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A Systematic Nomenclature for the *Drosophila* Ventral Nervous System

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Abstract

Insect nervous systems are proven and powerful model systems for neuroscience research with wide relevance in biology and medicine. However, descriptions of insect brains have suffered from a lack of a complete and uniform nomenclature. Recognising this problem the Insect Brain Name Working Group produced the first agreed hierarchical nomenclature system for the adult insect brain, using *Drosophila melanogaster* as the reference framework, with other insect taxa considered to ensure greater consistency and expandability (***Ito et al., 2014***). ***Ito et al.*** (***2014***) purposely focused on the gnathal regions that account for approximately 50% of the adult CNS. We extend this nomenclature system to the sub-gnathal regions of the adult *Drosophila* nervous system to provide a nomenclature of the so-called ventral nervous system (VNS), which includes the thoracic and abdominal neuromeres that was not included in the original work and contains the neurons that play critical roles underpinning most fly behaviours.

Background

Insect nervous systems are proven and powerful model systems for neuroscience research with wide relevance for biology and medicine. Although vast anatomical, physiological and molecular data are already available, integrating this information into a common analytical framework would generate an even more powerful resource. Computational analysis combined with digital microscopy now make it possible to consolidate data from multiple techniques and transform how we analyse nervous system function (***Jenett et al., 2006***; ***Dance, 2015***; ***Boettiger et al., 2016***). It is no longer sufficient to use 2D labelled diagrams and photomicrographs to identify and define anatomical structures, as it is now possible to use multilayer microscopy with computational reconstruction to precisely define and allocate boundaries and structures in 3D. This requires a systematic and consistent nomenclature to precisely define anatomical structures and boundaries. Furthermore the definitions and nomenclature need to be rationalised as multiple names can be used for the same structure. The precise inter relationships between structures also need to be specified. Once this is complete, new findings can be more easily added to this framework allowing

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a complete picture of all knowledge to be more readily accessed and enabling consistent analysis of anatomical data from different studies. Recognising this problem, a consortium of neurobiologists studying arthropod brains, the insect brain name working group (***Ito et al., 2014***) (IBNWG), was established and produced the first agreed hierarchical nomenclature system for the insect brain, using *Drosophila melanogaster* as the reference framework, with other insect taxa considered to ensure greater consistency and expandability (***Ito et al., 2014***). ***Ito et al.*** (***2014***) purposely focused on the gnathal regions that account for approximately 50% of the total adult Central Nervous System. Here we extend this nomenclature system (***Ito et al., 2014***) to the sub-gnathal regions of the adult *Drosophila* nervous system to provide a nomenclature of the so-called ventral nervous system (VNS), which includes the thoracic and abdominal neuromeres that was not included in the original work (***Ito et al., 2014***) and contains the neurons that play a critical role in effecting most fly behaviors. The VNS is an often overlooked but vital part of the insect central nervous system that is necessary for nearly all motor output (***Burrows, 1996***; ***Büschges et al., 2008***; ***Robertson et al., 1982***), and will be increasingly important as interest in behavior grows (***Lemon et al., 2015***; ***Pitmon et al., 2016***; ***Seeds et al., 2014***). ***Power*** (***1948***), ***Merritt and Murphey*** (***1992***) and ***Boerner and Duch*** (***2010***) all made previous attempts to provide anatomical frameworks for the fly VNS. These core works, plus many other additional studies, have created a rich catalogue of anatomical detail but the inconsistent approach to nomenclature and definitions has created ambiguity and confusion. The aim of the *Drosophila* adult VNS working group (DAVWG) was to create a nomenclature, definitions and spatial boundaries for the key anatomical entities of the *Drosophila* VNS.

Organization of the Working Group

The initial phase of work was similar to that adopted by the original Insect Brain Name Working Group (IBNWG) to create the nomenclature for the *Drosophila* brain (***Costa et al., 2013***; ***Ito et al., 2014***). We gathered together researchers who are leading relevant work or those who have made recognized historical contributions to the field of *Drosophila* VNS. We also included some individuals who have an expertise in development, some with knowledge of the VNS in other insect species, as well as representatives of the original IBNWG 2014. Prior to the workshop, a small group compiled a document listing all of the named regions found in the published literature and from the existing *Drosophila* anatomy ontology (***Costa et al., 2013***). The group also created additional representative anatomical datasets where required. These datasets and a list of definitions were assembled into a working document and shared online prior to the workshop. At the workshop held at Janelia Research Campus on October 2013 the task group systematically reviewed the published anatomical data and unpublished data provided by the participants, while compiling a proposed list of of nomenclature that was agreed upon by all members. Afterwards, the working document and its content were updated and reviewed by the task group. The document was next distributed to a wider constituency of individuals with a broader species based knowledge as well as any interested parties for feedback. Feedback and amendments were agreed, ultimately resulting in this publication.

Establishing the anatomical framework

Establishment of a systematic nomenclature requires two steps: (1) a clear morphological and spatial definition of all the structures to be named and (2) a standard and clear naming scheme. The neuropilar regions of the VNS are typically regarded as being 'unstructured' or 'tangled', or having a fine, granular appearance in sections (***Merritt and Murphey, 1992***) with different regions distinguished only by general spatial terms such as, 'intermediate' (***Merritt and Murphey, 1992***) or 'dorsal'. Despite this, different volumes of VNS neuropil can be defined in relation to fixed landmarks such as the longitudinal tracts, commissures (***Shepherd et al., 2016***). Recently, ***Shepherd et al.*** (***2016***) have used the primary projections of neuronal hemilineages to provide a vital organizational principle for defining the substructure of the neuropil. Although these landmarks may not always correspond to

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the underlying functional organisation, they provide a consistent means of structurally defining neuropil regions.

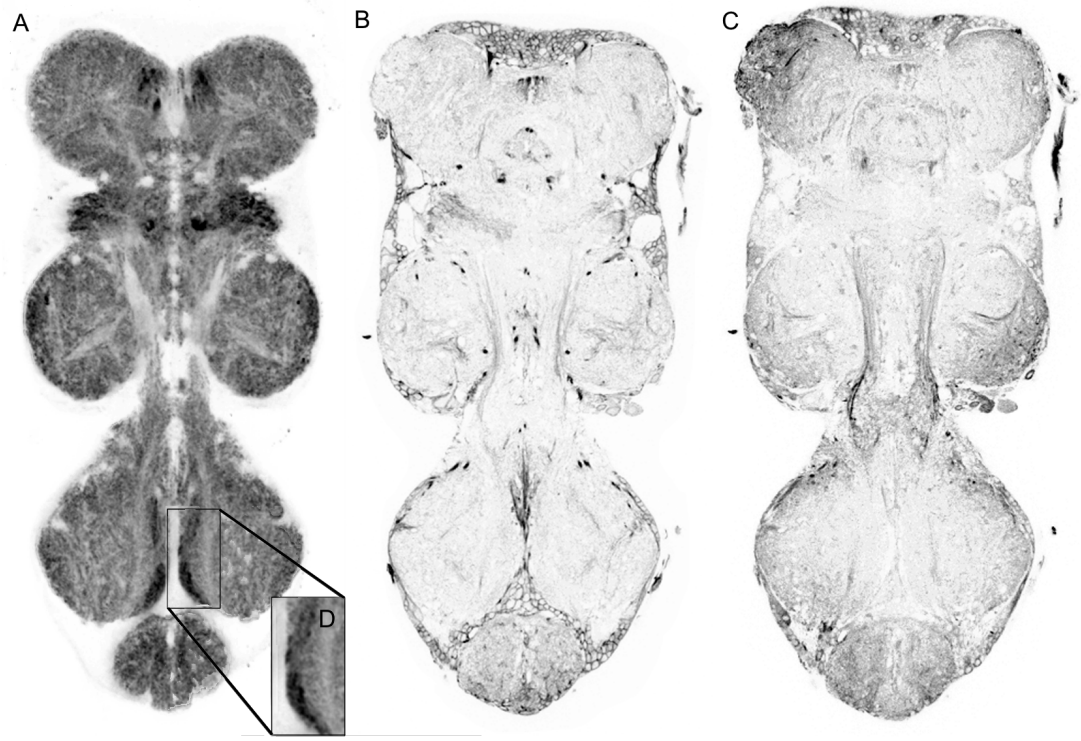

Figure 1. Frontal section through midsection of the adult VNS. **A** shows the neuropil marked by N-cadherin expression in the synapses, **B** shows neuroglian expression demonstrating the high intensity points of the tracts that guide lineage neurite projection, **C** shows tubulin expression used to highlight tracts and **D** highlights the dense neuropil regions associated with sensory connections. All levels are inverted for the purposes of clarity in print.

To provide an initial framework for establishing distinct boundaries within the VNS, we created confocal datasets that reveal various aspects of VNS structure, including tracts and neuropil. An anti alpha tubulin antibody (***Figure 1***C) was used to reveal fibrous structures such as longitudinal tracts and commissures. An anti-Drosophila N-cadherin (DNx8) was used to visualize neuropils according to the density of an active-zone-specific protein, mirroring exactly the structures revealed by nc82 (bruchpilot) immunostaining (***Wagh et al., 2006***). This allowed us to distinguish between neuropils that are poor in synapses, such as regions occupied by axons, primary neurites, and glial processes, which are left unlabeled (***Figure 1***A), and synapse-rich regions such as the primary sensory neuropils (***Figure 1***D). The anti-neuroglian antibody (***Figure 1***B) was used to reveal the projections of clonally related neurons in neuroblast (NB) hemilineages (***Shepherd et al., 2016***). The images obtained with these labeling methods are available via the Brain Explorer function of the FlyBrain Neuron Database (http://ndb.flybrain.org). To ensure consistency with previous and ongoing studies, we took into account the known projection patterns of various neurons, including those of single identified neurons (giant fiber neuron) and the trajectories of their fiber bundles (sensory afferents from the halteres). Information about VNS organization in other insect species was integrated wherever relevant.

The naming scheme

The key principle for the rationalisation of the nomenclature was to, wherever possible, integrate the current terminology into the newly developed standard. In doing so, we reused the existing terminology as much as possible and changes were only made where there was a need to remove

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ambiguity. When multiple names for an anatomical entity had been used in the literature, the principle was to give preference to the name that was in most popular usage. Where there was a need to create new terms we developed a standard and clear naming scheme (e.g. naming of the newly identified structures). We also sought consistency with terms used for earlier developmental stages and in other insects but in all cases avoiding the implication of any homology. We were also driven by the need to separate the naming scheme, driven by morphology, from function. Details about the latter, however, have been included in the definition, whenever known.

In developing a standard and clear naming scheme, we wished to encourage a more consistent use of terms. We recognize, however, that users may be inclined to continue using the nomenclature of their choice. In acknowledging this issue, we have captured all known synonyms for each term, including references. Users will be able to search for terms in the DAO using any of these synonyms.

Abbreviations

We adopted a systematic approach when developing abbreviations for each named anatomical entity based on the following points. The first was to adopt abbreviations that are unique across the whole CNS, avoiding abbreviations already in use for unrelated regions in the VNS and the brain (for disambiguation purposes, the letter case is ignored). The second was to create a system in which related entities would be easily recognizable (for example, all commissures). Finally, we tried to be consistent with standard neuroanatomical nomenclature e.g. BrainName (***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, at this level for "nerve" and the 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 had developed for specific structures the abbreviation that provided the clearest indication with least likelihood of confusion was agreed by consensus.

Known abbreviations were captured as synonyms and references were added whenever possible.

Axis orientation

Unlike the cephalic neuromeres (Ito et al., 2014) the issue of specifying general axis orientation for the VNS is relatively straightforward. The neuroaxis and the body axis are the same, with the prothoracic neuromere being anteriormost and the abdomen (abdominal ganglionic complex) being the most posterior. In the dorsal/ventral plane the tectulum is superior or dorsal and the leg nerves inferior or ventral. The left and right sides are applied as if the sample is viewed from the above (superior/dorsal). The axis is shown in all figures by arrows pointing towards the anterior (A), superior/dorsal (S) and the right (R).

Identifying and defining structures

Neuromere Boundaries

The thoracic neuromeres consist of three paired enlargements at the anterior end of the VNS, corresponding to the prothoracic, mesothoracic and metathoracic segments, and a single posterior, dorsally located mass, the abdominal neuromeres, that is composed of all the fused abdominal neuromeres.

The scaffold of axon fibres revealed by neuroglian expression provides a tool to define the internal segmented structure of the neuropils of the VNS. Since each neuromere is founded by a specific set of NBs, the neuroglian bundles produced by their progeny create a neuromere specific

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Table 1. Summary of the nomenclature for VNS structures.

Major neuromeres and Neuropil	**Described Tracts**
Prothoracic neuromere (ProNm)	Dorsal lateral tract (DLT)
Mesothoracic neuromere (MesoNm)	Intermediate tract of dorsal cervical fasciculus (ITD)
Accessory mesothoracic neuropil (AMNp)	Dorsal Medial Tract (DMT)
Tectulum (Tct)	Dorsal lateral tract of ventral cervical fasciculus (DLV)
Metathoracic neuromere (MetaNm)	Ventral lateral tract (VLT)
Abdominal neuromeres (ANm)	Ventral median tract of ventral cervical fasciculus (VTV)
The Commissures	Median dorsal abdominal tract (MDT)
Prothoracic Anterior Ventral Anterior Commissure (ProAVAC)	Ventral cervical fasciculus (VCF)
Prothoracic Anterior Ventral Posterior Commissure (ProAVPC)	Dorsal cervical fasciculus (DCF)
Mesothoracic Anterior Ventral Anterior Commissure (MesoAVAC)	Ventral ellipse (VE)
Mesothoracic Anterior Ventral Posterior Commissure (MesoAVPC)	**The peripheral nerves**
Metathoracic Anterior Ventral Anterior Commissure (MetaAVAC)	Cervical connective (CvC)
Metathoracic Anterior Ventral Posterior Commissure (MetaAVPC)	Cervical nerve (CvN)
Prothoracic Anterior Intermediate Commissure (ProAIC)	Dorsal prothoracic nerve (DProN)
Mesothoracic Anterior Intermediate Ventral Commissure (MesoAIVC)	Prosternal nerve (PrN)
Mesothoracic Anterior Intermediate Dorsal Commissure (MesoAIDC)	Prothoracic chordotonal nerve (ProCN)
Metathoracic Anterior Intermediate Ventral Commissure (MetaAIVC)	Prothoracic accessory nerve (ProAN)
Metathoracic Anterior Intermediate Dorsal Commissure (MetaAIDC)	Ventral prothoracic nerve (VProN)
Prothoracic Posterior Intermediate Anterior Commissure (ProPIAC)	Prothoracic leg nerve (ProLN)
Prothoracic Posterior Intermediate Posterior Commissure (ProPIPC)	Anterior dorsal mesothoracic nerve (ADMN)
Prothoracic Posterior Intermediate Anterior Dorsal (ProPIDC)	Posterior dorsal mesothoracic nerve (PDMN)
Prothoracic Posterior Intermediate Anterior Ventral (ProPIVC)	Mesothoracic accessory nerve (MesoAN)
Mesothoracic Posterior Intermediate Anterior Commissure (MesoPIAC)	Mesothoracic leg nerve (MesoLN)
Mesothoracic Posterior Intermediate Posterior Commissure (MesoPIPC)	Dorsal metathoracic nerve (DMetaN)
Metathoracic Posterior Intermediate Anterior Commissure (MetaPIAC)	Metathoracic leg nerve (MetaLN)
Metathoracic Posterior Intermediate Posterior Commissure (MetaPIPC)	First abdominal nerve (AbN1)
Prothoracic Posterior Dorsal Commissure (ProPDC)	Second abdominal nerve (AbN2)
Mesothoracic Posterior Dorsal Commissure (MesoPDC)	Third abdominal nerve (AbN3)
Metathoracic Posterior Dorsal Commissure (MetaPDC)	Fourth abdominal nerve (AbN4)
Commissure of the fine fibers of the intermediate tract of the dorsal cervical fasciculus (CFF)	Abdominal nerve trunk (AbNT)
Commissure of prothoracic neuromeres (CPN)	**Individual Neurons**
Dorsal accessory commissure of the mesothoracic neuromeres (DAM)	Giant fiber neuron (GF)

set of anatomical markers that provide a framework to define each neuromere as well as the tectulum and many of the commissures.

Tracts and Commissures

Power (***1948***) defined, later volumetrically clarified by ***Boerner and Duch*** (***2010***), the major commissures of the adult VNS. ***Truman et al.*** (***2004***) showed that in the larva the postembryonic secondary lineages crossed the midline via specific and invariant commissural pathways. Using the neuroglian labelled tracts ***Shepherd et al.*** (***2016***) were able to link the larval commissures to their adult counterparts to reveal the developmental origins of many of the commissures in the adult (VNS). This work not only confirmed the developmental origins of the commissures identified by ***Power*** (***1948***) but also identified several smaller commissures not previously observed.

The Tectulum

In the transverse plane the neuroglian tracts also help to define another substructure within the VNS: the tectulum. ***Power*** (***1948***) defined this region as a "distinct subdivision of the thoracic regions of the [VNS]. The region forms a saddle-like structure located dorsally primarily over the mesothoracic neuropil but extending over the posterior-most region of the prothoracic neuromere and the anteriormost region of the metathoracic neuropil". Despite the subsequent work done on the VNS this remained the best definition of the tectulum.

The neuroglian framework of lineage derived from primary neurite tracts provides a clear and easily recognisable boundary that more precisely circumscribes the tectulum (***Shepherd et al., 2016***). The tectulum is the dorsal region of the neuropil posterior to the anterior most limits of the T1 hemilineage 12B neuroglian tract but dorsal to the tracts from the T1 hemilineages 12B, 6A and B, 23 17 and 18. It extends posteriorly through T2 remaining dorsal to the tracts from hemilineages 3B, 6A and B and 23 to the entry point of hemilineage 3A in T3.

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The Peripheral Nerves

Most of the peripheral nerves have been historically defined by ***Power*** (***1948***) in which case the definitions were simply collated. However for some of the abdominal nerves we decided to use later terms (***Shepherd and Smith, 1996***) which names each nerve according to its neuromere of origin, giving them a more consistent naming scheme than the original.

Discussion

In this project, we addressed two primary issues in order to create a clearer understanding of the VNS structure and to promote dialogue 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 VNS. The second was to create a clear and consistent naming scheme for each anatomical entity. The detailed VNS map we provide will be invaluable for integrating past and future work into a common space, thereby contributing to new lines of investigation. In addition, our effort will also inform researchers working with other insects, providing them with a template that could be adapted to their own model organism.

Although the nomenclature developed in this project will serve as the standard, we acknowledge that to be useful it must be maintained as a 'living' process and evolve as our understanding of the VNS structure and function grows. Future revisions and additions will be required and this will be handled via the existing online system for posting anatomy ontology suggestions located at github.com/FlyBase/drosophila-anatomy-developmental-ontology/issues and maintained by VirtualFlyBrain.org.

Unlike the brain, the VNS in insects demonstrates extraordinary diversity in its gross organisation and structure (***Niven et al., 2008***). There is, however, a large anatomical literature for several insect groups that exhibit markedly different VNS structures (e.g. grasshoppers, crickets and moths) that sometimes use the same terms as used for Drosophila. Whilst it is desirable to describe the correspondence of terminology between species, as was done for the more conserved insect brain, for the VNS this is not so readily achievable and only possible for major structures (neuromere, tract, nerve). To create consistent cross species terminology for more detailed structures requires a programme of work to confirm homology rather than rely on inference from similar structure. For this reason we elected to not expand this nomenclature to other insects. It is intended that our work will provide a framework to then explore cross species homologies in the VNS.

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References

Bacon JP, Strausfeld NJ. The dipteran 'Giant fibre' pathway: neurons and signals. Journal of Comparative Physiology A. 1986; 158(4):529–548. http://dx.doi.org/10.1007/BF00603798, doi: 10.1007/BF00603798.

Bodenstein D. In: The postembryonic development of Drosophila. Wiley; 1950. p. 275–367.

Boerner J, Duch C. Average shape standard atlas for the adult Drosophila ventral nerve cord. J Comp Neurology. 2010; 518(13):2437–2455. doi: 10.1002/cne.22346.

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Boettiger AN, Bintu B, Moffitt JR, Wang S, Beliveau BJ, Fudenberg G, et al. Super-resolution imaging reveals distinct chromatin folding for different epigenetic states. Nature. 2016; doi: 10.1038/nature16496.

Burrows M. The neurobiology of an insect brain. OUP Oxford; 1996.

Büschges A, Akay T, Gabriel J, Schmidt J. Organizing network action for locomotion: insights from studying insect walking. Brain research reviews. 2008; .

Costa M, Reeve S, Grumbling G, David O. The Drosophila anatomy ontology. J Biomed Semantics. 2013; 4(1):32. doi: 10.1186/2041-1480-4-32.

Dance A. Core Concept: CRISPR gene editing. Proceedings of the National Academy of Sciences of the United States of America. 2015; doi: 10.1073/pnas.1503840112.

Ghysen a. The projection of sensory neurons in the central nervous system of Drosophila: choice of the appropriate pathway. Developmental biology. 1980; 78(2):521–541. doi: 10.1016/0012-1606(80)90351-6.

Ito K, Shinomiya K, Ito M, Armstrong J, Boyan G, Hartenstein V, et al. A systematic nomenclature for the insect brain. Neuron. 2014; 81(4):755–65. doi: 10.1016/j.neuron.2013.12.017.

Jenett A, Schindelin JE, Heisenberg M. The Virtual Insect Brain protocol: creating and comparing standardized neuroanatomy. BMC Bioinformatics. 2006; 7(1):544. doi: 10.1186/1471-2105-7-544.

Kamikouchi A, Inagaki HK, Effertz T, Hendrich O, Fiala A, Göpfert MC, et al. The neural basis of Drosophila gravity-sensing and hearing. Nature. 2009; 458(7235):165–71. http://dx.doi.org/10.1038/nature07810, doi: 10.1038/nature07810.

Leise EM. Evolutionary trends in invertebrate ganglionic structure. Seminars in Neuroscience. 1991; 3(5):369–377. http://dx.doi.org/10.1016/1044-5765(91)90027-L, doi: 10.1016/1044-5765(91)90027-L.

Lemon W, Pulver S, Höckendorf B, K M. Whole-central nervous system functional imaging in larval Drosophila. Nature Communications. 2015; 6:7924. doi: 10.1038/ncomms8924.

Lundquist T, Nässel DR. Substance P-, FMRFamide-, and gastrin/cholecystokinin-like immunoreactive neurons in the thoraco-abdominal ganglia of the flies Drosophila and Calliphora. The Journal of comparative neurology. 1990; 294(2):161–178. http://dx.doi.org/10.1002/cne.902940202, doi: 10.1002/cne.902940202.

Merritt D, Murphey R. Projections of leg proprioceptors within the CNS of the fly Phormia in relation to the generalized insect ganglion. J Comp Neurol. 1992; 322(1):16–34. doi: 10.1002/cne.903220103.

Middleton CA, Nongthomba U, Parry K, Sweeney ST, Sparrow JC, Elliott CJH. Neuromuscular organization and aminergic modulation of contractions in the Drosophila ovary. BMC biology. 2006; 4:17. http://dx.doi.org/10.1186/1741-7007-4-17, doi: 10.1186/1741-7007-4-17.

Miller A, Demerec M. In: The internal anatomy and histology of the imago of Drosophila melanogaster. Wiley; 1950. p. 420.

Niven JE, Graham CM, Burrows M. Diversity and evolution of the insect ventral nerve cord. Annu Rev Entomol. 2008; 53(1):253–71. doi: 10.1146/annurev.ento.52.110405.091322.

Pfluger HJ, Braunig P, Hustert R. The Organization of Mechanosensory Neuropiles in Locust Thoracic Ganglia. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences. 1988; 321(1202):pp. 1–26. http://www.jstor.org/stable/2396782, doi: 10.1098/rstb.1988.0090.

Pitmon E, Stephens G, Parkhurst S, Wolf F, Kehne G, Taylor M, et al. The D1 Family Dopamine Receptor, DopR, potentiates hindleg grooming behavior in Drosophila. Genes Brain Behav. 2016; 15(3):327–334. doi: 10.1111/gbb.12264.

Power ME. The thoracico-abdominal nervous system of an adult insect, Drosophila melanogaster. J Comp Neurol. 1948; 88(3):347–409.

Robertson R, Pearson K, Reichert H. Flight interneurons in the locust and the origin of insect wings. Science. 1982; 217(4555):177–9. doi: 10.1126/science.217.4555.177.

Seeds AM, Ravbar P, Chung P, Hampel S, Midgley FM, Mensh BD, et al. A suppression hierarchy among competing motor programs drives sequential grooming in Drosophila. Elife. 2014; 3:e02951.

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Shepherd D, Smith S. Central projections of persistent larval sensory neurons prefigure adult sensory pathways in the CNS of Drosophila. Development. 1996; 122(8):2375–84.

Shepherd D, Harris R, Williams D, Truman JW. Postembryonic Lineages of the Drosophila Ventral Nervous System: Neuroglian expression reveals the adult hemilineage associated fiber tracts in the adult thoracic neuromeres. J Comp Neurology. 2016; 524(13):2677–95. doi: 10.1002/cne.23988.

Strausfeld NJ, Seyan HS. Convergence of visual, haltere, and prosternai inputs at neck motor neurons of Calliphora erythrocephala. Cell and Tissue Research. 1985; 240(3):601–615. http://dx.doi.org/10.1007/BF00216350, doi: 10.1007/BF00216350.

Trimarchi JR, Schneiderman AM. Giant fiber activation of an intrinsic muscle in the mesothoracic leg of Drosophila melanogaster. Journal of Experimental Biology. 1993; 177(0022-0949 SB - IM):149–167. http://jeb.biologists.org/cgi/content/abstract/177/1/149.

Truman JW, Schuppe H, Shepherd D, Williams DW. Developmental architecture of adult-specific lineages in the ventral CNS of Drosophila. Development. 2004; 131(20):5167–84. doi: 10.1242/dev.01371.

Tyrer NM, Gregory GE. A Guide to the neuroanatomy of locust suboesophageal and thoracic ganglia. Philosophical Transactions of the Royal Society of London B, Biological Sciences. 1982; 297(1085):91–123. http://rstb.royalsocietypublishing.org/content/297/1085/91, doi: 10.1098/rstb.1982.0035.

Wagh DA, Rasse TM, Asan E, Hofbauer A, Schwenkert I, Dürrbeck H, et al. Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in Drosophila. Neuron. 2006; 49(6):833–44. doi: 10.1016/j.neuron.2006.02.008.

Yu JY, Kanai MI, Demir E, Jefferis GSXE, Dickson BJ. Cellular Organization of the Neural Circuit that Drives Drosophila Courtship Behavior. Current Biology. 2010 sep; 20(18):1602–1614. http://linkinghub.elsevier.com/retrieve/pii/S0960982210010158, doi: 10.1016/j.cub.2010.08.025.

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Appendix 1

Overview of nomenclature

Here we provide the agreed anatomy terms, each with a short description. We also highlight any changes we have made from what was considered the classical definition with the group's reasoning for making the change.

Ventral Nervous System (VNS)

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 often referred to as the ventral nerve cord (VNC) although the VNC would include the suboesophageal ganglion (***Niven et al., 2008***). The VNS 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 thoracico-abdominal 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. However, this structure includes the suboesophageal ganglion. Ultimately, ventral nervous system (VNS) was agreed upon with all alternatives recorded as synonyms.

Synonyms:

- VNS (Proposed by group),
- 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

Dense neuropil, as referred to later 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 imaged using standard immunofluorescence confocal microscopy targeting either NC82 or N-cadherin. See ***Figure 1***(D) for an example.

Thoracic Neuromeres

The three thoracic neuromeres are segmentally homologous and share common structural features. The detailed organisation 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 VNS, 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.

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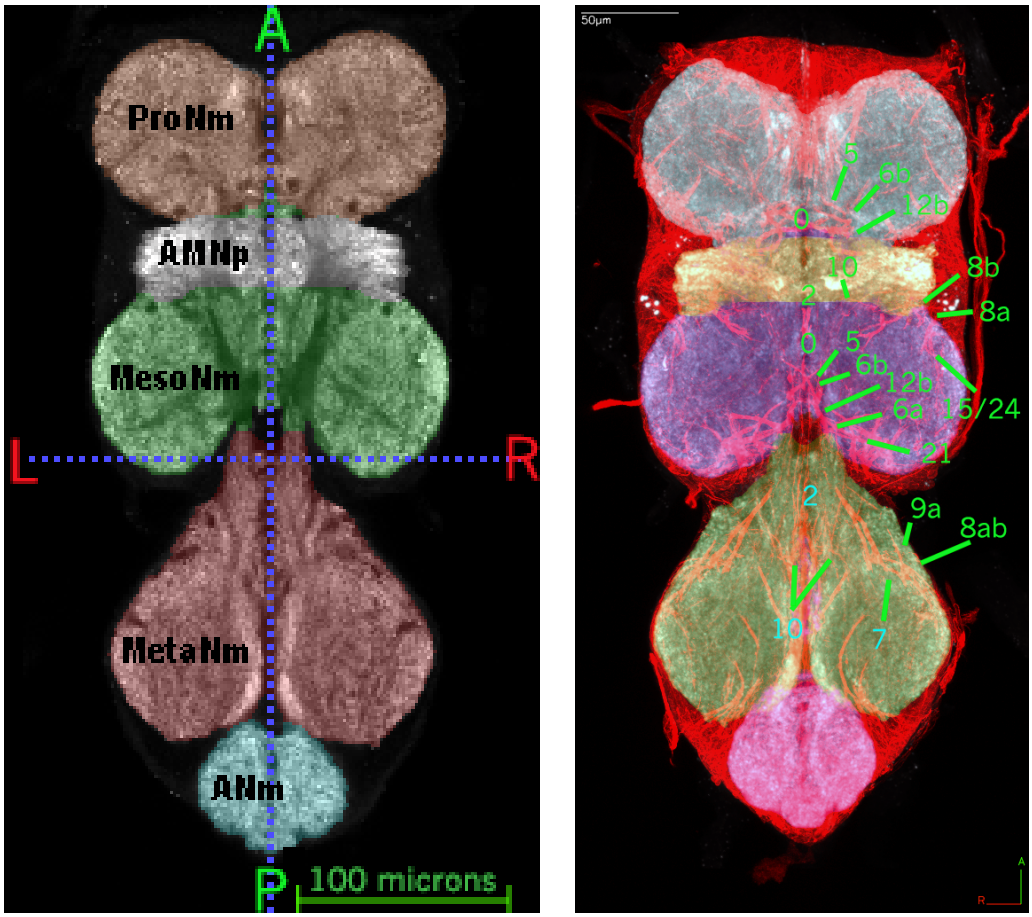

(a) Neuromeres (b) Lineage

Appendix 1 Figure 1. Frontal section through midsection of adult VNS template with painted neuromeres also showing lineage tract boundaries. Shown in ***Figure 1(a)*** is the labelled neuromeres: Prothoracic Neuromere - ProNm (described in Prothoracic Neuromere (ProNm)), Accessory Mesothoracic Neuropil - AMNp (described in Accessory Mesothoracic Neuropil (AMNp)), Mesothoracic Neuromere - MesoNm (described in Mesothoracic Neuromere (MesoNm)), Metathoracic Neuromere - MetaNm (described in Metathoracic Neuromere (MetaNm)) and Abdominal Neuromeres - ANm (described in Abdominal Neuromeres (ANm)). To show the lineage basis for internal boundaries ***Figure 1(b)*** shows a thick (22 µm) midsection slice of neuroglian (red) with the labelled neuropil (painted greyscale), showing the painted neuromeres with labelled examples of the lineage tracts defining the internal boundaries (see definitions in Prothoracic Neuromere (ProNm) - Accessory Mesothoracic Neuropil (AMNp) & Metathoracic Neuromere (MetaNm) - Abdominal Neuromeres (ANm)).

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Prothoracic Neuromere (ProNm)

Defined region shown in ***Appendix 1 Figure 2***

The anteriormost of the 4 major neuropils that makes up the VNS 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 and prothorax. The posterior margin is defined by the neuroglian tracts from the posterior T1 hemilineages 0, 2, 3, 6, 11 and 19, all of which project anteriorly into the neuromere (***Figure 1(b)***). The paired neuropil is formed as two more or less spherical masses.

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***).

Mesothoracic Neuromere (MesoNm)

Defined region shown in ***Appendix 1 Figure 3***

The 2nd (anterior-posterior) of the 4 major neuropils of the VNS 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 and mesothorax. The mesothoracic neuromere is closely associated with two distinct subdivisions of the thoracic neuropils: the accessory mesothoracic neuromere and the tectulum. The mesothoracic neuromere is contained anteriorly by the neuroglian tracts hemilineages from the anterior T2 hemilineages 2, 7, 8, 9, 10, 15 and 16, all of which project posteriorly into the neuromere and define the anterior margin of the neuropil (***Figure 1(b)***). Similarly the posterior margin of the neuromere is defined by the neuroglian tracts from the posterior hemilineages 0, 3, 6, 11, 19, and 21, all of which project anteriorly into the neuromere (***Figure 1(b)***).

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, 1992***),
- MN (***Power, 1948***).

Accessory Mesothoracic Neuropil (AMNp)

Defined region shown in ***Appendix 1 Figure 4***

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 NC82 or N-cadherin staining) largely from the sensory afferents entering the VNS via the Anterior Dorsal Median Nerve (ADMN) (***Power, 1948***). 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 posterior T1 hemilineages 0, 5, 6, 11, 19 and 23 and posteriorly by the anterior T2 hemilineages 1, 2, 7, 8, 9, 15, and 16 (***Figure 1(b)***).

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

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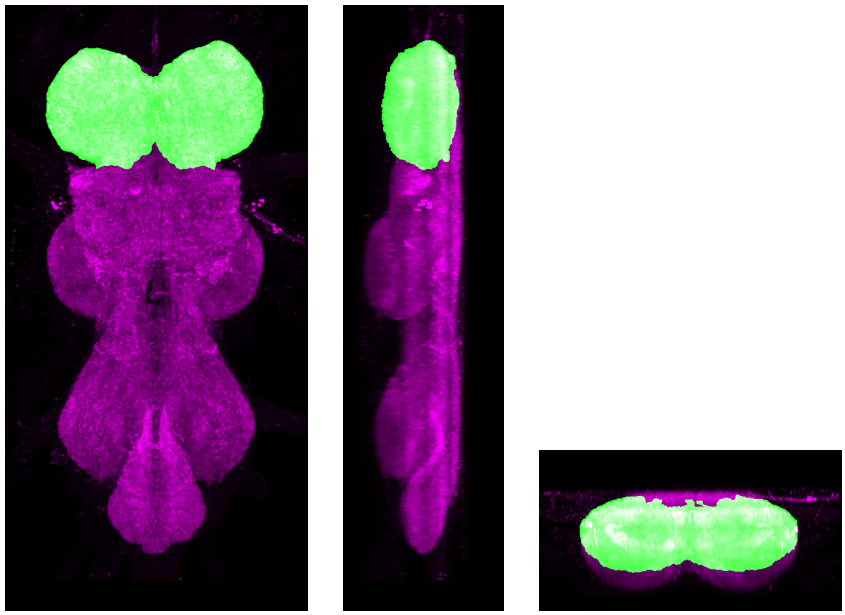
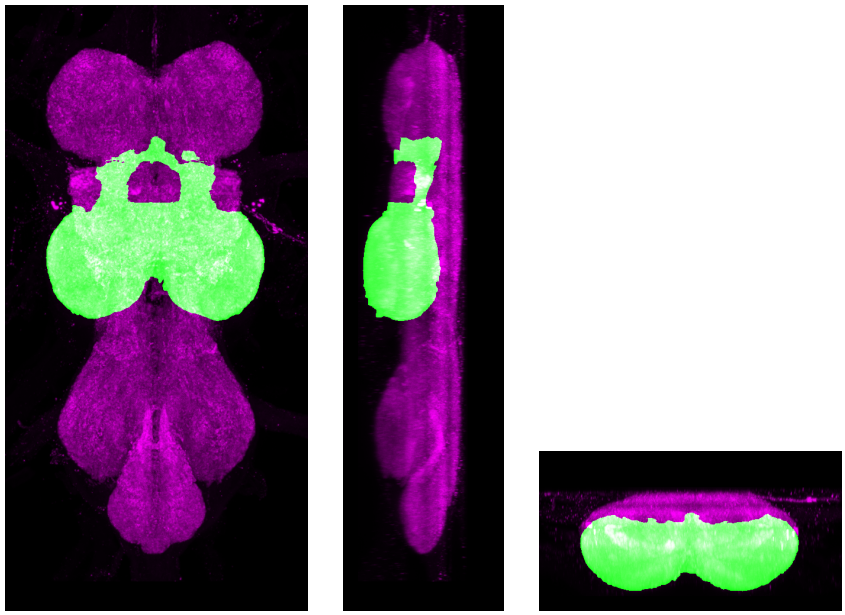


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 2. The proposed volume definition for the Prothoracic Neuromere (ProNm). Maximum projections from the labelled orthogonal plane with ProNm shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 3. The proposed volume definition for the Mesothoracic Neuromere (MesoNm). Maximum projections from the labelled orthogonal plane with MesoNm shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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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 (Tct)

Defined region shown in *Appendix 1 Figure 5*

The tectulum is a distinct subdivision of the thoracic regions of the VNS. 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 posterior-most region of the prothoracic neuromere (ProNm) and the anteriormost region of the metathoracic neuromere (MetaNm). Its internal boundaries within the VNS can be defined as the dorsal region of the neuropil posterior to the anteriormost limits of the hemilineage 12B neuroglian bundle in ProNm, but dorsal to the bundles from 12B, 6B, 23 17, 18B. Extending posteriorly through MesoNm to the entry point of hemilineage 3 in MetaNm.

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***).

Synonyms:

- Tct (Proposed by group),
- T (***Power, 1948***),
- flight neuropil RELATED (***Power, 1948***; ***Leise, 1991***).

Flight Neuropil (FNp)

Neuropil that contains branches from neurons that drive wing elevators and depressors but also receives projections from flight proprioceptors wing hinge receptors and wing sensory hairs (***Leise, 1991***).

Synonyms:

- FNp (Proposed by group).

Metathoracic Neuromere (MetaNm)

Defined region shown in *Appendix 1 Figure 6*

The 3rd (anterior-posterior) of the 4 major neuropils that make up the VNS (pro-, meso- and metathoracic neuromeres and ANm). 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 and metathorax.

The metathoracic neuromere is contained anteriorly by the neuroglian tracts associated with the anterior T3 hemilineages 2, 7, 8, 9, 10, 15 and 16, all of which project posteriorly into the neuromere and define the anterior margin of the neuropil (***Figure 1(b)***). Similarly, the posterior margin of the neuromere is defined by the neuroglian tracts from the posterior hemilineages 0, 3, 6, 19, 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:

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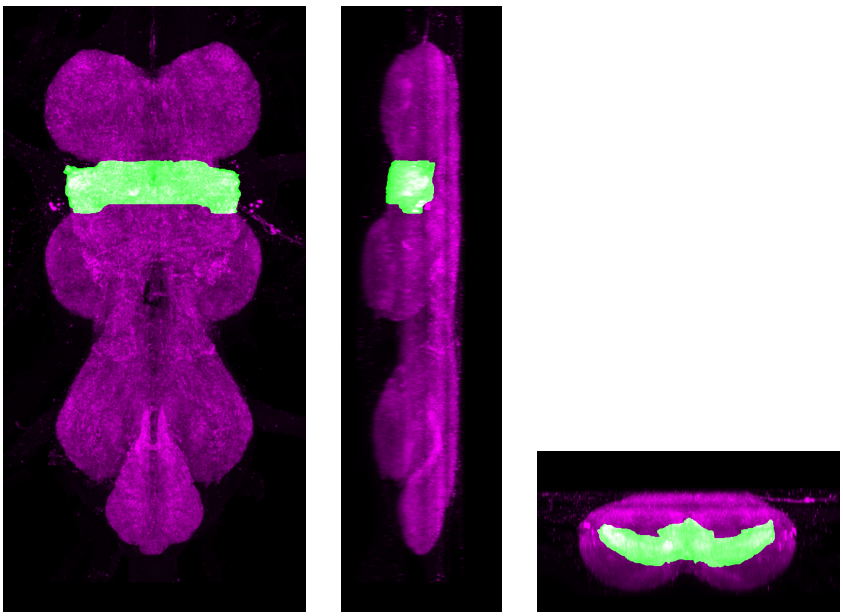
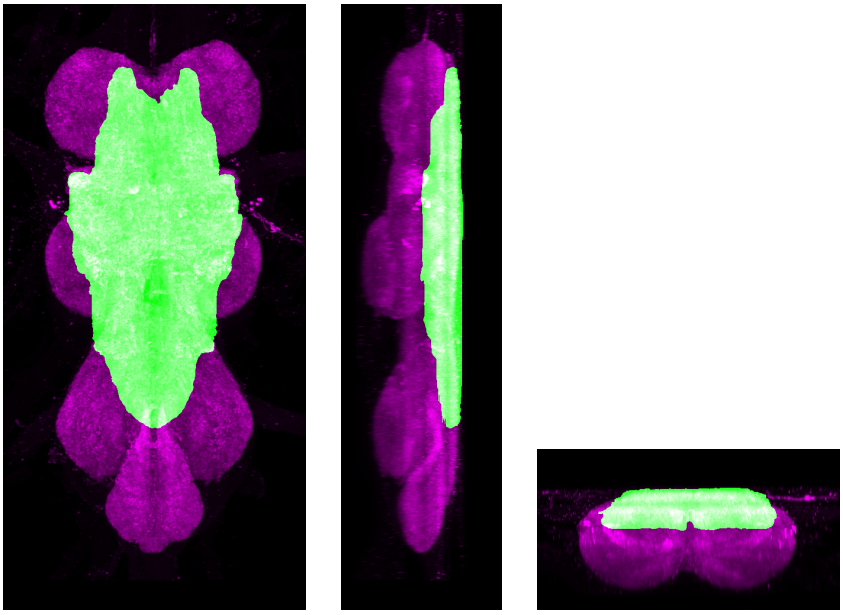


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 4. The proposed volume definition for the Accessory Mesothoracic Neuropil (AMNp). Maximum projections from the labelled orthogonal plane with AMNp shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 5. The proposed volume definition for the Tectulum (Tct). Maximum projections from the labelled orthogonal plane with Tct shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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- MetaNm (Proposed by group),
- Meta (***Merritt and Murphey, 1992***),
- MtN (***Power, 1948***).

Abdominal Neuromeres (ANm)

Defined region shown in *Appendix 1 Figure 7*

The 4th major neuropil situated at the posterior end of the VNS, 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***).

The Commissures

The neuroglian tracts and lineage specific green florescent protein (GFP) expression reveal the developmental origins of many of the commissures in the adult VNS. In the larval VNS there are 5 major commissures in each neuromere: the anterior (aD) and posterior (pD) dorsal commissures, the anterior (aI) and posterior (pI) intermediate commissures, and the anterior ventral (aV) commissure (***Truman et al., 2004***). The postembryonic hemilineages of the larval VNS cross the midline in specific and invariant commissures (***Truman et al., 2004***). Identification of the postembryonic hemilineages in the adult provides a definitive marker to link larval and adult VNS commissures (***Power, 1948***; ***Merritt and Murphey, 1992***). From this, it is possible to develop a nomenclature for some of the adult commissures that recognises their larval origins (***Shepherd et al., 2016***).

Prothoracic Anterior Ventral Anterior Commissure (ProAVAC)

ProAVAC is a ventral commissure formed by the axons of the ProNm hemilineage 1A as they cross the midline at the anteriormost margin of the prothoracic neuromere. The ProAVAC is ventrally located and lies at the ventralmost margins of the VNS, outside of the neuropil and cell cortex, crossing the midline anterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- ProAVAC (Proposed by group).

Prothoracic Anterior Ventral Posterior Commissure (ProAVPC)

The ProAVPC is a ventral commissure formed by the axons of ProNm hemilineage 13B as they cross the midline at the anterior of the prothoracic neuromere. The ProAVPC is ventrally located and crosses the midline posterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- ProAVPC (Proposed by group).

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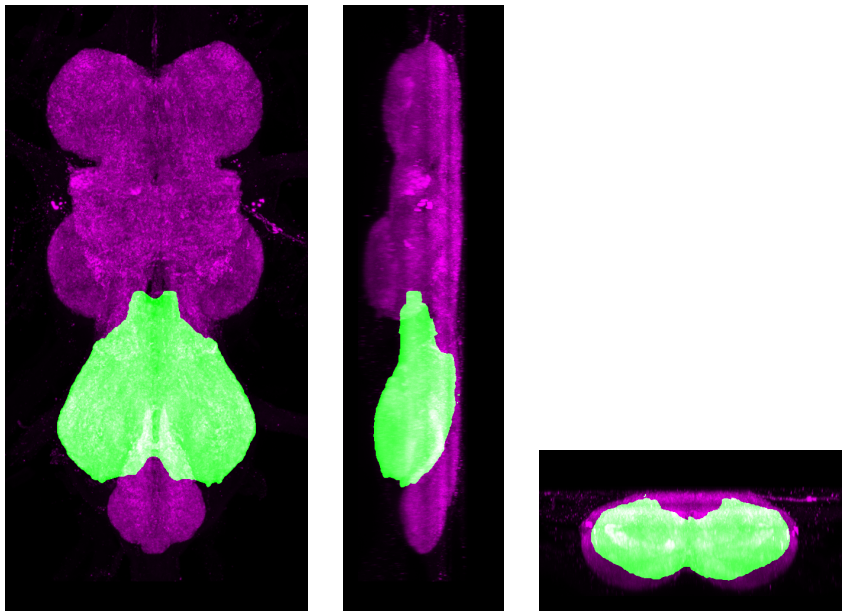
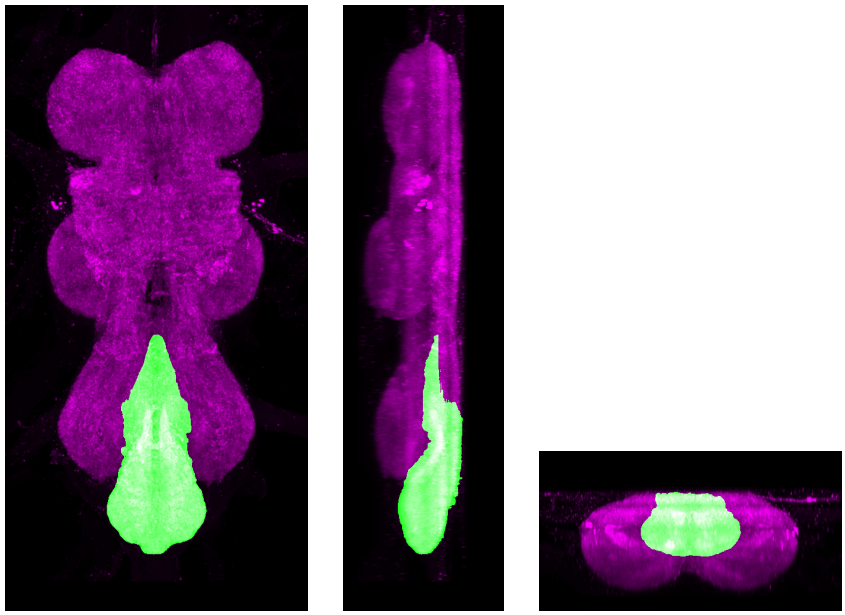


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 6. The proposed volume definition for the Metathoracic Neuromere (MetaNm). Maximum projections from the labelled orthogonal plane with MetaNm shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 7. The proposed volume definition for the Abdominal Neuromeres (ANm). Maximum projections from the labelled orthogonal plane with ANm shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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Mesothoracic Anterior Ventral Anterior Commissure (MesoAVAC)

The MesoAVAC is a ventral commissure formed by the axons of the MesoNm hemilineage 1A as they cross the midline at the anterior of the mesothoracic neuromere. The MesoAVAC s ventrally located and lies at the ventralmost margins of the VNS, outside of the neuropil and cell cortex, crossing the midline anterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

This commissure was originally identified by ***Power*** (***1948***) as the Accessory Prothoracic Commissure.

Synonyms:

- MesoAVAC (Proposed by group),
- Accessory Prothoracic Commissure (***Power, 1948***),
- APC (***Power, 1948***).

Mesothoracic Anterior Ventral Posterior Commissure (MesoAVPC)

The MesoAVPC is a ventral commissure formed by the axons of the MesoNm hemilineage 13B as they cross the midline at the anterior mesothoracic neuromere. The commissure is ventrally located and crosses the midline posterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

This commissure was originally identified by ***Power*** (***1948***) as the Ventral Accessory Commissure of the Mesothoracic Neuromere.

Synonyms:

- MesoAVPC (Proposed by group),
- Ventral Accessory Commissure of the Mesothoracic Neuromere (***Power, 1948***),
- VAC (***Power, 1948***).

Metathoracic Anterior Ventral Anterior Commissure (MetaAVAC)

The MetaAVAC is a ventral commissure formed by the axons of the MetaNm hemilineage 1A as they cross the midline at the anterior of the metathoracic neuromere. The MetaAVAC is ventrally located and lies at the ventralmost margins of the VNS, outside of the neuropil and cell cortex, crossing the midline anterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

This commissure was originally identified by ***Power*** (***1948***) as the Accessory Commissure of the Metathoracic Neuromere.

Synonyms:

- MetaAVAC (Proposed by group),
- Accessory Commissure of the Metathoracic Neuromere (***Power, 1948***),
- ACM (***Power, 1948***).

Metathoracic Anterior Ventral Posterior Commissure (MetaAVPC)

The MetaAVPC is a ventral commissure formed by the axons of the MetaNm hemilineage 13B as they cross the midline at the anterior of the mesothoracic neuromere. The commissure is ventrally located and crosses the midline posterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- MetaAVPC (Proposed by group).

Prothoracic Anterior Intermediate Commissure (ProAIC)

The ProAIC is a ventral commissure formed by the axons of the ProNm hemilineage 10B as they cross the midline in the anterior of the prothoracic neuromere. The commissure crosses the midline posterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***). The ProAIC is posterior to the ProAVAC and unlike ProAVAC lies within the neuropil.

Synonyms:

- ProAIC (Proposed by group).

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Mesothoracic Anterior Intermediate Ventral Commissure (MesoAIVC)

The MesoAVIC is a ventral commissure formed by the axons of the MesoNm hemilineage 10B as they cross the midline in the anterior of the mesothoracic neuromere. The commissure crosses the midline posterior to the axon bundles from hemilineage 2A (***Shepherd et al., 2016***). The MesoAVIC is posterior to the MesoAVAC and unlike MesoVAC lies within the neuropil.

Synonyms:

- MesoAIVC (Proposed by group).

Mesothoracic Anterior Intermediate Dorsal Commissure (MesoAIDC)

MesoAIDC is a dorsally located commissure formed by the axons of the mesothoracic hemilineage 18B as they cross the midline in the anterior of the mesothoracic neuromere. The axons cross the midline anterior to the axons from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- MesoAIDC (Proposed by group).

Metathoracic Anterior Intermediate Dorsal Commissure (MetaAIDC)

The MetaAIDC is a dorsally located commissure formed by the axons of the metathoracic hemilineage 18B as they cross the midline in the anterior of the metathoracic neuromere. The axons cross the midline anterior to the axons from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- MetaAIDC (Proposed by group).

Prothoracic Posterior Intermediate Anterior Commissure (ProPIAC)

ProPIAC is a dorsally located commissure formed by the axons of the prothoracic hemilineage 8B as they cross the midline in the anterior of the prothoracic neuromere. The axons cross the midline posterior to the axons from hemilineage 2A (***Shepherd et al., 2016***).

Synonyms:

- ProPIAC (Proposed by group).

Prothoracic Posterior Intermediate Posterior Commissure (ProPIPC)

The ProPIPC is a posterior commissure formed by the axons of hemilineages 6B and 12B as they cross the midline in the prothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- ProPIPC (Proposed by group).

Prothoracic Posterior Intermediate Anterior Dorsal Commissure (ProPIDC)

The ProPIDC is a dorsally located commissure formed by the axons of hemilineage 7B as they cross the midline in the anterior prothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- ProPIDC (Proposed by group).

Prothoracic Posterior Intermediate Anterior Ventral Commissure (ProPIVC)

The ProPIVC is a commissure at a mid dorso-ventral plane formed by the axons from hemilineage 5B as they cross the midline in the prothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- ProPIVC (Proposed by group).

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Mesothoracic Posterior Intermediate Anterior Commissure (MesoPIAC)

The MesoPIAC is an anterior commissure formed by the axons of hemilineages 7B and 8B as they cross the midline in the mesothoracic neuromere (***Shepherd et al., 2016***). This commissure was originally identified by ***Power*** (***1948***) as the Commissure of the Mesothoracic Neuromere.

Synonyms:

- MesoPIAC (Proposed by group),
- Commissure of the Mesothoracic Neuromere (***Power, 1948***),
- CMN (***Power, 1948***).

Mesothoracic Posterior Intermediate Posterior Commissure (MesoPIPC)

The MesoPIPC is a posterior commissure formed by the axons of hemilineages 5B, 6B and 12B as they cross the midline in the mesothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- MesoPIPC (Proposed by group).

Metathoracic Posterior Intermediate Anterior Commissure (MetaPIAC)

The MetaPIAC is an anterior commissure in the metathorax formed by the axons from hemilineages 7B and 8B as they cross the midline in the metathoracic neuromere. The commissure is posterior to the axons from hemilineage 2A (***Shepherd et al., 2016***).

This commissure was originally identified by ***Power*** (***1948***) as the Haltere Commissure.

Synonyms:

- MetaPIAC (Proposed by group),
- Haltere Commissure (***Power, 1948***),
- cHIN (***Merritt and Murphey, 1992***; ***Tyrer and Gregory, 1982***; ***Pfluger et al., 1988***),
- ITD-HC (***Boerner and Duch, 2010***),
- HC (***Power, 1948***).

Metathoracic Posterior Intermediate Posterior Commissure (MetaPIPC)

The MetaPIPC is a posterior commissure formed by the axons of hemilineages 5B, 6B and 12B as they cross the midline in the mesothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- MetaPIPC (Proposed by group).

Prothoracic Posterior Dorsal Commissure (ProPDC)

The ProPDC is a posterior commissure formed by the axons from hemilineage 6A as they cross the midline in the dorsal prothoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- ProPDC (Proposed by group).

Mesothoracic Posterior Dorsal Commissure (MesoPDC)

The MesoPDC is a dorsal commissure formed by the axons of hemilineage 6A as they cross the midline in posterior mesothoracic neuromere (***Shepherd et al., 2016***). This commissure was originally identified by ***Power*** (***1948***) as the Posterior Dorsal Mesothoracic Decussation.

Synonyms:

- MesoPDC (Proposed by group),
- Posterior Dorsal Mesothoracic Decussation (***Power, 1948***),
- PDD (***Power, 1948***).

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Metathoracic Posterior Dorsal Commissure (MetaPDC)

The MetaPDC is a dorsal commissure formed by the axons form hemilineage 6A as they cross the midline in the posterior metathoracic neuromere (***Shepherd et al., 2016***).

Synonyms:

- MetaPDC (Proposed by group).

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

A chiasma formed by the inner tracts of the intermediate tract of the dorsal cervical fasciculus (ITD-CFF), as they cross the midline above the mesothoracic neuromere and terminate on the opposite side. The chiasma of fine fibers is anterior to the haltere commissure (MetaPIAC).

Synonyms:

- CFF (***Power, 1948***).
- ITD-CFF BROAD (***Boerner and Duch, 2010***).

In addition to the commissures that can be related to the projections of specific hemilineages there are commissures that do not relate to specific clusters of central neurons.

Commissure of Prothoracic Neuromeres (CPN)

A transverse bundle of fibers that runs across the left and right prothoracic neuromeres. The fibers bow posteriorly and are located dorsal to the dorsal lateral tracts of the ventral cervical fasciculus (DLV). 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 runs across the dorsoposterior region of the left and right mesothoracic neuromeres. It is located ventrally to the roots of the dorsal metathoracic (haltere) nerves, dorsal to the ventral ellipse, and ventroanterior to the haltere chiasma.

synonyms:

- DAM (***Power, 1948***).

Described Tracts

Several tracts are described by ***Power*** (***1948***) and later clarified by ***Merritt and Murphey*** (***1992***) . They were later put in a volumetric context by ***Boerner and Duch*** (***2010***), who kindly made their data available for inclusion in this project.

Dorsal Lateral Tract (DLT)

Defined region shown in ***Appendix 1 Figure 8***

The DLT derives from the lateral bundles of the cervical connective. It has coarser fibres than the other tract derived from the ventral bundles of the cervical connective (DLV, VLT and VTV). The tract projects posteriorly and superficially at the dorsal lateral edge of the neuropil and terminates in the metathoracic neuromere.

Synonyms:

- DLT (***Power, 1948***)
- MTD (***Merritt and Murphey, 1992***),
- Median tract of dorsal cervical fasciculus (***Merritt and Murphey, 1992***).

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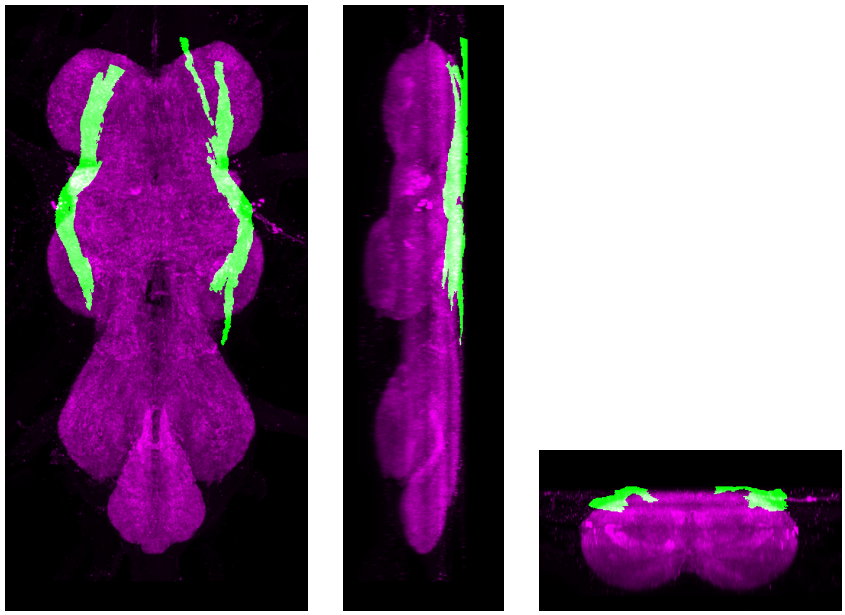
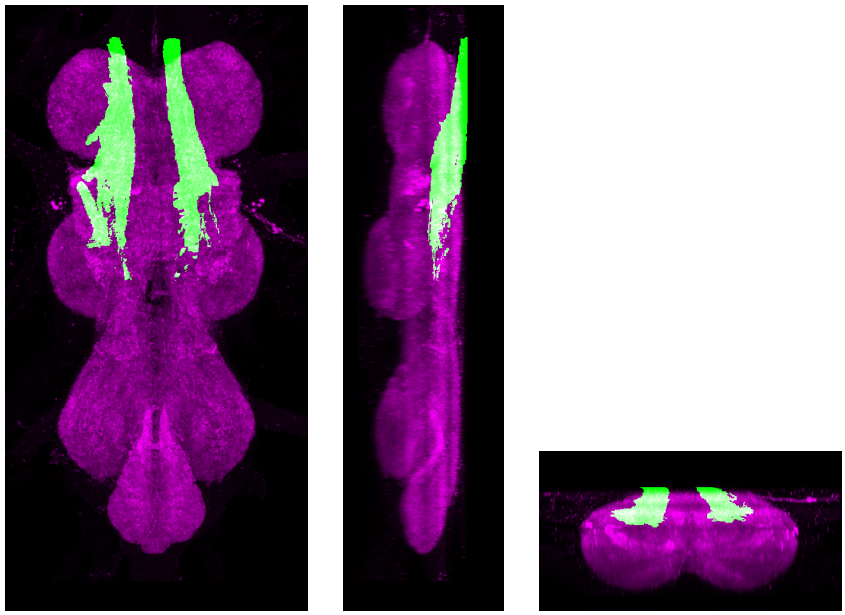


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 8. The proposed volume definition for the Dorsal Lateral Tract (DLT). Maximum projections from the labelled orthogonal plane with DLT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 9. The proposed volume definition for the Intermediate Tract of Dorsal Cervical Fasciculus (ITD). Maximum projections from the labelled orthogonal plane with ITD shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.

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Intermediate Tract of Dorsal Cervical Fasciculus (ITD)

Defined region shown in *Appendix 1 Figure 9*

The intermediate tracts of the dorsal fasciculus derive from the dorsal fibers in the connective but are much broader than the DLT and located more medial. The tract passes posteriorly, and tends to separate into three adjacent tracts. The inner subdivision (ITD-CFF) turns medially in the mesothoracic neuromere, crosses its homologue of the opposite side, and forms the chiasma of fine fibers of the intermediate tracts of the dorsal cervical fasciculus (CFF). The fibers of this chiasma pass dorsal to the median tracts of the dorsal fasciculus, and immediately over the very deep roots of the haltere nerves. Some of the fibers (ITD-HC) bend anterolaterally, while others turn ventrally and straggle into the dorsolateral part of the mesothoracic neuromere where they are quickly lost. There seems to be no consistency in which of the two bundles of the chiasma crosses above the other; sometimes the right tract passes above the left, and in other specimens the left is dorsal to the right, but in all cases, each tract retains its unity. In other words, the component elements of the two do not mix by intermeshing. The ITD as described by ***Power*** (***1948***) includes a third bundle of fibers (ITD-HT) that enter the VNS via the Dorsal Metathoracic Nerve (Haltere Nerve) and is the tract referred to as the Haltere Tract in ***Merritt and Murphey*** (***1992***). This haltere tract is the most lateral component of the intermediate tract and is composed of many large-diameter fibres that can be traced as a bundle running into the cervical connective. It is composed of sensory afferent axons (***Ghysen, 1980***; ***Strausfeld and Seyan, 1985***).

Synonyms:

- ITD (***Power, 1948***),
- ITD-HC NARROW (***Boerner and Duch, 2010***),
- ITD-CFF NARROW (***Boerner and Duch, 2010***),
- ITD-HT NARROW (***Boerner and Duch, 2010***).

Inner Tracts of the Intermediate Tract of the Dorsal Cervical Fasciculus (ITD-CFF)

Defined region shown in *Appendix 1 Figure 10*

An inner subdivision of the Intermediate tract of dorsal cervical fasciculus (ITD) that turns medially in the mesothoracic neuromere, crosses its homologue of the opposite side, and forms the chiasma of fine fibers of the intermediate tracts of the dorsal cervical fasciculus (CFF). The fibers of this chiasma pass dorsal to the median tracts of the dorsal fasciculus, and immediately over the very deep roots of the haltere nerves.

Synonyms:

- ITD-CFF (***Boerner and Duch, 2010***),
- CFF NARROW (***Power, 1948***),
- ITD BROAD (***Power, 1948***).

Haltere Tract (HT)

Defined region shown in *Appendix 1 Figure 11*

The haltere tract 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 (***Power, 1948***; ***Merritt and Murphey, 1992***). The tract has small arborizations with some of the fibres 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***). The haltere tract is the most lateral component of the intermediate tract (ITD) and is composed of many large-diameter fibres that can be traced as a bundle running into the cervical connective (***Power, 1948***).

Synonyms:

- ITD-HT (***Boerner and Duch, 2010***),

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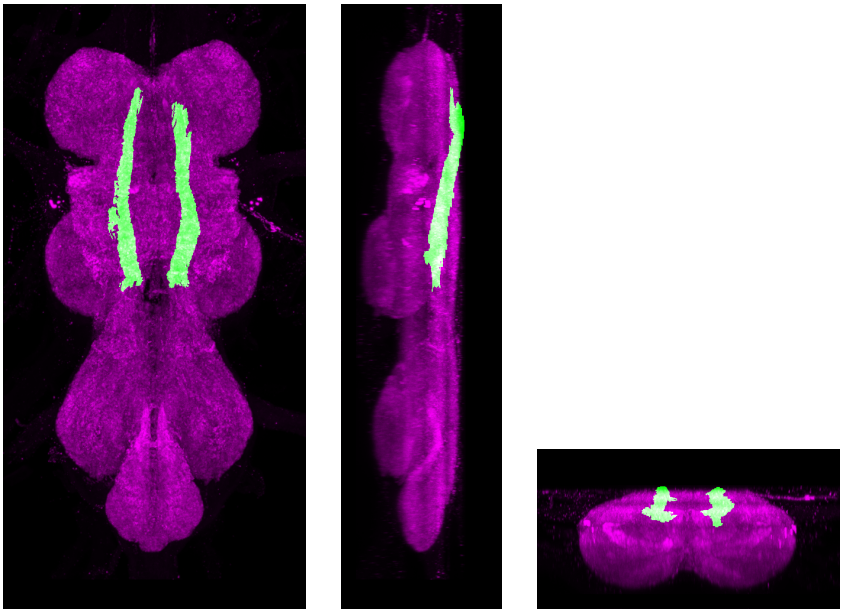
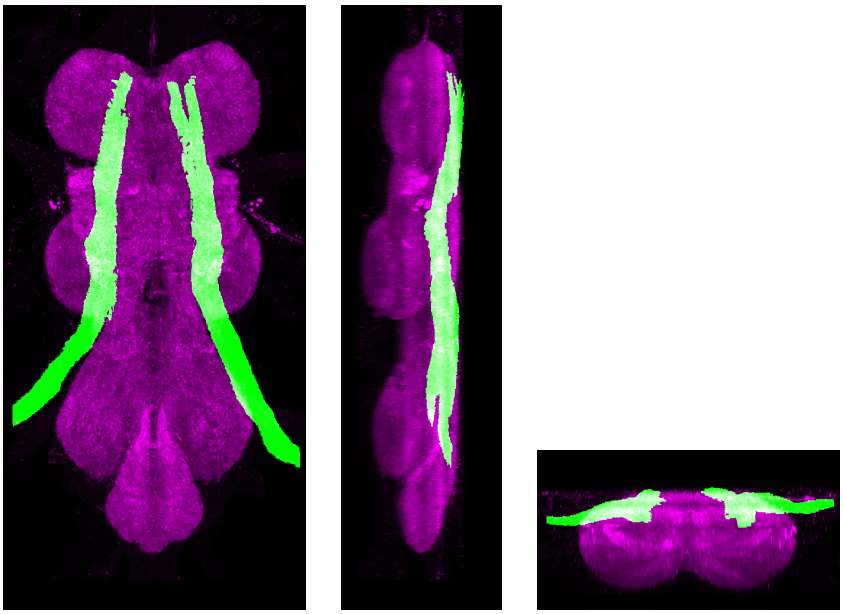


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 10. The proposed volume definition for the Inner Tracts of the Intermediate Tract of the Dorsal Cervical Fasciculus (ITD-CFF). Maximum projections from the labelled orthogonal plane with ITD-CFF shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 11. The proposed volume definition for the Haltere Tract (HT). Maximum projections from the labelled orthogonal plane with HT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.

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- HT (***Merritt and Murphey, 1992***),
- ITD BROAD (***Power, 1948***).

Dorsal Medial Tract (DMT)

Defined region shown in *Appendix 1 Figure 12*

A tract which derives from the dorsal bundles of the cervical connective. The tract extends posteriorly and bows laterally slightly, in the mesothoracic region, bending again medially, towards each other, in the narrow area between the meso- and metathoracic neuromeres and passing ventrally to the chiasma of fine fibers. The dorsal medial tract turns lateroposteriorly at the level and dorsal to the haltere commissure, enters the metathoracic neuromere, forming collaterals and joins the metathoracic leg nerve (***Power, 1948***).

Synonyms:

- MTD (***Power, 1948***),
- median tracts of the dorsal cervical connective (***Power, 1948***).

Dorsal Lateral Tract of Ventral Cervical Fasciculus (DLV)

Defined region shown in *Appendix 1 Figure 13*

A tract which derives from the ventral bundles of the cervical connective. It has coarser fibres than the ventral tract derived from the ventral bundles of the cervical connective (DLV, VLT and VTV). The tract spreads out as it extends posteriorly, passing dorsally to the commissure of the prothoracic neuromere, until it terminates in the mesothoracic neuromere. Two larger fibers occupy the lateral and medial borders of the tract. The lateral fibers send out a branch into the prothoracic neuromere, turn sharply posteriorly and enters the mesothoracic neuromere dorsally, producing a small median branch and joining the mesothoracic leg nerve. The medial fiber extends posteriorly until the level at which the giant fibers turn laterally, and turns slightly laterally into the mesothoracic neuromere. Ventral to these projections, the tract forms a fibrous loop (ventral ellipse) which runs from the prothoracic neuromere to the region between the meso- and metathoracic neuromeres. A right and left arm of fibers turn lateroposteriorly from the posterior end of the ellipse and join the metathoracic leg nerve. Ventral to the ellipse, fibers extend lateroanteriorly into the pro- and mesothoracic neuromeres.

Synonyms:

- DLV (***Power, 1948***).

Ventral Lateral Tract (VLT)

Defined region shown in *Appendix 1 Figure 14*

A thin bundle of fibers which derives from the ventral bundles of the cervical connective. It is lateral to the ventral ellipse. The ventral lateral tract extends from the prothoracic neuromere posteriorly into the anterolateral sides of the metathoracic neuromere. At the level of the anterior dorsal mesothoracic nerve, the fibers bow sharply laterally but quickly turn medially and continue extending posteriorly, above the mesothoracic commissure (***Power, 1948***).

Synonyms:

- ventral lateral tract of the ventral cervical fasciculus (***Power, 1948***),
- VLT (***Power, 1948***).

Ventral Median Tract of Ventral Cervical Fasciculus (VTV)

Defined region shown in *Appendix 1 Figure 15*

A bundle of fine fibers which derives from the ventral bundles of the cervical connective. They extend posteriorly along the medial plane until they bend dorsally and terminate in the ventroanterior region of the abdominal ganglion. A few fibers branch out in each neuromere before the tract reaches the abdominal ganglion (***Power, 1948***).

Synonyms:

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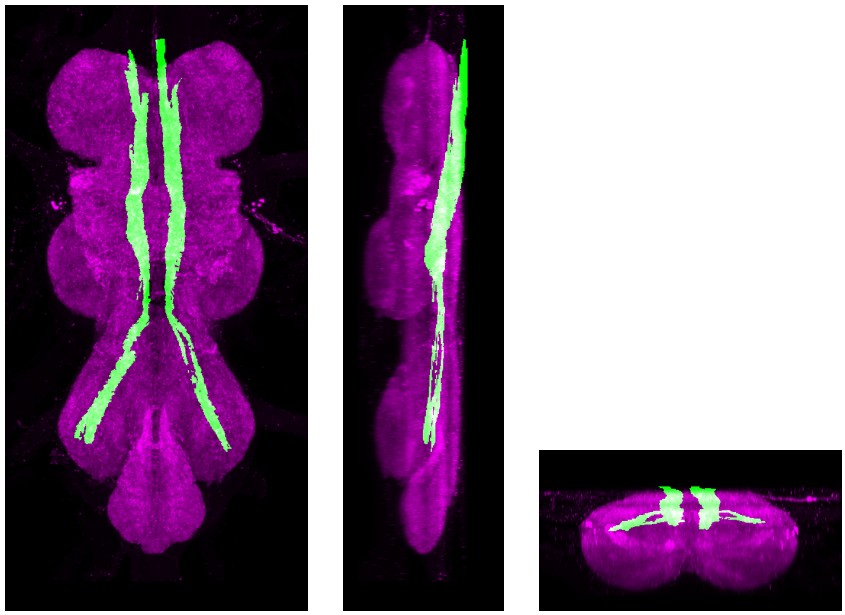
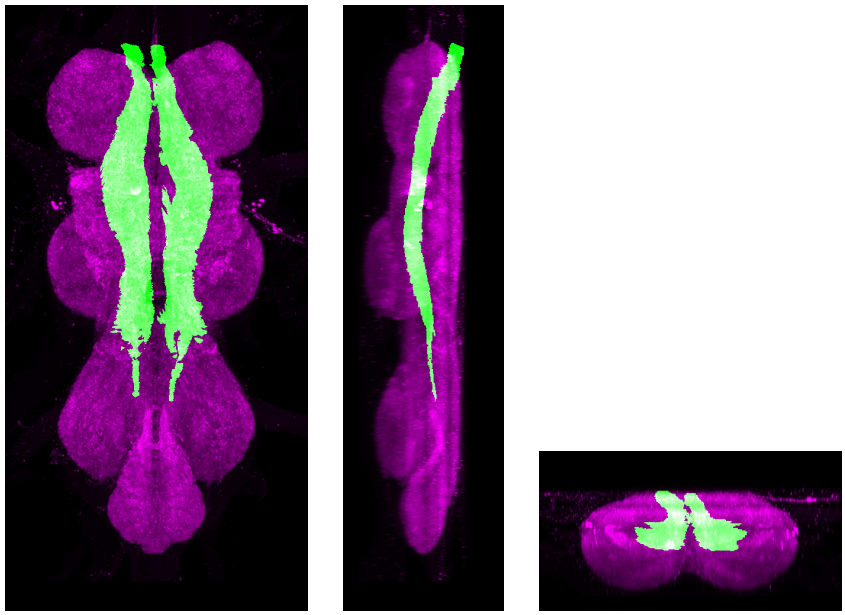


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 12. The proposed volume definition for the Dorsal Medial Tract (DMT). Maximum projections from the labelled orthogonal plane with DMT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 13. The proposed volume definition for the Dorsal Lateral Tract of Ventral Cervical Fasciculus (DLV). Maximum projections from the labelled orthogonal plane with DLV shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.

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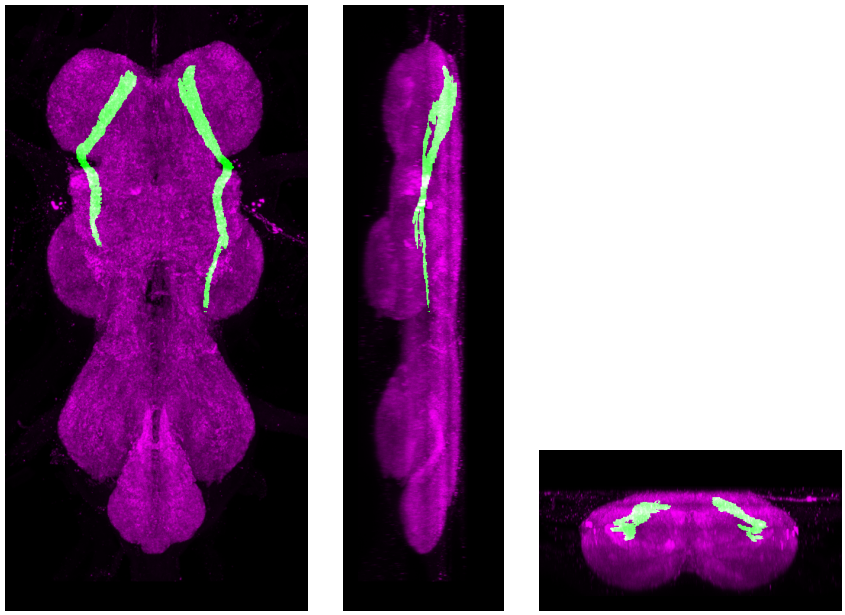
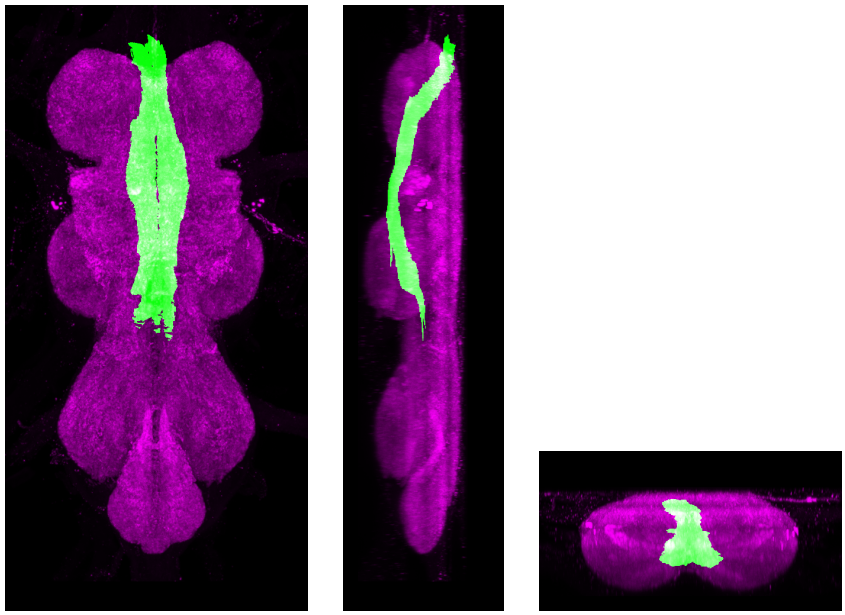


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 14. The proposed volume definition for the Ventral Lateral Tract (VLT). Maximum projections from the labelled orthogonal plane with VLT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 15. The proposed volume definition for the Ventral Median Tract of Ventral Cervical Fasciculus (VTV). Maximum projections from the labelled orthogonal plane with VTV shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.

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- VTV (***Power, 1948***).

Median Dorsal Abdominal Tract (MDT)

Defined region shown in *Appendix 1 Figure 16*

The dorsal-most tract, close to midline (***Merritt and Murphey, 1992***) running along the tectulum (***Boerner and Duch, 2010***). The median-most pair of three small dorsal tracts which connects the thoracic and abdominal neuromeres. It runs from the dorsal region of the tectulum along through the thoracico-abdominal mass, immediately under the giant fibers. It continues posteriorly after the giant fibers have turned laterally, passing above the haltere chiasma into the abdominal neuromeres (***Power, 1948***).

Synonyms:

- MDT (***Merritt and Murphey, 1992***),
- MDA (***Power, 1948***),
- median dorsal tract (***Merritt and Murphey, 1992***).

Intermediate Dorsal Abdominal Tract (IDT)

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

Synonyms:

- IDT (Proposed by group).

Lateral Dorsal Abdominal Tract (LDT)

The lateral pair of three small dorsal tracts which connects the thoracic and abdominal neuromeres dorsal of 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 VNS 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 and extends from the middle of the prothoracic neuromeres 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

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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.

Cervical Connective (CvC)

Defined region shown in *Appendix 1 Figure 17*

Major axon tract connecting the posterior-most subesophageal ganglion to the VNS 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)

Defined region shown in *Appendix 1 Figure 18*

A nerve that projects latero-anteriorly from the antero-lateral corner of the ventral nervous system (VNS) (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***).

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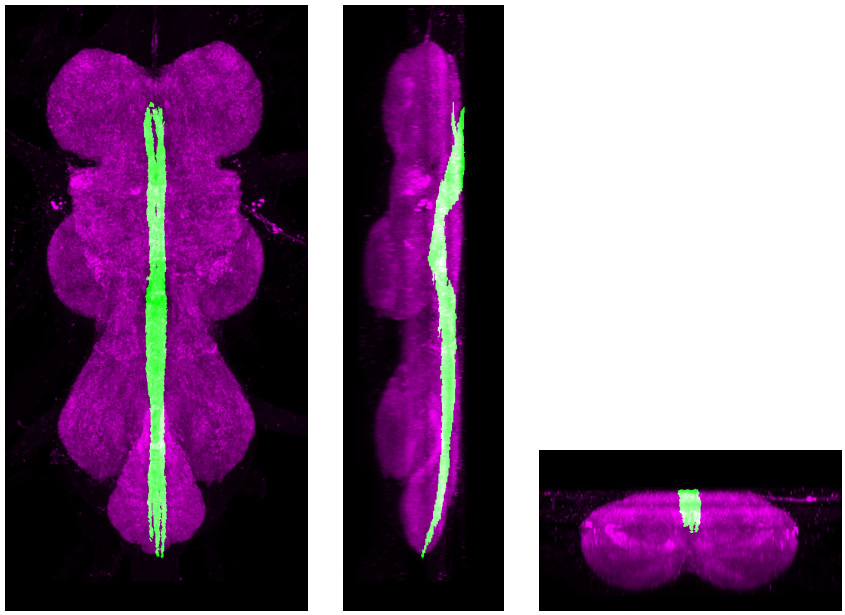
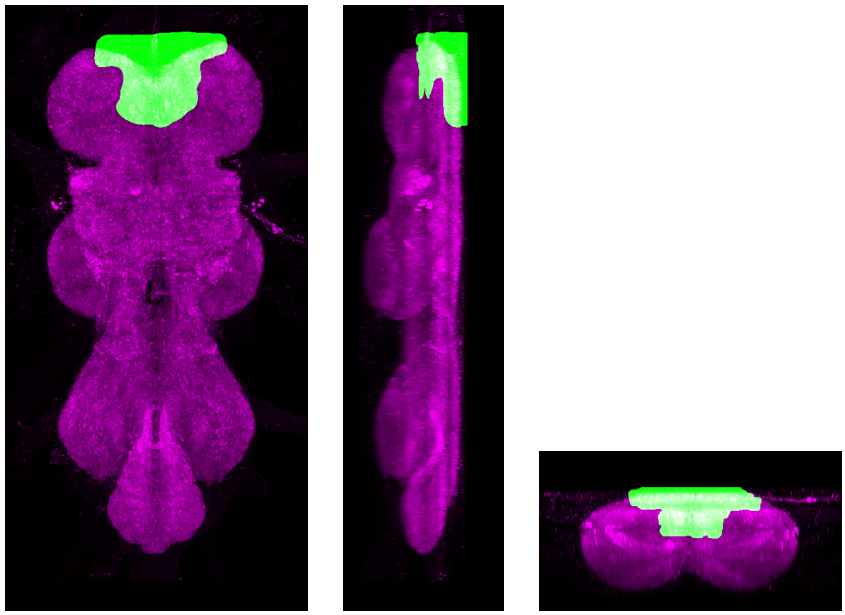


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 16. The proposed volume definition for the Median Dorsal Abdominal Tract (MDT). Maximum projections from the labelled orthogonal plane with MDT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on data provided by ***Boerner and Duch*** (***2010***) as well as literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 17. The proposed volume definition for the Cervical Connective (CvC). Maximum projections from the labelled orthogonal plane with CvC shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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Prosternal Nerve (PrN)

A slender nerve that projects anteriorly from the ventral nervous system (VNS), 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)

Defined region shown in *Appendix 1 Figure 19*

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)

Defined region shown in *Appendix 1 Figure 20*

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)

Defined region shown in *Appendix 1 Figure 21*

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***),

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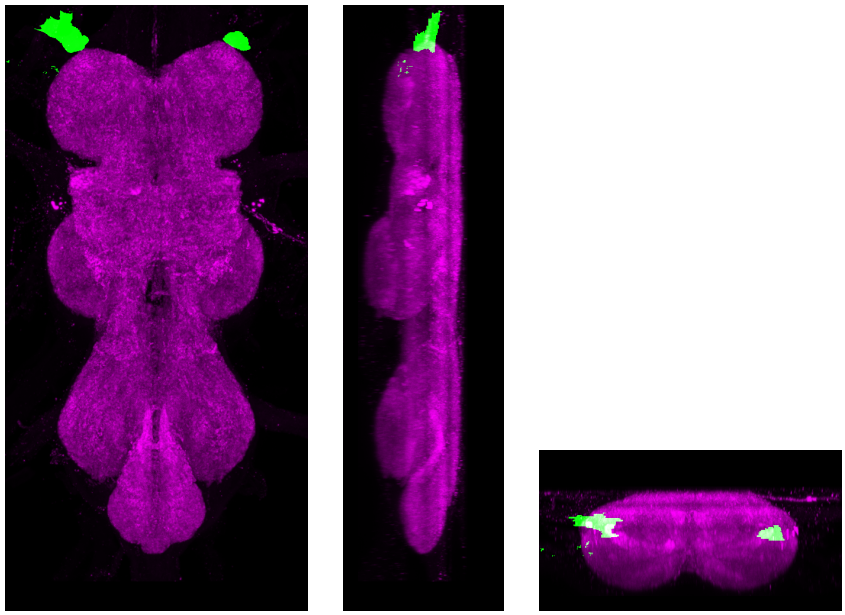
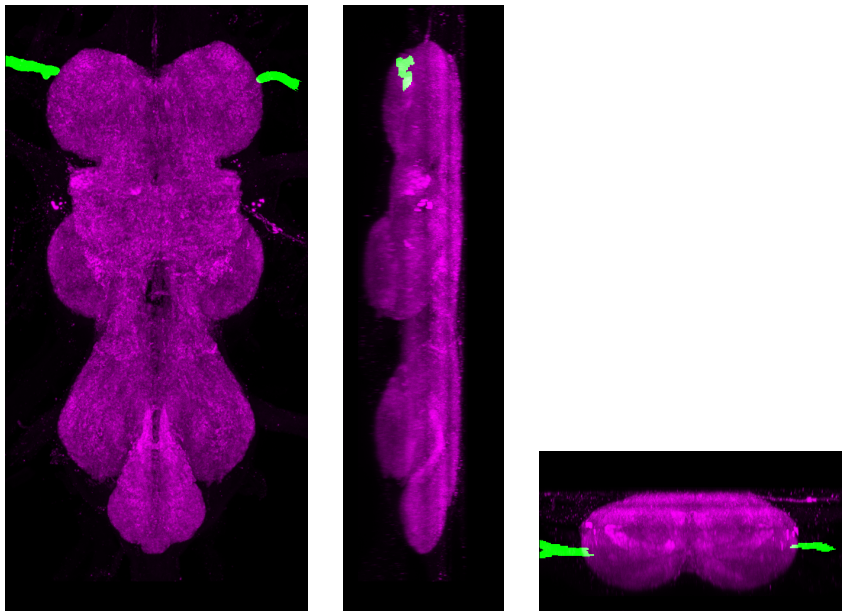


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 18. The proposed volume definition for the Dorsal Prothoracic Nerve (DProN). Maximum projections from the labelled orthogonal plane with DProN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 19. The proposed volume definition for the Prothoracic Accessory Nerve (ProAN). Maximum projections from the labelled orthogonal plane with ProAN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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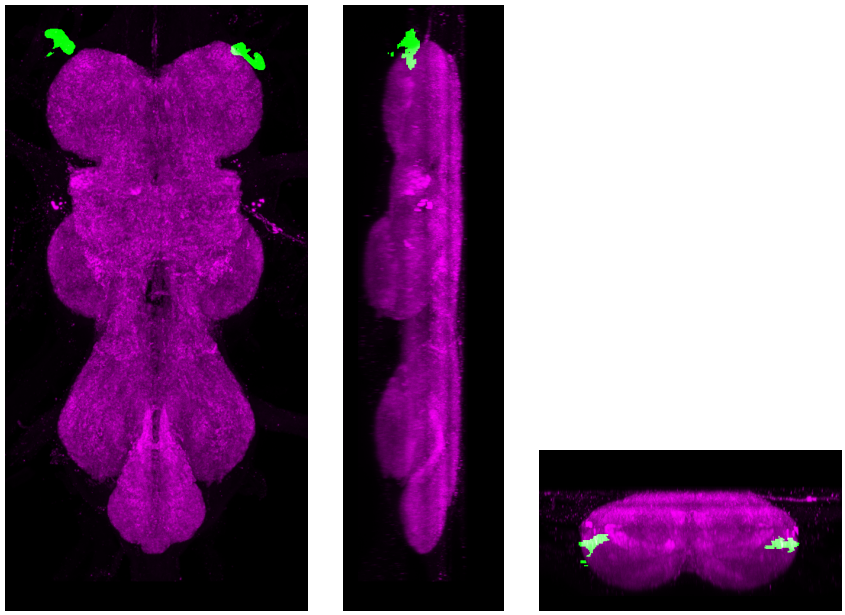
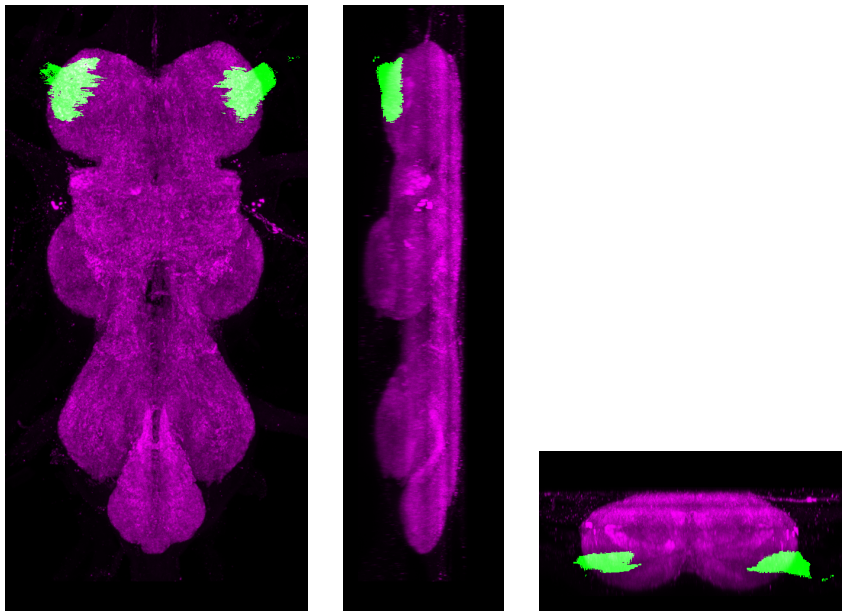


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 20. The proposed volume definition for the Ventral Prothoracic Nerve (VProN). Maximum projections from the labelled orthogonal plane with VProN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 21. The proposed volume definition for the Prothoracic Leg Nerve (ProLN). Maximum projections from the labelled orthogonal plane with ProLN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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- T1LN (***Merritt and Murphey, 1992***),
- first ventral nerve (***Miller and Demerec, 1950***),
- VNv1 (***Miller and Demerec, 1950***).

Anterior Dorsal Mesothoracic Nerve (ADMN)

Defined region shown in *Appendix 1 Figure 22*

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)

Defined region shown in *Appendix 1 Figure 23*

A nerve that arises from the ventral nervous system (VNS), 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)

Defined region shown in *Appendix 1 Figure 24*

A nerve that arises from the lateroposterior side of the mesothoracic neuromere at a point in 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)

Defined region shown in *Appendix 1 Figure 25*

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

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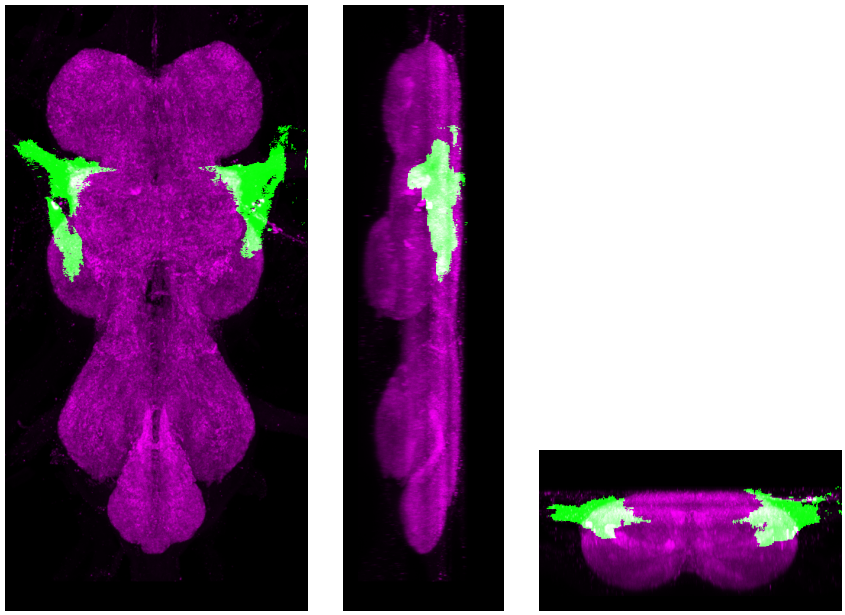
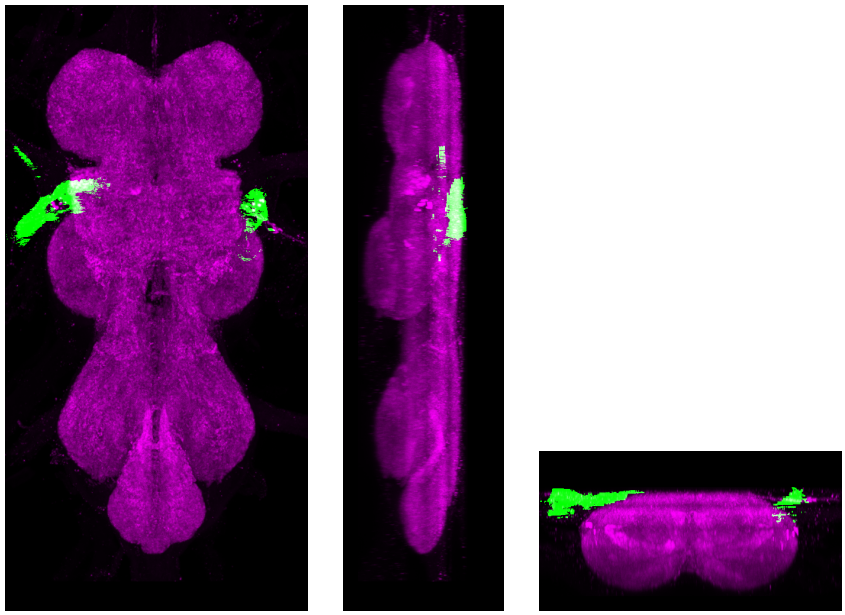


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 22. The proposed volume definition for the Anterior Dorsal Mesothoracic Nerve (ADMN). Maximum projections from the labelled orthogonal plane with ADMN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 23. The proposed volume definition for the Posterior Dorsal Mesothoracic Nerve (PDMN). Maximum projections from the labelled orthogonal plane with PDMN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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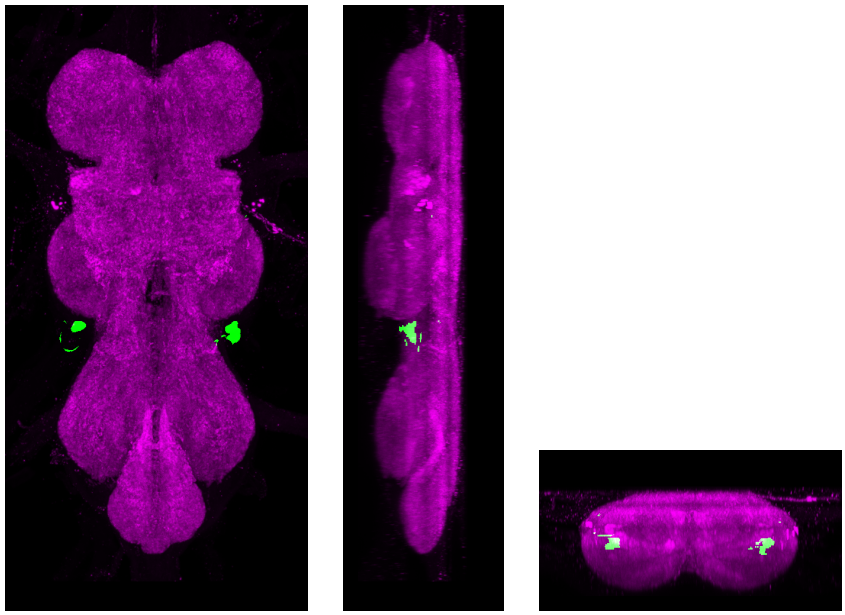
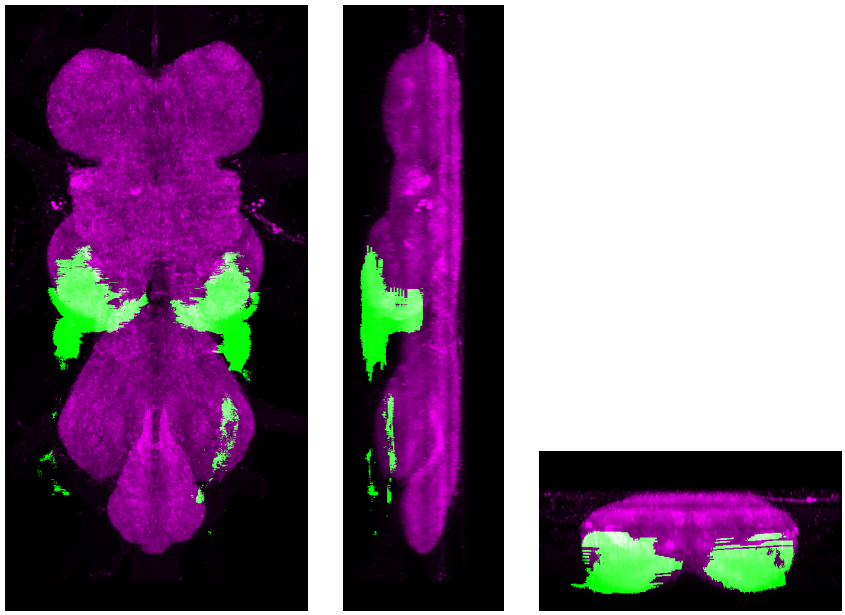


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 24. The proposed volume definition for the Mesothoracic Accessory Nerve (MesoAN). Maximum projections from the labelled orthogonal plane with MesoAN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 25. The proposed volume definition for the Mesothoracic Leg Nerve (MesoLN). Maximum projections from the labelled orthogonal plane with MesoLN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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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)

Defined region shown in ***Appendix 1 Figure 26***

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)

Defined region shown in ***Appendix 1 Figure 27***

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)

Defined region shown in ***Appendix 1 Figure 28***

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 first abdominal segment (***Miller and Demerec, 1950***),
- Ab1Nv (***Miller and Demerec, 1950***).

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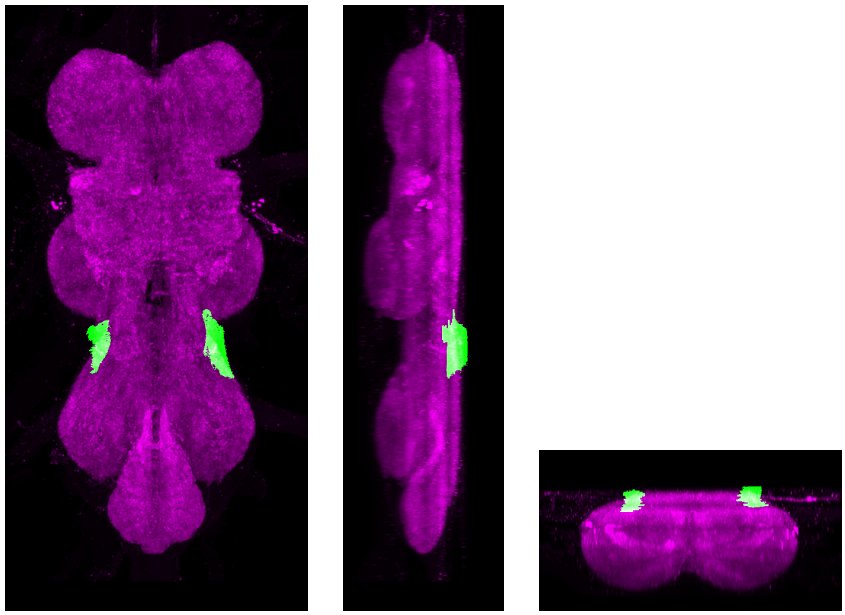
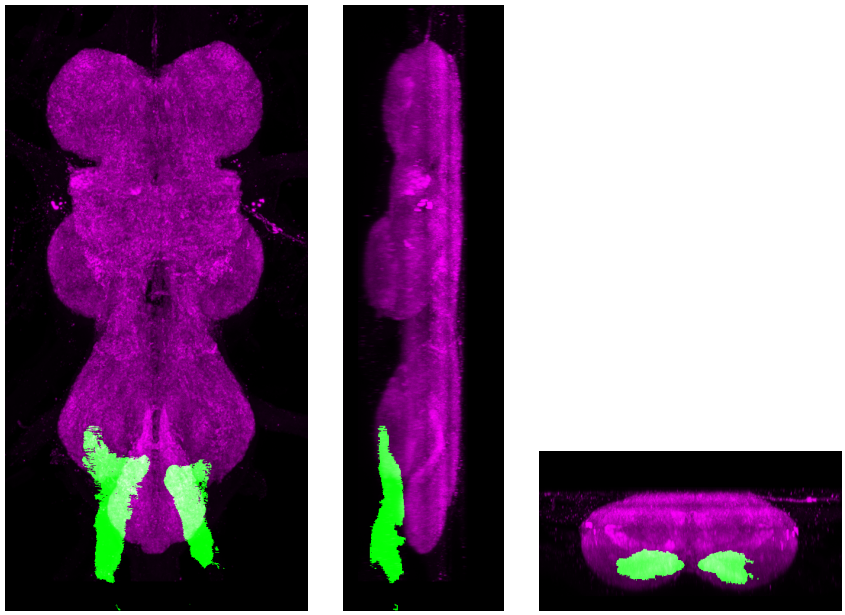


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 26. The proposed volume definition for the Dorsal Metathoracic Nerve (DMetaN). Maximum projections from the labelled orthogonal plane with DMetaN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 27. The proposed volume definition for the Metathoracic Leg Nerve (MetaLN). Maximum projections from the labelled orthogonal plane with MetaLN shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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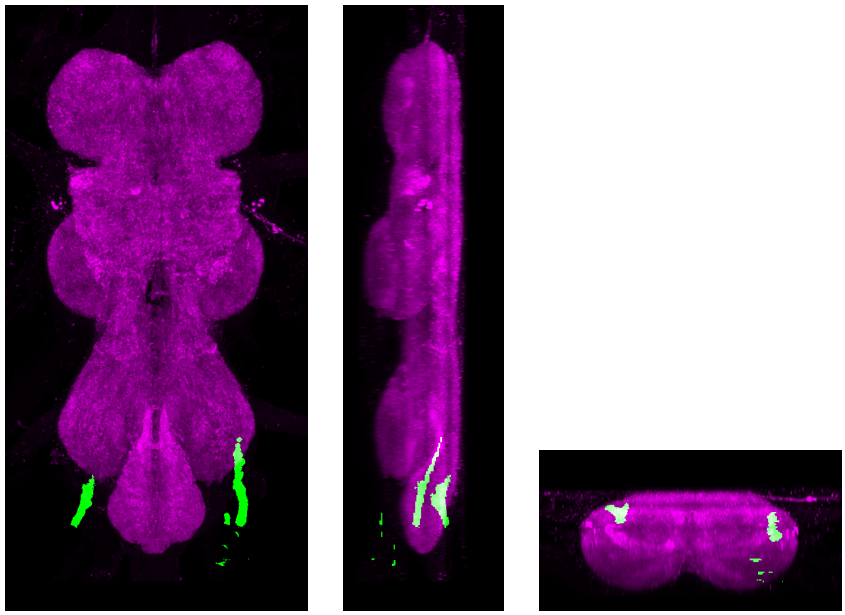
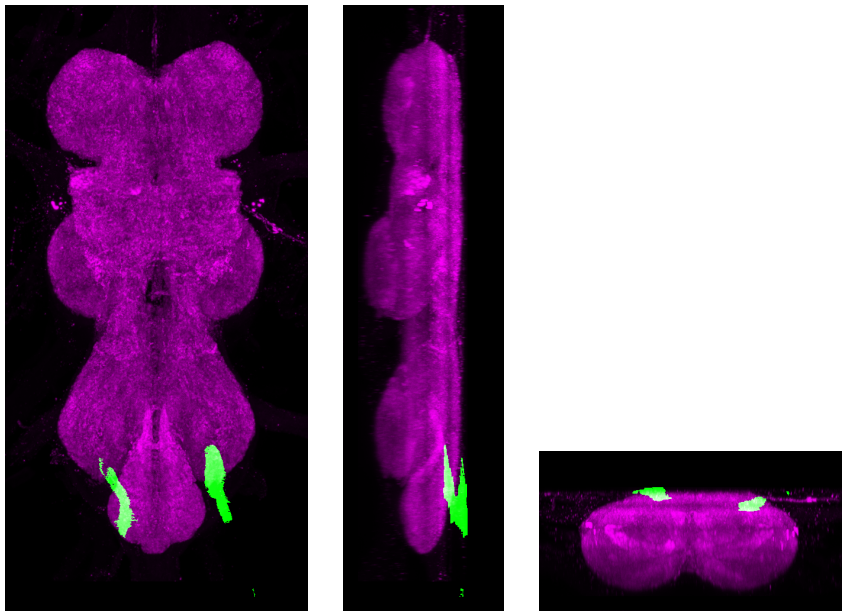


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 28. The proposed volume definition for the First Abdominal Nerve (AbN1). Maximum projections from the labelled orthogonal plane with AbN1 shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 29. The proposed volume definition for the Second Abdominal Nerve (AbN2). Maximum projections from the labelled orthogonal plane with AbN2 shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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Second Abdominal Nerve (AbN2)

Defined region shown in ***Appendix 1 Figure 29***

A nerve that apparently emerges in the dorsal-most 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)

Defined region shown in ***Appendix 1 Figure 30***

Lateral-most 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)

Defined region shown in ***Appendix 1 Figure 31***

Medial-most of the two bilaterally paired nerves connected to the abdominal neuropil. It is thin, containing only fine fibers (***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)

Defined region shown in ***Appendix 1 Figure 32***

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***).

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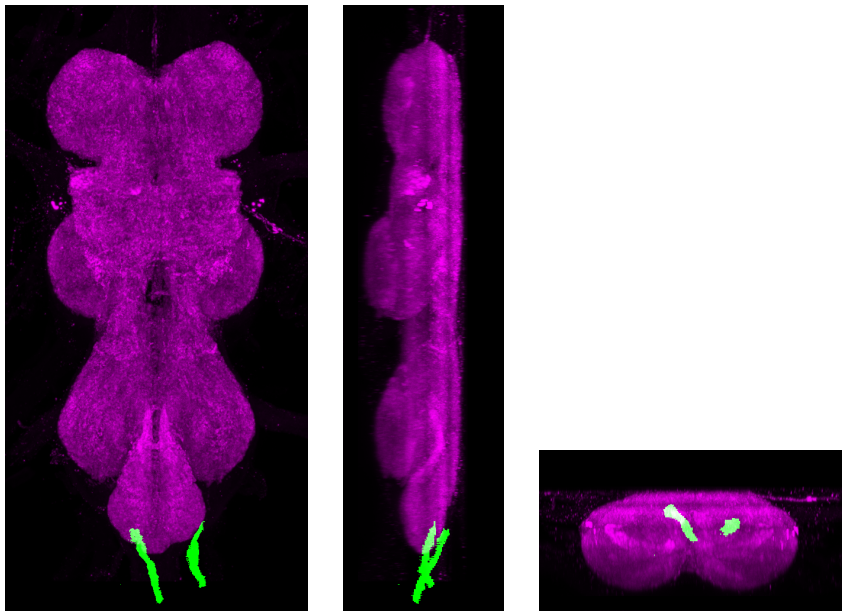
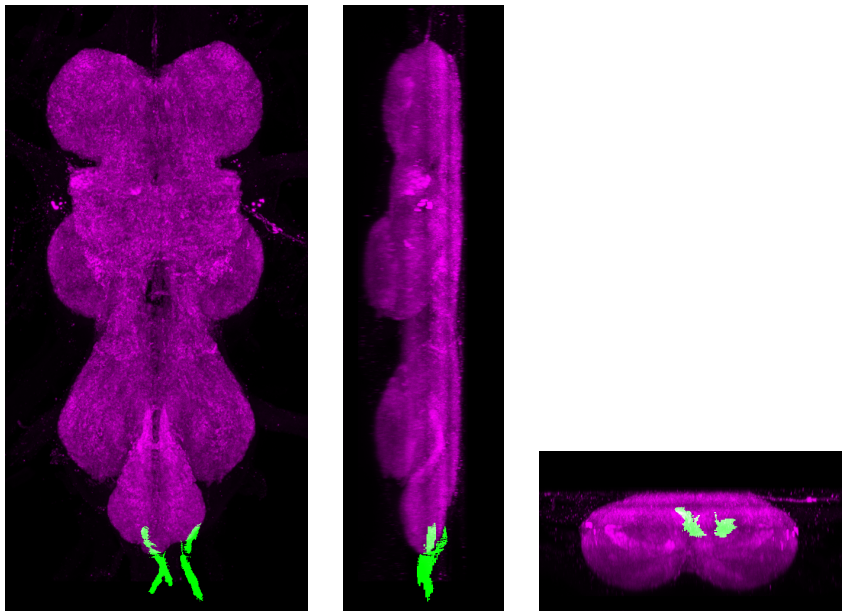


(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 30. The proposed volume definition for the Third Abdominal Nerve (AbN3). Maximum projections from the labelled orthogonal plane with AbN3 shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.



(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 31. The proposed volume definition for the Fourth Abdominal Nerve (AbN4). Maximum projections from the labelled orthogonal plane with AbN4 shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.

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Individual Neurons

Whilst we did not want to expand our remit to cover individual neurons in this first consolidation we thought it would be a good idea to include an example to demonstrate how easily this could be done.

Giant Fiber Neuron (GF)

A large, descending neuron that controls jump escape behaviour (***Trimarchi and Schneiderman, 1993***). Each adult has a bilaterally symmetric pair of these neurons, each with a large cell body at the posterior of the protocerebrum and a long primary neurite that branches to forms an extensive dendritic tree in the brain and an axon that projects, via the cervical connective, to the mesothorax. The axon enters the ventral nervous system (VNS) on the dorsal region and slopes ventrally, reaching the ventral mesothoracic neuromere. Targets of dendritic arborization include zone A of the antennal mechanosensory and motor center (AMMC) (***Kamikouchi et al., 2009***) and the inferior ventro-lateral protocerebrum. In the mesothorax, the axon forms electrical synapses with tergotrochanteral muscle motor neuron (TTMn) and peripherally synapsing interneurons (PSI)) of the mesothorax that in turn synapse to the motor neurons of the dorso-longitudinal flight muscles.

Synonyms:

- GF (***Power, 1948***),
- giant fiber (***Power, 1948***),
- GFN (***Kamikouchi et al., 2009***),
- giant fibers.

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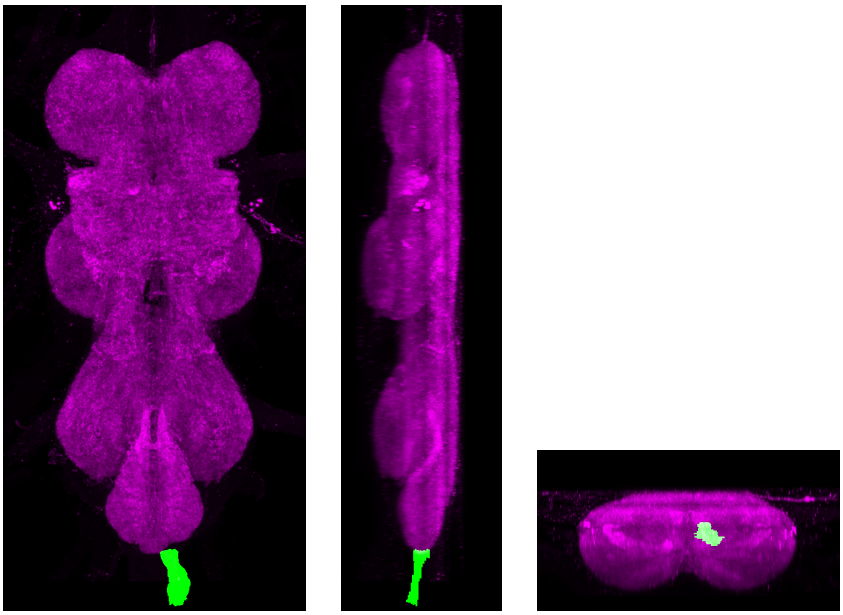

(a) Frontal (b) Sagittal (c) Transverse

Appendix 1 Figure 32. The proposed volume definition for the Abdominal Nerve Trunk (AbNT). Maximum projections from the labelled orthogonal plane with AbNT shown in green and neuropil (Bruchpilot) shown in magenta. Regions were painted using ITK-SNAP by Robert Court based on literature and group discussions to clarify boundaries.