Published in final edited form as:

*Cell Tissue Res*. 2021 January ; 383(1): 91–112. doi:10.1007/s00441-020-03386-4.

# Untangling the Wires: Development of Sparse, Distributed Connectivity in the Mushroom Body Calyx

**Vanessa M. Puñal**[1,2], **Maria Ahmed**[1], **Emma M. Thornton-Kolbe**[1,3], **E. Josephine Clowney**[1]

[1]Department of Molecular, Cellular & Developmental Biology, The University of Michigan, Ann Arbor, MI 48109, USA

[2]Department of Molecular & Integrative Physiology, The University of Michigan, Ann Arbor, MI 48109, USA

[3]Neuroscience Graduate Program, The University of Michigan, Ann Arbor, MI 48109, USA

## Abstract

Appropriate perception and representation of sensory stimuli poses an everyday challenge to the brain. In order to represent the wide and unpredictable array of environmental stimuli, principle neurons of associative learning regions receive sparse, combinatorial sensory inputs. Despite the broad role of such networks in sensory neural circuits, the developmental mechanisms underlying their emergence are not well understood. As mammalian sensory coding regions are numerically complex and lack the accessibility of simpler invertebrate systems, we chose to focus this review on the numerically simpler, yet functionally similar, *Drosophila* mushroom body calyx. We bring together current knowledge about the cellular and molecular mechanisms orchestrating calyx development, in addition to drawing insights from literature regarding construction of sparse wiring in the mammalian cerebellum. From this, we formulate hypotheses to guide our future understanding of the development of this critical perceptual center.

### Keywords

Calyx; mushroom body; sparse wiring; development; perception

## Introduction

Detection of environmental stimuli is essential for survival. A significant challenge of development is to build neural circuits capable of processing sensory inputs which are unpredictable and nearly limitless in number. Let us consider the case of olfaction. Odor-dependent behaviors—such as locating food, finding a mate, or avoiding predators—require the olfactory system to detect and distinguish between an expansive array of distinct, unforeseen odorants that sometimes are overlapping in quality. Coding capacity can be increased through growth of the repertoire of receptor genes, as has occurred in several insect clades. Regardless of the size of the receptor repertoire, coding capacity can be

Compliance with Ethical Statements

On behalf of all authors, the corresponding author states that there is no conflict of interest.

further increased by orders of magnitude through higher-brain perceptual mechanisms that are sensitive to combinatorial activation of receptors. This is achieved by a relatively larger number of principal neurons in higher order perception centers receiving sparse, combinatorial inputs from a select number of peripheral sensors. For example, in the *Drosophila melanogaster* mushroom body (MB) calyx, ~2,000 Kenyon cells (KCs) each receive three to ten discrete inputs out of the possible 52 distinct olfactory channels, such that subsets of KCs respond to unique combinations of sensory inputs. The utility of this brand of wiring, called an expansion layer, is reflected in its use by multiple other brain regions, including cerebellum and hippocampus, to enhance perceptual processing power (Olshausen and Field 2004). At present, it is not understood what developmental mechanisms give rise to the input structure necessary for such sensory amplification.

The fly MB calyx is a tractable model for illuminating the cellular and molecular mechanisms driving the formation of such wiring logic. Studying neural development in invertebrates has often hastened the discovery of similar mechanisms in vertebrates (Lichtneckert and Reichert 2005). The MB is structurally similar to the hippocampus and cerebellum and functionally analogous to piriform cortex. Additionally, the mammalian and fly olfactory systems have similar overall designs (Ache and Young 2005). The numerical simplicity of the MB has allowed higher resolution interrogation of its form and function. Moreover, developmental neuroscience in the fly benefits from an array of genetic tools unavailable in most other model organisms. In addition, publicly available, single-synapse resolution connectomic data generated from whole brain electron microscopy (EM) in both the adult and larva allow for complete reconstruction of any brain region (Eichler et al. 2017; Zheng et al. 2018). Together, these advantages create a unique opportunity for utilizing the fly calyx to gain a general understanding of expansion layer formation.

In this review, we discuss progress in our understanding of cellular and molecular mechanisms which make way for sparse, distributed connectivity in the calyx. Developmental processes shaping neural circuits determine the final behavioral repertoires of the adult organism. Understanding the conditions which guide construction of a circuit can highlight features of the functioning mature circuit. Similarly, a complete picture of the adult circuit facilitates the generation of hypotheses regarding what developmental mechanisms might produce it. With this in mind, here we juxtapose current knowledge regarding adult calyx design and function to presently understood cellular and molecular events in development leading up to its adult form. We have further taken a holistic approach in this discussion, knitting together the adult qualities and developmental timelines of each cellular member of the calyx. From this tapestry, we draw insights as to what events in which cellular members may direct or constrain the form and function of the emerging adult calyx, and highlight significant knowledge gaps for the field to explore.

## Functional role of the calyx in odor processing

Here, we provide a brief overview of odor processing in the fly and describe the context of the calyx within the olfactory processing circuit. Understanding the function of the calyx is the primary step towards thinking about the unique developmental features that wire this region and generate its function.

The *Drosophila* olfactory epithelium is composed of two major sensory organs, the antennae and maxillary palps, which house olfactory sensory neurons (OSNs) expressing receptors from the odorant receptor (OR) and ionotropic receptor (IR) families (Fig. 1A). Most natural smells are made up of a combination of odor molecules, each of which bind a few receptor types (Hallem and Carlson 2006). ORs and IRs are expressed by 52 types of olfactory sensory neurons (OSNs). OSNs usually express one, and occasionally two or three, ORs; or as many as four IRs (Vosshall et al. 1999; Couto et al. 2005; Fuss and Ray 2009; Gomez-Diaz et al. 2018). As a result, a single scent becomes represented by the activity of a unique subset of OSNs. OSNs expressing the same receptor converge onto a single glomerulus in the antennal lobe (AL), where they synapse with olfactory projection neurons (PNs) (Vosshall et al. 2000; Gao et al. 2000; Jefferis et al. 2001; Fishilevich and Vosshall 2005; Jacobson and Friedrich 2013) (Fig. 1A). Each glomerulus is innervated by 10–65 OSNs (average of 30) (Grabe et al. 2016). A large percentage of PN classes, each containing 1–10 PNs (median of 3), are uniglomerular thus forming synapses with OSNs from one glomerulus (Yu et al. 2010; Bates et al. 2020). The outcome is 52 distinct odor channels (Marin et al. 2002; Wong et al. 2002; Yu et al. 2010; Bates et al. 2020). In the AL, axon terminals of OSNs receive GABAergic inhibitory input from local interneurons, which scale inhibition with increased OSN activity to provide gain control. This allows faithful transmission of odor information and mitigates saturation of PN responses as odorant concentrations increase (Olsen and Wilson 2008; Root et al. 2008; Chou et al. 2010; Olsen et al. 2010). For further information on olfactory processing in the AL and its regulation by interneurons, readers are directed to other reviews in this issue.

PN axons relay sensory information from the AL to two higher brain regions: the lateral horn (LH) and MB calyx. The LH is thought to orchestrate innate behaviors (Heimbeck et al. 2001; Jefferis et al. 2007; Ruta et al. 2010; Kohl et al. 2013; Jeanne et al. 2018; Chin et al. 2018). However, as much of the sensory world is unpredictable in nature, animals benefit from combining genetically programmed, innate behaviors with experience-dependent behaviors. Sensory inputs to the MB calyx allow for stimulus representation, while dopamine-mediated plasticity of MB output synapses in the MB lobes produces associations between stimulus and context to adjust behavioral responses (Hige 2018; Handler et al. 2019). In addition, a recent study reported experience-dependent plasticity in calyceal responses to a particular odor upon long-term conditioning (Baltruschat et al. 2020). This review will focus on how sensory representations are set up in the calyx, and will not cover mechanisms underlying synaptic plasticity. For an in-depth reading on learning mechanisms operating at MB output synapses, see (Hige 2018) and other reviews in this issue.

The MB of each hemisphere has about 2,000 KCs (Aso et al. 2009) and ~150 uniglomerular PNs (Bates et al. 2020). The connectivity between olfactory PNs and KCs in the calyx displays two important features to cope with the diversity of sensory cues in the environment: 1) combinatorial expansion of a small set of PN inputs onto a much larger population of postsynaptic KCs; and 2) variability in the sets of inputs to each KC. Sparsely wired expansion layers are a frequently observed feature across regions involved in sensory perception and associative learning (Cayco-Gajic and Silver 2019). As the MB has ~2000 KCs with 52 possible glomerular inputs, the PN:KC synaptic layer carries an expansion

ratio (i.e. number of postsynaptic cells to the number of inputs) of 40 (Laurent 2002; Litwin-Kumar et al. 2017) (Fig. 1B). Seven of these glomerular inputs also relay thermo/hygrosensory information to KCs (Marin et al. 2020), which will be discussed in more detail in the next section. Another example of a sparsely connected expansion layer is found in the rat cerebellum where a much larger set (~200,000) of postsynaptic granule cells (GCs) have ~7,000 estimated mossy fiber (MF) inputs, resulting in a similar expansion ratio of 30 (Marr 1969; Harvey and Napper 1991; Tyrrell and Willshaw 1992; Litwin-Kumar et al. 2017). Despite the large differences in cell numbers, each MB KC and cerebellar GC receives only four to five sensory inputs on average. This sparse representation of sensory information has been theorized to optimize discriminability of stimuli by reducing overlap between their sensory representations (Kanerva 1988; Rolls and Treves 1990; Laurent 2002; Fiete et al. 2004; Cayco-Gajic et al. 2017). As a result, there is an increased dimensionality of sensory representations, allowing organisms to discriminate orders of magnitude more odors than the number of receptors encoded in the genome. Minimizing overlap between sensory representations also increases associative memory capacity by allowing learning-associated synaptic plasticity at the KC output synapses to be stimulus-specific.

While 50% of the PN population can respond to a single odor, sparse wiring between PNs and KCs results in only ~10% of KCs responding to an odor (Fig. 1B) (Perez-Orive et al. 2002; Wilson et al. 2004; Wang et al. 2004; Keene and Waddell 2007; Turner et al. 2008; Murthy et al. 2008; Honegger et al. 2011; Lin et al. 2014). Coding capacity is further enhanced when KCs are considered as a population, as distinct and overlapping populations of KCs are stimulated by different odors. The requirement of combinatorial inputs to KCs is evident in electrophysiological studies: more than half of the olfactory inputs to a KC need to be activated to elicit a spiking response, with the exception of a few cases where activation of a single KC claw is sufficient (Gruntman and Turner 2013). Together, these studies highlight that the number of olfactory inputs received by KCs is a crucial parameter in sensory coding.

Individual neurons in the expansion layer receive distinct combinations of inputs. In contrast to the stereotyped organization of the AL where each odor activates a topographically fixed set of glomeruli, the MB calyx has a surprisingly “flexible” organization. Characterization of the sets of PN inputs to individual KCs through multiple methods including electrophysiological recordings of KC odor responses, dye tracing of the inputs, and larval and adult EM studies have shown that the sets of inputs to individual KCs are largely unstructured (Murthy et al. 2008; Caron et al. 2013; Eichler et al. 2017; Zheng et al. 2018). This means that in most cases, knowing one input to a KC does not aid prediction of other inputs that the same cell receives. As a result, the PN:KC connectivity map is not expected to be consistent from fly to fly. That brings up the question: at this stage of odor processing, why would the olfactory system discard the stereotypy and structure preserved from OSNs to PNs? One explanation is that predictable wiring is required for innate processing, while unstructured connectivity may be “good enough” in circuits where meaning is instilled not by genetic hardwiring but through learning. If the KC input sets found in different flies are truly different (and differences between the hemispheres of a single individual suggest they will be), then the coding capacity across the population of flies could also be larger than that of an individual fly (Eichler et al. 2017; Marin et al. 2020). Nevertheless,

despite the expected variability in connectivity from animal to animal, the representation of different odors is still stable within each animal. Therefore, at the population level, KC soma responses can reliably convey odor identity (Honegger et al. 2011; Campbell et al. 2013; Elkahlah et al. 2020).

While the sets of inputs to individual KCs are unpredictable, they are not fully random. First, while some glomeruli, such as DA3, are represented in the calyx by just one PN forming a few boutons, others, such as DA1, are represented by multiple PNs forming more than 20 boutons in total (Caron et al. 2013). KCs receive more of their inputs from glomeruli that are represented by more boutons; this bias is observed more strongly in the inputs to α/β and α'/β' KCs than those to γ KCs (Li et al. 2020a). Second, boutons of PNs of different types occupy different characteristic radial positions in the calyx, and individual KCs tend to be co-innervated by bouton types that tend to be near each other in space (Tanaka et al. 2004; Zheng et al. 2020; Li et al. 2020a). One analysis suggests that boutons responding to food odors are located near one another in the calyx and co-innervate local KCs, resulting in a "food fovea" (Zheng et al. 2020). Third, 20% of the inputs to the calyx are non-olfactory, and different types of KCs receive input from different sensory modalities. As described further below, visual PNs innervate visual-only KCs in the dorsal and ventral accessory calyces, while hygrosensory, thermosensory, and gustatory PNs can innervate both dedicated KCs in the lateral accessory calyx and olfactory KCs in the main calyx (Tanaka et al. 2008; Aso et al. 2014; Vogt et al. 2016; Eichler et al. 2017; Zheng et al. 2018; Bates et al. 2020; Li et al. 2020a, b). Much like the distinction between dorsal and ventral olfactory bulb glomeruli in the mouse, where dorsal glomeruli are tuned to especially salient cues and ventral glomeruli are less constrained (Kobayakawa et al. 2007), these biases in the combinatorial code to KCs help highlight ethologically critical percepts and provide clues and constraints in our search for mechanisms shaping calyx development. Overall, the sparse density of inputs to KCs, together with the variability in those inputs, prepare the olfactory system for unfamiliar or evolutionarily novel environments.

Most of what we know about how development instructs wiring is from studies in spatially mapped or highly structured brain areas such as those used in vision and proprioception, or at the neuromuscular junction. In contrast to the flexible organization of olfactory inputs to the calyx, these sensory regions have a stereotyped organization that is consistent across animals. Additionally, KC responses are sparse regardless of the nature and complexity of an odor, while other sensory systems show enhanced responses to naturally occurring and behaviorally relevant cues (Rieke et al. 1995; Machens et al. 2001; Yu et al. 2005; Garcia-Lazaro et al. 2006; Honegger et al. 2011). This suggests that different developmental mechanisms might underlie the connectivity logic in the MB calyx as compared to other sensory systems. Quasi-randomized connectivity likely occurs in large swathes of the mammalian brain, including the sensory inputs to the cortex, hippocampus, and cerebellum.

## Cellular members of the calyx

In the past 25 years, light and electron microscopy have illuminated the morphology, projections, and synaptic architecture of the cells that contribute to the calyx. As a result of these efforts, we can appreciate that the calyx is made up of more cells than just its

protagonists. Here, we consider calyx architecture from a holistic vantage point. This is done to facilitate later discussions as to mechanisms which may drive sparse wiring between PNs and KCs.

## Kenyon cells

The MB is made up of KCs, whose mushroom-like plume of cell bodies and axonal stalk give the region its name. Their cell bodies are located in the dorsal posterior of the brain. KC dendrites make up the primary post-synaptic component of the calyx, while axons converge ventrally into the pedunculus before bifurcating anterodorsally to give rise to the α and α' lobes, and medially to give rise to the β, β', and γ lobes (Fig. 1A).

Individual KCs are primarily classified by the lobe their axons contribute to: γ KCs innervate the γ lobe, α/β KCs the α and β lobes, and α'/β' KCs the α' and β' lobes. Dendrites of different types of KCs are not as stratified as their axons but are also not uniformly distributed across the calyx. First, KCs occupy four territories in the calyx, reflecting the four neuroblasts from which they arise (Zhu et al. 2003). Each neuroblast gives rise to every type of KC, and within each neuroblast territory, dendrites of different types of KCs occupy loosely defined and overlapping regions: γ KC dendrites mostly make up the calyx core, while α/β and α'/β' make up most of the periphery (Figs. 2B–D) (Zhu et al. 2003; Leiss et al. 2009; Aso et al. 2014; Zheng et al. 2018) Despite this, it is common for the claws of several different classes of KCs to receive input from a single PN bouton, supporting the idea that KC regions within the main calyx have open borders (Figs. 2G,I) (Caron et al. 2013; Baltruschat et al. 2020). Dendrites from specific subsets of each of the three classes of KCs—γd, α'/β' ap and α/β p—make up the ventral, lateral and dorsal accessory calyces (vACA, lACA and dACA), respectively. These receive primarily non-olfactory sensory information from visual, thermosensory, hygrosensory, and gustatory PNs (Figs. 2B–F) (Tanaka et al. 2008; Aso et al. 2014; Vogt et al. 2016; Zheng et al. 2018; Li et al. 2020a, b). A recent preprint further subdivides KCss based on anatomic characteristics that can be observed by EM; the functional roles of these KC subtypes have yet to be tested (Li et al. 2020a).

## Projection Neurons

An estimated 80% of the combinatorial inputs received by KCs from PNs are olfactory, while the remaining fraction bring visual, hygrosensory, thermosensory, and gustatory information to the MB (Zheng et al. 2018; Bates et al. 2020).

**Olfactory PNs—**Most olfactory PNs innervating the calyx are cholinergic and project axons to the LH and calyx via the medial antennal lobe tract (mALT).(previously called the inner antennocerebral tract). A smaller number of olfactory inputs arrive via the outer and inner middle ALT after first projecting to the LH and pedunculus (Tanaka et al. 2008, 2012; Bates et al. 2020; Li et al. 2020a). There is also a smaller subset of GABAergic and cholinergic PNs targeting the calyx that receive multiglomerular input directly from OSNs, via local interneurons, or even from other PNs (Bates et al. 2020).

Olfactory PNs that innervate the same glomerulus are stereotyped in cell number, as well as in localization of their dendrites and axons (Grabe et al. 2016). PN cell bodies are divided into dorsal-anterior, lateral, and ventral clusters relative to the AL (Jefferis et al. 2001). Most glomeruli are innervated by three PNs, but this number can range from one to ten (Wong et al. 2002; Yu et al. 2010; Grabe et al. 2016; Bates et al. 2020). Axons of PNs that receive input from the same glomerulus have similar branching patterns and numbers of boutons, resulting in radial regions of the calyx which are more likely to contain terminals of certain PNs (Fig. 2E) (Wong et al. 2002; Marin et al. 2002; Jefferis et al. 2007; Zheng et al. 2018, 2020). PN axon and dendrite morphology are maintained in adults even when olfactory organs are removed at eclosion, indicating developmental pre-programming (Wong et al. 2002).

**Visual, Thermosensory, Hygrosensory and Gustatory PNs—**Visual inputs from the lobula and the medulla innervate both the γd KCs of the vACA and the α/β p KCs of the dACA (Fig. 2F) (Butcher et al. 2012; Aso et al. 2014; Vogt et al. 2016; Li et al. 2020a, b). Unlike in the vACA, visual inputs to the dACA come primarily from local visual interneurons (Li et al. 2020a).

The calyx includes axonal projections from 16 types of thermo/hygrosensory PNs that receive input from heat, cool, and humidity sensory neuron dendrites in seven glomeruli of the ventroposterior AL (Marin et al. 2020). These uni- and bi-glomerular PNs project primarily via the medial ALT to the lACA and anterior part of the main calyx (Frank et al. 2015; Marin et al. 2020). In the lACA, three α'/β' ap KCs (also called α'/β'-ap1 KCs by Li et al 2020a) exclusively receive thermo- and hygrosensory input, while two γd KCs and 24 additional α'/β' ap KCs combine thermo/hygrosensory inputs with olfactory inputs (Fig. 2F) (Caron et al. 2013; Marin et al. 2020). These olfactory inputs tend to come from PNs sensitive to odors of decaying fruit or danger, suggesting a degree of structuring in development (Marin et al. 2020).

Gustatory PNs project broadly and sparsely in the main calyx, while gustatory signals from the subesophageal zone also appear to be carried to the dACA via the multimodal interneuron MB-CP2 (Kirkhart and Scott 2015; Li et al. 2020a). Gustatory inputs to the calyx are spatially segregated by tastant (i.e. sugar or bitter) and gustatory sense organ (i.e. proboscis or leg) (Kirkhart and Scott 2015) (Fig. 2F). Activity of the γ lobe is required for aversive taste conditioning while the dACA is not (Kirkhart and Scott 2015). About half of KCs responsive to tastants in the main calyx also respond to odors.

## The microglomerulus: the PN to KC synapse

The calyx is made up of an array of microglomeruli. At the center of each microglomerulus is a single PN terminal bouton that is encased in the claws of several KCs (Figs. 2G,I) (Yasuyama et al. 2002; Leiss et al. 2009). About half of PN boutons are what Butcher *et al.* describe as clustered, in which multiple terminals cluster together (Fig. 2I). The other half of boutons are split evenly between being unilobed (a single round bouton) or elongated (a 'beads-on-a-string' formation) (Butcher et al. 2012). These three morphological types are found together on single PNs and it is unclear whether there are functional differences

between them. Each is encased by an average of 11 specialized, actin-rich claws that form the terminals of KC dendrites (Lee et al. 1999; Leiss et al. 2009; Butcher et al. 2012). These claws are either simple (i.e. a single claw wrapping a single presynaptic bouton) or complex, with more than one claw or a dense network of tiny claws or spine-like processes (Lee et al. 1999; Butcher et al. 2012).

The microglomeruli are synapse dense. A single bouton has between 24 T-bar ribbon synapses and 69 non-ribbon synapses, in a roughly 1:3 ratio. These synapses are divergent polyads meaning that multiple (1–14) postsynaptic profiles underlie each PN presynapse. Indeed, multiple postsynaptic profiles originating from the same claw sometimes underlie the same presynaptic active zone (Butcher et al. 2012; Baltruschat et al. 2020). The synaptic density and redundancy acts to ensure faithful transmission between PNs and KCs. PN inputs distributed amongst claws sum linearly and about half must be active for the KC to spike (Gruntman and Turner 2013).

As described above, PN bouton positioning in the calyx, while less stereotyped than projections to the LH, is still quite orderly (Marin et al. 2002; Wong et al. 2002; Tanaka et al. 2004; Jefferis et al. 2007; Zheng et al. 2018, 2020). Nevertheless, KCs receive inputs willy-nilly from locally available PNs (Caron et al. 2013; Zheng et al. 2020; Li et al. 2020a). These findings together suggest that the promiscuity and variability in KC inputs is programmed in KCs, which approach an orderly landscape of PN boutons in a chaotic manner.

## Other calyx members

**Anterior Paired Lateral (APL) Neurons—**The APL neurons are the primary GABAergic input to the MB, providing inhibition to the entire MB structure. Each hemisphere contains one APL neuron whose cell body is located near the LH (Tanaka et al. 2008; Liu and Davis 2009; Wu et al. 2013). Dendrites and presynaptic terminals are distributed throughout the network of APL fibers with slightly more presynaptic sites at α'/β' KCs and the heel of the pedunculus (Fig. 2G–I) (Liu and Davis 2009; Wang et al. 2019). APL is both pre- and post- synaptic to PNs and KCs (Yasuyama et al. 2002). It does not fire action potentials and shows localized responses to odors at its presynaptic terminals in the calyx (Wang et al. 2019). APL can mediate all-to-all inhibition of KCs or can restrict inhibition to a single more active lobe which helps to sparsen odor representations (Lin et al. 2014; Inada et al. 2017). Inhibition of the MB is important for olfactory reversal learning and serves to suppress the original memory (Wu et al. 2012). While APL is primarily GABAergic, it has also been shown to use octopamine, which is essential for the formation of anesthesia resistant memory (Wu et al. 2013).

**Odd skipped—**Three of the 76 neurons defined by expression of the transcription factor *odd-skipped* elaborate a few dendrites across the adult calyx where they could be postsynaptic to both KCs and PNs, and project axons to the inferior, ventromedial and posterior lateral protocerebrum (Levy and Larsen 2013). In larvae, *odd* neurons primarily innervate the calyx but these dendrites are pruned during metamorphosis (Levy and Larsen

2013; Slater et al. 2015). These neurons receive and respond to odor input from PNs and are necessary for chemotaxis behaviors (Slater et al. 2015).

**Anatomically Identified Calyx Neurons—**Several other neurons which innervate the calyx were identified through a GAL4 screen (Table 1) (Tanaka et al. 2008). The whole brain EM volume done by Zheng and colleagues also found these and other neurons innervate the calyx (Table 1) (Zheng et al. 2018). While the anatomical features of these neurons are well characterized, not much is known about their functional contributions.

**Glial Members of the Calyx—**There are three glia types which interact with the component cells of the *Drosophila* calyx: cortex glia, astrocytes, and ensheathing glia (EG). Cortex glia are not found in the calyx itself but instead wrap the cell bodies of calyx member neurons within the cortical region (Hoyle 1986; Pereanu et al. 2005; Awasaki et al. 2008; Spindler and Hartenstein 2010; Kremer et al. 2017). In general, they are believed to provide trophic support but also show activity-dependent calcium oscillations that mediate neuronal activity (Stork et al. 2012; Weiss et al. 2019). Though these glia could play a role in KC development and function, this review will not focus on cortex glia but instead will only discuss the glial types that directly contribute to calyx architecture: astrocytes and EG.

Astrocyte cell bodies are found at the boundary between cortical and neuropil regions. Their processes are localized to the neuropil, with processes from a single astrocyte often spanning multiple neuropil (Awasaki et al. 2008; Kremer et al. 2017). Many of the morphological, functional and genetic hallmarks of mammalian astrocytes are shared with astrocytes of *Drosophila*, including their requirement for appropriate synaptogenesis (Fig. 2G–I) (Tasdemir-Yilmaz and Freeman 2014; Stork et al. 2014; Muthukumar et al. 2014; Yildirim et al. 2019). Such evidence suggests that the developmental roles of mammalian astrocytes may be shared in the fruit fly (see below).

EG wrap axon bundles as well as the calyceal neuropil. EG enter the calyx alongside the PNs they wrap and stay with them until PNs branch to form boutons (Kremer et al. 2017). KC axons are also wrapped in EG, with the EG compartmentalizing axons of each KC subtype through the peduncle and into the lobes (Kremer et al. 2017). The entire calyx neuropil is encased by EG, which also extend processes into the calyx where they somewhat compartmentalize groups of microglomeruli (Fig. 2G–I) (Leiss et al. 2009; Kremer et al. 2017).

## Cellular events of calyx wiring

### The calyx is built twice

The adult calyx is not the first instantiation of an odor perception center in *Drosophila*, as PNs and KCs also form a calyx within the larval olfactory circuit (Masuda-Nakagawa et al. 2005). The odor-dependent behaviors of larvae and adult flies are not equivalent. Unlike larvae, adult flies must detect long-range odors and integrate visual input in order to find food or engage in reproductive behaviors. Given this, development of the emerging adult calyx is intertwined with and dependent on the reorganization of the larval calyx to

make way for adult-specific circuit requirements. Such modifications take place during a developmental period between the larval and adult stages called metamorphosis.

A salient pressure on calyx re-wiring is the installation of additional cells to the olfactory system (Fig. 3A, B). Peripheral odor detection relies on just 21 OSNs in the larva as compared to ~1400 in the adult, an expansion of ~60-fold. This increase in peripheral odorant sensors results in an expanded number of glomeruli, from 21 in the first instar larva to 52 in the adult. 21 embryonically born olfactory PNs (hereafter, “E-PNs”) each receive input from a single glomerulus in the larval circuit (Ramaekers et al. 2005; Eichler et al. 2017). Before odorant channel expansion occurs in the pupa, the PN population grows in size. This PN expansion is a result of neuroblast division from the L1 larval stage through just before onset of metamorphosis (larval-born PNs referred to as L-PNs hereafter) (Jefferis et al. 2001). L-PNs will only go on to innervate the adult calyx (Jefferis et al. 2004). Thus, what once were just ~21 E-PNs innervating the larval calyx becomes a mixture of ~150 E-PNs and L-PNs in the adult (Marin et al. 2005; Bates et al. 2020) (Fig. 3B). This 7-fold increase in PN number is paralleled by a ~25-fold increase in KCs, as the L1 larval count of ~75 mature KCs innervating the calyx jumps to ~2,000 in the adult circuit (Ramaekers et al. 2005; Eichler et al. 2017) (Fig. 3A).

KCs are born sequentially from four neuroblasts which continuously divide throughout development: γ KCs are born embryonically through the mid-3rd instar stage; α′/β′ KCs are generated from the mid-3rd instar stage to just before puparium formation (PF); and α/β KCs are generated immediately after PF (Lee et al. 1999) (Fig. 3A). Despite the ongoing increase of KC number, the ~75 embryonically born KCs (part of the γd KC subset) are sufficient for larval odor-driven behaviors as demonstrated by ablation experiments (Pauls et al. 2010). This suggests that larval-born KCs, although extending some dendrites into the larval calyx, do not meaningfully contribute to olfactory perception until metamorphosis. Thus, larval calyx circuitry appears to be a stable wiring of PNs and KCs that are born together in the embryo, while wiring of the emerging adult calyx is hallmarked by a continuous process of integration as KC population expansion continues long after PN birth has terminated.

## Kenyon Cells as drivers

**Innervation of the calyx by PNs—**When KCs are ablated during development, the adult calyx is not innervated by PN collaterals (Stocker et al. 1997; Elkahlah et al. 2020). Adult KC claw number, however, is unaffected by similar developmental alterations to the overall number of PNs (Elkahlah et al. 2020). This suggests KCs themselves may be a primary driver of PN collateral sprouting. Such results prompt us to ask: what are the source and temporal dynamics of KC-derived cues to PNs? Because E-PNs prune their processes from 4–12 hours APF and L-PNs also wait to form collaterals in these early pupal stages (Fig. 3B), the KC-derived cue must either be absent in these early pupal stages or PNs must be insensitive to it (Jefferis et al. 2004; Marin et al. 2005). Embryonic and larvally born KCs also undergo axon and dendrite pruning after metamorphosis. Between 4–18hrs APF, γ KC dendrites are completely eliminated and α’/β’ dendrites become shortened in length. By at least 24hrs APF, new KC processes are observed to branch into the calyx (Lee et al. 1999).

Overlapping with these events, PNs initiate sprouting of primary collaterals into the calyx between 6–18hrs APF (Jefferis et al. 2004).

KC sprouting signals need not be broadcast by dendrites per se, but could be secreted from KC somata, which are local to the calyx, or by KC primary processes traversing the nascent calyx field. It is curious that larvally born γ and α'/β' KCs expend energy to form dendrites only to be pruned after little, if any, meaningful integration into the larval calyx. Pruning of larval γ dendrites is a result of process degeneration (Watts et al. 2003). In the AL, degenerating larval OSN axons leave behind semaphorins as a map for proper targeting of incoming adult PN dendrites (Sweeney et al. 2011). Thus, perhaps degeneration of unused larval KC dendrites is itself a deposition of KC-derived cues required by PNs for collateral sprouting during pupal stages (KC pruning: 4hrs-18hrs APF, PN sprouting: 6hrs-18hrs APF).

**PNs match bouton production to KC population size—**KC ablation experiments by Elkahlah *et al.* also found that PN bouton production scales to KC population size: twice as many KCs results in a doubling of PN bouton number, while halving the KC population results in a proportionate loss of PN boutons. Individual PNs also show compensatory scaling in their bouton production in response to graded changes in overall PN number. In this case, bouton scaling is inverted: twice as many PNs results in halving of PN bouton number, and vice versa. An important detail is that, under all of these conditions, KCs generate similar median numbers of claws per cell. Such findings suggest KCs provide signals to PNs to scale bouton number to KC population size. Such a mechanism could ensure the appropriate degree of sparse input required for odor perception regardless of any disproportionate population expansion between partners, and could potentially support sparse coding across the very different PN:KC ratios found in the larval versus adult MB.

Changes in PN bouton number in response to alterations of the KC population, and indeed the dependence of PNs on KC signals generally, could be due to changes in bouton and/or primary collateral production or stabilization. Another open question concerns what aspect of the KC population PNs are sensing in order to make their quantitative match. Like sprouting cues, signals from KCs setting PN bouton quantity could emanate from KC somata, dendritic primary branches, or un-innervated claws. In addition, quantitative matching could be set by the clonal structure of the calyx as PNs first innervate it, with the progeny of four KC neuroblasts arranging their primary processes in concentric rings. Alternatively, it could be determined later in pupation, at the level of higher-order branching and bouton production in relation to the absolute number of KCs.

Regardless, as KC dendritic claw production remains stable upon perturbation of the PN population, cell-autonomous mechanisms must operate in KCs to set median claw number. Though many factors that affect KC dendrite branching have been identified (see below), it is not clear how cell identity mechanisms program the actions of a variety of growth and branching factors to achieve mature dendrite number and claw shape. Whether feedback mechanisms signal that the process of forming the microglomerulus is complete and/or "lock in" these anatomic units will require future study.

Does neuronal activity, i.e. cholinergic signaling from PNs to KCs or activity dependent feedback from KCs to PNs, influence calyx development? Both experimental and theoretical work are consistent with the hypothesis that PNs of different types may use neuronal activity-dependent mechanisms to compete with each other in contributing boutons to the calyx, with more active PNs winning the competition (Kremer et al. 2010; Kennedy 2019). Activity-dependent microglomerular plasticity has also been observed in the adult (Leiss et al. 2009; Kremer et al. 2010; Pech et al. 2015; Doll et al. 2017; Baltruschat et al. 2020). Studies of the role of FMRP in PN bouton production also suggests an early-adult critical period during which activity-dependent refinement may occur (Doll et al. 2017). However, because PN innervation of the calyx and LH is relatively mature before olfactory receptor expression and synaptic maturation, the role of neuronal activity in olfactory circuit development prior to eclosion is debated. Nevertheless, neurotransmission and intrinsic neuronal activity both seem to precede formation of mature synapses (Hume et al. 1983; Akin et al. 2019). Here, we consider findings that constrain the ways in which activity mechanisms can contribute to development of this synapse.

It is currently unclear when synaptic machinery such as neurotransmitters and their receptors are first localized to their respective synaptic compartments in the calyx. Despite this knowledge gap, we at least know that PNs and KCs must complete sparse wiring and develop mature synapses prior to eclosion as KCs display sparse odor responses in 1-day-old adults (Murthy et al. 2008). Studies in other brains regions (i.e. AL, MB dorsal lobes, and optic lobes) suggest that synaptogenesis initiates throughout the brain between 50hrs and 60hrs APF, with mature synapses first observed between 77hr and 84hrs APF. (Devaud et al. 2003; Chen et al. 2014; Muthukumar et al. 2014; Akin et al. 2019). It was recently discovered in the developing fly optic lobe that spontaneous, patterned neuronal activity can be observed by 55hrs APF. This activity occurred in three stages: a periodic stage (55hrs to 65hrs APF); a turbulent stage (70hrs APF to the end of metamorphosis); and an adult stage, which coexisted with mature stimulus responses for at least the first five days of adult life. Such patterned activity was unique to each cell type, stimulus-independent, and correlated between neurons destined to be adult partners. This study also demonstrated that the central brain is generating spontaneous activity in the same developmental window as the optic lobe (Akin et al. 2019). Consistent with this, KCs appear capable of activity during metamorphosis (Mayseless et al. 2018), but no studies to date have examined the details of whether such KC activity: 1) is cell-autonomous, or correlated with its pre-synaptic partners; 2) occurs spontaneously, or is instead stimulus-driven; and 3) kick-starts in sync with what appears to be a brain-wide onset of spontaneous activity.

Just as with KCs, the nature and timing of activity in PNs of the pupal brain is presently not understood. Despite these knowledge gaps, we can reason that KC activity driven by environmental stimuli likely does not occur until quite late in metamorphosis, given the following: 1) ORs are not localized to OSN dendrites until around 80hrs APF (Larsson et al. 2004); 2) OSN-to-PN synapses do not become mature until sometime between 60hrs and 84hrs APF (Muthukumar et al. 2014); and 3) there are likely limited opportunities for OSN activation by odor stimuli due to encasement of the pupa within a chrysalis. Thus, if activity plays any role in shaping calyx development, conditions appear favorable for this activity to be spontaneous. If we assume the aforementioned timelines of synaptogenesis and waves

of spontaneous activity are true for the calyx, then initial stages of PN primary collateral production would not rely on mechanisms dependent on any form of KC activity as these PN events all occur well before 55hrs APF. Bouton-bearing PN collaterals first become evident at 50hrs APF (Jefferis et al. 2004). Thus, it could be possible that patterned, spontaneous activity in the calyx is instructive for bouton stabilization. Such a mechanism may be a way to quantitatively match the number of PN inputs to KC claws by restricting bouton numbers to only a select stabilized few, and/or promote synapse formation between KC claws and PN boutons.

## Don’t forget about the glia!

Are KCs the only possible source of wiring factors driving calyx assembly? The answer is very likely no. As described above, two glial types directly contribute to the calyx neuropil: astrocytes and EG (Fig. 3C).

In the fly, astrocytes are generated in two waves: first in the late embryo (“primary astrocytes”), and again just before the third instar larval stage (“secondary astrocytes”) (Fig. 3C). Embryonically generated primary astrocytes do not incorporate into the adult neuropil. Instead, they are steadily and completely eliminated via apoptosis between the mid-larval stage and the end of metamorphosis (Fig. 3C). Secondary astrocytes begin migrating from their progenitor zones at the start of pupation and maintain a migratory morphology until ~48hrs APF (Omoto et al. 2015) (Fig. 3C). Little is known about astrocyte development in the calyx. Studies of astrocyte morphogenesis in the AL demonstrate that (likely secondary) astrocytes undergo a process of morphological maturation from 48hrs APF until eclosion. During this period, astrocyte processes intercalate finer and finer crevices of the AL neuropil (Fig. 3C). Timing of secondary astrocyte process infiltration in the AL coincides with what appears to be the brain-wide onset of synaptogenesis (Muthukumar et al. 2014) (Fig. 3C). Furthermore, synapse number in adult AL and MB dorsal lobe are significantly reduced when primary and secondary astrocytes are ablated before 48hrs APF (Muthukumar et al. 2014). In the mammalian nervous system, it is well documented that astrocytes express a large repertoire of secreted and contact-mediated molecules that modulate synaptogenesis (Baldwin and Eroglu 2017; Allen and Eroglu 2017). Together, these data suggest astrocytes provide synaptogenic factors to regulate developmental circuit wiring across the fly central brain. Some of these factors could limit synapse production and thus instruct sparse wiring in the calyx (Kucukdereli et al. 2011). Astrocytes could sparsen connectivity or contribute to PN:KC quantitative matching in several other ways. For example, astrocytes could eat excess PN boutons (Lee and Chung 2019), or modulate spontaneous neural activity often required for synapse refinement (Akin et al. 2019). Whether and how astrocytes sculpt input number between PNs and KCs necessitates further study.

As with astrocytes, little is known about the role of EG during calyx development. However, we do have some understanding of EG development brain-wide and in the AL. Such knowledge may elucidate how EG development interfaces with sparse wiring of PN:KC connections in the calyx. EG are generated and culled in a similar manner and timeline as astrocytes (Omoto et al. 2015) (Fig. 3C). In the AL, EG wrap individual glomeruli and undergo morphological maturation during key OSN:PN wiring events from 24–72h APF

(Fig. 3C). When normal EG morphogenesis is disrupted via obstruction of FGF signaling, OSNs no longer restrict their axons to specific glomeruli, resulting in loss of glomerular borders and mixing of OSN inputs (Wu et al. 2017).

EG are thought to be oligodendrocyte-like cells (Yildirim et al. 2019). In the developing mouse, immature oligodendrocyte progenitor cells make functional synapses with neurons, and thus are well positioned to influence synapse development (Bergles et al. 2000; Lin and Bergles 2004; Berret et al. 2017). While it is still unknown what roles oligodendrocytes play in synaptogenesis, it was recently observed that oligodendrocytes can at a minimum influence developing synapses via regulation of synaptic transmission and presynaptic plasticity (Jang et al. 2019). It remains to be determined whether EG ever make contacts with synapses or instruct synapse development in the fly. While EG processes have not been found to infiltrate adult calyx microglomeruli, their processes do permeate the adult calyx (Kremer et al. 2017) (Fig. 3C). The mere proximity of EG processes to adult PN:KC synapses creates cause for exploring whether EG contribute in any way to the development of sparse connectivity in the calyx.

### What about the other neurons?

Among the secondary neurons innervating the calyx, the pair of GABAergic APL neurons have the broadest innervation (Fig. 3D). APL neurons participate in both the adult and larval calyx, and are likely born embryonically given Gal4 drivers allow for visualization of these neurons in first instar larva. During metamorphosis, APL processes which innervated the larval MB are pruned starting at 6hrs APF, immediately after the onset of PN and KC pruning events in the calyx. By 12hrs APF, only primary APL processes remain in the calyx and lobes. Regrowth of APL processes is first observed as a few APL neurites innervating the calyx at 18hrs APF, with extensive regrowth into the calyx by 24hrs APF (Fig. 3C). 24hrs APF also marks the start of APL process innervation out of the calyx and into the lobes (Fig. 3C). The relationship between APL and calyx development has not been explored beyond these morphological descriptions. However, we at least know that APL development is tightly coupled with MB lobe development, with KCs appearing to lead the way. Pruning of APL processes in the lobes does not occur if γ KC axon pruning is blocked. Furthermore, both reducing neurotransmission in KCs and increasing adhesion between KCs and PNs results in the persistence of larval APL processes in the MB lobes. Moreover, ablation of APL neurons or cell-autonomous blockade of APL process pruning demonstrates that morphogenesis of KC lobes is not dependent on APL neurons (Mayseless et al. 2018). If such rules hold true in the calyx, then perhaps KCs provide instructions to multiple, if not all, presynaptic partners as a master driver of calyx wiring decisions. Furthermore, these results highlight that KCs can provide activity-dependent instructions to their partners, at least post-synaptically.

As for all other secondary neurons which contribute to calyx architecture, nothing is known of their development.

## Molecular mechanisms driving sparse wiring in the calyx

We have limited insight as to molecular mechanisms important for generating sparse wiring in the calyx. Currently identified genes could impact the degree of sparse coding by KCs via regulation of PN collateral or KC dendrite targeting, morphogenesis (i.e. extension and branching), and pruning. However, molecules which quantitatively match PN inputs to KC number have yet to be identified.

For sensory information about the outside world to make it to higher order perceptual centers, interneuron axons must find their targets and then generate the correct number of inputs. What molecules allow PNs to achieve these tasks? Semaphorin-1A (Sema1a) is the only cell-surface molecule thus far identified to draw PN axon collaterals into the calyx (Komiyama et al. 2007). When *Sema1a* is absent in single PNs, collaterals which usually innervate the calyx become mistargeted. Not all PNs utilize *Sema1a* to appropriately target their collaterals. PNs with dorsolateral dendritic targeting highly express *Sema1a* and demonstrate the most penetrant axon collateral targeting phenotypes in the absence of *Sema1a*, with the contrary being true for PNs with ventromedial dendritic targeting. PN collaterals which do mistarget outside of the calyx also become abundantly over-branched. This suggests that signals local to the calyx may limit PN input quantity. Elimination of the transmembrane cell-adhesion molecule N-cadherin in individual PNs increases the number of higher-order axon terminal branches without impacting collateral targeting (Zhu and Luo 2004), hinting at the adhesion of PN collaterals to calyx-specific structures as a mechanism for restricting input number to KCs.

Insight into the identities of other cell-surface PN molecules which regulate axon collateral targeting and branching could be gleaned by further studies of three molecules: the transcription factor Lola; the SUMO protease Velo, whose substrates in PNs have been proposed to be transcription factors or chromatin organizers; and the serine/threonine-protein kinase Unc-51. PNs mutant for either *lola* or *velo* present similar phenotypes: reduced axon collateral branching, and thus likely reduced bouton number, and sometimes the complete lack of collateral innervation into the calyx (Spletter et al. 2007; Mochizuki et al. 2011; Berdnik et al. 2012). *lola* defects in mutant PNs extend beyond these phenotypes and can include ectopic branching outside of the calyx or axon bifurcation prior to calyx entry. Variations in *lola* mutant phenotypes may be due to differential usage of its many isoforms by PNs. For example, overexpressing the *lola L* isoform in single PNs frequently increases collateral branching within the calyx and *lola T* overexpression induces significant ectopic PN innervation of brain regions beyond the calyx, while *lola A* overexpression has no effect. This suggests the collection of Lola transcription factors could regulate expression of several genes important for different steps of generating the appropriate degree of PN input sparsity to KCs. In contrast to *lola* and *velo* mutants, single-cell PN clones mutant for *unc-51* demonstrate increased collateral branching into the calyx (Mochizuki et al. 2011). This could be due to Unc-51 regulating the localization of unknown cell-surface proteins utilized by PNs to restrict calyx input number, given many molecules require Unc-51 for their appropriate subcellular localization (Lai and Garriga 2004; Sakamoto et al. 2005; Ogura and Goshima 2006; Watari-Goshima et al. 2007). Together, such results suggest that multiple molecules acting on different morphogenic steps in PNs work together to set the

number of PN inputs to KCs, and that extrinsic factors which instruct PN input sparsity are local to the calyx.

Sparse coding requires that KC dendrites are equipped to receive the correct number of inputs and have found the appropriate presynaptic partners. As a result, fine control over KC dendritic morphogenesis is needed. While molecular mechanisms behind how KCs make their dendrites and claws are poorly understood, some groundwork has been laid. Single larval KCs mutant for *Trio*, which encodes a GEF known to activate Rho family GTPases, are found to have a bundle of overextended putative dendrites traveling beyond the calyx (Awasaki et al. 2000). *RhoA* mutant larval KCs also have overextended dendrites which are increased in length and number, although they do not fasciculate as occurs in *Trio* mutants (Lee et al. 2000b). Opposite of *Trio* and *RhoA* larval phenotypes, $Rac1^{J11}$ $Rac2^{\Delta}$ $Mtl^{\Delta}$ mutant adult KC clones experience a reduction in dendritic length (Ng et al. 2002). Trio is known to activate RhoA and Rac1 (van Rijssel and van Buul 2012). Thus, the aforementioned mutants may be disrupting shared signaling pathways involved in limiting KC dendrite extension which together determine KC dendrite length and number. Such a mechanism could impact from who and to what degree sparse inputs are received by KCs. What cell-surface proteins on KCs could be transmitting these signals? Work on KC axon development found that Trio and Rac1 may be downstream effectors of the L1-type cell adhesion molecule Neuroglian (NRG) (Goossens et al. 2011). Axon guidance defects in NRG-overexpressing KCs were suppressed by *Trio* mutations and enhanced by *Rac1* mutations, suggesting Trio is directly downstream of NRG and in the same pathway, while Rac1 does not directly interact with NRG and could be part of multiple pathways. Mutagenized alleles of *Nrg*, originally called *"central brain deranged" (*$ceb^{1}$, $ceb^{2}$), are described as having enlarged and misshapen calyces (de Belle and Heisenberg 1996). Whether these calyx abnormalities are due to the axon stalling phenotypes of *Nrg* mutants described by Goossens *et al.* or instead a direct result of KC dendrite abnormalities is unknown. The only KC dendritic phenotype in *Nrg* mutants thus far noted is from a short description by Goossens *et al.*, which mentions the occasional observation of KCs devoid of dendrites.

While deciphering the roles of *Nrg* in KC dendrite morphogenesis requires further study, these results do point to a gene network which may include signaling through NRG via Trio, RhoA, and Rac1 to control multiple aspects of KC dendrite morphogenesis. A variety of other genes could be part of this network. *Flamingo* (a transmembrane cadherin), *Unc-51* (a serine/threonine-protein kinase), *Klc* (a kinesin light chain), *Short stop* (a spectraplakin that cross-links actin and microtubules), *Heron* (mutagenized allele, gene unknown), and *Kali* (mutagenized allele, gene unknown) mutants all have excessively long KC dendrites (Reuter et al. 2003; Mochizuki et al. 2011). Slight phenotypic differences exist across these highly similar mutants, suggesting multiple intersecting pathways are at play in controlling KC dendrite length.

KCs mutant for the negative translational regulator *Dfmr* have increased branch and claw numbers without changes in dendritic length, suggesting DFMR works in pathways dedicated only to controlling dendritic elaboration (Pan et al. 2004). Mutants with diminished KC dendrite length have also been observed. These include *Lis1* (a centrosomal

protein that regulates microtubule organization), *Dhc64c* (a dynein heavy chain), and *Roadblock* (a dynein light chain) (Liu et al. 2000; Reuter et al. 2003). Both *Lis1* and *Dhc64c* mutants additionally displayed reduced branch and claw numbers (the opposite of *Dfmr* mutants), and *Roadblock* mutants have thus far been shown to display a reduction in dendritic branch point number. The similar phenotypes of *Lis1, Dhc64c*, and *Roadblock* suggest they work together to promote dendrite length and branching.

Overall, achieving sparsely wired connections between KCs and PNs may require balancing signals that promote and restrict KC dendrite length, number and branching. It is notable that the aforementioned mutants for motor protein components (i.e. kinesin mutant: *Klc;* dynein mutants: *Dhc64c* and *Roadblock*) have opposing phenotypes. Identifying the cargo transported by kinesin motors to restrict KC dendrite length, and by dynein motors to increase KC dendrite length and claw number, could aid in further elucidating molecular mechanisms of KC dendrite development.

Because the calyx is built twice, pruning and re-extension of PN collaterals and KC dendrites is requisite for sparsely wiring the adult calyx. PNs and KCs appear to share molecular mechanisms to coordinate pruning of their larval connections. These synaptic partners both: 1) express EcR-B1 at the third instar stage; 2) signal through the same TGF-β type 1 receptor (Baboon) for induction of EcR-B1 expression; and 3) require the EcR co-receptor ultraspiricle (USP) for ecdysone-dependent pruning to occur (Lee et al. 2000a; Zheng et al. 2003; Marin et al. 2005). The identity of genes downstream of EcR-B1/USP-driven pruning signals is not clear. Following pruning, KCs mutant for *Cullin3* (encoding a component of ubiquitin ligase complexes) re-extend dendrites which are short and often lack claws (Zhu et al. 2005). Thus, perhaps *Cullin3* and other ubiquitin ligase complex components are one set of transcriptional targets of EcR-B1/USP signaling. *Cullin3* mutant phenotypes also suggest that degradation of presently unidentified proteins set dendrite length and claw number, at least after pruning. Whether *Cullin3* generally controls KC dendrite length and claw number would be interesting to explore. A large percentage of *Baboon* mutant PNs lack adult-type collaterals, a phenotype not observed in KC dendrites (Marin et al. 2005). Whether this occurs through an EcR–independent pathway is unclear. If the *Baboon* mutant phenotype is a result of obstructed EcR-signaling, it may be that some downstream pathways utilized for ecdysone-dependent calyx re-wiring are different between PNs and KCs.

Several open questions remain regarding molecular mechanisms driving sparse wiring in the calyx. First, do molecules derived from glia or secondary neurons regulate calyx wiring in any way? No study to date has demonstrated this. Second, what happens to synaptic partners in the above mutants? For example, do mistargeted PNs or KCs find new partners, or do their usual partners follow them? Answers to such questions would help demystify whether calyx wiring follows the rules of Sperry (i.e. synaptic partners express a unique set of complementary molecules that match them together), Peters (i.e. neurons synapse with any available partners, and global positioning cues are all that narrow down possible partner choices), or a mixture of both (Langley 1895; Sperry 1963; Peters and Feldman 1976; Kutsarova et al. 2016; Rees et al. 2017). Third, how do PNs and KCs sense where to elaborate their processes? Given the large number of KC dendrite overextension mutants,

it could be that KCs utilize extrinsic “stop cues” to terminate dendrite extension inside the calyx. In comparison, results for *Sema1a* mutant PNs suggest extra-calyx repulsive cues keep PN collaterals confined to the calyx. Fourth, do KCs provide feedback to PNs to limit final bouton number? While no such molecular signaling between KCs and PNs has yet been identified, work in the cerebellum provides insight as to what such signaling might look like. This is the subject of discussion in the following section.

## Developmental insights from the cerebellum

The PN:KC synapse is remarkably similar to the MF:GC synapse in the mammalian cerebellum. Cerebellar MFs and GCs can be thought of as being analogous to the *Drosophila* olfactory PNs and KCs, respectively. MFs originate from several sources, with the majority arising from pontine nuclei. Despite having 50 billion cerebellar GCs in the human brain, each GC only receives input from around four MFs, similar to KCs receiving four to five inputs on average (Eccles et al. 1966; Llinás and R 1998). In addition to sharing the feature of sparse input density with PN:KC connections of the *Drosophila* MB calyx, the cerebellar GC inputs are also observed to be largely unpredictable (Huang et al. 2013; Kennedy et al. 2014; Chabrol et al. 2015; Ishikawa et al. 2015). Cerebellar GCs also share the unique dendritic claw-like structure with KCs (Palay and Chan-Palay 1974; Leiss et al. 2009). Owing to these striking similarities in connectivity, we turn to the cerebellum to derive insights for mechanisms dictating sparse wiring in the fly. The aim of this section is not to offer an exhaustive list of molecular mechanisms underlying development of the MF:GC synapse, but to offer ideas to test unexplored mechanisms that can potentially apply to sparse connectivity in the calyx.

Some parallels to the molecular mechanisms dictating connectivity between PNs and KCs in the previous section can be seen in the cerebellum. Cerebellar GCs also use transcriptional regulators to control dendrite morphogenesis (i.e. FoxO6, NeuroD) (Gaudillière et al. 2004; de la Torre-Ubieta et al. 2010). Similar to N-cadherins and Flamingo in the calyx (Reuter et al. 2003; Zhu and Luo 2004), Cadherin-7 (Cdh7) mediates axon targeting (Kuwako et al. 2014). Cdh7 is expressed in both cerebellar GCs and pontine nuclei-derived MFs and plays a unique role in both inhibiting axon extension beyond the target region and mediating synaptic specificity. Knocking down Cdh7 in pontine nuclei neurons reduced the number of MF:GC synapses (Kuwako et al. 2014). This selective requirement of a single cell-adhesion factor for a sparsely wired synapse brings up the question: would PNs and KCs in the calyx rely on such a single cell surface molecule to wire with the correct partners, or would they use combinatorial expression of multiple cell surface molecules to allow for variability in the sets of connections?

Just as KCs appear to utilize ubiquitin ligase complexes to control dendritic morphogenesis at least after pruning (Zhu et al. 2005), cerebellar GCs also express Cul7-Fbxw8 which regulates dendritic number, growth, and elaboration in the developing rat cerebellum (Litterman et al. 2011). Given this highly comparable role of ubiquitin ligases, knowing the target proteins degraded by these complexes in GCs will be informative in thinking about what allows a GC dendrite to end up with a particular number of branches. If similar

proteins are targeted by *Drosophila* ubiquitin ligases, this could offer a crucial starting point into deciphering what sets up the number of KC inputs.

There is little known about axon guidance of MFs onto GCs; however, observations from manipulations to cerebellar GCs can offer clues as to what sets up the MF:GC synapse. One such observation comes from studying Semaphorins, which have well-defined roles in nervous system development especially for neuronal migration and axon guidance (Fiore and Püschel 2003). Loss of Sema6A leads to ectopic GC migration to the molecular layer instead of the internal granule layer (Kerjan et al. 2005). Strikingly, MFs are still able to locate and synapse with GCs at those ectopic sites (Kerjan et al. 2005). Furthermore, *in vitro* studies show that GCs exhibit partner-specific connectivity to MFs: in a culture with axons derived from different neural regions, GCs are able to identify their MF partners and do not synapse with other axons (Ito and Takeichi 2009). Altogether, these studies bring up the idea that GC-intrinsic mechanisms may be dictating the MF:GC connectivity, rather than the MFs. This could indicate MFs rely on attractant cues secreted by GCs.

As Semaphorins serve as both attractants and repellants and have been identified in vertebrates as well as invertebrates (Raper 2000), it will be of interest for future studies to test these genes for a role in establishing contact between PNs and KCs. One such study is discussed in the previous section, which shows that loss of Sema1a leads to mistargeting of PNs from the calyx (Komiyama et al. 2007). However, they do not investigate localization of KC dendritic claws. If KCs are seen to extend dendrites to the ectopic location of the PN axons, that would illustrate that KCs use partner-derived cues from PNs. If, in contrast, they do not go to the new location, that would rule out dependence of KCs on partner-derived cues, at least with respect to the establishment of synaptic connections.

In thinking about partner-derived cues, another interesting gene family to consider are the fibroblast growth factors (FGFs). FGFs have been shown to be important for mediating presynaptic differentiation at the MF:GC synapse (Umemori et al. 2004). Specifically, FGF22 is expressed by cerebellar GCs at the time of synaptogenesis, and its main partner receptor, FGFR2b, is expressed in pontine and vestibular MFs (Miki et al. 1992; Ornitz et al. 1996; Umemori et al. 2004). Inhibition of FGF22 in cerebellar GCs inhibits clustering of synaptic vesicles at the MF:GC synapse, emphasizing the role of FGF22 as a retrograde signal that regulates synapse formation (Umemori et al. 2004). This finding suggests that postsynaptic cerebellar GCs (and thus KCs) provide some kind of feedback to presynaptic MFs (and thus PNs). Other presynaptic organizing factors have been identified at this synapse, such as WNT-7a (Hall et al. 2000) and neuroligin (Scheiffele et al. 2000). A question for future studies to ask is: could such a feedback mechanism set the number of connections, and thus control sparse wiring in the cerebellum and MB calyx? Answering this question in the calyx will require screening for retrograde presynaptic organizing molecules and observing effects of their manipulation on sparse connectivity and sparse coding.

## Conclusion

How does the brain build neural circuits capable of perceiving unpredicted stimuli in a constantly changing environment? Sensory information processing relies on a general principle of expanding limited inputs onto a larger number of neurons via sparse, combinatorial wiring. One example of this coding strategy is the MB calyx of the fly olfactory system. Here, ~2,000 KCs each receive information from a select number of odorant receptor channels via sparse, unstructured combinatorial inputs from olfactory PNs, such that select numbers of KCs respond to unique combinations of sensory inputs. In this review, we took a holistic approach in discussing our current knowledge regarding the development of sparse connectivity in the calyx. While our understanding of this is currently limited, we do know that PNs scale bouton number in response to changes in KC number during development, and that this response is graded. Further, individual PNs also compensate in their bouton production for changes in overall PN number, while KC cell or claw number do not change. Development of APL inputs to the calyx appear to be similarly dependent on KCs. These results suggest KCs drive calyx wiring. While we don't know much about the molecular mechanisms instructing sparse wiring, we have suggested hypotheses based on the results of a small collection of experiments. Future progress in our understanding of calyx development will require elucidating: 1) whether KCs truly are the drivers of calyx wiring; 2) the role of activity, if any, in determining the number of PN:KC connections; and 3) what influence glia and secondary neurons have on the decision for PNs and KCs to make sparse, combinatorial connections. Importantly, what we learn from how the fly brain constructs a sparsely wired olfactory circuit will likely also have direct applications to understanding how sensory systems build circuits across taxa.

## Acknowledgements

We thank Shahara Miah and Donnell Williams for their assistance compiling literature. We also thank Caitlin Paisley and Cameron Prigge for critical reading of this manuscript. This work was made possible by funding from the National Institutes of Health (R01DC018032) to EJC, and a University of Michigan Institutional Research and Academic Career Development Award (IRACDA) (K12-GM111725) to VMP. EJC is an Alfred P Sloan Research Fellow in Neuroscience and the Rita Allen Foundation Milton Cassell Scholar.

## References

Ache BW, Young JM (2005) Olfaction: diverse species, conserved principles. Neuron 48:417–30. 10.1016/j.neuron.2005.10.022 [PubMed: 16269360]

Akin O, Bajar BT, Keles MF, et al. (2019) Cell-type-Specific Patterned Stimulus-Independent Neuronal Activity in the Drosophila Visual System during Synapse Formation. Neuron 101:894–904.e5. 10.1016/j.neuron.2019.01.008 [PubMed: 30711355]

Allen NJ, Eroglu C (2017) Cell Biology of Astrocyte-Synapse Interactions. Neuron 96:697–708. 10.1016/j.neuron.2017.09.056 [PubMed: 29096081]

Aso Y, Grübel K, Busch S, et al. (2009) The mushroom body of adult Drosophila characterized by GAL4 drivers. J Neurogenet 23:156–72. 10.1080/01677060802471718 [PubMed: 19140035]

Aso Y, Hattori D, Yu Y, et al. (2014) The neuronal architecture of the mushroom body provides a logic for associative learning. Elife 3:e04577. 10.7554/eLife.04577 [PubMed: 25535793]

Awasaki T, Lai S-L, Ito K, Lee T (2008) Organization and postembryonic development of glial cells in the adult central brain of Drosophila. J Neurosci 28:13742–53. 10.1523/JNEUROSCI.4844-08.2008 [PubMed: 19091965]

Awasaki T, Saito M, Sone M, et al. (2000) The Drosophila trio plays an essential role in patterning of axons by regulating their directional extension. Neuron 26:119–31. 10.1016/s0896-6273(00)81143-5 [PubMed: 10798397]

Baldwin KT, Eroglu C (2017) Molecular mechanisms of astrocyte-induced synaptogenesis. Curr Opin Neurobiol 45:113–120. 10.1016/j.conb.2017.05.006 [PubMed: 28570864]

Baltruschat L, Ranft P, Prisco L, et al. (2020) Circuit reorganization in the Drosophila mushroom body calyx accompanies memory consolidation. bioRxiv. 10.1101/2020.08.20.259135

Bates AS, Schlegel P, Roberts RJ V, et al. (2020) Complete Connectomic Reconstruction of Olfactory Projection Neurons in the Fly Brain. Curr Biol 30:3183–3199.e6. 10.1016/j.cub.2020.06.042 [PubMed: 32619485]

Berdnik D, Favaloro V, Luo L (2012) The SUMO protease Verloren regulates dendrite and axon targeting in olfactory projection neurons. J Neurosci 32:8331–40. 10.1523/JNEUROSCI.6574-10.2012 [PubMed: 22699913]

Bergles DE, Roberts JD, Somogyi P, Jahr CE (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187–91. 10.1038/35012083 [PubMed: 10821275]

Berret E, Barron T, Xu J, et al. (2017) Oligodendroglial excitability mediated by glutamatergic inputs and Nav1.2 activation. Nat Commun 8:557. 10.1038/s41467-017-00688-0 [PubMed: 28916793]

Butcher NJ, Friedrich AB, Lu Z, et al. (2012) Different classes of input and output neurons reveal new features in microglomeruli of the adult Drosophila mushroom body calyx. J Comp Neurol 520:2185–2201. 10.1002/cne.23037 [PubMed: 22237598]

Campbell RAA, Honegger KS, Qin H, et al. (2013) Imaging a population code for odor identity in the Drosophila mushroom body. J Neurosci 33:10568–81. 10.1523/JNEUROSCI.0682-12.2013 [PubMed: 23785169]

Caron SJC, Ruta V, Abbott LF, Axel R (2013) Random convergence of olfactory inputs in the Drosophila mushroom body. Nature 497:113–117. 10.1038/nature12063 [PubMed: 23615618]

Cayco-Gajic NA, Clopath C, Silver RA (2017) Sparse synaptic connectivity is required for decorrelation and pattern separation in feedforward networks. Nat Commun 8:1116. 10.1038/s41467-017-01109-y [PubMed: 29061964]

Cayco-Gajic NA, Silver RA (2019) Re-evaluating Circuit Mechanisms Underlying Pattern Separation. Neuron 101:584–602. 10.1016/j.neuron.2019.01.044 [PubMed: 30790539]

Chabrol FP, Arenz A, Wiechert MT, et al. (2015) Synaptic diversity enables temporal coding of coincident multisensory inputs in single neurons. Nat Neurosci 18:718–27. 10.1038/nn.3974 [PubMed: 25821914]

Chen Y, Akin O, Nern A, et al. (2014) Cell-type-specific labeling of synapses in vivo through synaptic tagging with recombination. Neuron 81:280–93. 10.1016/j.neuron.2013.12.021 [PubMed: 24462095]

Chin SG, Maguire SE, Huoviala P, et al. (2018) Olfactory Neurons and Brain Centers Directing Oviposition Decisions in Drosophila. Cell Rep 24:1667–1678. 10.1016/j.celrep.2018.07.018 [PubMed: 30089274]

Chou Y-H, Spletter ML, Yaksi E, et al. (2010) Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. Nat Neurosci 13:439–49. 10.1038/nn.2489 [PubMed: 20139975]

Couto A, Alenius M, Dickson BJ (2005) Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr Biol 15:1535–47. 10.1016/j.cub.2005.07.034 [PubMed: 16139208]

de Belle JS, Heisenberg M (1996) Expression of Drosophila mushroom body mutations in alternative genetic backgrounds: a case study of the mushroom body miniature gene (mbm). Proc Natl Acad Sci U S A 93:9875–80. 10.1073/pnas.93.18.9875 [PubMed: 8790424]

de la Torre-Ubieta L, Gaudillière B, Yang Y, et al. (2010) A FOXO-Pak1 transcriptional pathway controls neuronal polarity. Genes Dev 24:799–813. 10.1101/gad.1880510 [PubMed: 20395366]

Devaud J-M, Acebes A, Ramaswami M, Ferrús A (2003) Structural and functional changes in the olfactory pathway of adult Drosophila take place at a critical age. J Neurobiol 56:13–23. 10.1002/neu.10215 [PubMed: 12767029]

Doll CA, Vita DJ, Broadie K (2017) Fragile X Mental Retardation Protein Requirements in Activity-Dependent Critical Period Neural Circuit Refinement. Curr Biol 27:2318–2330.e3. 10.1016/j.cub.2017.06.046 [PubMed: 28756946]

Eccles JC, Llinás R, Sasaki K (1966) The mossy fibre-granule cell relay of the cerebellum and its inhibitory control by Golgi cells. Exp brain Res 1:82–101. 10.1007/BF00235211 [PubMed: 5910945]

Eichler K, Li F, Litwin-Kumar A, et al. (2017) The complete connectome of a learning and memory centre in an insect brain. Nature 548:175–182. 10.1038/nature23455 [PubMed: 28796202]

Elkahlah NA, Rogow JA, Ahmed M, Clowney EJ (2020) Presynaptic developmental plasticity allows robust sparse wiring of the Drosophila mushroom body. Elife 9:. 10.7554/eLife.52278

Fiete IR, Hahnloser RHR, Fee MS, Seung HS (2004) Temporal sparseness of the premotor drive is important for rapid learning in a neural network model of birdsong. J Neurophysiol 92:2274–82. 10.1152/jn.01133.2003 [PubMed: 15071087]

Fiore R, Püschel AW (2003) The function of semaphorins during nervous system development. Front Biosci 8:s484–99. 10.2741/1080 [PubMed: 12700098]

Fishilevich E, Vosshall LB (2005) Genetic and functional subdivision of the Drosophila antennal lobe. Curr Biol 15:1548–53. 10.1016/j.cub.2005.07.066 [PubMed: 16139209]

Frank DD, Jouandet GC, Kearney PJ, et al. (2015) Temperature representation in the Drosophila brain. Nature 519:358–61. 10.1038/nature14284 [PubMed: 25739506]

Fuss SH, Ray A (2009) Mechanisms of odorant receptor gene choice in Drosophila and vertebrates. Mol Cell Neurosci 41:101–12. 10.1016/j.mcn.2009.02.014 [PubMed: 19303443]

Gao Q, Yuan B, Chess A (2000) Convergent projections of Drosophila olfactory neurons to specific glomeruli in the antennal lobe. Nat Neurosci 3:780–5. 10.1038/77680 [PubMed: 10903570]

Garcia-Lazaro JA, Ahmed B, Schnupp JWH (2006) Tuning to natural stimulus dynamics in primary auditory cortex. Curr Biol 16:264–71. 10.1016/j.cub.2005.12.013 [PubMed: 16461279]

Gaudillière B, Konishi Y, de la Iglesia N, et al. (2004) A CaMKII-NeuroD signaling pathway specifies dendritic morphogenesis. Neuron 41:229–41. 10.1016/s0896-6273(03)00841-9 [PubMed: 14741104]

Gomez-Diaz C, Martin F, Garcia-Fernandez JM, Alcorta E (2018) The Two Main Olfactory Receptor Families in Drosophila, ORs and IRs: A Comparative Approach. Front Cell Neurosci 12:253. 10.3389/fncel.2018.00253 [PubMed: 30214396]

Goossens T, Kang YY, Wuytens G, et al. (2011) The Drosophila L1CAM homolog Neuroglian signals through distinct pathways to control different aspects of mushroom body axon development. Development 138:1595–605. 10.1242/dev.052787 [PubMed: 21389050]

Grabe V, Baschwitz A, Dweck HKM, et al. (2016) Elucidating the Neuronal Architecture of Olfactory Glomeruli in the Drosophila Antennal Lobe. Cell Rep 16:3401–3413. 10.1016/j.celrep.2016.08.063 [PubMed: 27653699]

Gruntman E, Turner GC (2013) Integration of the olfactory code across dendritic claws of single mushroom body neurons. Nat Neurosci 16:1821–9. 10.1038/nn.3547 [PubMed: 24141312]

Hall AC, Lucas FR, Salinas PC (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell 100:525–35. 10.1016/s0092-8674(00)80689-3 [PubMed: 10721990]

Hallem EA, Carlson JR (2006) Coding of odors by a receptor repertoire. Cell 125:143–60. 10.1016/j.cell.2006.01.050 [PubMed: 16615896]

Handler A, Graham TGW, Cohn R, et al. (2019) Distinct Dopamine Receptor Pathways Underlie the Temporal Sensitivity of Associative Learning. Cell 178:60–75.e19. 10.1016/j.cell.2019.05.040 [PubMed: 31230716]

Harvey RJ, Napper RM (1991) Quantitative studies on the mammalian cerebellum. Prog Neurobiol 36:437–63. 10.1016/0301-0082(91)90012-p [PubMed: 1947173]

Heimbeck G, Bugnon V, Gendre N, et al. (2001) A central neural circuit for experience-independent olfactory and courtship behavior in Drosophila melanogaster. Proc Natl Acad Sci U S A 98:15336–41. 10.1073/pnas.011314898 [PubMed: 11742061]

Hige T (2018) What can tiny mushrooms in fruit flies tell us about learning and memory? Neurosci Res 129:8–16. 10.1016/j.neures.2017.05.002 [PubMed: 28483586]

Honegger KS, Campbell RAA, Turner GC (2011) Cellular-resolution population imaging reveals robust sparse coding in the Drosophila mushroom body. J Neurosci 31:11772–85. 10.1523/JNEUROSCI.1099-11.2011 [PubMed: 21849538]

Hoyle G (1986) Glial cells of an insect ganglion. J Comp Neurol 246:85–103. 10.1002/cne.902460106 [PubMed: 3700719]

Huang C-C, Sugino K, Shima Y, et al. (2013) Convergence of pontine and proprioceptive streams onto multimodal cerebellar granule cells. Elife 2:e00400. 10.7554/eLife.00400 [PubMed: 23467508]

Hume RI, Role LW, Fischbach GD (1983) Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes. Nature 305:632–4. 10.1038/305632a0 [PubMed: 6621712]

Inada K, Tsuchimoto Y, Kazama H (2017) Origins of Cell-Type-Specific Olfactory Processing in the Drosophila Mushroom Body Circuit. Neuron 95:357–367.e4. 10.1016/j.neuron.2017.06.039 [PubMed: 28728024]

Ishikawa T, Shimuta M, Häusser M (2015) Multimodal sensory integration in single cerebellar granule cells in vivo. Elife 4:. 10.7554/eLife.12916

Ito S, Takeichi M (2009) Dendrites of cerebellar granule cells correctly recognize their target axons for synaptogenesis in vitro. Proc Natl Acad Sci U S A 106:12782–7. 10.1073/pnas.0906653106 [PubMed: 19622730]

Jacobson GA, Friedrich RW (2013) Neural circuits: random design of a higher-order olfactory projection. Curr Biol 23:R448–51. 10.1016/j.cub.2013.04.016 [PubMed: 23701688]

Jang M, Gould E, Xu J, et al. (2019) Oligodendrocytes regulate presynaptic properties and neurotransmission through BDNF signaling in the mouse brainstem. Elife 8:. 10.7554/eLife.42156

Jeanne JM, Fişek M, Wilson RI (2018) The Organization of Projections from Olfactory Glomeruli onto Higher-Order Neurons. Neuron 98:1198–1213.e6. 10.1016/j.neuron.2018.05.011 [PubMed: 29909998]

Jefferis GSXE, Marin EC, Stocker RF, Luo L (2001) Target neuron prespecification in the olfactory map of Drosophila. Nature 414:204–8. 10.1038/35102574 [PubMed: 11719930]

Jefferis GSXE, Potter CJ, Chan AM, et al. (2007) Comprehensive Maps of Drosophila Higher Olfactory Centers: Spatially Segregated Fruit and Pheromone Representation. Cell 128:1187–1203. 10.1016/j.cell.2007.01.040 [PubMed: 17382886]

Jefferis GSXE, Vyas RM, Berdnik D, et al. (2004) Developmental origin of wiring specificity in the olfactory system of Drosophila. Development 131:117–30. 10.1242/dev.00896 [PubMed: 14645123]

Kanerva P (1988) Sparse Distributed Memory. Cambridge MA

Keene AC, Waddell S (2007) Drosophila olfactory memory: single genes to complex neural circuits. Nat Rev Neurosci 8:341–54. 10.1038/nrn2098 [PubMed: 17453015]

Kennedy A (2019) Learning with naturalistic odor representations in a dynamic model of the Drosophila olfactory system. bioRxiv 783191. 10.1101/783191

Kennedy A, Wayne G, Kaifosh P, et al. (2014) A temporal basis for predicting the sensory consequences of motor commands in an electric fish. Nat Neurosci 17:416–22. 10.1038/nn.3650 [PubMed: 24531306]

Kerjan G, Dolan J, Haumaitre C, et al. (2005) The transmembrane semaphorin Sema6A controls cerebellar granule cell migration. Nat Neurosci 8:1516–24. 10.1038/nn1555 [PubMed: 16205717]

Kirkhart C, Scott K (2015) Gustatory learning and processing in the Drosophila mushroom bodies. J Neurosci 35:5950–8. 10.1523/JNEUROSCI.3930-14.2015 [PubMed: 25878268]

Kobayakawa K, Kobayakawa R, Matsumoto H, et al. (2007) Innate versus learned odour processing in the mouse olfactory bulb. Nature 450:503–8. 10.1038/nature06281 [PubMed: 17989651]

Kohl J, Ostrovsky AD, Frechter S, Jefferis GSXE (2013) A bidirectional circuit switch reroutes pheromone signals in male and female brains. Cell 155:1610–23. 10.1016/j.cell.2013.11.025 [PubMed: 24360281]

Komiyama T, Sweeney LB, Schuldiner O, et al. (2007) Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. Cell 128:399–410. 10.1016/j.cell.2006.12.028 [PubMed: 17254975]

Kremer MC, Christiansen F, Leiss F, et al. (2010) Structural long-term changes at mushroom body input synapses. Curr Biol 20:1938–44. 10.1016/j.cub.2010.09.060 [PubMed: 20951043]

Kremer MC, Jung C, Batelli S, et al. (2017) The glia of the adult Drosophila nervous system. Glia 65:606–638. 10.1002/glia.23115 [PubMed: 28133822]

Kucukdereli H, Allen NJ, Lee AT, et al. (2011) Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. Proc Natl Acad Sci U S A 108:E440–9. 10.1073/pnas.1104977108 [PubMed: 21788491]

Kutsarova E, Munz M, Ruthazer ES (2016) Rules for Shaping Neural Connections in the Developing Brain. Front Neural Circuits 10:111. 10.3389/fncir.2016.00111 [PubMed: 28119574]

Kuwako K-I, Nishimoto Y, Kawase S, et al. (2014) Cadherin-7 regulates mossy fiber connectivity in the cerebellum. Cell Rep 9:311–323. 10.1016/j.celrep.2014.08.063 [PubMed: 25284782]

Lai T, Garriga G (2004) The conserved kinase UNC-51 acts with VAB-8 and UNC-14 to regulate axon outgrowth in C. elegans. Development 131:5991–6000. 10.1242/dev.01457 [PubMed: 15539493]

Langley JN (1895) Note on Regeneration of Prae-Ganglionic Fibres of the Sympathetic. J Physiol 18:280–4. 10.1113/jphysiol.1895.sp000566

Larsson MC, Domingos AI, Jones WD, et al. (2004) Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43:703–14. 10.1016/j.neuron.2004.08.019 [PubMed: 15339651]

Laurent G (2002) Olfactory network dynamics and the coding of multidimensional signals. Nat Rev Neurosci 3:884–95. 10.1038/nrn964 [PubMed: 12415296]

Lee E, Chung W-S (2019) Glial Control of Synapse Number in Healthy and Diseased Brain. Front Cell Neurosci 13:42. 10.3389/fncel.2019.00042 [PubMed: 30814931]

Lee T, Lee A, Luo L (1999) Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126:4065–76 [PubMed: 10457015]

Lee T, Marticke S, Sung C, et al. (2000a) Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in Drosophila. Neuron 28:807–18. 10.1016/s0896-6273(00)00155-0 [PubMed: 11163268]

Lee T, Winter C, Marticke SS, et al. (2000b) Essential roles of Drosophila RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. Neuron 25:307–16. 10.1016/s0896-6273(00)80896-x [PubMed: 10719887]

Leiss F, Groh C, Butcher NJ, et al. (2009) Synaptic organization in the adult Drosophila mushroom body calyx. J Comp Neurol 517:808–24. 10.1002/cne.22184 [PubMed: 19844895]

Levy P, Larsen C (2013) Odd-skipped labels a group of distinct neurons associated with the mushroom body and optic lobe in the adult Drosophila brain. J Comp Neurol 521:3716–40. 10.1002/cne.23375 [PubMed: 23749685]

Li F, Lindsey J, Marin EC, et al. (2020a) The connectome of the adult Drosophila mushroom body: implications for function. bioRxiv 2020.08.29.273276. 10.1101/2020.08.29.273276

Li J, Mahoney BD, Jacob MS, Caron SJC (2020b) Visual Input into the Drosophila melanogaster Mushroom Body. Cell Rep 32:108138. 10.1016/j.celrep.2020.108138 [PubMed: 32937130]

Lichtneckert R, Reichert H (2005) Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. Heredity (Edinb) 94:465–77. 10.1038/sj.hdy.6800664 [PubMed: 15770230]

Lin AC, Bygrave AM, de Calignon A, et al. (2014) Sparse, decorrelated odor coding in the mushroom body enhances learned odor discrimination. Nat Neurosci 17:559–68. 10.1038/nn.3660 [PubMed: 24561998]

Lin S, Bergles DE (2004) Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat Neurosci 7:24–32. 10.1038/nn1162 [PubMed: 14661022]

Litterman N, Ikeuchi Y, Gallardo G, et al. (2011) An OBSL1-Cul7Fbxw8 ubiquitin ligase signaling mechanism regulates Golgi morphology and dendrite patterning. PLoS Biol 9:e1001060. 10.1371/journal.pbio.1001060 [PubMed: 21572988]

Litwin-Kumar A, Harris KD, Axel R, et al. (2017) Optimal Degrees of Synaptic Connectivity. Neuron 93:1153–1164.e7. 10.1016/j.neuron.2017.01.030 [PubMed: 28215558]

Liu X, Davis RL (2009) The GABAergic anterior paired lateral neuron suppresses and is suppressed by olfactory learning. Nat Neurosci 12:53–9. 10.1038/nn.2235 [PubMed: 19043409]

Liu Z, Steward R, Luo L (2000) Drosophila Lis1 is required for neuroblast proliferation, dendritic elaboration and axonal transport. Nat Cell Biol 2:776–83. 10.1038/35041011 [PubMed: 11056531]

Llinás R (1998) Cerebellum. Synaptic Organ Brain 255–288

Machens CK, Stemmler MB, Prinz P, et al. (2001) Representation of acoustic communication signals by insect auditory receptor neurons. J Neurosci 21:3215–27 [PubMed: 11312306]

Marin EC, Büld L, Theiss M, et al. (2020) Connectomics Analysis Reveals First-, Second-, and Third-Order Thermosensory and Hygrosensory Neurons in the Adult Drosophila Brain. Curr Biol 30:3167–3182.e4. 10.1016/j.cub.2020.06.028 [PubMed: 32619476]

Marin EC, Jefferis GSXE, Komiyama T, et al. (2002) Representation of the glomerular olfactory map in the Drosophila brain. Cell 109:243–55. 10.1016/s0092-8674(02)00700-6 [PubMed: 12007410]

Marin EC, Watts RJ, Tanaka NK, et al. (2005) Developmentally programmed remodeling of the Drosophila olfactory circuit. Development 132:725–37. 10.1242/dev.01614 [PubMed: 15659487]

Marr D (1969) A theory of cerebellar cortex. J Physiol 202:437–70. 10.1113/jphysiol.1969.sp008820 [PubMed: 5784296]

Masuda-Nakagawa LM, Tanaka NK, O'Kane CJ (2005) Stereotypic and random patterns of connectivity in the larval mushroom body calyx of Drosophila. Proc Natl Acad Sci U S A 102:19027–32. 10.1073/pnas.0509643102 [PubMed: 16357192]

Mayseless O, Berns DS, Yu XM, et al. (2018) Developmental Coordination during Olfactory Circuit Remodeling in Drosophila. Neuron 99:1204–1215.e5. 10.1016/j.neuron.2018.07.050 [PubMed: 30146303]

Miki T, Bottaro DP, Fleming TP, et al. (1992) Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci U S A 89:246–50. 10.1073/pnas.89.1.246 [PubMed: 1309608]

Mochizuki H, Toda H, Ando M, et al. (2011) Unc-51/ATG1 controls axonal and dendritic development via kinesin-mediated vesicle transport in the Drosophila brain. PLoS One 6:e19632. 10.1371/journal.pone.0019632 [PubMed: 21589871]

Murthy M, Fiete I, Laurent G (2008) Testing odor response stereotypy in the Drosophila mushroom body. Neuron 59:1009–23. 10.1016/j.neuron.2008.07.040 [PubMed: 18817738]

Muthukumar AK, Stork T, Freeman MR (2014) Activity-dependent regulation of astrocyte GAT levels during synaptogenesis. Nat Neurosci 17:1340–50. 10.1038/nn.3791 [PubMed: 25151265]

Ng J, Nardine T, Harms M, et al. (2002) Rac GTPases control axon growth, guidance and branching. Nature 416:442–7. 10.1038/416442a [PubMed: 11919635]

Ogura K-I, Goshima Y (2006) The autophagy-related kinase UNC-51 and its binding partner UNC-14 regulate the subcellular localization of the Netrin receptor UNC-5 in Caenorhabditis elegans. Development 133:3441–50. 10.1242/dev.02503 [PubMed: 16887826]

Olsen SR, Bhandawat V, Wilson RI (2010) Divisive normalization in olfactory population codes. Neuron 66:287–99. 10.1016/j.neuron.2010.04.009 [PubMed: 20435004]

Olsen SR, Wilson RI (2008) Lateral presynaptic inhibition mediates gain control in an olfactory circuit. Nature 452:956–60. 10.1038/nature06864 [PubMed: 18344978]

Olshausen BA, Field DJ (2004) Sparse coding of sensory inputs. Curr Opin Neurobiol 14:481–7. 10.1016/j.conb.2004.07.007 [PubMed: 15321069]

Omoto JJ, Yogi P, Hartenstein V (2015) Origin and development of neuropil glia of the Drosophila larval and adult brain: Two distinct glial populations derived from separate progenitors. Dev Biol 404:2–20. 10.1016/j.ydbio.2015.03.004 [PubMed: 25779704]

Ornitz DM, Xu J, Colvin JS, et al. (1996) Receptor specificity of the fibroblast growth factor family. J Biol Chem 271:15292–7. 10.1074/jbc.271.25.15292 [PubMed: 8663044]

Palay SL, Chan-Palay V (1974) Cerebellar Cortex: Cytology and Organization

Pan L, Zhang YQ, Woodruff E, Broadie K (2004) The Drosophila fragile X gene negatively regulates neuronal elaboration and synaptic differentiation. Curr Biol 14:1863–70. 10.1016/j.cub.2004.09.085 [PubMed: 15498496]

Pauls D, Selcho M, Gendre N, et al. (2010) Drosophila larvae establish appetitive olfactory memories via mushroom body neurons of embryonic origin. J Neurosci 30:10655–66. 10.1523/JNEUROSCI.1281-10.2010 [PubMed: 20702697]

Pech U, Revelo NH, Seitz KJ, et al. (2015) Optical dissection of experience-dependent pre- and postsynaptic plasticity in the Drosophila brain. Cell Rep 10:2083–95. 10.1016/j.celrep.2015.02.065 [PubMed: 25818295]

Pereanu W, Shy D, Hartenstein V (2005) Morphogenesis and proliferation of the larval brain glia in Drosophila. Dev Biol 283:191–203. 10.1016/j.ydbio.2005.04.024 [PubMed: 15907832]

Perez-Orive J, Mazor O, Turner GC, et al. (2002) Oscillations and sparsening of odor representations in the mushroom body. Science 297:359–65. 10.1126/science.1070502 [PubMed: 12130775]

Peters A, Feldman ML (1976) The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. I. General description. J Neurocytol 5:63–84. 10.1007/BF01176183 [PubMed: 1249593]

Ramaekers A, Magnenat E, Marin EC, et al. (2005) Glomerular maps without cellular redundancy at successive levels of the Drosophila larval olfactory circuit. Curr Biol 15:982–92. 10.1016/j.cub.2005.04.032 [PubMed: 15936268]

Raper JA (2000) Semaphorins and their receptors in vertebrates and invertebrates. Curr Opin Neurobiol 10:88–94. 10.1016/s0959-4388(99)00057-4 [PubMed: 10679438]

Rees CL, Moradi K, Ascoli GA (2017) Weighing the Evidence in Peters' Rule: Does Neuronal Morphology Predict Connectivity? Trends Neurosci 40:63–71. 10.1016/j.tins.2016.11.007 [PubMed: 28041634]

Reuter JE, Nardine TM, Penton A, et al. (2003) A mosaic genetic screen for genes necessary for Drosophila mushroom body neuronal morphogenesis. Development 130:1203–13. 10.1242/dev.00319 [PubMed: 12571111]

Rieke F, Bodnar DA, Bialek W (1995) Naturalistic stimuli increase the rate and efficiency of information transmission by primary auditory afferents. Proceedings Biol Sci 262:259–65. 10.1098/rspb.1995.0204

Rolls ET, Treves A (1990) The relative advantages of sparse versus distributed encoding for associative neuronal networks in the brain. Netw Comput Neural Syst 1:407–421. 10.1088/0954-898X_1_4_002

Root CM, Masuyama K, Green DS, et al. (2008) A presynaptic gain control mechanism fine-tunes olfactory behavior. Neuron 59:311–21. 10.1016/j.neuron.2008.07.003 [PubMed: 18667158]

Ruta V, Datta SR, Vasconcelos ML, et al. (2010) A dimorphic pheromone circuit in Drosophila from sensory input to descending output. Nature 468:686–90. 10.1038/nature09554 [PubMed: 21124455]

Sakamoto R, Byrd DT, Brown HM, et al. (2005) The Caenorhabditis elegans UNC-14 RUN domain protein binds to the kinesin-1 and UNC-16 complex and regulates synaptic vesicle localization. Mol Biol Cell 16:483–96. 10.1091/mbc.e04-07-0553 [PubMed: 15563606]

Scheiffele P, Fan J, Choih J, et al. (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 101:657–69. 10.1016/s0092-8674(00)80877-6 [PubMed: 10892652]

Slater G, Levy P, Chan KLA, Larsen C (2015) A central neural pathway controlling odor tracking in Drosophila. J Neurosci 35:1831–48. 10.1523/JNEUROSCI.2331-14.2015 [PubMed: 25653345]

Sperry RW (1963) Chemoaffinity in the orderly growth of nerver fiber patterns and connections. Proc Natl Acad Sci U S A 50:703–10. 10.1073/pnas.50.4.703 [PubMed: 14077501]

Spindler SR, Hartenstein V (2010) The Drosophila neural lineages: a model system to study brain development and circuitry. Dev Genes Evol 220:1–10. 10.1007/s00427-010-0323-7 [PubMed: 20306203]

Spletter ML, Liu J, Liu J, et al. (2007) Lola regulates Drosophila olfactory projection neuron identity and targeting specificity. Neural Dev 2:14. 10.1186/1749-8104-2-14 [PubMed: 17634136]

Stocker RF, Heimbeck G, Gendre N, de Belle JS (1997) Neuroblast ablation in Drosophila P[GAL4] lines reveals origins of olfactory interneurons. J Neurobiol 32:443–56. 10.1002/(sici)1097-4695(199705)32:5<443::aid-neu1>3.0.co;2-5 [PubMed: 9110257]

Stork T, Bernardos R, Freeman MR (2012) Analysis of glial cell development and function in Drosophila. Cold Spring Harb Protoc 2012:1–17. 10.1101/pdb.top067587 [PubMed: 22194269]

Stork T, Sheehan A, Tasdemir-Yilmaz OE, Freeman MR (2014) Neuron-glia interactions through the Heartless FGF receptor signaling pathway mediate morphogenesis of Drosophila astrocytes. Neuron 83:388–403. 10.1016/j.neuron.2014.06.026 [PubMed: 25033182]

Sweeney LB, Chou Y-H, Wu Z, et al. (2011) Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting. Neuron 72:734–47. 10.1016/j.neuron.2011.09.026 [PubMed: 22153371]

Tanaka NK, Awasaki T, Shimada T, Ito K (2004) Integration of Chemosensory Pathways in the Drosophila Second-Order Olfactory Centers Nobuaki. Curr Biol 14:449–457. https://doi.org/10.1016 [PubMed: 15043809]

Tanaka NK, Endo K, Ito K (2012) Organization of antennal lobe-associated neurons in adult Drosophila melanogaster brain. J Comp Neurol 520:4067–130. 10.1002/cne.23142 [PubMed: 22592945]

Tanaka NK, Tanimoto H, Ito K (2008) Neuronal assemblies of the Drosophila mushroom body. J Comp Neurol 508:711–55. 10.1002/cne.21692 [PubMed: 18395827]

Tasdemir-Yilmaz OE, Freeman MR (2014) Astrocytes engage unique molecular programs to engulf pruned neuronal debris from distinct subsets of neurons. Genes Dev 28:20–33. 10.1101/gad.229518.113 [PubMed: 24361692]

Turner GC, Bazhenov M, Laurent G (2008) Olfactory representations by Drosophila mushroom body neurons. J Neurophysiol 99:734–46. 10.1152/jn.01283.2007 [PubMed: 18094099]

Tyrrell T, Willshaw D (1992) Cerebellar cortex: its simulation and the relevance of Marr's theory. Philos Trans R Soc Lond B Biol Sci 336:239–57. 10.1098/rstb.1992.0059 [PubMed: 1353267]

Umemori H, Linhoff MW, Ornitz DM, Sanes JR (2004) FGF22 and its close relatives are presynaptic organizing molecules in the mammalian brain. Cell 118:257–70. 10.1016/j.cell.2004.06.025 [PubMed: 15260994]

van Rijssel J, van Buul JD (2012) The many faces of the guanine-nucleotide exchange factor trio. Cell Adh Migr 6:482–7. 10.4161/cam.21418 [PubMed: 23076143]

Vogt K, Aso Y, Hige T, et al. (2016) Direct neural pathways convey distinct visual information to Drosophila mushroom bodies. Elife 5:1–13. 10.7554/eLife.14009

Vosshall LB, Amrein H, Morozov PS, et al. (1999) A spatial map of olfactory receptor expression in the Drosophila antenna. Cell 96:725–36. 10.1016/s0092-8674(00)80582-6 [PubMed: 10089887]

Vosshall LB, Wong AM, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102:147–159. 10.1016/S0092-8674(00)00021-0 [PubMed: 10943836]

Wang G, Zhou B, Wang S, et al. (2019) The reconstruction and functional mapping of a recurrent microcircuit in Drosophila mushroom body. bioRxiv. 10.1242/dev.180224

Wang Y, Guo H-F, Pologruto TA, et al. (2004) Stereotyped odor-evoked activity in the mushroom body of Drosophila revealed by green fluorescent protein-based Ca2+ imaging. J Neurosci 24:6507–14. 10.1523/JNEUROSCI.3727-03.2004 [PubMed: 15269261]

Watari-Goshima N, Ogura K, Wolf FW, et al. (2007) C. elegans VAB-8 and UNC-73 regulate the SAX-3 receptor to direct cell and growth-cone migrations. Nat Neurosci 10:169–76. 10.1038/nn1834 [PubMed: 17237778]

Watts RJ, Hoopfer ED, Luo L (2003) Axon pruning during Drosophila metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteasome system. Neuron 38:871–85. 10.1016/s0896-6273(03)00295-2 [PubMed: 12818174]

Weiss S, Melom JE, Ormerod KG, et al. (2019) Glial Ca2+signaling links endocytosis to K+ buffering around neuronal somas to regulate excitability. Elife 8:1–29. 10.7554/eLife.44186

Wilson RI, Turner GC, Laurent G (2004) Transformation of olfactory representations in the Drosophila antennal lobe. Science 303:366–70. 10.1126/science.1090782 [PubMed: 14684826]

Wong AM, Wang JW, Axel R (2002) Spatial representation of the glomerular map in the Drosophila protocerebrum. Cell 109:229–41. 10.1016/s0092-8674(02)00707-9 [PubMed: 12007409]

Wu B, Li J, Chou Y-H, et al. (2017) Fibroblast growth factor signaling instructs ensheathing glia wrapping of Drosophila olfactory glomeruli. Proc Natl Acad Sci U S A 114:7505–7512. 10.1073/pnas.1706533114 [PubMed: 28674010]

Wu C-L, Shih M-FM, Lee P-T, Chiang A-S (2013) An octopamine-mushroom body circuit modulates the formation of anesthesia-resistant memory in Drosophila. Curr Biol 23:2346–54. 10.1016/j.cub.2013.09.056 [PubMed: 24239122]

Wu Y, Ren Q, Li H, Guo A (2012) The GABAergic anterior paired lateral neurons facilitate olfactory reversal learning in Drosophila. Learn Mem 19:478–86. 10.1101/lm.025726.112 [PubMed: 22988290]

Yagi R, Mabuchi Y, Mizunami M, Tanaka NK (2016) Convergence of multimodal sensory pathways to the mushroom body calyx in Drosophila melanogaster. Sci Rep 6:29481. 10.1038/srep29481 [PubMed: 27404960]

Yasuyama K, Meinertzhagen IA, Schürmann F-W (2002) Synaptic organization of the mushroom body calyx in Drosophila melanogaster. J Comp Neurol 445:211–26. 10.1002/cne.10155 [PubMed: 11920702]

Yildirim K, Petri J, Kottmeier R, Klämbt C (2019) Drosophila glia: Few cell types and many conserved functions. Glia 67:5–26. 10.1002/glia.23459 [PubMed: 30443934]

Yu H-H, Kao C-F, He Y, et al. (2010) A complete developmental sequence of a Drosophila neuronal lineage as revealed by twin-spot MARCM. PLoS Biol 8:e1000461. 10.1371/journal.pbio.1000461 [PubMed: 20808769]

Yu Y, Romero R, Lee TS (2005) Preference of sensory neural coding for 1/f signals. Phys Rev Lett 94:108103. 10.1103/PhysRevLett.94.108103 [PubMed: 15783530]

Zheng X, Wang J, Haerry TE, et al. (2003) TGF-beta signaling activates steroid hormone receptor expression during neuronal remodeling in the Drosophila brain. Cell 112:303–15. 10.1016/s0092-8674(03)00072-2 [PubMed: 12581521]

Zheng Z, Lauritzen JS, Perlman E, et al. (2018) A Complete Electron Microscopy Volume of the Brain of Adult Drosophila melanogaster. Cell 174:730–743.e22. 10.1016/j.cell.2018.06.019 [PubMed: 30033368]

Zheng Z, Li F, Fisher C, et al. (2020) Structured sampling of olfactory input by the fly mushroom body. bioRxiv 2020.04.17.047167. 10.1101/2020.04.17.047167

Zhu H, Luo L (2004) Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons. Neuron 42:63–75. 10.1016/s0896-6273(04)00142-4 [PubMed: 15066265]

Zhu S, Chiang A-S, Lee T (2003) Development of the Drosophila mushroom bodies: elaboration, remodeling and spatial organization of dendrites in the calyx. Development 130:2603–10. 10.1242/dev.00466 [PubMed: 12736205]

Zhu S, Perez R, Pan M, Lee T (2005) Requirement of Cul3 for axonal arborization and dendritic elaboration in Drosophila mushroom body neurons. J Neurosci 25:4189–97. 10.1523/JNEUROSCI.0149-05.2005 [PubMed: 15843622]

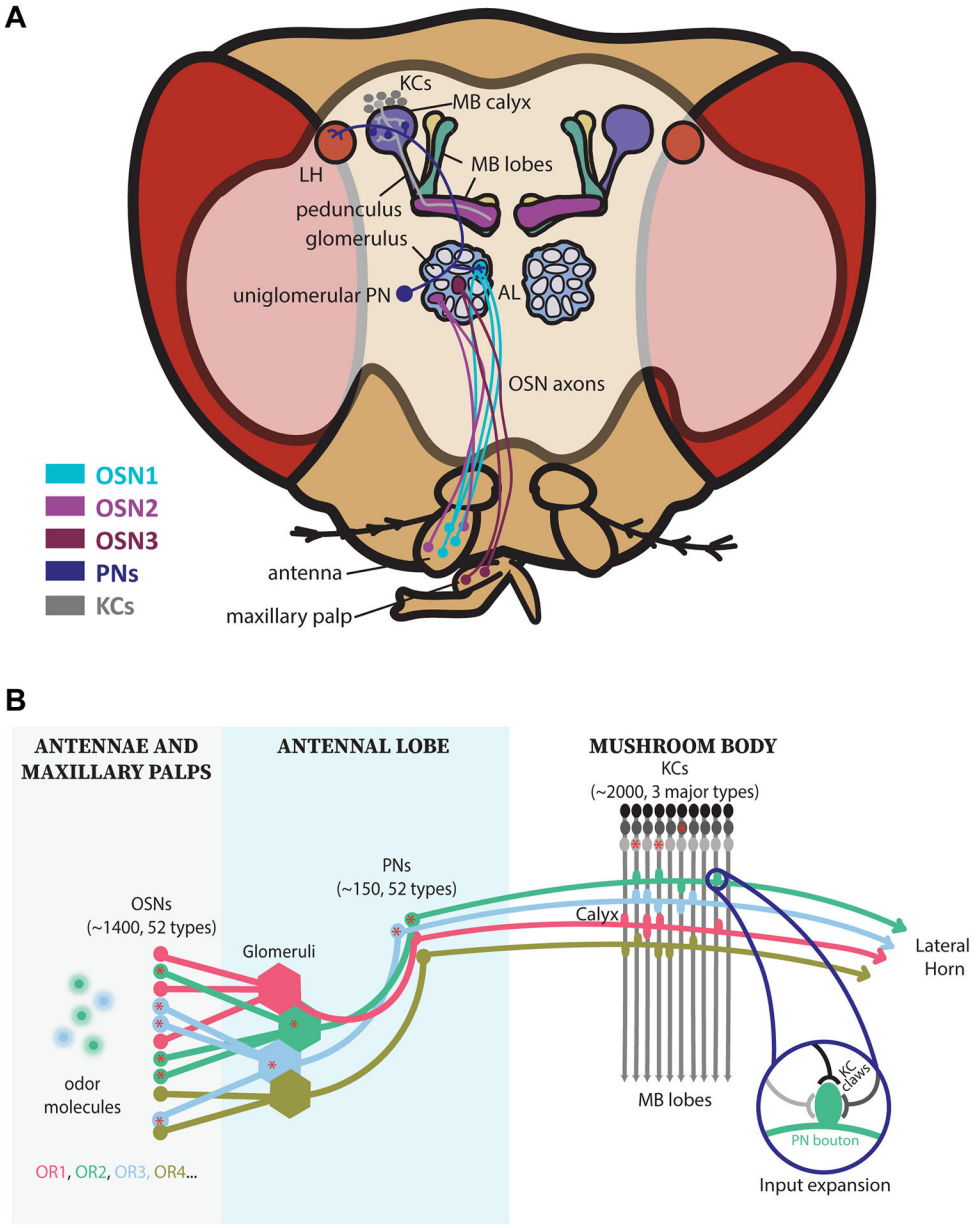


**Fig. 1: Organization of the fly olfactory system.**

**(A)** Anterior view of the adult *Drosophila* head highlighting brain regions involved in olfactory processing. The antennae and maxillary palps carry OSNs. The binding of odorants to OSNs is the first stage of odor processing. Axonal projections from OSNs expressing the same odor receptor (represented here as the same color) come together in a distinct location of the AL called a glomerulus. A representative uniglomerular PN sends dendrites to a single glomerulus where it synapses with OSNs of a single type. PNs then relay odor information to two higher brain regions – the MB and LH for learned and innate behaviors, respectively. Inside the MB calyx, KC dendritic claws synapse with PN axon boutons. The axons of KCs form the MB lobes, shown here in different colors to represent the three major KC types: α/β (teal), α‘/β‘ (yellow), and γ (purple).

**(B)** Representation of odor information at each layer of odor processing in the adult olfactory system. A single scent is composed of multiple distinct odor molecules and can thus activate a subset of OSNs. Olfactory PNs are densely activated by an odor (~50% of PNs respond). Most PNs which innervate the calyx are uniglomerular (52 types of uniglomerular PNs). KC firing depends on multiple PNs providing input at the same time. Thus, odor representation is sparse at the level of KCs (~10% of KCs respond to a given odor). Inset shows input expansion of PNs onto KCs. Activated neurons and glomeruli are represented by red asterisks.

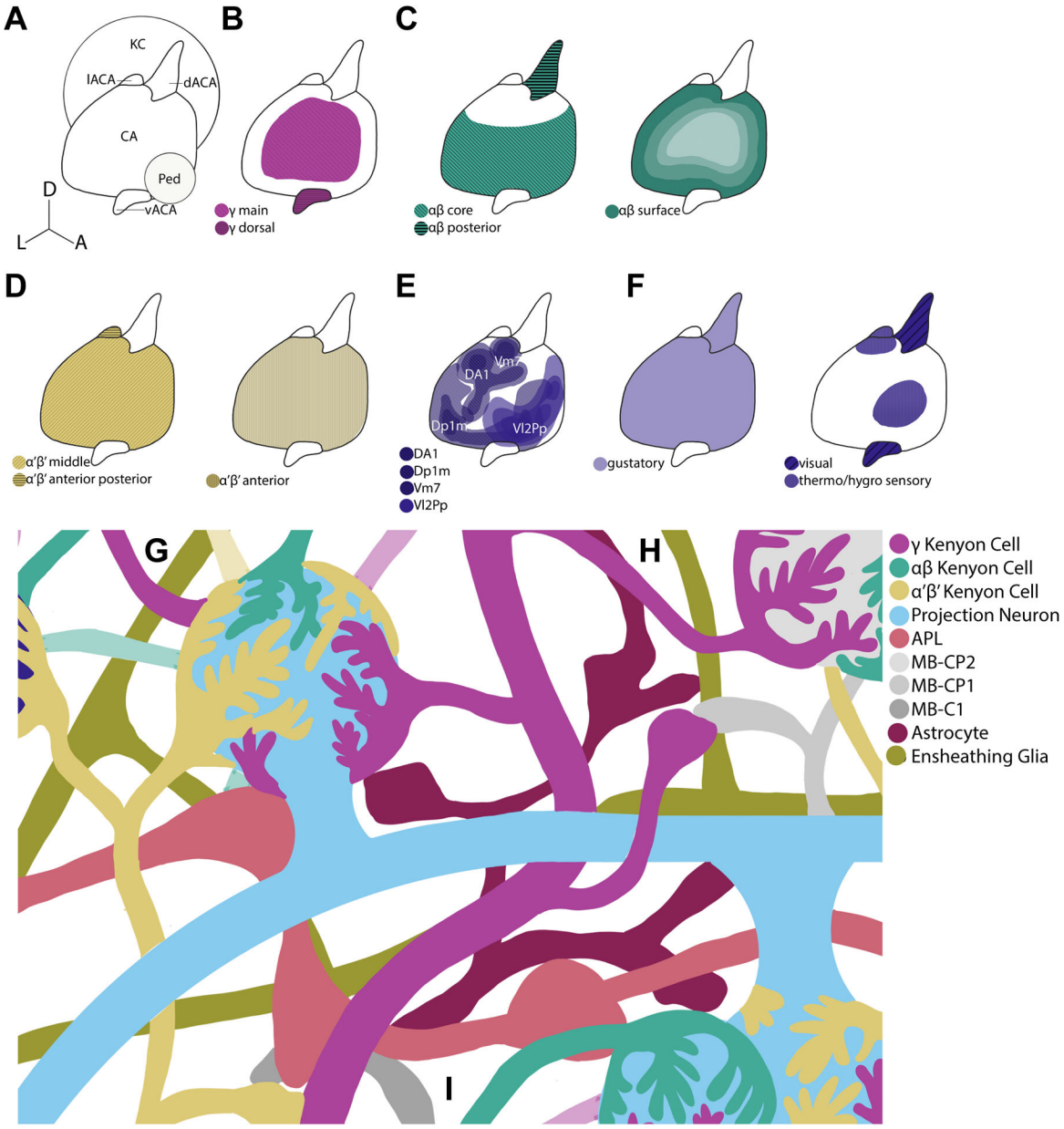


**Figure 2: Regionalization and connections of calyx member cells.**

**(A)** Diagram of the adult calyx from an anterior view. The plume of KC cell bodies is represented by a large circle (annotated as KC) behind the calyx (CA) diagram. The pedunculus (Ped), where KC axons converge, is represented by a small circle. D=dorsal; L=lateral; A=anterior.

**(B-D)** KC dendrites occupy loosely defined regions of the calyx.

**(B)** γ main dendrites are found mostly in the central area of the main calyx (see Fig. 3 of Tanaka et al. 2008). γ dorsal (γd) KCs elaborate dendrites only in the vACA (Aso et al. 2009; Butcher et al. 2012; see Figs. 1,2 of Vogt et al. 2016; see Fig. 3 of Yagi et al. 2016).

**(C)** Left: α/β core dendrites are ubiquitous in the main calyx and become sparse in the dorsal most region of the calyx (see Fig. 3 of Tanaka et al. 2008). αβ posterior dendrites

make up the dACA (see Fig. 3 of Tanaka et al. 2008; see Fig. 3 of Yagi et al. 2016). Right: α/β surface (sometimes called shell) dendrites are found throughout the calyx – they are most dense at the periphery, and progressively less dense centrally (see Fig. 3 of Tanaka et al. 2008).

**(D)** Left: α'/β' middle dendrites are ubiquitous throughout the main calyx. α'/β' anterior posterior dendrites innervate the lACA (see Fig. 3 of Tanaka et al. 2008). Right: α'/β' anterior dendrites are sparse throughout the calyx (see Fig. 3 of Tanaka et al. 2008).

**(E-F)** PN axon terminals in the calyx are mostly from olfactory projection neurons. Due to stereotyped morphology, they also occupy loosely defined regions.

**(E)** The bouton density of four different classes of olfactory PNs are shown (see Fig. 4 of Jefferis et al. 2007). Darker areas indicate a higher bouton density. Regions are slightly overlapping and some are quite broad.

**(F)** Bouton distribution of non-olfactory PNs, which make up about 20% of the total input to the calyx. Left: Gustatory inputs are dispersed sparsely throughout the main calyx and dACA. Right: Visual inputs are received in the vACA and dACA, and thermo- and hygro-sensory inputs are received primarily in the central region of the main calyx and in the lACA (see Figs. 4,5 of Kirkhart and Scott 2015; see Fig. 3 of Vogt et al. 2016; see Figs. 3,5 of Li et al. 2020b; see Fig. 4 of Marin et al. 2020).

**(G-I)** The cells within the calyx make a variety of connections with one another.

**(G)** A unilobed light blue PN bouton is grasped by the yellow, purple, and teal claws of the three different types of KCs (Butcher et al. 2012; Caron et al. 2013). The claws of the yellow α'β' KC grasps boutons from two different PNs (shown in dark blue and light blue). The maroon astrocyte is associated with a PN:KC synapse (from Figs. 13, 15 of Kremer et al. 2017). A beads-on-a-string extension of the pink APL is postsynaptic to PN boutons and KC dendrites (Yusuyama et al. 2002; from Fig. 4 of Tanaka et al. 2008; from Fig. 7 of Zheng et al. 2018; from Fig. 1 of Wang et al. 2019).

**(H)** The gray bouton of a MB-CP2 neuron is wrapped by KC dendritic claws (Zheng et al. 2018). A purple non-claw ending of a KC is presynaptic to the light gray MB-CP1 (see Fig. 7 of Zheng et al. 2018).

**(I)** A light blue clustered PN bouton is encased in KC claws (Butcher et al. 2012). A purple KC axon receives axonal input from another yellow KC (see Fig. 7 of Zheng et al. 2018). The purple KC is also presynaptic to gray putative inhibitory neuron MB-C1 (see Fig. 7 of Zheng et al. 2018).

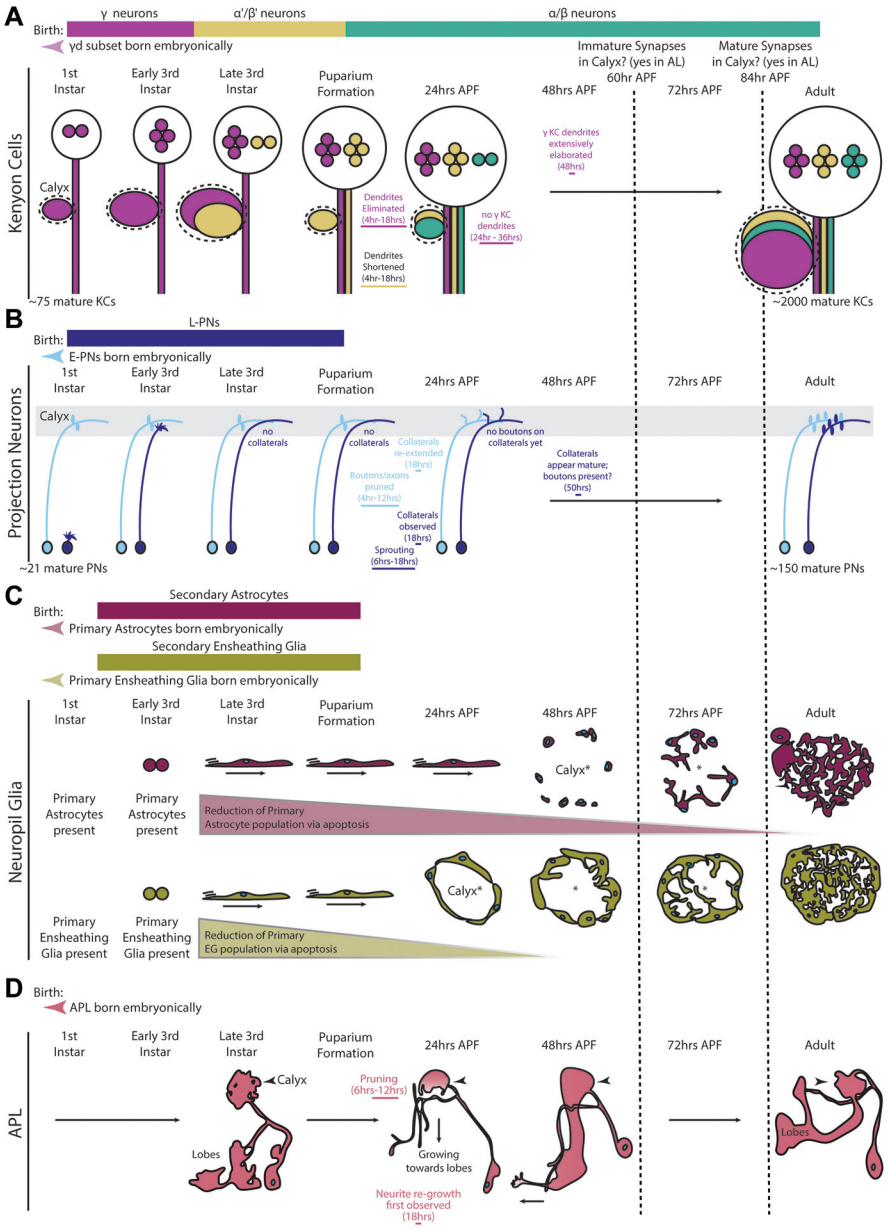


**Fig. 3: Developmental timelines of each major cellular member of the calyx.**

**(A)** Top: KCs are born continuously in the embryo through to the end of metamorphosis, and in sequential order: γd in the embryo (light purple), γ main during the first half of larval life (dark purple), α'/β' during the second half of larval life (yellow), and α/β throughout metamorphosis (teal). Vertical dashed lines at 60hrs and 80hrs APF represent the timepoints when immature or mature synapses, respectively, are first observed in the AL. Whether this synaptogenic timeline applies to the calyx is unknown. Bottom: Cortical region containing KC cell bodies represented by solid-lined circle. Calyx represented by dashed-line circle. Calyx size grows as the larva ages due to expansion of γ and α'/β' KC populations and dendrites, although it is believed that only γd KCs form functional synapses in the larval calyx. After initiation of metamorphosis, γ KC dendrites are completely eliminated and

α'/β' dendrite length is reduced. α/β KC dendrites are elaborated by 24hrs APF, while γ KC elaborations are not seen until 48hrs APF. The adult calyx is significantly larger in size than the larval calyx due to massive expansion of the KC population.

**(B)** Top: PNs are born in two waves: in the embryo (E-PNs; light blue) and throughout larval life (L-PNs; dark blue). Bottom: Only E-PNs innervate the larval calyx (calyx depicted as gray bar across width of figure) as L-PN axons do not reach the calyx until the 3rd instar stage and do not sprout collaterals until after the start of metamorphosis. E-PN boutons are eliminated and axons pruned back just after puparium formation. Both E-PNs and L-PNs innervate the calyx with immature collaterals by 18hrs APF. At least for L-PNs, mature collaterals that may bear boutons are observed by 50hrs APF. Both E-PNs and L-PNs functionally contribute to the adult calyx.

**(C)** Top: Astrocytes (magenta) and EG (green) are born in two waves: in the embryo (Primary; lighter colors) and throughout larval life (Secondary; darker colors). Bottom: Primary astrocytes and EG are present in the 1st instar larva brain, and begin undergoing apoptosis just after the start of the 3rd instar. Primary astrocytes and EG are completely eliminated by the end of metamorphosis or just before 48hrs APF, respectively. Secondary Astrocytes and EG begin migrating from their progenitor zones to colonize brain neuropil during the 3rd instar stage. It is not presently understood when secondary astrocytes and EG reach the calyx nor what the dynamics of astrocyte and EG process infiltration into the calyx are during metamorphosis. Thus, these developmental events are inferred here from studies in the AL (asterisks indicate data from AL applied to the calyx; only adult schematics represent data obtained from the calyx). If AL developmental events apply to the calyx, then the morphogenesis of both secondary astrocytes and EG coincides with calyx synaptogenesis.

**(D)** Top: APL neurons are born embryonically. Bottom: Little is known about APL development in the calyx. APL processes make functional connections in the larval calyx (calyx indicated by arrowhead). These larval connections are dismantled after the start of metamorphosis. APL process pruning starts immediately after the onset of γ KC dendrite and E-PN bouton/axon pruning. By 24hrs APF, APL processes are again present in the calyx. APL process growth proceeds from the calyx towards the lobes and is complete before eclosion. Direction of process growth indicated by arrows.

**Table 1.**

Anatomically Identified Calyx Neurons

| Neuron Name | # per hemisphere | Location and Projections |
|---|---|---|
| Mushroom Body Calyx 1 (MB-C1) | 1 | Cell body in posterior lateral cell body region. Projections arborize in ventral part of the LH and throughout the main calyx (Fig. 2I) (Tanaka et al. 2008). |
| Mushroom Body Calyx 2 (MB-C2) | 2–3 | Cell bodies in posterior superior medial protocerebrum. Projections extend into posterior and superior lateral protocerebrum in addition to a few projections into the dorsal accessory calyx that connect it to the ventral periphery of the main calyx (Tanaka et al. 2008). |
| Mushroom Body Calyx 3 (MB-C3) | 0–1 | Hypothesized to exist based on projections near MB-C2 that run more ventrally and innervate the superior posterior slope, both the middle and posterior superior lateral protocerebrum in addition to the ventral periphery of the main calyx (Tanaka et al. 2008). |
| Mushroom Body Calyx Pedunculus 1 (MB-CP1) | 2 | Cell bodies in posterior lateral protocerebrum. Arborizes in the calyx, pedunculus, and along the medial lobe (Fig. 2H) (Tanaka et al. 2008). Could be *odd* neurons (Levy and Larsen 2013). |
| Mushroom Body Calyx Pedunculus 2 (MB-CP2) | 1 | Cell body near the LH. Extends branches throughout a given hemisphere. Receives inputs at all its output synapses though has dedicated input synapses where it is postsynaptic to multimodal sensory and motor neurons. Its terminal boutons are presynaptic to all olfactory KC types in the main calyx and presynaptic to α/β p KCs in the dACA (Fig. 2H). In the pedunculus it receives input from γ main and γd KCs from the vACA. It likely relays multi-modal, non-olfactory input to KCs and provides feedback from KC axons in the pedunculus to KC dendrites in the calyx (Zheng et al. 2018). |
| Ventral Unpaired Medial Cluster | unknown | Cell bodies are clustered about the subesophageal ganglion.<br>Project via the mALT with PNs to innervate the dACA, main calyx, MB lobes, and LH. Most extensive arborizations are in the area around the esophagus (Tanaka et al. 2008). |