

Supplementary Information

Supplementary Table 1. KC γ -d Visual Inputs.

A list of all FlyWire visual projection neurons (VPNs) and local visual interneurons (LVINs) with direct or indirect input to KC γ -d neurons (≥ 5 synapse threshold). FlyWire neuron IDs are given for each neuron from the version 630 snapshot of the data, and when applicable, the updated version of the ID as of October 5, 2023. When able, we supply the ID of the neuron match in the opposing hemisphere (i.e. Mirror Twin). NBLAST cluster IDs for all direct inputs as well as left hemisphere indirect inputs are given.

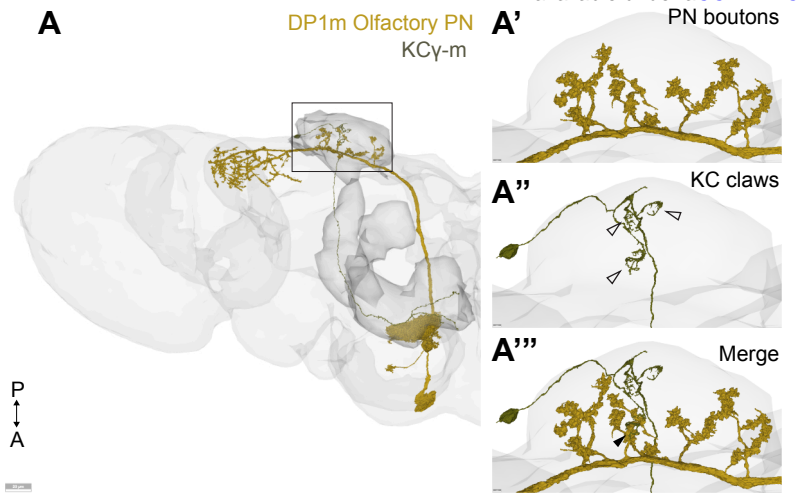
Supplementary Table 2. KC $\alpha\beta$ -p Visual Inputs.

A list of all FlyWire visual projection neurons (VPNs) and local visual interneurons (LVINs) with direct input to KC $\alpha\beta$ -p neurons (≥ 5 synapse threshold). FlyWire neuron IDs are given for each neuron from the version 630 snapshot of the data, and when applicable, the updated version of the ID as of October 5, 2023. We identify the cells that are also inputs to KC γ -d.

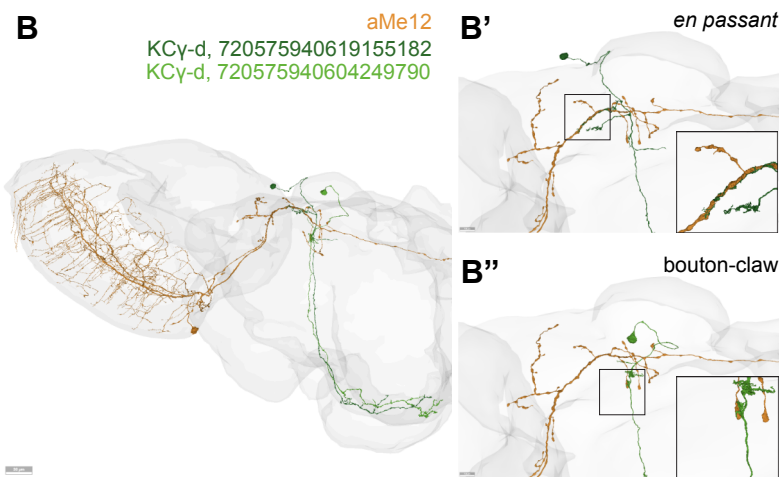
Supplementary Table 3. Hemibrain-FlyWire Matches.

A list of all FlyWire visual projection neurons (VPNs) and local visual interneurons (LVINs) with direct or indirect input to KC γ -d neurons (≥ 5 synapse threshold) along with their putative matches in the Hemibrain dataset obtained by computing NBLAST scores. Raw NBLAST scores are reported. The total number of direct

synapses each neuron makes onto the KC γ -d population in both the FlyWire and Hemibrain datasets as well as the Hemibrain Types are included. FlyWire neuron IDs for each neuron are from the version 630 snapshot.



Visual PNs and KCs form bouton-claw and *en passant* synapses



LVINs and KCs form bouton-claw and *en passant* synapses

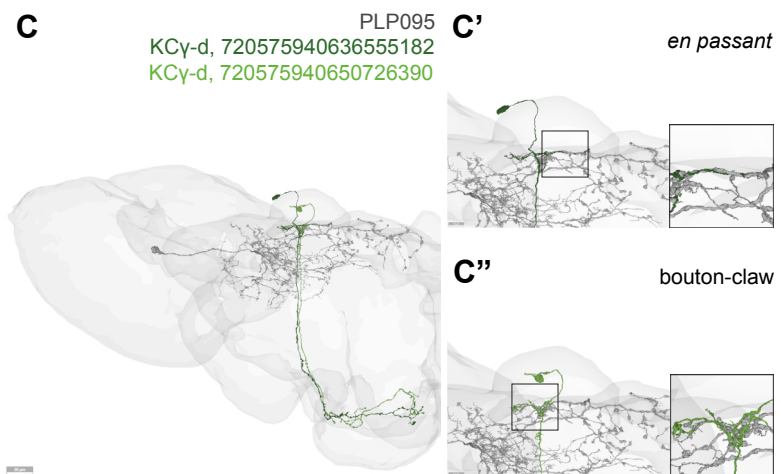


Figure 1 Supplement 1. KCy-d's form putative bouton-claw synapses and *en passant* synapses with VPNs and LVINs.

A. Olfactory projection neurons and olfactory Kenyon cells form bouton-claw synapses that are typical of the olfactory calyx. (A') Zoom in of the mushroom body calyx, showing PN boutons. (A'') KCy-m cell with dendritic claws indicated with open arrowheads. (A''') Merge of A' and A'' with bouton-claw contacts indicated by arrowheads. Scale bar for A, 20 μ m. Scale bar for A'-A''', 2 μ m.

B. Example of a VPN, aMe12, that form putative *en passant* synapses with one KCy-d (B') and a putative bouton-claw synapse with another KCy-d (B''). Insets show a zoomed in view of the boxed regions. Scale bar for A, 20 μ m. Scale bar for B'-B'', 7.5 μ m.

C. Example of an LVIN, PLP095, that forms putative *en passant* synapses with one KCy-d (C') and a putative bouton-claw synapse with another KCy-d (C''). Scale bar for A, 20 μ m. Scale bar for C'-C'', 7.5 μ m.

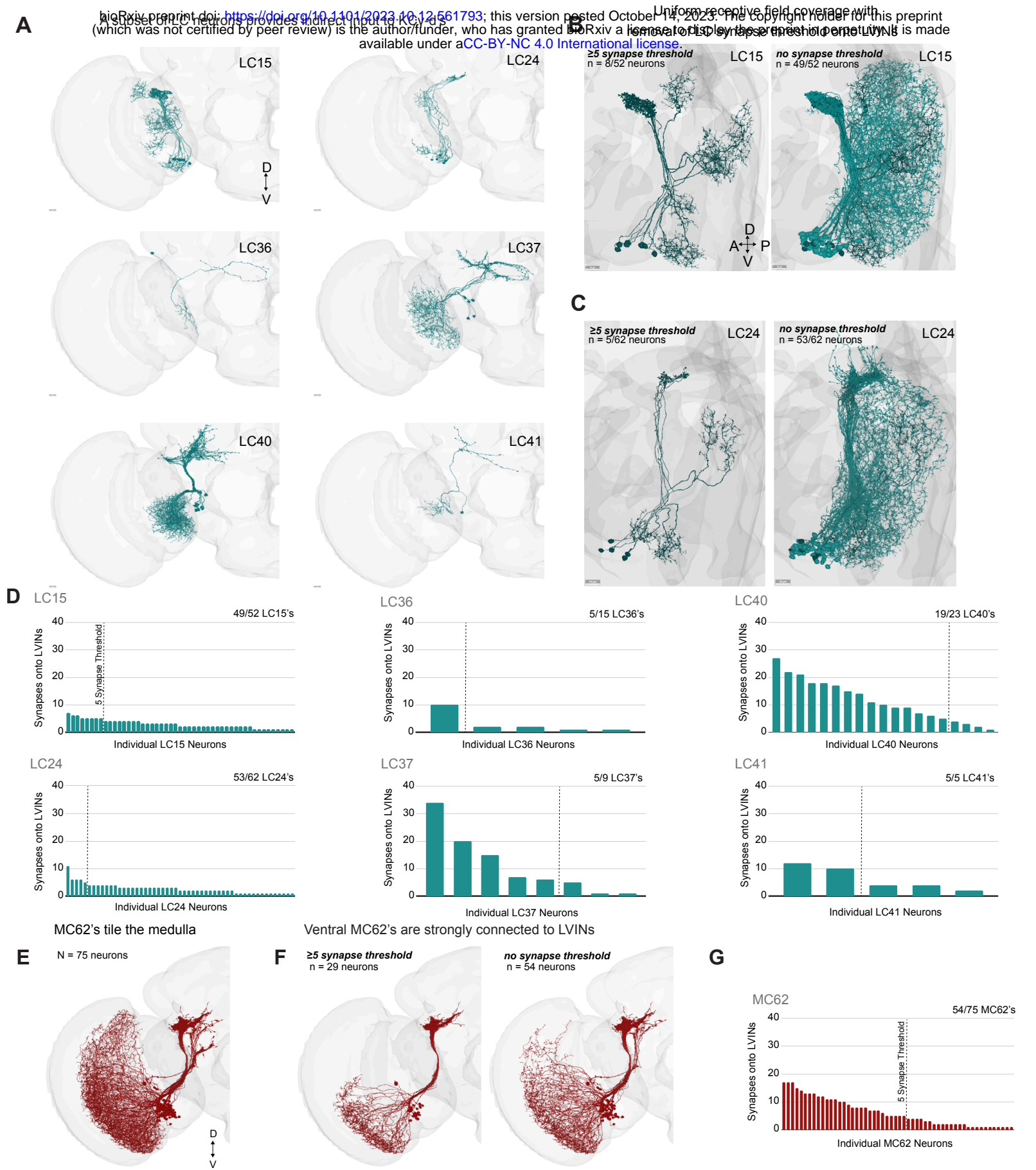
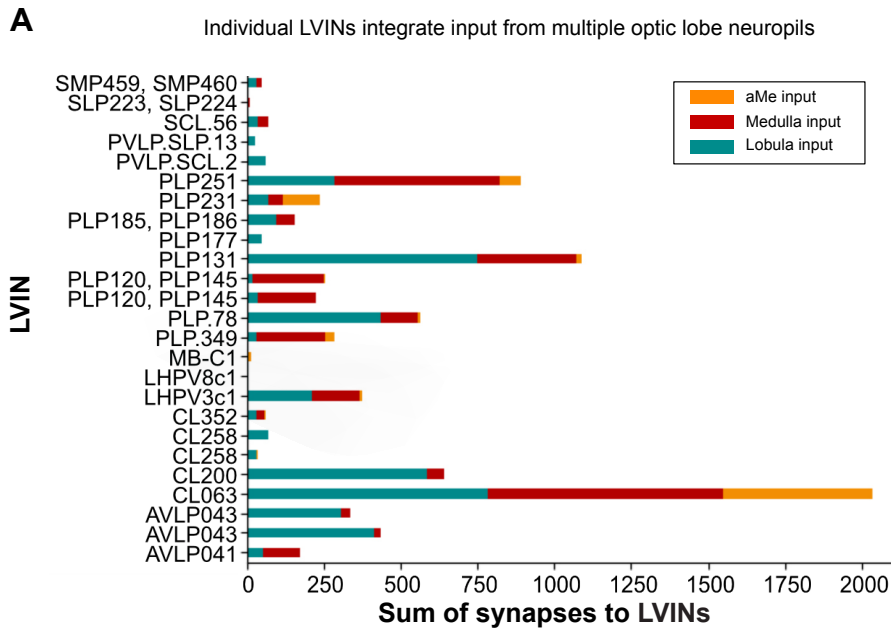
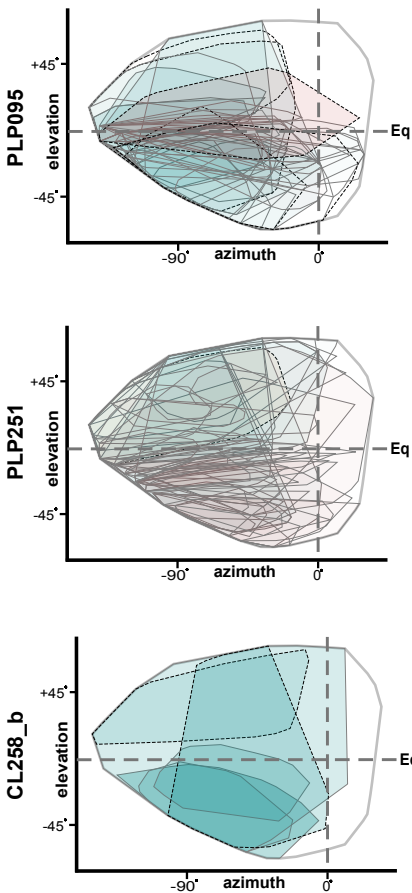


Figure 3 Supplement 1. The FlyWire standard synapse threshold masks patterns of visual input.

- A.** A subset of lobula columnar neurons (LCs) provide indirect inputs to KCy-d's via LVINs. Scale bars, 10µm.
- B.** Left, 8/52 LC15 neurons form ≥ 5 synapses with LVINs, creating a sporadically filled receptive field. Right, when the synapse threshold is removed, 49/52 LC15's are now found to synapse with LVINs, creating a uniform coverage of the receptive field. Scale bars, 10µm.
- C.** Left, 5/62 LC24 neurons form ≥ 5 synapses with LVINs. Right, when the synapse threshold is removed, 53/62 LC24's are now found to synapse with LVINs. Scale bars, 10µm.
- D.** Number of synapses made by individual unique LC neurons onto LVINs. Dashed lines indicate the 5-synapse threshold. The fraction of total LCs that make at least one synapse with an LVIN is indicated in the upper right of each graph.
- E.** The population of 75 MC62 neurons annotated in FlyWire have dendrites that tile the entire medulla. Scale bar, 10µm.
- F.** Left, 29/75 MC62 neurons synapse with LVINs when using a ≥ 5 synapse threshold. These neurons cover the ventral half of the medulla. Right, when the synapse threshold is removed 54/75 MC62's are now found to synapse with LVINs but coverage is lacking in the dorsal-most region of the medulla. Scale bars, 10µm.
- G.** Number of synapses made by individual unique MC62 neurons onto LVINs. Dashed line indicates the 5-synapse threshold. The fraction of total MC62s that make at least one synapse with an LVIN is indicated in the upper right of the graph.



B Receptive fields of top LVINs



C % of input synapses to top LVINs

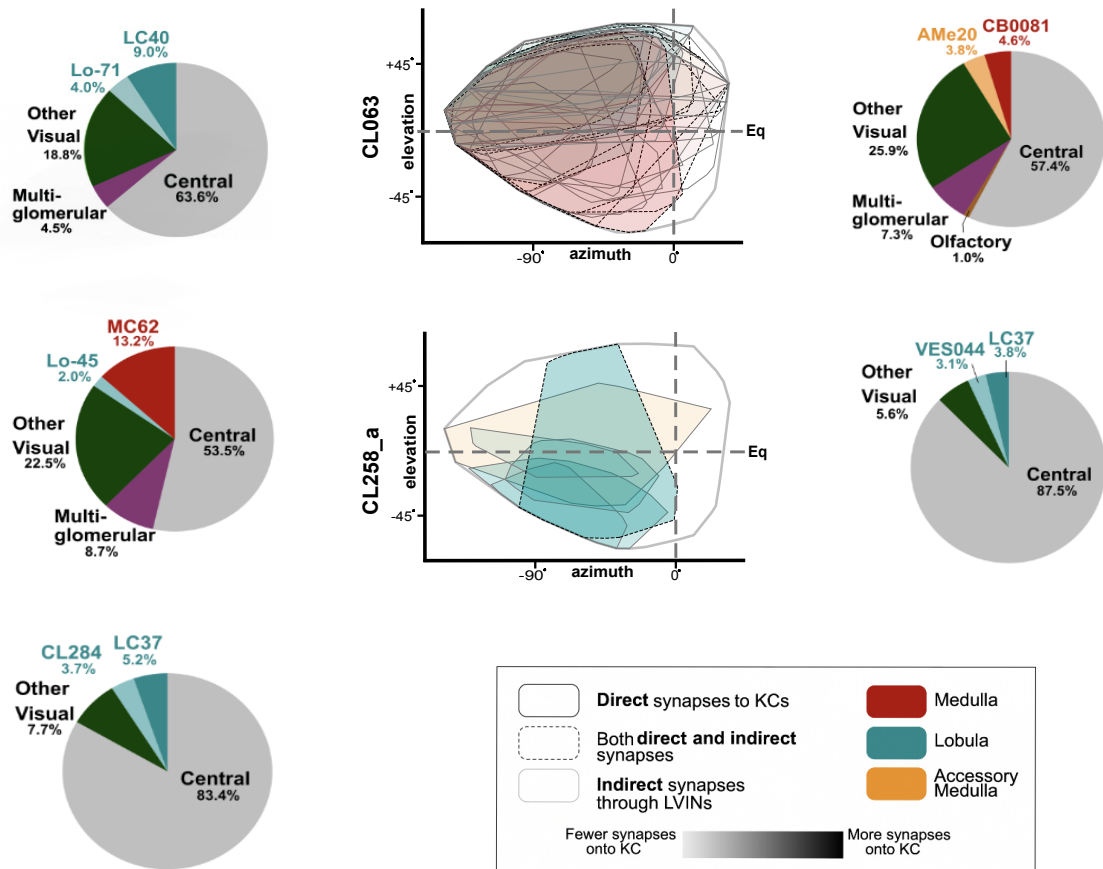


Figure 3 Supplement 2. LVINs integrate diverse sets of inputs.

A. Proportion of synapses from the primary sources of optic lobe input (aMe, medulla, and lobula) onto each individual LVIN. Most LVINs integrate a combination of input from different optic lobe neuropils.

B. The effective receptive fields of the top 5 LVINs (selected by considering the sum of synapses onto KCy-d's) are a combination of individual input VPN receptive fields. Color indicates source input neuropil and shading indicates the strength of connectivity (sum of synapses) onto the LVIN. LVINs receive multiple visual inputs with distributed weights.

C. Percentages of direct synaptic input received from the top two input VPN classes as well as all other visual inputs to the top 5 LVINs shown in (B). Also shown are the percentages of direct synaptic input from other sensory origins (multi-glomerular, olfactory) and the central brain.

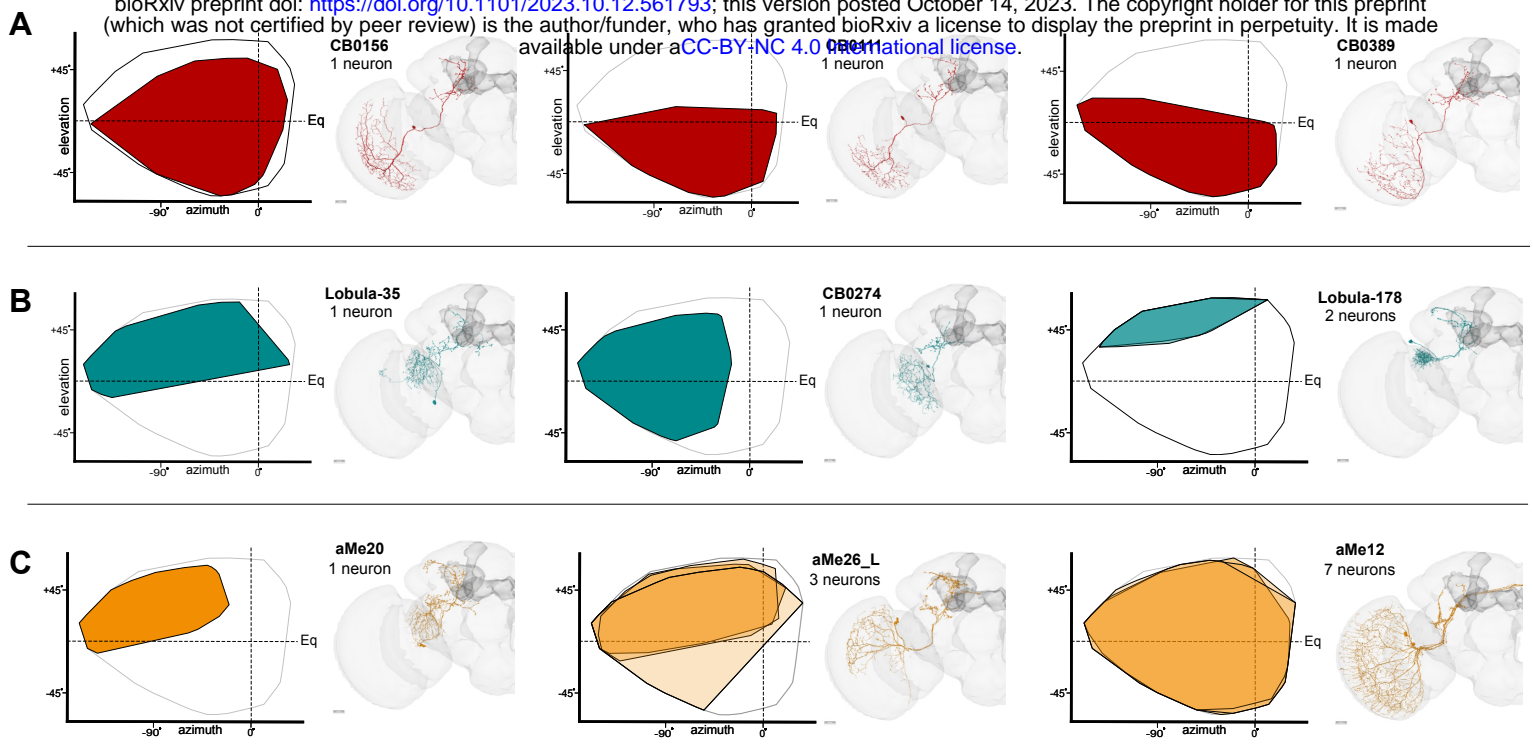


Figure 4. Supplement 1. Receptive fields of top morphological classes that contact KCy-d's.

A. Estimated receptive fields (Mollweide projections of ommatidial viewing angles) of the top three VPN input classes that carry visual information from the medulla to KCy-d's. For each morphological class, the receptive fields of individual neurons are overlaid, and shading indicates what proportion of the class's total synaptic input to the KCy-d population is accounted for by that neuron.

B. Estimated receptive fields as in (A) of the top three VPN input classes that carry visual information from the lobula to KCy-d's.

C. Estimated receptive fields as in (A) of the top three VPN input classes that carry visual information from the accessory medulla to KCy-d's.

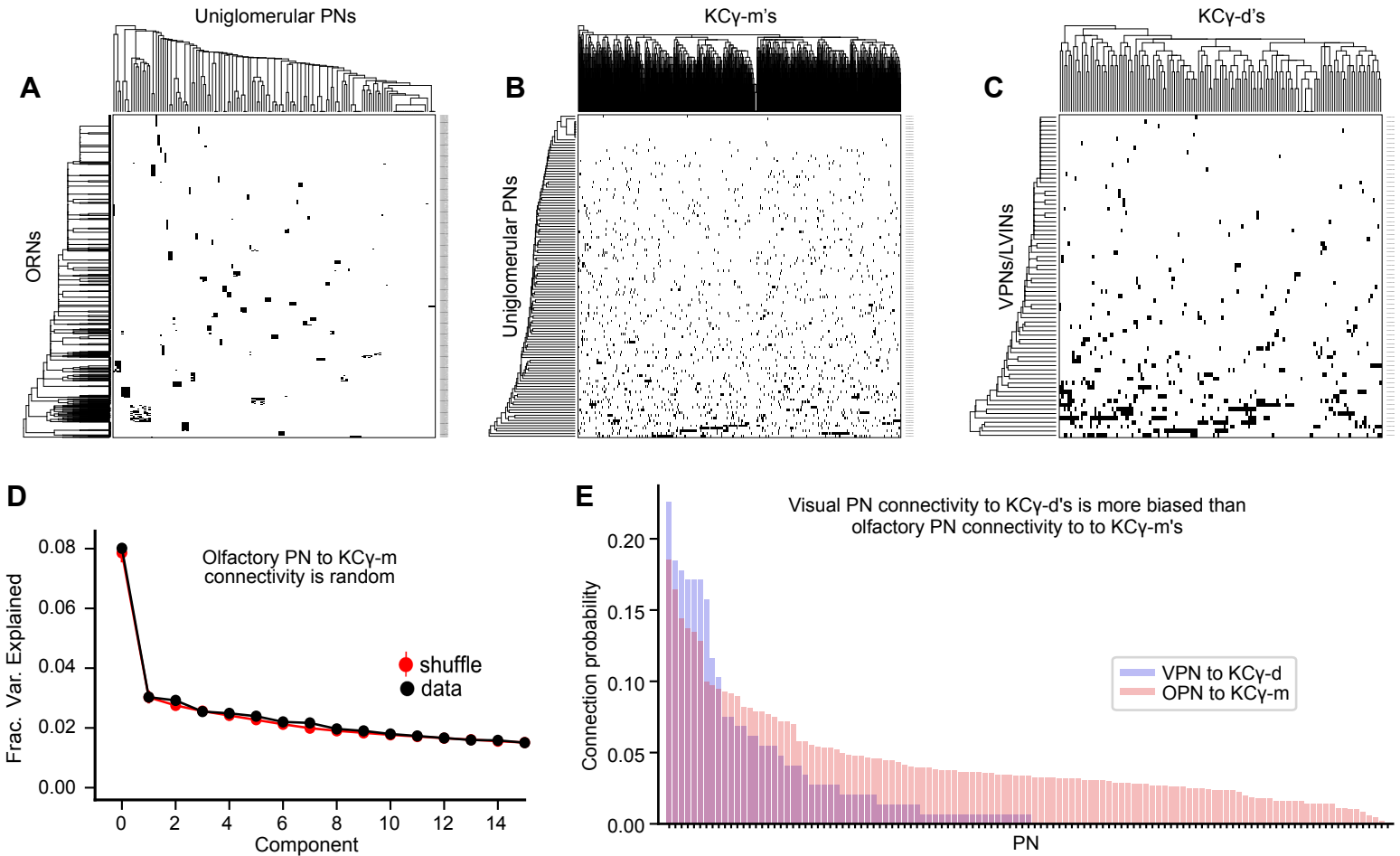


Figure 5 Supplement 1. Olfactory and visual PNs display analogous patterns of connectivity with Kenyon cells.

- A.** Olfactory receptor neurons (ORNs, rows) make largely discrete connections with uniglomerular olfactory projection neurons (PNs, columns). Connections were binarized using a 5 synapse threshold. Black cells indicate contacts ≥ 5 synapses. Rows and columns are hierarchically clustered based on partner similarity.
- B.** Heatmap showing binarized Uniglomerular PN-KCy-m synaptic contacts. PNs make distributed inputs onto KCy-d's.
- C.** Heatmap showing binarized VPN/LVIN-KCy-d synaptic contacts. VPN and LVIN inputs onto KCy-d's have a connectivity structure that is qualitatively similar to olfactory PNs and KCy-m.
- D.** Principal components analysis of uniglomerular olfactory projection neuron to KCy-m connectivity. Red circles and bars represent mean and 95% confidence intervals for variance explained by the principal components of shuffled connectivity matrices. Top PC components do not account for higher proportions of variance when compared to shuffled matrices preserving ORN connection probability and number of inputs to each KCy-m.
- E.** The sorted connection probabilities of individual VPNS onto the KCy-d population (blue) compared to the sorted connection probabilities of individual ORNs onto the KCy-m population (red). The top VPNS have higher, biased connection probabilities onto the KCy-d population when compared to the top ORNs connecting to the KCy-m population.
- Data in this figure examine connectivity in the left hemisphere, using a ≥ 5 synapse threshold.