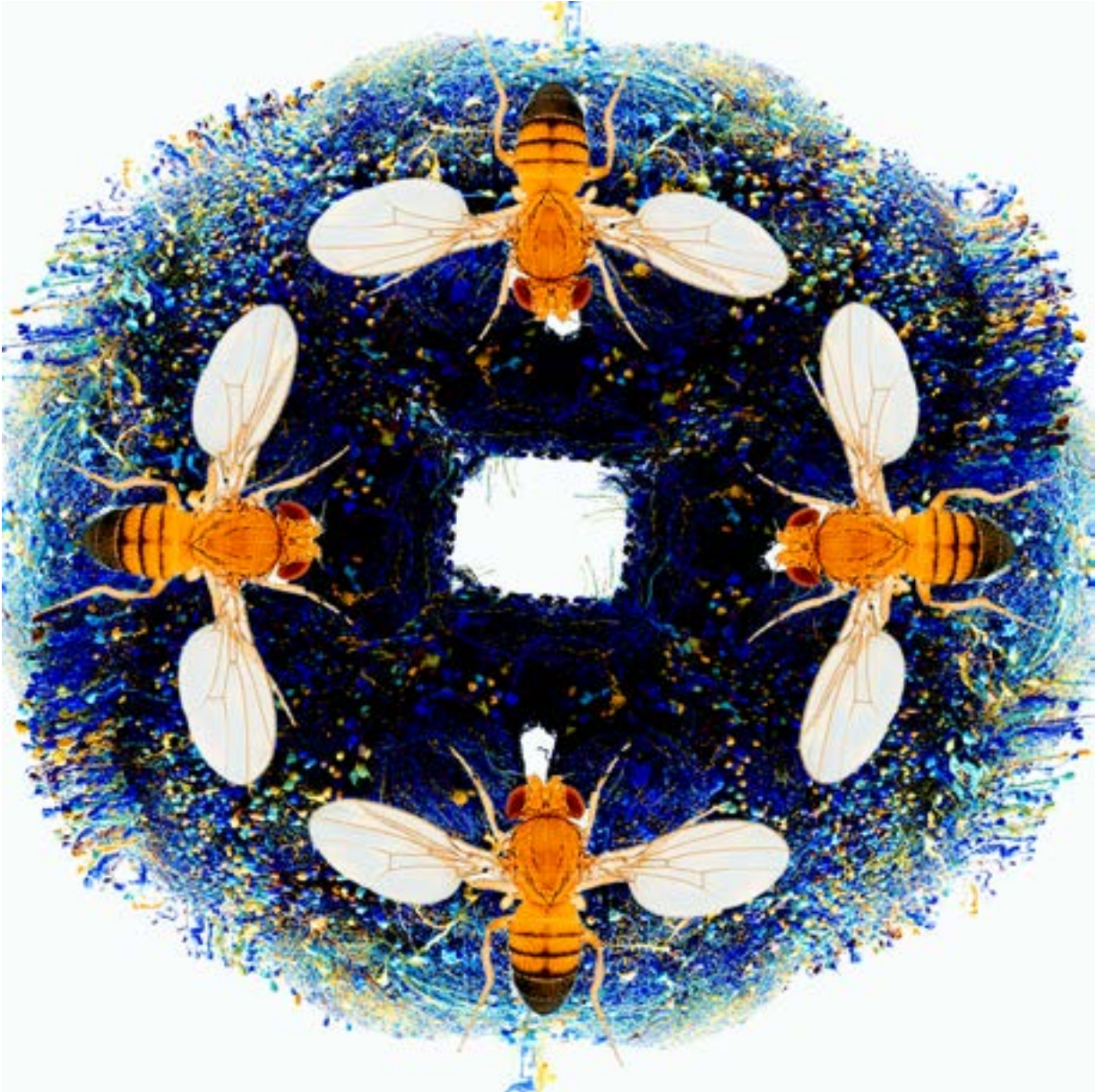


Exploring Olfactory Circuits in *Drosophila*: Connectomic Insights into Neural Connectivity, Behavioral Outputs, and Neurotransmitter Pathways

Nicholas Sangvai, Jinchen Wen, Joanna Kang, Walter Kornfeld, Talia Penn





Graphic by Monet Shum

Exploring Olfactory Circuits in Drosophila: Connectomic Insights into Neural Connectivity, Behavioral Outputs, and Neurotransmitter Pathways

Nicholas Sangvai, Jinchen Wen, Joanna Kang, Walter Kornfeld, Talia Penn

Duke University
<https://doi.org/10.55894/dv4.12>

Abstract

Using the FlyWire connectome, we conducted a comparative analysis of olfactory pathways in the female *Drosophila melanogaster* brain to investigate the relationship between neuronal structure, connectivity, and behavioral function. Our findings confirmed functional segregation at early neuronal levels and revealed conserved higher-order connectivity across attractive pheromonal and aversive food-odorant pathways, with distinct differences for the Ir64a receptor, potentially linked to its role as an acid-sensing ionotropic receptor. Connectivity to serotonergic, dopaminergic, insulin-producing, and circadian circuits highlighted potential roles in mediating innate and social behaviors, such as mating and aggression. While the static nature of the *Drosophila* connectome limits insights into developmental and sex-specific variations, this study provides a foundation for future research into connectomics and the structural and connective bases of behavior.

Introduction

Before the advent of the fly connectome, the understanding of olfactory connections and odor responses relied on single-cell labeling and manual tractography analysis of individual flies through complicated experimentation (Jefferis et al., 2004). With the release of the electron microscopy dataset of a full adult fly brain, researchers relied on manual skeletonization to reconstruct neural circuits from existing data (Cardona et al., 2012). FlyWire, in contrast, enables true three-dimensional interactive proofreading and serves as a searchable data bank of all neuronal connections in a *Drosophila melanogaster* hemibrain using Electron Microscopy and deep learning. The connectome, a comprehensive collection of neuronal connections of the hemibrain, is of increasing interest because it serves as a foundational framework to study individual circuits and their relevance to behavioral outputs at an unprecedented level of detail (Scheffer et al., 2020). The focus of the fly connectome specifically is of significance due to its role of *D. melanogaster* as a model organism in circuit neuroscience. Understanding neuronal connectivities in *Drosophila* may prove crucial to advancing our knowledge

and understanding of universal principles of brain function, neuroplasticity, learning and memory, and disease that can be subsequently applied to various organisms including the human brain.

To evaluate these circuits, we performed a connectomic analysis of the female *Drosophila* brain using FlyWire. Five unique olfactory receptors were chosen for their unique glomerular targets and involvement in opposing behavioral processes. For each receptor, comprehensive profiles of the downstream neuronal partners were constructed in a class-based manner, from the peripheral receptor neuron to third-order connections. From these profiles, representative neurons were selected to build olfactory circuit motifs and pathways that both localize to the antenna lobe and span the entire hemibrain. Individually constructing representative circuit pathways that extend beyond the projection neuron enabled a comparative visual analysis of these pathways across the five receptors. This revealed architectural differences that, along with the structure and function of the circuit-terminating neuron, may provide the basis for differences in the behaviors mediated by these receptors.

Background

Exploratory research was conducted using FlyWire.AI, an AI-segmented whole-brain connectome of a 5-day-old female of wild-type Canton S strain *Drosophila melanogaster*. The connectome itself was constructed through a joint effort of researchers and engineers to reconstruct neurons and chemical synapses using Focused Ion Beam Scanning Electron Microscopy, machine-learning algorithms, and 50-person years of proofreading effort to create neuron skeletons, synapse locations, and connectivity graphs (Scheffer et al., 2020). To conduct the investigation, five well-known olfactory receptors (Ors) that engage different social processes were explored along with their respective neural circuits.

FlyWire was used to determine the connectivity between individual neurons and neuronal classes to trace paths from peripheral sensory neurons to higher olfactory centers. For each receptor, the numbers of olfactory receptor neurons (ORNs), projection neurons (PNs), and local neurons (LNs) were found using FlyWire’s Search and Explore (Cell Type) toolbar. This analysis was only performed on the left side of the fly brain. From this, preliminary neuronal circuit motifs that localize to the antennal lobe (AL) neuropil of the *Drosophila* brain were identified. Next, available PNs were analyzed and representative PNs were then selected for each PN type—on the basis of neurotransmitter type, morphology, and connectivity—to further explore pathways reaching third-order neurons. FlyWire’s 3D and Connectivity (Pathways) tools were used to map from the representative PN’s glomerulus to the mushroom body cortex (MB), lateral horn (LH), and third-order brain regions, noting patterns of innervation and synaptic density, as well as the strength of synapses and the number of hops from the PN to the third-order neuron.



Table 1: Distance table between a representative neuron and downstream partners

Pathways to terminal neurons expressing different neurotransmitter types (dopamine, serotonin, insulin) in addition to CRY-expressing clock neurons, used to develop and stabilize the circadian rhythm, were also explored using FlyWire’s Connectivity (Pathways) tool. Individual pathways were superimposed on a 3D-represented modular fly brain to visually represent complete neural circuits for each receptor. These pathways were then layered to compare differences in region, connectivity, and morphology between each circuit. Together these approaches enabled a more complete representation and understanding of the neural pathways in *D. melanogaster*.

Connectomic analysis was performed using FlyWire’s pathway connectivity tool under *Connectivity/Pathway*. The minimum synapse count was set to 10 to represent a robust synapse and reduce noise. A representative starting PN was then picked for each glomerulus and the target cells were those expressing the specific neurotransmitter of interest. This generated a table (Table 1.) of connection counts between different neuron types and the number of hops necessary for connection.

Results

By understanding the differences between olfactory circuit structures across different behavioral contexts, we can begin to elucidate the relationships between neuronal connectivity and behavioral function. Ultimately, deciphering these pathways may lead to a greater overall understanding of systems neuroscience and lead to advancements in more complex organisms.

General Findings

ORNs gather information from the periphery and

LEFT SIDE ONLY	Or47b (VA1v) Attractive -> Pheromone ->	Or88a (VA1d)	Or67d (DA1) - cVA	Or56a (DA2) Repulsive -> Food ->	Ir64a (DC4)
ORNs	48 NT breakdown: 28 ACH 5 SER 15 unclassified	48 NT breakdown: 29 ACH 3 SER 14 unclassified	60 NT breakdown: 49 ACH 4 SER 7 unclassified	17 NT breakdown: 16 ACH 1 unclassified	11 NT breakdown: 11 ACH
PNs	6 adPN: 4 (ACH) vPN: 2 (GABA)	3 adPN: 2 (ACH) vPN: 1 (GABA)	9 IPN: 8 (ACH) vPN: 1 (GABA)	5 IPN: 5 (ACH)	3 adPN: 1 (ACH) vPN: 2 (GABA)
LNs	From 1 adPN: (AL.LH.61) 18 upstream 28 downstream From 1 vPN: (AL.LH.100) 20 upstream 0 downstream	From 1 adPN: (AL.MB_CA.43) 35 upstream 36 downstream From 1 vPN: (AL.LH.118) 5 upstream 0 downstream	From 1 IPN: (AL.MB_CA.129) 16 upstream 18 downstream From 1 vPN: (AL.LH.97) 12 upstream 0 downstream	From 1 IPN: (AL.LH.112) 6 upstream 2 downstream	From 1 adPN: (AL.LH.23) 61 upstream 53 downstream From 1 vPN: (AL.LH.271) 0 upstream 0 downstream
MB Paths	From 1 adPN: (AL.LH.61) 39 Kenyon Cells 1 MBIN From 1 vPN: (AL.LH.100) 0 Kenyon Cells	From 1 adPN: (AL.MB_CA.43) 87 Kenyon Cells 1 MBIN From 1 vPN: (AL.LH.118) 0 Kenyon Cells	From 1 IPN: (AL.MB_CA.129) 38 Kenyon Cells From 1 vPN: (AL.LH.97) 1 Kenyon cell 1 MBIN	From 1 IPN: (AL.LH.112) 21 Kenyon Cells	From 1 adPN: (AL.LH.23) 74 Kenyon Cells 1 MBIN From 1 vPN: (AL.LH.271) 0 Kenyon Cells
LH Paths	From 1 adPN: (AL.LH.61) 27 LHLNs 1 LHCENT 54 No Class From 1 vPN: (AL.LH.100) 13 LHLNs 53 No Class	From 1 adPN: (AL.MB_CA.43) 22 LHLNs 1 LHCENT 70 No Class From 1 vPN: (AL.LH.118) 12 LHLNs 43 No Class	From 1 IPN: (AL.MB_CA.129) 2 LHLNs 49 No Class From 1 vPN: (AL.LH.97) 3 LHLNs 2 LHCENT 62 No Class	From 1 IPN: (AL.LH.112) 7 LHLNs 36 No Class	From 1 adPN: (AL.LH.23) 31 LHLNs 2 LHCENT 106 No Class From 1 vPN: (AL.LH.271) 4 LHLNs 1 LHCENT 4 No Class

Table 2: Profiles of five olfactory receptors (Or) with their corresponding ORNs, projection neurons (PNs), local interneurons (LNs), Mushroom Body neurons, and Lateral Horn neurons. LNs, MB path, and LH path neuron counts are totaled from a representative projection neuron of the respective receptor. Only neurons on the left side of the hemibrain are counted.

convert the chemical reception into electrical signals transmitting a centralized signal to their respective (Couto et al., 2005). For all five receptor neuron classes, acetylcholine is the primary neurotransmitter utilized in synaptic transmission. The three receptors involved in pheromonal sensing, Or47b, Or88a, and Or67d, which also are all attractive, also utilize serotonin, however to a lesser extent. In addition to a more diversified neurotransmitter profile, the three pheromone-sensing ORN classes have a greater average neuron count ($n = 52$) compared to the two non-pheromonal receptor neurons, which are all aversive, ($n = 14$) (Table 2).

PNs are the acting relay centers between higher orders of the brain and sensory neurons—such as those in the antennae—that act in both bottom-up and top-down processing and determine responses to external stimuli (Marin et al., 2002). There are three classes of PNs that are defined by both the neuropil(s) to which they project and their respective neurotransmitter profiles. Anterodorsal PNs (adPNs) are cholinergic. They project to lateral horn (LH) neurons ($n=28$ on average) and mushroom body (MB) neurons via the inner antennocerebral tract (iACT), as well as to AL local neurons. Ventral PNs (vPNs) are gabaergic and their axons project through the medial antennocerebral tract (mACT). They typically have fewer connections to LH neurons ($n = 8$); they do not project to MB neurons or AL local neurons. Lastly, lateral PNs (IPNs) are cholinergic and their axons project through the iACT onto LH neurons and MB neurons, and back onto AL local neurons. However, they project to fewer LH neurons ($n=4.5$) than either adPNs or vPNs (Table 2).

Extracellular serotonin concentration at the steady state was graphed on the x-axes, and the number of individuals in a given population of 1000 for a given range of *eht* values was graphed on each y-axis. While there were some visible differences between the three populations, the distribution of extracellular serotonin at the steady state was roughly the same, with an approximately bell-curved shape with maximum frequencies near 60 nM. It should be noted well that the numerical values of extracellular serotonin concentration are not as important, since again, the steady state values and enzyme expression levels are known to differ across different projection

regions [6]. While we focused on the CA2 region of the hippocampus in these simulations, the steady state values of extracellular serotonin could very well be distributed around 40 nM or 80 nM in other regions of the brain.

Local neurons (LNs) in the AL mediate the responsiveness of ORNs and PNs to olfactory stimuli (Chou et al., 2010). Among the three PN classes, adPNs have the highest average number of LNs both upstream ($n = 38$) and downstream ($n = 39$). Compared to adPNs, IPNs have fewer numbers of upstream ($n = 11$) and downstream LNs ($n = 10$), on average. The vPNs have a comparable average number of upstream LNs ($n = 9.25$) to IPNs, but no downstream LN partners ($n = 0$) (Table 2).

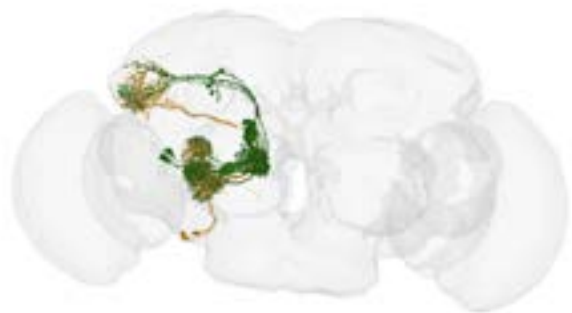


Figure 1: Nine representative projection neurons from the five olfactory receptors and respective glomeruli. Colored by neurotransmitter type: GABA neurons are orange, and acetylcholine neurons are green. All four vPNs (AL.LH.100, AL.LH.118, AL.LH.97, AL.LH.271) take the mACT tract, bypass the Mushroom Body, innervate the Lateral Horn, and use GABA. The three adPNs (AL.LH.61, AL.MB_CA.43, AL.LH.23) and two IPNs (AL.MB_CA.129, AL.LH.112) share a spatial and neurotransmitter profile; they take the iACT tract, innervating the Mushroom Body and the Lateral Horn in a bifurcated branch pattern, and use acetylcholine.

Attraction

ORNs expressing Or47b localize to the VA1v glomerulus to mediate sociosexual interactions via pheromone sensing (Dweck et al., 2015). Forty-eight ORNs synapse onto the left VA1v glomerulus and adPNs and vPNs project from this glomerulus. The representative adPN, AL.LH.61, projects to 4 types of higher-order classified cells in the MB and LH, including an MB interneuron (MBIN), Kenyon cells, and LH centrifugal neurons (LHCENT; Table

2). ORNs expressing Or88a localize to the VA1d glomerulus and are responsible for mediating attraction in male and female flies (Dweck et al., 2015). Forty-eight ORNs synapse onto the left VA1d glomerulus, and adPNs and vPNs also project from this glomerulus. ORNs expressing Or67d localize to the DA1 glomerulus and mediate responses to the male sex pheromone cis vaccenyl-acetate (cVA) (Kurtovic et al., 2007). Sixty ORNs synapse onto the left DA1 glomerulus and IPNs and vPNs project from this glomerulus. These three glomeruli—VA1v, VA1d, DA1—are clustered in the Antenna Lobe (Figure 2).

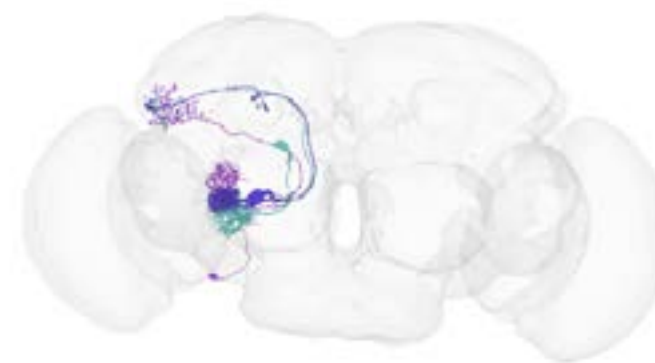


Figure 2: Three representative projection neurons from Or47b (VA1v, teal), Or88a (VA1d, blue), and Or67d (DA1, purple). These three olfactory receptors receive attractive pheromonal cues. The three respective glomeruli cluster on the lateral edge of the antenna lobe.

Aversion

ORNs expressing Or56a localize to the DA2 glomerulus and mediate avoidance behaviors to substances produced by potentially harmful microbes; one example is geosmin, a substance produced by fungi that is not itself toxic but co-occurs with other harmful substances (Stensmyr et al., 2012). Seventeen ORNs synapse onto the left DA2 glomerulus; IPNs project from this glomerulus. Receptor neurons that express Ir64a localize to the DC4 glomerulus and similarly to Or56a mediate avoidance behaviors to acidic pH that is harmful to the fly (Ai et al., 2010). Eleven ORNs synapse into the DC4 glomerulus, all of which are cholinergic; adPNs and vPNs project from this glomerulus. These two glomeruli—DA2 and DC4—are clustered in the Antenna Lobe (Figure 3).

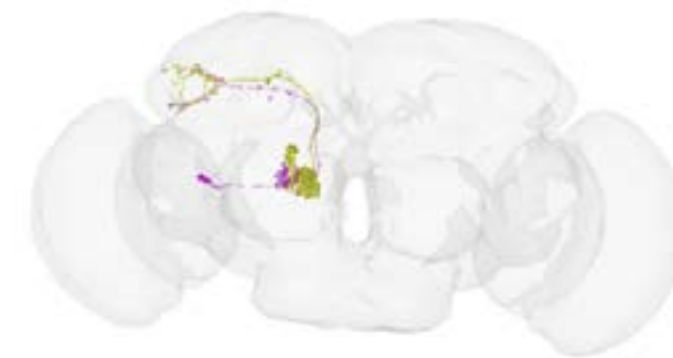


Figure 3: Two representative projection neurons from Or56a (DA2, pink) and Ir64a (DC4, yellow). These two olfactory receptors receive repulsive food-odor cues. The two respective glomeruli cluster in the antenna lobe.

Neurotransmitter Specific Pathways

Dopamine

Dopamine is involved in locomotion, learning & memory, sleep, and arousal cycles, as well as sexual behavior in courtship (Yamamoto et al., 2014). In the VA1v glomerulus the Or47b ORN, whose primary function is to detect methyl laurate (ML), a pheromone for copulation behavior, we see very few, if any, higher-order neurons that utilize Dopamine as its neurotransmitter (Dweck et al., 2015). This is evidenced by a search query of a representative adPN (AL.LH.61) as well as a representative vPN (AL.LH.100) in Flywire of the number of hops required to reach a dopamine-producing third-order neuron with a minimum synapse count of 10. For the representative Or47b adPN, the modal hop count is 4 interconnects. For the representative Or47b vPN, the modal hop count is also 4 interconnects.

Similarly, Or88a, which coexists with Or47b in the same sensory neuron (at4) and responds to methyl laureate (ML), methyl myristate (MM), and methyl palmitate (MP), also utilizes dopamine minimally in third-order neurons (Dweck et al., 2015). For the representative Or88a adPN (AL.MB_CA.43), the modal hop count is 3 interconnects before reaching a dopamine-expressing neuron. For representative Or88a vPN (AL.LH.118), the modal hop count is 4 interconnects

In the DA1 glomerulus, Or67d utilizes dopamine to mediate the detection of male pheromone cVA



Figure 4a: Dopamine neuron, SCL.WED.1, of neuron class Dnp32. This neuron produces neuropeptide dromyosuppressin (DMS). **Figure 4b:** Projection neuron AL.LH.97 from olfactory receptor Or67d synapses directly onto SCL.WED.1 (one hop, 6 synapses).



Figure 5a: Serotonin neuron, SLP.GNG.3, of neuron class Dnp62. This neuron produces neuropeptide drosulfakinin (DSK). **Figure 5b:** Projection neuron AL.LH.97 from olfactory receptor Or67d synapses onto lateral horn neuron, LH.SLP.802, which synapses onto SLP.GNG.3 (two hops).

and subsequently instigate a response in drosophila (Kurtovic et al., 2007). In females, cVA acts as an attractive pathway encouraging mating whereas cVA is a repulsive pathway in male drosophila (Kurtovic et al., 2007). Again, we see minimal Dopamine expressing higher-order neurons. For both of the representative Or67d IPN (AL.MB_CA.129) and Or67d vPN (AL.LH.97), the modal hop count is 4 interconnects.

From the Or67d glomerulus, a unique class of dopamine neurons was identified—Dnp32—that synapses directly onto the representative projection neuron AL.LH.97. This neuron, SCL.WED.1, has arborizations that span half the hemibrain and produces the neuropeptide dromyosuppressin (DMS).

Serotonin

Serotonin is involved in behavioral states including aggression & stress response, learning & memory, and mediating the circadian rhythm & sleep

cycles (Casture et al., 2018). We utilized the same exploratory search used to define the modal hop count for dopaminergic neurons from representative PNs originating in the VA1v glomerulus and synapsing with Or47b ORNs. From the representative Or47b adPN, (AL.LH.61), we found the modal hop count is 4 interconnects before reaching a serotonin-expressing neuron. For representative Or47b vPN (AL.LH.100), the modal hop count is 3 interconnects.

In the VA1d glomerulus, for the representative Or88a adPN (AL.MB_CA.43), the modal hop count is 4 interconnects before reaching a serotonin-expressing neuron. For representative Or88a vPN (AL.LH.118), the modal hop count is 5 interconnects.

In the DA1 glomerulus, for the representative Or67d IPN (AL.MB_CA.129), the modal hop count is 4 interconnects before reaching a serotonin-expressing neuron. For the representative Or67d vPN

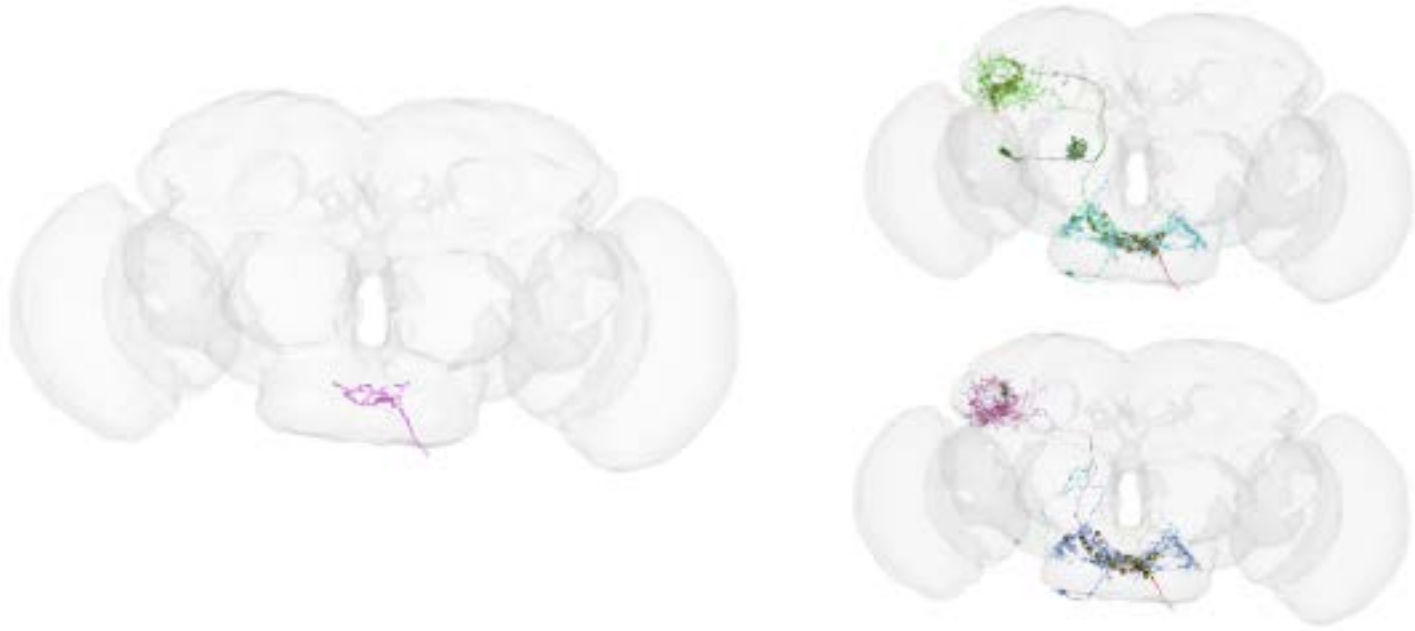


Figure 6a. Serotonin neuron, GNG.1894. This neuron is localized to the Gnathal Ganglia neuropil and has a projection that enters/exits the brain. **Figure 6b.** Pathway from DA2 representative projection neuron AL.LH.112 from olfactory receptor Or56a. **Figure 6c.** Pathway from DC4 representative projection neuron AL.LH.271 from ionotropic receptor Ir64a. Both the DA2 and DC4 pathways terminate in three-neuron pathway LH.SLP.20, GNG.13, GNG.1894 (15+ hops).

(AL.LH.97), the modal hop count is 5 interconnects before reaching a serotonin-expressing neuron. From the Or67d glomerulus, a unique class of serotonin neurons was identified—Dnp62—that is two hops away from the representative projection neuron AL.LH.97. This neuron, SLP.GNG.3 has arborizations that span the hemibrain and produces the neuropeptide drosulfakinin (DSK).

These scatterplots provide further evidence that In the DA2 glomerulus the Or56a ORN, for the representative Or56a IPN (AL.LH.112), the modal hop count is 4 interconnects before reaching a serotonin-expressing neuron. Similarly, in the DC4 glomerulus the Ir64a receptor neuron, for the representative Ir64a adPN (AL.LH.23), and the modal hop count is 4 interconnects before reaching a serotonin-expressing neuron. Interestingly, for Ir64a vPN (AL.LH.271), the modal hop count is 6 interconnects.

From the DA2 and DC4 glomeruli, a conserved three-neuron pathway, terminating in a serotonin neuron was identified, starting two hops away from the respective projection neurons AL.LH.112 (DA2)

and AL.LH.271 (DC4). This terminating serotonin neuron, GNG.1894, has a projection that exits (or enters) the Gnathal Ganglia (GNG) neuropil and may be involved in gustatory reception.

Insulin

Insulin is primarily involved in metabolism & growth, feeding behavior, development, and stress resistance (Viola et al., 2023). Across different glomeruli, the connectivity patterns reveal varying degrees of interaction with insulin-expressing neurons.

In the VA1v glomerulus (Or47b ORN), the representative adPN (AL.LH.61) requires 5 interconnects to reach an insulin-expressing neuron, while the vPN (AL.LH.100) requires 4 interconnects. Similarly, in the VA1d glomerulus (Or88a ORN), both the adPN (AL.MB_CA.43) and vPN (AL.LH.118) exhibit a modal hop count of 5 interconnects to insulin-expressing neurons.

For the DA1 glomerulus (Or67d ORN), both the representative IPN (AL.MB_CA.129) and vPN (AL.LH.97) show a modal hop count of 5 interconnects.

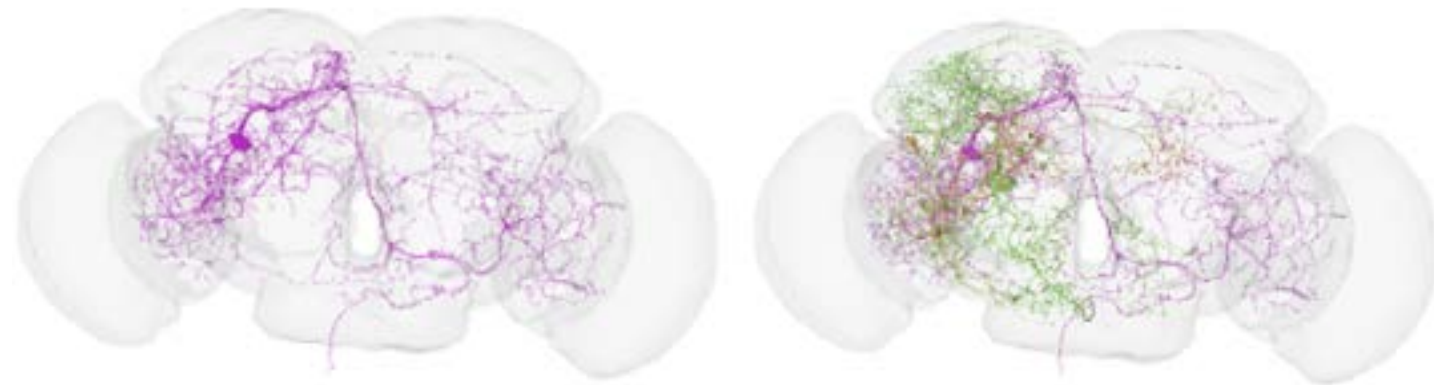


Figure 7a: Clock neuron, PLP.79. This neuron spans the entire hemibrain and has a projection that enters/exits the brain. **Figure 7b:** Pathway from DMS-expressing SCL.WED.1 (green) to allatostatin-C-expressing PLP.79 (purple), through ICL.3 (orange) (2 hops).

The same is observed in the DA2 glomerulus (Or56a ORN), where the representative IPN (AL.LH.112) also requires 5 interconnects to reach an insulin-expressing neuron.

In the DC4 glomerulus (Ir64a receptor neuron), the representative adPN (AL.LH.23) exhibits a modal hop count of 4 interconnects, whereas the vPN (AL.LH.271) requires 6 interconnects, the highest among the analyzed pathways. This variability underscores the distinct connectivity dynamics of the Ir64a pathway relative to other glomeruli.

Clock Neurons

Clock neurons are involved in regulating the natural sleep and wake cycles of the fly (Yao et al., 2016). In the VA1v glomerulus (Or47b), for the representative adPN (AL.LH.61), the modal hop count is 5 interconnects before reaching a clock neuron. For representative Or47b vPN (AL.LH.100), the modal hop count is 4 interconnects. In the VA1d glomerulus for the representative Or88a adPN (AL.MB_CA.43), the modal hop count is 5 interconnects before reaching a clock neuron. For representative Or88a vPN (AL.LH.118), the modal hop count is 4 interconnects.

In the DA1 glomerulus, the representative Or67d IPN (AL.MB_CA.129), the modal hop count is 5 interconnects before reaching a clock neuron. For representative Or67d vPN (AL.LH.97), the modal hop count is 5 interconnects.

A unique class of trissin-expressing clock

neurons was identified, DNp27, that also expresses allatostatin-C, which suppresses gonadotropin production. This clock neuron, PLP.79 is two hops away from the DMS-expressing SCL.WED.1 dopamine neuron, which synapses onto an Or67d PN.

In the DA2 glomerulus, the representative Or56a IPN (AL.LH.112), the modal hop count is 4 interconnects before reaching a clock neuron. Similarly, in the DC4 glomerulus the clock representative Ir64a adPN (AL.LH.23), the modal hop count is 4 interconnects before reaching a clock neuron. For representative Ir64a vPN (AL.LH.271), the modal hop count is 6 interconnects.

Discussion

This paper aimed to explore the relationship between neuronal structure, connectivity, and their impacts on function in the female fly brain. Using connectomic data analysis, our research described various odorant pathways and potential contributions of different factors such as neurotransmitter type, connectivity partners, and density of connections on aversion/attraction and pathway specificity.

Consistent with research conducted to build the connectome, we confirmed that functional segregation exists at the first and second-order neuron levels. This supports the established understanding of the organization of the olfactory system in *Drosophila*. In addition to this, it was noted that there existed no significant difference in the neuronal connectivity

of the five ORNs (with the exception of Ir64a) with serotonergic, dopaminergic, insulin-producing, and circadian circuits. This suggests a common underlying architecture connecting most odorant pathways to higher-order neurons while highlighting the potential for specialized connectivity in specific cases.

Quantitatively, we found no significant difference in the number of modal hops required to reach a neuron producing a specific neurotransmitter for all vPNs except for the Ir64a receptor neuron. The modal hop count for the Ir64a vPN is notably higher than all other representative PNs, averaging six modal hops, before reaching the nearest serotonergic dopaminergic, insulin-producing, or clock neuron. This could be due to Ir64a's identity as an ionotropic, acid-sensing receptor, which may explain differences in connectivity to higher-order neurons compared to pathways from olfactory receptors.

Our findings are consistent with previous research on the *Drosophila* olfactory system. The identification of distinct structural differences and pathways for different odorants, as well as the role of specific neurotransmitters in mediating attraction and aversion behaviors, confirms previous observations. However, the use of the FlyWire connectome allowed for a more comprehensive and detailed analysis of these pathways, revealing new insights into their organization and complexity.

While some behaviors, such as geosmin detection and avoidance, may be strictly innate, others—such as the sociosexual behaviors mediated by Or67d and Or47d—appear to have the capacity for learning and adaptation. For example, wild-type flies raised in isolation exhibit increased aggression, mating, and feeding behaviors, but these behaviors normalize upon reintroduction to social populations, highlighting a dynamic interplay between innate predispositions and environmental influences (Deanhardt et al., 2023). Similarly, the special case of the SCL.WED.1 neuron (Figure 7b), which expresses the neuropeptide DMS to mediate muscle contraction, offers further insights into the intersection of innate and learned behaviors (Nassal 2018). Its direct synaptic connection to an Or67d projection neuron suggests a potential role in egg-laying or mating-

related muscle mechanisms, bridging the gap between sensory input and motor output. This connection challenges the traditional dichotomy of innate versus learned behaviors, particularly in flies with mutations or natural variations in fruitless behavioral phenotypes. This example shows how the connectome can be leveraged to formulate hypotheses about structure- and connectivity-driven behaviors or social processes. For example, silencing Or67d in a fly mutant and subsequently counting the number of eggs laid may provide insights into the function of this neuron, beyond its spatial arrangement and synaptic partners.

The serotonergic neuron SLP.GNG.3 (Figure 5a) may be another neuron of interest in understanding mating-related and other social behaviors; it is downstream of Or67d and expresses drosulfakinin—a neuropeptide involved in aggression and sexual arousal regulation. This cell could drive further hypotheses surrounding mating behavior and aggression.

When examining food odorant pathways, the serotonergic neuron, GNG.1894 (Figure 6), contains processes that enter the hemibrain from below. It is well known that *Drosophila* have gustatory neurons in the extremities of its body, allowing it to “taste” their surroundings more effectively. This, in addition to the role of Or56a and Ir64a in processing food-odor information, may help to explain the interconnectedness of olfactory and gustatory information processing, especially relating to aversive food odors in the immediate environment.

Lastly, the clock example may explain the role that circadian cycles can affect social processes. Clock neuron PLP.79 (Figure 7a) expresses allatostatin-C, which is involved in the suppression of gonadotropin production, and as previously mentioned, SCL.WED.1 expresses DMS which may have a role in egg-laying. Because these two neurons are only two hops away, this is a point of interest that could lead to further investigation into the role of pheromones in mating-related social behaviors.

Together, the neurons of interest identified from our comparative connectomic analyses may enable further research involving downstream genetic

experiments to better understand the roles of olfaction and odor processing in facilitating social and behavioral processes.

The research and subsequent analysis were limited by the connectome itself. While the research done to build the connectome was groundbreaking, because it only represents one time point (5 days old), it does not offer an accurate depiction of neuronal growth and death in the fly brain over time. Former research has implied that certain neuronal pathways form and are more active during adolescence but later are unused in adulthood; the lack of versatility throughout age ranges causes the connectome to fall short in exhibiting the changes in plasticity (Sugie et al., 2018). Future revisions of the connectome should focus on capturing *Drosophila* neuronal connectivity across different time points, under different conditions, such as social isolation, and analysis of the male brain. A new and comprehensive connectome for *Drosophila* is currently under development, aiming to address these limitations by mapping neuronal connectivity across various life stages and incorporating dynamic changes in synaptic plasticity.

Similarly, comparing neuronal differences of male drosophila was not possible due to the research done to build the connectome only being done on a female fly. As such, the connectome offers minimal insight into pathways that show sexual dimorphism, particularly fruitless & gender-specific aversive and attractive pathways (Kurtovic et al., 2007). While we were able to observe the attractive pathways for cVA in female *Drosophila*, these pathways could not be observed in a connectome for male *Drosophila* in the context of attractive pathways, which are expected to differ in males given that cVA acts as a repellent to wild-type male drosophila (Kurtovic et al., 2007).

Further observations are needed to determine whether or not this neuronal network is conserved in *Drosophila* species and mammals, as the current connectome lacks the opportunity to cross-compare species. Notably, even though the mammalian olfactory bulb shows similar functional organization as the antennal lobe, these structures evolved independently (Farris, 2015). Given the

lack of common origin, the application of insect olfactory logic to mammal olfactory logic may be limited. However, understanding of the fly neuronal system may unlock higher-order insights into the mammalian nervous system. The *Drosophila* hemibrain connectome could serve as a leap forward in the connectomic field, bringing us one step closer to a human connectome and ultimately providing us a greater understanding of the impact of neuronal circuitry on innate behaviors and beliefs in comparison to learned behaviors and values. Combined with further advancements in other disciplines, connectomics serves as a biological basis of human action.

References

1. Ai, M., Min, S., Grosjean, Y., Leblanc, C., Bell, R., Benton, R., & Suh, G. S. (2010). Acid sensing by the *Drosophila* olfactory system. *Nature*, 468(7324), 691–695. <https://doi.org/10.1038/nature09537>
 2. Blakely, T., Bogovic, J., Dolafi, T., ... Plaza, S. M. (2020). A connectome and analysis of the adult drosophila central brain. *eLife*. <https://elifesciences.org/articles/57443>
 3. Cardona, A., Saalfeld, S., Schindelin, J., Arganda-Carreras, I., Preibisch, S., Longair, M., Tomancak, P., Hartenstein, V., & Douglas, R. J. (2012). TrakEM2 software for neural circuit reconstruction. *PloS one*, 7(6), e38011. <https://doi.org/10.1371/journal.pone.0038011>
 4. Chou, Y. H., Spletter, M. L., Yaksi, E., Leong, J. C., Wilson, R. I., & Luo, L. (2010). Diversity and wiring variability of olfactory local interneurons in the *Drosophila* antennal lobe. *Nature Neuroscience*, 13(4), 439–449. <https://doi.org/10.1038/nn.2489>
 5. Couto, A., Alenius, M., & Dickson, B. J. (2005). Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Current biology: CB*, 15(17), 1535–1547. <https://doi.org/10.1016/j.cub.2005.07.034>

6. Deanhardt, B., Duan, Q., Du, C., Soeder, C., Morlote, A., Garg, D., Saha, A., Jones, C. D., & Volkan, P. C. (2023). Social experience and pheromone receptor activity reprogram gene expression in sensory neurons. *G3 (Bethesda, Md.)*, 13(6), jkad072. <https://doi.org/10.1093/g3journal/jkad072>
 7. Dweck, H. K., Ebrahim, S. A., Thoma, M., Mohamed, A. A., Keesey, I. W., Trona, F., Lavista-Llanos, S., Svatoš, A., Sachse, S., Knaden, M., & Hansson, B. S. (2015). Pheromones mediating copulation and attraction in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 112(21), E2829–E2835. <https://doi.org/10.1073/pnas.1504527112>
 8. Farris, S. M. (2015). Evolution of brain elaboration. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1684), 20150054. <https://doi.org/10.1098/rstb.2015.0054>
 9. Jefferis, G. S., Vyas, R. M., Berdnik, D., Ramaekers, A., Stocker, R. F., Tanaka, N. K., Ito, K., & Luo, L. (2004). Developmental origin of wiring specificity in the olfactory system of *Drosophila*. *Development (Cambridge, England)*, 131(1), 117–130. <https://doi.org/10.1242/dev.00896>
 10. Kasture, A. S., Hummel, T., Sucic, S., & Freissmuth, M. (2018). Big Lessons from Tiny Flies: *Drosophila melanogaster* as a Model to Explore Dysfunction of Dopaminergic and Serotonergic Neurotransmitter Systems. *International journal of molecular sciences*, 19(6), 1788. <https://doi.org/10.3390/ijms19061788>
 11. Kurtovic, A., Widmer, A., & Dickson, B. J. (2007). A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature*, 446(7135), 542–546. <https://doi.org/10.1038/nature05672>
 12. Marin, E. C., Jefferis, G. S., Komiyama, T., Zhu, H., & Luo, L. (2002). Representation of the glomerular olfactory map in the *Drosophila* brain. *Cell*, 109(2), 243–255. [s0092-8674\(02\)00700-6
 13. Scheffer, L. K., Xu, C. S., Januszewski, M., Lu, Z., Takemura, S., Hayworth, K. J., Huang, G. B., Shinomiya, K., Maitlin-Shepard, J., Berg, S., Clements, J., Hubbard, P. M., Katz, W. T., Umayam, L., Zhao, T., Ackerman, D., Stensmyr, M. C., Dweck, H. K., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S., Wicher, D., Sachse, S., Knaden, M., Becher, P. G., Seki, Y., & Hansson, B. S. \(2012\). A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila*. *Cell*, 151\(6\), 1345–1357. <https://doi.org/10.1016/j.cell.2012.09.046>
 14. Sugie, A., Marchetti, G. & Tavoisanis, G. Structural aspects of plasticity in the nervous system of *Drosophila*. *Neural Dev* 13, 14 \(2018\). <https://doi.org/10.1186/s13064-018-0111-z>
 15. Viola, C.M., Frittmann, O., Jenkins, H.T. et al. Structural conservation of insulin/IGF signalling axis at the insulin receptors level in *Drosophila* and humans. *Nat Commun* 14, 6271 \(2023\). <https://doi.org/10.1038/s41467-023-41862-x>
 16. Yamamoto, S., & Seto, E. S. \(2014\). Dopamine dynamics and signaling in *Drosophila*: an overview of genes, drugs and behavioral paradigms. *Experimental animals*, 63\(2\), 107–119. <https://doi.org/10.1538/expanim.63.107>
 17. Yao, Z., Bennett, A. J., Clem, J. L., & Shafer, O. T. \(2016\). The *Drosophila* Clock Neuron Network Features Diverse Coupling Modes and Requires Network-wide Coherence for Robust Circadian Rhythms. *Cell Reports*, 17\(11\), 2873–2881. <https://doi.org/10.1016/j.celrep.2016.11.053>](https://doi.org/10.1016/

</p></div><div data-bbox=)