

RESEARCH ARTICLE

Recurrent synapses between CO₂-sensitive olfactory sensory neurons enable robust CO₂ detection in *Aedes aegypti* mosquitoes

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Abstract

The mosquito *Aedes aegypti*'s human host-seeking behavior depends on the integration of multiple sensory cues. One of these cues, carbon dioxide (CO₂), gates odorant and heat pathways and activates host-seeking behavior. The neuronal circuits underlying processing of CO₂ information remain unclear. We used automated serial-section transmission electron microscopy (EM) to image and reconstruct the circuitry of the glomeruli that are innervated by the *Ae. aegypti* maxillary palp, including the glomerulus that responds to CO₂. Notably, CO₂-sensitive olfactory sensory neurons (OSNs) make high levels of recurrent synaptic connections with one another, while making a low density of feedforward synapses. At some of these contacts between CO₂ OSNs, we observe ribbon-like presynaptic structures, which may further enhance recurrent signaling. We compared both feedforward and recurrent connectivity with all olfactory glomeruli in *Drosophila melanogaster*, and we found more recurrent connections between the *Ae. aegypti* CO₂-responsive OSNs than in any *D. melanogaster* glomeruli. We developed a computational circuit model that demonstrates recurrent synapses are necessary for robust CO₂ detection under normal physiological conditions. Together, elevated levels of recurrent connectivity and ribbon-like structures may amplify sensory information detected by CO₂-sensitive OSNs to support mosquito activation and sensitization by CO₂, even in the presence of high levels of other odorants in the environment. We propose that this circuit organization supports the salience of CO₂ as a mosquito host cue.

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Abbreviations: ALs, antennal lobes; ATUM, automated tape-collecting microtome; EM, electron microscopy; GR, gustatory receptor; iACT, inner antennocerebral tract; LNs, local neurons; MD, medio-dorsal; MGs, multiglomerular neurons; mPN, multiglomerular PN; OSNs, olfactory sensory neurons; PBS, phosphate-buffered saline; PNs, projection neurons; ROIs, regions of interest; uPNs, uniglomerular PNs.

Introduction

Mosquito-borne disease claims over half a million lives every year. The mosquito *Aedes aegypti* is a globally distributed disease vector responsible for thousands of deaths annually [1], with fatalities projected to increase over time [2,3]. Adult female mosquitoes innately seek blood to obtain the protein required for egg maturation [4] and they integrate a range of sensory cues to detect their preferred host, humans. These cues include skin-derived odorants [5–7], body heat [8,9], water vapor [10,11], visual cues [8,12], and carbon dioxide (CO₂) [6,7,13]. The host cue CO₂ drives notable changes in behavior, including increased locomotion, evident from both increases in take-off and flight time, as well as increased upwind flight [6,14]. These behaviors are presumed to initiate the search for a host [6], a state change termed *activation* [6,14]. An activated mosquito investigates host cues such as body odor, heat, and visual contrast. These synergize to drive host-seeking and biting behaviors, which include landing and probing with the proboscis [6]. We refer to the priming of other sensory channels during behavioral activation as CO₂ *sensitization* [6,15] (Fig 1A). We sought to discover how CO₂ activation and sensitization mechanisms are reflected in the neuronal circuitry that detects CO₂ and integrates it with other host cues.

Many principles of chemosensory wiring and coding were established in the model fly, *Drosophila melanogaster*. Olfactory sensory neurons (OSNs) in the antennae and maxillary palps [19,20] innervate ~50 glomeruli in the antennal lobes (ALs) [21,22], the first olfactory processing center of the brain. Multiple OSNs express a single olfactory receptor and all OSNs expressing the same receptor converge on a single glomerulus [19,20], which can be defined as an anatomically distinct local circuit. Local neurons (LNs) project among antennal lobe glomeruli, transforming information through a combination of excitatory and inhibitory interactions [23]. From antennal lobe glomeruli, projection neurons (PNs) send their axons to higher brain centers. Synapse-resolution reconstructions of the *D. melanogaster* olfactory system [24–26] and connectomes of the whole brain have recently been generated [27–30]. This work revealed circuit mechanisms of sensory coding [31–33], innate behavior [34,35], and learning and memory [34,36]. *Ae. aegypti* and *D. melanogaster* are both dipteran insects, but they have evolved dramatically different feeding strategies during the more than ~150-million-year timespan since they shared a common ancestor [37,38]. This has led to different specializations in chemosensory information processing. Recent work identified the co-expression of multiple olfactory receptors in many *Ae. aegypti* OSNs [39,40], and it is likely that additional differences in the chemosensory circuit architecture support their diet of primarily human blood.

Information processing in the olfactory system, like other neuronal circuits, relies on synapses, which are the main sites of information transmission between neurons. At chemical synapses, neurotransmitters are packaged into synaptic vesicles and released from specialized presynaptic active zones for detection by receptors at postsynaptic sites. Vesicular release machinery traffics vesicles to the presynaptic membrane, docks vesicles at the active zone, and drive vesicular exocytosis [41,42]. The structure of presynaptic active zones is diverse across species and synapse

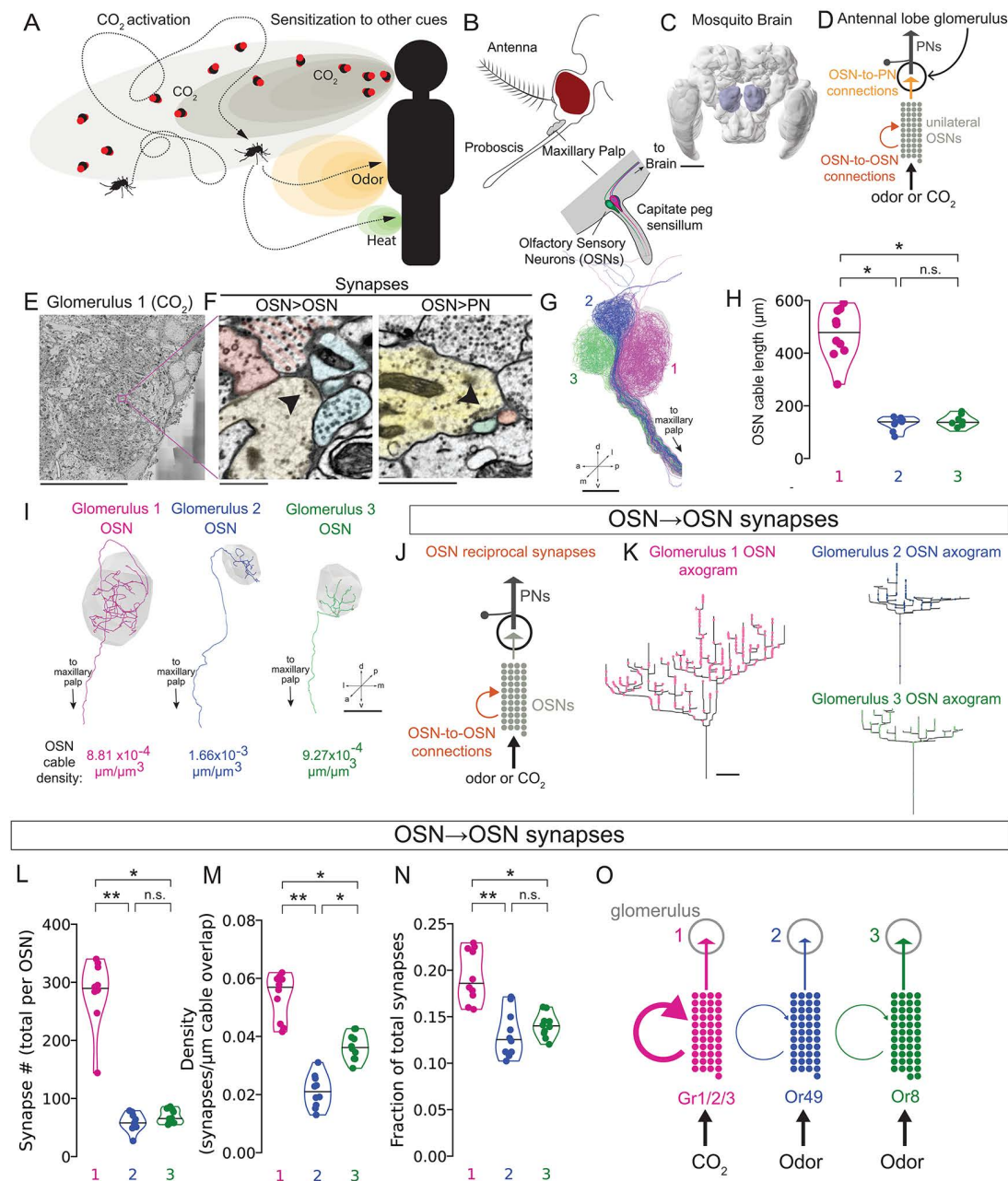


Fig 1. EM reconstruction of *Aedes aegypti* mosquito maxillary palp glomeruli. (A) Schematic of CO₂ activation and sensitization in *Ae. aegypti* host-seeking. (left) Quiescent adult female mosquitoes become activated upon CO₂ detection. (right) Sensitization to additional host cues (e.g., odor, heat, etc.) drives host-seeking behavior. (B) Diagrams (top) of an adult female *Ae. aegypti* head and (bottom) a capitate peg sensillum on maxillary palp (gray) which contain dendritic processes of olfactory sensory neurons (OSNs), including CO₂-sensitive Gr3-expressing OSNs. OSN axons project centrally to the antennal lobes in the brain. (C) Volumetric rendering of the mosquito brain neuropils including the antennal lobes (light blue) [16,17]. Scale bar 100 μm. (D) Schematic of an antennal lobe glomerulus and the circuit components analyzed here. Multiple OSNs selective to a chemosensory cue (e.g., odor or CO₂) converge on a glomerulus where they make synapses onto a cognate projection neuron (PN). OSNs can also make reciprocal synapses onto other OSNs. (E) Coronal 40 nm-thick section through maxillary palp Glomerulus 1, acquired and aligned using large-scale, tape-based transmission electron microscopy (EM) [18]. Scale bar 25 μm. Magenta inset expanded in (F) (left) Example OSN (yellow) to OSN (blue) synapse (black arrowhead). (right) Polyadic synapse (black arrowhead) in a Glomerulus 1 from an OSN (yellow) to a postsynaptic multiglomerular neuron (MG; red) and uniglomerular projection neuron (uPN; green). Scale bar 1 μm. (G) Sagittal view of reconstructed maxillary palp nerve OSNs: Glomerulus 1 (pink), 2 (blue), and 3 (green). Scale bar 25 μm. a, anterior; d, dorsal; l, lateral; m, medial; p, posterior; v, ventral. (H) Cable length of OSNs (Kruskal–Wallis test

(two-tailed), $p = 6.20 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.01$, ** $p \leq 0.001$). (I) Coronal view of individual OSNs that innervate Glomerulus 1, 2, or 3 (gray surface meshes). Scale bar 25 μm . (J) Schematic with reciprocal OSN-to-OSN connections highlighted. (K) Axograms of representative OSNs from each glomerulus and their reciprocal output synapses to other OSNs. Scale bar 25 μm . (L) Total number of outgoing OSN-to-OSN synapses contained within Glomerulus 1, 2, and 3 for 10 fully reconstructed OSNs (Kruskal–Wallis test (two-tailed), $p = 1.28 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$, * $p \leq 0.05$; ** $p \leq 0.001$). (M) Synapse density of outgoing OSN-to-OSN synapses per micrometer of cable overlap (length of postsynaptic OSN axon within 2 μm of presynaptic OSN axon, see [Methods](#)) (Kruskal–Wallis test (two-tailed), $p = 0.0003$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.001$). (N) Reciprocal OSN-to-OSN synapses as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p = 0.0002$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$, * $p \leq 0.01$, ** $p \leq 0.001$). (G) Schematic of reciprocal connectivity with arrow thickness reflecting synapse number. The data underlying this Figure can be found in [S1–S4 Data](#).

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types, and related to functional properties of different synapses [43–46]. In insects, synapses typically have a small electron-dense structure referred to as a T-bar (which appears as a T when cut in cross-section) that promotes vesicle docking and release [45,47]. In contrast, vertebrate hair cells and neurons in the retina have large presynaptic specializations called ribbon synapses, which are elongated ribbon-shaped electron-dense structures extending intracellularly from the presynaptic active zone. Ribbon synapses are thought to support sustained and graded synaptic vesicle release [44,46]. Ribbon synapses include the vertebrate-specific protein Ribeye [48–50] and have not previously been identified in invertebrates. The existence of ribbon-like synapses in invertebrates would imply a convergently evolved mechanism for sustained or graded synaptic vesicle release across species.

In *Ae. aegypti*, the maxillary palp ([Fig 1B](#)) is the sensory appendage that contains neurons that sense CO_2 . To examine the circuit architecture that underlies CO_2 sensitivity, we generated a synapse-resolution wiring diagram of the three glomeruli that are innervated by maxillary palp OSNs. We used automated transmission electron microscopy (EM) [18] to generate an EM volume that includes all three glomeruli innervated by the OSNs that arise from the maxillary palp. For each of the three glomeruli, we reconstructed and annotated every synapse in 10 OSNs and their cognate uniglomerular PNs (uPNs) that exit these glomeruli and project in the inner antennocerebral tract (iACT). We found that CO_2 -sensitive OSNs had high levels of recurrent connections, a motif that may be used to amplify CO_2 detection. To compare chemosensory coding strategies in animals with divergent feeding behaviors, we analyzed the recurrent and feedforward connectivity of all *D. melanogaster* olfactory glomeruli including the CO_2 glomerulus, Glomerulus V, using FlyWire [28]. We did not find recurrent or feedforward connectivity to be similar between CO_2 -sensitive glomeruli in *Ae. aegypti* and *D. melanogaster*. Rather, there were far more recurrent connections between CO_2 -sensitive OSNs in *Ae. aegypti* than the OSNs that innervate any glomerulus in the fly. We identified a novel structure located specifically in CO_2 -sensitive OSNs reminiscent of mammalian ribbon synapses [44,46,51–53], and propose that these ribbon-like structures may supplement excitatory axo-axonic signaling. Lastly, we adapted a biophysical model from *D. melanogaster* to include recurrent connections. Using this model and previously published measurements, we found that recurrent connections supported robust CO_2 detection, even in the presence of strong background odors that otherwise limit CO_2 detection sensitivity. Together, this work suggests that elevated levels of recurrent connectivity and ribbon-like synapses may amplify incoming CO_2 cues to support mosquito activation and sensitization to CO_2 .

Results

EM reconstruction of olfactory sensory neurons reveals high recurrent connectivity among CO_2 neurons

Ae. aegypti rely on multiple sensory cues to locate humans ([Fig 1A](#)), and some of these cues, including CO_2 , are first detected in the maxillary palp ([Fig 1B](#)) by OSNs that innervate the antennal lobes ([Fig 1C](#) and [1D](#)). To compare the circuits that detect and process CO_2 to those dedicated to other odorants, we set out to image and reconstruct multiple antennal lobe glomeruli with electron microscopy (EM). We used automated serial-section transmission EM [18] to image the posterior two-thirds of the antennal lobes ([Fig 1C–1F](#)) of an adult female *Ae. aegypti* brain ([S1A Fig](#)) at $4 \times 4 \times 40 \text{ nm}^3$ /

voxel resolution. This region of the antennal lobe includes the three glomeruli that are innervated by olfactory sensory neurons of the maxillary palp: the CO₂-responsive glomerulus, Glomerulus 1, and two other olfactory glomeruli, Glomeruli 2 and 3, which respond to odorants [39,54].

In order to analyze the connectivity of these circuits, we manually reconstructed neurons in Glomeruli 1, 2, and 3 on the right side of the brain (Fig 1G–1J). We identified neurons as olfactory sensory neurons (OSNs), uPNs, or multiglomerular neurons (MGs) by their morphology, axon tracts, and which glomeruli they innervated. Most MGs extended beyond the imaged volume, and we therefore could not determine their cell type. We therefore focus our analysis on OSN and uPN circuitry (see Methods for details).

To examine the morphology of individual OSNs, we first annotated the processes of every OSN projecting from the right maxillary palp nerve by creating a skeleton reconstruction of each OSN that includes all its branches (Fig 1G and 1I). We identified 136 OSNs that innervate Glomeruli 1, 2, and 3. Each capitata peg sensillum on the maxillary palp contains 3 OSNs [55], and each OSN is thought to project to a single glomerulus. We found that 45 OSNs that project to Glomerulus 1, 45 to Glomerulus 2, and 46 to Glomerulus 3. Consistent with previous work [39,54], we saw that the axonal arbors of each OSN projected to a single glomerulus (Figs 1G, 1I, S3, and S4).

From this population of OSNs, we chose 10 OSNs innervating each glomerulus that represented the morphologies of the total population by visual inspection (Figs 1G, 1I, and S3). We then tested whether these were representative of the morphologies of the total OSN population using NBLAST [56] and found that they were not significantly different from the overall population (Welch's *t* test, $p=0.21$, $p=0.07$, $p=0.19$ for Glomerulus 1, 2, and 3, respectively). The number of excitatory synapses between two cells is indicative of the amplitude and probability of depolarization in a postsynaptic neuron after the presynaptic neuron fires [24,57]. We therefore reconstructed these 30 OSNs (10 per glomerulus) to completion, meaning that all synapses made by these neurons onto any postsynaptic partner were annotated. We quantified the length of all neurites (cable length) and branching pattern of these fully reconstructed OSNs and found that Glomerulus 1 OSN cable length was over twice as long and their arbors were more complex compared to Glomerulus 2 or 3 OSNs (Figs 1H and S5A–S5C). Due to the large volume of Glomerulus 1, its neurite density is lower than Glomerulus 2 or 3 despite the elevated cable length and branching complexity (Fig 1H and 1I).

One mechanism for signal amplification in neural networks is recurrent or reciprocal excitatory connectivity [58,59]. We next asked if *Ae. aegypti* OSNs have recurrent connectivity that could amplify the detection of host cues. We examined recurrent connections between the OSNs that innervate each glomerulus (Fig 1J–1N). We define a recurrent synapse as any OSN-to-OSN synapse where both OSNs send axons to the same glomerulus. We found that Glomerulus 1 OSNs had 283.4 ± 54.3 recurrent synapses per neuron, while Glomerulus 2 and 3 OSNs had only 58.9 ± 14.5 and 68.7 ± 11.1 , respectively (Figs 1L and S5F). We considered the possibility that there are more recurrent synapses in Glomerulus 1 OSNs simply because their processes are longer than Glomerulus 2 or 3 OSNs (Fig 1I). Because the opportunities for neurons to be synaptically connected are limited to locations where axons and dendrites are sufficiently close to make synapses, we computed the density of recurrent synapses per micrometer of overlapping axonal cable length (when axons are within 2 μ m of each other or cable overlap) in each glomerulus (Fig 1M). Glomerulus 1 OSNs had the highest recurrent synapse density with 0.053 ± 0.008 synapses per micrometer, while Glomerulus 2 OSNs had 0.021 ± 0.006 and Glomerulus 3 OSNs had 0.036 ± 0.005 (Figs 3D and S5G). To account for the difference in neurite length, we also computed the fraction of the total OSN output synapses that are recurrent synapses. We also found that Glomerulus 1 OSNs had the highest fraction of recurrent synapses (Fig 1N). The higher number of recurrent connections among Glomerulus 1 OSNs may provide a neural substrate for amplifying excitation by CO₂ cues in the mosquito brain (Fig 1O).

CO₂-sensitive OSNs have more recurrent than feedforward synapses

To study feedforward connectivity, we reconstructed antennal lobe uPNs (Fig 2A). We identified the uPNs that innervate Glomerulus 1, 2, and 3, which have large-caliber axons that project dorsally in the iACT. These were classified as iACT

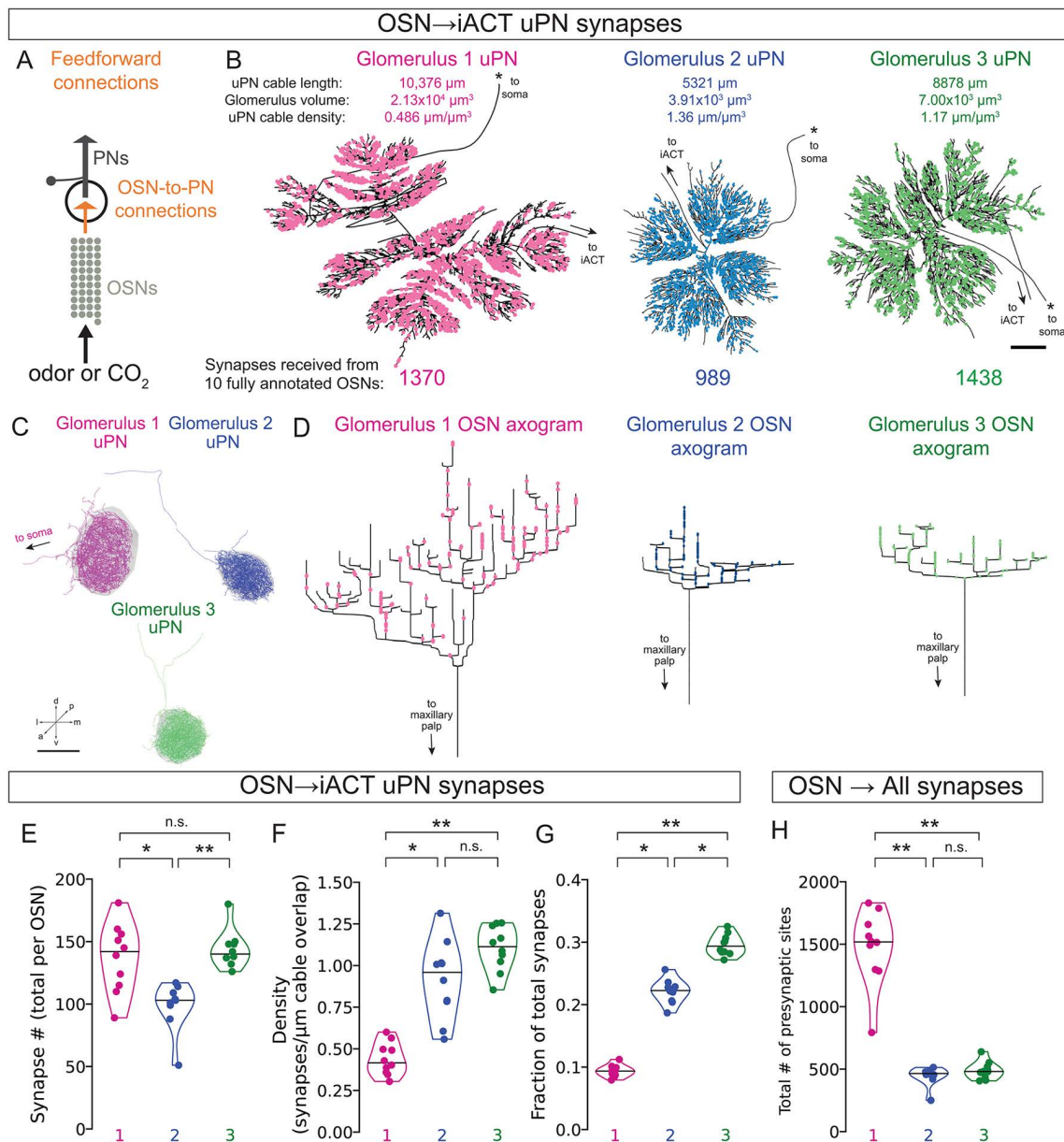


Fig 2. Feedforward connectivity of maxillary palp glomeruli. (A) Schematic with feedforward OSN-to-PN connections highlighted. (B) Dendrograms (flattened 2D representations of the 3D dendritic arbor preserving distance information) of uPNs and their incoming feedforward synapses from OSNs. * denotes branch leading to soma, and arrow denotes branch leading to the inner antennocerebral tract (iACT). (top) uPN cable length, glomerular volume, and cable density. Scale bar 100 μm . (C) Coronal view of uPN reconstructions. Scale bar 25 μm . (D) Axograms (flattened 2D representations of the 3D axonal arbor preserving distance information) of representative OSNs with their feedforward synapses to uPNs shown as colored dots. Scale bar 25 μm . (E) Number of OSN-to-uPN synapses from 10 fully reconstructed OSNs within Glomerulus 1, 2, and 3 (Kruskal–Wallis test (two-tailed), $p=0.0004$, pairwise comparisons using Dunn’s post-hoc test with Bonferroni correction: n.s., $p>0.05$; * $p\leq 0.05$; ** $p\leq 0.001$). (F) Density of OSN-to-uPN synapses per μm of cable overlap (length of uPN dendrite within 2 μm of OSN axon, see Methods) (Kruskal–Wallis test (two-tailed), $p=3.34 \times 10^{-5}$, pairwise comparisons using Dunn’s post-hoc test with Bonferroni correction: n.s., $p>0.05$; * $p\leq 0.01$; ** $p\leq 0.001$). (G) Feedforward OSN to uPN synapses as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p=2.49 \times 10^{-6}$, pairwise comparisons using Dunn’s post-hoc test with Bonferroni correction: * $p\leq 0.05$, ** $p\leq 0.001$). (H) Total number of outgoing OSN synapses to all cell types (Kruskal–Wallis test (two-tailed), $p=4.5 \times 10^{-5}$, pairwise comparisons using Dunn’s post-hoc test with Bonferroni correction: n.s., $p>0.05$; * $p\leq 0.01$; ** $p\leq 0.001$). The data underlying this Figure can be found in [S5–S8 Data](#).

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uPNs because of their axonal projections and because their dendrites did not extend beyond the glomerulus (S1B Fig). We found that only one ipsilateral iACT uPN innervated each glomerulus (Fig 2B and 2C). As with the OSNs, the Glomerulus 1 uPN cable length was greater, but the neurite density was lower than for Glomerulus 2 and 3 uPNs (Fig 2B).

To determine the number of feedforward synapses formed between OSNs and uPNs, we annotated all synapses onto each ipsilateral iACT uPN (Fig 2A–2C). We found that each Glomerulus 1 OSN (Figs 2D, 2E, and S5D), made 137.0 ± 26.0 (mean $\pm$ SD) synapses onto the Glomerulus 1 uPN. This uPN received 1,370 synapses from the 10 fully annotated OSNs, and therefore receives an estimated 6,000–7,000 synapses from all OSNs. Although the Glomerulus 1 OSNs had larger and more complex arbors than Glomerulus 3 OSNs (Fig 2B), Glomerulus 1 and 3 uPNs received a comparable number of synapses from each OSN, 137.0 ± 26.0 and 143.8 ± 14.0 per OSN, respectively (Fig 2E). Glomerulus 2 uPNs received the lowest number of feedforward synapses (98.9 ± 17.7). To account for the large volume of Glomerulus 1, we again calculated the feedforward connectivity as the density of feedforward synapses per micrometer of axon-to-dendrite cable (Figs 2F and S5E), the fraction of the total output synapses (Fig 2G), and the total number of OSN output synapses (Fig 2H). Glomerulus 1 OSNs had the lowest density and fraction of feedforward synapses. Taken together, the Glomerulus 1 OSNs made more recurrent synapses than feedforward synapses onto the uPN that we sampled, while Glomerulus 2 and 3 OSNs made more feedforward than recurrent synapses (Figs 1L and 2E).

High levels of recurrent connectivity are specific to *Ae. aegypti* CO₂ OSNs

While CO₂ initiates an activated search state in female *Ae. aegypti* mosquitoes, it has a different impact on *D. melanogaster* behavior. For *D. melanogaster*, CO₂ is aversive when the fly is at rest, and CO₂ can be attractive when flies are already in motion [60–64]. Although most of the olfactory systems of both insects utilize odorant receptors (ORs) and ionotropic receptors (IRs), CO₂ is detected by a gustatory receptor (GR) that is composed of Gr1, Gr2, and Gr3 subunits in *Ae. aegypti*, which are orthologues of Gr21a and Gr63a subunits in *D. melanogaster* [65,66]. We sought to determine if the organization of the CO₂ glomerulus is dictated by the receptor expressed in its OSNs or if ethological significance is more predictive of circuit structure. To this end, we compared circuit features of the *Ae. aegypti* CO₂ glomerulus to all *D. melanogaster* olfactory glomeruli, including the CO₂ glomerulus V (Figs 3 and 4).

We first compared feedforward synaptic connections between *Ae. aegypti* Glomerulus 1, and all *D. melanogaster* glomeruli. We leveraged our *Ae. aegypti* dataset and the *D. melanogaster* Full Adult Fly Brain dataset [30] using segmentations and synapse predictions available in FlyWire [28,67]. OSN projections to all *Ae. aegypti* glomeruli are unilateral, as are OSN projections to *D. melanogaster* glomerulus V. All other *D. melanogaster* olfactory glomeruli receive feedforward input from bilateral OSNs (Figs 3A, S6, and S7). To account for these morphological differences, we restricted our analysis to neurites and synapses within each right glomerulus in FlyWire. To compare feedforward connectivity across these glomeruli, we quantified the number of synapses each OSN makes onto its cognate uPNs (Fig 3B). We found that the feedforward synapse number (Fig 3B), feedforward synapse density (Fig 3C), and feedforward fraction of synapses (Fig 3D) of Glomerulus 1 were similar to many of the fly glomeruli, despite the large size of this glomerulus, however, the neurite length was greater than any of the fly glomeruli (Fig 3E). The total number of feedforward synapses in Glomerulus 1 was on the high end of the distribution, but was not significantly different from some of the fly glomeruli, including DC4 and DP1m, which have both been implicated in acid sensing [68,69].

The *D. melanogaster* V glomerulus is innervated by dendrites from a unilateral uPN in each hemisphere, and two bilateral uPNs, one with a soma in the left hemisphere and one in the right hemisphere (Fig 3A). In order to compare feedforward OSN-to-uPN connectivity between Glomerulus 1 and the fly V glomerulus, we included the three uPNs that innervate the right V glomerulus in the analysis: the right uPN, right bilateral uPN, and left bilateral uPN (S9A–S9C Fig). In glomerulus V, the unilateral uPN received 5.4 ± 4.0 synapses from each OSN, and the right and left bilateral uPNs received 22.4 ± 10.0 and 19.9 ± 10.3 , respectively (S9B Fig). The total number of feedforward synapses was 47.7 ± 22.1 , which was lower in number (Fig 3B) and density (Fig 3C) than Glomerulus 1. The total fraction of feedforward synapses in

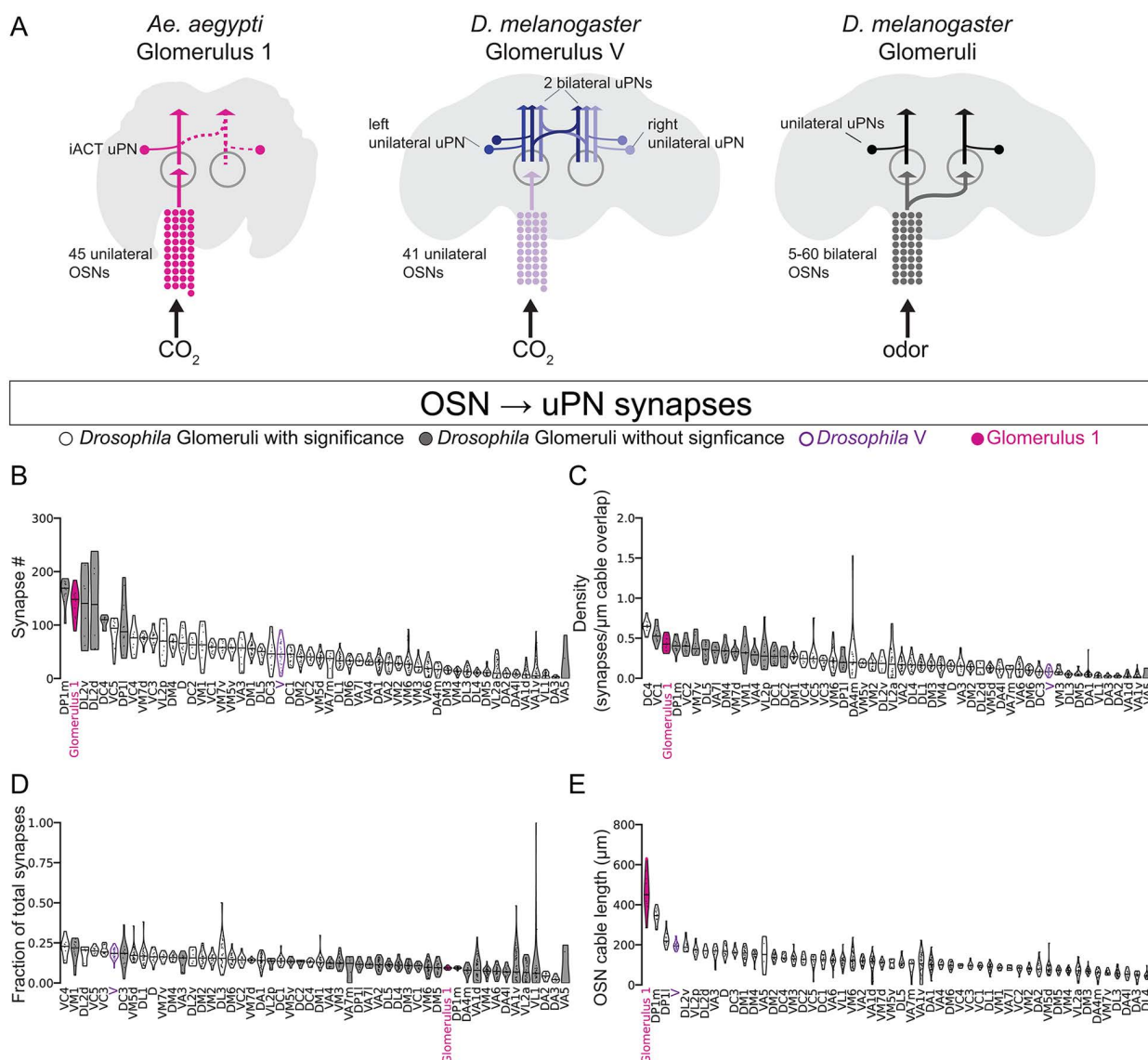


Fig 3. Feedforward connectivity of *Ae. aegypti* Glomerulus 1 compared to *D. melanogaster* antennal lobe glomeruli. (A) Schematics of cells and connectivity compared between *Ae. aegypti* and *D. melanogaster*. (left) The *Ae. aegypti* CO₂-sensitive Glomerulus 1 with 45 unilateral OSNs and the 1 uPN that projects ipsilaterally through the iACT to higher brain regions. Dashed lines indicate areas beyond the main reconstruction. (middle) The *D. melanogaster* CO₂-sensitive glomerulus V with 41 unilateral OSNs and 3 uPNs: one unilateral projecting uPN and two bilateral projecting uPNs, with one bilateral uPN on each side of the brain. (right) Representation of *D. melanogaster* glomeruli that contain a single uPN. These contain between 5 and 60 bilateral OSNs, which each innervate the corresponding glomerulus on both sides of the brain and have 1 uPN that projects ipsilaterally to higher brain regions. (B) Number of feedforward OSN-to-uPN synapses for each glomerulus (Kruskal–Wallis test (two-tailed), $p = 3.93 \times 10^{-56}$). Filled data are non-significantly different from Glomerulus 1 using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (C) Density of feedforward OSN-to-uPN synapses per μm of cable overlap within each glomerulus (Kruskal–Wallis test (two-tailed), $p = 1.39 \times 10^{-45}$). Filled data N.S. are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (D) Feedforward OSN-to-uPN synapses as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p = 4.87 \times 10^{-38}$). Filled data are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (E) OSN axonal cable length within each glomerulus. (Kruskal–Wallis test (two-tailed), $p = 1.03 \times 10^{-62}$). Filled data are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. The data underlying this Figure can be found in [S1](#), [S5–S7 Data](#).

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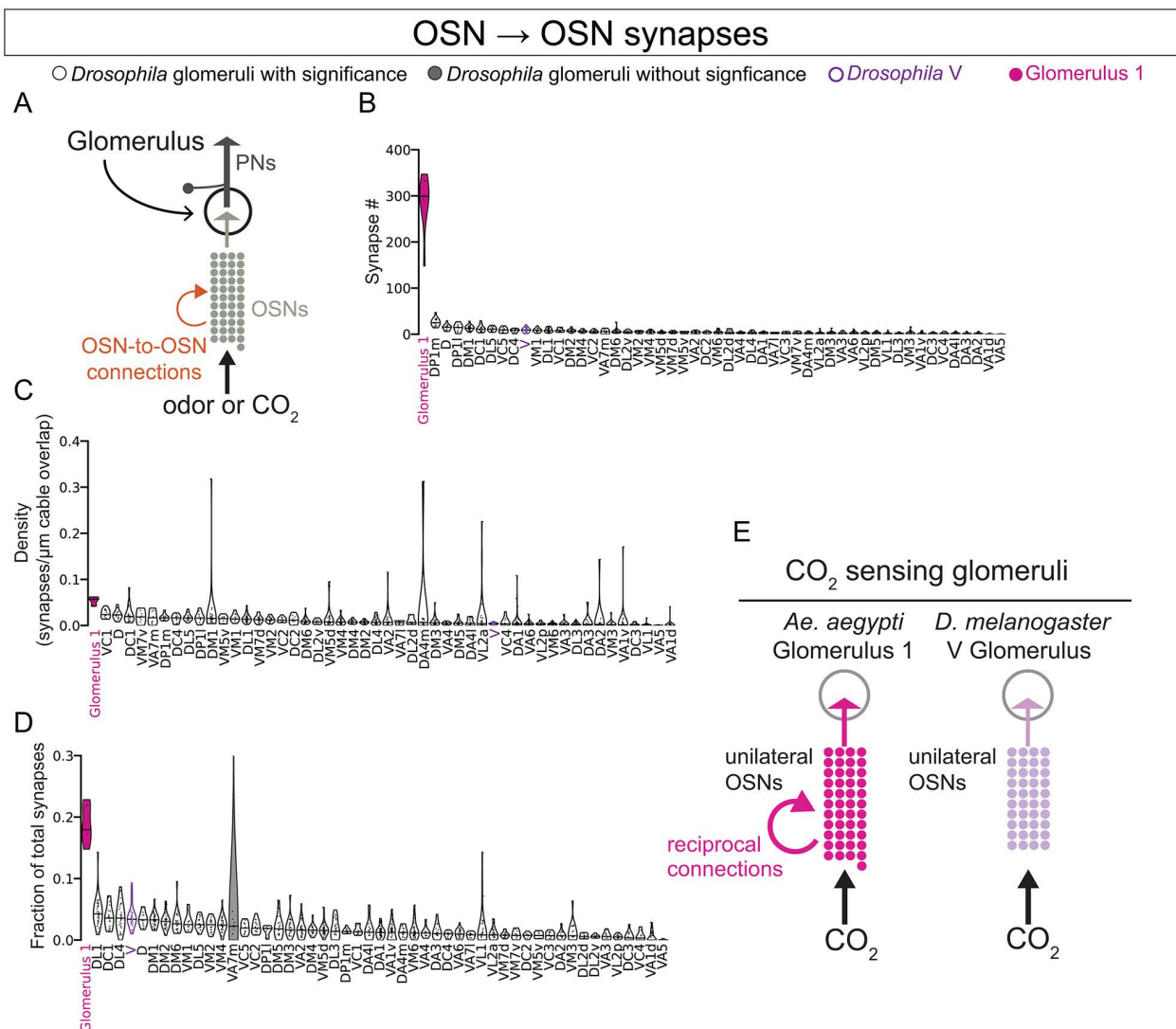


Fig 4. *Ae. aegypti* Glomerulus 1 has elevated reciprocal OSN-to-OSN connectivity. (A) Schematic with reciprocal OSN-to-OSN connections highlighted. (B) Total number of outgoing OSN-to-OSN synapses contained within each glomerulus (Kruskal–Wallis test (two-tailed), $p = 1.63 \times 10^{-98}$). Ten fully reconstructed OSNs are included for *Ae. aegypti* CO₂-sensitive Glomerulus 1. Filled data are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (C) Density of reciprocal OSN-to-OSN synapses per μm of cable overlap within each glomerulus (Kruskal–Wallis test (two-tailed), $p = 1.39 \times 10^{-72}$). Filled data are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (D) Reciprocal OSN-to-OSN synapses as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p = 1.13 \times 10^{-72}$). Filled data are non-significantly different using Dunn's post-hoc test with Bonferroni correction: $p \leq 0.01$. (E) Schematic summarizing difference between CO₂-selective OSN connectivity between *Ae. aegypti* and *D. melanogaster*. The data underlying this Figure can be found in [S2–S4 Data](#).

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V was higher than in Glomerulus 1 (Fig 3D). In summary, although Glomerulus 1 and V respond to the same sensory cue, the feedforward connectivity of Glomerulus 1 does not resemble V relative to other fly olfactory glomeruli.

We next compared recurrent OSN connectivity in *D. melanogaster* glomeruli to Glomerulus 1 (Fig 4A). We found that Glomerulus 1 OSNs made ~11-times more recurrent synapses (283.4 ± 53.4) than OSNs of any fly glomeruli (Fig 4B), and Glomerulus 1 had elevated OSN-to-OSN synapse density (Fig 4C) and fraction (Fig 4D) compared to *D. melanogaster* glomeruli. V was not similar to Glomerulus 1 in any of these metrics. We also found that all three *Ae. aegypti* maxillary

palp glomeruli had higher numbers of recurrent connections than the fly glomeruli (S8 Fig), although we note that none compared to the high levels seen in Glomerulus 1. Based on snRNA-seq expression data [70], these OSNs are cholinergic, and the CO₂-sensing CpA neurons express orthologues of four of the nicotinic acetylcholine receptor subunits (S9D and S9E Fig and S1 Table), suggesting these are excitatory connections. Taken together, Glomerulus 1 has comparable levels of feedforward connectivity, but far more recurrent connectivity than olfactory glomeruli in *D. melanogaster*, including the CO₂-sensing V glomerulus (Fig 4E).

Putative ribbon-like synapses are specifically between *Ae. aegypti* CO₂ sensory neurons

Synapses in *D. melanogaster*, and most other insects are typified by presynaptic structures called T-bars, named for their recognizable “T” shaped presynaptic density when viewed in cross-section [45,47]. We were surprised to identify two morphologically distinct categories of electron-dense specializations in *Ae. aegypti*. The majority of presynaptic structures resembled T-bars in the fruit fly (Fig 5A and 5D), although in *Ae. aegypti*, the base or “pedestal” of the T-bars appears shorter in our specimen. The “table-tops” of the T-bars are oriented parallel to the presynaptic membrane and are lined with synaptic vesicles on the side distal to the membrane. T-bars make up the vast majority of synapses in our dataset and occur between all combinations of cell types we examined.

We also found a number of electron-dense structures that were oriented perpendicularly to the cell membrane and resemble ribbon synapses found in mammalian retina and vertebrate hair cells, which are named for their elongated ribbon-like morphology (Figs 5B, 5E, S10, and S11 and S2 Table) [44,46,51–53]. These densities were lined with vesicles on both sides. These putative ribbon-like synapses were only seen in OSNs that innervate Glomerulus 1 and are only located at recurrent contacts between CO₂-sensitive OSNs. Within the reconstructed OSNs, we saw a total of 60 ribbon-like specializations, around 30 on each side of the brain. These ribbon-like structures are located most often near the primary branches of OSNs close to the point where the axon first enters the glomerulus, while T-bars are found throughout the entire OSN arbor in the glomerulus (Fig 5C, 5G, and 5H). We did not detect ribbon-like structures in OSNs or PNs in any other glomeruli we analyzed, in neither *Ae. aegypti* nor *D. melanogaster*, suggesting they may be specific to CO₂-sensitive Glomerulus 1 OSNs in the mosquito.

To determine whether these ribbon-like structures could be presynaptic specializations that participate in synaptic transmission, we looked for the presence of vesicles that were “docked” at the presynaptic membrane. Docked vesicles contact the presynaptic membrane in preparation for rapid exocytosis to release neurotransmitter into the synaptic cleft [41]. To identify putative docked vesicles, we re-imaged 19 ribbon-like structures at higher magnification (0.32 × 0.32 nm² per pixel and 0.4 × 0.4 nm² per pixel). We identified instances where vesicles were in contact with the presynaptic plasma membrane (Fig 5F), which is a signature of docked vesicles. This supports the hypothesis that these ribbon-like structures may function as presynaptic specializations involved in synaptic transmission.

Modeling reveals a role for recurrent connections in enhancing CO₂ detection

To better understand the impact of recurrent connections on olfactory encoding, we constructed a mathematical model that captured key elements of olfactory processing (Fig 6A). We envisioned an otherwise standard *D. melanogaster* olfactory system, with a single specialized CO₂ glomerulus with excitatory recurrent connections. Following existing approaches [71,72], we modeled the OSN-to-PN circuit including inhibitory gain normalization. In *D. melanogaster*, inhibitory local neurons (LNs) in the antennal lobe mediate interglomerular inhibition upon odorant activation (Fig 6A). GABA receptors are expressed in OSNs, including the CO₂-sensing CpA neurons, consistent with the presence of lateral inhibition by LNs in the *Ae. aegypti* antennal lobe (Fig 6B and S1 Table). We considered how LN inhibition impacts Glomerulus 1 PN activity when other glomeruli, which lack recurrent connections, are activated by background odorants, thereby increasing LN activity. This model took the following form:

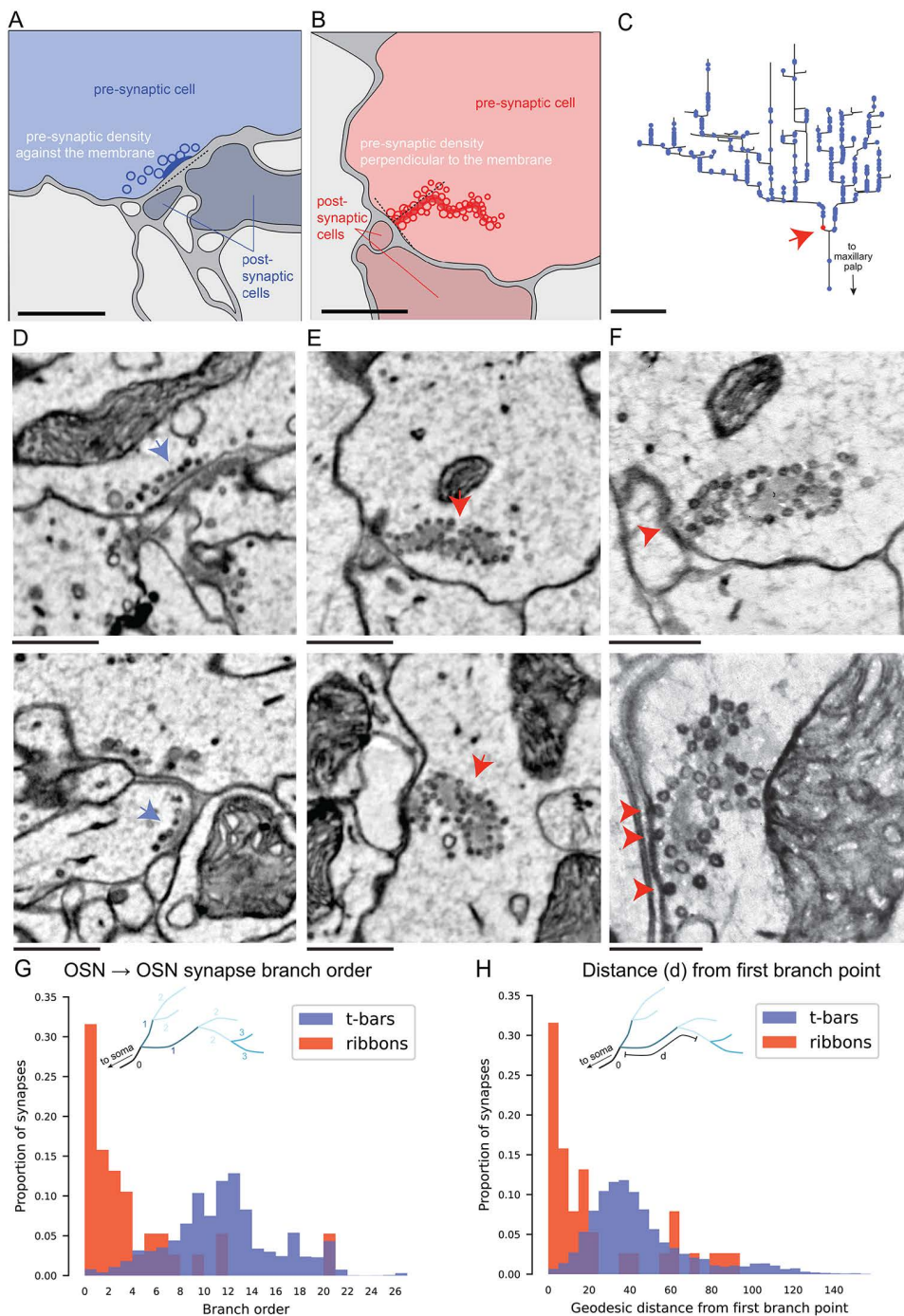


Fig 5. Ribbon-like synapses among CO₂ OSNs. (A) Schematic of a canonical T-bar synapse and (B) a putative ribbon-like synapse in *Ae. aegypti* Glomerulus 1. Scale bars 500 nm. (C) Axogram of an example Glomerulus 1 OSN with reciprocal output T-bar (blue) and putative ribbon-like (red) synapses represented by colored dots. Arrow points to a putative ribbon-like synapse from this OSN. Scale bar 25 μ m. (D) EM micrographs of canonical T-bar (blue arrows) synapses. Scale bars 500 nm. (E) EM micrographs of putative ribbon-like (red arrows) synapses. Scale bars 500 nm. (F) Higher magnification (0.5 nm/pixel) EM micrographs of putative ribbon-like synapses with vesicles docked to the presynaptic membrane (arrowheads). Scale bars 200 nm. (G) Histogram of putative ribbon-like and T-bar OSN-to-OSN synapse branch order (Mann–Whitney U test, $p = 5.93 \times 10^{-17}$). Inset: illustration of synapse branch order. (H) Histogram of distances (cable length) from the first branch point to putative ribbon-like and T-bar OSN-to-OSN synapses (Mann–Whitney U test, $p = 6.78 \times 10^{-9}$). Inset: illustration of quantified distance (geodesic distance or cable length) of synapse from the first branch point. The data underlying this Figure can be found in [S9](#) and [S10 Data](#).

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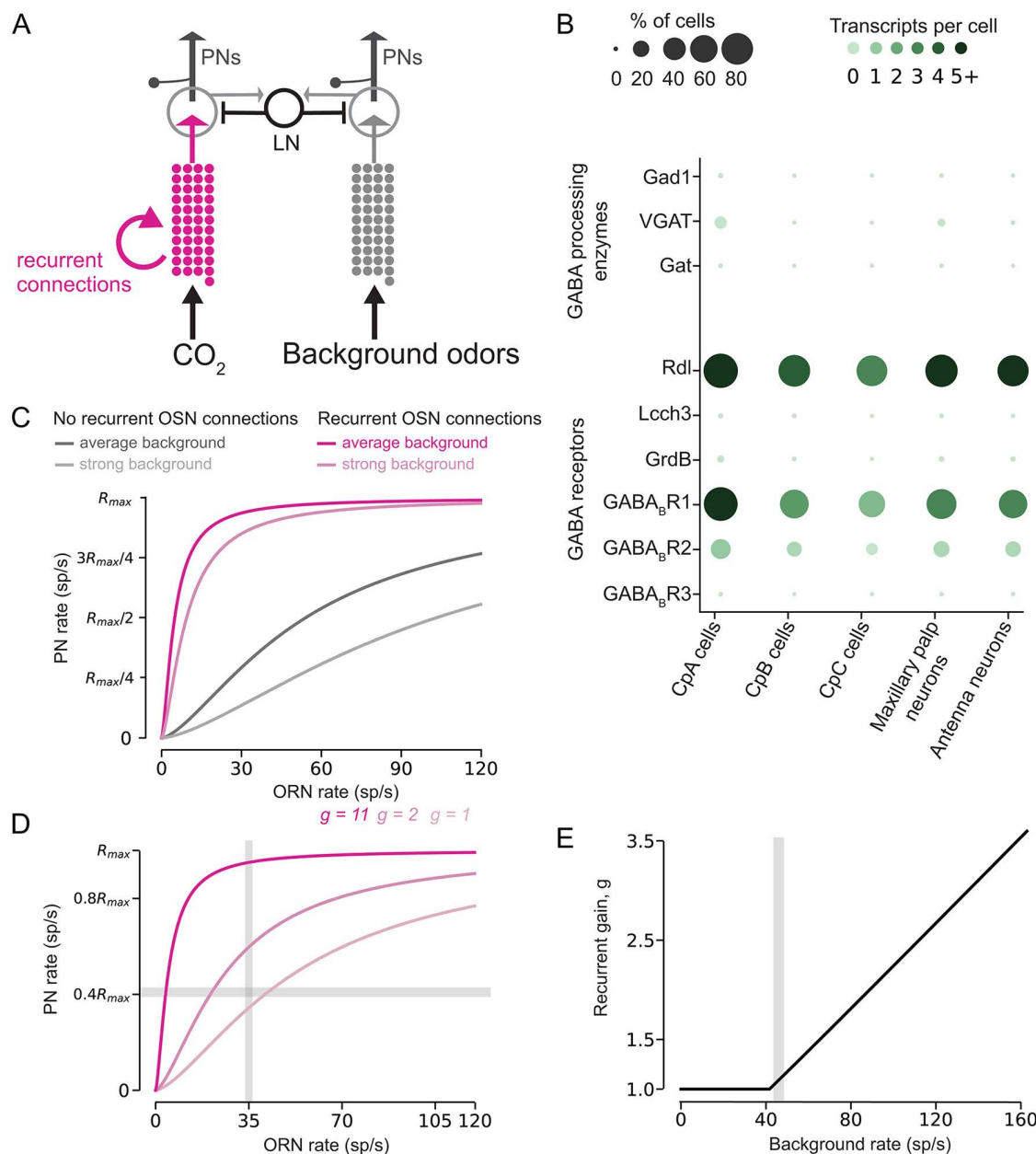


Fig 6. Connectomically-informed network model reveals recurrent connectivity specifically enhances stimulus detection. (A) Model architecture. CO₂ is processed by Glomerulus 1, which includes recurrent connections. Co-occurring odorants ('background') activate glomeruli which inhibit other glomeruli through inhibitory local neurons (LNs). (B) snRNAseq data replotted from ref [70]. (C) OSN to PN response curves for OSNs with and without recurrent connections, for two different background odor strengths (average and strong, see Methods). (D) OSN to PN response curve for differing values of g for an average background odor strength. Vertical gray shaded region indicates OSN response rates for which CO₂ should be detected (35 sp/s), and horizontal gray shaded region indicates the PN response strength that would correspond to CO₂ detection ($0.4R_{max}$). (E) Minimum value of g required to ensure CO₂ detection for varying background odor strengths. Vertical gray shaded region indicates an average level of background odor.

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$$r_{PN} = R_{max} \left(\frac{r_{ORN}^{1.5}}{r_{ORN}^{1.5} + s^{1.5} + \sigma^{1.5}} \right)$$

where R_{max} is the maximum PN response rate, s mediates inter-glomeruli divisive inhibition via a weighted average of all glomeruli to a given odorant, and σ defines the OSN value that achieves half-max activity. Values of σ , R_{max} , and the exponent of 1.5 were adopted from [71]. Recurrent connections were incorporated as a multiplicative gain g applied to OSN activity ($r_{ORN} \rightarrow g \cdot r_{ORN}$). We viewed this as the collective gain increase due to anatomical connections as well as their effective strength, which we estimated from EM data. We simulated our model under different scenarios to illustrate the impact of recurrent OSN connections on PN activity. To simulate CO₂ in the presence of other odors ('background odors'), we set $s=50$ spikes/s, consistent with previous models [71–73]. We refer to background odors of this strength as 'average' background.

We simulated PN responses to different OSN levels when recurrent connections were present (Fig 6C, magenta) and were not present (Fig 6C, gray). Recurrent connections were simulated by increasing g by a factor of 11, simulating the presence of 11-times the number of recurrent connections. Recurrent connections led to a sharp increase in the PN response curve, yielding a significantly larger PN response to smaller OSN activity. To examine the sensitivity of this gain to increases in the background odor, we doubled s to 100 spikes/s (Fig 6C, 'strong background'), demonstrating that global OSN activity decreases individual PN response due to lateral inhibition. These results are consistent with a hypothesis that recurrent connections amplify weak OSN input for the important ethological cue of CO₂.

Our primary hypothesis is that recurrent connections in Glomerulus 1 facilitate CO₂ detection. To illustrate this, we considered a detection task where a PN response greater than a threshold $R_{thresh} = 0.4R_{max}$ indicated the presence of CO₂ (Fig 6D). For accurate CO₂ detection, PN activity should reach R_{thresh} for CO₂ concentrations found to elicit weak OSN responses. Based on published results [74], we identified 35 sp/s as the OSN threshold for CO₂ response detection, for which PNs should become robustly active.

We explored values of g that achieved a PN response greater than or equal to R_{thresh} for OSN responses equal to 35 sp/s, for an average background odor (Fig 6D; $s=50$). We also identified the minimum value of g that achieved this for a range of background odor strengths (Fig 6E). For average background odor strength, both $g=11$ and $g=2$ (Fig 6D, dark and medium magenta, respectively) produced responses greater than $0.4R_{max}$ for OSN values of 35 sp/s, while $g=1$ (Fig 6D, light magenta) did not. We found that for average or stronger background odor strengths ($s=50$ or greater) g needed to be greater than one to achieve detection of transient CO₂ increases (Fig 6E), indicating that recurrent connections are necessary even under normal environmental conditions to ensure robust detection. Empirically, we measured a relatively large value for g that may suggest: (1) an extremely strong preference for detecting low levels of CO₂, even at the expense of false alarms; (2) inhibition is significantly stronger than excitation in this circuit, and/or (3) the recurrent synapses we identified are effectively weak despite their number. Taken together, our modeling results demonstrate how recurrent connections between OSNs can amplify weak sensory responses to achieve robust stimulus detection even in the presence of realistic background odor levels.

Discussion

To investigate the organizational principles of neuronal networks supporting innate sensory-driven behavior, we present the first central microcircuit connectome in the *Ae. aegypti* mosquito. We used automated tape-based transmission EM [18] and manual reconstruction to trace and annotate the wiring and connectivity of all three sensory glomeruli innervated by the maxillary palp, including the CO₂-sensitive glomerulus (Glomerulus 1). We found that each glomerulus had an array of 45–46 OSN inputs, and that the OSNs that innervate the CO₂-responsive Glomerulus 1 had a uniquely high level of recurrent OSN-to-OSN connectivity. Among the recurrent connections were structures that resemble ribbon synapses, which may couple OSNs to one another. We identified a single uPN that projects through the ipsilateral iACT in each palp

glomerulus and analyzed feedforward connectivity onto these neurons. Using comparative connectomics, we found that the feedforward wiring of the *Ae. aegypti* CO₂-sensitive glomerulus is similar to glomeruli in *D. melanogaster*, but with far higher recurrent connectivity. We found no obvious similarities between Glomerulus 1 and the V glomerulus, which share related receptors for CO₂. Our results suggest that highly recurrent excitatory connectivity between CO₂-sensitive OSNs in *Ae. aegypti* may amplify CO₂ detection to enable behavioral activation and sensitization. We further suggest that similar features of behavioral salience may predict the architecture of microcircuits across species. In support of our experimental findings, we demonstrated with network modeling how recurrent connections improve stimulus detection under normal environmental conditions and in the face of strong background odors which might make detection harder.

From the periphery to the brain

Recent work used serial block-face scanning EM to characterize the morphology of the dendrites, cell bodies, and axons of same OSN types in the periphery, at the level of the sensory organ [75]. The CO₂-sensing neuron, often referred to as CpA (capitate peg sensillum A), and the two odor-sensitive neurons, CpB and CpC (capitate peg sensillum B and C), are all housed in the basiconic (also called capitate peg) sensilla of the maxillary palp. A recent study identified flattened dendritic sheets that fold into elaborate lamellae, creating a major expansion in the dendritic surface area only in the CO₂-sensing CpA dendrites [75]. Our work complements this anatomical report of the dendrites with an analysis of the synaptic organization of their axons in the brain. Both studies highlight differences between CO₂-sensing neurons in *Ae. aegypti* and odorant-sensing neurons and together these findings suggest that in *Ae. aegypti*, CO₂ is uniquely processed as a sensory cue consistent with its potent role in behavior.

Recurrent excitatory connectivity

What is the functional role of recurrent synapses between OSNs? We predict that excitatory recurrent connections between Glomerulus 1 OSNs could support signal amplification, propagation, persistent activity, or a combination of these processes. Recurrent amplification has been reported in multiple sensory systems. In the mammalian sensory cortex, excitatory pyramidal neuron responses are thought to arise from selective amplification of thalamocortical signals [76–78] through recurrent connections between similarly selective excitatory neurons [59,79–82]. In *D. melanogaster*, a recurrent connectivity motif supports sustained activity for olfactory learning in the mushroom body [83]. It may be surprising that amplification would occur so early in sensory processing; however, CO₂ is a particularly important cue for *Ae. aegypti*, and the location of these recurrent connections would provide a mechanism to amplify CO₂ signaling prior to inhibition by LNs. This may create a mechanism that could surmount the strong and broad inhibitory tone provided by LNs in the presence of other chemosensory input [84]. By amplifying weak inputs, recurrent connections lower the effective detection threshold, which increases sensitivity, but also increases the risk of false alarms from noise or transient fluctuations in OSN activity. This is a classic example of a sensitivity-specificity trade-off and may reflect a circuit-level prioritization of detection over discrimination for CO₂ in *Ae. aegypti*.

Reliable signal propagation across long distances is a general challenge of nervous systems [85]. Notably, the reconstructed OSNs and their recurrent connections are far from their sensory endings in the maxillary palp. Given that these unmyelinated axons travel a long distance along the maxillary palp nerve and subsequently branch and ramify as they enter the glomerulus, one possibility is that recurrent synapses between OSNs may provide a mechanism to mitigate signal propagation failures and increase the robustness of CO₂ detection. Consistent with this idea, recurrent connections in the mammalian cortex enhance the reliability of sensory signals for subnetworks with more recurrent connectivity, and loss of a few neurons in the recurrent network results in degraded stimulus encoding [81].

Recurrent connectivity has been widely associated with sustained and persistent activity, a hallmark of short-term working memory [86,87]. In the mammalian cortex, recurrent connections are hypothesized to facilitate this process, providing a neural substrate for maintaining information over short timescales. However, examples of recurrence extend beyond the

cortex. In invertebrates, one notable case is the sustained bump of activity in the ring attractor networks of the *D. melanogaster* central complex, which plays a key role in spatial navigation [88,89]. Persistent activity in the CO₂ sensory system is an intriguing possibility in female *Ae. aegypti*, where CO₂ elicits a prolonged activated behavioral state. CO₂ sensitizes the organism and, when integrated with other host cues, drives innate host-seeking behavior that typically lasts minutes [90]. Notably, Glomerulus 3 OSNs respond to the host odorant 1-octen-3-ol and also have recurrent OSN-to-OSN connections, although fewer than between CO₂-sensitive OSNs, while Glomerulus 2 OSNs have relatively few recurrent connections and are not clearly characterized as host-detecting neurons [39]. The role of recurrent connections in host detection and neuron physiology remains to be seen, as virtually all mosquito OSN electrophysiology has been performed at the sensory periphery, far from the recurrent connections we describe [91].

A recent analysis of a *D. melanogaster* hemibrain connectome [27] reported the presence of recurrent axo-axonic connections between OSNs in *D. melanogaster* [92]. Analysis of this connectome diverged from our findings using the FlyWire connectome. In this work, network modeling demonstrated how activity-dependent recurrent connections for every glomerulus could lead to greater odor discrimination by pattern decorrelation, effectively supporting the functional role long established for divisive inhibition [71,72]. Our work demonstrates an alternative, but complementary role for recurrent connections for a single, high-priority glomerulus: odor detection via signal amplification. Further experimental investigation is necessary to understand how species-specific olfactory systems balance these two computations, perhaps as a function of ethological niche.

Invertebrate ribbon-like synapses

We identified structures specifically between CO₂-sensitive OSNs that resemble ribbon synapses seen in vertebrates. Examples of vertebrate ribbon synapses include retinal photoreceptors and bipolar cells [53,93], auditory hair cells [51], and fish lateral line hair cells [52], where ribbon synapses scaffold vesicle pools that enable sustained, graded release [44,46] (S10 Fig). Presynaptic ribbon-like structures have not previously been reported in insects and have not been reported in recently generated *D. melanogaster* connectomes. If these structures are indeed ribbon synapses, they would be the first identified in invertebrates, although we note that their function in neurotransmission remains to be studied.

Vertebrate ribbon synapses contain a protein encoded by the *ribeye* gene [50], which makes up the electron-dense structure of the ribbon [50,94,95]. The Ribeye protein contains two domains: the A domain that is believed to confer ribbon structure at active zones, and the B domain that is orthologous to the transcriptional repressor with enzymatic activity, CtBP2 [46,50]. Although there are invertebrate gene orthologues of the CtBP2 B domain, there are no orthologues of the *ribeye* A domain that is required for ribbon assembly. The absence of the *ribeye* domain from invertebrate genomes has led to the assertion that ribbon synapses are a vertebrate specialization. We speculate that the development of ribbon-like synapses in vertebrates and invertebrates may be a case of convergent evolution. Alternatively, invertebrates may have a Ribeye-like scaffold protein encoded by a divergent DNA sequence that hinders detection with DNA alignment algorithms, but nonetheless has a similar protein structure. Further study is needed to investigate the proteins that compose these structures.

We observe ribbon-like structures exclusively at recurrent contacts between CO₂-sensitive OSNs and only at the most proximal axon branches, which correspond to the entry point of axons into Glomerulus 1. The locations of these putative ribbon-like synapses suggest that they may amplify recurrent connectivity specifically among CO₂ sensory neurons.

Finally, it remains possible that these are not ribbon synapses, but rather a structure that appears similar when imaged with transmission EM. It is a challenge to explore their function because they are located deep in the antennal lobe, they are in small axo-axonic connections, and we currently lack an identified protein component such as Ribeye to gain genetic access to these structures. Given their close proximity with synaptic vesicles, one alternative is that they are specializations involved in trafficking or otherwise organizing vesicles in CO₂-selective OSNs. It is notable that these specializations have, to our knowledge, not been previously reported in insects and appear specifically in *Ae. aegypti* OSNs selective for CO₂. Future work is needed to uncover their functional role in mosquito CO₂ processing.

Circuit logic is not based on receptor alone

Ae. aegypti females are obligate blood drinkers, while *D. melanogaster* are dietary generalists that feed on decaying plant matter. The two Dipteran species diverged over 150 million years ago [37,38] and retain the same gross brain morphology [54,96]. Here, we compared the connectivity of the olfactory system of these divergent feeders at synaptic resolution. We observed a number of similarities between both organisms, including the prevalence of T-bar-like synapses as the predominant presynaptic specialization, although we note slight differences in the morphology of the T-bars. Some additional differences were clear at the onset of this study. *Ae. aegypti* OSNs send unilateral projections to the ipsilateral glomerulus while those in *D. melanogaster* largely send bilateral projections to mirror symmetric glomeruli in both the ipsi- and contralateral antennal lobes. One exception to this rule is the CO₂-sensitive OSN projections to the *D. melanogaster* V glomerulus, which are exclusively ipsilateral. It is notable that CO₂-sensitive OSNs in *Ae. aegypti* are housed in the maxillary palp, a different sensory organ than their location in the *D. melanogaster* antenna.

The role of CO₂ differs between these species. In *D. melanogaster*, behavioral response to CO₂ is context-dependent and can be either aversive or attractive [60–64]. Flies avoid regions of high CO₂ concentration, which is a signature of inhospitable environments [63]. CO₂ is also a component of an odorant mixture that *D. melanogaster* emits when stressed, and conspecifics innately avoid this mixture [61]. However, CO₂ can also be attractive, as decaying food sources release this cue [60,64]. While *D. melanogaster* is attracted to CO₂ during active foraging, attraction relies on a separate receptor and circuit that utilizes the subunit Ir25a [64]. The feedforward connectivity of CO₂ neurons in *Ae. aegypti* Glomerulus 1 and *D. melanogaster* glomerulus V at the first synaptic relay in the brain were not particularly similar compared to all other glomeruli in *D. melanogaster*. An underlying relationship between this sensory architecture and the valence or salience of sensory cues may exist, but we did not see a clear correlation. However, the recurrent connectivity of CO₂-sensitive OSNs was strikingly different, suggesting differential signal processing between species.

Beyond CO₂ sensitization: Host cue integration in the *Ae. aegypti* brain

Effective host-seeking behavior requires integration of multiple sensory cues [5–10,12,39]. Our analysis of the CO₂ system lays the groundwork for studying the integration of CO₂ with other host cues in the *Ae. aegypti* brain. The boundaries of the EM volume analyzed here did not allow for the analysis of the complete CO₂ circuit. There are likely additional uPNs and MGs that innervate these glomeruli, including a network of LNs. Our feedforward analysis is restricted to only part of the circuit, and future work to describe the entire CO₂ pathway will be a major step towards understating how multiple cues are integrated in the mosquito brain. In *D. melanogaster*, PNs mainly project to two higher-order brain areas: the mushroom body, crucial for learning and memory, and the lateral horn, thought to be critical for sensory-driven innate behavior [97–99]. As Glomerulus 1 is innervated by an ipsilateral iACT-projecting uPN, we predict that multiple sensory pathways converge in the mosquito lateral horn to support innate host-seeking behavior. Examining such hypotheses awaits an *Ae. aegypti* whole-brain connectome.

Methods

Experimental model and subject details

Aedes aegypti wild-type laboratory Liverpool mosquitoes (LVPib12 strain) were maintained and reared at 25–28 °C, 70%–80% relative humidity, with a photoperiod of 14 hr light:10 hr dark as previously described [100]. Adult mosquitoes were provided constant access to 10% sucrose. Male and female mosquitoes were housed together. The specimen used for this EM dataset was an adult female that was dissected nine days post-eclosion.

Specimen preparation

Procedures involving animals were conducted in compliance with NIH guidelines and approved by the IACUC at Rockefeller University and Harvard Medical School. We fixed and stained the brain of one adult female *Aedes aegypti*. After the

mosquito was immobilized on ice, the head was removed and fixed for three hours in 2.5% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer (0.1 M cacodylate buffer at pH 7.4 with 0.04% CaCl_2). The brain was dissected out of the head capsule in ice-cold phosphate-buffered saline (PBS) and stored in fresh fixative overnight.

The dissected brain was then washed three times for 10 min each with 0.02 M 3-amino-1,2,4-triazole (A-TRA) [30,101] in cacodylate buffer, stained in 1% osmium tetroxide, 0.1 M A-TRA in cacodylate buffer for 90 min on ice, washed in cold cacodylate buffer, and brought up to room temperature. Following a second round of staining in 2% OsO_4 (aqueous), the specimen was rinsed in filtered double distilled water (ddH_2O), placed in 1% thiocarbohydrazide (aqueous) at 40 °C for 8 min, then rinsed in ddH_2O again. The brain was rinsed in maleate buffer (pH 5.15) and stained in 1% uranyl acetate in maleate buffer overnight at 4 °C.

The following day, the brain was washed in maleate buffer (pH 5.15) followed by ddH_2O . It was stained in lead aspartate for 3 hours at 60 °C and washed in ddH_2O . The brain was dehydrated in a graded ethanol series on ice, infiltrated, and embedded in resin (LX-112, Ladd Research). The embedded brain was polymerized at 60 °C for ~48 hours.

Automated ultramicrotomy

The embedded specimen was automatically serially sectioned onto a tape-based collection substrate as described previously [18]. Briefly, the resin block with the brain was trimmed (Trim 90 diamond knife, Diatome) into an oblong hexagonal shape. The block was sectioned onto a Kapton substrate, GridTape (Luxel Corp), along the anterior–posterior axis using a customized an automated tape-collecting microtome (ATUM) [18,102] system attached to an ultramicrotome (Leica UC7). We targeted 40 nm section thickness. The automatically collected sections were accurately positioned (within approximately 200 μm from slot to slot) onto GridTape slots.

Annotation of regions of interest

We used a custom-made tape-handling machine with in-line illumination and a reel-to-reel system to capture overview images of slots and annotate regions of interest (ROIs) containing the brain. We specified regions of interest for EM imaging using a custom MATLAB script.

Automated transmission EM imaging

The GridTape reel containing the serial sections was placed into a custom reel-to-reel sample stage for continuous automatic acquisition [18] on a transmission EM (JEOL 1200 EX) with a 2×2 array of sCMOS cameras (Zyla 4.2, Andor). Magnification was 2,500 $\times$ on the microscope, accelerating potential was 120 kV, and the beam current was ~90 microamperes through a tungsten filament. Using the annotated ROIs, micrographs of 1,747 sections spanning the posterior antennal lobes and including the three palp glomeruli were acquired at a resolution of 4×4 nm per pixel.

Across the series of 1,747 imaged sections comprising the dataset, there were 29 single-section losses, with no consecutive section losses. Staining artifacts were usually small and did not adversely affect annotation, especially in large neuronal processes. Small, <1 μm membrane breaks occurred in some large caliber axonal processes, but were straightforward to overcome by trained annotators.

For the posterior 746 sections, ROIs were not annotated prior to imaging. Instead, taking advantage of consistent section placement, we created a “blind” ROI that was ~1.75 $\times$ the width of the previously placed ROIs, which added imaging time but captured all the tissue. This approach yielded the same results as placing smaller ROIs prior to imaging.

Image data alignment

Raw images were aligned using a custom pipeline based on AlignTK (<https://mmbios.pitt.edu/aligntk-home>) on the O2 computing cluster at Harvard Medical School as described previously [18,24,103,104]. First, camera image tiles were stitched into continuous two-dimensional montages, and then consecutive montages were aligned into a

three-dimensional volume. Every 50th section was used as a global constraint on the full dataset's alignment. For sections with small artifacts that would warp or misalign with the standard elastic alignment, rigid constraints were applied (absolute_maps in AlignTK), and manually placed control points were used to map corresponding features in neighboring sections.

Ribbon synapse manual imaging at 25,000× magnification

We performed higher-resolution re-imaging to examine the ultrastructure of ribbon synapses in greater detail. We targeted 18 out of 57 ribbons, which appeared to exhibit vesicle docking to the presynaptic membrane. Following 25 months in a dessicator (Secador, EMS), the reel was reloaded into the microscope. Single camera images at 25,000× were captured manually. Background was subtracted (rolling ball radius 50 pixels, light background) and local contrast was enhanced (histogram equalization, saturated pixels 0.4%) in ImageJ (version 1.52a) at a resolution of 0.4 × 0.4 nm per pixel.

Anatomical nomenclature

Throughout this work, we use the nomenclature proposed for glomeruli in [39]. We refer to the maxillary palp glomeruli as Glomerulus 1, 2, and 3. These glomeruli are referred to in previous literature as medio-dorsal (MD) glomeruli MD1, MD2, and MD3, respectively [54,105]. The glomeruli in *Ae. aegypti* were originally named [54] using a set of coordinate axes that is different from what is now commonly used in *D. melanogaster* [106], and these glomeruli are not notably dorsal in the coordinate space that we use here. We have therefore chosen the naming scheme that refers to them as Glomerulus 1, 2, and 3 (see [39] for a more complete description of antennal lobe glomeruli nomenclature).

Reconstruction of *Ae. aegypti* neurons

Neurons were manually reconstructed in the EM dataset as described previously [18,24,79]. We imported the data into Collaborative Annotation Toolkit for Massive Amounts of Image Data (CATMAID, release 2018.11.09) [107] for distributed web-based reconstruction and annotation. To reconstruct neurons, we manually placed points, or nodes, down the center of each process, creating skeletonized models of neuron morphology.

We identified the maxillary palp nerve bundle on the right side of the brain, with corroboration from light-level images, then completed tracing of 45 Glomerulus 1 OSNs, 45 Glomerulus 2 OSNs, and 46 Glomerulus 3 OSNs with axons in the bundle. For each OSN, the ventroposterior point of entry into the dataset was tagged as the root node, and the first branch point of the axonal arbor was tagged “first branch point”. The OSNs' first branch point was typically close to where the OSN entered and started to branch and ramify in its home glomerulus. We identified the uPN axons in the projection nerve bundle (iACT, S1B Fig) from the antennal lobe to higher brain centers. Tracing these back until they entered glomeruli, we found one densely branching uPN innervated each of the three maxillary palp glomeruli. PNs projecting through other tracts were not included in this analysis. Each uPN had a large-caliber process leading laterally to a cortical soma cluster, though only Glomerulus 1 uPN's soma was included in the bounds of the dataset. A multiglomerular PN (mPN) was identified by an axon in the iACT with a process projecting laterally towards a soma, and innervation of two different antennal lobe glomeruli.

In OSNs and PNs, T-bar synapses were identified and annotated using ultrastructural criteria: a presynaptic T-bar, presynaptic vesicles, synaptic cleft, and postsynaptic densities [45,47]. We identified *en face* cut synapses when a section contained a presynaptic specialization and an adjacent section(s) contained vesicle pools, and putative postsynaptic cells contained postsynaptic densities. Putative ribbon synapses consisted of a presynaptic density oriented non-parallel to the presynaptic membrane that was surrounded on all sides by vesicles (S10 and S11 Figs and S2 Table). The vast majority occurred at lower branch orders (Figs 5, S10, and S11). Ribbon synapses were differentiated from obliquely cut synapses by vesicle pools present on the same sections as the presynaptic density and the lack of putative postsynaptic cells on sections adjacent to the presynaptic density.

To increase our annotation accuracy, each fully reconstructed OSN and uPN was proofread by a minimum of three trained annotators, who marked all synapses with a connector node and all postsynaptic partners with a new skeleton. We reconstructed all postsynaptic partners of the 30 fully reconstructed OSNs, 10 randomly selected for each glomerulus, to identify their cell type. To empirically validate that the selected OSNs morphologies were representative, we used NBLAST to generate similarity scores for all OSNs in each glomerulus [56]. Neurons not identified as OSNs or uPNs are referred to here as MGs. Operationally, MGs were reconstructed cells that extended beyond their home glomerulus. Most of these are likely LNs, because this cell type is the most numerous and broadly arborizing of the MGs in the insect antennal lobe [108,109]. Additionally, some of these may be mPNs, or neurons that project into the antennal lobe from other regions. Some presynaptic specializations (<10%) appeared to contact glia or to interstitial space between cells.

Connectivity analysis

Analysis was performed in Python using pymaid (version 2.4.0, <https://pymaid.readthedocs.io>) and NAVis (version 1.5.0, <https://navis.readthedocs.io>). Data visualization and plotting were performed using matplotlib (version 3.8.3) and seaborn (version 0.13.2) packages and statistics were performed using SciPy (version 1.12.0), with posthoc tests performed using scikit-posthocs (version 0.9.0).

To measure cable length and distances between synapses and branch points, we computed geodesic or “along the arbor” distances. Because the opportunities for neurons to be synaptically connected is limited to regions where neuron processes are close enough to make synapses, we control for this by computing synapse density. We define synapse density as the number of presynaptic output synapses normalized by the cable overlap, which is the length of presynaptic neurite within sufficiently close proximity (<2 μm) of the postsynaptic neurite to make a synapse.

To control for differences in neurite lengths from each glomerulus to where axons reach the boundaries of the EM volume, we used two approaches to restrict our analysis to within glomeruli themselves. First, we generated meshes that defined the 3D boundary of the glomeruli and limited our analyses to neurites and synapses within the bounds of the 3D meshes. Second, for OSNs, we restricted our analysis to branches and synapses distal from the first branch point of the OSN axon, which was typically near where the axon enters the glomerulus.

Axogram and dendrograms

Axograms and dendrograms were generated with Neuroboom [36] (version 0.3.55, <https://github.com/markuspleijzier/neuroboom>). OSNs were plotted vertically (dot progression), whereas PNs were plotted radially (neato progression) to embed their more complex arbors.

Additional technical notes on reconstructed neurons

We identified a single putative mPN that sparsely innervates Glomerulus 1 and another unidentified glomerulus. This neuron has an axon tract that exits the antennal lobe in iACT, and may be an mPN. The branches were reconstructed, but it was not used in inter-glomerular comparative analyses because we were unable to do a complete survey of mPNs without a larger EM volume. We do not rule out the existence of other PN types that may exit through separate tracts [33,110,111].

Analysis of *D. melanogaster* neurons in Flywire

To compare *Ae. aegypti* glomerular wiring and connectivity to that of *D. melanogaster*, we first identified antennal lobe glomeruli in the Flywire dataset [28,67]. We then proofread OSNs of the CO₂-selective glomerulus V and appetitive glomerulus DM1 on the right side of the brain for merge and split errors; we selected the right side because at the time it contained better-proofread neurons. Subsequent proofreading by the FlyWire consortium completed the rest of the OSNs and PN types [28,29]. We analyzed fly OSNs, PN types, and their connectivity in the right hemisphere using the glomerular

boundary meshes made previously [32]. We used fafbseg (version 3.0.10; <https://fafbseg-py.readthedocs.io>) to pull data from the 3D reconstructions and the skeletonization function to generate skeletons from 3D neuron meshes. Specifically, OSNs were identified using the hemibrain_type field for all glomeruli except VC3, VC5, and VM6 which utilized the cell_type field due to changes in FlyWire's data structure. Additionally, we removed cells ($N=33$) labeled as OSNs from the analysis if they did not have any synapses onto a PN. For the purposes of comparison, we used a previously compiled list of uPNs [33]. We then used the flywire.skid_to_id function to recover the root_id in the FlyWire dataset.

In Flywire, a synapse prediction network [112] assigns each predicted synapse a cleft score, higher cleft scores reduce the chance of a false positive result, though the likelihood of a false negative result also increases. To estimate a cleft score for Flywire antennal lobe synapses with optimal and comparable accuracy, we manually inspected a sample of 19 feedforward OSN to PN synapses in V. We found a cleft score = 65 that provided the highest f -score = 0.91, with precision = 0.88 and recall = 0.94. This means ~6% of manually annotated synapses are missed, with slightly more false positives. We used a cleft score of 65 for the purposes of this analysis. To remove instances of nonexistent autapses, we set the parameter filter = True regardless of cleft score.

Quantification and statistical analysis

Unless otherwise stated, we used two-tailed Kruskal–Wallis tests and Dunn's posthoc tests with Bonferroni correction for pairwise comparisons. We implemented an algorithm (Piepho, 2004, [113]) that utilizes pairwise comparisons to identify significance groupings (S7 and S8 Figs). We used the non-parametric Kruskal–Wallis test to statistically compare across more than two groups because we do not assume normality and it is more robust to outliers. Some *D. melanogaster* glomeruli exhibit extreme values (Figs 3, 4, S7, and S8). Since normality was not assumed, a formal statistical test for outliers was not applicable. However, we inspected all data for points where synapse density was greater than two median absolute deviations ($2 \times \text{MADs}$) from the median. All feedforward synapses that were $2 \times \text{MAD}$ from the median that we visually inspected were verified as synapses, and these data were included in all analyses. We identified only 4 false-positive recurrent synapses among the outlier population. To most accurately reflect the morphology, these synapses were removed from the data presented throughout the paper. This difference did not change any of the statistically significant findings. The locations of the synapses in the FlyWire dataset that were removed are:

glomerulus	presynaptic cell_ID	presynaptic cell_ID	x location	y location	z location
VL2a	720575940627452668	720575940629918459	105268	59425	1582
DL3	720575940630014917	720575940621914392	110655	51685	1236
VM3	720575940638434357	720575940628897280	136581	64867	846
VL1	720575940629587031	720575940631025103	107970	65322	1650

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Network model

We simulated our model in Python using custom software. A notebook for simulating the modeling results are available on GitHub or Zenodo: https://github.com/htem/aedes_public (or <https://doi.org/10.5281/zenodo.21403889>). Model parameters were selected based on previous work [71–73]. We used values of $\sigma = 12$ spikes/s and $R_{max} = 165$ spikes/s based on OSN and PN models fit to experimental data [71]. The exponent 1.5 was used to simulate CO_2 in the presence of other odors ('background odors') that broadly activated many glomeruli [71]. We set $s = 50$ spikes/s. This value was consistent with models [71,72], and also with a weighted average OSN response of 43 sp/s calculated from 186 odor responses from 24 *D. melanogaster* glomeruli [73]. To select PN and OSN threshold values for our detection task, we used previous experimental findings. To estimate the PN threshold, we computed the average PN response across a distribution of odors [73] and selected this threshold to be two standard deviations larger than this mean. We assumed PN responses around this

mean were Gaussian distributed with previously derived variance parameters [72]. To compute the OSN threshold of 35 sp/s, we considered experiments with multiple mosquito species including *Ae. aegypti* that found that in an environment devoid of background CO₂, OSNs were unresponsive to CO₂ concentrations less than 200 ppm CO₂ but responded at a rate of approximately 35 sp/s for concentrations of 300 ppm [114,115], which they noted corresponds to the ambient CO₂ level in natural environments. Additionally, experimental work also found OSN response rates of around 35 sp/s for CO₂ concentrations 200 ppm greater than background CO₂ concentrations, for a variety of background levels [74]. Values of *g* identified in Fig 6E were found using scipy's root-finding method, `root_scalar`.

Gene expression of Acetylcholine and GABA in olfactory sensory neurons

We analyzed levels of gene expression in OSNs from neurons in both the maxillary palps and antennae by retrieving snRNA-seq data found in the Mosquito Cell Atlas [70]. Cells were categorized by their previously annotated subtypes (CpA cells, CpB cells, CpC cell) along with the tissue that they were isolated from (maxillary palps or antennae). To identify whether a given gene was expressed or not by a particular subtype, we used the average raw expression level per cell, where anything above 10 counts per cell is labelled as 10+ and anything less than one is labelled as 0. We restricted our analysis to the orthologues of the *Drosophila melanogaster* Acetylcholine and GABA receptors along with their associated processing enzymes. The information for each gene is provided in S1 Table.

Supporting information

S1 Fig. MicroCT and PNs with axons in iACT. (A) X-ray micro-computed tomography (microCT) of the *Ae. aegypti* brain analyzed in this study (anterior view). Scale bar 100 μ m. (B) Coronal (left) and sagittal (right) views of all PNs on the left side of the brain that contained axons that fasciculate into the ipsilateral iACT. Ipsilateral iACT uPNs were traced to completion while putative multiglomerular cells were not. a, anterior; d, dorsal; l, lateral; m, medial; p, posterior; v, ventral. Scale bar 50 μ m. (EPS)

S2 Fig. Examples of T-bar synapses and postsynaptic partners in *Aedes aegypti*. (A) Zoomed out (top) and zoomed in (bottom) canonical T-bar synapses, indicated by blue and red arrows respectively. Here, both synapses are characterized by the clustering of vesicles, postsynaptic densities, and presynaptic T-bar structures. (B) Example of one presynaptic cell making two closely located synapses. These are considered separately as one (blue arrow) synapse's characteristics disappear for at least 40 nm before appearing for the subsequent nearby synapse (red arrow). (C) Example of one T-bar synapse (blue arrow) and its postsynaptic partners. Presynaptic cell in yellow, postsynaptic partners in blue. Partners not considered postsynaptic for this synapse - based on contact with the synaptic cleft—are marked in red. Scale bars 500 nm. (EPS)

S3 Fig. Reconstructed OSN morphologies. Skeletonized neuron morphologies are shown for the 10 OSNs fully reconstructed from Glomerulus 1, 2, and 3. Labels are neuron IDs corresponding to the CATMAID database. Neuron 7,136 had a proximal branch that projects dorsoposteriorly toward higher brain areas. Colored dots indicate synapse type (key bottom-right). a, anterior; d, dorsal; l, lateral; m, medial; p, posterior; v, ventral. Scale bar 25 μ m. (EPS)

S4 Fig. Dorso-posteriorly projecting tract of OSN processes co-fasciculates with the iACT. (A) Rendering of 5 OSNs (colored traces) innervating Glomerulus 1 and Glomerulus 2 (gray volumes represent glomerular boundaries; Glomerulus 3 is also depicted, but none of the extra-projectional OSNs innervate it) that send small-caliber neurites along the iACT (medial uPN tract, Supplementary Fig 1B) to higher-order brain regions. (top) Right side of brain. (bottom) Left

side of brain. Dashed lines indicate locations of EM micrographs shown in (B). a, anterior; d, dorsal; l, lateral; m, medial; p, posterior; v, ventral. Scale bar 25 μm . **(B)** EM micrograph at dashed line locations in (A) Co-fasciculating neurites indicated by arrows. Scale bars 1 μm .

(EPS)

S5 Fig. Analyses of connectivity including extraglomerular OSN and uPN axons. **(A)** OSN cable length within each glomerulus. (Kruskal–Wallis test (two-tailed), $p = 1.81 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.01$; ** $p \leq 0.001$). **(B)** Number of branches for OSNs in each glomerulus. (Kruskal–Wallis test (two-tailed), $p = 6.32 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.01$; ** $p \leq 0.001$). **(C)** Mean number of branches at each branch order for OSNs in each glomerulus. **(D)** Number of OSN-to-uPN synapses (Kruskal–Wallis test (two-tailed), $p = 0.0002$, pairwise comparisons using Dunn's posthoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.01$). **(E)** Density of OSN-to-uPN synapses (Kruskal–Wallis test (two-tailed), $p = 3.61 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.001$). **(F)** Total number of outgoing OSN-to-OSN synapses restricted to within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 1.28 \times 10^{-5}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.001$). **(G)** Density of OSN-to-OSN synapses to within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 3.65 \times 10^{-6}$, pairwise comparisons using Dunn's post-hoc test with Bonferroni correction: n.s., $p > 0.05$; * $p \leq 0.01$). The data underlying this Figure can be found in [S1–S3](#), [S5](#), [S6](#), [S9](#), and [S10 Data](#).

(EPS)

S6 Fig. Visualization of statistical significance and median difference of morphological metrics of *D. melanogaster* and Glomerulus 1. Representation of each glomerulus is composed of two parts: size and color. The size of each point is the linearly scaled absolute difference of each metric. The color of each point is the significance of the pairwise statistical test for each glomerulus. **(A)** Color bar where significance from 0 to 0.05 is scaled at a different linear rate than from 0.05 to 1 to emphasize different degrees of significance. Red hues represent significance and blue hues represent non-significance. **(B)** Median difference of cable length within each glomerulus from Glomerulus 1, significance as indicated in (A). **(C)** Median difference of feedforward metrics of each glomerulus from Glomerulus 1, significance as indicated in (A). **(D)** Median difference of recurrent metrics of each glomerulus from Glomerulus 1, significance as indicated in (A). The data underlying this Figure can be found in [S1–S7 Data](#).

(EPS)

S7 Fig. Pairwise analysis of feedforward connectivity for all glomeruli. **(A)** Number of OSN-to-uPN synapses restricted to within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 9.21 \times 10^{-140}$, different letters mark whether glomeruli are significantly different using Dunn's posthoc test with Bonferroni correction: $p \leq 0.01$). **(B)** Density of OSN-to-uPN synapses within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 1.32 \times 10^{-137}$, compact letter display as in (A)). **(C)** OSN-to-uPN synapses restricted to within glomerular boundaries as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p = 1.63 \times 10^{-91}$, compact letter display as in (a)). The data underlying this Figure can be found in [S5–S7 Data](#).

(EPS)

S8 Fig. Analyses of recurrent connectivity for all glomeruli. **(A)** Number of OSN-to-OSN synapses restricted to within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 7.88 \times 10^{-105}$, different letters mark whether glomeruli are significantly different using Dunn's posthoc test with Bonferroni correction: $p \leq 0.01$). **(B)** Density of OSN-to-OSN synapses within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 6.96 \times 10^{-78}$, compact letter display as in (A)). **(C)** OSN-to-OSN synapses restricted to within glomerular boundaries as a fraction of overall OSN output synapses (Kruskal–Wallis test (two-tailed), $p = 1.03 \times 10^{-79}$, compact letter display as in (a)). The data underlying this Figure can be found in [S2–S4 Data](#).

(EPS)

S9 Fig. Reconstructions of OSNs and uPNs of *D. melanogaster* glomerulus V. (A) V OSNs and uPNs reconstructed in Flywire [28,29,33]. Lighter shades of orange and purple correspond to OSNs, while darker shades correspond to uPNs. Scale bars 50 μ m. **(B)** Number of OSN to uPN synapses restricted to within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 5.17 \times 10^{-17}$, different letters mark whether glomeruli are significantly different using Dunn's posthoc test with Bonferroni correction: $p \leq 0.01$). **(C)** Density of OSN to uPN synapses within glomerular boundaries (Kruskal–Wallis test (two-tailed), $p = 1.62 \times 10^{-09}$, letters display significant differences as in (B)). **(D)** snRNAseq data replotted from [70], for acetylcholine processing enzymes and (E) receptors. The data underlying this Figure can be found in [S5](#) and [S6 Data](#).

(EPS)

S10 Fig. Examples of ribbon synapses in vertebrates. (A–C) Schematics of ribbon synapses across vertebrate species. (A) Ribbon synapse in rabbit retina between rod bipolar cells and amacrine cells [53]. (B) Ribbon synapse in a zebrafish lateral line hair cell [52]. (C) Ribbon synapse in mouse auditory hair cell [51].

(EPS)

S11 Fig. Serial-sections of ribbon-like structures. Serial electron micrographs through ribbon-like structures in Glomerulus 1 OSNs. Labels are connector IDs corresponding to the CATMAID database. Scale bars 1 μ m. See [S2 Table](#) for links to view image data for each putative ribbon synapse in CATMAID. Scale bars 1 μ m.

(EPS)

S1 Table. This table includes the full gene names for the genes that are analyzed in the snRNAseq plots, gene abbreviations, corresponding AAEL numbers for identification in the *Ae. aegypti* genome, and FlyBase Gene Accession Numbers for the nearest gene orthologues in *D. melanogaster*.

(XLSX)

S2 Table. Links to view aligned EM image data with putative ribbon synapses in [S11 Fig](#).

(XLSX)

S1 Data. Data in [Figs 1H](#), [3E](#), [S5A](#), and [S6B](#).

(CSV)

S2 Data. Data in [Figs 1L](#), [4B](#), [S5F](#), [S6D](#), and [S8A](#).

(CSV)

S3 Data. Data in [Figs 1M](#), [4C](#), [S5G](#), [S6D](#), and [S8B](#).

(CSV)

S4 Data. Data in [Figs 1N](#), [4D](#), [S6D](#), and [S8C](#).

(CSV)

S5 Data. Data in [Figs 2E](#), [3B](#), [S5D](#), [S6C](#), [S7A](#), and [S9B](#).

(CSV)

S6 Data. Data in [Figs 2F](#), [3C](#), [S5E](#), [S6C](#), [S7B](#), and [S9C](#).

(CSV)

S7 Data. Data in [Figs 2G](#), [3D](#), [S6C](#), and [S7C](#).

(CSV)

S8 Data. Data in [Fig 2H](#).

(CSV)

S9 Data. Data in [Figs 5G](#) and [S5C](#).

(CSV)

S10 Data. Data in [Figs 5H](#) and [S5B](#).

(CSV)

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