Figures and figure supplements

The functional organization of descending sensory-motor pathways in *Drosophila*

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Figure 1. Strategy for identifying descending neurons. (A, B) The fly central nervous system includes the brain, located in the fly’s head, and a ventral nerve cord (VNC), located in the fly’s thoracic cavity. These are connected by a population of descending neurons (DNs, example in green), which have cell bodies in the brain. Arrow and dark line indicate area of the neck connective illuminated to selectively label the populations of descending and ascending neurons in a transgenic line pan-neuronally expressing photoactivatable GFP (PA-GFP). Dashed square indicates field of view for imaging results in C, D. (C, D) Anterior and posterior views of PA-GFP-labeled DN cell bodies. Black dotted circles represent location of identifiable brain neuropil structures, labeled bilaterally: antennal lobes (AL) and calyx. Blue, light grey, and pink dotted lines enclose separate clusters of DN cell bodies labeled unilaterally: A (anterior dorsal), B (anterior ventral), C (pars intercerebralis), D (outside anterior cluster), G (gnathal ganglion, GNG, shown with dotted line). The uncircled cell bodies in (D) are all considered part of the large posterior cluster (P). (E) Expression of VNC neurons is suppressed by expression of GAL80 under the teashirt promotor. This operation facilitates analysis of DN axonal projection patterns. (F) Example of intersection method used to generate split-GAL4 drivers for DNs. VT063736-GAL4 in attP2 (left) and R24A03-GAL4 in attP2 (center) both show expression in DNp02, when crossed to pJFRC2-10XUAS-IVS-mCD8::GFP in attP2. The enhancer fragments from these lines were used to generate the fly line JRC-SS01053 carrying both VT063736-p65ADZp in attP40 and R24A03- ZpGAL4DBD in attP2 (right).

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Figure 2. Reconstruction of identified descending neurons. Morphology of descending neurons identified in the present study. Neurons (black) and neuropil regions of the brain and VNC are shown (transparent). A total of 98 different cell types are shown. Neurons on both sides of the brain are Figure 2 continued on next page.
Figure 2 continued

shown in some cases (asterisk). Segmentation of neuron volume was performed using GAL4 lines with sparse expression and reconstructed with volume rendering. Figure 2—figure supplements 1–13 shows confocal images masked for individual neurons.

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Figure 2—figure supplement 1. Morphology of DNs, group a. Morphology of identified DNs, group a, whose somata are clustered dorsally on the anterior surface of the brain. For Figure 2—figure supplements 1–13, images are masks for single DNs made from maximum intensity projections of confocal images for sparse split lines. Shown here as horizontal views of the brain (top) and VNC (bottom)

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Figure 2—figure supplement 2. Morphology of DNs, group b. Morphology of identified DNs, group b, whose somata are clustered ventrally on the anterior surface of the brain.

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Figure 2—figure supplement 3. Morphology of DNs, group c. Morphology of identified DNs, group c, whose somata are located in the pars intercerebralis.
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Figure 2—figure supplement 4. Morphology of DNs, group d. Morphology of identified DNs, group d, whose somata are located in an outside cluster on the anterior surface of the brain.

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Figure 2—figure supplement 5. Morphology of DNs, group p (DNp01-11). Morphology of identified DNs, group p, whose somata are located on the posterior surface of the brain (1/4).

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Figure 2—figure supplement 6. Morphology of DNs, group p (DNp12-22). Morphology of identified DNs, group p, whose somata are located on the posterior surface of the brain (2/4).
DOI: https://doi.org/10.7554/eLife.34272.009
Figure 2—figure supplement 7. Morphology of DNs, group p (DNp23-29). Morphology of identified DNs, group p, whose somata are located on the posterior surface of the brain (3/4). DOI: https://doi.org/10.7554/eLife.34272.010
Figure 2—figure supplement 8. Morphology of DNs, group p (DNp30-35). Morphology of identified DNs, group p, whose somata are located on the posterior surface of the brain (4/4).
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**Figure 2—figure supplement 9.** Morphology of DNs, group g (DNg01-12) Morphology of identified DNs, group g, whose somata are located on the gnathal ganglion (1/4).

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Figure 2—figure supplement 10. Morphology of DNs, group g (DNg13-24). Morphology of identified DNs, group g, whose somata are located on the gnathal ganglion (2/4).
DOI: https://doi.org/10.7554/eLife.34272.013
Figure 2—figure supplement 11. Morphology of DNs, group g (DNg25-34). Morphology of identified DNs, group g, whose somata are located on the gnathal ganglion (3/4).

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Figure 2—figure supplement 12. Morphology of DNs, group g (DNg35-41). Morphology of identified DNs, group g, whose somata are located on the gnathal ganglion (4/4).

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Figure 2—figure supplement 13. Morphology of DN, group x. Morphology of identified DN, group x, whose somata is located outside the brain.
DOI: https://doi.org/10.7554/eLife.34272.016
Figure 3. Unique and population descending neurons. (A) Three example morphologies of DNs that are uniquely identifiable (DNa05, DNp25 and DNp06). Maximum intensity projection images for brain (top) and VNC (bottom) are shown. (B) Three examples of population DNs, with individual neurons revealed by multicolor flip-out (DNp17, DNg02 and DNg12). Each neuron of the same DN type shows similar morphology and we do not discriminate individual DNs for these population types.

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Figure 3—figure supplement 1. Further examples of population DNs. (A) An example of the morphology of the DNg07 population. (B) Confocal image of brain innervation for the sample with six pairs of DNg07 type neurons. (C) Confocal image of brain innervation for the sample with a single DNg07 cell body.
Figure 3—figure supplement 1 continued

DNg07. (D) Multicolor flip-out of the DNg07 population shows two different neurons in that type. (E–F) The corresponding VNC projection images for samples with six or a single DN, as in B-D. (G–H) Morphology of individual neurons in population DN types (G) DNg12 and (H) DNg01. Individual morphologies and merged image are shown.

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Figure 4. Anatomical compartments of the brain and VNC in Drosophila. (A–D) Identified brain neuropils labeled with different colors superimposed on an aligned confocal image. Depth from the anterior surface is indicated in top-right of each image. The data is from Virtual Fly Brain, http://www.virtualflybrain.org/. Neuropil names are from Ito et al., 2014 and name abbreviations are summarized in Supplementary file 6. (E, F) Sagittal view of VNC confocal images through a lateral (E) and medial (F) plane. The colors represent our divisions of the recognized domains in the VNC: AMN (accessory mesothoracic neuropil), AS (abdominal segment), mVAC (medial ventral association center), VAC (ventral association center). (G) Schematic of the neuropils in the VNC. T1 (prothoracic segment), T2 (mesothoracic segment), T3 (metathoracic segment). (H) The axis and sections used to describe VNC anatomy. The body axis is used.

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Figure 4—figure supplement 1. Morphology of interneurons innervating the wing neuropil. (A–E) Isolated morphologies of wing muscle motor neurons for (A) steering muscle b1 (mnb1), (B) steering muscle b2 (putative), (C) a dorsoventral power muscle (DVM), as well as two wing neuropil local interneurons (D, E). The horizontal view of whole VNC (top) and the frontal view of T2 segment are shown (bottom). The area of the wing neuropil is shaded with light blue in A2. The areas of tectulum and lower tectulum are indicated with white (A2) and red (B2–E2) dotted lines, respectively. These example moto- and interneurons from the wing neuropil rarely innervate the lower tectulum. Driver lines for these images are specified in Supplementary file 4.
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Morphology of interneurons innervating the lower tectulum. (A–E) Isolated morphologies for motoneurons and interneurons innervating the lower tectulum, including two neurons postsynaptic to the giant fiber (the tergotrochanteral muscle motor neuron, TTMn (A) and the peripheral synapsing interneuron, PSI (B), two intersegmental interneurons (C–D), and unknown motoneurons (E). Images as in Figure 4—figure supplement 1. The gross position of the lower tectulum is shown with an asterisk and orange dotted line. The smooth processes (inputs) of A–D are confined within the lower tectulum; E has smooth processes in wing neuropil and lower tectulum. Genotype information for lines used to make these images is available in Supplementary File 4.

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Figure 5. Distinguishing DN inputs and outputs. (A) The morphology of DNp02. The DN neurites have a smooth appearance in the posterior ventral lateral protocerebrum (PVLP) and anterior mechanosensory motor center (AMMC), and varicose processes in the gnathal ganglia (GNG) and the VNC. Inset shows a magnified view of the DN innervation in the VNC prothoracic ganglia, which have varicose appearance. (B) The morphology of DNp02 with nc82 counter-staining. (C–E) We determined polarity of DNp02 by cell-specific co-expression of membrane-bound GFP (green) and the presynaptic reporter synaptotagmin-smGFP-HA (magenta). Co-expression (white) is observed in the GNG and VNC, but not in the cerebral ganglion, indicating the DN is post-synaptic in the brain and pre-synaptic in the GNG and VNC. (F) Innervation profile of DNs in the brain and VNC. In each row, a filled pixel indicates innervation by the corresponding DN of the CNS neuropil corresponding to the filled column. Green indicates innervation by smooth process, magenta indicates innervation by varicose processes, and black indicates the region receives both types of processes. Smooth and varicose process of DNs are intermingle in the brain. The innervation in the gnathal ganglia (GNG) is mostly by varicose processes. Innervation into the VNC shows varicose endings in all cases.

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Figure 5—figure supplement 1. DN presynaptic terminals in the gnathal ganglion. DN innervation in the brain (A1–J1) and VNC (A2–J2) obtained with the multicolor flip-out technique, and the corresponding result of synaptotagmin labeling in the GNG (A3–J3). Ten examples of DNs with varicose process in the GNG are shown.

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Figure 5—figure supplement 2. DN presynaptic terminals in the brain. The result of synaptotagmin labeling of split-GAL4 lines targeting four DN types with prominent varicose endings in the brain. Insets show magnification of regions indicated by orange dotted lines. (A) Morphology of DNp29. A neuron with similar morphology has been described in Drosophila (SP1; Nääs, 1993, Figure 4). The DN has both smooth and varicose processes in the superior lateral protocerebrum. Varicose processes are dominant in other regions. (B) Morphology of DNp32. A neuron with similar morphology in the brain has been described in Drosophila (DN1, Tanaka et al., 2012, Figure 5—figure supplement 2 continued on next page
Figure 5—figure supplement 2 continued

Figure 8. The DN has smooth process in the superior and inferior clamp, superior lateral protocerebrum and posterior lateral protocerebrum. Varicose processes are dominant in other regions. (C) Morphology of DNg30. Both smooth and varicose process are observed in the superior lateral protocerebrum and anterior ventral lateral protocerebrum. Varicose processes are dominant in other regions. The morphologies in the brain and VNC are similar to those of the natalisin-positive neurons, called inferior contralateral interneurons (Jiang et al., 2013, Figure 2) (D) Morphology of DNp27. Smooth processes are only observed in the superior medial protocerebrum. The morphology is partially similar to a known DN (GAMDN, Mu et al., 2014), but DNp27 lacks the innervation to the antennal mechanosensory motor center.

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Figure 5—figure supplement 3. DNs in the pars intercerebralis. (A–D) Morphology of DNc01 and c02. Whole morphology (A, C) and horizontal views of VNC (B, D) are shown. Both cell types have their cell body located in the pars intercerebralis, where Hsu and Bhandawat (2016) also observed DNs. c01, but not c02, has varicose process in the mushroom body lobe, and both have varicose process in the fan-shaped body, protocerebral bridge and the mushroom body calyx. c02, but not c01, has varicose process in the lobula. Both c01 and c02 project to leg neuropils as well as dorsal VNC neuropils and the medial ventral association center. However, c02 projects to the dorsal part of the leg neuropils, where c01 has no innervation. DOI: https://doi.org/10.7554/eLife.34272.025
Figure 5—figure supplement 4. DN presynaptic terminals in the brain. (A) Synaptotagmin labeling of DNp04 (green, membrane-bound GFP; magenta, synaptotagmin), shown as a maximum intensity projection of the whole morphology in the brain. Inset shows magnification of innervation in the GNG. (B) Innervation of p04 in the ventral lateral protocerebrum near the optic glomeruli. Three consecutive confocal stacks are shown with depth from the anterior brain surface indicated in top-right. p04 innervates the LC4 glomerulus with smooth processes and has presynaptic branches outside the glomerulus (C) Examples of synaptotagmin labeling in the brain for DNa05, p02, p05, g29 (both DNs in pair shown), b01, b06, and p09.

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Figure 5—figure supplement 5. Determining polarity of DNα05 by cell-specific co-expression of a membrane-bound GFP (green) and the presynaptic reporter synaptotagmin-smGFP-HA (magenta). Images of the brain (A) and VNC (B) are shown.

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Figure 5—figure supplement 6. Reproducibility of neuronal labeling in Split-Gal4 lines. Three samples are shown for DNp10 (SS01580), DNg07 (SS01074) and DNp01 (SS00727).
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Figure 6. The number of identified DNs and their neurite distribution. (A) The number of DNs innervating individual brain regions with smooth process (top) and varicose process (bottom). The inset is a heat map of DN innervation in the brain: sagittal and frontal views show brain neuropils in which the number of DNs with processes in each compartment are represented with pseudo-color. Polarity was determined based on their terminal morphology, and confirmed by synaptotamin expression in 55 cell types (see Figure 5). Neuropils of the caudal part of the brain, including the superior and inferior posterior slope (SPS, IPS) and GNG, contain smooth processes from the largest number of DNs. The GNG contains varicose processes from the largest number of DNs. The IPS and inferior bridge (IB) also contain varicose processes of many DNs. (B) The distribution of DNs Figure 6 continued on next page
Figure 6 continued

running through different descending tracts. Inset shows the heat map. The majority of DNs run through either the median tract of the dorsal cervical fasciculus (MTD) or the intermediate tract of the dorsal cervical fasciculus (ITD). The segmented image is modified from Boerner and Duch, 2010. Anatomical detail including the position and name for individual tracts are shown in Figure 3—figure supplement 1. (C) The distribution of DNs innervating individual VNC regions with varicose process. Inset shows the heat map. The number of DNs is greater for the dorsal side than ventral side in the VNC. The tectulum receive the largest descending input. (D–F) A histogram of the number of brain (D–E) and VNC (F) regions innervated by different DN types. Note that panels A-C quantify DN neurons individually, including the number of DNs in a given population type, whereas D-F count DN types, not individual neurons.

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Figure 6—figure supplement 1. Laterality and extent of DNs axonal projections. (A–C) The number of DN types innervating individual brain regions (A), tracts (B), and VNC regions (C) divided into ipsilateral- (green) and contralateral- (magenta) projecting DNs. (D) The number of DN types innervating each of the four different VNC segments. The majority of DNs terminated in the T3 segment. DNs reaching the abdominal segment (AS) were rare.

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Figure 7. DNs differentially address wing/neck/haltere and leg motor systems. (A) Clustering of VNC neuropils based on patterns of innervation by each DN. Filled pixels indicate that we observed varicose processes in the neuropil represented by the corresponding row, for the DN in the corresponding column. Pixel color indicates VNC compartment grouping: Dorsal neuropils (blue), tectulum (green), lower tectulum (red), leg neuropils (yellow), or other regions (AMN, AS, VAC, mVAC; gray). (B) Autocorrelation matrix of innervation pattern in the VNC. For each pair of VNC compartments, the Pearson’s correlation coefficient between DN innervation profiles was calculated. The strongest correlation was amongst compartments within the same grouping (see colors above) but in different segments. (C–F) Examination of DN varicose processes in the brain gnathal ganglia. (C) Three example DNs from different split-GAL4 lines aligned to a standard brain template and overlaid. Neurons are colored according to which VNC compartments they innervate (wing neuropil, blue; leg neuropils, yellow; lower tectulum, red). (D) Sagittal view of axonal projections within the VNC of a subset of the DN population. (E) Transverse view of DN terminals in the different VNC segments: metathoracic (E1), mesothoracic (E2) and prothoracic (E3). (F) Horizontal view of DN innervation in the GNG. Magnified view of dashed box in C, shows images of the three example DNs (left). A group of 15 DNs for which aligned VNC data were available are also shown in the same view (right). The varicose processes of DNs targeting the same compartments in the VNC also form separate clusters in the GNG.

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Figure 7—figure supplement 1. Putative octopaminergic DNs. (A–B) Morphology of DNd02. Whole cell morphology (A) and frontal sections of VNC (B) are shown. The cell body is located in the GNG and the axon descends ipsilaterally through tract DLV. d02 has smooth process in the GNG, wedge, vest, anterior ventral lateral protocerebrum and posterior ventral lateral protocerebrum, and varicose process in the wedge and GNG. d02 has dense innervation to the leg neuropils including the ventral association center (VAC), but avoids the dorsal part of the VNC, including neck motor, wing and haltere neuropils. A few processes project within the medial ventral association center. Axonal projections to the VNC return back into the posterior neck connective. The morphology is similar to an octopaminergic DN, termed the OA-VL1 (Busch et al., 2009). (C–D) Morphology of DNd03. Whole cell morphology (C) and frontal sections of VNC (D) are shown. The cell body is located in the GNG, and the axon descends ipsilaterally through tract DLT. The DN has smooth process in the GNG, wedge, vest, anterior ventral lateral protocerebrum, posterior ventral lateral protocerebrum, saddle, flange and posterior lateral protocerebrum, and varicose process in the wedge and GNG. The morphology is similar to an octopaminergic DN, termed the OA-VL2 (Busch et al., 2009).

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Figure 7—figure supplement 2. DN projecting to both wing and leg neuropil. (A) DNg17 has smooth process in the saddle and GNG, and varicose process in the GNG. The DN descends contralaterally in the neck connective and projects to both leg and wing neuropils. (B,C) Labeling of DNg17 with membrane (green) and presynaptic (magenta) markers in the brain (B) and VNC (C). The DN has a few process in the GNG, which show a positive signal for the presynaptic marker, and dense projections in the dorsal part of the prothoracic leg neuropil, and wing neuropil on the ipsilateral side.
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**Figure 7—figure supplement 3.** DN projecting to the ventral association center. (A) Morphology of DNg20. The DN has smooth processes in the saddle and GNG, and smooth and varicose processes in the GNG. The cell body is located on the GNG and the DN descends the contralateral neck connective. (B) Frontal view of DNg20 innervation in the VNC. The number in the top left of each image corresponds to the number shown in the VNC in panel (A). The innervation is localized in the ventral most area of the VNC, which corresponds to the sensory area. The DN also projects to the accessory mesothoracic neuromere (AMN) (B2).

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Figure 7—figure supplement 4. DN projecting outside the VNC. (A) Lateral view of DNg28 morphology. The DN has smooth processes in the GNG, and varicose processes in the nerve fiber. The axon mainly runs through the dorsal surface of the VNC. (B) Four examples of DNg28 morphology. The smooth processes in the brain are similar, whereas the axon pathways are highly variable.

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Figure 8. Brain innervation by DNs. (A) Clustering of brain neuropils based on patterns of DN innervation. Both brain neuropils (rows) and DNs (columns) were sorted by hierarchical clustering based on Pearson’s correlation as a metric and average linkage for calculating distances. Only brain compartments with DN innervation are shown. (B) Autocorrelation matrix shows the similarity of DN innervation pattern among brain regions. For each pair of brain compartments, the Pearson’s correlation coefficient between DN innervation profiles was calculated.

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Figure 9. DN connectivity between the brain and VNC. (A) Dendritic distribution of DNs grouped by output. Heat map colors indicate the number of DN types innervating each brain neuropil for different groups of DNs (A1–A8) defined by their projection to a specified VNC neuropil. The brain innervation pattern is similar among DN groups projecting to the different dorsal VNC neuropils (neck motor, A1; wing, A2; haltere, A3) and among DN groups projecting to the different segmental leg neuropils (foreleg, A5; middle leg, A6; hindleg, A7). The distribution pattern for DNs projecting to the

Figure 9 continued on next page
lower tectum is different from others, with the largest number of DNs emanating from the posterior ventral lateral protocerebrum (PVLP). (B) Distribution of DN axonal projections grouped by input. Heat map colors indicate the number of DN types innervating each VNC neuropil for different groups of DNs (B2–B6) defined by their projection from a specified brain neuropil. The VNC atlas is shown in the left panel. Innervation biased for the leg neuropils is observed in DNs from the GNG, for the lower tectum from the PVLP, and for dorsal neuropils from the AMMC, IPS and SPS. (C) The connectivity matrix shows with pseudocolor the number of DNs that innervate both a given brain (columns) and VNC (rows) neuropil. Rich connections are observed from inferior and posterior slope (IPS and SPS) to the dorsal neuropils and from the gnathal ganglia (GNG) to the leg neuropils. For neuropil abbreviations, see Supplemental file 6.

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Figure 9—figure supplement 1. Innervation profile of DNs sorted by innervation clusters in the VNC. In each row, a filled pixel indicates the presence of innervation by the corresponding DN in the corresponding CNS neuropil column. Smooth process in the brain and varicose endings in the VNC are shown.

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Figure 9—figure supplement 2. Innervation profile of DNs sorted by innervation clusters in the brain. In each row, a filled pixel indicates the presence of innervation by the corresponding DN in the corresponding CNS neuropil column. Smooth process in the brain and varicose endings in the VNC are shown.

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Figure 9—figure supplement 3. Neuronal pathways of DNs. (A) Location of the DN tracts. Horizontal view of the whole VNC (left) and frontal sections of each segment are shown (right). Each frontal plane corresponds to the depth shown in dotted lines in the left panel. The tracts are labeled by color. (B) Reconstruction of neuronal pathways in the VNC. Segmentation data used is from Boerner and Duch, 2010. (C) Examples of DNs running through eight different neuronal pathways. For each image, a DN on one side was reconstructed from confocal stacks. (D–E) The relationship between the brain (D) or VNC (E) innervation and tract usage. The number of DN types are shown with pseudo-color.

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Figure 10. Tract-based analysis of DN axonal projection patterns in leg neuropils. (A) Major DN types projecting to leg neuropils have different terminal patterns that segregate by descending tract. Horizontal (A1) and frontal (A2) views of overlaid aligned DNs running through VLT, ITD (green), or DLV and MTD (magenta) illustrate these two disparate patterns. (B–F) Individual examples of axonal projections to leg neuropil for DNs running

*axon through oblique tract*
through the (B) VLT, (C) DLT, (D) ITD, (E) oblique via MTD, or (F) oblique via DLV tracts. Transverse sections of the prothoracic (top), mesothoracic (middle) and metathoracic (bottom) neuromere are shown. In most cases, the termination zone of axons were similar among the different segments and for DNs within the same tract. DNs do not innervate the hindleg neuropil in some cases. Note DNg14 was the only DN identified in this study that traverses the DLT tract. (G) Examples of neuronal polarity of DN axonal projection in leg neuropils. The synaptotagmin signal (magenta) was observed in terminals all along the oblique tract (bottom row).

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Figure 10—figure supplement 1. DNs running through the oblique tract. (A) Morphologies of six DNs with axons in the oblique tract. (B–D) Merged horizontal (B), sagittal (C), and frontal (D) views of the six DNs shown in panel (A), showing overlap and formation of the oblique tract. Individual data
are compared by registration to a standard VNC. The DNs running through the MTD tract (DNp07, p05, p18, p11, and p10) run dorsally to the DN running through the DLV (DNp02).

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Figure 10—figure supplement 2. Potential overlap between DNs and leg motor neurons. (A) Leg motor neuron dendrites form a myotopic map in each leg neuropil. Dendritic regions for motor neurons corresponding to different leg segments are schematized as different colored regions, based on Brierley et al. (2012). (B) Axonal projection of DNg38 running through the VLT tract. Right panel shows potential overlap between the DN (black) and dendrites of leg motor neurons for proximal leg joints (color). The DN may not overlap with motor neurons for distal segments, such as tibia (red). (C) Axonal projection of DNg11 running through the MTD and oblique tracts. Left panels shows potential overlap between the DN (black) and dendrites of leg motor neurons (color). The DN may overlap leg motor neurons for all segments.

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Figure 11. Tract-based analysis of DN axonal projection patterns in the wing neuropil. (A) Sagittal view of two example DN types targeting wing neuropil (blue dashed line) via the MTD tract. The axon of DNp03 (green) travels ventrally, with the volume of its major axon in the MTD. In contrast, DNg03 runs through the dorsal surface of the VNC from T1 to the middle of T2 segments, and enters the MTD tract in T2 (magenta). (B–C) Frontal view

Figure 11 continued on next page
of more example axonal projections (B) for DNs running through the ventral route (left) and dorsal route of the MTD (right). Merged images of DNs running through ventral and dorsal MTD route (C) shown for a middle section of the prothoracic neuropil, anterior and posterior sections of the mesothoracic neuropil, and an anterior section of the metathoracic neuropil illustrate how the two groups target different sublayers in the wing neuropil. (D–H) Individual examples of DN axonal projections in the wing neuropil for DNs running through the (D) MTD ventral, (E) MTD dorsal, (F) MDA, (G) DLT, and (H) ventral route tracts. Shown are a horizontal view of the whole VNC (top), and frontal sections of the prothoracic (2nd panel), mesothoracic (3rd) and metathoracic neuromeres (4th). Synaptotagmin labeling is shown at the bottom for the bilateral pair. Note DNg27 is the only DN identified in this study which runs through the MDA.

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Figure 11—figure supplement 1. Innervation in the wing neuropil of DNs and interneurons. (A,B) Confocal stacks of power muscle (green) and steering muscle (magenta) motor neurons (MNs). Horizontal (A) and frontal (B) views are shown. For each image, the depth of the horizontal plane is shown in the top-right (A). The depth in anterior-posterior axis shown in (B) and corresponds to the line markers shown in (A). The innervation profile of power and steering MNs is largely segregated. SS31997 was used to visualize power muscle motor neurons and SS00737, SS04528 and SS31976 were used to visualize steering muscle motor neurons. The motor neurons were classified based on their dendritic morphology (Trimarchi and Namiki et al. eLife 2018;7:e34272. DOI: https://doi.org/10.7554/eLife.34272)
Schneiderman, 1994. (C) Maximum intensity projection of the T2 segment for steering muscle MNs (left), power muscle MNs (center) and both with counterstaining (right). (D) Three-dimensional reconstruction of dendritic innervation of power and steering muscle motor neurons. (E,F) Confocal stacks of steering muscle MNs (magenta) and a group of type-I DNs (yellow). Horizontal (E) and frontal views are shown (F). SS01081, SS01546, SS01589, SS02536 were used to visualize type-I DNs. (G,H) Confocal stacks of populations of power muscle motor neurons (green) and a population of type-II DNs (yellow). Dorsal (G) and frontal (H) views are shown. SS02633, SS00733, SS02260, SS01557 and VT049125 were used to visualize type-II DNs.
Figure 11—figure supplement 2. Innervation in the wing neuropil of DNs and interneurons. (A) Reconstructed morphology of four different DNs running through the dorsal route of the MTD shown with pseudo-color for depth. Cold and hot colors indicate the dorsal and ventral part of the wing.
neuropil, respectively. (B) As in (A) for four DNs running through the ventral route of the MTD. The anterior-most branch mostly shows wider innervation, which is located deeper than those in DNs through dorsal tract of the MTD (A). (C) As in (A) for a power muscle MN for the dorsoventral muscle. (D) As in (A) for steering muscle MNs for basalar 1 (left) and 2 (right).

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Figure 12. DNs from optic glomeruli. (A) A matrix indicating DN innervation in the optic glomeruli. Neurite innervation in individual glomeruli was observed with a 63x objective. Black and gray pixel shading represent dense and sparse innervation, respectively. Many DNs were identified that innervate the LC4, and LC22/LPLC4 glomeruli. No DNs were identified that innervated about a half of the glomeruli. (B) The number of DNs innervating individual glomeruli is shown as pseudo-color onto the 3D-atlas of optic glomeruli. More DNs were found innervating the more posterior-ventral glomeruli.

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Figure 12—figure supplement 1. Morphology of DNs from optic glomeruli in the posterior ventral protocerebrum. High-resolution (63x) confocal images of the 10 different DNs innervating the optic glomeruli in the PVLP. Neurite morphology in the brain is shown, as well as images at the depth of Figure 12—figure supplement 1 continued on next page.
the optic glomeruli (insets). Seven DNs descend on the ipsilateral side and the others descend on the contralateral side. DNs have smooth process in one or a few optic glomeruli and most of DNs (9 out of 10) have varicose process in the GNG. DN cell membrane, green; nc82 neuropil stain, grey; yellow and red dotted circles indicate individual glomeruli.

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Figure 12—figure supplement 2. Morphology of DNs from optic glomeruli in the posterior lateral protocerebrum. High resolution (63x) confocal images of the six different DNs innervating the PLP optic glomeruli. Images as in Figure 10. The majority of these DNs (5 out of 6) descend contralaterally through the neck connective.

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Figure 12—figure supplement 3. Morphology of a DN innervating both olfactory and optic glomeruli. (A) Morphology of DNb05. (B) Segmentation of DNb05 on both sides of the CNS. The outer shapes of the brain and VNC are shown with gray. (C) Reconstruction of DN innervation in the antennal lobe. Neuron and glomeruli are shown in black and gray, respectively. The glomeruli innervated by the DN are shown in color. (D) Confocal stacks of DN innervation in the antennal lobe. Plane depth indicated in top-right of images. (E) Reconstruction of DN innervation to the optic glomeruli. Colors as in (C).

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Figure 13. DNs forming a dendritic cluster within the LC4 glomerulus project to the lower tectulum. (A) The morphologies of DNs which have dendritic innervation to the LC4 glomerulus ('LC4 DNs'). The maximum intensity projection of a confocal stack with 20x objective are shown. All DNs partially share input (LC4 glomeruli) and most of them have axonal projection into the lower tectulum. These DNs are comparable to the ‘descending neuron cluster’ reported in blowflies (Milde and Strausfeld, 1990). (B) Simultaneous labeling of two different DNs innervating the LC4 glomerulus, visualized using multicolor flip out (see Materials and methods). Three examples are shown. The shape of the LC4 glomerulus is shown with a dotted line. (C) Sagittal view of LC4 DN axonal projections within the VNC. All but one (8/9) have axon terminals in the lower tectulum region of the VNC. One DN does not innervate this region (DNp03). An example of simultaneous labeling of 2 DNs is shown (DNp04 and p06, right). (D) Frontal view of LC4 DN projections in the mesothoracic neuropil. Projections are focused in the central region of the VNC volume, in the lower tectulum layer.

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Figure 14. Anatomical organization of DNs in Drosophila. (A) Sagittal view schematic of brain and VNC illustrating the major descending pathways. Gross innervation areas of different DN types are shown with color. Inset shows number of each DN type targeting the three main VNC layers. (B) The wiring diagram between the brain and VNC via DNs. Only the major connections are shown. (C–D) Schematic of DN axonal projection into wing neuropil shown in sagittal (C) and frontal (D) views. DN populations that supply axons from the dorsal surface (type-II) provide more terminals than those contained within the more ventral MTD tract (type-I). See also Figure 11. Type-I DNs are more likely to project to the dorsal zone, whereas the type-II DNs are more likely to project to the ventral zone of the wing neuropil. (E–F) Schematic showing two types of DN innervation patterns in leg neuropils.
Figure 14 continued

for the whole VNC (E) and a single leg neuropil (F). The majority of DNs send projections to the medio-dorsal area of leg neuropil (magenta), whereas DNs running through oblique tract via MTD or DLV have fewer terminals and extend to the ventral part of the leg neuropil (green). In most cases, DNs do not innervate the ventral association center (VAC), the ventralmost part of the VNC, which is enriched for afferent sensory projections (F).

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Figure 15. Biased representation in the protocerebrum among DNs targeting wing and leg motor areas. (A) Morphology of DNs preferentially innervating anterior (DNg02, right) and posterior parts of the brain (DNg13, left). DNg02 projects to dorsal VNC, whereas DNg13 projects to ventral VNC. (B–C) Frontal view of brain neurite morphology for four DNs projecting to the (B) dorsal or (C) ventral neuropils. The dorsal-projecting DNs have neurites limited to the posterior side of the brain, whereas the neurites of ventral-projecting DNs extend to the anterior side of the brain. (D) Neurite distribution of the DNs. The relative density of neurites for each DN are shown in gray scale along the anterior-posterior axis based on aligning the data in the registered brain. The red circle indicates the center of mass of the neurite distribution. Along the x-axis, the DNs are arranged in by center of mass position from anterior to posterior. Neurite density was normalized by the maximum value for individual neurons. Table below shows DN projection neuropils in the VNC (blue, dorsal neuropils; yellow, leg neuropils). DNs with innervation toward the ventral side are more likely to project to leg neuropils. (E) Schematic of hypothesized information flow in visual descending pathways. The DN dendritic regions in the protocerebrum are not separated, rather there may be a gradient in the preference for axonal projection.

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