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A Systematic Nomenclature for the Insect Brain

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SUMMARY

Despite the importance of the insect nervous system for functional and developmental neuroscience, descriptions of insect brains have suffered from a lack of uniform nomenclature. Ambiguous definitions of brain regions and fiber bundles have contributed to the variation of names used to describe the same structure. The lack of clearly determined neuropil boundaries has made it difficult to document precise locations of neuronal projections for connectomics study. To address such issues, a consortium of neurobiologists studying arthropod brains, the Insect Brain Name Working Group, has established the present hierarchical nomenclature system, using the brain of *Drosophila melanogaster* as the reference framework, while taking the brains of other taxa into careful consideration for maximum consistency and expandability. The following summarizes the consortium's nomenclature system and highlights examples of existing ambiguities and remedies for them. This nomenclature is intended to serve as a standard of reference for the study of the brain of *Drosophila* and other insects.

INTRODUCTION

Thanks to their size, their relevance to medicine and agriculture, and their use for the development of novel molecular genetic techniques, insect brains have provided important insights in neuroscience research (Burne et al., 2011). Like those of vertebrates, the brains of insects consist of various regions that synergistically cooperate to achieve computational tasks. These regions are given names that facilitate descriptions of neural circuits, the projections of specific neurons, and the distribution of molecules associated with neural functions. Existing nomenclatures for describing insect brains have, however, suffered from the following crucial deficiencies: (1) comparable brain regions have been given different names depending on the species and the researcher studying them, (2) the same terms and words have been used to refer to different structures, (3) the boundaries of many brain regions have not been defined clearly, and (4) some volumes of the brain have no established names and thus, by definition, have no defined boundaries.

As in other sciences, neuroscience requires systematic and consistent nomenclature. Descriptions of brain and central nervous system are no exception, and many demand cartographic discipline. Linguistic inconsistencies detract from published studies or, worse, provide descriptions that are intelligible to only a few. Historically, insect neuroscience has focused on just a few well-known brain regions, such as those loosely defined as the "optic lobes," "antennal lobes," "mushroom bodies," and "central complex." However, comprehensive analyses of the development and connections of what are now recognized as elaborate and complex brains assume greater importance as whole-brain network analyses and behavioral genetics promise to link organization to function (Cachero et al., 2010; Yu et al., 2010; Chiang et al., 2011; Ito et al., 2013; Yu et al., 2013). A systematic and consistent nomenclature that can be applied across insect brains in general is a prerequisite for such studies. Researchers of avian brains have faced similar problems and have solved them by agreeing to a unified

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nomenclature (Jarvis et al., 2005). The present paper describes the results of a comparable nomenclature effort by a broad representation of neuroscientists working on the central nervous systems of insects and related arthropods.

RESULTS

Organization of the Working Group

In 2007, requests from two independent bodies associated with the Howard Hughes Medical Institute (HHMI) and the National Institutes of Health (NIH) resulted in the formation of a working group that discussed proposals for a systematic nomenclature of the insect brain. The Insect Neuroanatomy Meeting, held in March 2007 at the HHMI Janelia Farm Research Campus in Virginia, USA, brought together insect neuroscientists to discuss ongoing studies and problems in mapping neurons and their networks. This meeting acknowledged that a systematic nomenclature was urgently required, and an initial working group of seven individuals was formed. In the same year, the NIH Neuroscience Blueprint for Neuroscience Research, headed by Daniel Gardner and coordinated with the Society for Neuroscience Neuroinformatics Committee, invited a group of several neurobiologists working on the *Drosophila* brain to discuss a blueprint for a systematic nomenclature.

These two groups subsequently merged to form the Insect Brain Name Working Group. However, because its members mostly worked on the *Drosophila* nervous system, invitations to join the working group were extended to scientists studying the brains of other insects (e.g., locust, cockroach, moth, and honey bee) as well as crustaceans, the sister group of insects. The aim of the working group was expanded to evolve a nomenclature that could be used across arthropod species. The fly *Drosophila melanogaster* is the most commonly used species in insect neurobiology and has an acknowledged impact in neuroscience research in the broadest sense. It is a proving ground for developing a range of genetics-based techniques for structural and functional analyses, and it serves as a test bed for the development of methods and algorithms for brain connectomics. For these reasons, the group agreed to use the brain of *Drosophila melanogaster* as the basis taxon for developing a nomenclature system. However, because it was recognized that the brains of different insect species are a consequence of more than 400 million years of divergent evolution, other taxa had to be taken into consideration if the nomenclature would apply across Insecta and reflect, as far as possible, a ground pattern organization that is common to all.

Due to the working group's geographical dispersion, discussions were carried out via an online mailing list initiated in early 2007. In addition to the NIH Neuroscience Blueprint meeting that was held in New York in 2008, the group twice held 2-day face-to-face meetings in 2008 and 2010 at the Janelia Farm Research Campus. Online discussions throughout the process further fine-tuned the present nomenclature. During the entire time course of this project, the developing nomenclature was presented at international meetings attended by a broad representation of arthropod neuroscientists. This enabled opinions and comments from the wider community. Such events included the Janelia Farm Insect Neuroanatomy Meeting in 2008; the

Janelia Farm Meetings held between 2008 and 2011 on the mushroom body, visual system, brain evolution, and central complex; the Neurofly European *Drosophila* neurobiology meeting in 2008 (Würzburg) and in 2010 (Manchester); the Gordon Research Conference of Neuroethology in 2008 (Oxford); the European Symposium for Insect Taste and Olfaction (ESITO) in 2009 (Sardinia) and in 2011 (St. Petersburg); the Cold Spring Harbor Laboratory (CSHL) Banbury Conference on Evolution in 2009, Neuronal Circuits Meeting in 2010, and Neurobiology of *Drosophila* Course in 2012 (Cold Spring Harbor); the Japan *Drosophila* Research Conference in 2009 (Kakegawa) and Molecular Ethology Workshop in 2010 (Tokyo); the EMBO/ESF Minibrain Symposium in 2010 (Sant Feliu de Guixols); the EvoDevo Meeting in 2010 (Paris); the Max Planck Institute's Neuro-Evo Meeting in 2010 (Jena), the German Science Foundation's (DFG) focus working group meeting on "Metazoan Deep Phylogeny" in 2010 (Munich); the NCBS Maggot Meeting in 2010 (Bangalore); Fly Group Meetings at Edinburgh, London, Leicester, and Cambridge in 2011; the Cold Spring Harbor Asia Arthropod Neuroscience Meeting in 2012 (Suzhou); the SICSA/INCF Workshop on Atlas Informatics in 2012 (Madison); and the Nervous System of *Drosophila melanogaster* conference in 2013 (Freiburg). Throughout, the proposed nomenclature received strong support from the research community and benefited from many constructive suggestions that were incorporated into the nomenclature proposal.

Preparation of Brain Samples as a Framework

Establishment of a systematic nomenclature requires two steps: (1) a clear definition of the objects to be named and (2) finding the most appropriate names for them. To develop a nomenclature for the insect brain, its synapse-rich neuropils—where neuronal processes contact and form synaptic connections—needed to be distinguished as clearly defined volumes. However, except for a few neuropils, such as the antennal and optic lobes, mushroom bodies, and those of the central complex, many other brain regions have historically been referred to as "unstructured" or "diffuse," with different regions distinguished only by approximate cartographic terms such as, for example, "superior lateral" in the case of the protocerebrum (Strausfeld, 1976). Though boundaries between neuropil volumes had been provisionally established using neighbor-defining landmarks (Otsuna and Ito, 2006), those boundaries did not necessarily correspond to the organization of underlying neural architectures.

To establish distinct boundaries throughout the brain, we first prepared confocal as well as paraffin serial section images that visualize various aspects of neurons and glia. These provided an initial framework for analysis. Simultaneous panneuronal expression of cytoplasmic dsRed (Verkhusha et al., 2001), synaptic vesicle-targeted n-syb-GFP (Estes et al., 2000), and Rdl-type GABA-receptor-HA fusion protein (Sánchez-Soriano et al., 2005) driven by the *elav*-GAL4 driver (Lin and Goodman, 1994) were used to visualize neuronal fibers and the distribution of presynaptic and postsynaptic sites in the same brain sample (Figure 1A). Reduced-silver-stained paraffin sections (Blest, 1961) have traditionally been used for constructing brain atlases and were useful here for resolving the fibrous architecture (Figure 1B). The anti-Bruchpilot
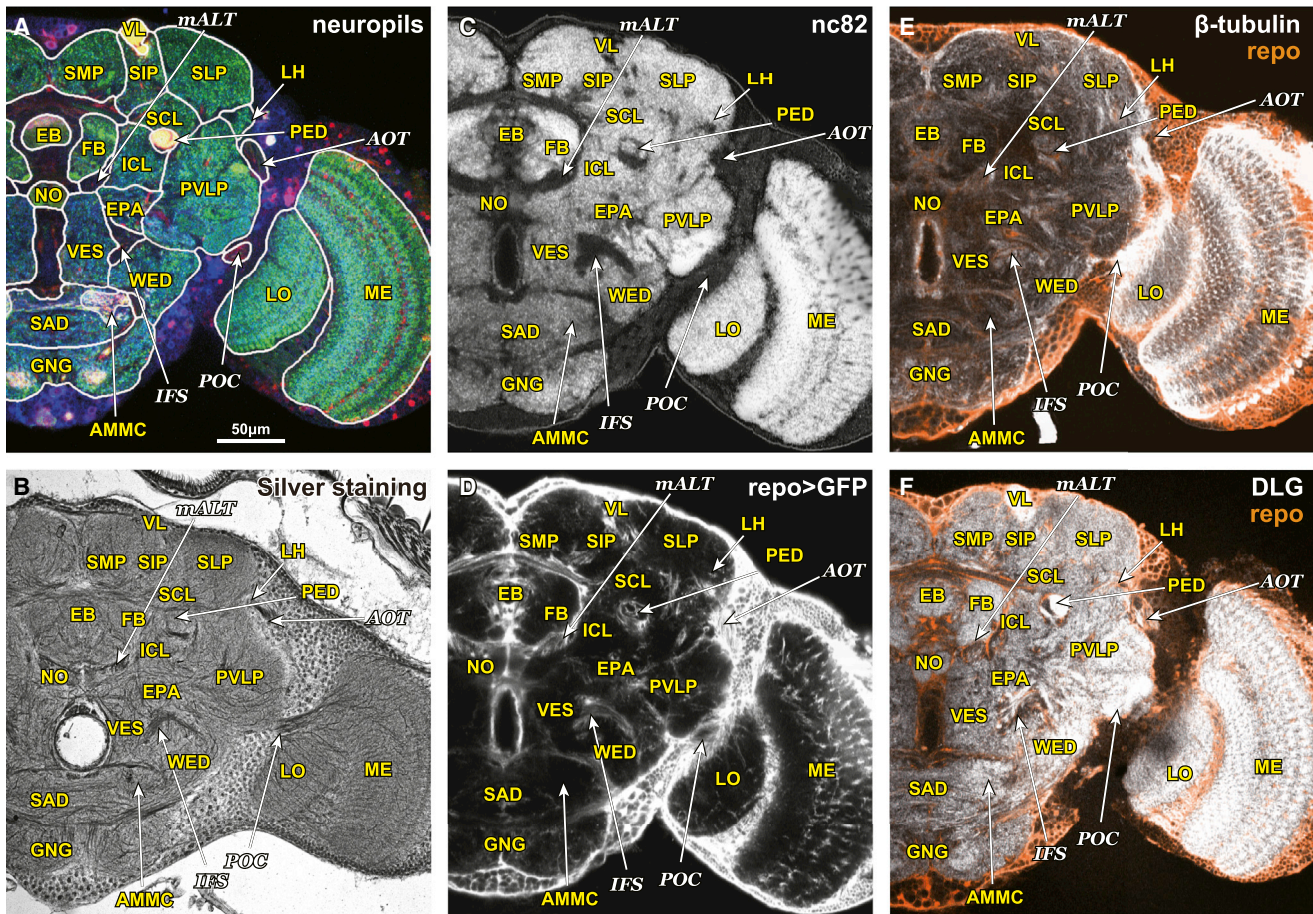
Figure 1. Sections of the Insect Brain Visualized with Various Markers

Frontal sections of the same region of the *Drosophila* brain are shown. Yellow and white characters denote the names of the synapse-rich neuropils and fiber bundles, respectively.

(A) Cytoplasmic dsRed (red), synaptic-vesicles-targeted n-syb-GFP (green), and GABA-receptor-targeted Rdl-HA (blue) expressed by the pan-neuronal *elav*-GAL4 expression driver, with outlines of neuropil boundaries superimposed.

(B) Silver stain (paraffin section) to visualize neural fibers.

(C) Anti-Bruchpilot nc82 antibody to visualize the density of synapses.

(D) Cytoplasmic GFP expressed by the pan-glial *repo*-GAL4 expression driver.

(E) Anti-β-tublin antibody to visualize fibrous structures of neurons and glia.

(F) Anti-Discs-large antibody to label membranes of neurons. (E) and (F) are superimposed with the *repo*-GAL4-driven GFP signal (orange) to visualize glial processes. Bar indicates 50 μm.

antibody (nc82), which visualizes neuropils according to the density of an active-zone-specific protein (Wagh et al., 2006), helped to distinguish synapse-rich neuropils, because regions occupied by neuronal axons, cell body fibers, and glial processes are left unlabeled (Figure 1C). GFP expressed by the pan-glial *repo*-GAL4 driver (Lai and Lee, 2006) visualizes glial cells and their processes, which form bounding sheaths surrounding many (but not all) neuropils (Figure 1D). Anti-β-tubulin antibody was used to visualize fibrous structures of both neurons and glial cells (Chu and Klymkowsky, 1989; Popodi et al., 2005) (Figure 1E), and anti-discs large (DLG) antibody was used to label membranes of neuronal cell bodies, neuronal fibers, and synapses by detecting DLG proteins required for septate junction structure (Parnas

et al., 2001) (Figure 1F). Serial section images obtained with these labeling methods are available via the Brain Explorer function of the Flybrain Neuron Database (http://ndb.flybrain.org).

To achieve maximum consistency with previous and ongoing studies, we took into account the known projection patterns of various types of neurons, including those of single identified neurons and the trajectories of their fiber bundles (e.g., Chiang et al., 2011; Crittenden et al., 1998; Fischbach and Dittrich, 1989; Hanesch et al., 1989; Otsuna and Ito, 2006; Shinomiya et al., 2011; Strausfeld, 1976; Stocker et al., 1990; Tanaka et al., 2008, 2012; Yang et al., 1995; Young and Armstrong, 2010; Yu et al., 2010), the distribution of glial processes (Awasaki et al., 2008; Younossi-Hartenstein et al., 2003), the projections of

Table 1. Summary of the Nomenclature System of the Insect Brain Structures

Synapse-rich neuropils

optic lobe	**OL**	*central complex*	**CX**	*antennal lobe*	**AL**
lamina	**LA**	central body	**CB**	AL glomeruli	GL
lamina dorsal rim area	LADRA	fan-shaped body	**FB**	AL hub	ALH
plexiform lamina	PLLA	upper division of CB in some species		*ventromedial neuropils*	*VMNP*
accessory lamina (*not in flies*)	**ALA**	ellipsoid Body	**EB**	ventral complex	**VX**
medulla	**ME**	lower division of CB in some species		vest	**VES**
medulla dorsal rim area	MEDRA	protocerebral bridge	**PB**	epaulette	**EPA**
plexiform medulla	PLME	noduli	**NO**	gorget	**GOR**
accessory medulla	**AME**	*lateral complex*	*LX*	posterior slope	**PS**
lobula complex	**LOX**	bulb	**BU**	superior posterior slope	**SPS**
lobula	**LO**	lateral accessory lobe	**LAL**	inferior posterior slope	**IPS**
lobula plate	**LOP**	*ventrolateral neuropils*	*VLNP*	*periesophageal neuropils*	*PENP*
mushroom body	*MB*	anterior optic tubercle	**AOTU**	saddle	**SAD**
calyx	**CA**	ventrolateral protocerebrum	**VLP**	antennal mechanosensory and	**AMMC**
accessory calyx	**ACA**	anterior VLP	**AVLP**	motor center	
pedunculus	**PED**	posterior VLP	**PVLP**	flange	**FLA**
pedunculus neck	PEDN	posteriorlateral protocerebrum	**PLP**	cantle	**CAN**
pedunculus divide	PEDD	wedge	**WED**	prow	**PRW**
spur	**SPU**	posterior optic tubercle (*not in flies*)	**POTU**	dorsal pharyngeal sensory center	DPS
vertical lobe	**VL**	*lateral horn*	*LH*	*gnathal ganglia*	*GNG*
α′ lobe (α′ division in some species)	α′L	*superior neuropils*	*SNP*	ventral pharyngeal sensory center	VPS
α lobe (α division in some species)	αL	superior lateral protocerebrum	**SLP**	anterior maxillary sensory center	AMS
α$_p$ lobe	α$_p$L	superior intermediate protocerebrum	**SIP**	posterior maxillary sensory center	PMS
vertical γ lobe (*not in flies*)	VγL	superior medial protocerebrum	**SMP**	labellar sensory center	LBS
medial lobe	**ML**	*inferior neuropils*	*INP*		
γ lobe (γ division in some species)	γL	crepine	**CRE**		
β′ lobe (β′ division in some species)	β′L	clamp	**CL**		
β lobe (β division in some species)	βL	superior clamp	**SCL**	**red:** level 1, supercategories	
β$_p$ lobe	β$_p$L	inferior clamp	**ICL**	**black:** level 2, neuropils	
Y tract (*not in flies*)	**YT**	inferior bridge	**IB**	blue: level 3, subregions	
Y lobe (*not in flies*)	**YL**	antler	**ATL**		

Landmark fiber bundles

AL-associated tracts		**other fascicles**		**commissures**	
medial antennal lobe tract	mALT	anterior optic tract	AOT	superior/inferior AL commissure	sALC, iALC
mediolateral antennal lobe tract	mlALT	pyriform fascicle	PYF	LAL commissure	LALC
lateral antennal lobe tract	lALT	posterior lateral fascicle	PLF	superior ellipsoid commissure	SEC
antenno-subesophageal tract	AST	anterior SLP fascicle	aSLPF	superior arch commissure	SAC
cerebro-cervical fascicles		horizontal VLP fascicle	hVLPF	superior PLP commissure	sPLPC
posterior cerebro-cervical fascicle	pCCF	vertical VLP fascicle	vVLPF	posterior PLP commissure	pPLPC
medial cerebro-cervical fascicle	mCCF	median bundle	MBDL	posterior optic commissure	POC
anterior cerebro-cervical fascicle	aCCF	medial equatorial fascicle	MEF	great commissure	GC
lateral cerebro-cervical fascicle	lCCF	lateral equatorial fascicle	LEF	superior AMMC commissure	sAMMCC
		fiber systems		WED commissure	WEDC
		superior fiber system	SFS		
		inferior fiber system	IFS		

Top panel: Hierarchical list of the synapse-rich neuropil names. Bottom panel: List of the landmark fiber bundle names. See Supplemental Information for detail.

clonally associated groups of neurons (Ito and Awasaki, 2008; Larsen et al., 2009; Ito et al., 2013, Yu et al., 2013), and developmental reorganization during the postembryonic period, notably during metamorphosis (Pereanu et al., 2010). Information about brain organization in other insect species was integrated wherever relevant (e.g., Boyan et al., 1993; Ehmer and Gronenberg, 2002; Farris and Sinakevitch, 2003; Flögel, 1876; Heinze and Reppert, 2012; Homberg, 2002; Mobbs, 1982; Rybak et al., 2010; Strausfeld et al., 2009; Strausfeld, 2012, Williams, 1975).

Identification and Drawing of Neuropil Boundaries

Different types of studies require different levels of spatial resolution. To satisfy diverse needs, we defined neuropils in a hierarchical manner (Table 1; Figures 2A and 2B). Large neuropil blocks (level 1 supercategories) and unit neuropils (level 2) are defined such that together they comprehensively partition the entire brain in a mutually exclusive fashion. Considering that synaptic markers like anti-Bruchpilot nc82 antibody are used as *de facto* standards to label neuropils, structures visible with these markers have been used to demarcate the level 1 and level 2 boundaries. In addition, wherever necessary, we have defined smaller substructures (level 3) within neuropils. Together, we defined 12 supercategories (level 1), 47 unit neuropils (level 2; 43 in case of *Drosophila*, because the other four are presumed absent in this species), and more than 40 subregions (level 3) with further subdivisions in some cases (see Supplemental Information for details).

Taking into account all the information obtained with various labeling methods, as well as previous studies reporting various
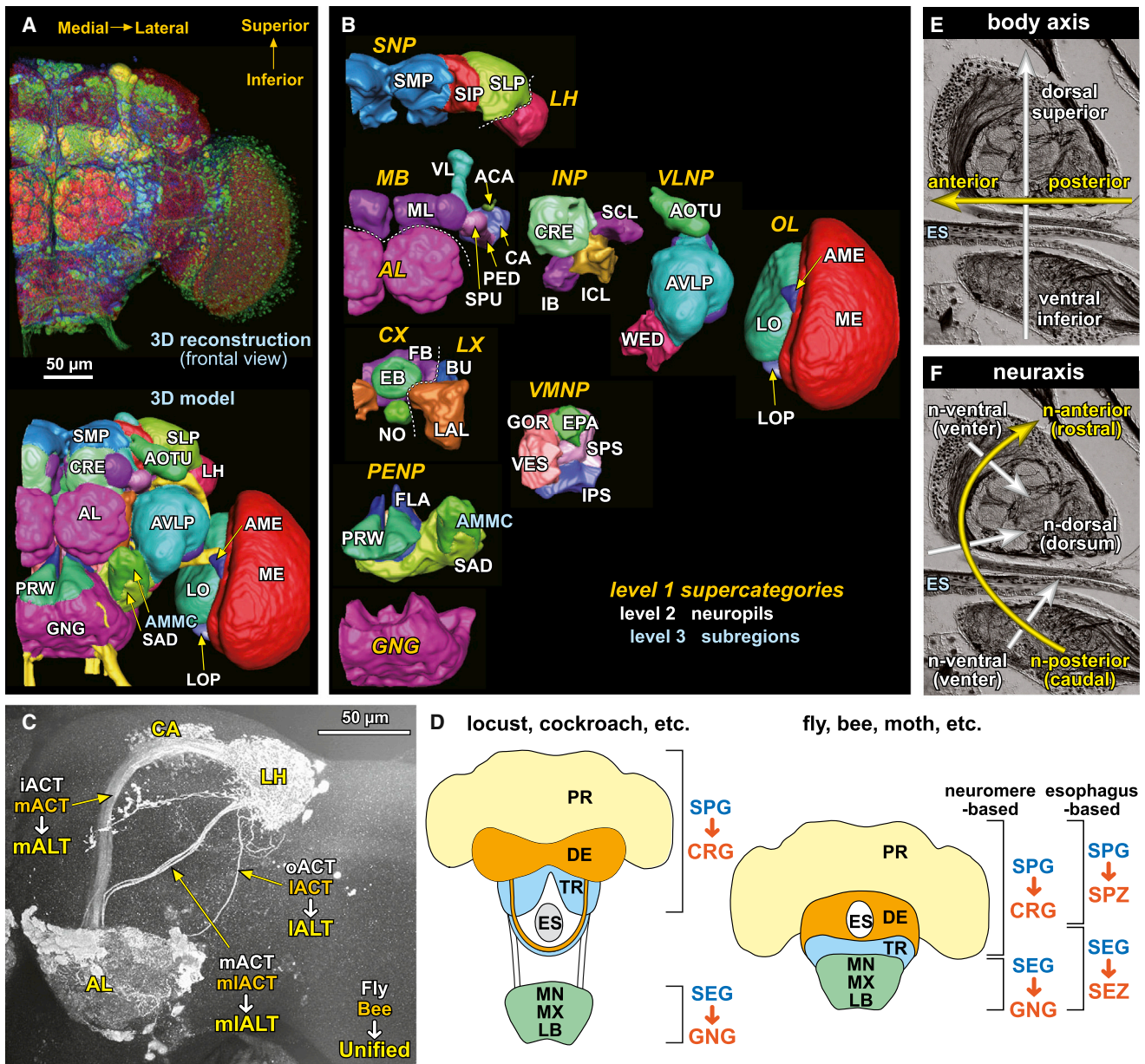
Figure 2. Summary of the Nomenclature System of the Insect Brain Structures

(A) Overview of the *Drosophila* brain. 3D reconstruction of the brain (top panel) and a 3D model with the defined synaptic neuropils (bottom panel).

(B) Hierarchical list of the defined synaptic neuropils. See Table 1 for abbreviations.

(C) Resolution of problematic nomenclature between species for the olfactory tract.

(D) Resolution of problematic nomenclature pertaining to brain ganglia. See text for explanation of abbreviations.

(E and F) Resolving confusing terms for the axis systems. See Supplemental Information for details. Bar indicates 50 μm.

types of neurons, identified neuropil boundaries were imposed onto serial section images of a representative brain labeled with *elav*-driven cytoplasmic dsRed, presynaptic sites-targeted n-syb-GFP, and postsynaptic sites-targeted Rdl-HA using the Amira software painting function. By comparing a pool of preparations of 5- to 10-day-old adult female brains, the most representative sample was selected. Images obtained from a single brain rather than averaged images from many brains were

used, because image averaging results in the loss of fine resolution. This tricolor-labeled series resolves all the landmarks required for demarcating level 1 and level 2 neuropils, because synaptic labeling of *elav*-driven n-syb-GFP closely mimics that of the nc82 antibody. Labeling of neuronal fibers and their presumed postsynaptic sites provided additional information for locating structural features suggested by other labeling methods.

Application of Boundaries to Other Specimens

To meet the diverse needs of researchers, the Supplementary Information demonstrate several ways for understanding and synthesizing information about neuropils: (1) the 84-page Supplemental Information provides detailed overviews of neuropils and landmark fiber bundles, together with how to name and abbreviate them, as well as discussion about conflicting terms and solutions; (2) serial section movies (Movies S1, S2, S3, and S4) provide dynamic images of synaptic labeling and maps of neuropils and fiber bundles; (3) Movies S5 and S6 provide interactive clickable 3D maps, with which users can visualize any combination of defined neuropils (with Movies S5 and S6) and fiber bundles (with Movie S5) from any desired angle. This assists understanding of the spatial relationships among neuropils. With these guidelines and tools, researchers will be able to locate neuropils in their own specimens of interest when these are counterlabeled with synaptic markers such as nc82.

In addition to these documents and movies, raw confocal image data files showing identified neuropils and underlying synaptic labeling are downloadable via FlyBase (http://flybase.org). They will be useful for spatially matching the provided neuropil images with the images of other brain samples using 3D registration software such as Computational Morphometry ToolKit (Jefferis et al., 2007) or BrainAligner (Peng et al., 2011). Researchers can either use the provided image file as a target template onto which their own image data would be morphed and registered or morph the provided image file to any target template used for their own particular study. For example, Virtual Fly Brain (http://www.virtualflybrain.org/), an annex project of FlyBase, has registered the images of over 10,000 single-neuron data by Chiang et al. (2011) onto the template neuropils described by us so that the projection patterns of these neurons can be understood in the framework of the current nomenclature. In addition, the official anatomy ontology and the existing anatomical literature index in FlyBase has been updated and reannotated with this new nomenclature via the Virtual Fly Brain project.

Assignments of Names to the Identified Structures

Arriving at a systematic nomenclature required extensive discussions about suitable names for neuropils. We have retained classic terminology whenever possible. But when several different names have been historically used to refer to an identical structure, or when a single name has historically been used to refer to different structures in different insect species, or by different researchers, the working group identified names that resolved such ambiguities (see below). Many regions of the insect brain had no established names at all. For those neuropils, the group had to devise names. For the sake of brevity and the convenience of electronic text searching, as well as for minimizing acronyms, we determined simple, unique names that are suggestive of the shapes or relative positions of neuropils. This follows established conventions for naming genes and mutants, as well as the history of naming vertebrate and invertebrate brain structures according to shapes, such as "Ammon's horn," "olive," "mushroom," "fan-shaped," etc. Crucially, names associated with neural functions are specifically avoided, because future studies are likely to reveal yet-unknown functions relating to neuropils. In addition, acknowledging that some names may be needed that reflect the relative positions of structures, we include lists of alternative position-based synonyms (see Supplemental Information).

Developmental and evolutionary studies agree that three segmental neuromeres compose the supraesophageal ganglia of insects (Hirth et al., 2003; Kumar et al., 2009). However, because the outgrowth of processes contributing to the adult brain obscures its embryonically defined segments, identifying neuromere boundaries in the mature brain is technically difficult. Considering the long history of debate on this issue, we have generally avoided neuromere-related terms. The resultant nomenclature system is neutral; it does not depend on the segmental nature of the arthropod brain.

Unlike in the vertebrate brain, most neuronal cell bodies are located in the cell body rind that encloses the brain's neuropil mass. The locality of a patch or cluster of neuronal cell bodies is most easily given by referring to the name of the next nearest (often adjacent) neuropils. Names describing the location of patches and clusters in the cell body rind are provided in Supplemental Information.

The insect brain also features abundant bundles of neuronal fibers (neurites) connecting different neuropils. We have defined and named the most prominent fiber bundles as well as those that form useful landmarks for determining the boundaries of synapse-rich neuropils. To make the terminology systematic, we defined "fascicles" and "tracts" as connecting two different brain regions ipsilaterally and "commissures" as connecting two regions contralaterally. Thus, a few fiber bundles historically referred to as "tracts" have been renamed as "commissures" when they connect neuropils in both brain hemispheres.

Establishment of Abbreviations

In publications, the names of brain structures are generally abbreviated. A systematic nomenclature would not serve well if abbreviations are not controlled. Toward this end, the working group established systematic abbreviations for all the provided nomenclature. As with naming genes, unique combinations of a few characters are preferred. Whenever possible, we kept existing abbreviations used in previous literature, but if a single abbreviation has been used to refer to more than one structure, we assigned a unique abbreviation for each structure to avoid ambiguity.

In addition, if we modified the definition of a neuropil so that it significantly departed from a previous description, we proposed an alternative abbreviation to distinguish the old and new terms. For example, the acronym "slpr" was once used to denote the superior lateral protocerebrum. Because its boundaries have been shifted in this documentation (see Supplemental Information for details), we employed the new abbreviation "SLP" to distinguish new definition from the previous one.

For the last character of any neuropil name, the letters C, T, and F are generally avoided because elsewhere in the document these refer to, respectively, "commissures," "tracts" and "fascicles."

Resolving Ambiguities of Terminology

A major task of the working group was to resolve ambiguities and confusion in existing terminology. We reviewed the names for

synapse-rich neuropils and fiber bundles used in previous literature and retained published terminology whenever possible so that past accounts do not lose their relevance with respect to present and future descriptions. However, there were over thirty instances where terms required clarification and revision. These are provided in Supplemental Information.

Different names that refer to an identical structure, or vice versa, cause immense confusion when discussing the results of past studies. In such cases, the working group decided on one name alone that was the least confusing and which best denoted the relevant structure. For example, the terms "ventral body," used in descriptions of dipteran brains, and "lateral accessory lobe," used in descriptions of the brains of locusts and moths, refer to homologous structures. Because the second term is used more often in studies suggesting possible roles of this neuropil, we excluded the first term and opted for "lateral accessory lobe." Likewise, two terms, "lateral horn" and "lateral protocerebrum," both refer to the same secondary olfactory center. However, because the latter term refers also to the lateral part of the protocerebrum in general, it was decided that only the first term would unambiguously refer to an olfactory center, irrespective of the species-specific shape of the protocerebrum.

The literature abounds with terms used without any clear definition. For example, the terms "central body" and "central complex" refer to combinations of neuropils in the central part of the brain without specifying which neuropils are, or should be, included. After examining the context in which these terms have been used in the past, we defined the former to mean the combination of the fan-shaped body and the ellipsoid body and the latter to mean the combination of these two neuropils and two closely interconnected regions: the protocerebral bridge and noduli. In addition, the bulb and the lateral accessory lobe, both neuropils closely associated with the central complex, are collectively defined as the "lateral complex."

In certain cases, a single name was used for different structures. To avoid confusion, it was decided to adopt entirely new terms for those structures, particularly if adopting just one of them would make comparisons of future accounts with already published ones difficult. For example, one of several ascending axon bundles from the antennal lobe was termed mACT, meaning the "middle" antennocerebral tract (ACT) seen in flies and moths (Stocker et al., 1990). Studies of honey bee brains used the same term to refer to the "medial" ACT, which corresponds to the "inner" ACT (iACT) in cockroaches and flies (Figure 2C) (Mobbs, 1982; Malun et al., 1993). Such inconsistencies make it impossible to compare the trajectories of antennal lobe projection neurons across species. Considering that the anatomical arrangement of the three pathways is better described as being medial, mediolateral, and lateral rather than inner, middle, and outer, we have adopted the convention used for the honey bee brain. To distinguish clearly the new unified terminology from previous ones, we selected the novel term ALT (antennal lobe tract) instead of the double-barreled ACT (antenno-cerebral tract). ALT best describes the tract with regard to its origin.

Likewise, structures referred to as the "lateral triangle" were not identical between flies and locusts. To remedy this, it was decided to employ the novel term "bulb" to refer to this structure, based on its characteristic organization of clustered microglomeruli.

It was also essential to resolve nomenclature for those ganglia that contribute to the brain (Figure 2D). Developmental and evolutionary evidence demonstrates that the insect (and malacostracan) brain comprises six neuromeres (Scholtz and Edgecombe, 2006): the protocerebrum (PR); deutocerebrum (DE); tritocerebrum (TR); and the mandibular (MN), maxillary (MX), and labial (LB) neuromeres. The terms supraesophageal and subesophageal ganglia (SPG and SEG) have been used, respectively, to denote the PR, DE, and TR separately from the MN, MX, and LB. However, SPG and SEG have also been used to generally refer to brain tissue above and below the level of the esophagus. The two employments of SPG and SEG are not, and cannot be, synonymous, however, because developmental studies show that the esophagus penetrates the deutocerebral neuromere during embryogenesis (Boyan et al., 2003). Two organizations can be found in the resulting adult. In many Hemimetabola, such as locusts and cockroaches, deutocerebral and tritocerebral neuropils below the esophagus are reduced to thin commissures, resulting in a clear distinction of three neuromeres above and three below the esophagus (Figure 2D, left). On the contrary, in many Holometabola, such as flies, bees, and moths, the deutocerebrum and tritocerebrum lie around and below the level of the esophagus. Thus, the terms SPG and SEG have contradictory meanings (Figure 2D, right).

To resolve this, we here employ the historical (and robust) terms "cerebral ganglia" (CRG) to replace the term "supraesophageal ganglion" (SPG) and "gnathal ganglia" (GNG) to replace the term "subesophageal ganglion" (SEG) for neuromere-based definitions that are independent of the location of the esophagus (Haeckel, 1896; Snodgrass, 1956). To generally refer to those parts above and below the esophagus, the word "ganglion" is avoided, because an arbitrary boundary might not match that of a neuromere and because of variation across species. After debating alternative terms, the group chose "supraesophageal zone" (SPZ) and "subesophageal zone" (SEZ), respectively, to refer to brain tissue above and below the level of the esophagus.

Introduction of new terms enables clear distinction of the studies that follow old or new nomenclature: literature that uses conventional but double-barreled terms like the ACT, lateral triangle, or SPG/SEG can be distinguished easily from studies that employ the new systematic nomenclature, which use terms like ALT, bulb, or CRG/GNG and SPZ/SEZ.

It was also important to avoid terms that can be applied to descriptions of the arthropod brain with terms used for describing the vertebrate brain. For example, the accumulation of neural cell bodies that cover the surface of the insect brain's neuropils has often been referred to as the "cortex." This term is inappropriate as it historically refers to sheet-like processing centers of the vertebrate forebrain. Unlike vertebrates, insect neurons rarely have synapses on their cell bodies, and therefore, neural computation seldom (if ever) occurs at that level. To avoid misleading implications of "cortex," we adopted the alternative term "cell body rind," which has a historical precedent (Strausfeld, 1976). The central volume of the insect brain, excluding the laterally extending optic lobes (the primary visual centers), is often referred to as the "central brain" or "midbrain." To avoid

confusion with the vertebrate midbrain, with its segmental implication, we have chosen the former term, "central brain." We have also tried to avoid terms that could be confused with current *Drosophila* gene and allele names.

Divergent spelling of the English language can pollute nomenclature. For example, US English simplifies the original spelling of "oesophagus" to "esophagus," a difference that has led to inconsistencies in abbreviating that part of the brain beneath the esophagus as either the SOG or the SEG (SEZ in the new nomenclature). Considering that most journals and coordinated ontologies follow US English spelling, it was decided to suggest the term "subesophageal" irrespective of the spelling policy of each journal.

To summarize, the working group identified and discussed 37 controversial issues relevant to unifying brain nomenclature. Resolution of these is provided in Supplemental Information.

Terms for Coordinate Axes

The working group also focused on the important issue of defining which axes of reference should be best used in descriptions of brain. Two systems previously employed are the body axes, determined by the longitudinal axis of the body (Figure 2E), or the neuraxis, which reflects the alignments of segmental neuromeres (Figure 2F). The two axes correspond in the thorax and abdomen, but because the adult brain undergoes a rotation of about 90°, the neuraxis is almost perpendicular to the body axis. Confusion has often occurred because the same directional terms (anterior/posterior and dorsal/ventral) have been used indiscriminately. We reached the consensus of adding the prefix "n-" to indicate directions that explicitly are based on the neuraxis. For example, in flies, the antennal lobes are directed forwards and are therefore anterior with regard to the body axis; however they are n-ventral with respect to the neuraxis. The prefix "b-" can be added to indicate a body axis descriptor explicitly (e.g., b-anterior). In addition, the terms "dorsum" and "venter" (n-dorsal/n-ventral) and "rostral" and "caudal" (n-anterior/n-posterior) are used specifically for describing locations according to the neuraxis, and "superior" and "inferior" are used specifically to indicate dorsal and ventral locations according to the body axis.

DISCUSSION

Many previous accounts have identified and named insect brain regions and their connections. Such studies have been useful guides for the present system of nomenclature, even though some of the terms used had to be abandoned in the development of this nomenclature. We acknowledge such earlier efforts, which include printed atlases of ganglia or brains (e.g., Power, 1943; Strausfeld, 1976; Tyrer and Gregory, 1982; Brandt et al., 2005; Otsuna and Ito, 2006; Kurylas et al., 2008) and web-based maps and databases, such as the "Flybrain" database (Armstrong et al., 1995) and other web-based databases that serve current research programs (Chiang et al., 2011; Shinomiya et al., 2011; Jenett et al., 2012, Milyaev et al., 2012). Each has provided invaluable information and guidance. In the present project, the Insect Brain Name Working Group has addressed two major concerns in

order to facilitate communication among diverse researchers working in insect neuroscience: (A) establishing a common framework to describe the brain's structural organization and (B) resolving inconsistency in nomenclature. A controlled nomenclature enables a common language and thus ease of communication among trainees and researchers alike, including those who are not yet familiar with insect neuroscience. The strategy of devising a standardized nomenclature through consensus has been employed most recently by a consortium of vertebrate neuroscientists for defining and naming regions of the avian brain (Jarvis et al., 2005), resulting in that nomenclature's universal acceptance. Considering the increasing importance of insect studies in neuroscience research, the present system of nomenclature should be helpful not only for those using *Drosophila* but also those working with other species. And, it is hoped, a controlled terminology will assist interdisciplinary collaborations between vertebrate and insect neuroscientists.

The common framework devised here for documenting brain organization demanded seven essential features. (1) Integrity: the framework had to include the entire brain relating all its parts to comparable spatial resolution. (2) Unambiguity: the boundaries of each brain region had to be clearly defined using landmarks that enable consistent identification across individuals. (3) Neutrality: the framework had to be detached from function, because rapidly progressing studies are likely to identify unexpected functions for neuropils. (4) Expandability: the framework had to be applicable to brains of other insects, and even crustacean species, with minimum alterations. (5) Consistency: comparisons with previous nomenclature had to be provided for the ease of transition to new terminologies. (6) Universality: a system of terms designed for broad use by the community. (7) Flexibility: a framework that permits its own evolution and adaptive integration of novel findings.

The nomenclature proposed here is comprehensive: 47 brain regions identified in this project comprise the entire brain. Volumes previously referred to as diffuse neuropils, which may occupy as large as 90% of the central brain, are here denoted by clearly defined names and boundaries. Without such notation, it is impossible to adequately describe locations and projections of neurons within such volumes. The integrity and unambiguity of the current nomenclature obviates such problems and provides essential support of research aimed at elucidating the organization, function, and development of neural circuits in not only a few well-known regions but also in the entire insect brain.

Generally, functional studies should not neglect underlying neural organization, and structural studies should consider possible functions. However, this has its risks: mushroom bodies, once thought to serve only olfactory learning due to their connections with the antennal lobes, have since been accorded a variety of other attributes. The working group has been keenly aware that implying functional associations would disadvantage a nomenclature designed for annotating brain architecture. Crucially, divorcing structure from function is essential if we consider that insect brains have undergone divergent evolution. A center identifiable in, for example, *Drosophila* that is also present in an aquatic beetle may support entirely different

functions in the two species. The nomenclature presented here provides a framework for the basis of future functional studies rather than proposing functional insights of its own.

A major aim of the project was the establishment of a nomenclature that would serve as a point of reference for the study of insect brains in general and those of their arthropod sister groups. Accordingly, in designing the present system of nomenclature, the working group integrated all available information about the structure of brains of other insect species to make it expandable to broad taxa. At the same time, it was acknowledged that a nomenclature cannot dissociate itself from history. This has resulted in the connection of past studies to the present system by providing relevant comparisons and look-up tables. In recognition of this need, the anatomical ontology and literature index in the FlyBase database has meanwhile been reannotated so that information pertaining to older terminologies can now be retrieved using the present nomenclature.

A nomenclature remains useless if it would be used only by a small fraction of researchers in the field. Throughout its development, this project has been openly discussed at many scientific meetings to gather opinions from the broad research community. It has been recognized by several key scientific endeavors, including FlyBase and its annex project The Virtual Fly Brain, and researchers involved in two of the newest GAL4 driver collection projects by the Rubin and Dickson groups, the FlyLight project (http://flweb.janelia.org/cgi-bin/flew.cgi). We are grateful to both groups for implementing the proposed nomenclature and neuropil definitions. Such a broad user base demonstrates the viability of the present framework.

In conclusion, standardized nomenclature provides the identifiers required to navigate brain space and organize data. However, while the current nomenclature will serve as the standard, the working group acknowledges that it is not set in stone and that it will evolve as our understanding of the brain's structure and function evolves. We anticipate that in the future certain revisions or the introduction of a novel term will be required. Accordingly, an online system for posting suggestions will be implemented in the Virtual Fly Brain for evaluation by a contemporaneous working group.

EXPERIMENTAL PROCEDURES

Antibodies and *Drosophila* Stocks

The following primary antibodies were used to visualize synapse-rich or fiber-bundle neuropils: anti-Bruchpilot nc82 (1:20; Wagh et al., 2006), anti-Synapsin 3C11 (1:1,000; Klagges et al., 1996), anti-DLG 4F3 (1:1,000; Parnas et al., 2001), and anti-β-tubulin E7 (1:1,000; Chu and Klymkowsky, 1989; Popodi et al., 2005). Each antibody was applied to brains expressing pan-glial membrane-bound GFP, using the *repo*-GAL4 driver (Awasaki et al., 2008; Lai and Lee, 2006) and UAS-mCD8-GFP reporter (Lee and Luo, 1999), so that labeling patterns of neuronal antigens could be observed simultaneously with the distribution of the glial processes. The pan-neuronal driver *elav*-GAL4 (C155; Lin and Goodman, 1994) was used to express cytoplasmic GFP with the UAS-GFP reporter (T2; Ito et al., 1998) or the combination of cytoplasmic dsRed (UAS-DsRed; Verkhusha et al., 2001), presynaptic GFP (UAS-n-syb-GFP; Estes et al., 2000), and postsynaptic Rdl-hemagglutinin (UAS-Rdl-HA; Sánchez-Soriano et al., 2005). Brains with expression of Rdl-HA were immunolabeled with anti-HA primary antibody (Covance; 1:1000). Alexa Fluor 488-, 568- and 647-conjugated anti-mouse IgG antibodies (Invitrogen; 1:250) were used for secondary antibodies.

Sample Preparation and Imaging

Five- to ten-day-old adult female brains were dissected, antibody labeled, and mounted as previously described (Otsuna and Ito, 2006). Frontal and horizontal serial optical sections of whole-mount brain samples were acquired at 1.2 μm z step intervals with a LSM510 (Zeiss) confocal laser scanning microscope with a 40× 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).

Boundary Drawing and 3D Rendering

To draw neuropil boundaries, frontal serial section images of the brain with *elav*-driven dsRed/n-syb-GFP/Rdl-HA labeling were imported to Amira (Mercury Inc.). 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 structural features best visible from these directions. Colors of the neuropils were chosen to maximize distinction between neighboring structures. Because of the large number of neuropils, however, it was inevitable that some of these colors resemble each other.

SUPPLEMENTAL INFORMATION

Supplemental Information includes 30 figures, 13 tables, four movies, two 3D neuroimaging files, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.12.017.

REFERENCES

Armstrong, J.D., Kaiser, K., Müller, A., Fischbach, K.F., Merchant, N., and Strausfeld, N.J. (1995). Flybrain, an on-line atlas and database of the *Drosophila* nervous system. Neuron *15*, 17–20.

Awasaki, T., Lai, S.L., Ito, K., and Lee, T. (2008). Organization and postembryonic development of glial cells in the adult central brain of *Drosophila*. J. Neurosci. *28*, 13742–13753.

Blest, A.D. (1961). Some modifications of Holmes's silver method for insect central nervous system. Q. J. Microsc. Sci. *102*, 413–417.

Boyan, G.S., Williams, L., and Meier, T. (1993). Organization of the commissural fibers in the adult brain of the locust. J. Comp. Neurol. *332*, 358–377.

Boyan, G., Reichert, H., and Hirth, F. (2003). Commissure formation in the embryonic insect brain. Arthropod Struct. Dev. 32, 61–77.

Brandt, R., Rohlfing, T., Rybak, J., Krofczik, S., Maye, A., Westerhoff, M., Hege, H.C., and Menzel, R. (2005). Three-dimensional average-shape atlas of the honeybee brain and its applications. J. Comp. Neurol. 492, 1–19.

Burne, T., Scott, E., van Swinderen, B., Hilliard, M., Reinhard, J., Claudianos, C., Eyles, D., and McGrath, J. (2011). Big ideas for small brains: what can psychiatry learn from worms, flies, bees and fish? Mol. Psychiatry 16, 7–16.

Cachero, S., Ostrovsky, A.D., Yu, J.Y., Dickson, B.J., and Jefferis, G.S. (2010). Sexual dimorphism in the fly brain. Curr. Biol. 20, 1589–1601.

Chiang, A.S., Lin, C.Y., Chuang, C.C., Chang, H.M., Hsieh, C.H., Yeh, C.W., Shih, C.T., Wu, J.J., Wang, G.T., Chen, Y.C., et al. (2011). Three-dimensional reconstruction of brain-wide wiring networks in Drosophila at single-cell resolution. Curr. Biol. 21, 1–11.

Chu, D.T., and Klymkowsky, M.W. (1989). The appearance of acetylated alpha-tubulin during early development and cellular differentiation in Xenopus. Dev. Biol. 136, 104–117.

Crittenden, J.R., Skoulakis, E.M., Han, K.A., Kalderon, D., and Davis, R.L. (1998). Tripartite mushroom body architecture revealed by antigenic markers. Learn. Mem. 5, 38–51.

Ehmer, B., and Gronenberg, W. (2002). Segregation of visual input to the mushroom bodies in the honeybee (Apis mellifera). J. Comp. Neurol. 451, 362–373.

Estes, P.S., Ho, G.L., Narayanan, R., and Ramaswami, M. (2000). Synaptic localization and restricted diffusion of a Drosophila neuronal synaptobrevin—green fluorescent protein chimera in vivo. J. Neurogenet. 13, 233–255.

Farris, S.M., and Sinakevitch, I. (2003). Development and evolution of the insect mushroom bodies: towards the understanding of conserved developmental mechanisms in a higher brain center. Arthropod Struct. Dev. 32, 79–101.

Fischbach, K.F., and Dittrich, A.P.M. (1989). The optic lobe of Drosophila melanogaster. - I. A Golgi analysis of wild-type structure. Cell Tissue Res. 258, 441–475.

Flögel, J.H.L. (1876). Über den feineren Bau des Arthropodengehirns. Tagebl. Versaml. Deutscher Naturforsch. Ärzte 49, 115–120.

Haeckel, E. (1896). Systematische Phylogenie. Zweiter Theil: Systematische Phylogenie der wirbellosen Thiere (Invertebrata). (Berlin: Reimer).

Hanesch, U., Fischbach, K.F., and Heisenberg, M. (1989). Neuronal architecture of the central complex in Drosophila melanogaster. Cell Tissue Res. 257, 343–366.

Heinze, S., and Reppert, S.M. (2012). Anatomical basis of sun compass navigation I: the general layout of the monarch butterfly brain. J. Comp. Neurol. 520, 1599–1628.

Hirth, F., Kammermeier, L., Frei, E., Walldorf, U., Noll, M., and Reichert, H. (2003). An urbilaterian origin of the tripartite brain: developmental genetic insights from Drosophila. Development 130, 2365–2373.

Homberg, U. (2002). Neurotransmitters and neuropeptides in the brain of the locust. Microsc. Res. Tech. 56, 189–209.

Ito, K., and Awasaki, T. (2008). Clonal unit architecture of the adult fly brain. Adv. Exp. Med. Biol. 628, 137–158.

Ito, K., Suzuki, K., Estes, P., Ramaswami, M., Yamamoto, D., and Strausfeld, N.J. (1998). The organization of extrinsic neurons and their implications in the functional roles of the mushroom bodies in Drosophila melanogaster Meigen. Learn. Mem. 5, 52–77.

Ito, M., Masuda, N., Shinomiya, K., Endo, K., and Ito, K. (2013). Systematic analysis of neural projections reveals clonal composition of the Drosophila brain. Curr. Biol. 23, 644–655.

Jarvis, E.D., Güntürkün, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D.J., Shimizu, T., et al.; Avian Brain Nomenclature Consortium (2005). Avian brains and a new understanding of vertebrate brain evolution. Nat. Rev. Neurosci. 6, 151–159.

Jefferis, G.S., Potter, C.J., Chan, A.M., Marin, E.C., Rohlfing, T., Maurer, C.R., Jr., and Luo, L. (2007). Comprehensive maps of Drosophila higher olfactory centers: spatially segregated fruit and pheromone representation. Cell 128, 1187–1203.

Jenett, A., Rubin, G.M., Ngo, T.T., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B.D., Cavallaro, A., Hall, D., Jeter, J., et al. (2012). A GAL4-driver line resource for Drosophila neurobiology. Cell Rep. 2, 991–1001.

Klagges, B.R., Heimbeck, G., Godenschwege, T.A., Hofbauer, A., Pflugfelder, G.O., Reifegerste, R., Reisch, D., Schaupp, M., Buchner, S., and Buchner, E. (1996). Invertebrate synapsins: a single gene codes for several isoforms in Drosophila. J. Neurosci. 16, 3154–3165.

Kumar, A., Fung, S., Lichtneckert, R., Reichert, H., and Hartenstein, V. (2009). Arborization pattern of Engrailed-positive neural lineages reveal neuromere boundaries in the Drosophila brain neuropil. J. Comp. Neurol. 517, 87–104.

Kurylas, A.E., Rohlfing, T., Krofczik, S., Jenett, A., and Homberg, U. (2008). Standardized atlas of the brain of the desert locust, Schistocerca gregaria. Cell Tissue Res. 333, 125–145.

Lai, S.L., and Lee, T. (2006). Genetic mosaic with dual binary transcriptional systems in Drosophila. Nat. Neurosci. 9, 703–709.

Larsen, C., Shy, D., Spindler, S.R., Fung, S., Pereanu, W., Younossi-Hartenstein, A., and Hartenstein, V. (2009). Patterns of growth, axonal extension and axonal arborization of neuronal lineages in the developing Drosophila brain. Dev. Biol. 335, 289–304.

Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22, 451–461.

Lin, D.M., and Goodman, C.S. (1994). Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron 13, 507–523.

Malun, D., Waldow, U., Kraus, D., and Boeckh, J. (1993). Connections between the deutocerebrum and the protocerebrum, and neuroanatomy of several classes of deutocerebral projection neurons in the brain of male Periplaneta americana. J. Comp. Neurol. 329, 143–162.

Milyaev, N., Osumi-Sutherland, D., Reeve, S., Burton, N., Baldock, R.A., and Armstrong, J.D. (2012). The Virtual Fly Brain browser and query interface. Bioinformatics 28, 411–415.

Mobbs, P.G. (1982). The brain of the honeybee Apis mellifera. I. The connections and spatial organizations of the mushroom bodies. Philos. Trans. R. Soc. B. 298, 309–345.

Otsuna, H., and Ito, K. (2006). Systematic analysis of the visual projection neurons of Drosophila melanogaster. I. Lobula-specific pathways. J. Comp. Neurol. 497, 928–958.

Parnas, D., Haghighi, A.P., Fetter, R.D., Kim, S.W., and Goodman, C.S. (2001). Regulation of postsynaptic structure and protein localization by the Rho-type guanine nucleotide exchange factor dPix. Neuron 32, 415–424.

Peng, H., Chung, P., Long, F., Qu, L., Jenett, A., Seeds, A.M., Myers, E.W., and Simpson, J.H. (2011). BrainAligner: 3D registration atlases of Drosophila brains. Nat. Methods 8, 493–500.

Pereanu, W., Kumar, A., Jennett, A., Reichert, H., and Hartenstein, V. (2010). Development-based compartmentalization of the Drosophila central brain. J. Comp. Neurol. 518, 2996–3023.

Popodi, E.M., Hoyle, H.D., Turner, F.R., and Raff, E.C. (2005). The proximal region of the beta-tubulin C-terminal tail is sufficient for axoneme assembly. Cell Motil. Cytoskeleton 62, 48–64.

Power, M.E. (1943). The brain of Drosophila. J. Morphol. 72, 517–559.

Rybak, J., Kuß, A., Lamecker, H., Zachow, S., Hege, H.C., Lienhard, M., Singer, J., Neubert, K., and Menzel, R. (2010). The digital bee brain: integrating and managing neurons in a common 3D reference system. Front. Syst. Neurosci. 4, 30.

Sánchez-Soriano, N., Bottenberg, W., Fiala, A., Haessler, U., Kerassoviti, A., Knust, E., Löhr, R., and Prokop, A. (2005). Are dendrites in Drosophila homologous to vertebrate dendrites? Dev. Biol. 288, 126–138.

Scholtz, G., and Edgecombe, G.D. (2006). The evolution of arthropod heads: reconciling morphological, developmental and palaeontological evidence. Dev. Genes Evol. *216*, 395–415.

Shinomiya, K., Matsuda, K., Oishi, T., Otsuna, H., and Ito, K. (2011). Flybrain neuron database: a comprehensive database system of the *Drosophila* brain neurons. J. Comp. Neurol. *519*, 807–833.

Snodgrass, R.E. (1956). Anatomy of the Honey Bee. (Ithaca: Comstock Pub. Associates).

Stocker, R.F., Lienhard, M.C., Borst, A., and Fischbach, K.F. (1990). Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. Cell Tissue Res. *262*, 9–34.

Strausfeld, N.J. (1976). Atlas of an Insect Brain. (Berlin, Heidelberg, New York, Tokyo: Springer-Verlag).

Strausfeld, N.J. (2012). Arthropod Brains: Evolution, Functional Elegance, and Historical Significance. (Cambridge: Harvard University Press).

Strausfeld, N.J., Sinakevitch, I., Brown, S.M., and Farris, S.M. (2009). Ground plan of the insect mushroom body: functional and evolutionary implications. J. Comp. Neurol. *513*, 265–291.

Tanaka, N.K., Tanimoto, H., and Ito, K. (2008). Neuronal assemblies of the *Drosophila* mushroom body. J. Comp. Neurol. *508*, 711–755.

Tanaka, N.K., Endo, K., and Ito, K. (2012). Organization of antennal lobe-associated neurons in adult *Drosophila melanogaster* brain. J. Comp. Neurol. *520*, 4067–4130.

Tyrer, N.M., and Gregory, G.E. (1982). A guide to the neuroanatomy of locust subesophageal and thoracic ganglia. Philos. Trans. R. Soc. B. *297*, 91–123.

Verkhusha, V.V., Otsuna, H., Awasaki, T., Oda, H., Tsukita, S., and Ito, K. (2001). An enhanced mutant of red fluorescent protein DsRed for double label-ing and developmental timer of neural fiber bundle formation. J. Biol. Chem. *276*, 29621–29624.

Wagh, D.A., Rasse, T.M., Asan, E., Hofbauer, A., Schwenkert, I., Dürrbeck, H., Buchner, S., Dabauvalle, M.C., Schmidt, M., Qin, G., et al. (2006). *Bruchpilot*, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in *Drosophila*. Neuron *49*, 833–844.

Wan, Y., Otsuna, H., Chien, C.-B., and Hansen, C. (2009). An interactive visu-alization tool for multi-channel confocal microscopy data in neurobiology research. IEEE Trans. Vis. Comput. Graph. *15*, 1489–1496.

Williams, J.L.D. (1975). Anatomical studies of the insect central nervous sys-tem: a ground-plan of the midbrain and an introduction to the central complex in the locust, *Schistocerca gregaria* (Orthoptera). J. Zool. *176*, 67–86.

Yang, M.Y., Armstrong, J.D., Vilinsky, I., Strausfeld, N.J., and Kaiser, K. (1995). Subdivision of the *Drosophila* mushroom bodies by enhancer-trap expression patterns. Neuron *15*, 45–54.

Young, J.M., and Armstrong, J.D. (2010). Building the central complex in *Drosophila*: the generation and development of distinct neural subsets. J. Comp. Neurol. *518*, 1525–1541.

Younossi-Hartenstein, A., Salvaterra, P.M., and Hartenstein, V. (2003). Early development of the *Drosophila* brain: IV. Larval neuropile compartments defined by glial septa. J. Comp. Neurol. *455*, 435–450.

Yu, J.Y., Kanai, M.I., Demir, E., Jefferis, G.S., and Dickson, B.J. (2010). Cellular organization of the neural circuit that drives *Drosophila* courtship behavior. Curr. Biol. *20*, 1602–1614.

Yu, H.-H., Awasaki, T., Schroeder, M.D., Long, F., Yang, J.S., He, Y., Ding, P., Kao, J.-C., Wu, G.Y., Peng, H., et al. (2013). Clonal development and organi-zation of the adult *Drosophila* central brain. Curr. Biol. *23*, 633–643.