Title

A Systematic Nomenclature for the Drosophila Ventral Nerve Cord

Journal

Neuron, 107(6)

ISSN

0896-6273

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Publication Date

2020-09-01

DOI

10.1016/j.neuron.2020.08.005

Peer reviewed

Neuron

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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

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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.

Court et al., 2020, Neuron *107*, 1071–1079
September 23, 2020
https://doi.org/10.1016/j.neuron.2020.08.005

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A Systematic Nomenclature for the *Drosophila* Ventral Nerve Cord

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https://doi.org/10.1016/j.neuron.2020.08.005

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 (Jenett 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

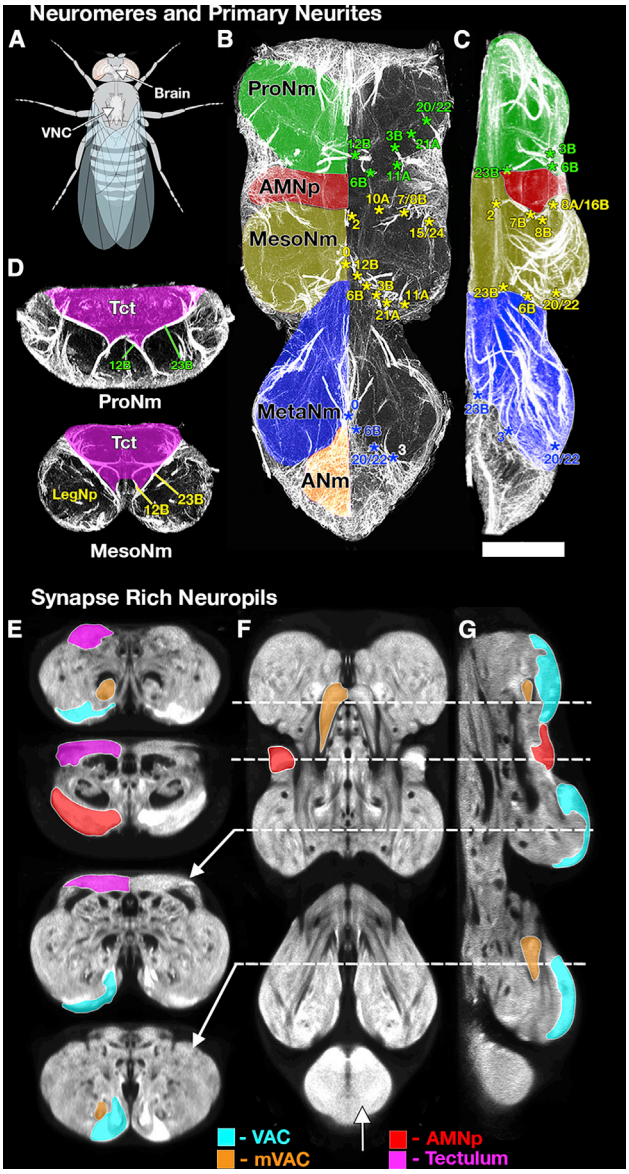

Figure 1. Selected Sections through an Adult VNC Illustrating the Tools Used to Define the Major Structures of the VNC

(A) Schematic of *Drosophila* illustrating the position of the VNC with respect to the body and brain.

(B–D) Neuroglian immunostaining showing neuromeres and Primary Neurite bundles in horizontal (B), lateral (C), and transverse (D) sections to reveal the tracts of the primary neurites of the postembryonic neuronal lineages. The pattern of labeled pathways is highly stereotyped; each pathway corresponds to the primary neurites of neurons derived from a single neuroblast. These tracts provide a robust basis for identifying the key structures of the VNC such as the following: (B and C) the neuromere boundaries (ProNm [green], MesoNm [yellow], MetaNm [blue], and ANm [red]) and (D) the tectulum (magenta—Tct). The numbers refer to specific hemilineage primary neurite bundles, with the color indicating their neuromere of origin.

(E–G) Brp-SNAP labeling (Bogovic et al., 2019) revealing the fine structure of the neuropil shown in transverse (E), horizontal (F), and lateral (G) sections. The *bruchpilot* (Brp) staining reveals characteristic regions of neuropil with high-density staining indicating synapse-rich neuropils. These synapse-rich neuropils can be used to define and segment specific neuropils such as the VAC (cyan), mVAC (orange), AMNp (red), and those of the tectulum (magenta, neck neuropil, wing neuropil, and haltere neuropil). The planes of the sections are indicated by the dotted lines. See also Video S1. A list of the abbreviations is given in Table 1. Scale 50 μm.

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 powerfully 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*

adult VNC working group (DAVWG) was to create a nomenclature, definitions, and spatial boundaries for the key anatomical entities of the *Drosophila* VNC that are consistent with the nomenclature used to describe the VNC in other insects.

RESULTS

Organization of the Working Group

The initial phase of work followed a similar format to that adopted by the original Insect Brain Name Working Group (IBNWG) to create the nomenclature for the *Drosophila* brain (Ito et al., 2014). We gathered researchers with expertise in the anatomy, development, and physiology of the VNC, hereafter referred to as the *Drosophila* Anatomy of the Ventral nerve cord Working Group (DAVWG) for a workshop at the Janelia Research Campus in October 2013. We discussed a document listing all of the named regions found in the published literature and from the existing *Drosophila* anatomy ontology (Costa et al., 2013), as well as representative anatomical images assembled by authors Court and Shepherd. After systematic review and debate, the participants compiled a working proposal for wider comment. Iterative revisions resulted in the current nomenclature described here.

Establishing the Anatomical Framework

Establishment of a systematic nomenclature requires a clear morphological and spatial definition of all the structures to be named and a standard naming scheme. The neuropil regions of the VNC are typically regarded as being "unstructured" or "tangled," or having a fine, granular appearance in sections with different regions distinguished only by general spatial terms (Merritt and Murphey, 1992). Despite this, fixed landmarks such as longitudinal tracts and commissures can be used to define the structure and organization of different volumes of VNC neuropil (Shepherd et al., 2016).

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). Shepherd et al. (2016) used the primary projections of neuronal hemilineages to provide an organizational principle for defining the substructure of the neuropil. Although these landmarks may not always correspond to the underlying functional organization, they provide a consistent means of structurally defining neuropil regions.

To provide an initial framework for establishing distinct boundaries within the VNC, we used confocal datasets that reveal various salient features, including tracts and neuropil. The anti-neuroglian antibody (Iwai et al., 1997) (Figures 1B–1D) was used to reveal the primary projections of clonally related neurons in neuroblast (NB) hemilineages (Shepherd et al., 2016). The detailed structure and textural details of the neuropil were based on VNCs labeled to visualize neuropils according to the density of active-zone-specific proteins using anti-*Drosophila* N-cadherin (Shepherd et al., 2016), anti-nc82 (bruchpilot [brp]) (Wagh et al., 2006), or brp-SNAP (Kohl et al., 2014) (Figures 1E–1G and Video S1). For most figures, we have used the high-resolution female VNC template produced by Bogovic et al., 2019, which provides the highest level of resolution and detail currently available. This template can be found at https://www.janelia.org/open-science/jrc-2018-brain-templates. These labels all reveal the fine details of texture and structure in the VNC neuropil, making it possible to distinguish between neuropils that are poor in synapses, such as regions occupied by axons; primary neurites; and glial processes and synapse-rich regions, such as the primary sensory neuropils and the dorsal neuropils associated with the neck, wings, and halteres (Figures 1E–1G). An anti-alpha tubulin antibody (data not shown) was used to reveal fibrous structures such as longitudinal tracts and commissures (Boerner and Duch, 2010). Other images obtained with these labeling methods are available on the Virtual Fly Brain (https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template).

Since all of these antibodies are available at low cost through the Developmental Studies Hybridoma Bank created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242, they can be used by future researchers to counterstain their own samples, identify neuropil regions described in this nomenclature, and computationally register them to our standard reference brains.

The Naming Scheme

All of the anatomical data used in this manuscript can be found on the Virtual Fly Brain GitHub repository https://github.com/VirtualFlyBrain/DrosAdultVNSdomains. All of the text definitions of the structures and synonyms considered in the nomenclature can be found on http://purl.obolibrary.org/obo/fbbt

A key principle was to integrate existing terminology into the standard nomenclature we propose here. We made changes only to remove ambiguity. When multiple names for an anatomical entity were used in the literature, we gave preference to the name that was most commonly used based on citations. While we sought to preserve consistency with terms used for earlier developmental stages and in other insects, we avoided the implication of homology. Most of the naming scheme relies on morphological features rather than functional data, which we incorporate in the definitions when known. We also include a look-up table of synonyms, prior terms, and references.

Abbreviations

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 neuromere 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 tectulum 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 1. List of the Major Structures and Their Abbreviations

Major Neuromeres and Neuropils	Longitudinal Tracts
Prothoracic neuromere (ProNm), Accessory Mesothoracic neuropil (AMNp), Mesothoracic neuromere (MesoNm), Metathoracic neuromere (MetaNm), Abdominal neuromere (ANm), Tectulum (Tct), Upper tectulum (UTct), Intermediate tectulum (IntTct), Lower tectulum (LTct), Wing tectulum (WTct), Haltere tectulum (HTct), Neck tectulum (NTct), Leg neuropil (LegNp), Intermediate neuropil (IntNp), Ventral Association Centre (VAC), Medial Ventral association centre (mVAC), Intermediate Lateral association centre (iLAC)	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), Median dorsal abdominal tract (MDT), Ventral cervical fasciculus (VCF), Dorsal cervical fasciculus (DCF), Dorsal median tract (DMT), Ventral ellipse (VE)
Commissures	**Peripheral Nerves**
anterior Anterior Ventral Commissure (aAV), posterior Anterior Ventral Commissure (pAV), Anterior Intermediate Commissure (AI), ventral Anterior Intermediate Commissure (vAI), Anterior Intermediate anterior Commissure (AIa), Anterior Intermediate posterior Commissure (AIp), dorsal Anterior Intermediate Commissure (dAI), anterior Posterior Intermediate Commissure (aPI), posterior Posterior Intermediate Commissure (pPI), dorsal PI Commissure (dPI), Posterior Dorsal Commissures (PD), Commissure of Fine Fibers of the Intermediate Tract of the Dorsal Cervical Fasciculus (CFF), Commissure of Prothoracic Neuromeres (CPN), Dorsal Accessory Commissure of the Mesothoracic Neuromeres (DAM), Ventral Ellipse (VE)	Cervical nerve (CvN), Dorsal prothoracic nerve (DProN), Prosternal nerve (PrN), Prothoracic chordotonal nerve (ProCN), 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)
Specific Neurons	**Other Structures**
Giant Fiber (GF), Contralateral haltere interneurons (cHIN)	Femoral chordotonal organ (FeCO), Cervical connective (CvC)

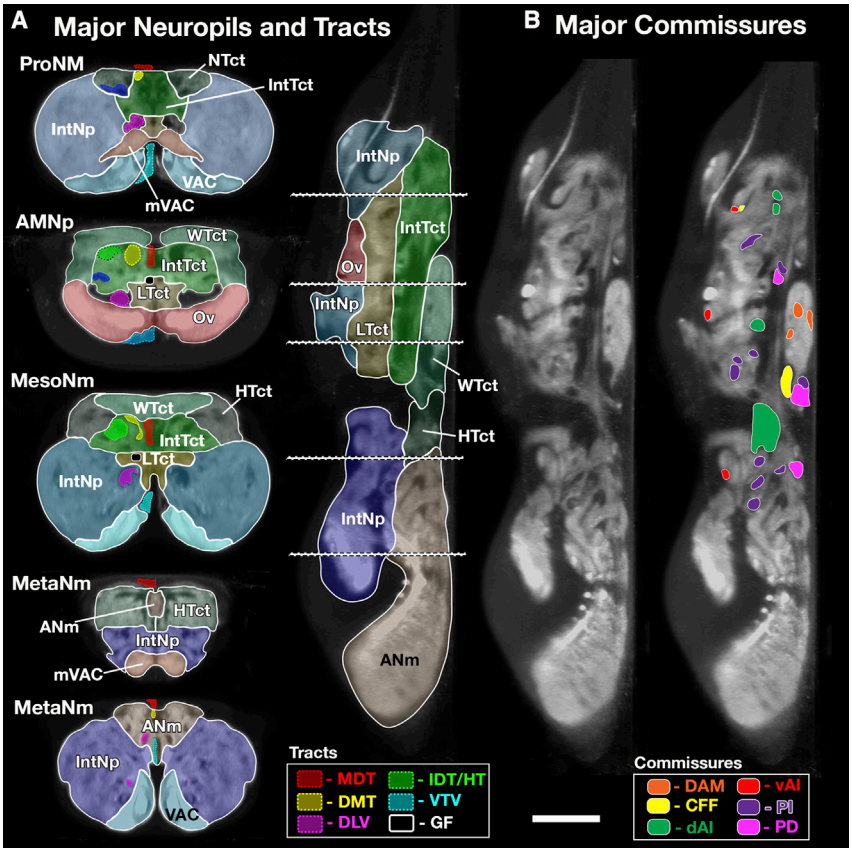

Figure 2. Major Neuropils, Tracts, and Commissures of the VNC

(A) Major Neuropils and Tracts—segmented VNC shown in transverse and lateral sections illustrating the outlines of the major neuropils and longitudinal tracts described in this study. The tectulum domains are shown in different shades of green, and the leg neuropil domains are shown in shades of blue. To further aid visualization, labeled tracts are only shown in the left half of the transverse sections. The plane of the transverse sections is indicated by dotted lines.

(B) The position of the major commissural pathways shown on a lateral section at the midline of the VNC. Tracts derived from the same larval commissure are shown in the same colors. An unlabeled section is provided to show the detail unhindered by labeling. See also Figure S1 and Video S2. A list of the abbreviations is given in Table 1. Scale 50 μm.

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 (Figures 1B and 1C). The neuroglian label also provides markers for other structures such as the tectulum (Tct [magenta], Figure 1D) and some commissures (Figure 2) (Shepherd et al., 2016).

Major Subdivisions of the Thoracic Neuropils

While the neuromeres divide the VNC along the anterior-to-posterior axis, there is also specialization on the dorso-ventral axis with a dorsal region called the tectulum (Tct) and a ventral region called leg neuropil (LegNp) (Figure 1D).

The Tectulum (Tct)

The tectulum (Tct) was described by Power (1948) as a discrete dorsal region of the VNC, overlying the mesothoracic neuromere like a saddle and extending from the posterior prothoracic to the anterior metathoracic neuromeres. The neuroglian positive primary neurites provide boundaries that precisely circumscribe the tectulum to define its boundaries (Figure 1D) (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, see Supplemental Information for details). Unlike the tectulum, the leg neuropils exhibit clear segmental boundaries and, although each thoracic neuromere is slightly different, they all conform to the same organizational principles (Figure 2A; Video S2). The legNps contain the sensory afferent endings of leg sensory neurons, the leg motor neurons, and local interneurons that control leg movement. The leg neuropils are best described in transverse section and can be partitioned into distinct regions along the dorsoventral axis (Figure 2A;

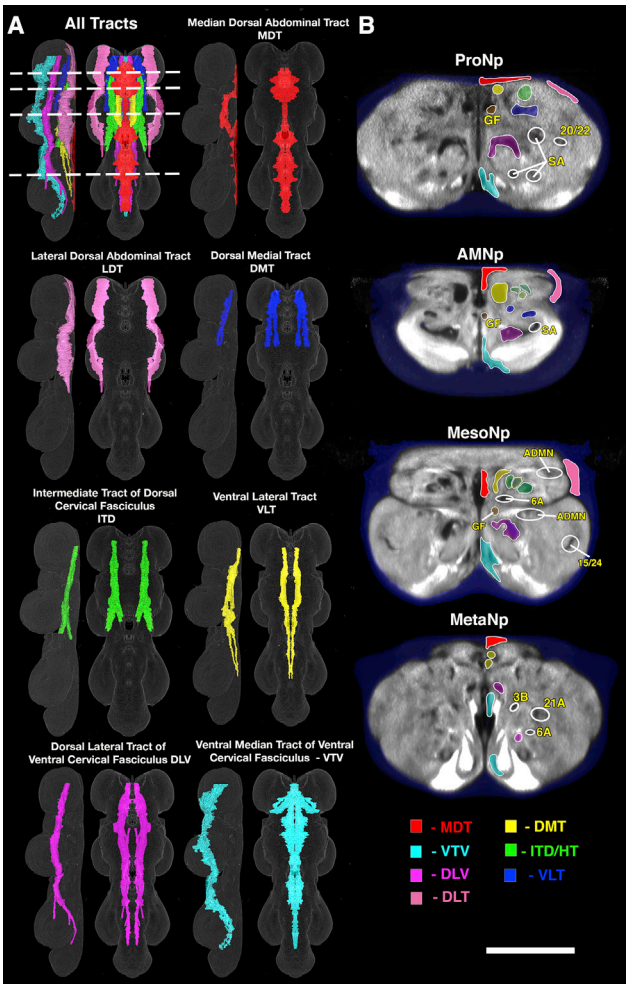

Figure 3. Major Longitudinal Tracts of the VNC

(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.

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 motorneurons, 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

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. Future 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 ontology suggestions located at https://github.com/FlyBase/Drosophila-anatomy-developmental-ontology/issues and maintained by 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 consistent nomenclature to other insects would provide a framework to explore cross-species homologies in the VNC, the evolution of neuronal networks, and the deep evolutionary conservation of the nervous system.

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.

ACKNOWLEDGMENTS

We thank Gerald M. Rubin, Janine Stevens, the Visiting Science and Conference Programs of the Howard Hughes Medical Institute's Janelia Research Campus for hosting the workshop. This work was initially conceived of as part of the Descending Interneuron Project Team at Janelia. This work was also supported, in part, by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (http://www.anc.ed.ac.uk/dtc) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC). Finally, by the Wellcome Trust as part of the "Virtual Fly Brain: a global informatics hub for *Drosophila* neurobiology" WT105023MA.

AUTHOR CONTRIBUTIONS

Conceptualization, W.K., J.W.T., and D.S.; Software, R.C.; Validation, All; Resources, D.S., R.C., and J.B.; Data Curation, R.C., M.C., and J.D.A; Writing—Original Draft, D.S. and R.C.; Writing—Review & Editing, M.C., M.D., R.K.M., A.M.S., J.H.S., T.S., J.C.T., J.W.T., and D.W.W.; Visualization, D.S. and R.C.; Funding Acquisition, All.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 13, 2020
Revised: June 30, 2020
Accepted: August 5, 2020
Published: September 14, 2020

SUPPORTING CITATIONS

The following references appear in the Supplemental Information: Bacon and Strausfeld (1986); Bodenstein (1950); Ghysen (1980); Lundquist and Nässel, (1990); Middleton et al. (2006); Pflüger et al. (1988); Shepherd and Smith (1996); Strausfeld and Seyan (1985); Yu et al. (2010).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-neuroglian	Developmental Studies Hybridoma Bank (Iwai et al., 1997)	Cat.no. BP 104 anti-neuroglian RRID:AB_528402
Anti-*Drosophila* N-cadherin	Developmental Studies Hybridoma Bank	Cat. no. DN-Ex 8 RRID:AB_528121
Deposited Data		
All datasets and segmented domains	https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template	This Paper
Experimental Models: Organisms/Strains		
Drosophila melanogaster brp-SNAP transgene	Kohl et al., 2014	Dmel\brp$^{SNAPf\text{-}tag}$
Software and Algorithms		
ITK-SNAP	Yushkevich et al., 2006	http://www.itksnap.org/pmwiki/pmwiki.php RRID:SCR_002010
Fluorender	Scientific Computing and Imaging Institute, University of Utah	https://www.sci.utah.edu/software/fluorender.html RRID:SCR_014303
FIJI	Schindelin et al., 2012	https://fiji.sc/ RRID:SCR_002285
Adobe Premiere	Adobe.com	N/A

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-*Drosophila* 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: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),

(https://www.janelia.org/open-science/jrc-2018-brain-templates). 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).

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Supplemental Information

A Systematic Nomenclature

for the *Drosophila* Ventral Nerve Cord

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Data S1 – Related to Figures 1, 2 and 3 and Table 1- Definitions of VNC structures

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Commissures derived from the larval pI commissure

ProNm

anterior Posterior Intermediate Commissure (aPI)
posterior Posterior Intermediate Commissure (pPI)

MesoNm and MetaNm

anterior Posterior Intermediate Commissure (aPI)
posterior Posterior Intermediate anterior Commissure (pPIa)
posterior Posterior Intermediate posterior Commissure (pPIp)

Commissures derived from the larval pD commissure

Posterior Dorsal Commissures (pD)

Commissures formed by descending neurons

Commissure of Fine Fibers of the Intermediate Tract of the Dorsal Cervical Fasciculus (CFF)

Other Commissures

Commissure of Prothoracic Neuromeres (CPN)
Dorsal Accessory Commissure of the Mesothoracic Neuromeres (DAM)

Longitudinal Tracts

Dorsal Lateral Tract (DLT)
Intermediate Tract of Dorsal Cervical Fasciculus (ITD)
Intermediate Tract of Dorsal Cervical Fasciculus – Haltere Chiasma (ITD-HC)
Haltere Tract ITD-(HT)
Dorsal Medial Tract (DMT)
Dorsal Lateral Tract of Ventral Cervical Fasciculus (DLV)
Ventral Median Tract of Ventral Cervical Fasciculus (VTV)
Median Dorsal Abdominal Tract (MDT)
Lateral Dorsal Abdominal Tract (LDT)
Ventral Cervical Fasciculus (VCF)
Dorsal Cervical Fasciculus (DCF)
Ventral Ellipse (VE)

The peripheral nerves

Cervical Connective (CvC)
Cervical Nerve (CvN)
Dorsal Prothoracic Nerve (DProN)
Prosternal Nerve (PrN)
Prothoracic Chordotonal Nerve (ProCN)
Prothoracic Accessory Nerve (ProAN)
Ventral Prothoracic Nerve (VProN)
Prothoracic Leg Nerve (ProLN)
Anterior Dorsal Mesothoracic Nerve (ADMN)

Wing Nerve
Posterior Dorsal Mesothoracic Nerve (PDMN)
Mesothoracic Accessory Nerve (MesoAN)
Mesothoracic Leg Nerve (MesoLN)
Dorsal Metathoracic Nerve (DMetaN)
Metathoracic Leg Nerve (MetaLN)

The abdominal nerves
First Abdominal Nerve (AbN1)
Second Abdominal Nerve (AbN2)
Third Abdominal Nerve (AbN3)
Fourth Abdominal Nerve (AbN4)
Abdominal Nerve Trunk (AbNT)

Definitions

Agreed definitions of the anatomical structures. Highlighting any changes from the classical definition with the group's reasoning for making the change. Synonyms for each structure are provided and unless stated otherwise these are exact synonyms.

The Ventral Nerve Cord (VNC)

The non-cephalic division of the central nervous system consolidated into a single ganglion located in the ventral thorax. The ganglion contains all of the thoracic and abdominal neuromeres (Figure 1). For this reason, it was called the thoracicoabdominal ganglion by Power (1948) but is most often referred to as the ventral nerve cord (VNC) although the VNC would include the suboesophageal ganglion (Niven et al., 2008). The VNC ganglion has three paired enlargements at its anterior end, thoracic neuromeres, which correspond to the prothoracic, mesothoracic and metathoracic segments, with a single posterior, dorsally located mass, the abdominal neuromeres that contains all the fused abdominal neuromeres.

Changes:

Although the name thoracicoabdominal ganglion (TAG) (Power, 1948) was initially agreed by the group, many who worked in both larvae and adult felt that ventral nerve cord (VNC) was more appropriate and indeed was often used in the adult and was agreed upon with all alternatives recorded as synonyms.

Synonyms:

- Ventral Nervous System (VNS),
- thoracico-abdominal ganglion (Power, 1948),
- thoracico-abdominal ganglia (Lundquist and Nässel, 1990),
- adult ventral nerve cord (Niven et al., 2008),
- VNC [BROAD] (Niven et al., 2008),
- TAC (Merritt and Murphey, 1992),
- TAG (Ito et al., 2014),
- ThGng (Miller and Demerec, 1950),
- thoracic + abdominal ganglion,
- T1 + T2 + T3 + A1 + A2 + A3 + A4 + A5 + A7 + A8,
- thoracico-abdominal center (Power, 1948; Merritt and Murphey, 1992),
- thoracic nerve center (Miller and Demerec, 1950),
- abdominal ganglion RELATED (Power, 1948).

Dense Neuropil/Synapse Rich Neuropil

Dense neuropil, as referred to in this document, indicates more tightly packed synaptic regions. It can be identified by a notably brighter signal than that from the general surrounding neuropil when imaging the VNC using confocal microscopy of probes targeting synapse associated proteins such as Bruchpilot or N-cadherin. See Figure 1 and video S1.

Major neuropil features of the VNC

The defined region of each neuropil is shown in Figure 2 and video S2.

Thoracic Neuromeres

The three thoracic neuromeres are segmentally homologous and share common structural features. The detailed organization of serially homologous tracts and neuropil regions within the leg neuropils, as well as the angle of entry of the relevant leg nerve into the VNC,

indicate that the thoracic neuromeres have undergone rotation during development. This is such that the prothoracic neuromeres are rotated anteriorly, the mesothoracic and metathoracic and neuromeres rotated posteriorly.

The Prothoracic Neuromere (ProNm; also called T1) is the anteriormost of the 4 major neuropils that makes up the VNC and derives almost completely from the somata and projections of central neurons derived from the prothoracic array of neuroblasts, as well as the axonal projections of sensory afferents from the prothoracic legs and prothorax. The paired neuropil is formed as two more or less spherical masses. Its anterior boundary defines the anterior extent of the VNC. Although the posterior boundary is less obvious, it can be defined by the extent of the primary neurite projections of the central neurons produced by the prothoracic NBs (0, 3, 6, 11, 21, 20/22 and 23). These neuroglian positive tracts project anteriorly into the neuromere and their entry points represent the posterior limit of the prothoracic neuropil.

Changes:
The abbreviation was changed to resolve the clash for the original abbreviation PN with those for both the prosternal nerve and the adult antennal lobe projection neuron. The internal boundaries were clarified in relation to lineage tracts (Shepherd et al., 2016).

Synonyms:
• ProNm (Proposed by group),
• Pro (Merritt and Murphey, 1992),
• PN (Power, 1948).

The Mesothoracic Neuromere (MesoNm; also called T2) The 2nd (anterior-posterior) of the 4 major neuropils of the VNC that derives almost completely from the somata and projections of central neurons derived from the mesothoracic array of neuroblasts as well as the axonal projections of sensory afferents from the mesothoracic legs and mesothorax. The mesothoracic neuromere is closely associated with two distinct subdivisions of the thoracic neuropils: the accessory mesothoracic neuromere and the tectulum. It is delimited anteriorly by the entry points of the neuroglian-positive tracts from the anterior mesothoracic lineages 2, 7, 8, 10, 15 and 16. The posterior margin is defined by the entry points of the neuroglian-positive tracts from the posterior mesothoracic lineages 0, 3, 6, 12, 11, 19, 21 and 23, all of which project anteriorly into the neuromere.

Changes:
The abbreviation was changed for consistency. The internal boundaries were clarified in relation to lineage tracts (Shepherd et al., 2016).

Synonyms:
• MesoNm (Proposed by group),
• Meso (Merritt and Murphey),
• MN (Power, 1948).

The Metathoracic Neuromere (MetaNm; also called T3) The 3rd (anterior-posterior) of the 4 major neuropils that make up the VNC. It derives almost completely from the somata and projections of central neurons derived from the metathoracic array of neuroblasts as well as the axonal projections of sensory afferents from the metathoracic legs and metathorax. Because the MetaNp is drawn anteriorly by morphogenetic changes the anterior metathoracic neuroblast primary neurites are not a reliable marker for the anterior margin of the MetaNp.

The anterior MetNm boundary is best defined by the neuroglian tracts associated with the posterior T2 hemilineages 0, 3, 6, 11, 12, and 21, which also define the posterior margin of the MesoNp. The posterior margin of the neuromere is defined by the neuroglian tracts from the posterior hemilineages 0, 3, 6, 20/22 and 21, all of which project anteriorly into the neuromere.

Changes:
The abbreviation was changed for consistency. The internal boundaries were clarified in relation to lineage tracts (Shepherd et al., 2016).

Synonyms:
• MetaNm (Proposed by group),
• Meta (Merritt and Murphey, 1992),
• MtN (Power, 1948).

Abdominal Neuromeres (ANm)

The 4th major neuropil situated at the posterior end of the VNC, composed of the fused neuromeres A1 through A8. The lineage composition of the abdominal neuromeres has not been undertaken but the anterior limit of the ANm is defined by reference to the neuroglian bundles delineating the posterior limit of the MetaNm. The neuropil regions posterior to the neuroglian tracts of hemilineages 0, 5, 6, 19 and 23 define the ANm.

Synonyms:
• ANm (Proposed by group),
• AbGng (Miller and Demerec, 1950),
• AG (Merritt and Murphey, 1992),
• ac (Power, 1948),
• A1+A2+A3+A4+A5+A6+A7+A8,
• abdominal ganglia (Merritt and Murphey, 1992),
• abdominal ganglion (Yu et al., 2010),
• abdominal center (Power, 1948),
• abdominal nerve center (Miller and Demerec, 1950).

Other major Neuropils

The fusion of the thoracic neuromeres created two additional major neuropil regions, the accessory mesothoracic neuropil (AMNp) and the tectulum, that do not conform to the evolutionarily ancestral segmental origins.

The accessory mesothoracic neuropil (AMNp) The accessory mesothoracic neuropil (AMNp) is a distinct subdivision of the mesothoracic neuromere. It is formed of dense neuropil (as seen by brighter signal in bruchpilot or N-cadherin labeling) largely from the sensory afferents from the wing and notum entering the VNC via the Anterior Dorsal Median Nerve (ADMN) (Power, 1948). This dense synaptic neuropil corresponds to a structure called the ovoid by Merritt and Murphey (1992). The AMNp was originally called the Accessory Mesothoracic Neuromere by Merritt and Murphey (1992) but we substitute *neuropil* to indicate that it is a region made up of incoming sensory afferents rather than intrinsic neurons of a common developmental origin. The AMNp sits at the interface between the pro- and mesothoracic neuromeres from which it is morphologically distinct with the tectulum (Tct) forming the dorsal boundary. The AMNp is bounded anteriorly by the primary neurites from

the posterior T1 hemilineages 0, 5, 6, 11, 19 and 23 and posteriorly by the primary neurites from the anterior T2 hemilineages 1, 2, 7, 8, 9, 15, and 16.

Changes:
After careful consideration it was concluded and agreed by all that the accessory region was indicated by the dense neuropil (see description) and that any area posterior to the prothoracic neuromere lineage boundary but not dense neuropil should be considered part of the mesothoracic neuromere. This also meant that the accessory region should more correctly be labelled neuropil rather than a neuromere. The internal boundaries were clarified in relation to lineage tracts (Shepherd et al., 2016).

Synonyms:
• AMNp (Proposed by group),
• accessory mesothoracic neuromere (Power, 1948),
• Acc Meso (Merritt and Murphey, 1992),
• AMN (Power, 1948),
• ovoid (Merritt and Murphey, 1992).

Tectulum

The tectulum is a distinct subdivision of the thoracic regions of the VNC. The region forms a saddle-like structure located dorsally, primarily over the accessory mesothoracic neuropil (AMNp) and the mesothoracic neuromere (MesoNm) but extending over the posteriormost region of the prothoracic neuromere (ProNm) and the anteriormost region of the metathoracic neuromere (MetaNm). Its internal boundaries within the VNC can be defined as the dorsal region of the neuropil posterior to the ventral ellipse in ProNm, but dorsal to the bundles from hemilineages 12B, 6B, 23 17, 18B. Extending posteriorly through MesoNm to the entry point of hemilineage 3 in MetaNm. The tectulum can be stratified into three regions (Namiki et al., 2018), which the working group renamed as upper, intermediate and lower tectulum.

The **upper tectulum** is the dorsalmost stratum of the tectulum sitting dorsal to the tracts DMT, MTD and ITD-HT. The upper tectulum can be further segregated on the basis of the synapse rich neuropil regions into three neuromere specific neuropils. A prothoracic neck neuropil (NTct), a mesothoracic wing neuropil (WTct) and a metathoracic haltere neuropil (HTct).

The **intermediate tectulum**, lies immediately ventral to the upper tectulum and dorsal to the lower tectulum and extends from the ProNm mVAC to the posterior margin of the MesoNm at the ITD-CFF commissure.

The **lower tectulum** is a region of the pro and mesothoracic neuromeres ventral to the intermediate tectulum extending posteriorly from the proNm mVAC and the posterior margin of the mesoNm at the ITD-CFF commissure. It is contained ventrally by the tract VTV and laterally by DLV tract.

Changes:
Abbreviation changed from Power's 'T' (Power, 1948) to avoid potential confusion. The internal boundaries were clarified in relation to lineage tracts (Shepherd et al., 2016). Agreed naming and definitions of the upper, intermediate and lower tectulum.

Synonyms:
• Tct (Proposed by group),
• T (Power, 1948),
• Flight neuropil RELATED (Leise, 1991; Power, 1948).

Leg Neuropil

Whilst the tectulum occupies the dorsal regions of the thoracic neuromeres, the remaining ventral portion is considered leg neuropils. Unlike the tectulum, the leg neuropils exhibit overt segmental boundaries and although each thoracic neuromere is slightly different the leg neuropils conform to the same organizational principles. The leg neuropils receive sensory inputs from the legs and contains the dendritic processes of the motor neurons that target leg muscles. The leg neuropils are best described in transverse section and can be readily partitioned into distinct regions along the dorsoventral axis.

Ventral Association Centre (VAC)

The ventralmost layer of leg neuropil, the Ventral Association Center (VAC) (Merritt and Murphey, 1992) is readily distinguishable as a unique region by the high expression levels of the synaptic proteins Bruchpilot and N-cadherin indicating high synaptic density in VAC. The VAC is innervated by sensory afferents from sensory neurons associated with tactile bristles on the leg which form a somatotopic projection (Murphey et al., 1989) within the VAC.

Medial Ventral Association Centre (mVAC)

Adjacent to the VAC of each leg neuropil is a paired globular structure, the medial Ventral Association Center (mVAC), a bilaterally symmetrical region that can be identified both by its fine textured appearance and the high expression levels of bruchpilot and N-cadherin (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 which also receive primary sensory afferents for leg chordotonal organs and is known as “auditory neuropil” (Oshinsky and Hoy, 2002; Pflüger et al., 1988; Römer et al., 1988).

Intermediate Neuropil (Int Np)

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 2 and video S2). The leg neuropil contains the dendritic branches of the leg motorneurons as well as premotor interneurons (Shepherd et al., 2019) and sensory afferent terminals 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 neurons are located dorsally 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., 1989; Tsubouchi et al., 2017).

The Commissures

Defined region of the commissures are shown in Figure S1.

Commissures derived from the larval aV commissure

The larval aV commissure is present in all three thoracic neuromeres. In the adult thoracic neuromeres it segregates into two distinct commissures which we called the **anterior Anterior Ventral Commissure** (aAV) and the **posterior Anterior Ventral Commissure** (pAV).

The **anterior Anterior Ventral Commissure** (aAV) is present in all three thoracic neuromeres and is formed by the primary neurite bundles of hemilineage 1A. It sits at the anterior of the neuromere at the ventralmost margins, outside the neuropil and cell cortex and anterior to the hemilineage 2A axons. The MesoTh aAV commissure was called the Accessory Prothoracic Commissure by Power (1948). The MetaTh aAV commissure was called the Accessory Commissure of the Metathoracic Neuromere by Power (1948).

Synonyms:
• Accessory Prothoracic Commissure (Power, 1948),
• APC (Power, 1948).
• Accessory Commissure of the Metathoracic Neuromere (Power, 1948),
• ACM (Power, 1948).

The **posterior anterior Ventral Commissure** (pAV) is present in all three thoracic neuromeres and is formed by the primary neurite bundles of hemilineages 13B and 14A. It sits at the anterior of the neuromere but crosses the midline posterior to the hemilineage 2A primary neurites (Shepherd et al., 2016). In the ProNm and MesoNm the axons form a single commissure but in MetaNm the axons of pAV are pulled apart to form two distinct commissures (pAVa and pAVp). The MesoNm pAV was called the Ventral Accessory Commissure of the Mesothoracic Neuromere by Power.

Synonyms:
• Ventral Accessory Commissure of the Mesothoracic Neuromere (Power, 1948),
• VAC (Power, 1948).

Commissures derived from the larval AI commissure

The larval anterior Intermediate Commissure (AI) is present in all three thoracic neuromeres and crosses the midline as two neurite bundles separated by the primary neurites of hemilineage 2A. The axon bundle anterior to 2A contains the fibers from hemilineages 10B and 18B, except in the ProNm where hemilineage 18B is not found. The neurite bundle posterior to 2A contains the neurites from hemilineages 7B and 8B Truman et al. (2004).

In the adult ProNm, the **Anterior Intermediate Commissure** (**AI**) segregates into three commissures. 1) The **ventral Anterior Intermediate Commissure (vAI)** formed by the 10B neurons which crosses anterior to the hemilineage 2A fibers. 2) the **Anterior Intermediate anterior Commissure** (**AIa**) formed by the 8B neurons and 3) the **Anterior Intermediate posterior Commissure** (**AIp**) formed by the 7B neurons. Both dorsal AI commissures cross the midline posterior to the 2A neurites with dAIa anterior to dAIp (Shepherd et al., 2016).

In the adult MesoNm aI commissure segregates into three separate commissures. 1) The **ventral Anterior Intermediate Commissure (vAI)** formed by the 10B neurons which

crosses the midline anterior to the 2A neurites. 2) A single **Anterior Intermediate Commissure** (**AI**) that contains fibers from both hemilineages 7B and 8B which crosses the midline posterior to the 2A neurites. The AI commissures in the MesoNm is one of the most prominent commissures of the VNC and was called the **Commissure of the Mesothoracic Neuromere** by Power (1948). 3) The **dorsal Anterior Intermediate Commissure** (**dAI**) containing the fibers of hemilineage 18B and crosses the midline anterior to hemilineage 2A.

In the MetaNm the AI commissure segregates into 2 commissures. 1) The **ventral Anterior Intermediate Commissure (vAI)** formed by the 10B neurons which crosses anterior to the 2A axons. 2) A single **Anterior Intermediate Commissure** (AI) that contains the fibers from hemilineages 7B, 8B and 18B to create the largest and most notable commissure of the VNC which was called the **Haltere Commissure** by Power (1948).

Synonyms:
• Commissure of the Mesothoracic Neuromere (Power, 1948),
• CMN (Power, 1948).
• Haltere Commissure (Power, 1948),
• cHIN (Merritt and Murphey, 1992; Pflüger et al., 1988; Tyrer and Gregory, 1982),
• ITD-HC (Boerner and Duch, 2010),
• HC (Power, 1948).

Commissures derived from the larval pI commissure

The larval posterior Intermediate Commissure (pI) is formed by neurites from hemilineages 5B, 6B, 12B, 19B and 23B. In all three thoracic neuromeres pI segregates into multiple commissures. In all three thoracic neuromeres the fibers of hemilineage 5B form the most anterior commissure the **anterior PI Commissure (aPI).** In the ProNm the fibers from hemilineages 6B, 12B and 23B remain closely bundled and cross the midline, slightly posterior and ventral to aPI, as a single commissure called the **posterior PI Commissure (pPI)**. The neurites from 19B form a separate, more dorsally located commissure called the **dorsal PI Commissure (dPI**) that crosses the midline close to, but anterior to the Posterior Dorsal Commissure (pD).

In the MesoNm and MetaNm the PI commissure segregates into four distinct commissural pathways. In addition to aPI formed by the 5B neurons and dPI by the 19B neuron, the pPI segregate into distinct commissures the **pPI anterior** (pPIa) commissure formed by hemilineage 6B neurites which is located just anterior to the **pPI posterior** (pPIp) commissure formed by 12B and 23B neurites.

None of these commissures has been previously identified.

Commissures derived from the larval pD commissure

The larval posterior Dorsal commissure (pD) is the dorsalmost commissure and is found in all three thoracic neuromeres. It contains the axons from hemilineage 6A. In the adult the commissure is the dorsalmost and located in the upper tectulum. Power (1948) only described this commissure in the mesothorax and called it the Posterior Dorsal Mesothoracic Decussation (PDD). We called all three commissures the **Posterior Dorsal Commissures (PD).**

Synonyms:
• MesoPDC (Proposed by group),

• Posterior Dorsal Mesothoracic Decussation (Power, 1948),
• PDD (Power, 1948).

Commissures formed by descending neurons

Commissure of Fine Fibers of the Intermediate Tract of the Dorsal Cervical Fasciculus (CFF)

In the MesoNm there is a robust commissure formed by the inner tracts of the intermediate tract of the dorsal cervical fasciculus (ITD-CFF), as they cross the midline, anterior to the haltere commissure in the upper tectulum above the MesoNm to terminate on the contralateral side.

Synonyms:
• CFF (Power, 1948).
• ITD-CFF BROAD (Boerner and Duch, 2010).

Other Commissures

In addition to the commissures that can be related to the projections of specific hemilineages there are two commissures described by Power (1948) that cannot be related to specific hemilineages.

Commissure of Prothoracic Neuromeres (CPN)
A transverse bundle of fibers that cross the midline in the ProNm. The fibers characteristically bow posteriorly and are dorsal to the dorsal lateral tracts of the ventral cervical fasciculus (DLV). The commissure crosses the midline just dorsal to the axons of the apI commissure. The arms of the bow are directed lateroanteriorly and extend almost to the lateral borders of the neuromeres.

Synonyms:
• CPN (Power, 1948),
• Prothoracic commissure (Bacon and Strausfeld, 1986).

Dorsal Accessory Commissure of the Mesothoracic Neuromeres (DAM)
A transverse thin bundle of fibers that crosses the dorsoposterior region of the MesoNm. It is ventral to the roots of the dorsal metathoracic (haltere) nerves, dorsal to the ventral ellipse, and ventroanterior to the haltere chiasma.

Synonyms:
DAM (Power, 1948).

Longitudinal Tracts

Defined region of the longitudinal tracts are shown in Figure 2 and Video S3

Dorsal Lateral Tract (DLT)

As its name implies the DLT is located in dorsal lateral neuropil, it is formed by fibers from the lateral bundles in the cervical (neck) connective and projects posteriorly and superficially at the dorsal lateral edge of the neuropil to terminate in the metathoracic neuromere. The tract contains the axons of descending neurons that innervate neck, wing, haltere and leg neuropils (Namiki et al, 2018). Histologically DLT has coarser fibers than the other tracts derived from the ventral bundles of the cervical connective described below.

Intermediate Tract of Dorsal Cervical Fasciculus (ITD)

The ITD is a dorsal tract, derived from the dorsal fibers in the connective and sits just medial to the DLT. According to Power (1948), the ITD projects posteriorly and separates into three adjacent tracts. The medialmost of these subdivisions, called ITD-CFF turns medially in the mesothoracic neuromere to cross its contralateral homolog to form the chiasma of fine fibers of the intermediate tracts of the dorsal cervical fasciculus (ITD-CFF). This longitudinal projection is what we now refer to as ITD. It contains the axons of many descending interneurons that terminate widely in neck, wing, haltere and leg neuropils (Namiki et al 2018). The other two subdivisions of ITD are now recognized as distinct tracts and called the Haltere Chiasma (ITD-HC) and the Haltere Tract (ITD-HT) respectively.

Intermediate Tract of Dorsal Cervical Fasciculus – Haltere Chiasma (ITD-HC)

ITD-HC is formed by the axons of the cHIN interneurons as they project anteriorly from the metathoracic neuromere. The axons originate from interneurons produced by metathoracic hemilineage 8B, the primary projections of which also form the major component of the Haltere Commissure. The tract itself extends anteriorly from the HC just lateral to the ITD and medial to the HC. This tract was termed cHIN by Merritt and Murphey (1992).

Haltere Tract ITD-(HT)

The haltere tract is the most lateral component of Power's (1948) ITD and is composed of many large-diameter fibers that can be traced as a bundle into the cervical connective. The HT is formed by the sensory afferent axons (Ghysen, 1980; Strausfeld and Seyan, 1985) from the dorsal metathoracic nerve (Haltere Nerve) entering the metathoracic neuromere and extending anteriorly through the cervical connective (Merritt and Murphey, 1992; Power, 1948). The tract has small arborizations with some of the Fibers bending anterolaterally to become part of the haltere commissure (HC) in the metathoracic neuromere, while others turn ventrally and straggle into the dorsolateral part of the mesothoracic neuromere where they are quickly lost (Power, 1948).

Dorsal Medial Tract (DMT)

The **Dorsal Medial Tract** was previously called the Median Tracts of the Dorsal Cervical Connective (MTD) by Power, (1948) and Merritt and Murphey (1992); we propose DMT as an alternative to be more consistent with the rest of the tract nomenclature. The DMT is derived from the dorsal bundles in the cervical connective and extends posteriorly and bows laterally slightly, in the mesothoracic region, bending again medially, towards each other, at the narrowing between the meso- and metathoracic neuromeres. The tract turns laterally and posteriorly at the level of the haltere commissure and enters the metathoracic neuromere, where it forms collateral fibers that merge with the oblique tract of the metathoracic leg nerve (Power, 1948). The DMT contains the projections of a large number of descending neurons

that innervate both the dorsal and ventral tectulum and the leg neuropils (Namiki et al., 2018).

Dorsal Lateral Tract of Ventral Cervical Fasciculus (DLV)

The DLV derives from the ventral bundles of the cervical connective. It contains coarser fibers than the other tracts derived from the ventral bundles of the cervical connective (DLV, VLT and VTV). The tract broadens as it extends posteriorly, in a medial position and just ventral to the tectulum to terminate in the mesothoracic neuromere merging with the Oblique Tract.

Ventral Median Tract of Ventral Cervical Fasciculus (VTV)

VTV is the ventralmost tract in the VNC. It runs adjacent to the midline and derives from the ventral bundles of the cervical connective. The tract extends posteriorly on either side of the midline just under the ventral most part of the lower tectulum until they bend dorsally and terminates in the ventral-anterior region of the abdominal ganglion. A few fibers extend laterally in into the leg neuropil of each neuromere before the tract terminates in the abdominal ganglion (Power, 1948).

Median Dorsal Abdominal Tract (MDT)

MDT is the dorsalmost tract in the VNC, sits close to midline (Merritt and Murphey, 1992) and runs dorsally along the length of the tectulum (Boerner and Duch, 2010) past the haltere chiasma to terminate into the abdominal neuromeres (Power, 1948). It is the medialmost of the three small dorsal tracts which connects the thoracic and abdominal neuromeres.

Lateral Dorsal Abdominal Tract (LDT)

The lateral pair of three small dorsal tracts which connects the thoracic and abdominal neuromeres dorsal to the metathoracic neuromeres (Power, 1948).

Synonyms:
• LDT (Proposed by group).

Ventral Cervical Fasciculus (VCF)

Ventral fascicle of the dorsal tectulum. It enters the thoracico-abdominal mass along with the dorsal bundles and takes a more ventral course. There are two of these fascicles. Each fascicle passes posteriorly between the prothoracic and at an intermediate position in these neuromeres, dividing into the dorsal lateral (DLV) and ventral median (VTV) tracts (Power, 1948).

Synonyms:
• VCF (Power, 1948).

Dorsal Cervical Fasciculus (DCF)

Dorsal fascicle of the dorsal tectulum. It occupies the uppermost region within the anterior part of the dorsal tectulum. The fibers slope gently ventrally, so that by the time they have passed over the anterior tips of the mesothoracic neuromeres, they have descended to a relatively lower position, below the mesothoracic decussations. There are two of these fascicles. Near the anterior end of the VNC each of the dorsal fasciculus separates forming three poorly defined longitudinal tracts: the dorsal lateral (DLT), intermediate (ITD) and dorsal median tract (DMT) (Power, 1948).

Synonyms:
• DCF (Power, 1948).

Ventral Ellipse (VE)
Flattened annulus of fibers which lies in a frontal plane that crosses the midline at the posterior edge of the prothoracic mVAC and extends axons posteriorly to the isthmus between the meso- and metathoracic neuromeres. It lies immediately below the dorsal decussations of the mesothoracic and metathoracic neuromeres. A right and left arm of fibers extends lateroposteriorly from the posterior end of the ellipse into the respective metathoracic neuromeres, and within them, joins the bundle which runs out into the third leg nerve. At the anterior end, the ventral ellipse incorporates the dorsolateral tracts of the ventral fasciculi (DLV) (Power, 1948).

Synonyms:
• VE (Power, 1948).

The peripheral nerves
Most nerves have been historically well defined by Power (1948) in which case the definitions are maintained, however, with some of the abdominal nerves we decided to utilise later terms (Shepherd and Smith, 1996) naming each nerve according to its neuromere of origin to give them a more consistent naming scheme than the original. Defined regions for each peripheral nerve are shown in Figure S3

Cervical Connective (CvC)
Major axon tract connecting the posteriormost subesophageal ganglion to the VNC in the adult central nervous system (Power, 1948).

Synonyms:
• CvC (Power, 1948),
• cephalo-thoracic cord (Bodenstein, 1950),
• cephalo-thoracic nerve strand,
• CC (Merritt and Murphey, 1992),
• CV (Ito et al., 2014),
• CvCon (Miller and Demerec, 1950).

Cervical Nerve (CvN)
A bilaterally paired nerve that connects laterally to the cervical connective, immediately posterior to where it enters the thorax. Each cervical nerve extends laterally, branching and innervating horizontal muscles of the anterior thorax (Power, 1948).

Synonyms:
• CvN (Power, 1948),
• nerve to crop (Miller and Demerec, 1950),
• CvNv (Miller and Demerec, 1950).

Dorsal nerves directly innervating the neuropil of the prothoracic neuromere
The dorsal prothoracic nerve, prosternal nerve, prothoracic chordotonal nerve, prothoracic accessory nerve and the ventral prothoracic nerve emerge closely adjacent to each other in

the anteriolateral corner of the prothoracic neuromere. Their proximity is such that in some preparations they appear to share a common exit point.

Dorsal Prothoracic Nerve (DProN)
A nerve that projects latero-anteriorly from the antero-lateral corner of the ventral nervous cord (VNC) (prothoracic neuromere). It splits into 4 or more branches before innervating various muscles (Power, 1948).

Synonyms:
• DProN (Proposed by group),
• ADN (Power, 1948),
• anterior dorsal nerve (Power, 1948),
• DPN (Merritt and Murphey, 1992),
• First dorsal nerve (Miller and Demerec, 1950),
• DNv1 (Miller and Demerec, 1950).

Prosternal Nerve (PrN)
A slender nerve that projects anteriorly from the ventral nerve cord (VNC), medial to the base of the dorsal prothoracic nerve to the prosternal sense organ (Power, 1948).

Synonyms:
• PrN (Power, 1948),
• PN (Merritt and Murphey, 1992).

Prothoracic Chordotonal Nerve (ProCN)
Very short and thick nerve that arises in the prothoracic neuromere, immediately below the anterior dorsal and prosternal nerves, and connects to each prothoracic chordotonal sense organ (Power,1948).

Synonyms:
• ProCN (Proposed by group),
• CN (Power, 1948).

Prothoracic Accessory Nerve (ProAN)
A mixed motor-sensory nerve that connects to the prothoracic neuromere, slightly posterior and ventral to the anterior prothoracic chordotonal organ and slightly dorsal to the root of the ventral prothoracic nerves. It extends laterally and dorsally almost to the lateral body wall before branching to innervate muscles (Power, 1948).

Synonyms:
• ProAN (Proposed by group),
• accessory prothoracic nerve,
• PAN (Power, 1948),
• First accessory nerve (Miller and Demerec, 1950),
• AcNv1 (Miller and Demerec, 1950).

Ventral Prothoracic Nerve (VProN)
A mixed motor-sensory nerve that carries axons from two clusters of microchaetae on the prothoracic coxa and to motor neuron fibers from lateral anterior muscles. It connects to the

prothoracic neuromere just dorsal to the root of the prothoracic leg nerve and branches about halfway along its length into a motor branch that stays within the body and a sensory branch that projects to the leg (Power, 1948).

Synonyms:
• VProN (Proposed by group),
• VPN (Power, 1948),
• prosternal sense organ (Miller and Demerec, 1950),
• PSO (Miller and Demerec, 1950).

Prothoracic Leg Nerve (ProLN)
A nerve that carries a mix of motor and sensory axons from the prothoracic leg to the adult prothoracic neuromere. Each nerve extends laterally and slightly anteriorly from the ventral anterior region of the prothoracic neuromere (Power, 1948).

Synonyms:
• ProLN (Proposed by group),
• PLN (Power, 1948),
• T1LN (Merritt and Murphey, 1992),
• First ventral nerve (Miller and Demerec, 1950),
• VNv1 (Miller and Demerec, 1950).

Anterior Dorsal Mesothoracic Nerve (ADMN)
A mixed sensory-motor nerve that is the thicker of the two dorsal nerves of the mesothorax. The nerve enters the mesothoracic neuromere slightly anterior and dorsal to the smaller PDMN. The ADMN projects anteriorly and dorsally (Power, 1948).

Synonyms:
• ADMN (Power, 1948),
• second dorsal nerve BROAD (Miller and Demerec, 1950),
• DNv2 BROAD (Miller and Demerec, 1950),
• wing nerve BROAD (Merritt and Murphey, 1992).

Wing Nerve
A nerve that carries sensory fibers from the sense organs of the wing, eventually joining the anterior dorsal mesothoracic nerve (ADMN) (Merritt and Murphey, 1992).

Posterior Dorsal Mesothoracic Nerve (PDMN)
A nerve that arises from the ventral nerve cord (VNC), just posterior to the root of the anterior dorsal mesothoracic nerve. It projects posterolaterally before branching, with one branch innervating the tergal depressor of the trochanter (jump muscle), while the other branch forms further, terminal branches that innervate targets including the dorsal medial muscle (dorsal longitudinal muscle) (Power, 1948).

Synonyms:
• PDMN (Merritt and Murphey, 1992),
• PDM (Power, 1948)
• second dorsal nerve BROAD (Miller and Demerec, 1950),
• DNv2 BROAD (Miller and Demerec, 1950).

Mesothoracic Accessory Nerve (MesoAN)

A nerve that arises from the lateroposterior side of the mesothoracic neuromere at a point anterior to the root of the haltere nerve. It extends posteriorly and slightly dorsally and laterally, around the anterior wings of the mesofurca, before branching. One branch innervates the furcoentopleural muscles (muscles 59 and 60) and the other innervates laterally placed muscles, anterior to the halteres (Power, 1948).

Synonyms:
- MesoAN (Proposed by group),
- MAC (Power, 1948),
- accessory mesothoracic nerve (Power, 1948)
- second accessory nerve (Miller and Demerec, 1950),
- AcNv2 (Miller and Demerec, 1950).

Mesothoracic Leg Nerve (MesoLN)

A mixed motor-sensory nerve that arises ventrally from the mesothoracic neuromere. It splits at its base, with a small number of axons innervating a ventral muscle that is posterior-lateral to the tergal depressor of the trochanter (jump muscle) and the rest projecting into the mesothoracic leg (Power, 1948).

Synonyms:
- MesoLN (Proposed by group),
- T2LN (Merritt and Murphey, 1992),
- second ventral nerve (Miller and Demerec, 1950),
- VNv2 (Miller and Demerec, 1950),
- ventral mesothoracic nerve.

Dorsal Metathoracic Nerve (DMetaN)

A thick nerve that primarily carries sensory axons from the haltere to the metathoracic neuromere. It extends anteriorly and somewhat medially to terminate in the center of the metathoracic neuromere. Its fibers extend anteriorly, contributing to the tectulum (Power, 1948).

Synonyms:
- DMetaN (Proposed by group),
- haltere nerve (Power, 1948),
- HN (Power, 1948),
- third dorsal nerve (Miller and Demerec, 1950),
- DNv3 (Miller and Demerec, 1950).

Metathoracic Leg Nerve (MetaLN)

A large sensory motor nerve that originates in the ventral metathoracic neuromere and innervates the metathoracic leg (Power, 1948).

Synonyms:
- MetaLN (Proposed by group),
- MLN (Power, 1948),
- T3LN (Merritt and Murphey, 1992),
- third ventral nerve (Miller and Demerec, 1950),
- VNv3 (Miller and Demerec, 1950),

• ventral metathoracic nerve.

The Abdominal Nerves

Emerging from the abdominal neuromeres are four paired nerves that extend posteriorly and laterally into the abdomen.

First Abdominal Nerve (AbN1)

The first abdominal nerve apparently emerges from the metathoracic neuromere but contains axons that originate/terminate in the first abdominal neuromere. The nerve exits laterally just dorsal to the exit of the metathoracic leg nerve (Shepherd and Smith, 1996).

Synonyms:
• AbN1 (Shepherd and Smith, 1996),
• accessory metathoracic nerve,
• MA (Power, 1948),
• metathoracic accessory nerve (Power, 1948),
• nerve of the 1rst abdominal segment (Miller and Demerec, 1950),
• Ab1Nv (Miller and Demerec, 1950).

Second Abdominal Nerve (AbN2)

A nerve that apparently emerges in the dorsalmost region of the metathoracic neuromere but contains axons that originate/terminate in the second abdominal neuromere. The nerve projects postero-laterally to the most posterior-lateral corner of the thorax, where it innervates transverse tubular muscles. This nerve also contains the afferent fibers from a multiscolophorous organ located on the ventral surface of the second abdominal segment (Shepherd and Smith, 1996).

Synonyms:
• AbN2 (Shepherd and Smith, 1996),
• extra metathoracic nerve (Power, 1948),
• EMN (Power, 1948),
• nerve of the second abdominal segment (Miller and Demerec, 1950),
• Ab2Nv (Miller and Demerec, 1950).

Third Abdominal Nerve (AbN3)

Lateralmost of the two bilaterally paired nerves connected to the abdominal neuropil. It is thin, containing only fine fibers (Shepherd and Smith, 1996).
Synonyms:
• AbN3 (Shepherd and Smith, 1996),
• First lateral abdominal nerve (Power, 1948),
• FLA (Power, 1948),
• nerve of the third abdominal segment (Miller and Demerec, 1950),
• Ab3Nv (Miller and Demerec, 1950).

Fourth Abdominal Nerve (AbN4)

Medialmost of the two bilaterally paired nerves connected to the abdominal neuropil. (Shepherd and Smith, 1996).

Synonyms:

• AbN4 (Shepherd and Smith, 1996),
• second lateral abdominal nerve (Power, 1948),
• SLA (Power, 1948),
• nerve of the fourth abdominal segment (Miller and Demerec, 1950),
• Ab4Nv (Miller and Demerec, 1950).

Abdominal Nerve Trunk (AbNT)

A fused terminal nerve that projects posteriorly along the midline from the posterior of the abdominal neuropil (Shepherd and Smith, 1996).

Synonyms:
• AbNT (Shepherd and Smith, 1996),
• abdominal median nerve trunk,
• median nerve trunk (Middleton et al., 2006),
• AbNvTr (Middleton et al., 2006),
• MAN (Power, 1948),
• median abdominal nerve (Power, 1948),
• terminal abdominal nerves (Miller and Demerec, 1950),
• AbTNv (Miller and Demerec, 1950).

Figure S1- Related to Figure 2

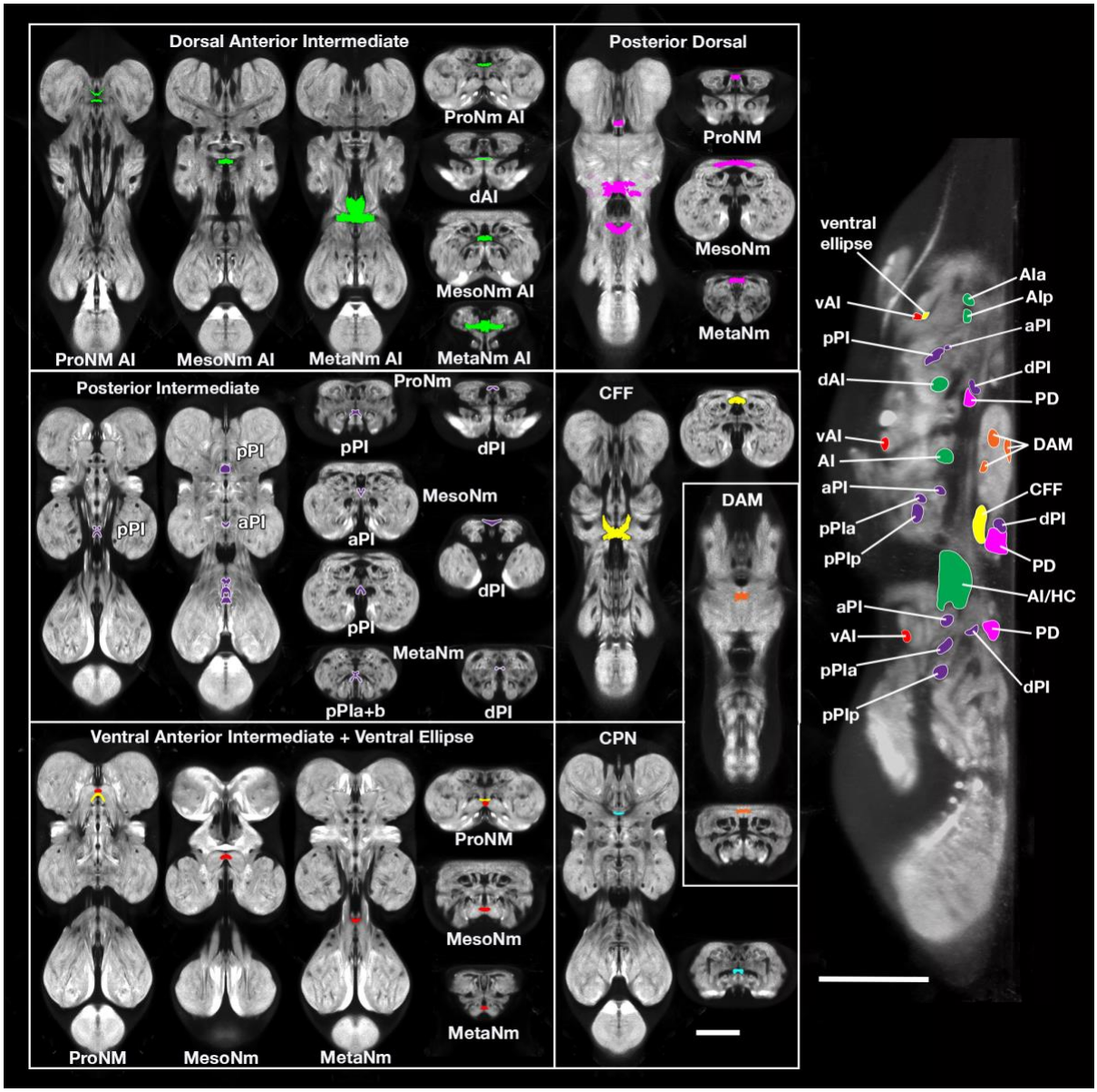

Figure S1- Related to Figure 2) The major identified commissures of the VNC – the positions of the major commissures of the VNC shown in horizontal, transverse and lateral sections. The horizontal and transverse sections are taken at planes that transect each commissure to reveal the commissural position against the neuropil structure. A list of the abbreviations is given in Table 1. Scale 50μm

Figure S2 – Related to Figure 1

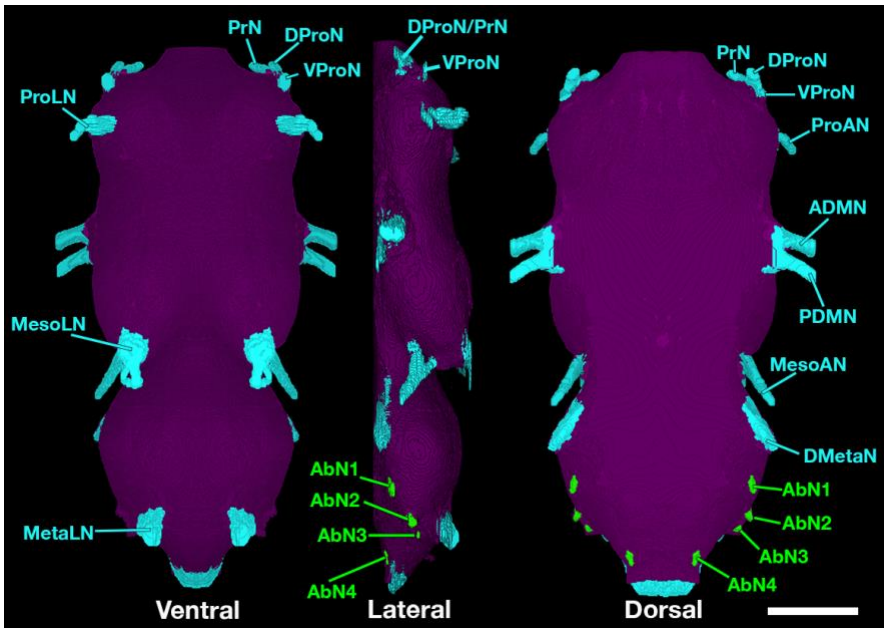

Figure S2 – Related to Figure 1) Peripheral nerves – Rendered volume of the VNC showing the location of the peripheral nerve roots of the VNC from ventral, lateral and dorsal view. A list of the abbreviations is given in Table 1. Scale 50µm