Supplementary Information for

A multiscale brain map derived from whole-brain volumetric reconstructions

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Supplementary Results: A more technical treatment of: 1. Anaylsis to establish bilateral homology (Extended Data Fig 1); 2. Similarity between homologous and proximal immediate neighborhoods (Extended Data Fig 2a); 3. Validation of core-variable synaptic model (Extended Data Fig. 3); 4. Functional identification of neuron process clusters (Fig 1c,d and Extended Data Fig 5); 5. Rich-club neurons exhibit meso-connectivity properites (Fig. 3, Extended Data Fig. 8 and 9) ; 6. Network motifs and arhitectural features of the brain map (Extended Data Fig. 9).

Supplementary Information 1: Membrane adjacency data scored in the L4 and adult nerve rings. We used custom software (https://github.com/cabrittin/parsetrakem2) to extract and quantify membrane contacts from the segmented TrakEM2 data. Mebrane contacts for both the adult and L4 dataset are provided in a single plain ASCII text (.csv) file, which can be opened with any text editor. Each row lists a membrane contact intance, occuring between two segmented cells in an EM (see Methods: *Extracting adjacency data*). A single cell can have multiple portions of its neurite present in the same EM section. To differentiate between two segmented neurites belonging to the same cell, we index each segmented neurite belonging to the same cell in a single EM (cell_index1, cell_index2). EM_section_numbers correspond to the numbers in the original JSH, N2U series (White *et al.* 1986[5]) with numbers increasing from anterior to the posterior. Membrane contact is given in number of pixels. To convert from pixels to membrane contact area in μm², scale by 4.5e-4 (for both the L4 and the adult). Each row (i.e. membrane contact instance) has the following format: `[dataset, cell1, cell2, cell1_index, cell2_index, EM_section_number, membrane_contact_in_pixels]`. 'dataset' is listed as either 'adult' or 'l4', indicating that the EM on which the adjacency was scored came from the adult (N2U) or L4 (JSH) series, respectively. For example, row `[l4,URXL,CEPDL,0,0,JSH001,175]` is an L4 membrane contact between cells URXL neurite segment 0 and CEPDL neurite segment 0 in EM section JSH001. This membrane contact has a membrane contact length of 175 pixels which we estimate as 0.07875 μm².

Supplementary Information 2: Membrane contact localization data for all bilateral cells. Extended Data Fig. 1d demsonstrates the local membrane reproducibility count for RIA. Here, we provide equivalent plots for all (80) the bilateral cell classes in our restricted dataset (see Methods for restricted dataset and Supplementary Results for definitions of the notation). Left: for each cell, the raster plot shows the reproducibility counts (δ, color) of all $\mathbb{M}^4$ contacts made with that cell at effective location $\hat{z}$ (horizontal axis). Each row in the raster counts membrane adjacencies with a different neighboring process. The number of rows is the number of $\mathbb{M}^4$ membrane contacts that are reproducible immediate neighbors of that cell. The rows are arranged in alphabetical order of the neighboring cell names (from the top). Color: reproducibility count. The maximum spatial reproducibility count, $\max(\delta)_z$ is defined as the highest reproducibility count across all locations, $\hat{z}$, per cell pair (i.e. in every row in the raster). Right: The distribution of $\max(\delta)_z$ for immediate neighbors of one cell. Plots generated with elegancebrainmap (https://github.com/cabrittin/elegansbrainmap).

Supplementary Information 3: $\mathbb{M}^\delta$, $\mathbb{C}^\delta$ and $\mathbb{G}^\delta$ reference graphs. All reference graphs are provided in spreadsheets in an xlsx file. The file contains 4 sheets. Sheet 1: 'restricted_cells' lists the cells in the restricted dataset (173 cells, including 80 bilateral cell pairs and 13 single cells, see Methods). Sheets 2-4: 'M', 'C' and 'G' give edge lists and associated reproducbility for the corresponding reference graphs $\mathbb{M}^\delta$, $\mathbb{C}^\delta$ and $\mathbb{G}^\delta$ (above threshold contacts only see Methods, Fig. 2a). Each row corresponds to a single edge (i.e. contact) and has the following format: [cell_1, cell_2, weight, delta]. 'delta' is the number of datsets in which the contact is observed, δ. Synaptic and gap junctions, $\mathbb{C}^\delta$ and $\mathbb{G}^\delta$, are restricted to those that occur on $\mathbb{M}^4$ contacts. $\mathbb{M}$: 'weight' is the mean aggregate membrane contact (i.e. the mean number of pixels across the δ datasets, see Methods) in pixels (scale by 4.5e-4 to convert to μm²). $\mathbb{C}$

and $\mathbb{G}$: 'weight' is the mean number of EM sections across δ datasets (see Methods). For synaptic contacts $\mathbb{C}$, 'cell_1' and 'cell_2' are the pre- and postsynaptic cells, repsec tively.

Supplementary Information 4: Cluster assignment and process morphologies and synaptic organization features. These data are provided as a single xlsx file with 4 sheets. Sheet 1: 'final_classifications' lists the consensus cluster assignments for every cell class in the restricted dataset, brain map assignment and reproducible spatial features associated with that cell. For each cell class (93 classes), we list the members of the class, 'Final' cluster assignment (Fig. 1d), the module and layer assignment in the *C. elegans* brain map (Fig. 4b) and the descriptions of any subcellular structures (examples in Fig. 3d-g and Extended Data Fig. 8). Sheet 2: 'perturbation_clusters' lists the cluster assignments for all spatial population models ($\widetilde{\mathbb{M}^4}$, $\widetilde{L4}$ and $\widetilde{Adult}$ and validations, see Extended Data Fig. 5i-l and Extended Data Fig. 6b). Sheet 3: 'fig1c_row_order' lists the row and column order for the $\widetilde{\mathbb{M}^4}$ cluster frequency matrix in Fig. 1c. Sheet 4: 'edfig5i_row_order' gives the row and column order for the $\widetilde{\mathbb{M}^4}$, cluster frequency matrix in Extended Data Fig. 5i.

Supplementary Information 5: Validation of our membrane contact data against membrane contacts scored by White *et al.* (1983)[18]. White *et al.* scored membrane contacts for 3 cells (AIAR, AIBR and AQR) in the L4 (JSH) EM dataset and only scored contacts in every 5th EM section. As validation, we compared the adjacent cells extracted by our algorithm for these 3 cells to White *et al.* We provide the bidirectional comparison in an xlsx file with 2 sheets. Sheet 1: 'white_scored_by_brittin' lists the membrane contacts scored by White *et al.* (1983) for the 3 cells and states whether the contact was scored by our algorithm. Sheet 2: 'brittin_scored_by_white' lists the membrane contacts scored by our algorithm for the 3 cells and states whether the contact was scored by White *et al.* (1983). Membrane contacts scored by us are further divided into three groups ('low', 'mid', 'high) based on membrane contacts areas (see Methods and Extended Data Fig. 2a).

Supplementary Table 1: Summary of volumetric and reconstruction and extracted reference datasets.

Supplementary Table 2: Error analysis of reconstruction.

Supplementary Table 3: Lateral synaptic contacts are not distinguishable from random contacts.

Supplementary Table 4: Classification of brain map $\mathbb{C}^4$ contacts.

Supplementary Video 1: A fly through of the segmented L4 (JSH) serial sectioned electron micrograph (EM) series. Neurites are manually segmented with TrakEM2. Segmented neurites are colored based on final clusters assignment (Fig. 1c). The series starts at the most anterior section of the nerve ring neuropil and proceeds posteriorly.

Supplementary Video 2: A fly through of the segmented Adult (N2U) serial sectioned EM series. Neurites are manually segmented with TrakEM2. Segmented neurites are colored based on final clusters assignment (Fig. 1c). The series starts at the most anterior section of the nerve ring neuropil and proceeds posteriorly.

Supplementary Video 3: Volumetric rendering of the complete L4 nerve ring neuropil. Neuronal processes are colored by final cluster assignment (Fig. 1c). The video starts with the anterior part of the neuropil facing left, the left side of the neuropil facing the viewer and the dorsal part of the neuropil facing up. The neuropil is then rotated 360° about the vertical (dorsoventral) axis. Note that the wider

anterior regions correspond to the nerve ring commissure shown in Fig 1a. The narrower region corresponds to the posterior lobe of the nerve ring neuropil.

Supplementary Video 4: Volumetric reconstruction of representative (L4) neurons SMBVL and SMBVR. Rotating images of the SMBVL and SMBVR neurons illustrate the bilateral symmetry of their respective processes and of specialized topographical features along these processes. At the start of the video, both left and right neurons are colored by final cluster assignment (sublateral, Fig. 1c). The pharynx (gray volume, ~16 μm) is included for spatial context. Midway through the video the pharynx is removed and the left and right cells are assigned different colors to distinguish between the cells.

Supplementary Video 5: Volumetric reconstruction of representative (L4) neurons RIBL and RIBR. Rotating images of the RIBL and RIBR neurons illustrate the bilateral symmetry of their respective processes and of specialized topographical features along these processes. At the start of the video, both left and right neurons are colored by final cluster assignment (taxis, Fig. 1c). The pharynx (gray volume, ~16μm) is included for spatial context. Midway through the video the pharynx is removed and the left and right cells are assigned different colors to distinguish between the cells.

Supplementary Video 6: Volumetric reconstruction of representative (L4) neuron class IL1. Rotating images of all six IL1 class members (IL1L/R, IL1VL/R,IL1DL/R) illustrate the bilateral symmetry of more complex processes. IL1 processes are closely aligned on both sides of the body. At the start of the video, all cells are colored by final cluster assignment (anterior, Fig. 1c). The pharynx (gray volume, ~16μm) is included for spatial context. Midway through the video the pharynx is removed and each of the 6 neurons is assigned a different color to distinguish between the cells.

Supplementary Video 7: Volumetric reconstruction of representative (L4) neurons RIML and RIMR. Rotating images of RIML and RIMR neurons illustrate the bilateral symmetry of their respective processes and of specialized topographical features along these processes. At the start of the video, both left and right neurons are colored by final cluster assignment (lateral, Fig. 1c). The pharynx (gray volume, ~16μm) is included for spatial context. Midway through the video the pharynx is removed and the left and right cells are colored differently to distinguish between the cells.