Figures and figure supplements

Conserved neural circuit structure across *Drosophila* larval development revealed by comparative connectomics

Stephan Gerhard *et al*
Figure 1. Structure of mdIV terminals through postembryonic development. (A) Cartoon comparison of the dendritic fields of the three nociceptive mdIV sensory neurons from a single hemisegment at first and third instar stages; sagittal view; anterior to left. (B) Dorsal view of EM reconstructions of all mdIV terminals from a single abdominal segment in the L1v (left; segment A1, first instar larva) and L3v (right; segment A3, third instar larva) data. Colors are as in A. The vertical extent of the gray box indicates the width of the neuropil; anterior to left; dashed line indicates midline. (C) Morphology of the terminals of each mdIV subtype, presented as in B. Unbranched primary projections from the nerve are cropped. (D) Dorsal view of a single vdaB terminal from the L1v and L3v, shown with synapses (outputs, red; inputs, cyan). Dashed line indicates midline. (E) Number of synaptic outputs on each mdIV terminal. L1v (solid bars), L3v (empty bars); left/right bar corresponds to left/right neuron. (F) Fold-change in synaptic outputs in the L1v and L3v. For each mdIV subtype, left and right neurons were averaged. (G) A standard polyadic synapse. In this example, taken from the L3v, the single pre-synaptic site (red arrowhead) has four post-synaptic contacts (cyan arrowheads). (H) Normalized histogram of number of post-synaptic contacts per pre-synaptic site on mdIV terminals (No significant difference; p=0.5641, two-sided Kolmogorov-Smirnov test). n.s. not significant; *p<0.05. **p<0.01. ***p<0.001.
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Figure 1—figure supplement 1. A new EM image volume from a third instar larva ventral nerve cord. (A) Schematic of the region of the third instar larva CNS sectioned and imaged for the L3v. Anterior is up. (B) A single section of L3v includes the complete neuropil (region inside white outline) and all soma (region outside white outline). Dorsal is up. (C) Ventromedial neuropile indicated in the blue outline in B. Neurite cross-sections highlighted in orange correspond to ipsilateral mdIV axons. (D–F') Example synapses from vdaB (E), v'ada (E,E'), and ddaC (F,F') terminals. Vesicles and pre-synaptic specializations highlighted by the red arrowhead. Post-synaptic neurons from LNs described in the main text are highlighted. Note the combination of small and dense core vesicles found in all three mdIV neurons.

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Figure 1—figure supplement 2. Reconstructions of mdIV terminals. (A, A'), Dorsal view of all mdIV terminals from the L1v (A) and L3v (A'), identities as labeled. Views are at the same scale. Dashed lines indicate lateral neuropil boundaries, solid line the midline. (B, B') ddaC terminals in the L1v (B) and L3v (B'), left and right shown separately for clarity, as in all subsequent panels. ddaC can be distinguished by a midline crossing where the axon initially approaches the midline from the nerve and a projection into the adjacent segment posterior with little to no midline crossing. (C, C'), v’ada terminals in the L1v (C) and L3v (C') can be distinguished by a lack of midline crossings and a projection into the adjacent segments anterior and, typically, posterior. D, D', vdaB terminals in the L1v (D) and L3v (D') can be distinguished by a midline crossing both where the axon initially approaches the midline and a second midline crossing in the adjacent segment anterior. Note that for all mdIV types, there is some variability — extra or missing branches, such as the missing posterior branch of the right L1v v’ada, are true reflections of the data — although certain features remain typical across most cell types.

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Figure 2. Morphology and properties of second-order nociceptive LNs. (A). Starting from the synapses of the mdIVs in segment A1 of the L1v, we reconstructed all synaptic partners (grays). See Figure 2—figure supplement 1 for details of each cell type. Dorsal view, gray outline indicates CNS boundary; anterior is to left. (B) Examples of the anatomy of all five classes of LNs from the L1v. Posterior view; gray outline indicates neuropile boundary, orange shows mdIV position. (C) Based on the mdIV reconstructions in the L3v (orange), we reconstructed the same populations of all mdIV LNs in segment A3 (grays; 12 LN cells in total). (D) Examples of the anatomy of all five classes of LNs from the L3v, shown as in B. (E) All neurons were split into axonal and dendritic compartments based on well-separated synaptic input and output domains. The example shown is the A02n from D. (F), Total dendritic cable length for all LNs. (G) Number of synaptic inputs onto LN dendrites. (H) Fold-change in dendritic cable length and dendritic synaptic inputs between the L1v and L3v LNs.

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Figure 2—figure supplement 1. The complete second-order mdIV network from the L1v. (A) All cell types synaptically connected to mdIV terminals in the L1v. Cell types were organized by spatial extent of the dendrites. Dorsal views of a single example of each interneuron cell type (black) and the mdIV terminals of segment A1 (orange), anterior to left. Outline indicates CNS boundary. Local neurons (LNs) had dendrites spanning 1–2 segments, regional neurons (RNs) had dendrites spanning 3+ segments but not the whole VNC, a descending neuron (DN) had dendrites in subesophageal zone (SEZ) and an axon in VNC, and ascending neurons (ANs) had cell bodies in the posterior tip and projections that spanned the entire VNC toward the brain. See Supplemental Atlas for more views of cell types. (B) Connectivity between individual cells in the mdIV network expressed as an adjacency matrix. Entries indicate the number of synaptic contacts from the column neuron to the row neuron. Black lines separate mdIV/LN/RN/DN/AN classes. Note that mdIV order is clockwise from ventral left. (C) Connectivity between cell types in the mdIV network. Each column indicates connections from cell types in the left category to all cell types. Line thickness indicates number of synapses. Connections not observed at least twice at a 3+ synapse level are not shown here. Additionally, to the LN networks discussed elsewhere, we also find a strong pathway for feedback regulation of mdIV terminals. The SEZ neuron SelN138 has an axonal projection descending through every abdominal segment, along which it both receives synaptic input from and outputs back onto mdIV terminals of all subtypes, offering a local axo-axonal feedback pathway across just a few microns of axonal arbor. Interestingly, SelN136 also receives dendritic input near the SEZ from two ascending mdIV projection neurons, A08m and TePn19, that receive mdIV input throughout the nerve cord. This mdIV—AN—DN—mdIV pathway could allow every mdIV terminal across the body to be presynaptically regulated by ascending nociceptive input coming from any one location on the body. No other cell type was strongly or consistently pre-synaptic to mdIV terminals, suggesting this is the only such direct pathway.

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Figure 2—figure supplement 2. Additional LN properties. (A) Total axonal cable length for A02n, A09l, and A10a. The LNs A09a and A09c had incomplete axons in the L3v due to the limited extent of the image volume and are omitted from axon-related analysis here. (B) Number of synaptic inputs onto LN axons. (C) Number of axonal outputs for LNs. (D) Number of synaptic outputs on the dendrites of each LN. All neuron types that exhibited dendritic outputs in the L3v also had them in the L1v, suggesting that all of the basic categories of connections are preserved. (E) Fold-change between the L1v and L3v for the properties in A–D. Colors correspond to cell types. Axonal cable scales significantly less than dendritic cable (p=0.009, two sided t-test with Bonferroni correction), though other differences between axonal and dendritic property scaling are not significant. (F) Segregation index for complete LNs, which measures the degree of input/output segregation of a neuron (one indicates a completely segregated neuron, with all outputs in one region and all inputs in another; 0 indicated a neuron with completely intermixed inputs and outputs. See Materials and methods for precise definition.) Note that segregation index is generally maintained as a cell type-specific property across larval stages. DOI: https://doi.org/10.7554/eLife.29089.007
Figure 3. Connectivity of second-order nociceptive LNs is topographically arranged and consistent across larval development. (A) Number of synaptic inputs onto LNs from mdIV terminals in the same segment. (B) Normalized dendritic synaptic input from mdIV terminals for each LN. (C) Fold-change in number of synapses and normalized synaptic inputs from mdIVs for each LN type. (D) Heatmap of normalized dendritic input from each mdIV terminal onto each LN for L1v (left) and L3v (right). Note that mdIV terminals are ordered clockwise from ventral left. (E) Normalized dendritic input from mdIVs onto LNs is strongly correlated across animals and developmental time points. Each data point corresponds to average normalized dendritic input from an mdIV type onto an LN type. (Pearson’s r = 0.77, p<0.001 to be different from zero). (F) Asymmetry between normalized mdIV synaptic input into left and right LNs, measured as coefficient of variation. Asymmetry in the L3v is significantly lower (p=0.006, paired two sided t-test). (G) Cartoon of the larval body wall viewed from posterior. The dendritic receptive field of each mdIV covers approximately 1/6 of the circumference of the animal. (H) Mean body wall orientation of mdIV input into each LN in the L1v (left) and L3v (right), computed as the average of unit vectors pointing at the center of each mdIV dendrite receptive field, weighted by number of synaptic inputs from that neuron. Arrow color corresponds to LN type. n.s. not significant; *p<0.05. **p<0.01. ***p<0.001.

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Figure 3—figure supplement 1. Topographically structured feed-forward connectivity between mdIV-related LNs. (A) Synaptic connectivity between LN cell types in the L1v (solid bars) and L3v (empty bars). Each bar plot depicts the number of synapses each cell of the post-synaptic cell type (rows) receives from all cells of the pre-synaptic cell type (columns). Cell types are labeled with spatial receptive fields from Figure 3H. Each cell type that was strongly connected in the L1v was again connected in the L3v. Strikingly, the dorsally oriented A09a targeted the dorsally oriented A02n and the ventrolaterally oriented A09c and A09l targeted the ventrolaterally oriented A10a, suggesting feed-forward topographic microcircuits. (B) Normalized synaptic connectivity between LNs. (C) Mean strength, measured as normalized synaptic inputs, for specific connections between cell types in the L1v and L3v. The number of data points is too small to make a statistical conclusion.

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Figure 4. Numerically strong connections are associated with stereotypically high filling fraction. (A) Description of ‘filling fraction’ for a connection from Neuron 1 (purple) to Neuron 2 (black). Neurons can only be connected where they are adjacent to one another in space. A pre-synaptic site on Neuron 1 is a potential synapse from Neuron 1 to Neuron 2 if any part of Neuron 2 passes within a given radius (dashed circles). Filling fraction is defined as the number of potential synapses (red and green dashed circles) that are actually connected (green dashed circles only). (B) Dependence of filling fraction on the potential synapse radius for four example connections. For subsequent figures, we chose $2\mu m$ (filled circles) as a compromise between the typical size of a terminal branch and a shoulder in the filling-fraction versus radius curve. (C–D) Mean filling fraction vs. mean number of synapses in the L1v (C) and L3v (D). Each data point represents the average value for connections from mdIV types onto LN types. The high correlation in both (L1v, Pearson $r = 0.99$, $p<0.001$ different from zero; L3v, Pearson $r = 0.93$, $p<0.001$) suggests that increased connection probability, not merely access to differing numbers of pre-synaptic synapses, helps set cell type-specific differences in synaptic counts. (E) Filling fraction of mdIV type to LN type connections in the L1v and L3v are significantly correlated with one another (Pearson $r = 0.64$, $p=0.009$).

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**Figure 5.** The structure of terminal dendritic branches across postembryonic development. (A) Definition of microtubule-containing ‘backbone’ (black) and microtubule-free, spine-like ‘twigs’ (red). (B) Example A02n cell (from the L3v) where all twigs are labeled, posterior view. (C) Number of twigs in each LN in the L1v and L3v. Inset: Fold-change in number of twigs between the L1v and L3v. (D) Fold-change in length of cable comprised of twigs or backbone in the L1v and L3v. Twigs increase more than backbone (two sided t-test). (E) Fraction of dendritic cable comprised of twigs for all LNs. (F) The average fraction of dendritic cable comprised of twigs per cell type was larger in L3v than L1v (two sided, paired t-test). (G) Input synapses that contact twigs as a fraction of all input synapses for all LNs. (H) The fraction of input synapses that are onto twigs increased significantly (two-sided, p=0.003, paired t-test). (I) Cartoon definition of Strahler order. Terminal tips are defined to have Strahler order 1. Where two branches with the same Strahler order converge, the value increments by one. The most core, proximal neurites thus have the highest Strahler order. (J) An example A09a cell from the L1v with branches labeled by Strahler order (Dorsal view). (K) Fraction of dendritic cable for each LN cell by Strahler order. The relative amount of cable with low Strahler order (i.e. distal) is approximately conserved between the L1v and L3v neurons. n.s. not significant; *p<0.05. **p<0.01. ***p<0.001.

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Figure 5—figure supplement 1. Twig and backbone morphology for all LNs. Backbones are shown in black, twigs with colors. Neurons from the L1v are shown to left (Regular letters), neurons from the L3v to right (Primed letters). Posterior view with dorsal up. Scales are consistent across all figures, scale bars are 20 µm.
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Figure 6. Twig properties across postembryonic development. (A) Distribution of morphological and synaptic properties of distinct twigs in the L1v and L3v LNs. Total twig length and maximum twig depth are in µm, branch points and synapses are integer. Boxes are interquartile intervals, blue dashes are median values, and whiskers correspond to 5/95 percentiles. Wilcoxon rank sum test with Bonferroni correction. (B) Number of distinct twigs in a connection versus number of synapses in the same connection. The blue line indicates a linear fit to connections with five or more synapses (slope shown). The gray region corresponds to the disallowed situation of more twigs connected than synapses. (C) Histogram of number of synapses per twig in each mdIV—LN connection. (D) The fraction of synapses in the connection from a v’ada to an A09a in the L1v (9 synapses onto twigs) and L3v (56 synapses onto twigs) recovered after simulated random omission of twigs (N = 5000 instances), as a function of omission probability. Thick lines show median value, shaded region the 5/95 percentile value. The dashed horizontal line indicates the 25% of synapses recovered. (E) Maximum error rate permitting the recovery of 25% of synapses with probability >0.05 for each observed mdIV—LN connection (i.e. where the horizontal dashed line crosses into the shaded area in D). Each data point is a single mdIV axon’s synapses with a single LN. The vertical line indicates the error rate for twigs found previously for manual annotation of motor neurons (Schneider-Mizell et al., 2016). n.s. not significant; *p<0.05. **p<0.01. ***p<0.001.

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Figure 6—figure supplement 1. Individual twig properties, broken down by LN cell type. For each panel, bars indicate interquartile intervals, whiskers show 5/95 percentile lines. White dashes indicate median. Each bar collects twigs from all cells in the cell type, and each twig was weighted equally. (A) Box
Figure 6—figure supplement 1 continued
plots of total cable length per twig by cell type and developmental stage. (B) Box plots of maximum twig depth (distance from distal tip to twig base) by cell type and developmental stage. (C) Box plots of number of branch points per twig by cell type and developmental stage. (D) Box plots of number of input synapses per twig by cell type and developmental stage. (E) Box plots of minimum distance between twig bases along neuronal backbone by cell type and developmental stage. *p<0.05, **p<0.01, ***p<0.001, n.s.: not significant, two-sided t-test with Bonferroni correction.
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