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Drosophila Olfaction

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Summary and Keywords

Olfactory systems are tasked with converting the chemical environment into electrical signals that the brain can use to optimize behaviors such as navigating towards resources, finding mates, or avoiding danger. *Drosophila melanogaster* has long served as a model system for several attributes of olfaction. Such features include sensory coding, development, and the attempt to link sensory perception to behavior. The strength of *Drosophila* as a model system for neurobiology lies in the myriad of genetic tools made available to the experimentalist, and equally importantly, the numerical reduction in cell numbers within the olfactory circuit. Modern techniques have recently made it possible to target nearly all cell types in the antennal lobe to directly monitor their physiological activity or to alter their expression of endogenous proteins or transgenes.

Keywords: Drosophila, olfaction, chemosensation, development, odor coding, sensory, modulation, antennal lobe, mushroom body, lateral horn, plasticity

Introduction to Drosophila as a Model for Olfaction

The use of the fruit fly, *Drosophila melanogaster*, for genetic research has proved invaluable and enduring. The fly has also emerged as a useful tool for addressing questions in neuroscience, especially in olfactory systems. This success is in large part due to the fly's genetic toolkit, its ease of husbandry, and its numerically reduced nervous system with identified neurons that can be found in every individual fly (Olsen & Wilson, 2008).

Early studies on olfaction arose from the use of *Drosophila* for studies on learning and memory in which odors were used as the conditioned stimulus. This was done by providing punishment (electric shock) in coordination with one of two odors, leading the fly to actively avoid the shock-paired odor (Quinn, Benzer, & Harris, 1974). The use of odor-based behavioral assays eventually led to the discovery of specific olfactory genes (Ayyub, Paranjape, Rodrigues, Siddiqi, & Siddiqi, 1990) as well as insect brain regions

associated with olfactory learning and memory (de Belle & Heisenberg, 1994; Heisenberg, Borst, Wagner, & Byers, 1985).

Due to the advancement in the study of *Drosophila* genetics, researchers were able to identify genetic mutants in relation to odor-detection at an early stage. Inspired by Seymour Benzer's work in generating *clock* mutants (Benzer & Konopka, 1971), Kikuchi demonstrated that genetic mutations in flies could be used to alter odor responses between parent generations and their offspring (Kikuchi, 1973). This discovery in part led to the search for the genes and brain structure organization responsible for olfaction. Differences in odor responses were observed between wild type populations of *Drosophila* based on geographic origin (Fuyama, 1976) as well as intrapopulational differences (Alcorta & Rubio, 1989). Odor responses were also determined to be ethologically critical for proper courtship (Averhoff & Richardson, 1974).

The prevalence of olfaction across *Drosophila* behavioral and ethological research made it clear that a cellular and physiological basis for odor perception was needed. In a landmark study on *Drosophila* olfactory structures, Stocker et al. used antennal cobalt dye fills to identify individual glomeruli in the antennal lobe (AL), the first olfactory relay in the insect brain (Stocker, Singh, Schorderet, & Siddiqi, 1983). This allowed them to theorize that "individual glomeruli might represent functional units, each receiving antennal input in a characteristic combination." As such, Stocker continued his work in structural study, further characterizing glomeruli as well as identifying neuronal types in the antennal lobe including local interneurons (LNs) (Stocker, Borst, Lienhard, & Fischbach, 1990).

While structural analyses of the Drosophila olfactory system were underway, electrophysiological study and other activity assays were being employed. Field potentials (Alcorta, 1991) were used to determine odor response variations in mutants (Venard & Pichon, 1984) as well as to determine possible olfactory genes (Borst, 1984). Activity assays such as 2-deoxyglucose mapping showed differing odors caused specific patterns of glomeruli to be activated (Rodrigues & Buchner, 1984), lending early credence to the neural coding of odor stimuli through the combination of glomerular responses.

The discovery of mammalian odorant receptors (Buck & Axel, 1991) paired with algorithms to search for these genes in the fully sequenced *Drosophila* genome (Adams et al., 2000; Clyne et al., 1999) led to the neuroscientific tools and methods used today that thrust *Drosophila* into the forefront of olfactory research. Coinciding with these advances was the advent of the GAL4/UAS binary expression system (Brand & Perrimon, 1993) in flies, allowing *Drosophila* to develop as a favorable organism for the study of olfaction. Structural analyses, activity assays, and behavior have been refined and are commonly implemented in modern studies, along with transgenic methods for monitoring and manipulating specific populations of neurons in the olfactory system.

Basic Organization of the Antennal Lobe

Olfaction in *Drosophila* begins with the binding of odor molecules to chemoreceptors expressed by olfactory receptor neurons (ORNs). ORNs are housed in one of three broad types of sensilla located on the surface of the third antennal segments or the maxillary palps (Fig. 1A). This organization of ORNs located on the external surface of the antennae and palps greatly facilitates direct recordings from ORNs, and has thus contributed to *Drosophila's* rapid rise as a model system for olfaction (Wilson, 2013). The sensilla classes are the basiconic, trichoid, and coeloconic sensilla and they are distinguished based on their anatomy, physical appearance, and the specific ORNs that they house (Clyne, Grant, O'Connell, & Carlson, 1997; Grabe et al., 2016; Stocker, 1994). Each class of sensillum is present in a specific spatial pattern on the antenna, but there is large overlap between the regions where each sensillum class is found. Basiconic sensilla are widely distributed over the surface of the antennae but are most concentrated at the anterior proximal region and absent at the distal tip. Trichoid sensilla are predominant at the distal tip and coeloconic sensilla are found at the greatest density on the posterior surface.

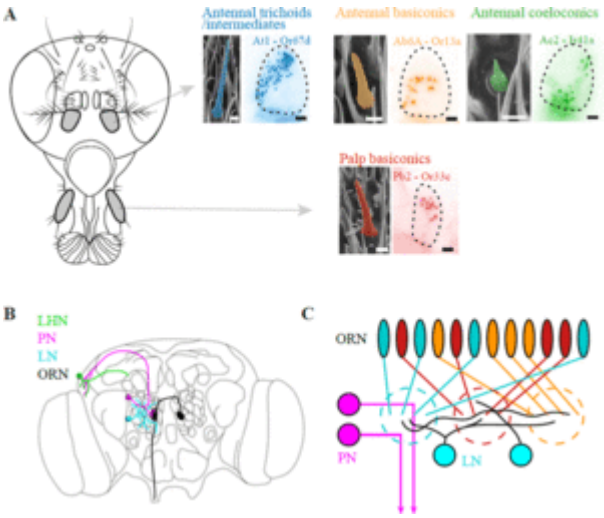


Figure 1. Organization of the *Drosophila* olfactory system. **A**. A schematic representation of the *Drosophila* head showing the antennae and maxillary palps shaded in light gray. Trichoid, basoconic, and coeloconic sensilla are distributed on the antenna in distinct yet overlapping regions. Each sensilla class houses unique ORNs and can be identified based on their morphology. Dashed outline in fluorescence image shows the boundaries of the antenna or palps. Scale bar = 2 μm in EM images and 20 μm in fluorescence images. Adapted from Grabe et al. (201 6), Elucidating the neuronal architecture of olfactory glomeruli in the drosophila antennal lobe. *CellReports*, *16*(12), 3401–3413, with permission. **B**. A schematic of the *Drosophila* brain showing ORN axons synapsing with PN dendrites in the AL. PNs then project to the LH where they synapse onto LHNs. ORN = olfactory receptor neuron, PN = projection neuron, LHN = lateral horn neuron. **C**. Within the glomerulus, every ORN that expresses the same olfactory receptor (denoted by the same color of ORN) projects to the same glomerulus. ORNs synapse with every PN in the glomerulus. Glomeruli are interconnected via multiglomerular LNs. LN = local interneuron.

Sensilla typically house two ORNs (though the range is one to four) per sensillum with each ORN expressing one or two chemoreceptors. A strict organization is observed where the same ORN types are reliably paired together. Based on the specific ORNs they possess, there are 4 types of trichoid sensilla and 4 types of coeloconic sensilla on the antenna. The maxillary palps contain only 6 classes of ORNs housed in three types of basiconic sensilla (and no trichoid and coeloconic sensilla). There are approximately 415 sensilla on the antenna and 57 sensilla on the maxillary palps (Grabe et al., 2016). This corresponds to approximately 1,150 ORNs per antenna and 120 ORNs per maxillary palps (Grabe et al., 2016). While most types of sensilla are represented equally between male and female flies, dimorphisms in a number of specific types of sensilla exist (Grabe et al., 2016; Shanbhag, Müller, & Steinbrecht, 2000; Stocker, 2001).

ORNs from the antenna and palps project their axons along the antennal nerve to spherical compartments of neuropil in the brain called glomeruli. In total, there are 56 glomeruli and a central region that serves as a hub for the axons and dendrites of neurons innervating the AL (Tanaka, Endo, & Ito, 2012). In the AL, ORN axons synapse onto the dendrites of projection neurons (PNs) (Couto, Alenius, & Dickson, 2005; Stocker, Lienhard, Borst, & Fischbach, 1990), broadly defined as any cells possessing dendrites in the AL and axons that convey olfactory information to higher order brain regions, such as the mushroom bodies (MB) or lateral horn (LH) of the protocerebrum (Fig. 1B) (Bargmann, 2006; Strausfeld & Hildebrand, 1999). Several different classes of PNs have

been described (see Projection Neurons), though the best-described, canonical PNs are those that innervate a single glomerulus, release the excitatory neurotransmitter acetylcholine, and project to the protocerebrum via the medial antennal lobe tract (mALT). In this review, the term PN in isolation of any description will refer specifically to these PNs.

Olfactory Receptor Neurons

At the heart of olfactory coding resides the ORN and the detection aspect of olfaction. *Drosophila* express three classes of chemosensory receptors based on sequence homologies within each class. These classes are the olfactory receptors (ORs), ionotropic receptors (IRs), and gustatory receptors (GRs). ORs are ligand-gated ion channels that share little sequence homology with mammalian olfactory G-protein coupled receptors (GPCRs) (Benton, Sachse, Michnick, & Vosshall, 2006; Clyne et al., 1999; Kaupp, 2010; Vosshall, 2009). Additionally, they are seven-pass transmembrane proteins but have an inverted membrane topology relative to GPCRs (Benton et al., 2006; Lundin et al., 2007). ORs were identified in the *Drosophila* genome by algorithmic sequence analysis (Clyne et al., 1999) as well as cloning (Vosshall, Amrein, Morozov, Rzhetsky, & Axel, 1999). Through cloning and spatial mapping, the receptors were shown to be expressed only in the antennae and maxillary palps, reinforcing their role as chemical receptors (Vosshall et al., 1999). OR-expressing neurons typically express a single OR in addition to the co-receptor termed *Orco* (formerly OR83b) (Benton, Vannice, Gomez-Diaz, & Vosshall, 2009; Couto et al., 2005; Fishilevich & Vosshall, 2005; Larsson et al., 2004). IRs are considered variant ionotropic glutamate receptors that bind to odors instead of glutamate (Benton et al., 2009). The mechanism of gustatory receptor mediated signaling is unclear; however, it is known that co-expression of the only two gustatory receptors used for olfaction is necessary to confer CO_2 sensitivity (Kwon, Dahanukar, Weiss, & Carlson, 2007).

ORN Axonal Targeting

Each ORN expressing a given class of ORs will project to the same glomerulus (Fig. 1C) (Couto et al., 2005; Vosshall, Wong, & Axel, 2000). An average of 30 ORNs converge on each glomerulus, but this number varies from 10 to 65 (Grabe et al., 2016). Each ORN synapses onto each PN within the glomerulus (Gaudry, Hong, Kain, de Bivort, & Wilson, 2013; Kazama & Wilson, 2009) and each individual ORN contributes equally to the total number of synapses in that glomerulus (Mosca & Luo, 2014). In order to achieve proper AL development, ORNs must target to their corresponding glomeruli. The PNs' contribution to glomerular formation is determined in space before ORN axon arrival in early stages of pupal formation by means of interdendritic interactions (Jefferis, 2004). These projections are target specific, meaning a given ORN will consistently project to its corresponding glomerulus (Gao, Yuan, & Chess, 2000), and the PN dendrites will consistently form the glomerular atlas (Jefferis, 2004). Interestingly, this level of specificity has been shown to be independent of OR gene expression (Dobritsa, Warr, Van der Goes van Naters, Steinbrecht, & Carlson, 2003). This is contrary to mammalian ORN

axonal targeting, which is dependent on the OR genes expressed in each neuron (Bozza, Feinstein, Zheng, & Mombaerts, 2002).

Although much remains unclear, one mechanism by which ORNs and PNs pair properly is by homophilic targeting using transmembrane proteins like Teneurin-a and Teneurin-m (Fig. 2A) (W. Hong, Mosca, & Luo, 2012), which are also involved in the formation of *Drosophila* neuromuscular junctions (Mosca, Hong, Dani, Favaloro, & Luo, 2012). In this process, ORNs will only pair with PNs expressing the same Teneurin profile (currently defined by classes of teneurins), and mismatches can be generated using overexpression or RNAi suppression of teneurin genes (W. Hong et al., 2012).

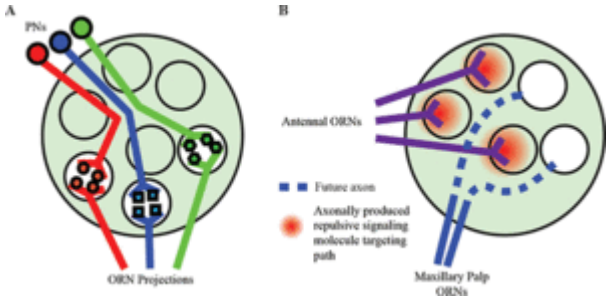


Figure 2. Aspects of ORN axon to PN dendrite targeting to glomeruli. **A**. Homophilic targeting of specific cell surface proteins is used by ORN axons to properly target PN dendrites. ORNs will only pair with PNs expressing the same cell surface marker profile. Markers can include proteins such as Teneurins and Toll receptors. Genetic changes to the expression levels of these proteins can cause mistargeting. Different shapes in the ORN to PR pairings represent different protein profiles. **B**. Temporal contributions to targeting are utilized by maxillary palps ORNs (blue). Antennal ORNs (purple) must arrive prior to maxillary palps ORNs during AL development in order for palps ORNs to properly target to their glomeruli. Antennal ORNs produce repulsive molecules such as Sema-1a which bind to PlexinA receptors on the palps ORNs, assisting in their guidance. The temporally delayed paths of the palps ORNs are represented by the dashed lines.

Other proteins, such as the Toll receptors, have also been implicated in ORN-PN pairing. RNAi knockdown screens for various proteins causing a misdirection in the DA1 and/or VA1d glomeruli identified Toll-6 and Toll-7 as being involved with proper targeting (Ward, Hong, Favaloro, & Luo, 2015). Although known for being the beginning of a well-characterized signaling cascade, the downstream signaling and even the entire cytosolic portion of the Toll-6 and Toll-7 receptors were deemed unnecessary for proper ORN-PN pairing in the aforementioned glomeruli (Ward et al., 2015). This implies the extracellular regions of Toll-6 and Toll-7 can be used for cell surface recognition.

Putatively known developmental proteins, like Wnt5 (Wu et al., 2014; Yao et al., 2007) and Hedgehog (Chou, Zheng, Beachy, & Luo, 2010B), as well as cell surface markers such as Dscam (Hummel et al., 2003), Ephrin (Sekine et al., 2013), and various semaphorins (Joo, Sweeney, Liang, & Luo, 2013; Sweeney et al., 2007, 2011) are also known to be involved in guiding ORN axons and PN dendrites toward proper glomerular formation. Aberrations in the production of these proteins produce phenotypes such as mistargeted ORN axons or PN dendrites. Interestingly, there appears to be a temporal aspect mediated by Semaphorin-1a and PlexinA between ORN axons in which antennal ORNs must develop

and begin patterning in order for maxillary palps ORNs to properly target glomeruli (Fig. 2B) (Sweeney et al., 2007). Furthermore, a broader spatial component is also involved. Differential expression of the transcription factors Atonal and Amos in the ORNs is necessary for proper development of the posterior and anterior glomeruli, respectively (Okumura, Kato, Miura, & Chihara, 2015). The combination of multiple cell surface proteins, temporal regulation, transcription factors, and developmental proteins shows the complexity of proper glomerular formation in the AL. Investigations into interactions between these contributors as well as concepts such as concentration differences of cell surface markers could begin to elucidate the details of this complex process.

ORN Physiology Is Mediated by Receptors

While proper OR expression may not be necessary for axonal targeting of ORNs, the olfactory receptor determines the physiological properties of the neuron. Or22a and Or22b are expressed in the ab3a neuron, and deletion of these genes ablates the sensitivity of this neuron to odor stimulation (Dobritsa et al., 2003). As suggested in the ORN Axonal Targeting section, the ab3A neuron still targets the DM2 glomerulus without issue, however it no longer responds to its preferred ligand, ethyl butyrate (Dobritsa et al., 2003). The Or22a/b deficient ab3a neuron has been repurposed to study other ORs using GAL4/UAS control. Various OR genes have thus been expressed in this “decoder” neuron in what is called the “empty neuron assay.” Expression of an OR in the empty neuron assay results in a change in odor sensitivity determined by the new OR (Hallem, Ho, & Carlson, 2004). Additionally, individual receptors can yield both excitatory and inhibitory responses by means of interacting with different odors (Hallem et al., 2004). Therefore, the odor response is dependent on the interaction between a given odor and receptor (Hallem & Carlson, 2006), so no single odor nor receptor can be classified as simply “excitatory” or “inhibitory.” However, it was demonstrated that esters and alcohols tended to produce strong excitatory responses, and aromatics were often found to be more inhibitory (Hallem & Carlson, 2006). The empty neuron assay has also been used to reveal the breadth of tuning of ORs. Broadly tuned ORs, such as Or67a and Or49b, were identified along with narrowly tuned ORs, like Or82a (Hallem & Carlson, 2006). As might be expected, when odor strength is increased, a wider variety of ORs are activated (Hallem & Carlson, 2006). As stated by Hallem, Ho, and Carlson (2004), “[receptors] vary widely in their breadth of tuning, and odorants vary widely in the number of receptors they activate,” indicating the importance of ligand/receptor interaction to ORN activity. Aside from OR receptor expression, few peripheral factors influence ORN olfactory responses. For example, ORN odor responses are invariant to the wind speed in which they are detected (Zhou & Wilson, 2012). One notable exception however is the emphatic coupling between ORNs residing in the same sensillum, where activity in one ORN can inhibit another (Su, Menuz, Reisert, & Carlson, 2012).

Ionotropic Receptors

In addition to ORs, *Drosophila* express a subfamily of ionotropic glutamate receptors (iGluRs) known as IRs. They have more variable extracellular regions than iGluRs, which bind to various olfactory ligands instead of glutamate (Benton et al., 2009). However, IRs maintain the ion-channel characteristics of traditional iGluRs (Abuin et al., 2011), but also confer odor responses much like ORs. For example, misexpression of an IR as per the "empty neuron assay" confers the IR-specific odor responsivity (Benton et al., 2009). IRs act in a complementary function to ORs with respect to odor representation; while ORs tend to respond to esters and alcohols, IRs mainly respond to amines and acids (Silbering et al., 2011). Furthermore, IRs and ORs have been shown to be evolutionarily distinct (Croset et al., 2010).

Representation of Food-Based Odors

Volatile products of fermentation are highly attractive to *Drosophila*. Importantly, a distinction is made that the response to the volatiles of the yeast causing the fermentation independent of the fruit are what cause the attraction behavior (Becher et al., 2012). The presence of the yeast has been found to encourage oviposition and larval development (Becher et al., 2012). Yeast byproducts such as ethanol, acetic acid, and 2,3-butanediol are overrepresented in the antennal lobe. Additionally, food deprivation increases signaling in ORNs sensitive to appetitive odors in a neuropeptide-dependent manner while decreasing sensitivity to aversive odors (Ko et al., 2015; Root, Ko, Jafari, & Wang, 2011). Odors related to food tend to be attractive to *Drosophila* and are generally overrepresented in the antennae (Hallem & Carlson, 2006), indicating their ethological relevance. Furthermore, *Drosophila* also detect and respond to odors that indicate potential harm associated with food. Geosmin, a product of toxic bacteria and fungi found on fruit, interacts with a single receptor and elicits a strong, aversive behavior (Stensmyr et al., 2012).

Projection Neurons

Projection neurons are defined as the output cells of the antennal lobe, transmitting olfactory information from the AL to the LH and MB. Originally, the term PN referred specifically to uniglomerular excitatory projection neurons that served a similar function to the mitral and tufted cells of the mammalian olfactory bulb. It is now appreciated that there are likely several types of neurons that convey olfactory information to other brain regions; some of these cells differ substantially from the originally described PNs.

Most projection neurons are derived from one of three neuroblasts named according to the position of their cell bodies: the anterodorsal, lateral, and ventral neuroblasts (Jefferis, Marin, Stocker, & Luo, 2001). The best described members of the anterodorsal and lateral neuroblast-derived PNs are uniglomerular, excitatory cholinergic neurons, which project to third order brain regions via the medial antennal lobe tract. These cells

have been referred to as ePNs (excitatory PNs), uPNs (uniglomerular PNs), mPNs (mALT-projecting PNs), and simply PNs. This nomenclature can create confusion as other neurons in the AL have been assigned similar abbreviations. For example, multiglomerular PNs have also been referred to as mPNs. For the purposes of this review, we will refer the canonical excitatory, uniglomerular PNs as ePNs and the GABAergic multiglomerular PNs as iPNs. We do this acknowledging that PN nomenclature is still not fully resolved and that the identification of future classes of PNs will likely necessitate revisiting how we refer to these neurons.

PN Anatomy

The dendrites of ePNs project into a single glomerulus ipsilateral to their somata. On average, 2 to 3 mPNs innervate each glomerulus, though the number ranges from 1 to 6 (Grabe et al., 2016). While the volume of the glomerulus is dictated primarily by the number of ORNs, it also correlates slightly with increasing ePN number (Grabe et al., 2016). It is generally believed that all of the ePNs that innervate a single glomerulus function identically, and stark differences between ePNs within a glomerulus have not been reported. There is also a correlation where glomeruli innervated by the highest number of ePNs receive their input from narrowly tuned ORNs (Grabe et al., 2016).

Within the glomerulus, ePNs receive synaptic input from ORNs, LNs, and from sister ePNs that innervate the same glomerulus. Interestingly, approximately one-fourth of the ePN synapses within the AL are outputs (Rybak et al., 2016). The ORN to ePN synapse is cholinergic and reliably blocked by the nicotinic receptor antagonist mecamylamine (Kazama & Wilson, 2008). Cholinergic, as well as electrical, synapses are also used between sister PNs to correlate their spontaneous activity and odor responses (Kazama & Wilson, 2009). ePNs make synaptic connections onto LNs (Wilson & Laurent, 2005) and in turn receive direct inhibition from several classes of LNs (Liu & Wilson, 2013).

The next best characterized group of PNs arises from the ventral neuroblast and have their somata located ventral to the AL. These PNs are inhibitory, utilize GABA, and project to the LH via the mlALT. Most of these ventrally positioned PNs innervate multiple glomeruli, though a smaller subset is uniglomerular (Shen et al., 2016; Tanaka et al., 2012). These PNs have also been referred to by a number of abbreviations including iPNs (inhibitory PNs), vPNs (ventral PNs), mPNs (multiglomerular PNs), and mlPNs (mediolateral tract PNs). Here, we refer to these cells as iPNs. iPNs have been further subdivided into three types based on their glomerular innervation patterns (Tanaka et al., 2012): Type 1 are uniglomerular, Type 2 are multiglomerular and represent the vast majority (~80%), and Type 3 are panglomerular. Further classes of iPNs have been recently identified and are thus poorly described in terms of physiology and function. The iPNs are densely interconnected with all of the other cells classes within the AL. Like the ePNs, they also receive direct excitation from ORNs but are generally more broadly tuned to odor input (K. Wang et al., 2014). This may be due to a combination of direct input from more ORN types (since iPNs typically innervate multiple glomeruli) or from lateral

excitatory connections. iPNs also make reciprocal connections with ePNs. They are excited by ePNs and in turn depolarize ePNs via electrical synapses.

Characterization of PNs

Initial studies into the organization of the AL focused on screening GAL4 promoter lines to identify cells that appeared most like the traditional PNs described in other insects; namely uniglomerular PNs. However, recently there have been more consistent efforts to fully characterize the projection neurons in glomeruli using unbiased approaches (H. H. Lin, Chu, Fu, Dickson, & Chiang, 2013; Tanaka et al., 2012). For example, photoactivatable GFP can be used to reveal all of the neurons innervating a glomerulus. This approach revealed a total of 12 PNs that innervate the CO_2-sensitive V glomerulus representing novel classes of PNs that project bilaterally and exclusively to the V glomerulus (H. H. Lin et al., 2013). Interestingly, these different classes of PNs responded uniquely to increasing concentrations of CO_2 and influenced concentration-dependent aversion to the odor. Thus while it is still assumed that classic ePNs within a glomerulus function identically, it is clear that different classes of PNs within a glomerulus function together to shape olfactory-mediated perceptions and behavior.

Local Interneurons

The characterization of *Drosophila* LNs has been among the most complicated for neurons of the antennal lobe. In part, this is due to the incredible heterogeneity in features used to classify them. Such features include neurotransmitter composition, anatomy as defined by glomerular projection, intrinsic cellular properties (Seki, Rybak, Wicher, Sachse, & Hansson, 2010), and responses to olfactory stimuli. The only common feature that unites all LNs in the AL is simply that their processes are restricted entirely to the antennal lobe. Otherwise, it is clear that AL LNs represent an extremely diverse and complex class of neurons.

LN Anatomy

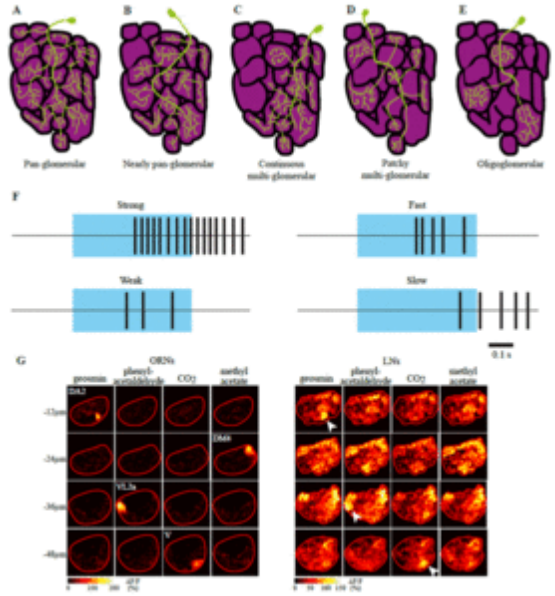


Figure 3. Diversity of individual LN morphologies and responses, and also populational LN responses to private odors. (A–E) The five classes of LN morphologies as defined by Chou et al. (2010A). These classes are panglomerular (A), nearly panglomerular (B), continuous multiglomerular (C), patchy multiglomerular (D), and oligoglomerular (E). Pan- and nearly panglomerular LNs innervate all and nearly all glomeruli, respectively. Multiglomerular LNs innervate many glomeruli, but not to the extent of nearly panglomerular LNs. Continuous LN innervate neighboring glomeruli while patchy LNs do not. Complimentary patchy LNs without overlap can be observed within a fly. Oligoglomerular LNs tend to innervate between one and three glomeruli and are the least common of the five categories. A weak inverse correlation exists between the number of glomeruli an LN innervates and the strength of its odor responses (Chou et al., 2010A). (F) Examples of spike trains demonstrating the diversity of LN responses.

Recordings were obtained from LNs labeled by the same GAL4 line, and responses are all to the same odor. Vertical lines represent individual LN spikes, and the blue rectangles denote odor delivery. Scale bar is 0.1 seconds. Top left, a strong odor response, compared to bottom left, a weak odor response. Top right and bottom right, a fast and slow response, respectively. Responses in LNs may also be strong with a long latency or weak and short in latency. These responses tend to vary more across cells rather than odors, allowing for the prediction of other odor responses given an observed response to a known odor. (G) Odors that elicit ORN signaling to a single glomerulus evoke LN activity in all other glomeruli. Left, the GCaMP3 signal in ORNs in response to four odors. Right, the corresponding GCaMP3 signal of LNs to the same odors. Arrowheads indicate additional LN activity in the glomerulus being activated. These data indicate activity in as little as one glomerulus is sufficient to elicit LN activity in all other glomeruli, and the resulting spatial intensity profile is similar across odors. Adapted from Hong and Wilson (2015), Simultaneous encoding of odors by channels with diverse sensitivity to inhibition. *Neuron*, *85*(3), 573–589, with permission.

Anatomically, virtually all LNs are multiglomerular and send their dendrites to several olfactory glomeruli. However, the specific glomeruli targeted by LNs can vary tremendously across cells. LNs can innervate (a) virtually all glomeruli and be panglomerular, (b) all but a few glomeruli, (c) a continuous region of the AL, (d) a patchy or discontinuous portion of the AL, or (e) only a few glomeruli and be oligoglomerular (Fig. 3A–E) (Chou et al., 2010A). It is generally believed that LNs will release GABA throughout their dendrites, making total innervation a reasonable proxy for GABA release into a glomerulus (Chou et al., 2010A; E. J. Hong & Wilson, 2015). Interestingly, while there are only approximately 100 LNs in each AL, a survey of nearly 1500 LNs (across different individual flies) revealed approximately 850 unique glomerular innervation patterns. Thus the specific glomerular pattern cannot be used to rigidly classify LNs as this feature is not stereotyped across flies. However, despite the great variability in LN projection patterns, their anatomical innervations are clearly not randomly distributed. Various GAL4 lines that label LNs show consistency in the glomeruli that they target. For example, LNs labeled by some GAL4 lines consistently avoid glomeruli whose cognate ORNs are selective for pheromones, while reliably targeting other glomeruli. Other GAL4 lines preferentially label patchy LNs or LNs that innervate a continuous region of the AL. Genetic multicolor labeling of two patchy LNs shows that their dendrites appear to repel one another, preventing them from occupying the same glomerulus (Chou et al., 2010A). Thus the logic of LN innervation in the AL is likely not a rigid stereotypy, but rather more likely regulated on an individual fly basis through developmental processes, such as LN to LN interactions.

LN Physiology

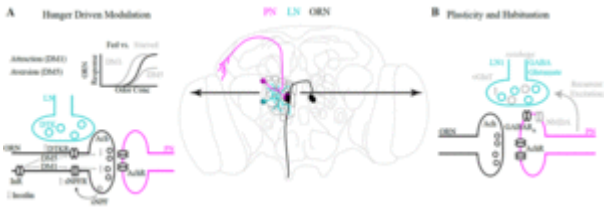
Similar to their diversity in morphology, LNs also demonstrate great variability in their physiology. Both the intrinsic properties and synaptic conductances of LNs vary across individual cells to give them unique odor responses (Chou et al., 2010A; Nagel & Wilson, 2016; Seki et al., 2010; Wilson & Laurent, 2005; Wilson, Laurent, & Turner, 2004; Yaksi & Wilson, 2010). Such odor responses can vary with regards to their odor tuning, sensitivity,

and temporal dynamics (Fig. 3F). While the physiology of LNs can vary between neurons, there are consistencies observed within and across cells' responses, much like their anatomy. Importantly, LN odor responses vary more across cells than they do across odors. This means that having information about an LN's responses to one stimulus can help predict its responses to untested odors. One of the most convincing means of grouping LNs into individual classes is to correlate patterns of anatomy with physiology. Indeed, such trends have been observed, suggesting that distinct classes of LNs are present in the AL (Chou et al., 2010A). For example, panglomerular LNs that innervate the most glomeruli show weaker odor responses than LNs innervating far fewer glomeruli. Additionally, LNs that selectively avoid pheromone processing glomeruli tend to have more transient odors responses compared with other LNs. Not surprisingly, such physiological differences thus also correlate with the GAL4 line used to label the LNs. Given the diversity of LN physiology and the likely presence of multiple classes of LNs, an important issue is how LNs respond to odor presentations at the population level. Because most of the variability in LN responses occurs across cells and not odors, most odors will elicit the same pattern of activity within LNs across the AL (at least at the resolution of calcium imaging) (Fig. 3G) (E. J. Hong & Wilson, 2015). This fixed pattern simply scales with the strength of the odor, defined as the total number of ORN spikes elicited. An exception to this observation is that some odors which selectively activate a single class of ORNs generally recruit additional intraglomerular LN activity within their targeted glomerulus.

The most common neurotransmitter utilized among LNs is the inhibitory transmitter GABA, although there is diversity in LN transmitter profile as well. GABAergic transmission from LNs hyperpolarizes and inhibits both presynaptic ORN terminals and PN dendrites (Olsen & Wilson, 2008; Root et al., 2008). In the intact olfactory circuit, presynaptic inhibition of ORN terminals is the dominant form of inhibition. Olfactory receptor neurons express both $GABA_A$ and $GABA_B$ receptors which allows them to be both rapidly inhibited by LN activity and to undergo a longer, slower form of inhibition that is likely mediated by GABA-spillover after LNs have stopped responding to olfactory stimulation. Pharmacological manipulations show that both forms of inhibition are important for shaping olfactory responses in the AL. Some LNs in the AL do not release GABA, but instead release other neurotransmitters. One example are the glutamatergic LNs (Glu-LNs) that reside in the ventral cluster of the AL (Das et al., 2011; Liu & Wilson, 2013). These LNs also show diverse anatomical profiles and broad odor responses. They inhibit virtually every class of neuron in the AL and make reciprocal connections with GABAergic LNs. Interestingly, Glu-LNs do not send their processes directly into the glomerulus, but rather seem to release glutamate into the space around the glomeruli. This suggest that that Glu-LNs may inhibit by bulk release and spillover of glutamate rather than forming traditional synapses in the AL. Consistent with a model of spillover, individual Glu-LN to PN synapses are not physiologically observed whereas as stimulation of large populations of Glu-LNs reliably hyperpolarize ePNs.

The AL also possess LNs that utilize acetylcholine as their neurotransmitter. The presence of an excitatory local network in the AL was implied from early experiments showing that PNs can respond to odors to which their cognate ORNs are insensitive (Bhandawat, Olsen, Gouwens, Schlief, & Wilson, 2007; Wilson & Laurent, 2005), or when the ORNs are silenced via mutation (Root, Semmelhack, Wong, Flores, & Wang, 2007; Shang, Claridge-Chang, Sjulson, Pypaert, & Miesenböck, 2007) or have been physically removed (Olsen, Bhandawat, & Wilson, 2007). These excitatory LNs (eLNs) surprisingly do not mediate lateral excitation via acetylcholine, but instead broaden odor responses via electrical coupling with PNs via gap junctions (Yaksi & Wilson, 2010). The eLNs are broadly tuned to odors and highly sensitive to even weak odor stimuli. This is likely mediated by both input from a large array of ORNs and reciprocal connections with PNs. While eLNs use gap junctions to mediate lateral excitation in the AL, they also make acetylcholine-mediated synapses with GABAergic LNs.

Modulation and Plasticity of LNs



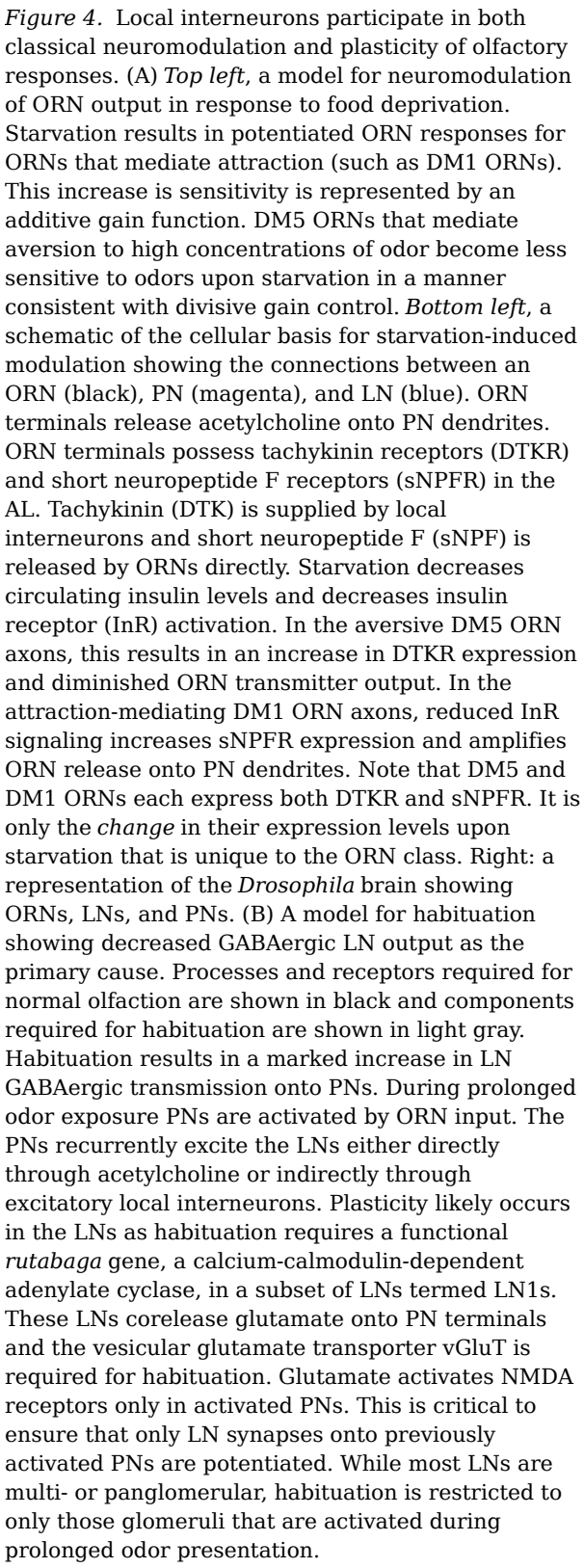


Figure 4. Local interneurons participate in both classical neuromodulation and plasticity of olfactory responses. (A) *Top left*, a model for neuromodulation of ORN output in response to food deprivation. Starvation results in potentiated ORN responses for ORNs that mediate attraction (such as DM1 ORNs). This increase is sensitivity is represented by an additive gain function. DM5 ORNs that mediate aversion to high concentrations of odor become less sensitive to odors upon starvation in a manner consistent with divisive gain control. *Bottom left*, a schematic of the cellular basis for starvation-induced modulation showing the connections between an ORN (black), PN (magenta), and LN (blue). ORN terminals release acetylcholine onto PN dendrites. ORN terminals possess tachykinin receptors (DTKR) and short neuropeptide F receptors (sNPFR) in the AL. Tachykinin (DTK) is supplied by local interneurons and short neuropeptide F (sNPF) is released by ORNs directly. Starvation decreases circulating insulin levels and decreases insulin receptor (InR) activation. In the aversive DM5 ORN axons, this results in an increase in DTKR expression and diminished ORN transmitter output. In the attraction-mediating DM1 ORN axons, reduced InR signaling increases sNPFR expression and amplifies ORN release onto PN dendrites. Note that DM5 and DM1 ORNs each express both DTKR and sNPFR. It is only the *change* in their expression levels upon starvation that is unique to the ORN class. Right: a representation of the *Drosophila* brain showing ORNs, LNs, and PNs. (B) A model for habituation showing decreased GABAergic LN output as the primary cause. Processes and receptors required for normal olfaction are shown in black and components required for habituation are shown in light gray. Habituation results in a marked increase in LN GABAergic transmission onto PNs. During prolonged odor exposure PNs are activated by ORN input. The PNs recurrently excite the LNs either directly through acetylcholine or indirectly through excitatory local interneurons. Plasticity likely occurs in the LNs as habituation requires a functional *rutabaga* gene, a calcium-calmodulin-dependent adenylate cyclase, in a subset of LNs termed LN1s. These LNs corelease glutamate onto PN terminals and the vesicular glutamate transporter vGluT is required for habituation. Glutamate activates NMDA receptors only in activated PNs. This is critical to ensure that only LN synapses onto previously activated PNs are potentiated. While most LNs are multi- or panglomerular, habituation is restricted to only those glomeruli that are activated during prolonged odor presentation.

LNs are also key players in modulating olfactory representations in the AL, as they can synthesize numerous neuropeptides and monoamines, and they possess several receptors for neuromodulators released by centrifugal neurons innervating the AL (Carlsson, Diesner, Schachtner, & Nässel, 2010; Kim, Su, & Wang, 2016; Sizemore & Dacks, 2016). A number of neuropeptides including tachykinin (Carlsson et al., 2010; Ignell et al., 2009; Winther, Siviter, Isaac, Predel, & Nässel, 2003), myoinhibitory peptide, and allostatin A localize to LNs. Tachykinin released by LNs inhibits the terminals of ORNs to depress odor activity (Fig. 4A) (Ignell et al., 2009). This suppression targets glomeruli tuned to aversive odors and is invoked during food deprivation. This form of modulation (along with additional ORN-mediated forms) enables flies to better navigate towards appetitive odors (Ko et al., 2015). Dopamine is also supplied to the AL by a pair of LNs that also likely release GABA (Chou et al., 2010A). However, the specific role of most LN-released peptides has not been thoroughly investigated. In addition to releasing modulating compounds, LNs are themselves the targets of neuromodulation. Modulation of LNs has been best characterized in regards to serotonin (Coates, Majot, Zhang, et al., 2017;

Dacks, Green, Root, Nighorn, & Wang, 2009; A. P. Singh et al., 2013; Sizemore & Dacks, 2016; X. Zhang & Gaudry, 2016). Each of the five *Drosophila* 5-HT receptors localize to at least some LNs. Endogenous serotonin hyperpolarizes both LNs and PNs and ultimately leads to reduced olfactory responses in most glomeruli (Zhang & Gaudry, 2016). In flies, only one serotonergic neuron innervates the AL. This cell is termed the contralaterally-projecting, serotonin-immunoreactive deutocerebral neuron (CSDn) (Dacks, Christensen, & Hildebrand, 2006). Manipulation of the CSDn's activity affects multiple odor-mediated behaviors such as CO_2 avoidance and sensitivity to the *Drosophila* pheromone 11-cis vaccenyl acetate (cVA) (A. P. Singh et al., 2013). However, some glomeruli do not receive input from the CSDn and yet remain sensitive to serotonergic pharmacology (X. Zhang & Gaudry, 2016). This suggests that some LNs or PNs may be able to respond to serotonin released extrasynaptically or potentially in the hemolymph.

In addition to altering odor representations via neuromodulation, LNs can also change odor responses through learning and plasticity (Fig. 4B). Long-term exposure to CO_2 induces an expansion specific to the V glomerulus, which is innervated by ORNs selective for CO_2. It is accompanied with an increased odor response in LN neurites within the V glomerulus, which decreases PN output resulting in behavioral habituation (Sachse et al., 2007). ORN activity remains the same after long-term odor exposure, and so habituation is thought to be mediated by increased LN inhibition of PN dendrites (Das et al., 2011; Larkin et al., 2010; Sadanandappa et al., 2013). This leads to an interesting question: if LNs typically innervate many glomeruli, how is modulation of LN output restricted to only the activated glomerulus? Current models propose that LN activity is enhanced in only in the portions of an LN's neurites that innervate the activated glomeruli (Ramaswami, 2014; Twick, Lee, & Ramaswami, 2014). In this model, ORN activity drives only postsynaptic PNs and activates NMDA receptors only on those PNs' dendrites. Because PNs activate LNs within the glomerulus in a dendrodendritic fashion, it is reasonable to believe that reciprocal PN-LN synapses could be potentiated locally within only that glomerulus. While the LNs will also inhibit other glomeruli (mediating lateral inhibition), the lack of co-PN activation in those glomeruli appears to prevent the strengthening of synaptic connections within those glomeruli. Consistent with this model, habituation fails to be induced (a) if NMDA receptors are knocked down in PNs (Das et al., 2011), (b) if *rutabaga*, a calcium dependent adenylate cyclase gene, is knocked down in LNs (Das et al., 2011), or (c) if PN activity is suppressed during the period of the habituating long-time odor-exposure (Sudhakaran et al., 2012).

Olfactory Coding

Early olfactory coding in the *Drosophila* brain is a result of ORN, LN, and PN properties, harmoniously working to modulate the inputs and outputs of the antennal lobe based on given odors. The main goal of this system is optimization of signal processing: to balance the discrimination of a plethora of odors while also keeping the system as fast as possible.

The result is conflicting components contributing to the olfactory system, leading to multiple, coexisting models of coding.

The simplest proposed model of olfaction is perhaps that of the antennal lobe being a simple relay of signal. That is, the ePNs receive excitatory inputs from ORNs and faithfully transmit to higher order brain regions (Ng et al., 2002; J. W. Wang, Wong, Flores, Vosshall, & Axel, 2003). The accuracy of neuronal activity imaging at the time, paired with the knowledge that ORNs expressing a given receptor converge at the same glomerulus (Gao et al., 2000), supported this faithful transmission model. However, it is now clear for multiple reasons that substantial processing of odor representations occurs both through lateral connections across olfactory channels and through the ORN to ePN synapse itself. First, ePNs are more broadly tuned to odors than their respective ORNs (Wilson et al., 2004). Additionally, ePNs are hyperpolarized and suppressed by GABAergic mechanisms and LNs can be heterogenous in glomerular innervation and odor responses (Wilson & Laurent, 2005). Lastly, ePN activity can still be observed from the corresponding glomerulus when recorded in the empty neuron assay, suggesting excitatory or disinhibiting lateral activity (Olsen & Wilson, 2008; Shang et al., 2007). Since blocking GABA receptors does not affect this ePN activity, this activity was deemed to be from excitatory LNs (Shang et al., 2007). Taken together, this evidence shows that profound odor processing indeed occurs in the antennal lobe and is accomplished through a variety of means.

Labeled Lines and Combinatorial Coding

Some olfactory circuits are more narrowly tuned than others. Often referred to as single-channel or labeled line circuits, these pathways are characterized by ORNs narrowly tuned to just a single odorant molecule along with similarly tuned PNs, although it is worth noting that some PNs may be broadly tuned despite being postsynaptic to narrowly tuned ORNs (Schlief & Wilson, 2007). These lines often represent ethologically relevant odors, such as those involved with mating, oviposition, and food-finding, indicating a possible correlation between efficiency of transmission, extent of processing, and importance of the odor to the fly. For example, one labeled line is that of the gustatory receptors Gr21a and Gr63a, which detect carbon dioxide and whose neurons project only to glomerulus V (Kwon et al., 2007; Suh et al., 2004). Other labeled line odors include geosmin, cVA, methyl laurate, and limonene. Geosmin interacts only with Or56a found on ORN ab4B, which projects to the DA2 glomerulus (Stensmyr et al., 2012). Methyl laurate and cVA are *Drosophila* pheromone signals that activate ORNs also projecting to dedicated glomeruli (VA1v [Dweck et al., 2015] and DA1 [Datta et al., 2008], respectively). Scents involved with preferential oviposition such as limonene and other *Citrus* terpenes excite the DC1 glomerulus and cause attraction (Dweck et al., 2015). Another potential labeled line is the Or83c-expressing ORNs projecting to the DC3 glomerulus, responding exclusively to farnesol, a behaviorally attractive odor for flies produced in ripe *Citrus* fruits (Ronderos, Lin, Potter, & Smith, 2014).

However, if single channels were to be used exclusively for *Drosophila* odor coding, the number of possible odors represented would be a reflection of the 56 glomeruli. An alternative means to represent odors is not through the activation of labeled lines, but rather through the relative activity across many glomeruli. This strategy, called combinatorial coding, allows for the representation of a greater number of odors as well as for more opportunities for transformations to occur through interglomerular interactions. It is clear that most odors are coded in this fashion, as many ORs are responsive to multiple odor molecules and a given odor molecule is likely to activate multiple ORs (Hallem et al., 2004; Hallem & Carlson, 2006). This also allows odors to be encoded across a wide range of intensities by recruiting new ORNs classes when other ORN responses might have saturated. A fly’s discrimination of odors is determined mainly by the distance between ePN activity patterns across the AL (Badel, Ohta, Tsuchimoto, & Kazama, 2016; Parnas, Lin, Huetteroth, & Miesenböck, 2013). Some glomeruli may play a distinct role within the context of combinatorial coding. For example some glomeruli are preferentially activated by odors that are attractive and may represent the positive valence of olfactory signals within the AL while others may signal aversion (Semmelhack & Wang, 2009).

ORN to ePN Synapse

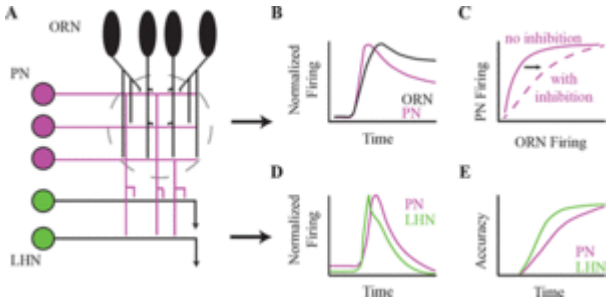


Figure 5. Transformations of odor representations across synapses in the early olfactory system. (A) A schematic representation of the synaptic connections of ORNs (black), PNs (magenta), and LHNs (green). Convergence and divergence occurs at each stage of processing as seen by the all-to-all connectivity from ORNs to PNs and from PNs to LHNs. Gray, dashed circle indicates the connections within the AL. (B) Normalized PN responses peak earlier relative to ORNs. This occurs because PNs rest near their firing threshold and receive strong synaptic input from every ORN innervating the same glomerulus. The strong synapse between ORNs and PNs also renders PNs sensitive to weak odors. (C) Inhibition in the AL prevents saturation of PN responses (solid line without lateral inhibition and dashed line with lateral inhibition) thus allowing them to code odors over a greater range of odor concentrations. Inhibition in the AL is provided primarily by GABAergic LNs. (D) The convergence of all PNs onto all LHNs also contributes to rapid olfactory discrimination. LHNs are most sensitive to the fast rising phase of PN responses. (E) A model of odor detection based on identifying spikes rate above spontaneous activity reveals that PNs and LHNs are equally able to detect odors given enough time. However, LHNs show greater accuracy in early phases of the response (*see* Jeanne & Wilson, 2015 for model). This occurs because PNs spike trains are noisier due to their high spontaneous activity. However, LHNs have low spontaneous activity and only fire when PN activity is high and correlated.

Significant transformations occur at the ORN to ePN synapse to ensure olfactory coding is fast and reliable. This holds true for ORN to ePN synapses used to encode odors represented by either labeled lines or combinatorial coding. The convergence of all ORNs onto all ePNs within a glomerulus (Fig. 5A), combined with a high probability of release at ORN terminals, allows ePNs to be rapidly depolarized above threshold (Bhandawat et al., 2007; Kazama & Wilson, 2008). The peak ePN response to a strong odor actually occurs before the peak ORN response (Bhandawat et al., 2007), essentially anticipating the ORN peak (Fig. 5B). Therefore, convergence represents an aspect of olfactory coding used to accelerate olfactory processing. This is important as *Drosophila* have been shown to respond behaviorally to olfactory stimulation within less than 100 ms (Gaudry et al., 2013). A high convergence ratio also makes ePNs exquisitely sensitive to dilute odor concentrations. Although individual ORNs are noisy (de Bruyne, Clyne, & Carlson, 1999), pooling their input makes ePNs more reliable than ORNs at predicting odors. ePN sensitivity is further enhanced through their correlated activity that arises both from the divergence of ORNs onto each PN and specific properties of the synapse (Kazama & Wilson, 2009). The high probability of release and high number of release sites at the ORN to ePN synapse means that there will be little variation in quantal content across release sites and across ePNs. While ORN-to-PN EPSC amplitudes vary greatly over time, they are strongly correlated with the

interval between spikes in the presynaptic ORN. Fast spiking in an ORN leads to rapid depression across all of its synapses, and longer spike intervals results in larger EPSC amplitudes. Correlated ORN input across all ePNs innervating a glomerulus helps correlate ePN activity to provide a more reliable signal for downstream circuits to decode (Franks, 2015; Jeanne & Wilson, 2015).

Lateral Interactions

Further processing occurs at the glomerulus through lateral input. Application of GABA to the *Drosophila* brain hyperpolarizes PNs and suppresses their odor responses (Wilson & Laurent, 2005). This suggests an inhibitory system in the antennal lobe which could serve as a means of global gain control in the AL, the source of which was found to be LNs (Ng et al., 2002). These inhibitory LNs (iLNs) mediate their action through presynaptic, GABAergic suppression of ORN activity (Olsen & Wilson, 2008). The mechanism of presynaptic inhibition has been supported by computational analysis, which indicated postsynaptic inhibition would not be able to contribute to proper gain control and accuracy (Oizumi, 2012). When direct ORN input is removed, lateral inhibition is eliminated and lateral excitation is observed instead. This demonstrates that glomeruli are connected via both lateral excitation and inhibition and that the locus of lateral inhibition must be presynaptic (Olsen & Wilson, 2008). Inhibition has been observed to be panglomerular in that stimulation of one glomerulus results in LN activity in all other glomeruli (E. J. Hong & Wilson, 2015). This pattern is invariant, with activation of any glomerulus always causing the same LN activity pattern. The strength of this activity tends to correlate with odor concentration (E. J. Hong & Wilson, 2015) and with the overall ORN response (Olsen & Wilson, 2008). It scales linearly with the logarithm of the ORN field potential, which is an indirect measure of the total amount of action potentials elicited across all ORNs (E. J. Hong & Wilson, 2015). Additional intraglomerular inhibition can be observed in cases of some private odors such as in DA2 activation from geosmin, VL2a activation by phenylacetaldehyde, and V activation from carbon dioxide (E. J. Hong & Wilson, 2015).

Although LN activity is panglomerular and invariantly patterned, the sensitivity of PNs to inhibition is variable (Olsen, Bhandawat, & Wilson, 2010). There is large but consistent variation in glomeruli sensitivity to GABA pharmacology and GABAergic LN activity, as defined by changes in PN output (E. J. Hong & Wilson, 2015). Furthermore, it is not likely that differential glomerular innervation or release by LNs contributes to the variable sensitivity (E. J. Hong & Wilson, 2015). This leads to each glomerulus having a characteristic level of sensitivity to lateral inhibition that is independent of odor tuning, and therefore being target- but not stimulus-specific. Interestingly, single odors will often activate multiple glomeruli, some representing high and others low sensitivity to inhibition (E. J. Hong & Wilson, 2015). This suggests a method of odor coding in which an odor may be identified by the pattern of activation of low-sensitivity glomeruli, and the concentration by glomeruli with high GABA sensitivity. Therefore, representations of odors in this model are independent of concentration while simultaneously allowing for a vast range of odor strengths. Taken together, it is apparent that lateral inhibition in the

antennal lobe serves as a means of gain control, preventing PN saturation when ORN activity is high (Fig. 5C).

eLNs also contribute to shaping olfactory responses in the AL. eLNs and ePNs can excite each other in a reciprocal fashion, although the eLN to ePN coupling is generally stronger (Huang, Zhang, Qiao, Hu, & Wang, 2010). Since lateral inhibition exists, it is possible that lateral excitation could stem from disinhibition as opposed to actual excitation. However, blocking GABA receptors in the antennal lobe has no effect on the lateral excitation (Shang et al., 2007), suggesting the activity is not mediated through disinhibition. Interestingly, the activity of eLNs could cause an indirect inhibition by exciting target PNs and thus activating associated iLNs (Yaksi & Wilson, 2010). The reciprocal excitation along with indirect inhibition could contribute to the simultaneous activation and silencing of multiple glomeruli during the presentation of a single odor. This leads to a possible mechanism for which strengths and identities of odors are coded in the early olfactory system. The functional consequence of these connections is that eLNs may also help boost PN responses to weak stimuli by distributing activity from the most active PNs to connected glomeruli. During strong odor activation, eLNs should help prevent PN responses from saturating by both distributing their activity and recruiting the GABAergic LN network through acetylcholine-mediated synaptic transmission.

Third Order Olfactory Processing

Olfactory information that has been processed by ePNs in the AL is next sent to third order neurons in the mushroom body and lateral horn of the protocerebrum. Generally, these structures are viewed as serving different functions in odor representation. Early experiments ablating the mushroom body late in development with hydroxy urea revealed that only the ability to undergo olfactory learning is compromised while innate olfactory behaviors remain intact (de Belle & Heisenberg, 1994). This general model is supported by behavioral, anatomical, and computational evidence. However, it is worth noting that connections between the mushroom bodies and lateral horn suggest there may be mechanisms allowing learned and innate olfactory representations to influence one another (Aso, Hattori, et al., 2014A; Schultzhaus, Saleem, Iftikhar, & Carney, 2017).

Mushroom Body

The mushroom body is anatomically divided into neuropil called the calyx and the lobes. ePN axons synapse in the calyx onto Kenyon cells (KC), which are the primary neurons of the MB. There are approximately 2300 Kenyon cells in the MB and approximately six ePNs make a connection onto one KC (Aso et al., 2014A, 2014B). These connections are sparse and KCs fire generally only a few action potentials in response to any given odor. The connectivity between a given ePN and a KC is thought to be random (Caron, Ruta, Abbott, & Axel, 2013; Murthy, Fiete, & Laurent, 2008), and thus Kenyon cells are not viewed as being stereotyped across individual flies in the same manner as an ePN. Kenyon cell axons collect and project from the calyx to the lobes via the peduncle. The

MB is divided into three lobes named according to the projection patterns of the three classes of KC that innervate the lobes. These are called the γ, α′/β′, and α/β lobes, and the KC that form these lobes share the same names. Here, KCs make synapses onto the 21 individual mushroom body output neurons (MBONs). The lobes of the MB can be divided into 15 functional and anatomical compartments where the MBONs send their dendrites. Each MBON will send its dendrites generally into one and rarely two of these compartments. In addition to the KC to MBON synapses, these compartments also contain the processes of 20 dopaminergic neurons (DANs) that target DA1-receptors on presynaptic KC terminals. Each DAN also only innervates one or at most two compartments. Thus each compartment is defined by a specific set of MBONs and DANs. Individual experience can then shape KC to MBON synaptic strengths through dopaminergic modulation mediated by DANs. In addition to contributing to long-term memory formation associated with odors, it is also likely that DAN activity can also modulate state-dependent representations of external stimuli (Aso, Sitaraman, et al., 2014B; Cohn, Morantte, & Ruta, 2015; Lewis et al., 2015; S. Lin et al., 2014; Shih et al., 2015).

Lateral Horn

The organization of the LH differs substantially from that of the MB. Unlike the seemingly random connections from ePNs onto KCs, LH neurons (LHNs) appear to be stereotyped and receive synaptic input that is consistent across individual flies (Datta et al., 2008; Fisek, 2014; Jefferis et al., 2007; Ruta et al., 2010; Tanaka, Awasaki, Shimada, & Ito, 2004). This difference in organization is consistent with the view of the LH as mediating innate behaviors versus the MB which mediates learned olfactory behaviors (de Belle & Heisenberg, 1994). While compartmentalization in the LH is not as discrete compared to the MB, regions exist where the axons of ePNs arising from precise glomeruli terminate into five broad zones within the LH (Jefferis et al., 2007). These zones are consistent across individual flies and encode specific olfactory signals. Glomeruli that process fruit odorants project to the posterior LH while pheromone processing ePNs send their axons to the anterior ventral LH (Jefferis et al., 2007). Ammonia, which is a byproduct of the fermentation process and thus attractive to flies, is processed with fruity odors in a region distinct from aversive odorants such as CO_2 and acids (Min, Ai, Shin, & Suh, 2013). This clustering of ePN axons in the LH is likely related to the integration of odorants with similar ethological relevance rather than merely chemical similarity. Indeed, the terminal arbor locations of ePNs appear related to the valence of the odors that drive those ePNs. There is one notable exception to the separation of food-derived odors and pheromone processing in the LH. PN axons sensitive to the plant and fruit-derived odorants phenylacetaldehyde and phenylacetic acid terminate within the pheromone processing region of the lateral horn (Grosjean et al., 2011). This sensory integration may serve to identify ideal mating sites that would also provide food for the resulting larvae. Interestingly, the olfactory receptor neurons that detect these specific fruit-derived odors also express the sexually dimorphic transcription factor gene, *fru*, making them unique among ORN classes detecting fruit derived odors.

The LH is also unique in its processing of odors as it is the first olfactory neuropil that shows sexually dimorphic responses to odors (Kohl, Ostrovsky, Frechter, & Jefferis, 2013; Ruta et al., 2010). While it has been documented that the size of pheromone-processing glomeruli differs between the sexes (Kondoh, Kaneshiro, Kimura, & Yamamoto, 2003; Stockinger, Kvitsiani, Rotkopf, Tirián, & Dickson, 2005), physiological responses to the pheromone cVA are identical in ORNs (Kurtovic, Widmer, & Dickson, 2007; Liu et al., 2011) and ePNs (Datta et al., 2008) in *Drosophila*. This is consistent with the fact that both male and female flies are sensitive to cVA and use it as an aggregation cue (Bartelt, Schaner, & Jackson, 1985). However, males and females also have unique behavioral responses to cVA as well (Kurtovic et al., 2007; L. Wang & Anderson, 2010; Yamamoto & Koganezawa, 2013). In the LH, ePNs from the DA1 glomerulus make synapses onto gender specific *fru*-positive third order neurons (Kohl et al., 2013). This organization allows both genders to be equally sensitive to the pheromone, and yet allows each gender to utilize this information to generate sex-specific behavior.

In addition to ePNs, iPNs also send axons to the LH and similarly display discretization of their terminals into zones, in part according their function. A recent study has shown that iPN terminals form at least two distinct regions in the LH; a posterior medial region (LH-PM) that encodes attractive odors in a concentration *independent* manner, and an anterior medial (LH-AM) region that encodes similar odors in a concentration *dependent* manner (Strutz et al., 2014). These axons arise from two distinct classes of iPNs defined not only by their target region in the LH, but also from the AL glomeruli innervated by their dendrites (Strutz et al., 2014). Typically, an AL glomerulus will be innervated by either class of iPN, but seldom both. iPNs play a critical role in the processing of attractive odors and RNAi-mediated knockdown of GABA synthesis in these cells reduces attraction in T maze assays (Strutz et al., 2014).

Only a few classes of intrinsic LH neurons have been described to date. One class has its cell bodies situated in a region distal to the LH, called the ventral lateral protocerebrum (vlPr) (Liang et al., 2013; Tanaka et al., 2004). These GABA-negative neurons send their dendrites to the LH and relay information into the vlPr. They receive inputs from ePNs and iPNs and respond to a variety of odors including pheromones, attractive odors, and aversive odors (Liang et al., 2013; Strutz et al., 2014). Ablation studies (transection of axon tracts) revealed that vlPr neuron responses to attractive odors are strongly inhibited by iPNs while pheromone-mediated responses are spared (Liang et al., 2013). These data suggest iPNs might regulate the gating of sensory information through the vlPr neurons. Alternatively, this inhibition may not simply suppress all vlPr neuron output, but instead might be critical for shaping vlPr responses temporally or ameliorating coding in another fashion. Indeed, suppressing iPN output hinders flies' abilities to discriminate between various nonpheromone odors (Parnas et al., 2013). vlPr neurons also project to a lateral region in the LH, (the LH-AL) where they encode aversive odors such as benzaldehyde. Interestingly, this region does not receive iPN input and thus RNAi-mediated GABA knockdown in iPNs does not affect aversion to odorants (Strutz et al., 2014).

Two additional classes of LH neurons have been described physiologically and anatomically. These are referred as Type 1 and Type 2 LH neurons (Fisek & Wilson, 2013). Type 1 neurons have somata located dorsal medial to the main LH neuropil and they send their dendrites into superior medial protocerebrum whereas Type 2 LH neurons have cell bodies located ventral lateral to the LH and send their dendrites into superior lateral protocerebrum. The two classes of neurons also display different odor tuning and response profiles. Type 1 neurons are broadly tuned to odors whereas Type 2 are narrowly tuned. A physiological screen suggests that Type 1 LH neurons integrate input from about 3 or 4 ePNs. Interestingly, Type 1 LH neurons can be excited and fire action potentials with the activation of even a single ePN. While these cells integrate input from multiple glomeruli, their responses do not saturate as quickly. Thus Type 1 LH neurons have an extended dynamic range compared to their individual PN inputs and can encode odors over a greater concentration range. As Type 2 neurons are selective for very few odors, it is not surprising that they receive input from few if not only one glomerulus. In paired recordings between a Type 2 LH neuron and its presynaptic PN, it was found that any odor that elicited activity in the LH neuron also drove the PN. This suggests that only one glomerulus connects to that particular LH neuron. However, some odors caused firing in the PN with little to no response in the LH neuron due to the recruitment of inhibition from other olfactory channels. This inhibition arises predominantly from local GABAergic neurons within the LH. Type 2 LH neurons that respond selectively to cVA show distinct adaptations to extract the most information from PN spike trains. While PNs have long integration windows allowing them to summate ORN spikes with great sensitivity, LH neurons have much shorter integration times thus reducing their sensitivity to noise while allowing them to function as coincidence detectors of presynaptic ePN firing (Figure 5D-E) (Jeanne & Wilson, 2015).

The level of interaction between ePNs and iPNs in the LH remains unclear. Synaptic boutons of ePNs and iPNs do not overlap in the LH (Y. C. Wang et al., 2013), suggesting that the ePN and iPN pathways from the AL to the LH may represent independent, parallel channels of processing. However, physical overlap of axons and dendrite may not be a good indicator of cellular interactions as extrasynaptic inhibition has been postulated in the AL with regards to both GABA and Glutamate (Liu & Wilson, 2013; Wilson & Laurent, 2005). Imaging studies have shown mixed results. Laser ablation of the iPN axons to the LH failed to disinhibit ePN axons when assessed via calcium imaging (Liang et al., 2013). However, thermogenetic activation of iPNs suppresses synaptophlourin signals in ePN terminals in the LH (Parnas et al., 2013). This implies that iPNs mediate gain control of LH input at the level of ePN axons. There is also some evidence that ePNs and iPNs may converge on the same LH neurons. Ablating ePN axons projecting to the LH eliminates odor-evoked excitation of the LH neuron and instead reveals slight inhibition. This inhibition is thought to be in part mediated by iPNs. A fine scale analysis of connectivity between neurons in the LH, including their electrophysiological and synaptic properties, still needs to be conducted before it is fully determined how much overlap exists between these two processing channels.

Conclusion

The use of *Drosophila* has elucidated a range of central issues in olfaction including the connectivity and function of different olfactory cell classes, the transformations of odor representations across brain regions, and the regulatory effects that allow proper development of a highly organized circuit. Specifically, interesting aspects of olfaction that resulted in part from *Drosophila* research include: the clear roles of inhibition and inhibitory networks, molecular systems for matching ORNs with their targets, and properties of individual cell classes previously thought to be the same. Future technologies, such as electron-microscopic reconstruction of the AL connectome, intersection strategies for targeting individual cell types, and real-time reporters of intracellular signaling cascades will further advance our understanding of *Drosophila* olfaction as well as olfaction in other species.

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References

Abuin, L., Bargeton, B., Ulbrich, M. H., Isacoff, E. Y., Kellenberger, S., & Benton, R. (2011). **Functional architecture of olfactory ionotropic glutamate receptors**. *Neuron*, *69*(1), 44–60.

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science*, *287*(5461), 2185–2195.

Alcorta, E. (1991). Characterization of the electroantennogram in *Drosophila melanogaster* and its use for identifying olfactory capture and transduction mutants. *Journal of Neurophysiology*, *65*(3), 702–714. Retrieved from **www.ncbi.nlm.nih.gov/pubmed/1904913**.

Alcorta, E., & Rubio, J. (1989). **Intrapopulational variation of olfactory responses in *Drosophila melanogaster***. *Behavior Genetics*, *19*(2), 285–299.

Aso, Y., Hattori, D., Yu, Y., Johnston, R. M., Iyer, N. A., Ngo, T.-T., et al. (2014a). **The neuronal architecture of the mushroom body provides a logic for associative learning**. *eLife*, *3*, e10806–e10847.

Aso, Y., Sitaraman, D., Ichinose, T., Kaun, K. R., Vogt, K., Belliart-Guérin, G., et al. (2014b). **Mushroom body output neurons encode valence and guide memory-based action selection in *Drosophila***. *eLife*, *3*, e04580.

Averhoff, W. W., & Richardson, R. H. (1974). Pheromonal control of mating patterns in *Drosophila melanogaster*. *Behavior Genetics*, *4*(3), 207–225.

Ayyub, C., Paranjape, J., Rodrigues, V., Siddiqi, O., & Siddiqi, O. (1990). **Genetics of olfactory behavior in *Drosophila melanogaster***. *Journal of Neurogenetics*, *6*(4), 243–262.

Badel, L., Ohta, K., Tsuchimoto, Y., & Kazama, H. (2016). **Decoding of context-dependent olfactory behavior in *Drosophila***. *Neuron*, *91*(1), 155–167.

Bargmann, C. I. (2006). **Comparative chemosensation from receptors to ecology**. *Nature*, *444*(7117), 295–301.

Bartelt, R. J., Schaner, A. M., & Jackson, L. L. (1985). ***cis*-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster***. *Journal of Chemical Ecology*, *11*(12), 1747–1756.

Becher, P. G., Larsson, M. C., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., et al. (2012). **Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition, and development**. *Functional Ecology*, *26*(4), 822–828.

Benton, R., Sachse, S., Michnick, S. W., & Vosshall, L. B. (2006). **Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo**. *PLoS Biology*, *4*(2), e20–18. .

Benton, R., Vannice, K. S., Gomez-Diaz, C., & Vosshall, L. B. (2009). **Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila***. *Cell*, *136*(1), 149–162.

Benzer, S., & Konopka, R. J. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, *68*(9), 2112–2116.

Bhandawat, V., Olsen, S. R., Gouwens, N. W., Schlief, M. L., & Wilson, R. I. (2007). **Sensory processing in the *Drosophila* antennal lobe increases reliability and separability of ensemble odor representations**. *Nature Neuroscience*, *10*(11), 1474–1482.

Borst, A. (1984). **Identification of different chemoreceptors by electroantennogram-recording**. *Journal of Insect Physiology*, *30*(6), 507–510.

Bozza, T., Feinstein, P., Zheng, C., & Mombaerts, P. (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *Journal of Neuroscience*, *22*(8), 3033–3034.

Brand, A. H., & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes, *118*, 401–415. Retrieved from **http://dev.biologists.org/content/develop/118/2/401.full.pdf**.

Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell*, *65*(1), 175–187.

Carlsson, M. A., Diesner, M., Schachtner, J., & Nässel, D. R. (2010). **Multiple neuropeptides in the *Drosophila* antennal lobe suggest complex modulatory circuits**. *Journal of Comparative Neurology*, *518*(16), 3359–3380.

Caron, S. J. C., Ruta, V., Abbott, L. F., & Axel, R. (2013). **Random convergence of olfactory inputs in the Drosophila mushroom body**. *Nature*, *497*(7447), 113–117.

Chou, Y.-H., Spletter, M. L., Yaksi, E., Leong, J. C. S., Wilson, R. I., & Luo, L. (2010a). **Diversity and wiring variability of olfactory local interneurons in the *Drosophila* antennal lobe**. *Nature Publishing Group*, *13*(4), 439–449.

Chou, Y.-H., Zheng, X., Beachy, P. A., & Luo, L. (2010b). **Patterning axon targeting of olfactory receptor neurons by coupled hedgehog signaling at two distinct steps**. *Cell*, *142*(6), 954–966.

Clyne, P., Grant, A., O'Connell, R., & Carlson, J. R. (1997). Odorant response of individual sensilla on the *Drosophila* antenna. *Invertebrate Neuroscience*, *3*(2–3), 127–135.

Clyne, P. J., Warr, C. G., Freeman, M. R., Lessing, D., Kim, J., & Carlson, J. R. (1999). A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron*, *22*(2), 327–338.

Coates K. E., Majot, A.T., Zhang, X., et al. (2017). **Identified serotonergic modulatory neurons have heterogeneous synaptic connectivity within the olfactory system of *Drosophila***. *Journal of Neuroscience*, *37*(31), 7318–7331.

Cohn, R., Morantte, I., & Ruta, V. (2015). **Coordinated and compartmentalized neuromodulation shapes sensory processing in *Drosophila***. *Cell*, *163*(7), 1742–1755.

Couto, A., Alenius, M., & Dickson, B. J. (2005). **Molecular, anatomical, and functional organization of the *Drosophila* olfactory system**. *Current Biology*, *15*(17), 1535–1547.

Croset, V., Rytz, R., Cummins, S. F., Budd, A., Brawand, D., Kaessmann, H., et al. (2010). **Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction**. *PLoS Genetics*, *6*(8), e1001064.

Dacks, A., Christensen, T., & Hildebrand, J. (2006). **Phylogeny of a serotonin-immunoreactive neuron in the primary olfactory center of the insect brain**. *The Journal of Comparative Neurology*, *498*(6), 727–746.

Dacks, A., Green, D., Root, C., Nighorn, A., & Wang, J. (2009). **Serotonin modulates olfactory processing in the antennal lobe of *Drosophila***. *Journal of Neurogenetics*, *23*(4), 1–13.

Das, A., Chiang, A., Davla, S., Priya, R., Reichert, H., VijayRaghavan, K., et al. (2011). **Identification and analysis of a glutamatergic local interneuron lineage in the adult *Drosophila* olfactory system**. *Neural Systems & Circuits*, *1*(1), 4.

Datta, S. R., Vasconcelos, M. L., Ruta, V., Luo, S., Wong, A., Demir, E., et al. (2008). **The *Drosophila pheromone* cVA activates a sexually dimorphic neural circuit**. *Nature*, *452*(7186), 473–477.

de Belle, J. S., & Heisenberg, M. (1994). Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science*, *263*(5147), 692–695.

de Bruyne, M., Clyne, P. J., & Carlson, J. R. (1999). Odor coding in a model olfactory organ: The *Drosophila* maxillary palp. *Journal of Neuroscience*, *19*(11), 4520–4532.

Dobritsa, A. A., Warr, C. G., Van der Goes van Naters, W., Steinbrecht, R. A., & Carlson, J. R. (2003). Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron*, *37*(5), 827–841.

Dweck, H. K. M., Ebrahim, S. A. M., Thoma, M., Mohamed, A. A. M., Keesey, I. W., Trona, F., et al. (2015). **Pheromones mediating copulation and attraction in *Drosophila***. *Proceedings of the National Academy of Sciences*, *112*(21), E2829–E2835.

Fisek, M. (2014). *Connectivity and computations in higher-order olfactory neurons in Drosophila* (Doctoral dissertation). Retrieved from **https://dash.harvard.edu/bitstream/handle/1/12274607/Fisek_gsas.harvard_0084L_11534.pdf;sequence=1**.

Fisek, M., & Wilson, R. I. (2013). **Stereotyped connectivity and computations in higher-order olfactory neurons**. *Nature Neuroscience*, *17*(2), 280–288.

Fishilevich, E., & Vosshall, L. B. (2005). **Genetic and functional subdivision of the *Drosophila* antennal lobe**. *Current Biology*, *15*(17), 1548–1553.

Franks, K. M. (2015). **I want it all and I want it now: How a neural circuit encodes odor with speed and accuracy**. *Neuron*, *88*(5), 852–854.

Fuyama, Y. (1976). Behavior genetics of olfactory responses in *Drosophila*. I. Olfactometry and strain differences in *Drosophila melanogaster*. *Behavioral Genetics*, *6*(4), 407–420.

Gao, Q., Yuan, B., & Chess, A. (2000). Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nature Neuroscience*, *3*(8), 780–785.

Gaudry, Q., Hong, E. J., Kain, J., de Bivort, B. L., & Wilson, R. I. (2013). **Asymmetric neurotransmitter release enables rapid odour lateralization in *Drosophila***. *Nature*, *493*(7432), 424–428.

Grabe, V., Baschwitz, A., Dweck, H. K. M., Lavista-Llanos, S., Hansson, B. S., & Sachse, S. (2016). **Elucidating the neuronal architecture of olfactory glomeruli in the *Drosophila* antennal lobe**. *Cell Reports*, *16*(12), 3401–3413.

Grosjean, Y., Rytz, R., Farine, J.-P., Abuin, L., Cortot, J., Benton, R., et al. (2011). **An olfactory receptor for food-derived odours promotes male courtship in *Drosophila***. *Nature*, *478*(7368), 236–240.

Hallem, E. A., & Carlson, J. R. (2006). **Coding of odors by a receptor repertoire**. *Cell*, *125*(1), 143–160.

Hallem, E. A., Ho, M. G., & Carlson, J. R. (2004). **The molecular basis of odor coding in the *Drosophila* antenna**. *Cell*, *117*(7), 965–979.

Heisenberg, M., Borst, A., Wagner, S., & Byers, D. (1985). ***Drosophila* mushroom body mutants are deficient in olfactory learning**. *Journal of Neurogenetics*, *2*(1), 1–30.

Hong, E. J., & Wilson, R. I. (2015). **Simultaneous encoding of odors by channels with diverse sensitivity to inhibition**. *Neuron*, *85*(3), 573–589.

Hong, W., Mosca, T. J., & Luo, L. (2012). **Teneurins instruct synaptic partner matching in an olfactory map**. *Nature*, *484*(7393), 201–207.

Huang, J., Zhang, W., Qiao, W., Hu, A., & Wang, Z. (2010). **Functional connectivity and selective odor responses of excitatory local interneurons in *Drosophila* antennal lobe**. *Neuron*, *67*(6), 1021–1033.

Hummel, T., Zipursky, S. L., Vasconcelos, M. L., Clemens, J. C., Fishilevich, Y., & Vosshall, L. B. (2003). **Axonal targeting of olfactory receptor neurons in *Drosophila* is controlled by Dscam**. *Neuron*, *37*(2), 221–231.

Ignell, R., Root, C. M., Birse, R. T., Wang, J. W., Nassel, D. R., & Winther, A. M. E. (2009). **Presynaptic peptidergic modulation of olfactory receptor neurons in Drosophila**. *Proceedings of the National Academy of Sciences*, *106*(31), 13070–13075.

Jeanne, J. M., & Wilson, R. I. (2015). **Convergence, divergence, and reconvergence in a feedforward network improves neural speed and accuracy**. *Neuron*, *88*(5), 1014–1026.

Jefferis, G. S. X. E. (2004). **Developmental origin of wiring specificity in the olfactory system of *Drosophila***. *Development*, *131*(1), 117–130.

Jefferis, G. S., Marin, E. C., Stocker, R. F., & Luo, L. (2001). **Target neuron prespecification in the olfactory map of *Drosophila***. *Nature*, *414*(6860), 204–208.

Jefferis, G. S. X. E., Potter, C. J., Chan, A. M., Marin, E. C., Rohlfing, T., Maurer, C. R., Jr., et al. (2007). **Comprehensive maps of *Drosophila* higher olfactory centers: Spatially segregated fruit and pheromone representation**. *Cell*, *128*(6), 1187–1203.

Joo, W. J., Sweeney, L. B., Liang, L., & Luo, L. (2013). **Linking cell fate, trajectory choice, and target selection: Genetic analysis of Sema-2b in olfactory axon targeting**. *Neuron*, *78*(4), 673–686.

Kaupp, U. B. (2010). **Olfactory signalling in vertebrates and insects: Differences and commonalities**. *Nature Reviews Neuroscience*, *11*, 1–13.

Kazama, H., & Wilson, R. I. (2008). **Homeostatic matching and nonlinear amplification at identified central synapses**. *Neuron*, *58*(3), 401–413.

Kazama, H., & Wilson, R. I. (2009). **Origins of correlated activity in an olfactory circuit**. *Nature Neuroscience*, *12*(9), 1136–1144.

Kikuchi, T. (1973). Genetic alteration of olfactory functions in *Drosophila melanogaster*. *Japanese Journal of Genetics*, *48*(2), 105–118.

Kim, S. M., Su, C.-Y., & Wang, J. W. (2016). **Neuromodulation of innate behaviors in *Drosophila***. *Annual Review of Neuroscience*, *40*(1), 327–348.

Ko, K. I., Root, C. M., Lindsay, S. A., Zaninovich, O. A., Shepherd, A. K., Wasserman, S. A., et al. (2015). **Starvation promotes concerted modulation of appetitive olfactory behavior via parallel neuromodulatory circuits**. *eLife*, *4*, 221.

Kohl, J., Ostrovsky, A. D., Frechter, S., & Jefferis, G. S. X. E. (2013). **A bidirectional circuit switch reroutes pheromone signals in male and female brains**. *Cell*, *155*(7), 1610–1623.

Kondoh, Y., Kaneshiro, K. Y., Kimura, K.-I., & Yamamoto, D. (2003). **Evolution of sexual dimorphism in the olfactory brain of Hawaiian *Drosophila***. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1519), 1005–1013.

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.

Kwon, J. Y., Dahanukar, A., Weiss, L. A., & Carlson, J. R. (2007). **The molecular basis of CO2 reception in *Drosophila***. *Proceedings of the National Academy of Sciences*, *104*(9), 3574–3578.

Larkin, A., Karak, S., Priya, R., Das, A., Ayyub, C., Ito, K., et al. (2010). **Central synaptic mechanisms underlie short-term olfactory habituation in *Drosophila* larvae**. *Learning & Memory*, *17*(12), 645–653.

Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H., & Vosshall, L. B. (2004). **Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction**. *Neuron*, *43*(5), 703–714.

Lewis, L. P. C., Siju, K. P., Aso, Y., Friedrich, A. B., Bulteel, A. J. B., Rubin, G. M., et al. (2015). **A higher brain circuit for immediate integration of conflicting sensory information in *Drosophila***. *Current Biology*, *25*(17), 2203–2214.

Liang, L., Li, Y., Potter, C. J., Yizhar, O., Deisseroth, K., Tsien, R. W., et al. (2013). **GABAergic projection neurons route selective olfactory inputs to specific higher-order neurons**. *Neuron*, *79*(5), 917–931.

Lin, H. H., Chu, L. A., Fu, T. F., Dickson, B. J., & Chiang, A. S. (2013). **Parallel neural pathways mediate CO2 avoidance responses in *Drosophila***. *Science*, *340*(6138), 1338–1341.

Lin, S., Owald, D., Chandra, V., Talbot, C., Huetteroth, W., & Waddell, S. (2014). **Neural correlates of water reward in thirsty *Drosophila***. *Nature Neuroscience*, *17*(11), 1536–1542.

Liu, W. W., & Wilson, R. I. (2013). **Glutamate is an inhibitory neurotransmitter in the *Drosophila* olfactory system**. *Proceedings of the National Academy of Sciences*, *110*(25), 10294–10299.

Liu, W., Liang, X., Gong, J., Yang, Z., Zhang, Y.-H., Zhang, J.-X., et al. (2011). **Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila***. *Nature Neuroscience*, *14*(7), 896–902.

Lundin, C., Käll, L., Kreher, S. A., Kapp, K., Sonnhammer, E. L., Carlson, J. R., et al. (2007). **Membrane topology of the DrosophilaOR83b odorant receptor**. *FEBS Letters*, *581*(29), 5601–5604.

Min, S., Ai, M., Shin, S. A., & Suh, G. S. B. (2013). **Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in Drosophila**. *Proceedings of the National Academy of Sciences*, *110*(14), E1321–E1329.

Mosca, T. J., & Luo, L. (2014). **Synaptic organization of the *Drosophila* antennal lobe and its regulation by the Teneurins**. *eLife*, *3*, 6264–6269.

Mosca, T. J., Hong, W., Dani, V. S., Favaloro, V., & Luo, L. (2012). **Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice**. *Nature*, *484*(7393), 237–241.

Murthy M, Fiete, I., & Laurent G. (2008). **Testing odor response stereotypy in the *Drosophila* mushroom body**. *Neuron*, *59*(6), 1009–1023.

Nagel, K. I., & Wilson, R. I. (2016). **Mechanisms underlying population response dynamics in inhibitory interneurons of the *Drosophila* antennal lobe**. *Journal of Neuroscience*, *36*(15), 4325–4338.

Ng, M., Roorda, R. D., Lima, S. Q., Zemelman, B. V., Morcillo, P., & Miesenböck, G. (2002). **Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly**. *Neuron*, *36*(3), 463–474.

Oizumi, M., Satoh, R., Kazama, H., & Okada, M. (2012). **Functional differences between global pre- and postsynaptic inhibition in the *Drosophila* olfactory circuit**, 1–10.

Okumura, M., Kato, T., Miura, M., & Chihara, T. (2015). **Hierarchical axon targeting of *Drosophila* olfactory receptor neurons specified by the proneural transcription factors Atonal and Amos**. *Genes to Cells*, *21*(1), 53–64.

Olsen, S., Bhandawat, V., & Wilson, R. I. (2008). Excitatory interactions between olfactory processing channels in the *Drosophila* antennal lobe. *Neuron*, *54*(1), 89–103.

Olsen, S. R., Bhandawat, V., & Wilson, R. I. (2010). **Divisive normalization in olfactory population codes**. *Neuron*, *66*(2), 287–299.

Olsen, S. R., & Wilson, R. I. (2008). **Cracking neural circuits in a tiny brain: New approaches for understanding the neural circuitry of *Drosophila***. *Trends in Neurosciences*, *31*(10), 512–520.

Parnas, M., Lin, A. C., Huetteroth, W., & Miesenböck, G. (2013). **Odor discrimination in *Drosophila*: From neural population codes to behavior**. *Neuron*, *79*(5), 932–944.

Quinn, W. G., Benzer, S., & Harris, W. A. (1974). Conditioned behavior in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA*, *71*(3), 708–712.

Ramaswami, M. (2014). **Network plasticity in adaptive filtering and behavioral habituation**. *Neuron*, *82*(6), 1216–1229.

Rodrigues, V., & Buchner, E. (1984). [3H]2-deoxyglucose mapping of odor-induced neuronal activity in the antennal lobes of *Drosophila melanogaster*. *Brain Research*, *324*(2), 374–378.

Ronderos, D. S., Lin, C. C., Potter, C. J., & Smith, D. P. (2014). **Farnesol-detecting olfactory neurons in *Drosophila***. *Journal of Neuroscience*, *34*(11), 3959–3968.

Root, C. M., Ko, K. I., Jafari, A., & Wang, J. W. (2011). **Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search**. *Cell*, *145*(1), 133–144.

Root, C. M., Masuyama, K., Green, D. S., et al. (2008). **A presynaptic gain control mechanism fine-tunes olfactory behavior**. *Neuron*, *59*(2), 311–321.

Root, C. M., Semmelhack, J. L., Wong, A. M., Flores, J., & Wang, J. W. (2007). **Propagation of olfactory information in Drosophila**. *Proceedings of the National Academy of Sciences*, *104*(28), 11826–11831.

Ruta, V., Datta, S. R., Vasconcelos, M. L., Freeland, J., Looger, L. L., & Axel, R. (2010). **A dimorphic pheromone circuit in *Drosophila* from sensory input to descending output**. *Nature*, *468*(7324), 686–690.

Rybak, J., Talarico, G., Ruiz, S., Arnold, C., Cantera, R., & Hansson, B. S. (2016). **Synaptic circuitry of identified neurons in the antennal lobe of *Drosophila melanogaster***. *Journal of Comparative Neurology*, *524*(9), 1920–1956.

Sachse, S., Rueckert, E., Keller, A., Okada, R., Tanaka, N. K., Ito, K., et al. (2007). **Activity-dependent plasticity in an olfactory circuit**. *Neuron*, *56*(5), 838–850.

Sadanandappa, M. K., Redondo, B. B., Michels, B., Rodrigues, V., Gerber, B., VijayRaghavan, K., et al. (2013). **Synapsin function in GABA-ergic interneurons is required for short-term olfactory habituation**. *Journal of Neuroscience*, *33*(42), 16576–16585.

Schlief, M. L., & Wilson, R. I. (2007). **Olfactory processing and behavior downstream from highly selective receptor neurons**. *Nature Neuroscience*, *10*(5), 623–630.

Schultzhaus, J. N., Saleem, S., Iftikhar, H., & Carney, G. E. (2017). **The role of the *Drosophila* lateral horn in olfactory information processing and behavioral response**. *Journal of Insect Physiology*, *98*(C), 29–37.

Seki, Y., Rybak, J., Wicher, D., Sachse, S., & Hansson, B. S. (2010). **Physiological and morphological characterization of local interneurons in the *Drosophila* antennal lobe**. *Journal of Neurophysiology*, *104*(2), 1007–1019.

Sekine, S. U., Haraguchi, S., Chao, K., Kato, T., Luo, L., Miura, M., et al. (2013). **Meigo governs dendrite targeting specificity by modulating Ephrin level and N-glycosylation**. *Nature Neuroscience*, *16*(6), 683–691.

Semmelhack, J. L., & Wang, J. W. (2009). **Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion**. *Nature*, *459*(7244), 218–223.

Shanbhag, S. R., Müller, B., & Steinbrecht, R. A. (2000). **Atlas of olfactory organs of *Drosophila melanogaster***. *Arthropod Structure and Development*, *29*(3), 211–229.

Shang, Y., Claridge-Chang, A., Sjulson, L., Pypaert, M., & Miesenböck, G. (2007). **Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe**. *Cell*, *128*(3), 601–612.

Shen, H.-C., Wei, J.-Y., Chu, S.-Y., Chung, P.-C., Hsu, T.-C., & Yu, H.-H. (2016). **Morphogenetic studies of the *Drosophila* DA1 ventral olfactory projection neuron**. *Plos One*, *11*(5), e0155384–e0155412.

Shih, C.-T., Sporns, O., Yuan, S.-L., Su, T.-S., Lin, Y.-J., Chuang, C.-C., et al. (2015). **Connectomics-based analysis of information flow in the *Drosophila* brain**. *Current Biology*, *25*(10), 1249–1258.

Silbering, A. F., Rytz, R., Grosjean, Y., Abuin, L., Ramdya, P., Jefferis, G. S. X. E., et al. (2011). **Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems**. *Journal of Neuroscience*, *31*(38), 13357–13375.

Singh, A. P., Das, R. N., Rao, G., Aggarwal, A., Diegelmann, S., Evers, J. F., et al. (2013). **Sensory neuron-derived Eph regulates glomerular arbors and modulatory function of a central serotonergic neuron**. *PLoS Genetics*, *9*(4), e1003452–14.

Sizemore, T. R., & Dacks, A. M. (2016). **Serotonergic modulation differentially targets distinct network elements within the antennal lobe of *Drosophila melanogaster***. *Scientific Reports*, 6, 1–14.

Stensmyr, M. C., Dweck, H. K. M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., et al. (2012). **A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila***. *Cell*, *151*(6), 1345–1357.

Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: A review. *Cell and Tissue Research*, *275*(1), 3–26.

Stocker, R. F. (2001). **Drosophila as a focus in olfactory research: Mapping of olfactory sensilla by fine structure, odor specificity, odorant receptor expression, and central connectivity**. *Microscopy Research and Technique*, *55*(5), 284–296.

Stocker, R. F., Borst, A., Lienhard, M. C., & Fischbach, K. F. (1990). **Neuronal architecture of the antennal lobe in *Drosophila melanogaster***. *Cell and Tissue Research*, *262*(1), 9–34.

Stocker, R. F., Singh, R. N., Schorderet, M., & Siddiqi, O. (1983). Projection patterns of different types of antennal sensilla in the antennal glomeruli of *Drosophila melanogaster*. *Cell and Tissue Research*, *232*(2), 237–248.

Stockinger, P., Kvitsiani, D., Rotkopf, S., Tirián, L., & Dickson, B. J. (2005). **Neural circuitry that governs *Drosophila* male courtship behavior**. *Cell*, *121*(5), 795–807.

Strausfeld, N. J., & Hildebrand, J. G. (1999). **Olfactory systems: Common design, uncommon origins?** *Current Opinion in Neurobiology*, *9*(5), 634–639.

Strutz, A., Soelter, J., Baschwitz, A., Farhan, A., Grabe, V., Rybak, J., et al. (2014). **Decoding odor quality and intensity in the *Drosophila* brain**. *eLife*, *3*, 17–21.

Su, C.-Y., Menuz, K., Reisert, J., & Carlson, J. R. (2012). **Non-synaptic inhibition between grouped neurons in an olfactory circuit**. *Nature*, *492*(7427), 66–71. .

Sudhakaran, I. P., Holohan, E. E., Osman, S., Rodrigues, V., VijayRaghavan, K., & Ramaswami, M. (2012). **Plasticity of recurrent inhibition in the *Drosophila* antennal lobe**. *Journal of Neuroscience*, *32*(21), 7225–7231.

Suh, G. S. B., Wong, A. M., Hergarden, A. C., Wang, J. W., Simon, A. F., Benzer, S., et al. (2004). **A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila***. *Nature*, *431*(7010), 854–859.

Sweeney, L. B., Chou, Y.-H., Wu, Z., Joo, W., Komiyama, T., Potter, C. J., et al. (2011). **Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting**. *Neuron*, *72*(5), 734–747.

Sweeney, L. B., Couto, A., Chou, Y.-H., Berdnik, D., Dickson, B. J., Luo, L., et al. (2007). **Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions**. *Neuron*, *53*(2), 185–200.

Tanaka, N. K., Awasaki, T., Shimada, T., & Ito, K. (2004). **Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers**. *Current Biology*, *14*(6), 449–457.

Tanaka, N. K., Endo, K., & Ito, K. (2012). **Organization of antennal lobe-associated neurons in adult *Drosophila melanogaster* brain**. *Journal of Comparative Neurology*, *520*(18), 4067–4130.

Twick, I., Lee, J. A., & Ramaswami, M. (2014). **Olfactory habituation in *Drosophila*—Odor encoding and its plasticity in the antennal lobe**. [Special issue: *Odor Memory and Perception*]. *Progress in Brain Research*, *208*, 3–38.

Venard, R., & Pichon, Y. (1984). **Electrophysiological analysis of the peripheral response to odours in wild type and smell-deficient olf C mutant of *Drosophila melanogaster***. *Journal of Insect Physiology*, *30*(1), 1–5.

Vosshall, L. B. (2009). **The evolution of chemosensory perception in insects**. *Developmental Biology*, *331*(2), 388.

Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A., & Axel, R. (1999). **A spatial map of olfactory receptor expression in the Drosophila antenna**. *Cell*, *96*(5), 725–736.

Vosshall, L. B., Wong, A. M., & Axel, R. (2000). **An olfactory sensory map in the fly brain**. *Cell*, *102*(2), 147–159.

Wang, J. W., Wong, A. M., Flores, J., Vosshall, L. B., & Axel, R. (2003). **Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain**. *Cell*, *112*(2), 271–282.

Wang, K., Gong, J., Wang, Q., Li, H., Cheng, Q., Liu, Y., et al. (2014). **Parallel pathways convey olfactory information with opposite polarities in *Drosophila***. *Proceedings of the National Academy of Sciences*, *111*(8), 3164–3169.

Wang, L., & Anderson, D. J. (2010). **Identification of an aggression-promoting pheromone and its receptor neurons in *DrosophilaI***. *Nature*, *463*(7278), 227–231.

Wang, Y. C., Yang, J. S., Johnston, R., Ren, Q., Lee, Y. J., Luan, H., et al. (2013). ***Drosophila* intermediate neural progenitors produce lineage-dependent related series of diverse neurons**. *Development*, *141*(2), 253–258.

Ward, A., Hong, W., Favaloro, V., & Luo, L. (2015). **Toll receptors instruct axon and dendrite targeting and participate in synaptic partner matching in a *Drosophila* olfactory circuit**. *Neuron*, *85*(5), 1013–1028.

Wilson, R. I. (2013). **Early olfactory processing in *Drosophila*: Mechanisms and principles**. *Annual Review of Neuroscience*, *36*(1), 217–241.

Wilson, R. I., & Laurent, G. (2005). **Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the *Drosophila* antennal lobe**. *Journal of Neuroscience*, *25*(40), 9069–9079.

Wilson, R. I., Laurent, G., & Turner, G. C. (2004). Transformation of olfactory representations in the *Drosophila* antennal lobe. Science, *303*(5656), 366–370. Retrieved from **http://science.sciencemag.org/content/303/5656/366**.

Winther, Å. M. E., Siviter, R. J., Isaac, R. E., Predel, R., & Nässel, D. R. (2003). **Neuronal expression of tachykinin-related peptides and gene transcript during postembryonic development of *Drosophila***. *Journal of Comparative Neurology*, *464*(2), 180–196.

Wu, Y., Helt, J. C., Wexler, E., Petrova, I. M., Noordermeer, J. N., Fradkin, L. G., et al. (2014). **Wnt5 and Drl/Ryk gradients pattern the *Drosophila* olfactory dendritic map**. *Journal of Neuroscience*, *34*(45), 14961–14972.

Yaksi, E., & Wilson, R. I. (2010). **Electrical coupling between olfactory glomeruli**. *Neuron*, *67*(6), 1034–1047.

Yamamoto, D., & Koganezawa, M. (2013). **Genes and circuits of courtship behaviour in *Drosophila* males**. *Nature Reviews Neuroscience*, *14*(10), 681–692.

Yao, Y., Hing, H., Wu, Y., Yin, C., Ozawa, R., Aigaki, T., et al. (2007). **Antagonistic roles of Wnt5 and the Drl receptor in patterning the *Drosophila* antennal lobe**. *Nature Neuroscience*, *10*(11), 1423–1432.

Zhang, X., & Gaudry, Q. (2016). **Functional integration of a serotonergic neuron in the Drosophila antennal lobe**. *eLife*, *5*, 2435.

Zhou, Y., & Wilson, R. I. (2012). **Transduction in *Drosophila* olfactory receptor neurons is invariant to air speed**. *Journal of Neurophysiology*, *108*(7), 2051–2059.

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