary of the *ICL* (Fig. S15C) and the massive fiber stream of the *medial equatorial fascicle* (Fig. S11G, S12F). Whereas the *posterior slope*, which lies below the *inferior bridge*, is contributed by rich arborizations of the neurons associated with the *cervical connective* (Strausfeld, 1976), the *inferior bridge* is relatively free from them. This difference was used to determine its inferior boundary.

- The *inferior bridge* was identified in the brain of a larger fly (*Musca*) (Strausfeld, 1976), but it has been regarded as a part of the *inferior medial protocerebrum* (*impr*) or the *posterior slope.* Because of its distinct fused structure across the midline, we defined it as a separate neuropil. It is not known whether comparable structures exist in other insects, but its specific characteristic of being a fused structure in the posterior brain, positioned near the *protocerebral bridge*, and its paucity of descending/ascending fibers, will help identification of its corresponding region in other species. A potentially corresponding structure has been described in the monarch butterfly and was called the *posterior medial protocerebrum* (*PMP*; Heinze and Reppert, 2012). It might roughly correspond to the combination of *IB* and *ATL*.

V-8-4 Antler (ATL)

The *antler* is a newly identified small neuropil. It has thin elongated arch-like architecture spanning from the *inferior bridge* to the posterior inferior edge of the *SLP* (Fig. S15D, S22A, B). The name is taken after its eponymous structure in deer. The *antler* extends through the space between the *protocerebral bridge* and the *fan-shaped body,* along the *mALT* medial surface

(in its medial part) and *mALT* superior surface (in the more lateral part). It is partially embedded in the cell body rind of the posterior brain. In spite of its thin bundle-like structure, the *antler* is not a fiber bundle but a genuine synapse-rich neuropil, because it features many synapses within it. (Fig. S11G)

- *Antler* is a newly determined neuropil in the fly brain. Whether comparable structure exists in other insects is likely to depend on the specific arrangements of the *mALT*, *protocerebral bridge, inferior bridge,* and *superior protocerebrum* in the brains of individual species.

V-9. Antennal lobe (AL)

The *antennal lobe* is so far the only neuropil that can unambiguously be ascribed to the deutocerebral neuromere because of its association with the first (uniramous) head appendage. It lies anterior to the protocerebral neuropils (*LAL* and *vest*), below the *crepine,* and above the *prow* and *flange.* Its surface is separated clearly from other structures by extensive glial sheaths. It has many glomerular components, each of which receives projections from specific types of olfactory sensory neurons (OSNs) on the surface of the *antennae* and the *maxillary palps*. Each glomerulus is separated by glial sheath. The *AL*'s posterior surface provides the roots of the *mALT* and *mlALT*. The *lALT* emerges from a more lateral position (Fig. S26C).

The *AL* consists of two types of neuropils. Most of its volume, especially along its surface, is occupied by the *AL glomeruli.* The center of the *AL* is essentially free from these glomeruli, and olfactory sensory neurons do not terminate in this region. It is not merely a mass of fiber bundles, though, because some neurons other than the OSNs terminate here. To distinguish this region clearly from the glomerular regions of the *AL*, we here give it a name *antennal lobe hub*.

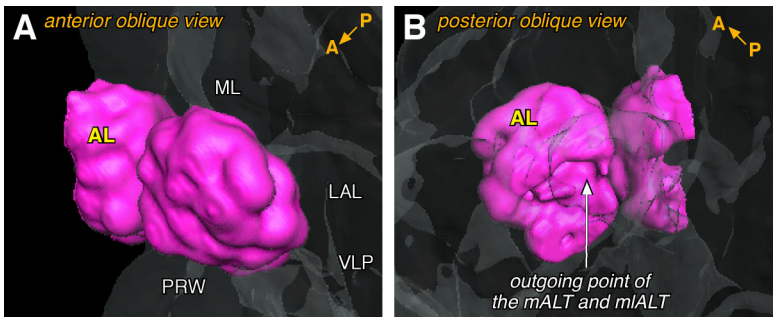


Figure S23. Antennal lobe

V-9-1 Antennal lobe glomeruli

Depending on species, the glomerular region of the *AL* can be classified into two parts, one with very large glomeruli (macroglomeruli) and the other with smaller ones (ordinary glomeruli).

Macroglomerular complex (MGC)

In butterflies, moths and cockroaches, glomeruli that receive information about pheromones are extremely large and form a distinct cluster, called the *MGC*, which is situated close to where olfactory sensory neuron axons enter the *antennal lobe* (Anton and Homberg, 1999; Schachtner et al., 2005). Similar specialized glomeruli presumably also exist in other insects, but they may not be so easily distinguished. For example, some of the glomeruli in *Drosophila* show sexual dimorphism in size (Kondoh et al., 2003). Whether they correspond to the *MGC* remains to be investigated.

Ordinary glomeruli (GL)

Glomeruli other than those of the MGC are traditionally called *ordinary glomeruli* or just *glomeruli*. Because each glomerulus is surrounded by glial sheaths, they are clearly delineated by synaptic markers such as nc82 (Laissue et al., 1999).

For an increasing number of species, it is recognized that almost all glomeruli can be routinely identified and are given specific names based on their positions in the *antennal lobe*. The number of glomeruli varies depending on the species. In some parasitic species, there may be fewer than 10 (Crespo and Vickers, 2012). In *Drosophila* there are about 50 (Tanaka et al., 2012a), and in the honey bee about 160 (Robertson and Wanner, 2006; Kirschner et al., 2006) In some taxa such as fungus growing ants (*Apterostigma magri*), there can be more than 600 glomeruli (Kelber et al., 2009) and the *AL* of desert locusts consists of about 2,000 extremely small glomeruli (Schachtner et al., 2005).

Other than in locusts and their relatives, each glomerulus provides its own uniglomerular projection neurons and receives axon terminals from a unique population of olfactory sensory neurons (OSNs) defined by the expression of a specific gene that encodes a specific olfactory receptor (OR) protein. The number of glomeruli relate to the expansion of the genome encoding the receptor protein family of that taxon (Robertson and Wanner, 2006) The exception is in one group of Orthoptera (the Acridiae) represented by locusts, in which any OSN usually innervate several, often many, minute glomeruli in the *AL*, and all projection neurons in the *AL* also arborize in numerous glomeruli (Ignell et al., 2001).

V-9-2 Antennal lobe hub (ALH)

The core of the *Drosophila AL* is non-glomerular. Fibers of the *antennal lobe* projection neurons and the connecting processes of many local neurons extend through this region. However, it has also synapses albeit at a much lower density than in the glomeruli (Tanaka et al., 2012a).

- The words *AL core* or *AL center* were avoided and *AL hub* was adopted, because *core* and *center* are sometimes used to refer to the central part of each glomerulus.

V-10. Ventromedial neuropils (VMNP)

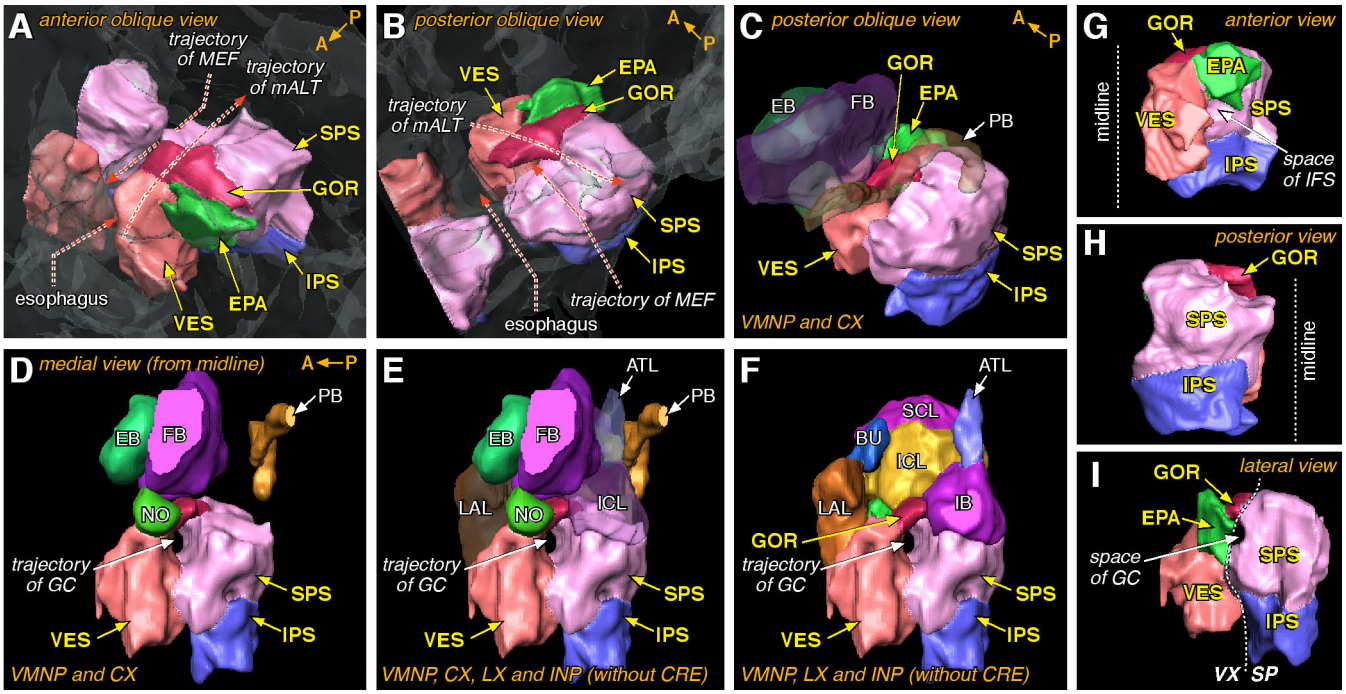


Figure S24. Ventromedial neuropils

The *ventromedial neuropils* are paired inferiormost (*n-posteriormost*) regions of the *CRG* above (*n-anterior*) and on both sides of the esophagus. Each VMNP lies posterior and inferior to the *antennal lobe* and below the *central complex, LAL, and inferior neuropils.* In its medial region, the fiber bundles of *mALT* and *medial equatorial fascicle* (*MEF*) cover its superior boundary. Its inferior region is flanked on one side by the esophagus foramen. The *VMNP* contains many neurons that are associated with the *cervical connective*.

The neuropils in this region appear to be rather confluent, with no clear glial boundaries that separate them into distinct subparts. However, the *great commissure* effectively divides the region into anterior and posterior parts. The posterior part has been called the *posterior slope* (*PS*). The anterior part, on the contrary, had no particular name. We here give it the name *ventral complex* (*VX*), as it consists of several structures that lie ventral (in body axis) to the *central complex*. The *ventral complex* also lies in the *n-ventral* part of the *VMNP*, thereby also making its name meaningful in the context of the neuraxis.

Another natural landmark in this region is the structure of fiber bundles newly termed here the *inferior fiber system* (*IFS*). This is a conjunction of many fiber bundles merging from various directions. In *Drosophila* it lies in the region inferior posterior to the *LAL*. Because of the paucity of synapses, the *IFS* appears dark with synaptic labeling (Fig. S11C-E).

- In spite of its location in the *ventromedial neuropils*, we call it the *inferior fiber system* rather than *the ventral fiber system* (*VFS*), because the acronym *VFS* has extensively been used to refer to the *vertical fiber system* of the *central complex* neurons.

Unlike the *antennal lobe* (part of the *deutocerebrum*) and the protocerebral neuropil regions explained so far, the precise neuromere assignments are not yet established in the *VMNP*. This region has often been regarded as a part of the *protocerebrum,* and the name *ventral protocerebrum* was sometimes used to refer to this region. However, at least some parts of it are likely to be deutocerebral. To leave the question about the segmental boundaries open for future study, neuropil names have been chosen so as not to infer any association with a particular neuromere.

V-10-1 Ventral complex (VX)

The *ventral complex* is a group of neuropils that lies below the *central body*. It lies behind the *LAL* and *flange,* in front of the *great commissure* and *posterior slope*, medial to the *VLP*, and above the *saddle* of the *periesophageal neuropils.* The organization of this brain region has, historically, hardly been analyzed.

In *Drosophila*, we identified one large and two small structures in the *ventral complex*. Their names – *vest*, *epaulette*, and *gorget* – are taken so that they are indicative of their relative arrangements, and because these unique identifiers do not appear in the regular context of any neurobiological paper (this provides an important feature for computer-aided text search). The *vest* is the largest region lying medially. The *epaulette* is a small neuropil lying superior and lateral to the vest. The *gorget* is a thin small neuropil lying above the *vest*. The *vest* and *epaulette* are separated by the *inferior fiber system*, the *gorget* is sandwiched between *fan-shaped body* and the *great commissure.*

- The *vest, epaulette, and gorget* are newly defined here from the present study of the *Drosophila* brain. The region of the *ventral complex* should exist in most insect species, and its main part, the *vest*, is likely to be situated similarly behind the *LAL* and below the *central body*. In species where *cerebral* and *gnathal ganglia* (*CRG* and *GNG*) are separated, the *vest* is unlikely to extend as far along the esophageal foramen. The arrangement and existence of the other two smaller neuropils might be more variable, because their separation from the *vest* depends on the specific arrangements of the *inferior fiber system, great commissure* and *central complex.*

V-10-1a Vest (VES)

Vest is the medial and largest neuropil of the *ventral complex*. It lies behind the *AL, LAL* and *flange*, in front of the *great commissure* and superior posterior slope, below the *mALT*, and above the *saddle.* The inferior boundary with the *saddle* is demarcated by glial sheaths, and the medial and lateral sides are flanked by the esophagus and *inferior fiber system*, respectively. The anterior and posterior boundaries are more contiguous with the neighboring neuropils, where we determined the boundaries based on the extent of the arborizations of several clonal units contributing broadly to this region (Fig. S15E, F).

V-10-1b Epaulette (EPA)

The *epaulette* is a tiny but easily recognizable region in *Drosophila*, lying superior-lateral to the *vest* and below the *inferior clamp*. The name is taken from the ornamental shoulder piece of certain uniforms. It lies behind the *LAL*, in front of the *great commissure* and *gorget,* below the *inferior clamp,* above and lateral to the *inferior fiber system,* and medial to the *AVLP* and *PVLP.* The *great commissure*, *inferior fiber system*, and the *horizontal VLP fascicle* are the prominent landmarks for identifying its locality. No clonal units have so far been identified that arborize preferentially within this neuropil, but clones arborizing in the neighboring neuropils often respect the neuropil boundary with the *epaulette* (Fig. S15G, H).

V-10-1c Gorget (GOR)

The *gorget* is defined as a thin plate-like region protruded medially from below the *inferior clamp* to the region between the *great commissure* and the *central body*. Its name is taken from an ornamental collar or a steel collar that protected the wearer's throat. The position of the *gorget* corresponds to the throat, if the region of the *vest* and *epaulette* are regarded as the body and shoulder, respectively. The *gorget* lies behind the *vest* and *epaulette* at the level of the anterior surface of the *great commissure*, in front of the *superior posterior slope* and *inferior bridge,* and medial to the *lateral equatorial fascicle* (*LEF*). The *mALT* and *medial equatorial fascicle* extend along its superior surface, and the *great commissure* extends below it. The medial tip of the *gorget* extends towards the *noduli*, but is separated from them. Although the *gorget* appears to be a cantilever-like protrusion of neuronal fibers with regard to its resolution by synaptic labeling (Fig. S11E, F), its tip is actually contiguous with non-synaptic neurites that project through the mass of connective fibers in the region around and below the *noduli* (Fig. S15I).

V-10-2 Posterior slope (PS)

The *posterior slope* is the inferior part of the posterior brain, covering the region between the *inferior bridge* and *GNG.* Like the *ventral complex,* extensive arborizations of descending/ascending neurons are observed in this region but not in the region above it (Strausfeld, 1976). The superior boundary was determined as the level above which such arborizations are not observed. The *posterior slope* is also characterized by the terminals of axons from the ocellar ganglia projecting via the ocellar nerve (Strausfeld, 1976).

The *posterior slope* is a rather uniform structure, but it can practically be divided into *superior* and *inferior posterior slope* at the level of two major commissures in this region: the *wedge commissure* and the *posterior optic commissure.* A part of arborizations of some clonal units contribute only to one of these two parts (Fig. S15J).

V-10-2a Superior posterior slope (SPS)

The *superior posterior slope* is the region behind the *great commissure, gorget, vest,* and *wedge*. There is no clear glial boundary with the surrounding neuropils. It lies below the *inferior clamp* and *inferior bridge* at the level of the *medial equatorial fascicle* and *lateral equatorial fascicle,* above the *cantle* and *inferior posterior slope,* and medial to the *PLP.*

V-10-2b Inferior posterior slope (IPS)

The *inferior posterior slope* is a region flanking both sides of the esophagus. It lies behind the *wedge,* below the *superior*

posterior slope, above the *GNG*, and medial to the *wedge.*

- The region of the *posterior slope* should exist in all insect species. Whether it can be separated into superior and inferior parts may depend on the species. The extent of the neuropil, i.e., whether it extends down on both sides of the esophagus, may be considerably different in insects, such as Orthoptera and Dictyoptera, in which the *CRG* is clearly separated from the *GNG* above and below the esophagus.

V-11. Periesophageal neuropils (PENP)

The *periesophageal neuropil* is the region of neuropil between the *antennal lobe/ventromedial neuropils* and *GNG*. Historically, its organization has hardly been studied other than suggesting relationships with the *median bundle* and the sympathetic nervous system (Melcher and Pankratz, 2005; Strausfeld, 2012). Despite its position around and below the esophagus, it is a part of the *CRG*. This region is a composite one, comprising several synaptic territories, most likely of deuto- or tritocerebral origin. Their precise neuromeric assignments are not yet established. Again, so as to leave open questions about segmental boundaries, neuropil names have been chosen so as not to infer associations with specific neuromeres.

We identified two major parts and two smaller annexes in the *periesophageal neuropils*. The *saddle* is its most major part, lying above the *GNG* like a saddle of a horse. It contains the volume of the *antennal mechanosensory and motor center* (*AMMC*) in it. The *prow* is the anteriormost region of the *periesophageal neuropils*, covering the anteriormost and superior region of the *GNG*. Unlike the *saddle,* the *prow* faces the anterior external surface of the brain at the frontal opening of the *esophageal foramen*. *Flange* and *cantle* are two small regions lying at the anterior and posterior ends of the *saddle*, respectively, separated by the *vest* of the *ventromedial neuropils*.

- The *saddle, prow, flange,* and *cantle* are newly defined entities in the *Drosophila* brain. It is likely that the region that corresponds to the *saddle* should exist in most insects with fused *CRG* and *GNG*. Because it is defined as the region surrounding the *AMMC*, its position and arrangement in other insects would depend on the arrangement of the mechanosensory axon terminals from the antennae. Whether the *prow* exists in other insects depends on the morphology of the *GNG* in front of the volume of the *saddle.* The existence, shape and position of the *flange* and *cantle* may also depend on the species.

Note: association of the PENP with the supraesophageal zone and subesophageal zone (SPZ/SEZ)

- To resolve ambiguity associated with the terms “supraesophageal” and “subesophageal” ganglia, due to the penetration of the esophagus within what is commonly called the *supraesophageal ganglion*, we have employed new terms. These are the *cerebral and gnathal ganglia* (*CRG* and *GNG*) and the *supraesophageal and subesophageal zones* (*SPZ* and *SEZ*). The former pair denotes segmental neuromeres; the latter denotes zones of the neural tissues, independent of their segmental origin, above or below the level of the esophageal foramen (see p. 3 and p. 11). In certain insect species, some of the neuropils in the *PENP* lie below the esophagus and have therefore been regarded as parts of the *SEZ* (or, in previous publications, the *SEG*). In *Drosophila*, the *saddle, AMMC,* and *prow* lie below the esophagus and can therefore be considered as parts of the *SEZ* (but not of the *GNG*).

V-11-1 Saddle (SAD)

The *saddle* is the neuropil that contains the *antennal mechanosensory and motor center* (*AMMC*). The *AMMC* is a distinct subregion of the *saddle* that is embedded within the latter. The name “saddle” was taken because of its shape, which rests on the “back” of the *GNG*. The *AMMC* is determined as the extent of terminal arborizations of the antennal mechanosensory neurons. Cerebral neurons innervating the *AMMC* arborize in a slightly larger region surrounding the *AMMC* (Fig. S15M, N). The *saddle* is determined to accommodate these neuropil regions around the *AMMC*.

The *saddle* consists of two parts: a lateral part that runs around the axons of the antennal mechanosensory neurons arising from the *antennal nerve*, and a medial part that lies above the *GNG* and below the esophagus. Because the *GNG* is bent upwards at its anterior end, the medial part of the *saddle* is situated behind the anterior dorsal part of the *GNG* and above the middle/posterior part of the *GNG*. It lies below the *AVLP, wedge*, *vest*, *flange,* and *cantle,* and medial to the *AVLP* and *wedge*.

Antennal mechanosensory and motor center (AMMC)

The *AMMC* is defined by the terminal projections of the axons of the *Johnston’s Organ* neurons projecting from the antennae as well as the dendritic region of the antennal motor neurons (Stocker et al., 1990; Kamikouchi et al., 2006). It is housed within the *saddle.* Unlike the *antennal lobe*, the other target of antennal sensory neurons, the *AMMC* is not covered by glial processes and has a complex shape with many extensions. From its superior-lateral and lateral aspect, the contour of the *AMMC* looks essentially like that of the *saddle*.

The *AMMC* in *Drosophila* is subdivided into 5 zones (zones A-E), each of which receives inputs from specific type of *Johnston’s Organ* sensory neurons. (Zone C forms a thin posterior protrusion from the rest of the zones. Although this protrusion belongs to the *AMMC*, and therefore it is within the *saddle*, it was not labeled in the neuropil map because of the diffi-

culty to trace their extent in the general labeling.) In addition, some branches of *Johnston's Organ* neuron axons project further dorsally to the *wedge* or ventro-laterally to the *GNG* (Kamikouchi et al., 2006).

Terminals of *Johnston's Organ* neurons are subdivided into 3 major branches in the honey bee brain (Ai et al., 2007). Structural and functional comparison between the *AMMC* of *Drosophila* and the honey bee are yet to be resolved.

- *AMMC* is the only abbreviation that ends with the letter C even though it is not the name of a commissure. This is to retain the frequently used acronym of this neuropil.

Note: neuromere identity of the AMMC and saddle

- The antennal nerve and the *antennal lobe* are considered to be the deutocerebral structures. Another terminal of the antennal nerve, the *AMMC* may therefore also be of deutocerebral origin. However, the medial-posterior tips of the *AMMCs* of both hemispheres are almost fused at the midline below the esophagus, and some if not many sensory neurons cross the midline to terminate in the contralateral side (Kamikouchi et al., 2006). This might suggest that at least some part of the *AMMC* could be tritocerebral. Future studies would answer this question.

Likewise, surrounding neuropil of the posterior medial *saddle* contains various commissural neurons below the esophagus. This is regarded as a characteristic feature of the *tritocerebral neuromere* (Scholtz and Edgecombe, 2006), though developmental studies suggest that the ventralmost part of the deutocerebrum may also lie beneath the esophagus (Boyan et al., 2003). It is yet to be determined whether the entire *saddle* is deutocerebral or tritocerebral, or whether there is a deuto-tritocerebral neuromere boundary within the *saddle* despite its contiguous appearance.

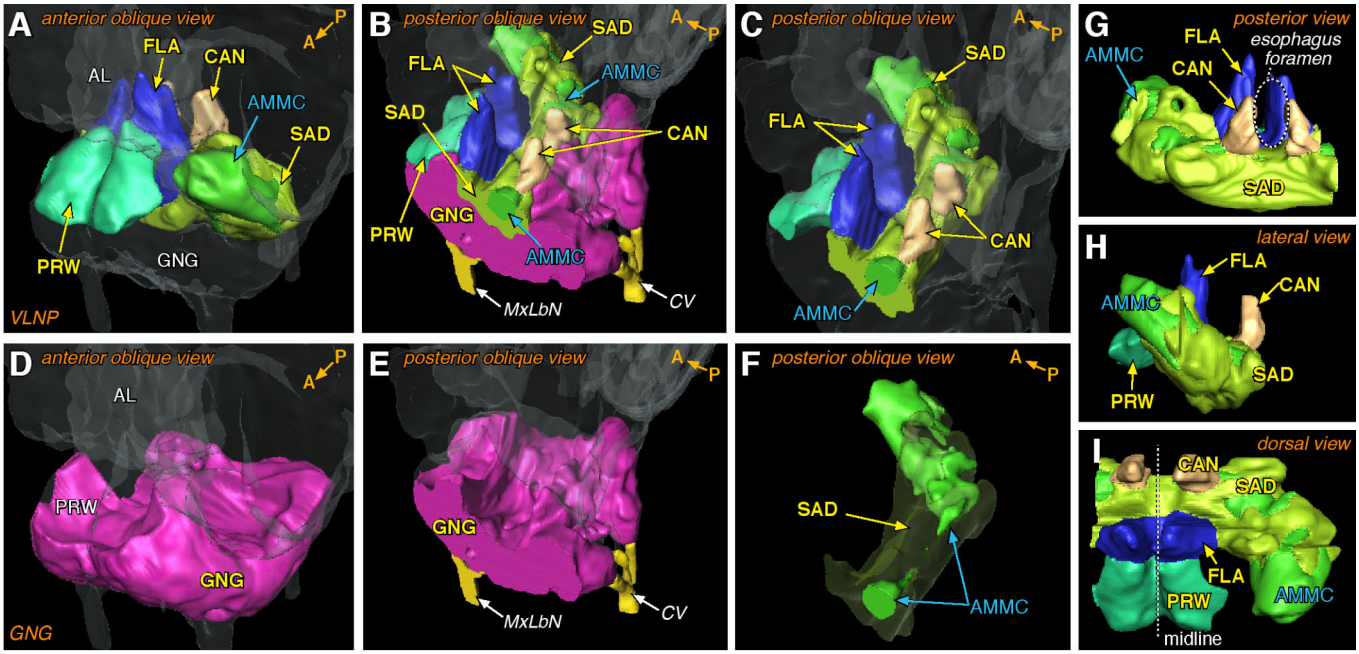


Figure S25. Periesophageal neuropils and *GNG*

V-11-2 Flange (FLA)

The *flange* is a triangular structure that lies above the anterior end of the *saddle*, on both sides of the esophagus. It lies at the root of the *median bundle,* behind the *prow*, anterior to as well as inferior-medial to the *vest*, above the *saddle*, and below the *AL.* The *flange* features arborizations of many neurons that contribute to the *median bundle* (Fig. S15K). The extent of these arborizations, as well as thin glial processes between this neuropil and the *vest*, are used to demarcate the neuropil. The clonal units arborizing in the *vest* hardly intrude the thin *flange* neuropil just in front of it (Fig. S15L). Because of this feature, the *flange* and the *vest* are categorized into different neuropil groups.

V-11-3 Cantle (CAN)

The *cantle* is a small triangular structure lying on the posterior end of the *saddle.* The name was taken from the protruding rear edge of an equestrian saddle. It is situated on both sides of the esophagus, behind the *vest,* in front of the *superior posterior slope,* and below the upper part of the *vest*. Unlike the *flange,* the *cantle* is clearly demarcated by glial boundaries. Although no neurons have so far been found to arborize specifically within this region, because of these glial boundaries we identified the *cantle* as a distinct small neuropil.

V-11-4 Prow (PRW)

The *prow* is the anteriormost and superior region of the brain tissue below the esophagus (*SEZ*). It lies inferior and anterior to

the opening of the esophageal foramen. Its superior surface is curved to accommodate the curved trajectory of the esophagus. The term *prow* was taken from the anteriormost part of the hull of a boat (assimilating the *SEZ*).

This region has been suggested to be a part of the *tritocerebrum*, but it appears contiguous with the *GNG* lying beneath it and *flange* lying behind it. The boundaries are not visible with synaptic markers such as nc82 antibody. The projection sites of the pharyngeal nerves (*SPhS*, see below) can be visualized as a higher density of signals with *elav*-GAL4 > UAS-n-syb-GFP (or with appropriate molecular markers and expression driver strains that label pharyngeal nerve neurons). The extent of the *prow* is determined as including the entire region of the *SPhS*.

Superior pharyngeal sensory center (SPhS)

Upon entering the brain, the *pharyngeal/accessory pharyngeal nerve* forms several branches (Miyazaki and Ito, 2010). Those that run superiorly enter the *prow* and form extensive terminals, which is newly termed here as the *superior pharyngeal sensory center* (*SPhS*).

- The *superior pharyngeal sensory center* is abbreviated as *SPhS*, not *SPS*, to avoid confusion with the superior posterior slope. The term *sensory center* could also be abbreviated as *SC* (e.g., *SPhSC*), but it would then become confusing as an acronym of a commissure. To avoid this, the last C was removed from the abbreviation.

V-12. Gnathal ganglia (GNG)

Developmentally, the *gnathal ganglia* (*GNG*) comprise three divisions: *mandibular*, *maxillary*, and *labial neuromeres*. Though they have clear segmental structures in the embryonic *GNG*, their segmental boundaries in the adult *GNG* neuropil are far less distinct. Two types of landmarks are helpful for demarcating the neuromeres. (1) The fiber bundles of the *VUM* (*ventral unpaired median*) *cluster neurons*, which exists on the midline of each neuromere. Because all three *VUM clusters* lie in the anterior half of the *Drosophila GNG*, it seems plausible that the *mandibular* and *maxillary neuropils* are rather anteriorly placed together (this arrangement may not always be applicable to other taxa). (2) The sensory terminals from the *pharyngeal/accessory pharyngeal nerve* (for the *mandibular neuropil*) and the *maxillary–labial nerve* (for the *maxillary* and *labial neuropils*). They show intense synaptic labeling on both sides of the neuropil and a commissural connection between them.

The neuromere boundaries should exist between these landmarks, but because of the highly contiguous nature of fiber arrangements, we did not try to draw boundaries of each neuromere. We instead defined the names of the terminal regions of each sensory nerve, which are recognizable by synaptic labels such as nc82.

- In *Drosophila*, neuropils of the *gnathal ganglia* occupy only the ventral part of the *subesophageal zone* (*SEZ*). The region that has been conventionally referred to as the *subesophageal ganglia* (abbreviated as *SEG* or *SOG*) sometimes includes neuropils in addition to those of the *GNG*, such as the *prow, saddle,* and *AMMC.*
- The word *suboesophageal* is often written with “oe”, but we suggest using “e” instead of “oe” (see p. 15).

Inferior pharyngeal sensory center (IPhS)
Anterior maxillary sensory center (AMS)
Posterior maxillary sensory center (PMS)
Labial sensory center (LS)

These sensory centers are embedded parts of the *GNG*, just like the *AMMC* is an embedded part of the *saddle*. Gustatory sensory neurons in the mouth and mechanosensory neurons in the inferior head capsule enter the brain via two peripheral nerves: the *pharyngeal/accessory pharyngeal nerve* and *maxillary-labial nerve*. The former provides superior and inferior branches, amongst which the first enters the *prow* to form the *superior pharyngeal sensory center* (*SPhS*) mentioned above. The inferior branch supplies the *inferior pharyngeal sensory center* (*IPhS*) in the anteriormost *GNG* (within the presumptive *mandibular neuromere*).

The *maxillary-labial nerve* of the fly bifurcates upon entering the *GNG*. The anterior branch forms two further branches that terminate in the *anterior maxillary sensory center* (*AMS*) and *posterior maxillary sensory center* (*PMS*) in the presumptive *maxillary neuromere*. The posterior branch extends posteriorly to terminate in the *labial sensory center* (*LS*) in the presumptive *labial neuromere.* These sensory centers are subdivided into various zones, some of which receive signals from specific types of gustatory neurons in the mouth.

- These *sensory centers* could also be abbreviated as *SC*, but it would then become confusing as an acronym of a commissure. To avoid this, the last C was removed from the abbreviation.

VI. Guide to landmark fiber bundles

We here define only fiber bundles that are most prominent and form useful landmarks for determining neuropil boundaries. See also Section III-3 (pp. 17-19) for the scheme of fiber bundle nomenclature.

VI-1 Fascicles, tracts, and bundles

VI-1-1 Antennal lobe-associated tracts

Tracts that connect the antennal lobe and other brain regions.

- The *m, ml, lALT* have been called *m, ml, lACT* in honey bees but *i, m, oACT* in flies. To obviate these inconsistencies, we chose *m*, *ml*, *l*, and changed the term *ACT* to *ALT* to distinguish the classic and new terminologies (see p. 14).

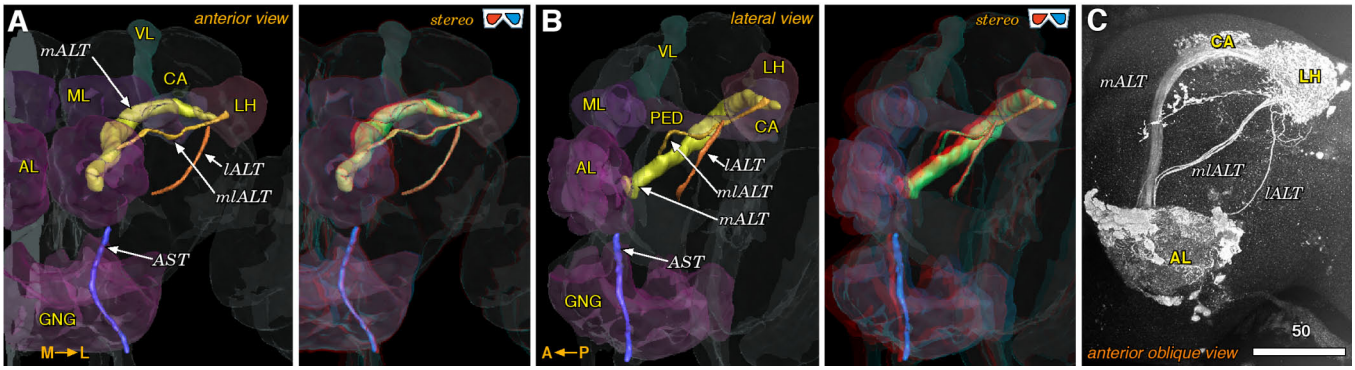


Figure S26. ALT and AST (red-cyan 3D color stereogram)

Abbrev-iation	Full Name Brief description	Classic names
mALT	**medial antennal lobe tract**	iACT in flies, mACT in honey bees

The *mALT* emerges from the posterior surface of the *antennal lobe* and terminates in the *lateral horn*. It contains axons of *antennal lobe* projection neurons. In *Drosophila* it demarcates the boundaries of the *LAL* (medial), *vest* (superior), *epaulette* (medial), *fan-shaped body* (lateral), *gorget* (superior), *inferior clamp* (lateral and superior-lateral), *inferior bridge* (superior-lateral), *antler* (lateral), *superior clamp* (posterior-superior), *MB calyx* (anterior), and *SLP* (inferior).

mlALT **mediolateral antennal lobe tract** — mACT in flies, mlACT in honey bees

The *mlALT* emerges from the posterior surface of the *antennal lobe* (same root with the *mALT*) and terminates in the *lateral horn*. It contains axons of *antennal lobe* projection neurons. In *Drosophila* it demarcates the boundaries of the *fan-shaped body* (anterior), *LAL* (posterior), *epaulette* (superior), *inferior clamp* (anterior-superior), and *MB pedunculus* (inferior).

lALT **lateral antennal lobe tract** — oACT in flies, lACT in honey bees

The *lALT* emerges from the inferior posterior lateral region of the *antennal lobe* and ends in the *lateral horn*. It is not used for demarcating neuropils but runs closely along the boundary between the *AVLP* and *PVLP*.

AST **antenno-subesophageal tract** — *same*

The *AST* emerges from the root of the *maxillary labial nerve* in the inferior *GNG* and ends at the inferior-posterior surface of the *antennal lobe*. Contrary to the word order of its nane, the *AST* contains axons projecting from the *GNG* to the *antennal lobe* (mostly from olfactory sensory neurons). It is not used for demarcating neuropils, but it is a useful lateral landmark of the *posterior maxillary sensory center* (*PMS*) of the *GNG* and the boundary between *saddle* and *GNG*.

VI-1-2 Fiber bundles in the cerebrum

AOT **anterior optic tract** — *same*

The *AOT* emerges from the medial part of the *lobula*, and one branch ends in the *anterior optic tubercle* whereas the other makes a medial turn to terminate in the superior medial *PVLP*. It demarcates the boundaries of the *PVLP* (lateral), *AVLP* (superior and lateral), *anterior optic tubercle* (inferior-lateral), and *lateral horn* (inferior).

PYF **pyriform fascicle** — *same*

The *PYF* emerges from the superiormost lateral region of the *SMP* and ends in the region between *SLP* and *lateral horn*. It demarcates the boundary between *SLP* and *lateral horn*.

PLF **posterior lateral fascicle** *same*

The *PLF* emerges from the *PLP,* runs near the anterior medial edge of the *lateral horn,* and ends in the *SIP*. It demarcates the boundary between the *superior clamp* and *lateral horn/SLP*.

aSLPF **anterior SLP fascicle** *newly introduced name*

The *aSLPF* features a characteristic J-shape with many cell body fibers. It emerges from the cells in the lateral cell body rind and ends at the boundary between *SLP* and *SIP* just posterior to the *anterior optic tubercle*. It demarcates the inferior boundary of *SLP* with *SIP, AVLP, PVLP*, and *superior clamp*.

hVLPF **horizontal VLP fascicle** *newly introduced name*

The *hVLPF* emerges from the cells in the *lateral cell body rind* and ends in the region between the *LAL, inferior clamp,* and *epaulette*. It contains many cell body fibers projecting to the *inferior* and *ventromedial neuropils.* It demarcates the boundaries of the *LAL* (posterior), *inferior clamp* (inferior), and *epaulette* (superior).

vVLPF **vertical VLP fascicle** *newly introduced name*

The *vVLPF* emerges from the cells in the lateral cell body rind and ends in the *SLP*. It contains many cell body fibers projecting towards *SLP.* It is not used for demarcating neuropils but is very prominent in this region together with the *hVLPF*.

MBDL **median bundle** *same*

The *MBDL* is the only fiber bundle that extends exactly along the brain's midline. It emerges from a broad region around the esophagus, merging at the tip of the *flange,* and terminates in the *SMP*. It contains axons having both ascending and descending polarities. Fibers from some of the large cell bodies in the *pars intercerebralis* extend ventrally in the *MBDL*, other axons project dorsally to the *SMP* and *crepine.* The *MBDL* is not used for demarcating neuropils but is very prominent in this midline location. It is the only fiber bundle with the word "bundle" in its name; we do not change the term in order to keep consistency.

MEF **medial equatorial fascicle** *protocerebral bridge - lateral protuberance tract*

The *MEF* is one of the thickest bundles in the *Drosophila* brain along with the *mALT*. It emerges from the cells in the medial posterior rind below the *MB calyx* and branches out in the region inferior-lateral to the *fan-shaped body*. It contains many cell body fibers as well as the fibers deriving from the *protocerebral bridge.* It demarcates the boundaries of the *inferior bridge* (lateral), *inferior clamp* (superior-medial), *superior posterior slope* (superior), *gorget* (superior), and *fan-shaped body* (inferior-lateral).

- The name is changed because the term *lateral protuberance* is not used in the current terminology system and because the fascicle contains many fibers that do not derive from the *protocerebral bridge.*

LEF **lateral equatorial fascicle** *newly introduced name*

The *LEF* is thinner and runs lateral to the *MEF*. It emerges from the cells in the lateral posterior rind and ends in the inferiormost part of the *inferior clamp.* It demarcates the boundaries of the *inferior clamp* (inferior-lateral), *PLP* (medial), *superior posterior slope* (superior-lateral), and *gorget* (lateral).

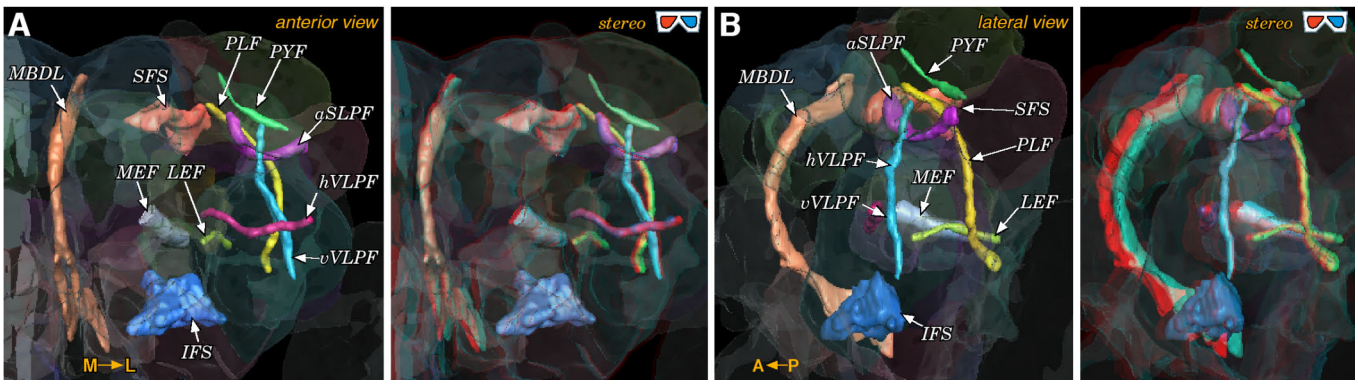


Figure S27. Fiber bundles in the cerebrum and fiber systems (red-cyan 3D color stereogram)

VI-1-3 Cerebro-cervical fascicles

Fascicles connecting the *cervical connective* and various brain neuropils.

- The superior and inferior parts of the *cervical connective* tend to contain descending and ascending axons, respectively, but there might be a mixture of ascending and descending fibers in each tract deriving from these bundles. We therefore avoided using names that infer particular directions of information.

pCCF **posterior cerebro-cervical fascicle** *newly introduced name*

The *pCCF* derives from a superior lateral part of the *cervical connective* (may be rich with descending neuronal

fibers) and ends in the *inferior fiber system*. It also contains the axon of the *giant fiber neuron* (*GF*). It demarcates the boundaries of the *SPS* (inferior), *IPS* (superior), and *wedge* (superior-medial).

mCCF **medial cerebro-cervical fascicle** *newly introduced name*

The *mCCF* derives from the superior medial part of the *cervical connective* (may be rich with descending neuronal fibers) and ends in the medial part of the posterior *GNG*. Neuronal fibers further extend from there, but the fascicle cannot be further traced from this level. It is not used for demarcating neuropils but is prominent in this region.

aCCF **anterior cerebro-cervical fascicle** *newly introduced name*

The *aCCF* derives from the inferior medial part of the *cervical connective* (may be rich with ascending neuronal fibers), runs by the *posterior maxillary sensory center* (*PMS*), and converges to the *inferior fiber system*. It demarcates the boundary between the *flange* and *saddle*.

lCCF **lateral cerebro-cervical fascicle** *newly introduced name*

The *lCCF* derives from the inferior lateral part of the *cervical connective* (may be rich with ascending neuronal fibers) and project towards the lateral surface of the *saddle* and *wedge,* demarcating their lateral boundaries.

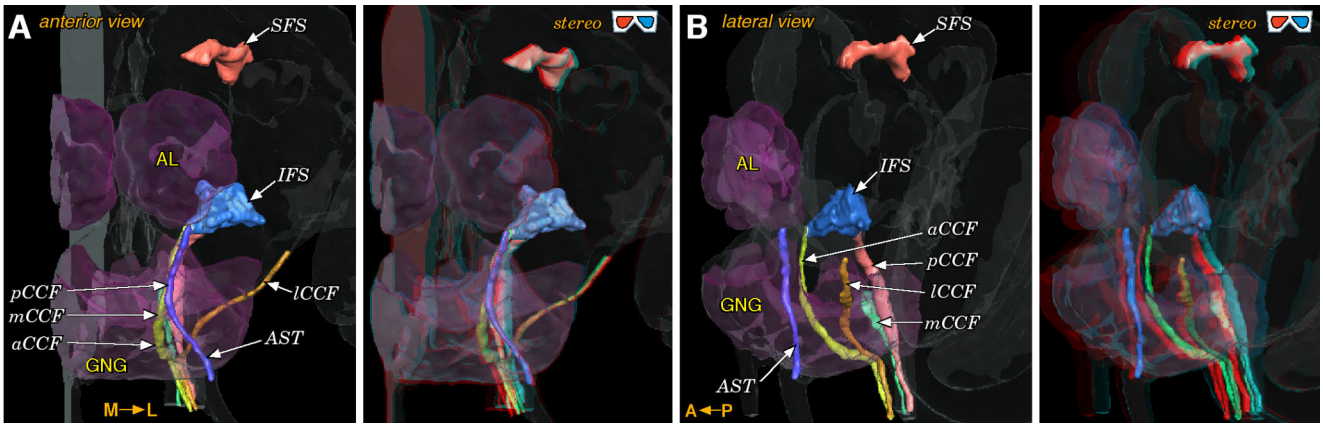


Figure S28. CCFs and fiber systems (red-cyan 3D color stereogram)

VI-2 Fiber systems

SFS **superior fiber system** *newly introduced name*

The *SFS* is a prominent fiber system in the superior brain. It lies superior-lateral to the *fan-shaped body* and anterior to the superior part of the *mALT* (around *calyx*). It demarcates the boundaries between *SLP* (posterior-medial), *SIP* (posterior), *SMP* (posterior-lateral), and *superior clamp* (superior-medial).

IFS **inferior fiber system** *newly introduced name*

The *IFS* is another major fiber system that resides in the inferior brain. It lies inferior-posterior to the *antennal lobe*. It is contributed by the *pCCF*, *aCCF*, and the *lALT* runs through it. It demarcates the boundaries between *AVLP* (inferior-medial), *PVLP* (inferior-medial), *wedge* (medial), *LAL* (inferior), *vest* (lateral), *epaulette* (inferior and medial), *superior posterior slope* (anterior), and *saddle* (superior).

VI-3 Commissures

sALC/iALC **superior/inferior AL commissure** inter antennal lobe tract

Commissures connecting the *antennal lobes* (two bundles). They extend in front of the *ellipsoid body*.

- The names are changed because they connect both hemispheres.

LALC **LAL commissure** inter ventral body commissure

The commissure connecting the lateral accessory lobes. It extends in front of the ellipsoid body. It demarcates the boundary between the upper LAL and lower LAL.

- The name is changed because the region of the *ventral body* is now called the *LAL*.

SEC **superior ellipsoid commissure** supra ellipsoid tract

The commissure above the *ellipsoid body*, connecting the *SMP*, *SIP*, *superior clamp*, *VLP,* etc. Some of its fibers span between the superior surface of the *central body* and *MB pedunculus*, and separate the *clamp* into *superior clamp* and *inferior clamp*. It demarcates the boundaries of the *ellipsoid body* (superior), *SMP* (inferior), *SIP* (inferior), *superior clamp* (inferior), *and inferior clamp* (superior).

- The name is changed because it connects both hemispheres.

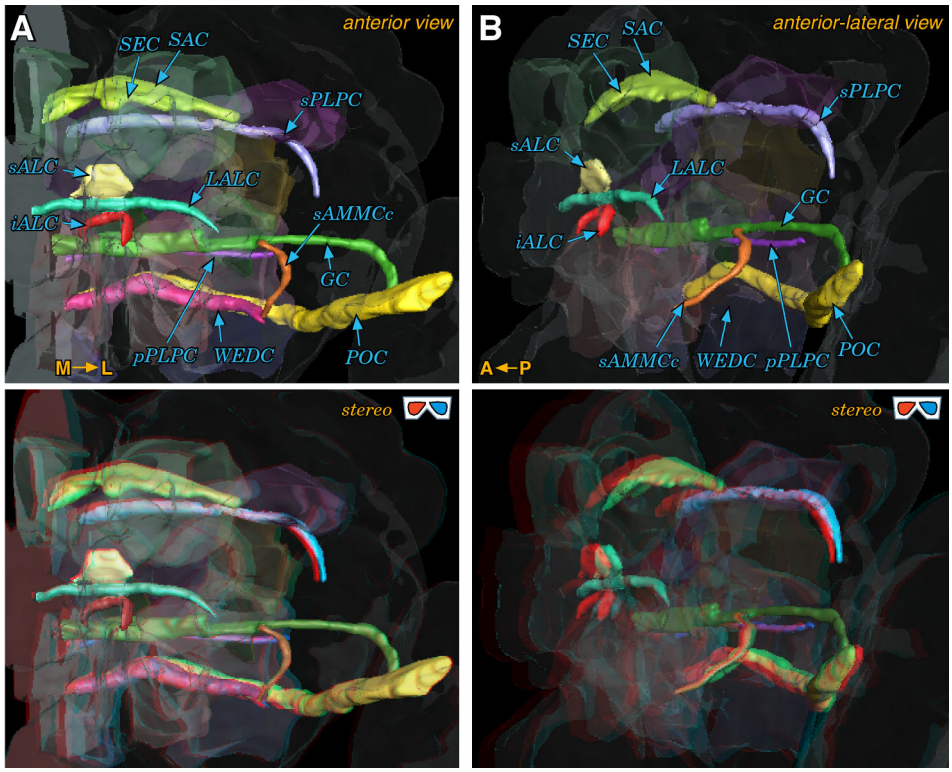


Figure S29. Commissures (red-cyan 3D color stereogram)

SAC **superior arch commissure** *same*

The commissure above the *fan-shaped body,* connecting the *SMP*, *superior clamp*, *inferior clamp*, etc. It demarcates the boundaries of the *fan-shaped body* (superior) and the *SMP* (inferior).

sPLPC **superior PLP commissure** commissure of lateral horn

The commissure running above the *MB pedunculus* and between the *fan-shaped body* and *antler*. It passes near the *lateral horn* but does not enter this neuropil as it was originally thought. Instead, it bends downwards in its lateral parts and connects superior regions of the *PLPs*. It demarcates the boundary between the *superior clamp* and *inferior clamp*.

- The name is changed because it does not actually project to the *lateral horn.*

pPLPC **posterior PLP commissure** *newly introduced name*

The commissure running above the esophagus and below the *inferior bridge*, connecting the middle levels of the *PLPs*. It demarcates the boundary between the *inferior bridge* and *superior posterior slope.*

POC **Posterior optic commissure** posterior optic tract

The *POC* emerges from the serpentine layer of the *medulla* and connects both *medullae*, crossing the posterior brain. It demarcates the boundaries of the *PLP* (posterior-inferior), *SPS* (posterior-inferior), and *IPS* (posterior-superior).

- The name is changed because it connects both hemispheres.

GC **great commissure** *same*

A massive compound pathway comprising many commissures that together extend inferior to the *fan-shaped body* and posterior to the *noduli.* The largest component connects the *lobula* of both *optic lobes*. The commissure also contains many other fibers that connect different neuropils. It demarcates the boundaries of the *PVLP* (posterior), *PLP* (anterior), *noduli* (posterior), *inferior bridge* (anterior), *SPS* (anterior), *vest* (posterior-superior), *epaulette* (posterior), and *gorget* (inferior).

sAMMCC **superior AMMC commissure** *same*

A component of the *great commissure*. It branches off anteriorly from the *great commissure* at the position posterior to the *epaulette* and ends in the *AMMC*.

WEDC **wedge commissure** *newly introduced name*

The *WEDC* lies posterior inferior to the *GC* and anterior to the *POC*, connecting *wedges* of both hemispheres. It demarcates the boundaries of the *SPS* (inferior) and *IPS* (superior).

VI-4 Chiasmata

OCH1 **first optic chiasma** *same*

The *OCH1* lies between the *lamina* and *medulla* of the *optic lobe*. Axons from the lamina reverse the anterior-posterior order of its columns in the *medulla*.

OCH2 **second optic chiasma** *same*

The *OCH2* lies between the *medulla* and *lobula*/*lobula plate* of the *optic lobe*. In insects with an undivided *lobula complex*, axons between the *medulla* and *lobula* reverse their linear order between these two neuropils to form the second chiasma. In flies, the separate *lobula* and *lobula plate* require that axons from the *medulla* to the *lobula* form a chiasma, whereas axons from the *medulla* to the *lobula plate* do not, neither do axons linking the *lobula* and *lobula plate*, whose facing retinotopic columns are in register (see Fig. S17).

ACH **anterior chiasma of the central complex** *same*

The *ACH* lies on the midline between the *FB* and *EB.* Some (not all) fibers from one side of the *FB* project to the contralateral side of the *EB* and *LAL.*

PCH **posterior chiasma of the central complex** *same*

The *PCH* lies on the midline between the *PB* and *FB.* Some (not all) fibers from one side of the *PB* project to the contralateral side of the *FB.*

VI-5 Nerves and connective

Here we provide the general list of the nerves and connectives in the head capsule of insects. In various species some of these nerves are fused to form single bundles. Such fused nerves are indicated with asterisks.

AN **antennal nerve** *same*

The *AN* contains axons associated with the antennae and upper half of the head capsule surface. It enters the brain to supply the *AL* and *AMMC*. A few fibers also project to the *GNG* and the *VLP*.

TgN **tegumentary nerve (anterior, posterior)** *same*

The *TgNs* contain axons from mechanosensory neurons on the head capsule. Axonal fibers target the *AMMC* and may continue into the *GNG* and the *thoracico-abdominal ganglia* (*TAG*).

LbrN **labral nerve** *same*

The *LbrN* is associated with the labrum. It enters the brain at the level of the *tritocerebrum* and is fused with the *frontal nerve* in several species, forming a labro-frontal nerve.

FrN **frontal nerve** *same*

The *FrN* contains a variety of fibers, largely from interneurons, and connects the *tritocerebrum* and the *frontal ganglion* (an unpaired structure on the anterior side of the esophagus).

LbrFrN* **labro-frontal nerve** *same*

The *LbrFrN* is a fused nerve of the *LbrN* and *FrN* observed in some species including *Drosophila*.

RcN **recurrent nerve** *same*

The *RcN* extends from the *frontal ganglion* posteriorly along the esophagus and foregut. It contains fibers of the enteric nervous system. (It therefore does not emerge directly from the brain.)

NCC I, II, III **corpora cardiaca nerve I, II, III** *same*

The *NCCs* contain axons from neurosecretory cells of the brain projecting to the *retrocerebral complex* (*corpora cardiaca* and *corpora allata*).

PhN **pharyngeal nerve** *same*

APhN **accessory pharyngeal nerve** *same*

The *PhN* and *APhN* contains axons associated with the lower half of the head capsule surface (anterior part) including sensory organs along the esophagus. The two nerves fuse and enter what is thought to be the *mandibular neuromere* of the *GNG*.

MnN **mandibular nerve** *same*

The *MnN* is a mixed sensory-motor nerve of the *GNG*. It contains axons from sensory cells of the mandibles entering the *mandibular neuromere* of the *GNG* and *GNG* motor axons to mandibular musculature.

MxN **maxillary nerve** *same*

The *MxN* is a mixed sensory motor nerve of the *GNG*. It contains axons from sensory cells of maxillary appendages entering the *maxillary neuromere* of the *GNG* and *GNG* motor axons to maxillary musculature.

LbN **labial nerve** labial nerve or labellar nerve

The *LbN* is a mixed sensory motor nerve of the *GNG*. It contains axons from sensory cells of the labium entering what is considered to be the *labial neuromere* of the *GNG* and *GNG* motor axons to labial musculature.

- The term *labial* rather than *labellar* is used because the *labellum,* which refers to the flat tip of the *labium* (mouth part), exists in only some insect species, whereas the *labium* exists in most species (see p. 15).

MxLbN* **maxillary-labial nerve** maxillary labial nerve or maxillary labellar nerve

The *MxLbN* is a fused nerve of the *MxN* and *LbN* observed in some species including *Drosophila*. It enters what we interpret as the *maxillary neuromere* of the *GNG* and then bifurcates to project to the *maxillary* and *labial neuromeres*.

OCN **ocellar nerve** *same*

The *OCN* contains axons from the ocellar ganglion. It enters the *superior posterior slope.* Depending on the number of ocelli, many insects (e.g., locusts) have three ocellar nerves, others (e.g. cockroaches) only two.

CV **cervical connective** *same*

The *CV* connects the brain with the *thoracico-abdominal ganglia* (*TAG*). It emerges from the posteriormost *GNG*.

VII. Comparison and Lookup Tables between terminology systems

VII-1 Unique and generic position-based neuropil names

There have been no established categorizations for the neuropils within the *INP*, *VMNP* and *PENP* (except for the *inferior bridge*, *posterior slope*, and *AMMC*). In this nomenclature system, newly defined neuropils are named with short and unique names that are suggestive of the shapes or relative positions of the neuropils but appear seldom in the regular context of neurobiological documents. This follows the naming convention of the genes, mutants, and classic neuropil names, and it will be beneficial for electronically searching phrases that mention these neuropils. On the other hand, there were also opinions among working-group members that generic names with the combination of supercategories and positional descriptors may also be useful. To accommodate both positions, a list of alternative position-based names (that are also uniquely defined) is provided as follows:

Table S10. Unique and position-based terminologies

Unique names			Position-based generic names	
inferior neuropils	**(INP)**		**inferior protocerebrum (IP)**	
- Crepine	(CRE)	=	Anterior inferior protocerebrum	(IPa)
- Superior Clamp	(SCL)	=	Medial and Lateral inferior protocerebrum	(IPm/IPl)
- Inferior Clamp	(ICL)	=	Ventral inferior protocerebrum	(IPv)
- Antler	(ATL)	=	Posterior inferior protocerebrum	(IPp)
- Inferior Bridge	(IB)	=	also Posterior inferior protocerebrum	(IPp)
ventromedial neuropils	**(VMNP)**		**ventromedial cerebrum (VMC)**	
- Gorget	(GOR)	=	Supracommissural VMC	(VMCs)
- Epaulette	(EPA)	=	Precommissural VMC	(VMCpr)
- Vest	(VES)	=	Precommissural VMC	(VMCpr)
- Superior Posterior Slope	(SPS)	=	Dorsal Postcommissural VMC	(VMCpod)
- Inferior Posterior Slope	(IPS)	=	Ventral Postcommissural VMC	(VMCpov)
ventrolateral neuropils	**(VLNP)**		**ventrolateral cerebrum (VLC)**	
- Anterior Ventrolateral Protocerebrum	(AVLP)	=	AVLP	
- Posterior Ventrolateral Protocerebrum	(PVLP)	=	PVLP	
- Posterior Lateral Protocerebrum	(PLP)	=	PLP	
- Wedge	(WED)	=	Inferior VLC	(VLCi)
- Anterior Optic Tubercle	(AOTU)	=	AOTU	
- Posterior Optic Tubercle	(POTU)	=	POTU	
periesophageal neuropils	**(PENP)**		**periesophageal neuropils (PENP)**	
- Cantle	(CAN)	=	Posterior PENP	(PENPp)
- Saddle	(SAD)	=	Lateral PENP/Venromedial PENP	(PENPl/PENPvm)
– AMMC		=	part of above	
- Flange	(FLA)	=	Anterior PENP	(PENPa)
- Prow	(PRW)	=	also Anterior PENP	(PENPa)

VII-2 Boundary differences between classic and current neuropil definitions

The neuropil names used in this terminology system are mostly different from those of the system published previously for the house fly (*Musca domestica*) brain (Strausfeld, 1976; extended for *Drosophila* by Otsuna and Ito, 2006). This is because the definition of some of the neuropils have been reorganized, and whereas Otsuna and Ito used a rectilinear coordinate system as the landmarks of neuropil boundaries, the current nomenclature system refers to internal, that is "natural" structures to demarcate the neuropils. Major differences between these classic terminologies and new terminologies are as follows:

1: Whereas the classic terminology divides the superior *protocerebrum* into *two* parts (lateral and medial: *slpr / ilpr* and *smpr / impr*), the new terminology divides it into *three* parts (lateral, intermediate and medial: *SLP / SIP / SMP*, Fig. S30A, F). The *SIP* is a specific region surrounding the *MB vertical lobe*.

2: Whereas the classic terminology divides the *medial* and *lateral protocerebra* (*slpr / ilpr* and *smpr / impr*) *parallel* to the longitudinal body axis, the new terminology uses *oblique* boundaries between *SLP* and *SIP*. Because of this, the posterior (*n-dorsal in neuraxis*) part of the new *SLP* extends more medially than the classic *slpr / ilpr*.

3: Short of easily recognizable horizontal boundaries across the brain, the classic terminology used the 50% height of the *MB vertical lobe* as the landmark for demarcating the *superior* and *inferior protocerebra*. In this definition the *ilpr* extended above the *VLP / PLP* (*vlpr / plpr*). Developmental studies suggest that the entire region above the *VLP / PLP* belongs to one group. We therefore moved downwards the inferior (*n-posterior*) boundary of the *SLP* so that it is now flanked directly by the *VLP / PLP* (Fig. S30C, H). Thus, the lateral part of the *ilpr* is now regarded as a part of the *SLP*.

Similarly, the inferior (*n-posterior*) boundary of the *SMP* is lowered so that it is now flanked directly by the *central complex*. Thus, the medial part of the *impr* above the *central complex* is now regarded as a part of the *SMP*.

4: The classic terminology separated the *protocerebrum* into three subregions antero-posteriorly (*n-dorso-ventrally*), i.e. the

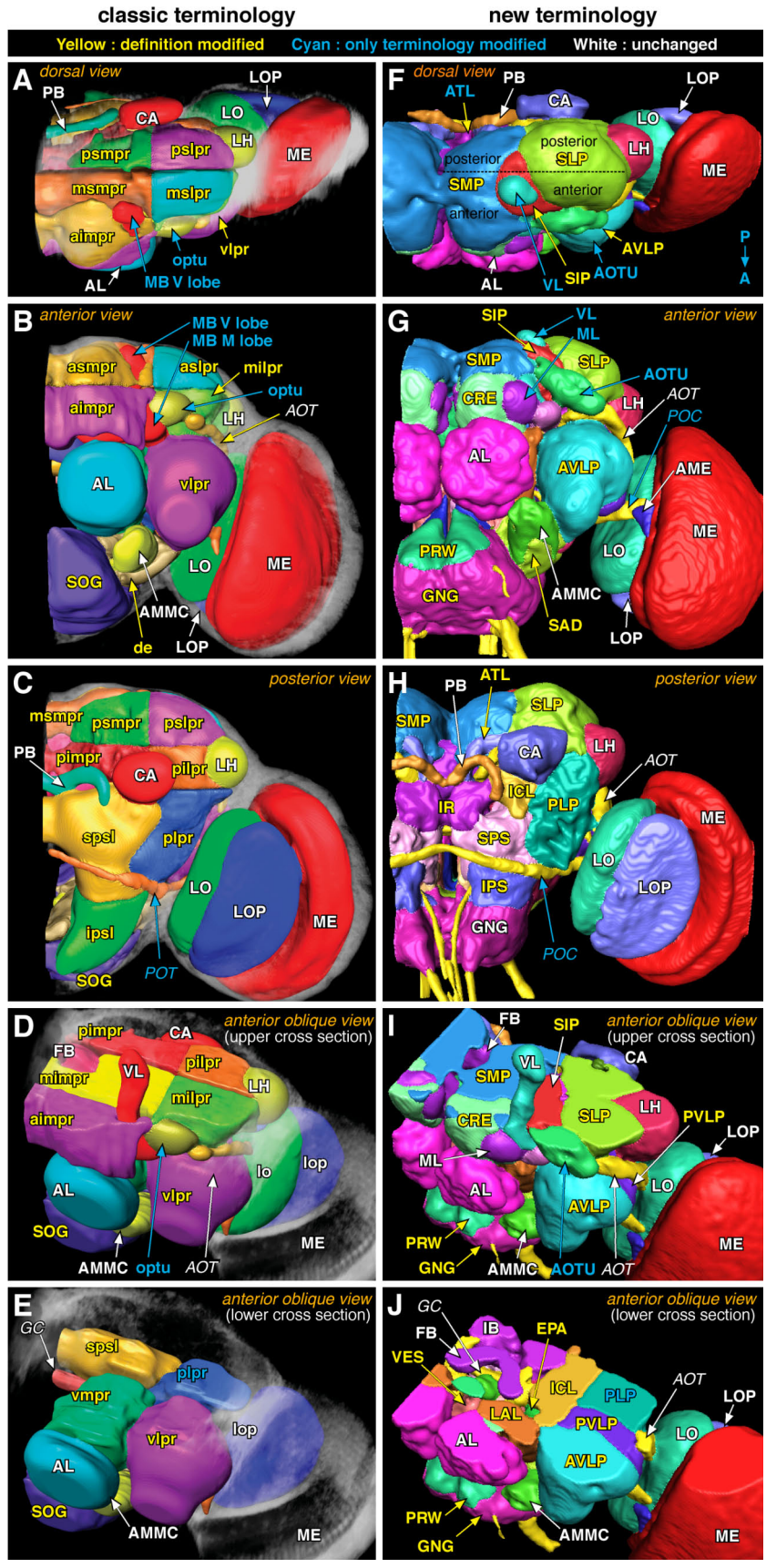


Figure S30. Comparison between classic and new terminologies

anterior, middle and *posterior protocerebra*. Because there is no clear natural boundary in this region, the new terminology does not explicitly divide the *protocerebra* in this direction (Fig. S30A, F). The level of the superiormost apex of the *fan-shaped body* and the plane above the boundary between *PVLP* and *PLP* can both be used as practical landmarks for separating each *protocerebrum* into anterior (*n-ventral*) and posterior (*n-dorsal*) regions. This roughly corresponds to the boundary between the *middle* (*msmpr, mslpr*) and *posterior* (*psmpr, pslpr*) subregions used in the classic terminology (compare Fig. S30A and F).

5: The specific region around the *MB medial lobe*, which essentially corresponds to the *aimpr* (*anterior inferior medial protocerebrum*) of the classic terminology, is defined as *crepine* in the new terminology (Fig. S30B, G).

6: The lateral half of the *ilpr* and medial half of the *impr* are now regarded as parts of the *SLP* and *SMP*, respectively, as explained above. The rest of the region around the *MB pedunculus* (i.e. medial *ilpr* and lateral *impr*) is now regarded as a part of the *clamp.*

7: Classic terminology used the inferior surface of the *MB pedunculus* as a practical landmark for demarcating the boundary between the *impr*/*ilpr* and underlying *vmpr* (*ventromedial protocerebrum*). In the new terminology the boundary in this region is defined according to the regions that are, respectively, richly or poorly supplied with the arborization of the neurons associated with the *cervical connective*. According to this new definition, the inferior (*n-posterior*) boundary of the *inferior neuropils* is lowered to the level of the superior surface of the great commissure (Fig. S30E, J).

8: Classic terminology included the *ventral body* (= *LAL*) as a part of the *vmpr.* New terminology regards the *LAL* as a distinct structure. The superiormost part of the *vmpr* flanking the *MB pedunculus* is now regarded as a part of the *clamp.* The rest of the *vmpr* is defined as the *ventral complex* and fine neuropil subregions are newly identified and named (Fig. S30E, J).

9: The *ventrolateral protocerebrum* (*vlpr* = *VLP*) is now divided into three subregions (*AVLP*, *PVLP*, and *wedge,* Fig. S30E, J).

10: The boundary between the *superior* and *inferior posterior slope* (*SPS* and *IPS*) in the new terminology is shifted more superiorly (*n-anteriorly*) than in the classic terminology (*spsl* and *ipsl,* Fig. S30C, H).

11: The region collectively called the *deutocerebrum* is renamed as the *periesophageal neuropils* because of the ambiguity in the neuromere identity. Fine neuropil subregions are newly identified and named.

12: The anterior superiormost part of the *SOG* is now regarded as a distinct region called the *prow* (Fig. S30B, G).

An approximate mapping between brain regions named according to the classic and new terminologies is provided below.

Table S11. Lookup Table between classic terminology and current terminology

Gray characters: only a small part of that neuropil is included because of slight differences of boundaries.

Classic terminology	Subdivisions		Current terminology
superior lateral protocerebrum (slpr)	- middle slpr (mslpr)	=	anterior superior *SLP*
	- posterior slpr (pslpr)	=	posterior superior *SLP*
superior medial protocerebrum (smpr)	- anterior smpr (asmpr)	=	lateral part : anterior part of superior *SIP* medial part : anterior part of superior *SMP*
	- middle smpr (msmpr)	=	lateral part : posterior part of superior *SIP* medial part : anterior part of superior *SMP*
	- posterior smpr (psmpr)	=	lateral part : posterior part of superior *SLP* medial part : posterior part of superior *SMP*
	* Inferior boundary of *smpr*/*slpr* was slightly higher than that of the *SLP*/*SIP*/*SMP*. Lateral boundary of *psmpr* was more lateral than that of the posterior *SMP*.		
inferior lateral protocerebrum (ilpr)	- middle ilpr (milpr)	=	anterior lateral part of *SCL* + anterior inferior part of *SLP* + (small superiormost part of *PVLP*)
	- posterior ilpr (pilpr)	=	posterior lateral part of *SCL* + posterior inferior part of *SLP* + (small superiormost part of *PLP*)
	* Lateral part of *ilpr* is now regarded as a part of the *SLP*. Boundary between *impr* and *ilpr* was more lateral than that between the *SCL* and *ICL*. Superior boundary of the *PVLP*/*PLP* was found to be higher than before.		
inferior medial protocerebrum (impr)	- anterior impr (aimpr)	=	(lateral part : very small anterior medial part of the *SCL*) medial part : *CRE*
	- middle impr (mimpr)	=	lateral part : anterior part of medial *SCL* medial part : anterior part of superior *ICL* + anterior inferior part of *SMP*, *SIP* (+ *BU*)

	- posterior impr (pimpr)	=	lateral part : posterior part of medial *SCL* + posterior part of superior *ICL* + posterior part of inferior *SIP* medial part : posterior part of inferior *SMP* + *ATL* + (small superiormost part of *PLP*)
	* Medialmost part of *impr* is now regarded as a part of *SMP*. * Boundary between *impr* and *smpr* was higher than that between *SCL* and *SMP/SIP*. Inferior boundary of *impr* was higher than that of *ICL*. *BU* (former *lateral triangle*) has been regarded as a distinct neuropil that is included in the volume of *impr*.		
ventrolateral protocerebrum (vlpr)		=	anterior part : *AVLP* posterior part : *PVLP* inferior part : anterior part of *wedge*
posterior lateral protocerebrum (plpr)		=	*PLP* + posterior part of *wedge*
	* The *wedge* is extended more posteriorly than *AVLP* and *PVLP*.		
ventromedial protocerebrum (vmpr)		=	(anterior superior part : *LAL*) posterior superior part : inferior part of *ICL* inferior part : *VX* (*EPA*, *GOR*, *VES*)
	* Superior boundary of *vmpr* was slightly higher than that of *VX*. *LAL* (former *ventral body*) has been regarded as a distinct neuropil but included in the volume of *vmpr*.		
posterior slope (psl)	- superior psl (spsl)	=	superior part : *SPS*, *IB* inferior part : superior part of *IPS*
	- inferior psl (ipsl)	=	superior part : inferior part of *IPS* inferior part : posterior part of *GNG*
	* Boundary between *superior* and *inferior psl* was slightly lower than in the new system. Previous *psl* included *IB* and a part of the posterior *GNG*.		
deutocerebrum other than the AL	-	=	*SAD* (including *AMMC*), *FLA*, *CAN*
	* The exact extent of the *deutocerebrum* is not yet determined. Some of the structures shown above may belong to the *tritocerebrum*, and some of the neuropils of the *VMNP* might belong to the *deutocerebrum*.		
SOG	-	=	*PRW* + *GNG* (except for the posteriormost part)
	* Anteriormost part of *SOG* is separated as *PRW*. *PRW* is likely to be a part of the *tritocerebrum* together with some neuropils of the *PENP*.		

VII-3 Comparison with observations of single neuron projection patterns

Chiang et al. (2011) proposed brain regions based on the comparison of the projection patterns of single neurons. Though the study did not define precise boundaries of the identified regions, an approximate comparison with the neuropils defined in the current nomenclature system is possible. They match to many regions of the brain, but differ in certain instances, primarily because of differences in identifying boundaries in contiguous neuronal projections. The study also used some unconventional terms and abbreviations. To enable understanding of the study in the framework of the current terminology, bidirectional lookup tables are provided below.

Table S12. Lookup Table from Chiang et al. (2011) to the current terminology

Chiang et al. Abbreviation	Full name	→ Current terminology
AL	Antennal Lobe	*AL*
AMMC	Antennal Mechanosensory and Motor Center	*AMMC* and surrounding *SAD*
Cal	Calyx	*CA* (subregion of *MB*)
CCP	Caudalcentral Protocerebrum	*ATL* + *IB*
CMP	Caudalmedial Protocerebrum	most part of *PS*
CVLP	Caudal Ventrolateral Protocerebrum	inferior part of *PLP*
DLP	Dorsolateral Protocerebrum	anterior *SLP* + *SIP*
DMP	Dorsomedial Protocerebrum	*ICL*
EB	Ellipsoid Body	*EB*
FB	Fanshaped Body	*FB*
FSPP	Frontal Superpeduncular Protocerebrum	posterior part of *SIP*
IDFP	Inferior Dorsofrontal Protocerebrum	*LAL* + posterior part of *CRE*
IDLP	Inner Dorsolateral Protocerebrum	posterior *SLP* + posterior *SMP*
LH	Lateral Horn	*LH*

Lob	Lobula	*LO*
LoP	Lobula Plate	*LOP*
Lat Tri	Lateral Triangle	*BU*
MB	Mushroom Body	*MB*
Med	Medulla	*ME*
Nod	Noduli	*NO*
OG	Optic Glomerulus	subregions of *VLP*
OPTU	Optic Tubercle	*AOTU*
PAN	Proximal Antennal Protocerebrum	anterior part of *VX* (*VES*)
PCB	Protocerebral Bridge	*PB*
SDFP	Superior Dorsofrontal Protocerebrum	anterior *SMP*
SOG	Subesophageal Ganglion	*GNG* + *FLA* + *PRW*
SPP	Superpeduncular Protocerebrum	superior part of *PLP* + *SCL*
VLP	Ventrolateral Protocerebrum	*VLP*
VMP	Ventromedial Protocerebrum	most part of *VX* (*VES* + *EPA* + *GOR*) + part of *PS*

Table S13. Lookup Table from the current terminology to Chiang et al. (2011).

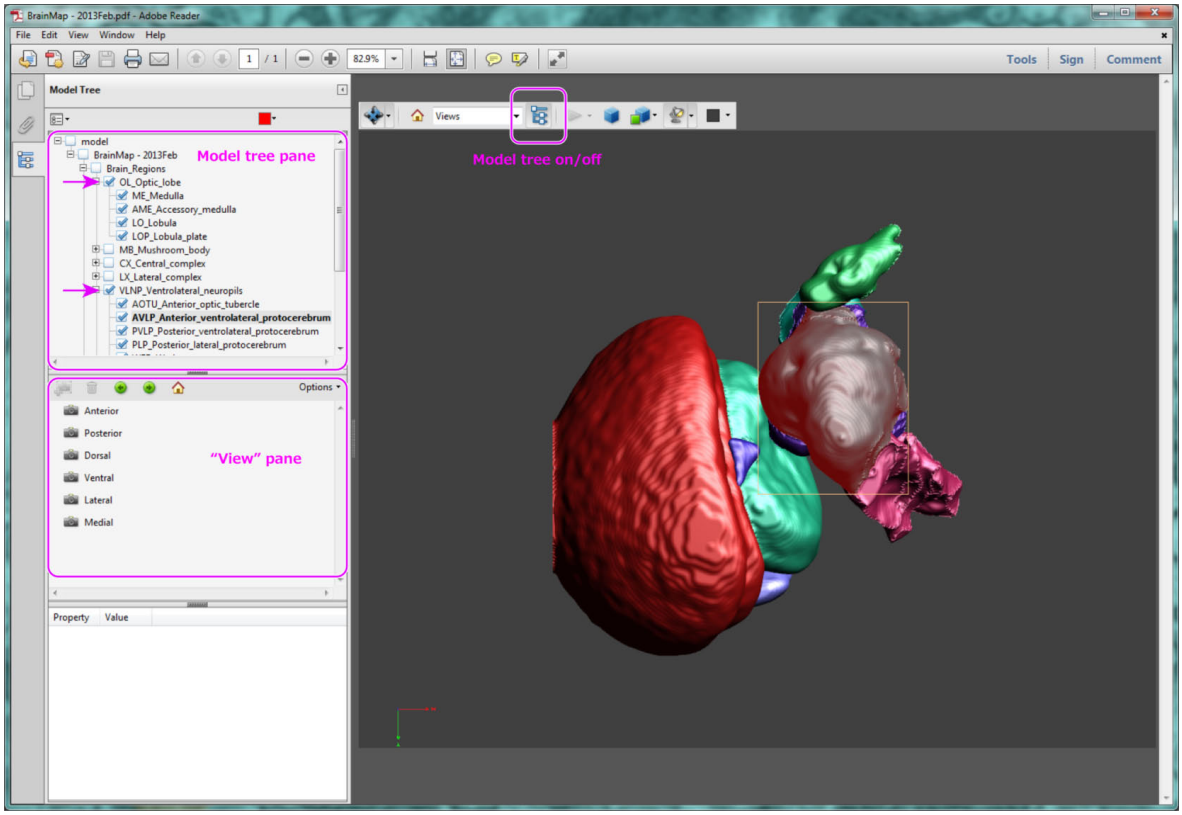
Current terminology	**→ Chiang, et al. (2011)**
LA	- not determined
ALA	- not determined
ME	Med
AME	- not determined
LO	Lob
LOP	LoP
MB	MB (Mushroom Body) + Cal (Calyx)
FB	FB
EB	EB
PB	PCB
NO	Nod
BU	Lat Tri (Lateral Triangle)
LAL	ventral part of IDFP (Inferior Dorsofrontal Protocerebrum)
AOTU	OPTU (Optic Tubercle)
VLP	VLP (Ventrolateral Protocerebrum) + OG (Optic Glomerulus)
PLP	CVLP (Caudal Ventrolateral Protocerebrum) + lateral part of SPP (Superpeduncular Protocerebrum)
LH	LH
SLP	lateral part of DLP (Dorsolateral Protocerebrum) + lateral part of IDLP (Inner Dorsolateral Protocerebrum)
SIP	medial part of DLP (Dorsolateral Protocerebrum) + FSPP (Frontal Superpeduncular Protocerebrum)
SMP	SDFP (Superior Dorsofrontal Protocerebrum) + medial part of IDLP (Inner Dorsolateral Protocerebrum)
CRE	anterior part: [not in Chiang et al.] posterior part: dorsal part of IDFP (Inferior Dorsofrontal Protocerebrum)
SCL	medial part of SPP (Superpeduncular Protocerebrum)
ICL	DMP (Dorsomedial Protocerebrum)
IB	ventral part of CCP (Caudalcentral Protocerebrum)
ATL	dorsal part of CCP (Caudalcentral Protocerebrum)
AL	AL
VX (*VES*, *EPA*, *GOR*)	most part of VMP (Ventromedial Protocerebrum) + PAN (Proximal Antennal Protocerebrum)
PS	CMP (Caudalmedial Protocerebrum), possibly including part of VMP (Ventromedial Protocerebrum)
SAD (including *AMMC*)	AMMC (Antennal Mechanosensory and Motor Center)
FLA	dorsoposterior part of SOG (Subesophageal Ganglion)
CAN	ventroposterior part of VMP (Ventromedial Protocerebrum)
PRW	dorsoanterior part of SOG (Suboesophageal Ganglion)
GNG	ventral part of SOG (Suboesophageal Ganglion)

VIII. How to use Interactive 3D Brain Maps (Supplemental Movies S5, S6)

This section provides a user's manual of the Interactive 3D Brain Maps, provided as separate supplementary materials called "Movie_S5_Interactive_Map_40x.pdf" and "Movie_S6_Interactive_Map_20x.wrl." These maps provide three-dimensional image data of all the synapse-rich neuropils (brain regions) and landmark fiber bundles of the fly *Drosophila melanogaster* described in this document. They enable users to interactively rotate, zoom, and turn on/off the display of any structure. This is meant to be a tool for gaining an intuitive understanding of the morphology and spatial relationship of brain regions.

Movie_S5_Interactive_Map_40x.pdf

This interactive map is based on a serial section image dateset acquired with a water-immersion 40x objective lens. Using 3D PDF format, the file can be viewed with common Adobe Reader or Adobe Acrobat software. It enables the visualization of the model in many different ways. However, visualization during rotation may be slow depending on the graphics power of the computer used. Because of the high-resolution original image, not only synapse-rich neuropils but also fiber bundles are traced. Only one half of the brain is covered, however.



Sample image: The model tree pane on the left can be turned on and off with the button (indicated with a rectangle) in the toolbar, which is shown above the image. Brain regions and fiber bundles can be selected either by clicking on the image or by clicking the names in the model tree pane. Display of each object (neuropils and fiber bundles) can be turned on and off by clicking the checkboxes in the model tree pane (arrows). In this sample image, only the *optic lobe* (*OL*) and the *ventrolateral neuropils* (*VLNP*) are turned on. Images can be rotated freely with a mouse-drag on the image. Different viewing angles can be selected from the "view" pane under the model tree pane.

How to open the file

First, download the PDF file and save it in your computer, and open it with *Adobe Reader* or *Adobe Acrobat* (version 8.0 or later). Use genuine software from Adobe. Note that PDF readers from other companies and web browser plug-ins may not be able to handle 3D PDF data. The latest version of Adobe Reader is available from the following website: http://get.adobe.com/reader/

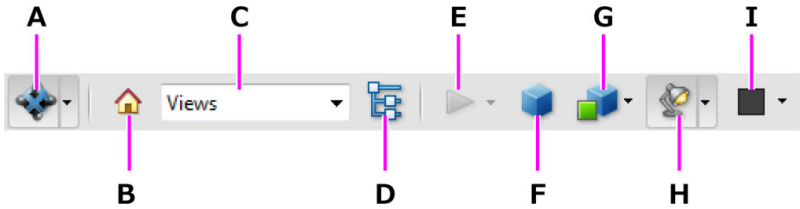
User instruction

A: Activating the file

After opening the file, click anywhere on the title page to activate the 3D Brain Map. Wait for a moment, as it may take up to a couple of minutes to activate the file. Depending on the setup of the Reader software, you may encouter yellow popup message that warns potential security risks. In this case, click the "Options" button and select "Trust this document always."

B: Toolbar buttons

After activation is complete, a toolbar appears by hovering the mouse pointer over the brain image. You can change orientation of the brain, projection mode, etc., by clicking the buttons.



A: Rotation mode
B: Back to default view (anterior view with all the neuropils displayed)
C: View selection (with all the neuropils displayed)
D: Toggle model tree display (see next section)
E: Play animation (disabled in this file)
F: Toggle perspective/orthographic projections (default: Orthographic)
G: Model render mode (default: Solid, change modes for different rendering effects)
H: Extra lightning (default: headlamp)
I : Background color

C: Model tree

The model tree of brain regions is shown on a pane left to the image (see Sample image). It can be turned off and on by clicking the button (**D**) of the toolbar. Users are recommended to keep the model tree open while using the brain map. In the model tree, synapse-rich neuropils and fiber bundles are listed hierarchically. The tree can be expanded by clicking the plus (⊞) or rightward triangle buttons to show the items within the category, and can be collapsed by clicking minus (⊟) or downward triangle buttons.

Display of individual brain regions can be turned on and off by clicking the checkboxes (☑) left to each item of the list. For example, when the checkbox for “Brain_Regions” is turned off, all synapse-rich neuropils will disappear and only fiber bundles will be displayed.

Viewing all the objects can slow down interactive movement of the map. For faster response, it is sometimes helpful to turn off all the objects first by clicking the checkboxes (☑) of “Brain_Regions” and “Fiber_Bundles”, and then turn on only the objects you want to examine by expanding the model tree and clicking the respective checkboxes.

- In the default setting, only a part of the image will be displayed during interactive rotation. If you want to view the entire image during rotation, go to the menu “Edit > Preferences > 3D & Multimedia”, locate the item called “Optimization Scheme for Low Frame Rate” in the box titled “Auto-Degrade Options”, and select “None.” You need a computer with a high-speed graphics processor for smooth visualization. Otherwise select “Bounding Box (default)” or “Drop Objects.”

D: Image handling

The 3D Brain Map can be rotated by left-click and dragging the mouse on the image, and zoomed in and out by right-click and dragging the mouse. Position of the image in the window can be moved by control-left-click and dragging the mouse.

Names of brain regions and fiber bundles will be listed on the model tree (when the model tree is open, see above). By clicking the objects in the image, the name of the corresponding brain region or fiber bundle will be highlighted in the model tree pane. Alternatively, clicking on the names in the model tree will highlight the corresponding part of the brain in the image. (Note: the highlighted object may not be visible if it is obscured by other objects.)

When the model tree is turned on, the “view pane” is also shown under the model tree. Users can switch the view to pre-set angles (Anterior, Posterior, Dorsal, Ventral, Lateral and Medial views) shown with camera icons (📷). By clicking the home (🏠) icon, the default view (the anterior view) is shown. (Note: by clicking these pre-set and default view buttons, all brain regions will be displayed even when you have selected specific objects to be displayed.)

E: Right-click menu on the object names on the model tree

By right-clicking individual object names on the model tree, the following menu will appear. Each object can be handled separately.

Show All Parts	--- Show all the brain regions
Fit Visible	--- Zoom into visible objects
Hide	--- Hide selected objects
Isolate	--- Hide all but selected objects
Zoom to part	--- Zoom into selected objects
Transparent	--- Make selected objects transparent
Part Render Mode	--- Change rendering mode of selected objects

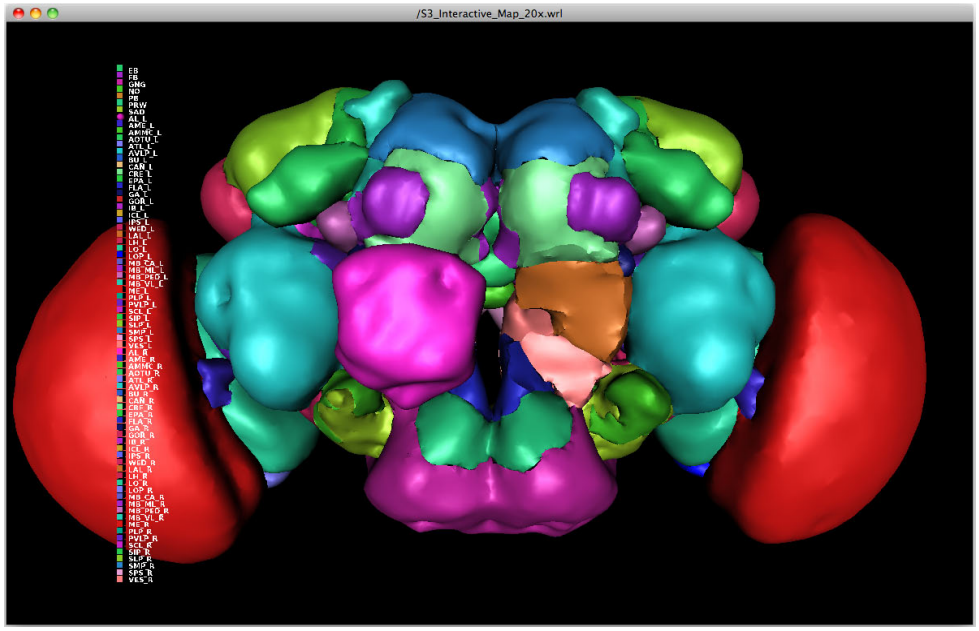
Copy Data --- Copy the name of the object
Export as XML --- Export the selected objects as a XML format file
Export as CSV --- Export the selected objects as a CSV format file

Copyright notice

When using images made from Movie_S5_Interactive_Map_40x.pdf for publication and other purposes, please mention that the images are generated using the Interactive 3D Brain Map developed by Kazunori Shinomiya and cite this paper.

Movie_S6_Interactive_Map_20x.wrl

This interactive map is based on a serial section image dateset acquired with a dry 20x objective lens. The model is complementary to the other one in several aspects. Specialized viewer software is required to view the wrl file. The viewer has limited visualization options but is faster even with computers with limited graphics capability. The model covers both brain halves, providing better overall view. Because of the lower resolution of the original image, however, fiber bundles and very small neuropil structure such as the accessory calyx are not described in this model.



Sample image: Neuropil list is seen on the right side of the window. Images can be rotated and zoomed freely with a mouse-drag on the image. Display of each object (neuropils on the left and right sides) can be turned on and off by clicking the colored symbols in the list, e.g., the antennal lobe on the left side (AL-L) is turned off in the above example.

How to open the file

First, download the wrl file and save it in your computer. Download also a viewer software of the wrl data. “FreeWRL” is the most popular software for this purpose, available for Windows, Mac, and Linux. It is downloadable from the following website: http://freewrl.sourceforge.net/ . Alternative standalone software and browser plugins can be found on the site of the web3Dconsortium: http://www.web3d.org/x3d/content/examples/X3dResources.html .

User instruction

After opening the file, the anteror view of the entire brain should be seen. To rotate the model, left-click the region of the window outside of the model (i.e. black area) and drag the mouse. To zoom in and out, right-click the black region and drag the mouse. If the neuropil list on the left side of the window is not shown, rotate or zoom the model to activate the list. If the list remains hidden, decrease the hight of the window (or increase the width of the window) to make its aspect ratio rather wide. (Neuropils are listed in an alphabetical order, from those around the midline followed by those on the left and right brain halves.) Click any part of the model will disable the visualization of the corresponding neuropil, showing the structures that lie behind. The square symbol of the corresponding neuropil in the list will become a sphere to show that the neuropil is hiddlen. Click the symbol of the neuropil (square or sphere) to toggle the visibility of the neiropil.

Copyright notice

When using images made from Movie_S6_Interactive_Map_20x.wrl for publication and other purposes, please mention that the images are generated with the Interactive 3D Brain Map developed by Anim Jenett and Kazunori Shinomiya, and cite this paper.

Experimental procedures

Antibodies and *Drosophila* stocks for labeling overall brain structures

The pan-neuronal enhancer-trap strain *elav*-Gal4 (C155; Lin and Goodman, 1994) was used to drive cytoplasmic reporter UAS-GFP (T2; Ito et al., 1998) or the combination of cytoplasmic UAS-DsRed (C6; Verkhusha et al., 2001), presynaptic UAS-n-Syb-GFP (Ito et al., 1998), and postsynaptic UAS-Rdl-HA (Sánchez-Soriano et al., 2005). Brains with expression of Rdl-HA were subsequently immunolabeled with anti-HA primary antibody (HA.11 Clone 16B12, mouse monoclonal, Covance; diluted 1:1000).

The following primary antibodies were used to visualize synapse-rich and fiber-bundle neuropils: anti-Bruchpilot nc82 (mouse monoclonal, gift from E. Buchner; diluted 1:20; Wagh et al., 2006), anti-Synapsin 3C11 (mouse monoclonal, Developmental Studies Hybridoma Bank; diluted 1:1000; Klagges et al., 1996), anti-DLG 4F3 (mouse monoclonal, Developmental Studies Hybridoma Bank; diluted 1:1000; Parnas et al., 2001), and anti-β Tubulin E7 (mouse monoclonal, Developmental Studies Hybridoma Bank; diluted 1:1000; Chu and Klymkowsky, 1989; Popodi et al., 2005). Each antibody was applied to the specimens of the flies carrying pan-glial *repo*-Gal4 expression driver (Awasaki et al., 2008; Lai and Lee, 2006) and membrane-bound reporter UAS-mCD8-GFP (LL6) (Lee and Luo, 1999) so that labeling patterns of the antibodies can be observed simultaneously with the distribution of the glial processes. For secondary antibodies, Alexa Fluor 488, 568 and 647-conjugated anti mouse IgG antibodies (Invitrogen; diluted 1:250) were used.

Drosophila stocks for labeling clonally associated neuron groups

Following genetic cross and heat shock conditions were used to label clones shown in Figs. S14 and S15: *elav*^{c155}-Gal4 *hs-FLP; FRT*^{G13} *tub*-GAL80 crossed with UAS-DsRed; *FRT*^{G13}; UAS-n-syb-GFP (heat shock at 36°C for 45 minutes; *elav*^{c155}-Gal4 *hs-FLP*; *FRT*^{G13} *tub*-GAL80 crossed with UAS-syt-HA; *FRT*^{G13} UAS-GFP; UAS-mCD8-GFP (heat shock at 36°C for 45 minutes); *hs-FLP tub*-GAL80 *FRT*^{19A}; *actin*-Gal4 crossed with UAS-DsRed *FRT*^{19A}; +; UAS-n-syb-GFP (heat shock at 36°C for 30 minutes); *hs-FLP tub*-GAL80 *FRT*^{19A}; *actin*-Gal4 crossed with *FRT*^{19A}; UAS-GFP (heat shock at 37°C for 30 minutes). In all cases heat shock was applied between 12-36 hours after egg laying. Following primary antibodies were used to visualize the labeled cells: rabbit anti-DsRed polyclonal antibody (Takara Bio; #632496, 1:1000), rat anti-GFP monoclonal antibody (Nacalai Tesque; #GF090R, 1:1000), rabbit anti-GFP polyclonal antibody (Molecular Probe; #A11122, 1:1000). The samples were also labeled with mouse nc82 monoclonal antibody (gift from E. Buchner and A. Hofbauer, 1:20), the signal of which is useful for the registration of different image datasets using 3D registration software such as Computational Morphometry ToolKit (Jefferis et al., 2007) or BrainAligner (Peng et al., 2011).

Sample preparation and imaging

Flies were raised at 25°C with 12-hour light/12-hour dark cycle. Five to ten day-old adult female brains were dissected, fixed and antibody labeled as previously described (Otsuna and Ito, 2004) and mounted in 100% glycerol. Frontal and horizontal serial optical sections of whole-mount brain samples were acquired at 1.41-μm z-step intervals with a LSM510 (Zeiss) confocal laser-scanning microscope with a 40x water-immersion C-Apochromat objective (n.a.=1.2). Serial images of between three and ten samples were taken for each combination of antibodies and reporters for comparison. 3D reconstruction of confocal images was performed with Fluorender software (Wan et al., 2009).

Silver staining and imaging

Silver stain was performed following the Holmes-Blest protocol (Blest, 1961) with 7-μm Paraffin serial sections. Sections were photographed with an Axioplan microscope (Zeiss) with a 40x oil-immersion Plan Apochromat objective (n.a. 1.4).

Boundary drawing and 3D rendering

To draw boundaries of identified neuropils, frontal serial section images of the brain with elav-driven DsRed/n-syb-GFP/Rdl-HA labeling were imported to Amira (Mercury Inc.), and regions that correspond to each synapse-rich or fiber-bundle neuropil were marked manually using Amira's painting function. Painted volumes were also examined and edited in the horizontally and sagittally resliced sections to compare with the structural features that were best visible from these directions. Neuropil volumes were visualized using the 3D rendering function of Amira. Colors of the neuropils were chosen to maximize distinction between neighboring structures. Because of the large number of neuropils, however, resemblance of certain colors occurred inevitably.

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Relevant databases past and present

FLYBRAIN. An Online Atlas and Database of the *Drosophila* Nervous System. *http://www.flybrain.org*

FLYBRAIN NEURON DATABASE. *http://ndb.flybrain.org*

VIRTUAL FLY BRAIN. *http://www.virtualflybrain.org*

Appendix: Detailed definition of neuropil boundaries in the *Drosophila* brain

This appendix explains in detail the landmarks used to demarcate boundaries between synapse-rich neuropils (see Section V as well as Movie_S1_, S2_, and S3_Neuropils.mov). In many cases, neuropil boundaries are determined by taking into account the arborization domains of clonally related groups of neurons or neurons labeled by various expression driver strains. Because such arborizations are not visible with synaptic markers, such as nc82 antibody labeling, we provide those landmark indicators here. Descriptions all refer to the brain of *Drosophila melanogaster*. Because the spatial arrangement of neuropils will vary depending on species, the present determinations are not immediately applicable to the brains of other insects. Nevertheless, information provided here should be helpful for locating corresponding boundaries in other taxa. To facilitate this, we provided useful ways to identify respective neuropils at the beginning of each section.

1. Optic lobe (OL)

The *lamina* (*LA*)*, medulla* (*ME*)*, lobula* (*LO*)*,* and *lobula plate* (*LOP*) are easy to identify, because they are separated clearly by the fibers of the *optic chiasmata* (*first optic chiasma* and *second optic chiasma*).

Accessory medulla (AME)

The *accessory medulla* is a small triangular neuropil slightly protruded from the anterior medial edge of the *medulla*. It is almost completely surrounded by cell bodies and thick glial walls. Its lateral side is flanked by the *medulla*, separated by thin glial processes.

2. Mushroom body (MB)

Most parts of the *mushroom body* are easy to identify, because they are separated from surrounding neuropils by extensive glial processes.

The *accessory calyx* (*ACA*) is the only part that is not clearly segregated from the surrounding neuropils by glia. It can be distinguished by; (1) the slight difference of synaptic density that can be visualized with synaptic markers (e.g., elav>n-syb-GFP, but not clearly visible with nc82), (2) using molecular markers such as GAL4 expression driver lines that label subsets of Kenyon cells arborizing in this region, or (3) tracing the extent of fibers protruding anteriorly from the calyx to the surrounding neuropils (*superior lateral protocerebrum* (*SLP*).

3. Central complex (CX)

The *fan-shaped body* (*FB*), *ellipsoid body* (*EB*), *protocerebral bridge* (*PB*) and *noduli* (*NO*) are easy to identify from each other and from the surrounding neuropils, because they are surrounded by extensive processes of glia that can be visualized with anti-glutamine synthetase or anti -repo immunolabeling as well as glial-specific expression drivers such as repo-GAL4.

4. Lateral complex (LX)

4-1 Bulb (BU)

The *bulb* is relatively easy to identify by (1) tracing the neuronal fibers from the *ellipsoid body* to the region where they form dendritic arborizations, or (2) its characteristic structure comprising tiny glomerulus-like volumes that can be visualized with synaptic markers such as nc82, elav>n-syb-GFP, etc., or (3) performing GABA immunolabeling to identify prominent GABA-immunoreactive structures with glomerular appearance in the vicinity of the *central complex* and *LAL*. The *bulb* lies outside of the *LAL* in flies but may be embedded within it in some species such as locusts.

4-2 Lateral accessory lobe (LAL)

The *LAL* is characterized with the terminals of columnar neurons from the *FB* as well as the arborizations of bilateral neurons that cross the midline via the *LAL commissure.* Tracing these neurons help locating the position of the *LAL,* especially in species in which the overall layout of the *lateral complex* is different (e.g. rotated) from that of *Drosophila*.

Boundaries in *Drosophila*

anterior Flanked by the *antennal lobe* (*AL*), separated by thick glial processes.

posterior Flanked by the *bulb* (superior half), *inferior clamp* (*ICL*), and *epaulette* (*EPA*) of the *ventral complex* (*VX*) (inferior half). Its posterior boundary lies at the level of the mid point of the *bulb* and the branching point of the *medial* and *mediolateral antennal lobe tracts* (*mALT* and *mlALT*).

lateral Flanked by the *anterior ventrolateral protocerebrum* (*AVLP*). Thick glial wall separates the *LAL* and *ventrolateral protocerebrum* (*VLP*).

superior medial

anterior half: situated beneath the *MB medial lobe*. However, the *LAL* and *MB lobe* are separated by the *crepine* (*CRE*), which lies in between and shows different characteristics of fiber arrangements from that of the *LAL*. (Below the *MB medial lobe* in the *crepine,* fibers tend to run antero-posteriorly. In the *LAL,* fibers run medio-laterally.)

posterior half: flanked by the *ellipsoid body*, separated by glial processes.

inferior medial: flanked by the *antennal lobe* (anterior half) and *vest* (*VES*) of the *ventral complex* (posterior half). The landmark boundary between *LAL* and *vest* is practically defined by the virtual plane interpolating the *medial antennal lobe tract* and *posterior cerebro-cervical fascicle* (*pCCF*).

5. Ventrolateral neuropils (VLNP)

5-1 Anterior optic tubercle (AOTU)

The *AOTU* can be identified by the terminal arborizations of neurons projecting from the *optic lobe* via the *anterior optic tract*.

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior Flanked by the *SLP* and *SIP*, separated by thick glial processes.

lateral Facing the cell body rind between the *cerebrum* and *optic lobe*.

medial Flanked by the *SIP* and *MB vertical lobe*, separated by thick glial processes.

superior Facing the superior cell body rind.

inferior Flanked by the *AVLP*, separated by thick glial processes.

inferior lateral Connected with the *anterior optic tract* (*AOT*).

5-2 Ventrolateral protocerebrum (VLP)

5-2a Anterior ventrolateral protocerebrum (AVLP)

The *AVLP* can be identified as the non-glomerular volume of *VLP* (lying between the *antennal lobe / LAL* and the *optic lobe*), which is devoid of glomerular structures with high synaptic density (i.e., extensive labeling with nc82).

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior Flanked by the *PVLP*. Because *PVLP* is glomerular whereas *AVLP* is not, the plane enveloping the anteriormost contour of the glomeruli demarcates the boundary. The *lateral antennal lobe tract* (*lALT*) runs through the posterior region of the *AVLP*, just in front of the boundary with the *PVLP*.

superior **anterior part:** flanked by the *anterior optic tubercle*, separated by glial processes.

posterior superior part: flanked by the *anterior optic tract*.

posterior part: flanked by the *superior lateral protocerebrum*. The *anterior SLP fascicle* (*aSLPF*) demarcates the boundary between *AVLP* and *SLP*.

inferior **anterior part:** flanked by the *saddle* (*SAD*), separated by glia.

posterior part: flanked by the *wedge* (*WED*). The boundary is rather contiguous and is practically defined with the plane interpolating the external indentation of the neuropil surface and the bottom region of the thick glial wall between *LAL* and *AVLP*. The region is defined so that the superior branches of the axons from the antennal mechanosensory neurons are contained within *wedge* and not *AVLP*.

lateral Facing the cell body rind between the *cerebrum* and *optic lobe*.

medial Flanked by the *LAL* with a thick glial wall in between. In the superiormost region, *AVLP* has a small boundary with the *inferior clamp*. The plane interpolating the lateral surface of *MB pedunculus* and the lateral edge of the *LAL* demarcates the boundary.

5-2b Posterior ventrolateral protocerebrum (PVLP)

The *PVLP* can be identified as the volume of the *VLP* that contains glomerular structures with high synaptic density. Only the region that is anterior (n-ventral) to the *great commissure* (*GC*) is included.

Boundaries in *Drosophila*

anterior Flanked by the *AVLP*. Because *PVLP* is glomerular whereas *AVLP* is not, the plane enveloping the anteriormost contour of the glomeruli demarcates the boundary.

posterior Flanked by the *posterior lateral protocerebrum* (*PLP*). The boundary is defined by the contour of the posteriormost glomeruli that are contributed by the *lobula* neurons. It roughly corresponds to the frontal (vertical) plane extrapolated from the posterior surface of the *great commissure.*

superior **lateral part:** flanked by the *lateral horn* (*LH*). Externally, the boundary with the *lateral horn* is demarcated by an indentation on the lateral neuropil surface where the *anterior optic tract* is partially embedded. The internal boundary with the *lateral horn* is defined by the high density of synapses in the *lateral horn*, which is visible in the elav>n-syb-GFP but appears contiguous with nc82. This boundary roughly corresponds to the plane interpolating the indentation of the neuropil surface and the trajectory of the *posterior lateral fascicle* (*PLF*).

medial part: flanked by the *SLP* (middle) and *superior clamp* (*SCL*, medially). The boundary is demarcated by the superior medial contour of the glomeruli.

inferior Flanked by the *wedge*. The contours of the most inferior glomerular structures demarcate the boundary. Externally, there is an indentation on the lateral neuropil surface that lies inferior to a few distinctive glomeruli.

lateral Facing the cell body rind between the *cerebrum* and *optic lobe*.

medial Flanked by the *inferior clamp* superiorly and *epaulette* inferiorly. The contours of the medialmost glomerular structures demarcate the boundary, roughly at the level of the medial surface of the *MB pedunculus*. The superiormost region of the medial boundary is flanked by the *MB pedunculus.*

5-3 Posterior lateral protocerebrum (PLP)

The *PLP* can be identified as the region that is posterior (n-dorsal) to the *PVLP* and *great commissure* and that contains glomerular structures with high synaptic density like the *PVLP*.

Boundaries in *Drosophila*

anterior Flanked by the *PVLP*. The boundary is defined by the contour of the posteriormost glomeruli that are contributed by the *lobula* neurons. It roughly corresponds to the frontal (vertical) plane extrapolated from the posterior surface of the *great commissure.*
At its inferiormost region it is flanked by the *wedge*. The anterior surface of the most inferior glomerular structure demarcates the boundary.

posterior Facing the posterior cell body rind.

superior **lateral part:** flanked by the *lateral horn*. Externally (along the neuropil surface), the boundary with the *lateral horn* is demarcated by a small indentation on the lateral neuropil surface (Fig. S11F). The boundary with the *lateral horn* is defined by the high density of synapses in the *lateral horn*, which is visible with elav>n-syb-GFP but appears contiguous with nc82. This boundary roughly corresponds to the plane interpolating the external indentation and the trajectory of the *posterior lateral fascicle.*

posterior middle part: flanked by the *SLP*. In the posteriormost region, the superior boundary of the *PLP* exceeds the level of the *MB pedunculus* and reaches the level of the inferior surface of the *medial antennal lobe tract* (Fig. S11G, S20C). This is because many visual neurons in the *PLP* run superiorly in this region.

medial part: flanked by the *superior clamp*, roughly at the level of the superior surface of the *MB pedunculus*. At the posteriormost region, the superior boundary of the *PLP* exceeds the level of the *MB pedunculus,* where it is flanked by the *superior clamp*.

inferior Flanked by the *wedge* anteriorly and *superior posterior slope* (*SPS*) posteriorly, at the level of the inferiormost glomerular structure, slightly above the level of the *wedge commissure*.

lateral Facing the cell body rind between the *cerebrum* and *optic lobe*.

medial Flanked by the *inferior clamp* superiorly and *superior posterior slope* inferiorly. The medial contour of the *optic glomeruli* demarcates the boundary, roughly up to the level of the medial surface of the *MB pedunculus*.

5-4 Wedge (WED)

The *wedge* can be identified as the region between the *VLP* and *saddle / GNG*, which features essentially no connection with the *optic lobe* but receives neurons connecting with the underlying *saddle.*

Boundaries in *Drosophila*

anterior **lateral and superior parts:** flanked by the *AVLP*, at the antero-posterior level where the glial sheath between *AVLP* and *saddle* disappears.

medial and inferior parts: flanked by the *saddle*, the contour of *antennal mechanosensory and motor center* (*AMMC*, fiber bundles deriving from the *antennal nerve*, *AN*) demarcates the boundary.

posterior **medial part:** flanked by the *superior posterior slope* and *inferior posterior slope* (*IPS*). At the lateral part of the boundary, the *wedge* extends posteriorly up to the level of the *wedge commissure*. At the medial part, the trajectory of the *posterior cerebro-cervical fascicle* and its extrapolated plane demarcate the boundary between *wedge* and *posterior slope*.
lateral part: flanked by the *PLP*. The boundary corresponds to the anterior surface of the inferiormost glomerular structure in the *PLP* (Fig. S12H, S13F).

superior **anterior part:** flanked by the *AVLP*. The boundary is contiguous, accommodating the region of arborization of the antennal mechanosensory neurons. It is practically defined by the plane interpolating the external indentation of the neuropil surface and the bottom region of the thick glial wall between *LAL* and *AVLP*.

posterior part: flanked by the *PVLP* and *PLP*. The inferior contour of the inferiormost glomeruli of the *PVLP* and *PLP* demarcates the boundary.

inferior **anterior part:** flanked by the *saddle*. The superior contour of the *AMMC* demarcates the boundary. An indentation in the lateral neuropil surface demarcates the boundary.

posterior part: flanked by the *gnathal ganglia* (*GNG*). The plane interpolating the lateral edge of the *AMMC* and the external indentation of the neuropil surface demarcates the boundary (Fig. S11E).

lateral Facing the cell body rind between the *cerebrum* and *optic lobe*.

medial Flanked by the *inferior fiber system* (*IFS*) and the *vest* of the *ventromedial neuropils* (*VMNP*). The boundary with the *vest* is determined by the plane extrapolated inferiorly from the corresponding end of the *inferior fiber system* (Fig. S11D).

5-5 Posterior optic tubercle (POTU)

In species with a prominent *POTU*, it can be identified by labeling large glomerulus-like arborizations emerging from the *posterior optic commissure* as well as by tracing tangential neurons of the *PB*.

Boundaries in *Drosophila*

The *POTU* is not clearly visible in *Drosophila*.

6. Lateral horn (LH)

The *LH* can be identified by mass innervation from *AL*-projection neurons via several *antennal lobe tracts* (*ALTs*).

Boundaries in *Drosophila*

anterior **lateral:** the boundary starts at the lateral protrusion of the neuropil surface. It is directly posterior to the root of the *anterior SLP fascicle* (Fig. S12C, D). The external boundary is also flanked by the *anterior optic tract*.

medial: flanked by the *SLP and PVLP*. The boundary is visible with high-intensity labeling with elav>n-syb-GFP but not clear with nc82. The boundary with the *SLP* corresponds to the plane interpolating the external indentation and the trajectory of the *pyriform fascicle.* The boundary with the *PVLP* is defined by the *posterior lateral fascicle*. The crossing point between the *posterior lateral fascicle* and *mediolateral antennal lobe tract* demarcates the anterior inferior medial edge of the *lateral horn.*

posterior **lateral part:** facing the posterior cell body rind.

medial part: extending almost to the posterior end of the neuropil surface. However, a small and slender region of the *SLP* neuropil extends along the posteriormost surface behind the *lateral horn* and *medial antennal lobe tract* to connect to the *PLP* (Fig. S12D).

superior Flanked by the *SLP*. Externally, a small indentation on the superior neuropil surface demarcates the boundary with the *SLP*. Internally, this boundary roughly corresponds to the plane extrapolated from the *pyriform fascicle* (*PYF*).

inferior Flanked by the *PVLP* and *PLP*. A small indentation on the lateral neuropil surface demarcates the boundary. The internal boundary corresponds to the plane interpolating this indentation and the intersection point of the *posterior lateral fascicle* and *mediolateral antennal lobe tract*.

lateral Facing the superior lateral cell body rind.

medial Flanked by the *superior clamp.* The *posterior lateral fascicle* demarcates the boundary.

7. Superior neuropils (SNP)

7-1 Superior lateral protocerebrum (SLP)

The *SLP* can be identified as that part of the superior-most neuropils that is more lateral to the center of the *MB calyx* and flanked by the *AOTU* and *LH.*

Boundaries in *Drosophila*

anterior Flanked by the *anterior optic tubercle.* Separated by fiber streams and glial processes.

posterior **lateral and superior parts:** facing the posterior cell body rind.

inferior medial part: flanked by the *MB calyx*, separated by glial processes. The *accessory calyx* of the *MB* invades the posterior region of the *SLP* (Fig. S9B, S12B), the boundary between *accessory calyx* and SLP is not visible with nc82.

In its posterior medial edge, the distal tip of the *antler* (*ATL*) is connected with the inferiormost region of the *SLP* (Fig. S11G).

lateral Facing the superior lateral cell body rind.

medial **anterior part:** flanked by the *SIP*, slightly lateral to the *MB vertical lobe*. In the superior part, the boundary corresponds to the small indentation on the neuropil surface. The boundary is also defined so that the *SIP* includes the entire part of the ring neuropil, which surrounds the tip of the *MB vertical lobes*.

posterior part: flanked by the SMP, but no glial boundary is observed between SLP and SMP. A virtual plane above the lateral edge of the *superior fiber system* (*SFS*), slightly medial to the medial surface of the *MB calyx* and beneath the indentation on the posterior neuropil surface, is taken as a practical landmark of the boundary. This plane also corresponds to the level of the superior lateral tip of the *antler* (S12B).

superior Facing the superior cell body rind.

inferior **medial part:** flanked by the *superior clamp.* Demarcated by the plane interpolating the inferior surface of the *superior fiber system* and the trajectory of the *posterior lateral fascicle*, *anterior SLP fascicle*, and *medial antennal lobe tract*.

lateral part: flanked by the *AVLP*, *PVLP, lateral horn,* and *PLP*. The *anterior optic tract* lies between *SLP* and *AVLP*. The *anterior SLP fascicle* demarcates the boundary with the *PVLP*. The boundary with the *lateral horn* corresponds to the small indentation on the superior neuropil surface and the plane extrapolated from the *pyriform fascicle*.

In the posteriormost region, the *SLP* extends as a thin region behind the *medial antennal lobe tract* and touches the *PLP* at the level of the inferior surface of the *medial antennal lobe tract* (Fig. S11G).

***anterior SLP* and *posterior SLP*:** A frontal plane extrapolated from the boundary between the *PVLP* and *PLP*, which corresponds to the posterior surface of the *great commissure*, can be used as a practical boundary between the *anterior* and the *posterior SLP* (*ASLP* and *PSLP*).

7-2 Superior intermediate protocerebrum (SIP)

The *SIP* can be identified as the region around and posterior to the *MB vertical lobe*, and which features many arborizations of the *MB* extrinsic neurons associated with the *vertical lobe*.

Boundaries in *Drosophila*

anterior **superior part:** facing the anterior cell body rind.

inferior part: flanked by the *anterior optic tubercle,* with thick glial processes in between.

posterior **superior part:** flanked by the *SMP*. The level above the anterior and lateral edges of the *superior fiber system* corresponds to the boundary.

inferior part: flanked by the *superior fiber system*.

lateral Flanked by the *SLP*, at the level slightly lateral to the *MB vertical lobe*.

superior part: the boundary corresponds to the small indentation on the neuropil surface. The boundary is defined so that the *SIP* includes the entire part of the *ring neuropil*, which surrounds the tip of the *MB vertical lobes.*

inferior part: the boundary corresponds to the trajectory of the *anterior SLP fascicle.*

medial Flanked by the *SMP*. The boundary is demarcated by the plane interpolating the medial limit of the *ring neuropil* around the *MB vertical lobe* and the center of the *superior fiber system*.

superior Facing the superior cell body rind.

inferior Flanked by the *superior clamp*. The boundary is demarcated by the plane interpolating the *posterior lateral fascicle* and the superior lateral edge of the *bulb*.

7-3 Superior medial protocerebrum (SMP)

The *SMP* can be identified as that part of the superior-most neuropils more medial to the center of the *MB calyx* and overlying the *central complex.*

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior Facing the posterior cell body rind.

lateral **anterior part:** flanked by the *SIP.* The boundary is demarcated by the plane interpolating the medial limit of the *ring neuropil* in the *SIP* around the *MB vertical lobe* and the center of the *superior fiber system*.

posterior part, superior half: flanked by the *SLP*, but no glial boundary is observed between *SLP* and *SMP*. A virtual plane above the *superior fiber system*, slightly medial to the medial surface of the *MB calyx* and beneath the indentation on the posterior neuropil surface, is taken as the boundary. This plane also corresponds to the level of the superior lateral tip of the *antler*.

posterior part, inferior half: flanked by the *superior clamp*. The plane interpolating the *superior fiber system* and the lateral edge of *fan-shaped body* demarcates the boundary.

medial Extends up to the midline.

superior Facing the superior cell body rind.

inferior **anterior part:** flanked by the *crepine* (*CRE*). The boundary is defined so that most of the arborizations of the identified MB extrinsic neurons associated with the *MB medial lobe* are included in the *crepine.* It roughly corresponds to the mid-line between the center of the *MB medial lobe* and the superior neuropil surface.

middle-posterior part: flanked by the *central complex*. The *supra ellipsoid commissure* (*SEC*) and the *superior arch commissure* (*SAC*) lie in between.

posteriormost part: flanked by the *antler* and the *medial antennal lobe tract*. The *antler* is protruded posteriorly and faces the cell body rind.

***anterior SMP* and *posterior SMP*:** The level of the superior apex of the *fan-shaped body* can be used for a practical boundary landmark between the *anterior* and *posterior SMP* (*ASMP* and *PSMP*).

8. Inferior neuropils (INP)

8-1 Crepine (CRE)

The *CRE* can be identified as the region around the *MB medial lobe*, and which features many arborizations of the *MB* extrinsic neurons associated with the *medial lobe*.

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior **superior part:** flanked by the *SMP*. It extends up to the level of the posterior surface of the *MB vertical lobe*.

inferior part: flanked by the *bulb*. Characteristic glomerular structure of the *bulb* demarcates the boundary.

superior Flanked by the *SMP*. The boundary is defined so that most of the arborizations of the identified MB extrinsic neurons associated with the *MB medial lobe* are included in the *crepine.* It roughly corresponds to the level of the centerline (i.e. 50% height) between the superior neuropil surface and the center of the *MB medial lobe*. Below this level (in *crepine*), many neuronal fibers run vertically. Above this level (in *SMP*), many neuronal fibers run horizontally. This region, rich with vertical fibers, extends superiorly towards the *MB vertical lobe*. Because of this, the superior boundary of the *crepine* is slanted superiorly in its lateral region.

inferior **anterior part:** flanked by the *antennal lobe*, separated by glial processes.

posterior part: flanked by the *LAL* and *bulb*. Neuronal fibers in the *crepine* surround the *MB medial lobe*. In the *crepine* below the *MB medial lobe,* fibers tend to run antero-posteriorly. In the *LAL,* fibers run medio-laterally. This level roughly corresponds to the level of the posterior surface of the *MB pedunculus.* The boundary with the *bulb* is determined by the characteristic glomerular structure of the *bulb*.

lateral At the level of the medial surface of the *MB vertical lobe*. Posterior to the lobes, the *crepine* is flanked by the inferiormost part of the *SIP*. The boundary corresponds to the centerline of the *MB vertical lobe*.

medial Extends up to the midline.

8-2 Clamp (CL)

The *clamp* can be identified as that region between the *central body* (*EB* and *FB*) and the *MB pedunculus,* which includes the volume above and below the *pedunculus*, but excludes the anterior (n-ventral) region occupied by the *LAL* and *bulb*. The neuropil is divided into *superior* and *inferior* parts in the species in which the thin sheet-like stream of neuronal fibers deriving from the *superior ellipsoid commissure* (*SEC*) and/or *superior arch commissure* (*SAC*) project through the *clamp*.

8-2a Superior clamp (SCL)

Boundaries in *Drosophila*

anterior **lateral part:** flanked by the *SLP*. Demarcated by the plane interpolating the *anterior SLP fascicle* and the *posterior lateral fascicle.*

medial part: flanked by the *SIP*. Demarcated by the plane interpolating the *posterior lateral fascicle* and the superior lateral edge of the *bulb*.

posterior Flanked by the *medial antennal lobe tract*.

superior medial anterior part: flanked by the *SIP*. Demarcated by the plane interpolating the *posterior lateral fascicle* and the superior lateral edge of the *bulb*.

posterior part: flanked by the *SMP*. The plane interpolating the *superior fiber system* and the lateral edge of the *fan-shaped body* demarcates the boundary.

superior lateral: flanked by the *SLP*. Demarcated by the plane interpolating the inferior surface of the *superior fiber system* and the trajectory of the *posterior lateral fascicle* and *medial antennal lobe tract*.

inferior Flanked by the *MB pedunculus* and *inferior clamp*. Fibers of the *superior arch commissure* demarcate the boundary.

lateral Flanked by the *lateral horn.* The *posterior lateral fascicle* demarcates the boundary.

inferior lateral: flanked by the *PVLP* and *PLP*. The outer contour of the superiormost optic glomeruli of the *PVLP*/*VLP* demarcates the boundary. The boundary with the *PLP* roughly corresponds to the level of the superior surface of the *MB pedunculus*. (In the posteriormost region, the superior boundary of the *PLP* exceeds the level of the *MB pedunculus* and reaches the level of the inferior surface of the *medial antennal lobe tract* (Fig. S11G) to flank the SCL. This is because many visual neurons in the *PLP* run superiorly in this region.)

medial Flanked by the *fan-shaped body*, separated by glial processes.

8-2b Inferior clamp (ICL)

Boundaries in *Drosophila*

anterior **medial part:** flanked by the *LAL* and *bulb*. The boundary with the *LAL* is rather contiguous. The *mediolateral antennal lobe tract* runs along the posterior surface of the *LAL* shortly after it branches off from the *medial antennal lobe tract* (Fig. S13D, E). The boundary with the *bulb* is demarcated by its characteristic glomerular architecture.

lateral part: flanked by the *AVLP*. The plane interpolating the lateral surface of the *MB pedunculus* and the lateral edge of the *LAL* demarcates the boundary.

posterior Facing the posterior cell body rind. The lateral end of the *protocerebral bridge* is located posterior to its posterior surface, near the root of the *medial equatorial fascicle* (*MEF*).

superior Flanked by the *superior clamp*. Neuronal fibers spanning the superior surface of the *central complex* and *MB pedunculus* (extension of the *superior arch commissure*) demarcate the boundary.

inferior **anterior protruded part:** flanked by the *LAL,* separated by glial processes.

anterior part: flanked by the *epaulette* and *gorget* (*GOR*), at the level of the inferior edge of the *fan-shaped body*.

posterior part (posterior to the *great commissure*): flanked by the *superior posterior slope*. The plane interpolating the *medial equatorial fascicle* and the *lateral equatorial fascicle* (*LEF*) demarcates the boundary.

lateral **anterior part:** flanked by the *PVLP*. The contour of the most medial surface of the optic glomeruli demarcates the boundary.

posterior part: flanked by the *PLP*. The contour of the most medial surface of the optic glomeruli demarcates the boundary. In the most posterior part, it corresponds to the plane interpolating the *lateral equatorial fascicle* and the lateral surface of the *MB pedunculus*.

medial **anterior protruded part:** flanked by the *LAL* and *bulb*, separated by glial processes.

anterior part: flanked by the *fan-shaped body*, separated by the *medial antennal lobe tract*.

posterior part: flanked by the *inferior bridge*. The boundary is rather contiguous. It is demarcated by the plane interpolating the *medial equatorial fascicle* and *medial antennal lobe tract*.

8-3 Inferior bridge (IB)

The *inferior bridge* can be identified as a fused structure in the posterior brain, positioned posterior (n-dorsal) to the *central body* and near the *protocerebral bridge*, and containing only a few descending/ascending fibers.

Boundaries in *Drosophila*

anterior **lateral part:** flanked by the *gorget*. The boundary is demarcated by the plane interpolating the posterior surface of the *great commissure* and the branching point between the *medial antennal lobe tract* and *medial equatorial fascicle*, near the medial edge of the *lateral horn*.

medial part: flanked by the crisscrossing neuronal fibers posterior to the *noduli.*

posterior Facing the posterior cell body rind.

superior **lateral part:** the *medial antennal lobe tract* runs above it.

medial part: the root of the *antler* extends from the *inferior bridge.*

midline part: the *protocerebral bridge* of the *central complex* lies just above it.

inferior Flanked also by the *superior posterior slope*, demarcated by the *posterior PLP commissure* (*pPLPC*).

inferior lateral: flanked by the *superior posterior slope*. At the region inferior medial to the *medial equatorial fascicle*, a few glial processes extend from the region around the *great commissure* to the posterior surface of the brain. The plane interpolating the *medial equatorial fascicle* and these glial processes demarcate the boundary.

lateral Flanked by the posterior part of the *inferior clamp*. The boundary corresponds to the plane interpolating the *medial equatorial fascicle* and *medial antennal lobe tract*.

medial Contiguous with the midline

8-4 Antler (ATL)

The *antler* can be identified as a thin neuropil protruding from the *inferior bridge* towards the *SLP*. Depending on the arrangement of the surrounding structures such as the *protocerebral bridge* and *medial ALT,* in some species the *antler* may not have a distinct volume but appears as part of the *inferior bridge*.

Boundaries in *Drosophila*

anterior **inferior part:** flanked by the *fan-shaped body*, with distinct glial boundary in between.

superior part: flanked by the *SMP*. The *antler* is protruded posteriorly towards the cell body rind. The boundary between the *antler* and *SMP* corresponds to the level of the *superior PLP commissure*.

posterior Facing the posterior cell body rind.

superior lateral Continues to the inferior medial part of the *SMP* and *SLP*.

inferior lateral Extends above the lateral and superior surface of the *medial antennal lobe tract*.

inferior Continues to the *inferior bridge.*

medial Facing the posterior cell body rind.

9. Antennal lobe (AL)

The *AL* can easily be identified by the glomerular architecture that is formed by the terminals of the olfactory sensory neurons as well as dendrites of the local and projection neurons.

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior Flanked by the *LAL* (medial part) *and vest* (lateral part), separated by thick glial processes.

superior Flanked by the *crepine*, separated by thick glial processes.

inferior **medial part:** flanked by the *prow* (*PRW*) and *flange* (*FLA*), separated by thick glial processes.

lateral part: flanked by the *GNG,* connected via the *antenno-subesophageal tract* (*AST*).

lateral Facing the anterior cell body rind.

medial Facing the anterior cell body rind on the midline.

10. Ventromedial neuropils (VMNP)

10-1 Ventral complex (VX)

10-1a Vest (VES)

The *vest* can be identified as the volume situated posterior (n-dorsal) to the *LAL*, ventral (n-posterior) to the *central body*, and anterior (n-ventral) to the level of the *great commissure*. In species where *cerebral* and *gnathal ganglia* (*CRG* and *GNG*) are separated, the *vest* is unlikely to extend as far along the esophageal foramen.

Boundaries in *Drosophila*

anterior **superior part:** flanked by the *antennal lobe* and *LAL*. The boundary with the *antennal lobe* is separated by glial processes. The boundary with the *LAL* is defined by the plane interpolating the *medial antennal lobe tract* and the superior medial surface of the *inferior fiber system*.

inferior part: flanked by the *flange*, separated by thin glial processes. Fibers of the *median bundle* (*MBDL*) project to the *flange* but not to the *vest*.

posterior **most superior part:** flanked by the *gorget*. Boundary corresponds to the anterior surface of the *great commissure.*

superior part: flanked by the *great commissure.*

middle part: flanked by the *superior posterior slope*. The boundary is rather contiguous; the posterior surface of the *inferior fiber system* and a slanted plane extrapolated from the trajectory of the *posterior cerebro-cervical fascicle* demarcate the boundary.

most inferior part: flanked by the *cantle* (*CAN*) of the *periesophageal neuropils* (*PENP*), demarcated by the glial sheaths around the *cantle.*

superior The *medial antennal lobe tract* runs above it.

inferior Flanked by the *saddle*, separated by glial processes. In the anteriormost part flanked by the *flange,* separated also by thin glial processes.

lateral Flanked mostly by the *inferior fiber system.*

most superior part: flanked by the *epaulette*. The *vest* and *epaulette* are separated in the posterior part by the *inferior fiber system*. In the anterior part they are separated by a fascicle connecting the *inferior fiber system* and the region lateral to the *fan-shaped body*.

most inferior part: flanked by the *wedge*. The plane interpolating the medialmost edge of the *inferior fiber system* and the *saddle* demarcates the boundary.

medial Flanked by the esophagus foramen and *median bundle.*

most superior part: lying on both sides of the *noduli*, but separated by thick glial processes and neuronal fibers.

10-1b Epaulette (EPA)

The *epaulette* can be identified as the volume between the dorsal *vest* and *VLP,* separated from the *vest* by the massive fiber streams of the *inferior fiber system*.

Boundaries in *Drosophila*

anterior Flanked by the *LAL*. The boundary is rather contiguous and is defined by the plane interpolating the *mediolateral antennal lobe tract* and the superior apex of the *inferior fiber system.* At its anterior lateral part, the medial end of the *horizontal VLP fascicle* (*hVLPF*) demarcates the boundary between the *LAL, inferior clamp,* and *epaulette* (Fig. S13E).

posterior Flanked by the *great commissure* and *gorget*. The boundary with the *gorget* corresponds to the anterior surface of the *great commissure*.

superior Flanked by the *inferior clamp,* at the level of the inferior edge of the *fan-shaped body*.

inferior Flanked by the *inferior fiber system*.

lateral Flanked by the *AVLP* and *PVLP*. The boundary corresponds to the plane interpolating the superior tip of the *inferior fiber system* and the lateral surface of the *MB pedunculus.*

medial Flanked by the *vest*. The *vest* and *epaulette* are separated at their anterior regions by a fascicle connecting the *inferior fiber system* and the region lateral to the *fan-shaped body*. In the posterior part they are separated by the *inferior fiber system* itself.

10-1c Gorget (GOR)

The *gorget* can be identified as a thin plate-like region protruded medially from below the *inferior clamp* to the region between the *great commissure* and the *central body*.

Boundaries in *Drosophila*

anterior **medial part:** flanked by the *vest*. The boundary corresponds to the anterior surface of the *great commissure.*

lateral part: flanked by the *epaulette*. The boundary corresponds to the anterior surface of the *great commissure*.

posterior Flanked by the *superior posterior slope and inferior bridge*. The boundary is demarcated by the plane interpolating the posterior surface of the *great commissure* and the branch point between *medial antennal lobe tract* and *medial equatorial fascicle*.

superior Flanked by the *medial antennal lobe tract*, *medial equatorial fascicle*, *inferior clamp*, and the *fan-shaped body*. Thick glial processes demarcate them except for the *inferior clamp.* Higher synaptic density (visualized with elav>n-syb-GFP but not with nc82) and intense projections from the inferior region of the brain (visualized with elav>DsRed and elav>GFP) in the *gorget* demarcates its boundary with the *inferior clamp.* This corresponds to the plane interpolating the *medial equatorial fascicle* and *lateral equatorial fascicle.*

inferior Flanked by the *great commissure.*

lateral Flanked by the *lateral equatorial fascicle*.

medial Flanked by the *noduli* and the crisscrossing neuronal fibers that occupy the region posterior to the *noduli*.

10-2 Posterior slope (PS)

The *posterior slope* can be identified as the region posterior (n-dorsal) to the level of the *great commissure*, which contains many ascending / descending fibers. The region is practically divided into superior and inferior parts at the level of the *wedge commissure* and *posterior optic commissure.*

10-2a Superior posterior slope (SPS)

Boundaries in *Drosophila*

anterior **superior part:** flanked by the *gorget* and *great commissure*. The boundary with the *gorget* is demarcated by the plane interpolating the posterior surface of the *great commissure* and the branching point between the *medial antennal lobe tract* and *medial equatorial fascicle*.

inferior part: flanked by the *inferior fiber system, vest,* and *wedge*. The boundary with the *vest* is rather contiguous, demarcated by the posterior surface of the *inferior fiber system* and a slanted plane extrapolated from the trajectory of the *posterior cerebro-cervical fascicle*. At the lateral region, the boundary with the *wedge* corresponds to the level of the *wedge commissure* (*WEDC*), while at the medial region, the trajectory of the *posterior cerebro-cervical fascicle* and its extrapolated plane demarcate the boundary.

posterior Facing the posterior cell body rind.

superior **medial part:** flanked by the *inferior clamp* and *inferior bridge*. The boundary with the *inferior clamp* corresponds to the plane interpolating the *medial equatorial fascicle* and *lateral equatorial fascicle.* The boundary with the *inferior bridge* corresponds to the plane interpolating the *medial equatorial fascicle* and a few glial processes that extend from the region around the *great commissure* to the posterior surface of the brain.

lateral part: flanked by the *PLP*. The boundary corresponds to the contour of the inferiormost glomerular structure of the *PLP*.

inferior **anterior part:** flanked by the *cantle*, separated by glial processes.

posterior part: flanked by the *inferior posterior slope*. The boundary corresponds to the plane interpolating the *wedge commissure* and *posterior optic commissure*.

lateral Flanked by the *PLP*. Contour of the medialmost glomeruli in the *PLP* demarcates the boundary.

In the posteriormost region, the lateral part of the *superior posterior slope* is extended to the region between the inferiormost glomerular structure of the *PLP* and the *posterior optic commissure*, so that the lateral boundary of the *superior posterior slope* reaches the lateral surface of the brain.

medial Separated on the midline by thick glial processes and connecting fibers above the esophagus foramen.

10-2b Inferior posterior slope (IPS)

Boundaries in *Drosophila*

anterior Flanked by the *wedge*.

lateral part: the boundary corresponds to the level of the *wedge commissure*.

medial part: the boundary corresponds to the trajectory of the *posterior cerebro-cervical fascicle* and its extrapolated plane.

posterior Facing the posterior cell body rind.

superior Flanked by the *superior posterior slope*. The boundary corresponds to the plane interpolating the *wedge commissure* and *posterior optic commissure*.

inferior Flanked by the *GNG*. The boundary corresponds to the inferior surface of the esophagus foramen.

lateral **anterior part:** flanked by the *wedge*.

posterior part: facing the lateral cell body rind.

medial Flanked by the esophagus foramen.

11. Periesophageal neuropils (PENP)

11-1 Saddle (SAD)

The *saddle* can be identified as the volume surrounding the *AMMC*. In species with fused *CRG* and *GNG*, the *saddle* is likely to lie ventrally to the *esophageal foramen,* separated from the underlying *GNG* by a glial sheath. In species with separated *CRG* and *GNG*, the *saddle* may lie above or around the *esophageal foramen,*

Boundaries in *Drosophila*

anterior **lateral part:** extends towards the root of the *antennal nerve* along the axons of the antennal mechanosensory neurons.

medial part: flanked by the *flange* and the anterior superior part of the *GNG,* without a clear glial boundary. The boundary with the *GNG* corresponds to the plane just posterior to the *antenno-subeso- phageal tract*. The boundary with the *flange* corresponds to the plane posterior to the thin glial processes that separate the *flange* and *vest*.

posterior Flanked by the posterior part of the *GNG*. The boundary is determined so that the *saddle* houses all the terminals of the *AMMC*. It roughly corresponds to the plane extrapolated from the posterior surface of the *cantle*.

superior **lateral part:** flanked by the *AVLP* and *wedge.* Separated from the *AVLP* by glial processes. The boundary with the *wedge* is determined by the superior contour of the *AMMC*. An indentation in the lateral neuropil surface demarcates the boundary externally.

medial part: flanked by the *flange*, *vest*, and *cantle*. The boundary with the *flange* is rather contiguous. It corresponds to the level posterior to the middle point of the *posterior maxillary sensory center* (*PMS*) in the *GNG*, which is labeled strongly with synaptic markers such as elav>n-syb-GFP and nc82. More laterally, the trajectory of the *anterior cerebro-cervical fascicle* (*aCCF*) also demarcates the boundary. The boundary with the *vest* is separated by glial processes. The boundary with the *cantle* corresponds to the superior surface of the *AMMC* and the inferior surface of the *vest*.

inferior Flanked by the middle and posterior parts of the *GNG*.

anterior part: somewhat contiguous with the *GNG*. A plane just superior to the loose commissural fibers within the *GNG* is recruited as the boundary, which roughly corresponds to the level of the inferior limit of the *posterior maxillary sensory center*.

posterior part: clearly separated from the *GNG* by a thick glial sheath. Externally, it corresponds to the indentation on the lateral surface of the neuropil, along which runs the *lateral cerebro-cervical fascicle* (*lCCF*).

lateral **superior part:** flanked by the *AVLP* and *wedge*. Separated from the *AVLP* by glial processes. The boundary with the *wedge* is determined by the superior contour of the *AMMC*. An indentation in the lateral neuropil surface demarcates the boundary externally.

inferior part: facing the lateral cell body rind. The *lateral cerebro-cervical fascicle* runs along its boundary.

medial Contiguous on the midline.

Antennal mechanosensory and motor center (AMMC)

The *AMMC* is determined by the terminals of the mechanosensory neurons deriving from the *antennal nerve*, with complex branched shape. The structure is recognizable with slightly intensive labeling with elav>n-syb-GFP, but nc82 antibody does not clearly demarcate the boundaries.

11-2 Flange (FLA)

The *flange* can be identified as a thin volume that houses the terminal / dendritic arborizations of *median bundle* neurons. Whether it lies above or below the level of the esophagus may vary depending on the arrangement of the *CRG* and *GNG*.

Boundaries in *Drosophila*

anterior Flanked by the *prow*. The boundary corresponds to the level of the *posterior maxillary sensory center* of the *GNG*, which is labeled strongly with synaptic markers such as elav>n-syb-GFP and nc82.

posterior Flanked by the *vest* and *saddle*. Separated from the *vest* by thin glial processes. The boundary with the *saddle* corresponds to the plane posterior to the thin glial processes that separate the *flange* from the *vest*.

superior Extends along both sides of the midline almost up to the superiormost level of the esophagus foramen. A thick glial wall demarcates the boundary with the *antennal lobe*.

inferior Flanked by the *GNG* and *saddle*. The boundary with the *GNG* corresponds to the superior edge of the *posterior maxillary sensory center* of the *GNG*. The boundary with the *saddle* is rather contiguous; it corresponds to the level posterior to the middle point of the *posterior maxillary sensory center* of the *GNG*. More laterally, the trajectory of the *anterior cerebro-cervical fascicle* also demarcates the boundary.

lateral Flanked by the *vest*. Separated by thin glial processes.

medial **superior part:** flanked by the esophagus foramen.
lateral part: contiguous with the midline.

11-3 Cantle (CAN)

The *cantle* can be identified as a small volume demarcated by glial boundaries at the posterior end of the *saddle*. It may not be apparent in the species with separated *CRG* and *GNG*.

Boundaries in *Drosophila*

anterior Flanked by the *vest* separated by glial processes.

posterior Flanked by the *superior posterior slope*, separated by glial processes.

superior Flanked by the superior posterior part of the *vest*, separated by glial processes.

inferior Flanked by the *saddle* and posterior *GNG*. Contiguous with the *saddle*. The boundary is demarcated as the superior surface of the *AMMC* and the inferior surface of the *vest*. Separated from the *GNG* by glial processes.

lateral Flanked by the *vest* and *posterior cerebro-cervical fascicle*, separated by glial processes.

medial Facing the esophagus foramen.

11-4 Prow (PRW)

The *prow* can be identified as a region of the supraesophageal neuromeres extending from the *flange* towards the anteriormost tip of the brain volume below the esophagus (*subesophageal zone, SEZ*). Theoretically it would not exist in species with clearly separated *CRG* and *GNG*.

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind below the level of the esophagus foramen.

posterior Flanked by the *flange*. The boundary corresponds to the level of the *posterior maxillary sensory center* of the *GNG*.

superior Facing the anterior cell body rind below the opening point of the esophagus foramen.

inferior Flanked by the *GNG*.
anterior part: the boundary, which should be between the *superior* and *inferior pharyngeal sensory centers* (*SPhS* and *IPhS*), corresponds to the plane superior to the entering point of the *pharyngeal nerve* to the *GNG*.

posterior part: the boundary is superior to the *posterior maxillary sensory center* in the *GNG*.

lateral **anterior part:** facing the lateral cell body rind.

posterior part: flanked by the *GNG*. The boundary is medial to the trajectory of the *antenno-subesophageal tract*.

medial Facing the esophagus foramen.

12. Gnathal ganglia (GNG)

The GNG are identified as the region of the subesophageal zone that contains pharyngeal, maxillary, and labial sensory and motor centers (terminal arborizations of respective nerves, which can be visualized as dense synaptic labeling) but does not contain the AMMC, which comprises the terminals of the deutocerebral antennal nerve.

Boundaries in *Drosophila*

anterior Facing the anterior cell body rind.

posterior Connected to the thoracic abdominal ganglia via the *cervical connective*.

boundary with the *saddle* Because the *saddle* is partially embedded in the superior part of the *GNG*, the anterior superior and posterior superior regions of the *GNG* are flanked by the *saddle* posteriorly and anteriorly, respectively. The boundary is determined so that the *saddle* contains all the terminals of the *AMMC*. The boundary of the former corresponds to the plane just posterior to the *antenno-subesophageal tract*, and the latter boundary corresponds to the plane extrapolated from the posterior surface of the *cantle*.

superior Flanked by the *antennal lobe, prow, flange, saddle, wedge, cantle* and *inferior posterior slope*. Glial boundaries separate the *GNG* from the *antennal lobe, cantle*, and the posterior part of the *saddle*. Other boundaries are more contiguous.

Boundary with the *antennal lobe*: Connected via the *antenno-subesophageal tract*.

Boundary with the *prow*:

anterior part: the boundary lies between the *dorsal* and *ventral pharyngeal sensory centers*, corresponding to the plane superior to the point of entry of the *pharyngeal nerve* to the *GNG*.

posterior part: the boundary corresponds to the superior edge of the *posterior maxillary sensory center*.

Boundary with the *flange*: The boundary corresponds to the superior edge of the *posterior maxillary sensory center*.

Boundary with the *saddle*:

anterior part: rather contiguous. A plane just superior to the loose commissural fibers within the *GNG* is recruited as the boundary, which roughly corresponds to the level of the inferior limit of the *posterior maxillary sensory center*.

posterior part: clearly separated from the *GNG* by a thick glial sheath. Externally, it corresponds to the indentation on the lateral surface of the neuropil, along which runs the *lateral cerebro-cervical fascicle*.

Boundary with the *wedge*: The plane interpolating the lateral edge of the *AMMC* and the external indentation of the neuropil surface demarcates the boundary.

Boundary with the *cantle*: Separated by glial processes.

Boundary with the *inferior posterior slope*: The boundary corresponds to the inferior surface of the esophagus foramen.

inferior Facing the cell body rind.

lateral Facing the cell body rind.

medial Contiguous with the midline.