A Systematic Nomenclature for the Drosophila Ventral Nerve Cord

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A Systematic Nomenclature for the *Drosophila* Ventral Nerve Cord

**Highlights**

- A framework defining the anatomy of the adult *Drosophila* ventral nerve cord (VNC)
- A clear and consistent naming scheme for the anatomy of the adult *Drosophila* VNC
- The framework is a tool for integrating past and future work into a common space
- Provides a template that can be adapted to other arthropod nervous systems

**Authors**

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**In Brief**

The ventral nerve cord (VNC) of *Drosophila* is an important model system for understanding how nervous systems generate locomotion. In this issue of *Neuron*, Court et al. define the structures of the adult VNC to provide an anatomical framework for analyzing the functional organization of the VNC.
A Systematic Nomenclature for the *Drosophila* Ventral Nerve Cord

NeuroResource

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SUMMARY

*Drosophila melanogaster* is an established model for neuroscience research with relevance in biology and medicine. Until recently, research on the *Drosophila* brain was hindered by the lack of a complete and uniform nomenclature. Recognizing this, Ito et al. (2014) produced an authoritative nomenclature for the adult insect brain, using *Drosophila* as the reference. Here, we extend this nomenclature to the adult thoracic and abdominal neuromeres, the ventral nerve cord (VNC), to provide an anatomical description of this major component of the *Drosophila* nervous system. The VNC is the locus for the reception and integration of sensory information and involved in generating most of the locomotor actions that underlie fly behaviors. The aim is to create a nomenclature, definitions, and spatial boundaries for the *Drosophila* VNC that are consistent with other insects. The work establishes an anatomical framework that provides a powerful tool for analyzing the functional organization of the VNC.

INTRODUCTION

Insects, and *Drosophila melanogaster* in particular, have made huge contributions to neuroscience research (Bellen et al., 2010). The powerful genetic tools and high-resolution neuroanatomy available in flies (Janett et al., 2012; Scheffer and Meinertzhagen, 2019) and the large number of research groups working on this model will ensure that the fly will remain a powerful tool for analyzing the function and development of complex nervous systems. Here we focus on the organization of an often-overlooked part of the *Drosophila* nervous system, the ventral nerve cord (VNC). The VNC is the insect analog of the vertebrate spinal cord and a significant part of the fly nervous system. The VNC is the locus for the reception and integration of sensory information and is involved in generating most of the locomotor actions that underlie fly behaviors such as walking (Bidaye et al., 2014; Mamiya et al., 2018; Mendes et al., 2013; Tuthill and Wilson, 2016; Wosnitza et al., 2013), grooming (Seeds et al., 2014), jumping (Card and Dickinson, 2008), flying (Dickinson and Muijres, 2016), courtship (Clyne and Miesenböck, 2008), and copulation (Crickmore and Vosshall, 2013; Pavlou et al., 2016). The VNC is, however, not a passive executive center receiving descending signals from the brain; it also sends significant major ascending projections to it (Tsubouchi et al., 2017). While the VNC in *Drosophila* is a complex fusion of all of the sub-gnathal neuromeres, it has a relatively simple and highly ordered structure. From external morphology, it is possible to recognize its constituent segmental neuromeres, the larger of...
which are the three thoracic ones, with the smaller, merged abdominal neuromeres protruding from the posterior end (Figure 1).

As with all arthropods, the neuronal cell bodies of the VNC form an outer cortex with neurons projecting processes centrally to form a dense fibrous central neuropil. The neuropil is stereotyped and highly ordered with functional segregation evident even at the level of the gross anatomy. The VNC is clearly subdivided in the dorso-ventral plane: ventral regions of the thoracic neuropils are innervated by neurons associated with the legs (Merritt and Murphey, 1992), whereas the dorsal neuropils are innervated by neurons associated with the wings and flight (Leise, 1991; Milde et al., 1989; Strausfeld, 1992) with intermediate regions serving to link legs and wing control (Namiki et al., 2018) (Figure 1). At a more detailed level, the neuropils exhibit a fine-grade functional order with modality-specific (Murphey et al., 1989a) and somatotopic (Murphey et al., 1989b) segregation of sensory afferent projections and myotopic organization of motor neuron dendrites (Baek and Mann, 2009; Brierley et al., 2012).

This functional organization of the neuropil provides a rigid anatomical framework against which it is possible to infer the function of neurons simply based on their anatomy. This framework is powerfuly informative and an essential tool to analyze how neurons control complex behaviors such as flying, courtship, and walking. Given the fundamental importance of this anatomical order, it is vital that this anatomical framework is robust, with a shared knowledge base to allow researchers to confidently and accurately place neurons within this framework.

To achieve this requires a systematic and consistent nomenclature and an anatomical template that precisely defines key anatomical structures, their boundaries, and the terms used to describe them. Recognizing the need for such consistent and robust anatomical framework, a consortium of neurobiologists studying arthropod brains (the insect brain name working group [IBNWG]), was established and produced a comprehensive hierarchical nomenclature system for the insect brain, using Drosophila melanogaster as the reference framework (Ito et al., 2014). This effort focused specifically on the brain and the gnathal regions of insects. In this work, we extend the development of a consistent nomenclature and anatomy to the Drosophila VNC.

Our work builds on previous descriptions of the Drosophila VNC (Power, 1948; Miller and Demerec, 1950; Merritt and Murphey, 1992; Boerner and Duch, 2010). It is also informed by the descriptions of the thoracic and abdominal ganglia of other insects such as grasshopper (Tyrer and Gregory, 1982) and stick insect (Kittmann et al., 1991). These comparative studies also point to clear evolutionary conservation of the basic elements of the Drosophila VNC. While these studies, plus many others, have created a rich catalog of anatomical detail, the inconsistent approach to nomenclature and definitions across the field has created ambiguity and confusion. The aim of the Drosophila...
Establishing the Anatomical Framework

Developmental origin provides an alternative organizational principle for defining the substructure of the neuropil. Neurons arise from neuroblasts whose first division results in A and B daughter cells. These undergo self-renewing divisions to produce clonal populations referred to as hemilineages. The neurons from a hemilineage tend to share properties, such as neurotransmitter identity and projection pattern—and even function (Harris et al., 2015; Lacin et al., 2019; Shepherd et al., 2019). We adopted a systematic approach when developing abbreviations for each named anatomical entity based on the following principles: (1) We adopted abbreviations that are unique across the whole CNS, avoiding abbreviations already in use for regions in the brain. (2) We created a system in which related entities would be easily recognizable. (3) We tried to be consistent with nomenclature established for the brain (Ito et al., 2014). The reasoning behind each abbreviation change was recorded and embedded in the definition. When referring to the neurorome and related structures, abbreviations were changed from a single letter or number to “Pro,” “Meso,” and “Meta.” This removed confusion with positional abbreviations such as...
posterior or medial. The use of the single letter “N,” which is used widely (neuromere, neuropil, nerve, neuron), was reserved for “nerve”; other larger gross anatomy structures differentiated with additional letters (e.g., “Nm” for neuromere and “Np” for neuropil). The letter “C” was used to identify commissures. In cases where multiple abbreviations already exist in the literature for specific structures, the abbreviation that provided the clearest indication with least likelihood of confusion was selected, and additional abbreviations were captured as synonyms. A list of abbreviations is given in Table 1.

**Axis Orientation**

The general axis of orientation for the VNC is straightforward. The neuroaxis and the body axis are the same, with the prothoracic neuromere being the most anterior and the abdomen (abdominal ganglionic complex) being the most posterior. In the dorsal/ventral plane, the tectum is dorsal and the leg nerves ventral. The dorsal/ventral axis is also sometimes referred to as superior/inferior, but dorsal and ventral are the preferred terms. The designation of left and right is assigned as if the sample is viewed from above (dorsal). The orientation in all figures is with anterior up for wholemount, lateral and horizontal views and dorsal up for transverse section views.

**Definition of the VNC**

The VNC is the region of the central nervous system posterior to the brain. It is connected to the brain by descending and ascending neurons that pass through the neck connective. The *Drosophila* VNC is a single consolidated ganglion located in the ventral part of the thorax. This ganglion contains all of the thoracic and abdominal neuromeres (Figure 1) and was called the thoracicoabdominal ganglion by Power (1948); see also synonyms in the supplemental section.

**Identifying and Defining the Neuropil Structures in the VNC**

Many insects have a ladder-like ventral nervous system composed of physically separated segmental neuromeres connected by longitudinal tracts (connectives), but in *Drosophila*, the thoracic and abdominal neuromeres are fused into a single complex (Niven et al., 2008) located within the thorax (Figure 1A). At the gross anatomical level, the segmental organization of the VNC can be resolved from external morphology. The thoracic neuromeres constitute the bulk of the VNC and are recognizable as three paired enlargements at the anterior of the VNC, corresponding to the prothoracic, mesothoracic, and metathoracic neuromeres (ProNm, MesoNm, and MetaNm, Figures 1B and 1C). At the posterior end is a small, dorsally located mass, the abdominal neuromeres, that is a fusion of all the abdominal neuromeres (ANm, Figure 1B).

Despite the evident external segmental organization, the fusion of multiple neuromeres means that identifying precise neuropil boundaries can be problematic. One of our aims was to define different regions of neuropil and provide landmarks to facilitate consistent identification and nomenclature for future studies. Although the VNC does not have the clearly defined compartmental structure found in the *Drosophila* central brain, it does have a clear architecture of tracts, commissures, and

<table>
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<th>Major Neuromeres and Neuropils</th>
<th>Longitudinal Tracts</th>
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<tr>
<td>Prothoracic neuromere (ProNm), Accessory</td>
<td>Dorsal lateral tract (DLT), Intermediate tract of dorsal cervical fasciculus (ITD), Dorsal lateral tract of ventral cervical fasciculus (DLV), Ventral lateral tract (VLT), Ventral median tract of ventral cervical fasciculus (VTV), Cervical nerve (CvN), Dorsal prothoracic nerve (DProN), Prosternal nerve (PrN), Prothoracic chordotonal nerve (ProCIN), Prothoracic accessory nerve (ProAN), Ventral prothoracic nerve (VProN), Prothoracic leg nerve (ProLN), Anterior dorsal mesothoracic nerve (ADMN), Posterior dorsal mesothoracic nerve (PDMN), Mesothoracic accessory nerve (MesoAN), Mesothoracic leg nerve (MesoLN), Dorsal metathoracic nerve (DMetaN), Metathoracic leg nerve (MetaLN), First abdominal nerve (AbN1), Second abdominal nerve (AbN2), Third abdominal nerve (AbN3), Fourth abdominal nerve (AbN4), Abdominal nerve trunk (AbNT)</td>
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<td>Mesothoracic neuromere (MesoNm)</td>
<td>Medial ventral association centre (MVC), Intermediate lateral association centre (ILAC)</td>
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<td>Metathoracic neuromere (MetaNm), Abdominal neuromere (ANm), Tectulum (Tct), Lower tectum ((LTct), Wing tectum (WTct), Haltere tectum (HTct), Neck tectum (NTct), Leg neuropil (LegNp), Intermediate neuropil (IntNp), Ventral Association Centre (VAC), Medial Ventral association centre (mVAC), Intermediate lateral association centre (ILAC)</td>
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<th>Specific Neurons</th>
<th>Other Structures</th>
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<tr>
<td>Giant Fiber (GF), Contralateral haltere interneurons (cHIN)</td>
<td>Femoral chordotonal organ (FeCO), Cervical connective (CvC)</td>
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axon bundles that provide the basis for defining different regions of neuropil. Cell body positions are not a reliable indicator of the segmental organization of the VNC. There are many examples of cell bodies being passively displaced during neuropil expansion at metamorphosis, resulting in somata being drawn across the midline or pulled into adjacent neuromeres (Shepherd et al., 2019).

Neuromere Boundaries
Although the VNC is a fusion of thoracic and abdominal neuromeres, it is possible to define neuromere boundaries using the scaffold of neuronal fibers revealed by neuroglian expression. The neuroglian positive bundles are the tightly fasciculated primary neurites from individual neuronal lineages, where somata from a lineage remain closely associated with each other. Since each neuromere is founded by a specific set of NBs, the lineage derived neuroglian bundles create a neuromere-specific set of markers, creating a robust framework that clearly outlines the neuropil within each neuromere and thus helps to define the neuropilar boundaries between each neuromere (Shepherd et al., 2016). Although Power (1948) defined the tectulum as a single neuropil without sub-divisions, the tectulum can be stratified into three layers in the dorsal ventral plane that the working group renamed as upper, intermediate, and lower tectulum (Figure 2A). The lower and intermediate tectulum show no overt signs of segmental barriers and are considered to lack a segmental organization. The upper tectulum, however, does have some segmental specializations and can be segregated on the basis of the synapse rich neuropils revealed by N-Cadherin/bruchpilot expression into three neuromere specific neuropils: neck, wing, and haltere tectulum for the ProNm, MesoNm, and MetaNm neuromeres, respectively (Figures 1B and 2A; Video S2).

The Leg Neuropil
The ventral portion of each thoracic neuropil outside of the tectulum is the leg neuropil (LegNp) (Figure 1D).

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Video S2). The ventralmost layer of leg neuropil, the ventral association center (VAC) (Merritt and Murphey, 1992) is readily distinguishable as synapse rich neuropils (VAC, Figures 1E–1G and 2A; Video S2). The VAC is innervated by sensory afferents from sensory neurons associated with tactile bristles on the leg (Murphey et al., 1989b). Adjacent to the VAC is a paired globular structure, the medial ventral association center (mVAC) (mVAC, Figures 1E–1G and 2A; Video S1). The mVAC is a bilaterally symmetrical neuropil region that can be identified both by its fine textured appearance and as dense synaptic neuropil (Merritt and Murphey, 1992). In Drosophila, the mVAC is innervated by a subset of femoral chordotonal organ (FeCO) sensory neurons which form a “club”-shaped projection that terminates in the mVAC (Phillis et al., 1996). The Drosophila mVAC is homologous to the mVAC described in locusts and other insects that also receive primary sensory afferents for leg chordotonal organs and is known as “auditory neuropil” (Oshinsky and Hoy, 2002; Römer et al., 1988).

The leg neuropil, between the VAC and the tectulum, is called “intermediate neuropil” (IntNp) because it occupies most of the central third of the dorsoventral area in transverse section (IntNp, Figure 2A; Video S2). The IntNp contains the dendritic branches of the leg motoneurons, premotor interneurons (Shepherd et al., 2019), and sensory afferents from leg campaniform sensilla, hair plates, and the “hook” and “claw” projection types from the FeCO (Mamiya et al., 2018). Like the tectulum, the leg neuropils exhibit clear functional segregation: motor neuron dendrites show clear spatial and functional organization (Maniates-Selvin et al., 2020), and the sensory modalities are partitioned into layers, with proprioception in intermediate neuropil and a somatotopic representation of tactile information in the ventralmost zone (Murphey et al., 1989b; Tsubouchi et al., 2017).

Tracts and Commissures

Building on studies of orthopterous insect ganglia such as the grasshopper (Tyrer and Gregory, 1982), Merritt and Murphey, (1992) and Boerner and Duch (2010) described the stereotyped patterns of longitudinal tracts and commissures in the adult Drosophila VNC (Figures 2A, 3, and S1; Video S3). Here we have reviewed these studies and nomenclatures and extended them by providing high resolution volumes for these structures. The nomenclature for the commissures has been redesigned to create a new consistent naming system that reflects the developmental origins of each adult commissure. Truman et al., (2004) showed that the larval VNC has just five commissures per neuromere and that the postembryonic neuronal lineages that cross the midline do so via a specific and invariant commissure (Truman et al., 2004). The five larval commissures split into additional pathways during metamorphosis due to the expansion and extension of the neuropil, so the adult fly has more commissures than the larva (Figures 3 and S1). Using lineage-based markers, Shepherd et al. (2016) linked the larval commissures to their adult counterpart (Power, 1948; Merritt and Murphey, 1992). These lineage-based definitions underlie the proposed nomenclature. Unlike the commissures, the longitudinal tracts were fully described by Power (1948) and Merritt and Murphey (1992) with a largely consistent and widely accepted nomenclature that we have retained.

DISCUSSION

With this nomenclature, we address two primary issues required to create a clearer understanding of the VNC structure and to facilitate dialog and data exchange among neuroscience researchers. The first was to establish a common anatomical framework to precisely define and describe, textually and spatially, the anatomical organization of the VNC. The second was to create a clear and consistent naming scheme for each anatomical entity. The detailed VNC map we provide is essential for integrating past and future work into a common space, thereby contributing to new lines of investigation. In addition, our effort will inform researchers working with other insects, providing them with a

Figure 3. Major Longitudinal Tracts of the VNC

(A) The major tracts of the VNC shown as rendered volumes from lateral and dorsal perspectives.

(B) Transverse section views of the tracts at selected points in the VNC. The areas outlined by white circles identify other key structures (GF, giant fiber; ADMN, sensory afferents entering from the ADMN; SA, sensory afferents entering from the leg nerve; the numbers refer to hemilineage-derived axon fascicles). The planes of section are indicated by dotted lines in (A). See also Video S3. A list of the abbreviations is given in Table 1. Scale (A), 100 μm; (B), 50 μm.
template that can be adapted to their own model organism. Although the nomenclature developed in this project will serve as an initial standard, we acknowledge that to remain useful it must be maintained as a “living” process and evolve as our understanding of the VNC structure and function grows. Further revisions and additions will be required, and there are regions of the neuropil that will benefit from further analysis to provide a clearer breakdown of the substructure. Most notably, the thoracic IntNp, which, although extremely important, still remains a broadly defined region that lacks detailed spatial information, particularly in relation to the spatial organization of sensory neurons and motor neuron dendrites. Such additions and improvements will be handled via the existing online system for posting anatomy terminology suggestions located at https://github.com/FlyBase/VirtualFlyBrain.org.

Unlike the brain, the VNC in insects demonstrates significant diversity in its gross organization and structure (Niven et al., 2008). However, there is, a large anatomical literature for several insect groups that exhibit markedly different VNC structures (e.g., grasshoppers, crickets, and moths) that often use the same terms as used for Drosophila. The differences among the VNCs of different insects are likely to be largely superficial and simply reflect the pattern of ganglionic fusion. While this fusion does create some anatomical confusion, the basic pattern of tracts and commissures is preserved throughout the insects. Considering the conservation of lineages, tracts, and commissures, insects do exhibit remarkably similar CNS structures despite the distortions imposed by ganglionic fusion. Consequently, it is important not only to have a consistent nomenclature to benefit Drosophila researchers but also to develop a nomenclature that can be used as broadly as possible across the insects to create a consistent cross-species terminology. While this would require some work to confirm homology rather than rely on inference from similar structure, extension of a nomenclature that can be used as broadly as possible across the insects to create a consistent cross-species terminology. Most notably, the thoracic IntNp, which, although extremely important, still remains a broadly defined region that lacks detailed spatial information, particularly in relation to the spatial organization of sensory neurons and motor neuron dendrites. Such additions and improvements will be handled via the existing online system for posting anatomy terminology suggestions located at https://github.com/FlyBase/VirtualFlyBrain.org.

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**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.neuron.2020.08.005.


**STAR METHODS**

**KEY RESOURCES TABLE**

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**RESOURCE AVAILABILITY**

**Lead Contact**
Further information and requests for data and resources should be directed to and will be fulfilled by the Lead Contact, David Shepherd (d.shepherd@bangor.ac.uk).

**Materials Availability**
This study did not generate any new reagents.

**Data and Code Availability**
All anatomical datasets and segmented domains have been deposited at Virtual Fly Brain (https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template) and are openly available.

**METHOD DETAILS**

**Anatomical Materials**
All images are based on previously published data and described methodologies. The anti-neuroglian antibody (Iwai et al., 1997) was used to reveal the primary projections of neuron hemilineages as described by (Shepherd et al., 2016). The structure of the neuropil was revealed using anti-<i>Drosophila</i> N-cadherin (Developmental Studies Hybridoma Bank; Cat. no. DN-Ex 8 RRID:AB_528121) as described by (Shepherd et al., 2016), anti-nc82 (Developmental Studies Hybridoma Bank; Cat.no. nc82 anti-Bruchpilot RRID:D:AB_2314866) as described by (Wagh et al., 2006) and the brp-SNAP transgene (Kohl et al., 2014) as described by (Bogovic et al., 2019).

**Boundary Drawing and 3D rendering**
Neuropil regions, tracts and commissures were manually painted using ITK-SNAP (Yushkevich et al., 2006, RRID:SCR_002010, http://www.itksnap.org/pmwiki/pmwiki.php) using the adult female VNC template produced by (Bogovic et al., 2019),
Surface rendered images were generated with Fluorender software (RRID:SCR_014303, https://www.sci.utah.edu/software/fluorender.html). Videos were created with Adobe Premiere from on TIFF stacks created in FIJI (Schindelin et al., 2012) RRID:SCR_002285).