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## Supplementary information

# Whole-brain annotation and multi-connectome cell typing of *Drosophila*

In the format provided by the authors and unedited

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## Supplementary Tables

| flow | superclass | class | sub class | type | side | hemilineage | neurotransmitter |
|---|---|---|---|---|---|---|---|
| afferent | sensory | olfactory | ALRN | ORN_DA1 | left | ALl1_ventral | acetylcholine |
| intrinsic | central | DAN | | PAM08 | left | CREa1_dorsal | dopamine |
| efferent | descending | | | DNa02 | right | WEDd1 | acetylcholine |

**Supplementary Table 1:** Sets of annotations for three example neurons.

| Field | Value | Ontology ID | Definition |
|---|---|---|---|
| flow | intrinsic | | Neurons fully contained within the brain. |
| | afferent | | Neurons that enter the brain from the periphery or the ventral nerve cord (VNC). |
| | efferent | | Neurons that leave the brain towards the periphery or the VNC. |
| superclass | central | FBbt_00059245 | Neurons fully contained within the central brain. |
| | ascending | FBbt_00048301 | Neurons entering the brain from the VNC. These can be sensory or interneurons. |
| | descending | FBbt_00047511 | Neurons with soma in the brain that exit the brain towards the VNC. |
| | endocrine | FBbt_00059246 | Neurons that exit the brain via the NCC towards the retrocerebral complex (corpora cardiaca and corpora allata). |
| | motor | FBbt_00005123 | Neurons that exit the brain towards the periphery (and are hence assumed to be motor neurons). |
| | optic | FBbt_00007577 | Neurons fully contained within the optic lobes or the ocellar ganglion. Includes some bilateral neurons (see class field). |
| | visual projection | FBbt_00048287 | Neurons that have dendrites in the optic lobes or the ocellar ganglion and axons in the central brain. |
| | visual centrifugal | FBbt_00059244 | Neurons that have dendrites in the central brain and axons in the optic lobes or the ocellar ganglion. |
| | sensory | FBbt_00005124 | Neurons that enter the brain from the periphery. Note that “ascending” also includes some sensory neurons that we are unable to distinguish from ascending interneurons with any certainty. |

**Supplementary Table 2:** Glossary for terms used in the top-most layers of the annotation hierarchy. Ontology ID refers to the Virtual Fly Brain database.

| Class | Subset of superclass | Definition |
|---|---|---|
| bilateral | optic | Optic lobe neurons with projections into the contralateral optic lobes. Sometimes also leave synapses in the central brain. |
| ocellar | visual centrifugal | Projection neurons with dendrites in the brain and axons in the ocellar ganglia. |
| | visual projection | Projection neurons with dendrites in the ocellar ganglia and axons in the brain. |
| | optic | Neurons intrinsic to the ocellar ganglia. Cell bodies can be inside the brain though. |
| ALIN | central | “Antennal lobe input neurons”: neurons with dendrites out- and axons inside the antennal lobes. Does not include sensory neurons. |
| ALPN | central | “Antennal lobe projection neurons”: neurons with dendrites in the antennal lobe and axonal projections into the protocerebrum, lateral horn or calyx. |
| ALON | central | “Antennal lobe output neurons”: neurons with dendrites in the antennal lobes and axonal projections outside of the brain that are not canonical ALPNs. |
| ALLN | central | “Antennal lobe local neurons”: neurons with both dendrites and axons contained to the antennal lobes. Can be bilateral. |
| CX | central | Central complex neurons as defined by Hulse *et al*.(Hulse et al. 2021) |
| DAN | central | Dopaminergic neurons (PAM and PPL) whose axons target the mushroom body lobes. |
| Kenyon Cell | central | Neurons with dendrites in the mushroom body calyx whose axons form the parallel fibre system of the mushroom body lobes. |
| MBIN | central | “Mushroom body input neuron”: APL or DPM. |
| LHCENT | central | “Lateral horn centrifugal neurons”: neurons with dendrites in the protocerebrum and axons in the lateral horn. |
| LHLN | central | “Lateral horn local neurons”: neurons with both dendrites and axons contained to the lateral horn. |
| ME | optic | medulla local neuron |
| ME>LO | optic | projection neuron with dendrites in medulla and axon in the lobula |

**Supplementary Table 3:** Selection of cell *classes*.

## Supplementary notes

This section provides detailed explanations for columns in the Supplemental Files.

### *Supplemental_file1_neuron_annotations.tsv* and *Supplemental_file2_non_neuron_annotations.tsv*

- *pos_x*, *pos_y*, *pos_z* are anchor coordinates in 4x4x40nm voxel space (typically on the backbone of the neuron) for given neuron
- *supervoxel_id* is the ID of the supervoxel the anchor coordinates map to
- *root_id* is the ID of the neuron in the FlyWire 783 release
- *soma_x*, *soma_y*, *soma_z* are soma coordinates in 4x4x40nm voxel space
- *nucleus_id* maps to the nucleus detection table (available through CAVE); neurons with *soma_x/y/z* but without nucleus_id have had their soma manually marked
- *flow, super_class, cell_class, cell_sub_class, cell_type, hemibrain_type* are the hierarchical annotations
- *ito_lee_hemilineage* and *hartenstein_hemilineage* provide the hemilineage in ItoLee and Hartenstein nomenclature; note that not all labels exist in the Hartenstein nomenclature
- *morphology_group* provides a coarse morphological grouping based on hemilineage clustering
- *top_nt* is the top predicted neurotransmitter for the given neuron, calculated by averaging confidences over the transmitter predictions for all presynapses for the given neuron and choosing the most confident transmitter
- *top_nt_conf* is the average confidence for the top neurotransmitter
- *side* refers to the soma side for brain-intrinsic neurons and the nerve-entry side for sensory/ascending neurons
- *fbbt_id* and *vfb_id* provide mappings to the database of the VirtualFlyBrain; the former is used for ontology terms (like cell types), the latter is an identifier for individual neurons
- *status* records a number of outliers: *outlier_seg* are neurons with segmentation issues (often due to dark cytosol); *outlier_bio* are neurons with small to medium sized differences (e.g. extra branches) compared with their contralateral or hemibrain homologues
- *known_nt* and *known_nt_source* provide information about whether there is information about a confirmed neurotransmitter for a given neuron/neuron type

### *Supplemental_file3_summary_with_ngl_links.csv*

- *ito_lee_hemilineage* and *hartenstein_hemilineage* provide the hemilineage in ItoLee and Hartenstein nomenclature; note that not all labels exist in the Hartenstein nomenclature.
- *notes* records some of our observations of select published clones.
- *ito_lee_lineage* and *hartenstein_lineage* provide the corresponding lineage names to the hemilineages.
- *hemibrain_map* contains the *rough* mapping of that hemilineage to a hemibrain cell body fiber.

- *is_hemilineage* contains our best guesses on whether the entry is a hemilineage or not. For instance, H(NT) means that we guess that this is a hemilineage (instead of a combination of two hemilineages of one lineage) based on the neurotransmitter information from Eckstein et al. (2023).
- *ngl_link (``, *left_*, `*right_*` and `*hemibrain_*`) columns contain neuroglancer links with the neurons in that hemilineage selected, on that side, and coloured based on the morphological groups. Each morphological group is in a separate layer (in addition, FlyWire and hemibrain neurons are in separate layers). There is also a de-selected layer that contains all neurons in that hemilineage in FlyWire.
- *ids_** (*left*, *right*, *center*, *hb*) columns contain the neuron ids for that hemilineage in that side.
- *id_count_** (*left*, *right*, *center*, *hb*) columns contain the neuron count for that hemilineage in that side.
- *n_clusters_** (*left*, *right*, *hb*) columns contain the number of clusters for that hemilineage in that side.
- *shape_truncated* and *number_truncated* columns contain information on whether the hemilineage is truncated in shape/number in the hemibrain.

### *Supplemental_file4_hemilineages_clustering.csv*

- *root_id* is the ID of the neuron in the FlyWire 783 release.
- *persistent_cluster* column contains the side_cluster labels of neurons that cluster together across one-, two- and three-hemisphere clustering (see the Morphological groups section in Methods).
- *side* is the hemisphere (left/right/hemibrain) the neuron is in.
- *ito_lee_hemilineage* and *hartenstein_hemilineage* provide the hemilineage in ItoLee and Hartenstein nomenclature; note that not all labels exist in the Hartenstein nomenclature.
- *hemilineage_group* records the morphological groups.
- *supervoxel_id* corresponds to the supervoxel_ids in the *Supplemental_file1_neuron_annotations.tsv* file. The value is NA if the neuron is from the hemibrain.
- *nps* column contains the top-three innervated neuropils, alphabetically sorted, for that neuron.

### *Supplemental_file5_hemibrain_meta.csv*

The following columns correspond 1:1 to columns shown/available for download through neuPrint: *bodyId, type, notes, status, cellBodyFiber, somaLocation, pre, post* and *cropped*.

We additionally added the following columns:

- in *morphology_type* we collapsed connectivity types back into morphology types (i.e. removed the _a, _b, etc. suffixes)

- *cell_class* contains labels analogous to those the cell class labels we provide for FlyWire neurons
- *side* refers to the soma side and is principally based on the instance column where an _L and _R typically indicates the side; we did however make a sizeable number of manual adjustments
- *pre_con2* contains the number of outgoing connections for a given neuron whereas pre contains the number of presynapses (remember that insect synapses are polyadic); these numbers were used to compare hemibrain vs FlyWire presynapse counts
- *fbbt_id* contains a FBbt ID that maps to the entry for a given hemibrain type in the VirtualFlyBrain database
- *ito_lee_hemilineage* provide identified hemilineages in ItoLee nomenclature
- *morphology_group* provides a coarse morphological grouping based on hemilineage clustering