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The olfactory circuit of the fruit fly *Drosophila melanogaster*

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The olfactory circuit of the fruit fly *Drosophila melanogaster* has emerged in recent years as an excellent paradigm for studying the principles and mechanisms of information processing in neuronal circuits. We discuss here the organizational principles of the olfactory circuit that make it an attractive model for experimental manipulations, the lessons that have been learned, and future challenges.

olfactory receptor neurons, projection neurons, local interneurons, antennal lobe, mushroom body, lateral horn, behavior, information processing

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Elucidating the principles of information processing is a topic of increasing interest and has emerged as a central goal in modern neuroscience. This is due in part to the recent development of tools to label, record, inactivate or activate genetically defined neuronal populations [1]. These tools have been applied to investigate a number of neural circuits, ranging from *Caenorhabditis elegans* to the primate brain. The olfactory circuit of the fruit fly, *Drosophila melanogaster*, has provided one of the best models for neural circuit analysis thanks to the genetic tools available, the elegant nature of glomerular organization being similar from insects to mammals, and the efforts of a large number of skilled investigators working in the field.

Here we review the olfactory circuit of *Drosophila melanogaster* with an emphasis on its role as a model for analyzing the principles of neural information processing. We encourage readers to consult several other excellent recent reviews on fly olfaction for additional details and examples [2,3].

1 Input to the olfactory circuit: olfactory receptor neurons (ORNs)

Olfaction is initiated when odorants bind to odorant receptors on the dendrites of olfactory receptor neurons (ORNs, also called olfactory sensory neurons), which are housed in hair-like structures called sensilla on the antenna and maxillary palp (Figure 1). The identification of the odorant receptors that are functionally expressed in each ORN class is nearly complete in fruit flies, providing an important foundation at the level of input of this sensory system, as well as providing genetic tools to access these input neurons.

Most ORNs express a specific odorant receptor (originally identified to be encoded by the Or gene family) [4-6] and a common co-receptor Or83b [7]. These include ORNs housed in large basiconic sensilla (ab1-3), small basiconic sensilla (ab4-10), trichoid sensilla (at1-4), and all maxillary palp basiconic sensilla (pb1-3), as well as a single coeloconic class expressing Or35a [8]. One of the few exceptions is the CO_2-detecting ORNs (ab1c), which express Gr21a and Gr63a [9,10] that share sequence similarity with the

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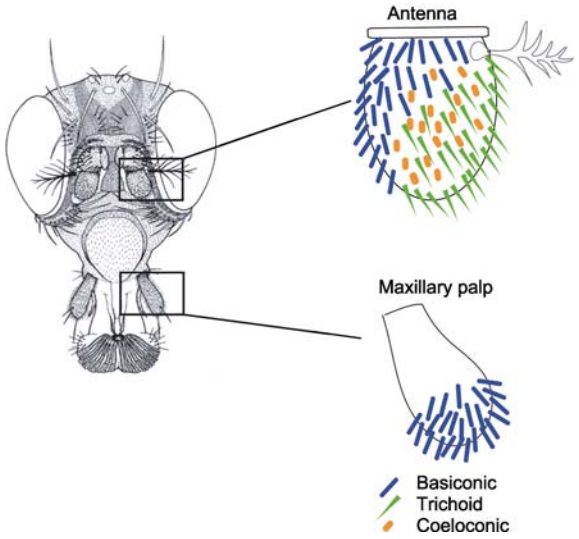

Figure 1 Scheme of odorant sensillar organization. The distribution of basiconic, trichoid and coeloconic sensilla on the antenna and maxillary palp are shown. Only basiconic sensilla are present on the maxillary palp.

gustatory receptors (hence the name Gr). In addition, different types of receptors, encoded by the ionotropic receptor gene family, are expressed in the coeloconic sensilla (Figure 1). Some odorant receptors are only expressed in larval ORNs, others are expressed only in adult ORNs, and others are expressed in both larval and adult ORNs [11,12] (shown in Table 1).

Carlson and colleagues have mapped the odor specificity for the vast majority of the Or family of the odorant receptors using the *in vivo* "empty neuron" system [13] against a panel of 10-100 pure odorants and some natural products. These include receptors that are expressed in the antenna [14,15], in the maxillary palp [16], or in the larva [12]. In addition, before odorant receptors were mapped to ORN classes with distinct glomerular targets (see below), they also mapped odor responses of identifiable sensilla in the maxillary palp [17], antenna basiconics [18], and coeloconics [19]. Subsequent receptor→ORN maps confirmed this early physiological data. While there is much potential for obtaining useful data from these comprehensive descriptions, the physiological ramifications of the odor space (the constellation of odorants that activate specific odorant receptors) remains to be explored. Some odorants excite many odorant receptors, while others excite few. Likewise, some ORNs respond to many odorants, while others are more selective. In general, trichoid ORNs are not responsive to general odorants found in the environment, consistent with the notion that they are specialized for detecting pheromones. Indeed, some trichoid ORNs even exhibit high basal firing rates that are generally inhibited by odorant application (e.g., Or88a and Or47b). Trichoid ORNs generally respond to odorants (pheromones) given off by other flies [20].

Interestingly, pheromonal odorant detection may be more complicated than a simple binding of odorant to receptor. Or67d has been identified as the receptor for cVA (11-cis-vaccenyl acetate) [20–22]. In studying this class of pheromone and its receptor, additional co-factors have been identified, including the odorant binding protein LUSH [23] and a transmembrane protein SNMP [24,25]. In fact, activated LUSH (activated by cVA binding), rather than cVA itself, is the most likely ligand for Or67d [26]. Whether other classes of pheromones or even general odorants involve such elaborate cofactors awaits future investigation.

In summary, the sensory neurons and odorant receptors in the fly olfactory system have been almost entirely characterized. The physiological responses of most ORN classes have been characterized to some extent. Importantly, the promoters of genes encoding odorant receptors (including Ors, Grs or Irs) provide genetic access to individual olfactory input channels for labeling and genetic manipulating most ORN classes. For example, almost all Or promoters have been used to drive the yeast transcription factor Gal4 for binary expression [1] of various UAS-effector transgenes for the specific labeling of each ORN. In addition, loss-of-function mutations in a number of these receptor genes can be used to perturb specific olfactory input channels. Taken together, these findings have revealed much about how odorant information is received by the primary sensory neurons in the olfactory circuit. The next step is to determine how this information is transmitted and transformed by the rest of the olfactory circuit.

2 Anatomical organization of the central olfactory circuit

2.1 Glomerular organization and ORNs

Stocker and colleagues originally described 43 stereotyped glomeruli in the antennal lobe based on their size, shape and relative position [27]. Following the initial finding that ORNs expressing a given odorant receptor converge their axons to a common glomerulus [28,29], there is now an almost complete map detailing which ORNs project to which glomeruli in the antennal lobe for Or-expressing ORNs [8,11] (Table 1). The glomeruli that still do not have known corresponding ORNs are predominantly targets of Ir-expressing coeloconic ORNs [30]. ORNs project their axons via the antennal (antennal ORNs) or labial (maxillary palp ORNs) nerves to terminate in ipsi- and contra-lateral target glomeruli (Figure 2). All examined ORN classes (by unilateral removal of antenna or maxillary palp) target their axons bilaterally to their cognate glomeruli in the antennal lobes with the exception of Gr21a expressing ORNs, which only target the ipsi-lateral V glomerulus [28,31]. Some unidentified ORN classes (coeloconics) may also project their axons unilaterally as judged from early Golgi staining [32].

Table 1 Correspondence of ORN Classes — Glomeruli — PN Classes[a)]

OR	Sensillum	Larva	Glomerulus	PN Class	GH146	OR	Sensillum	Larva	Glomerulus	PN Class	GH146
Or1a		+				Or74a		+			
Or2a	at3	+	DA4m		+	Or82a	ab5	+	VA6	ad	+
Or7a	ab4	+	DL5	ad	+	Or83a		+			
Or9a	ab8		VM3	ad	+	Or83b	ab,at,pb,ac3	+			
Or10a	ab1		DL1	ad		Or83c	at2		DC3	ad	+
Or13a	ai1	+	DC2	ad	+	Or85a	ab2		DM5	l	+
Or19a	at3		DC1	ad	+	Or85b	ab6		VM5d		+
Or19b	at3		DC1	ad	+	Or85c		+			
Or22a	ab3	+	DM2	l	+	Or85d	pb3	+	VA4		+
Or22b	ab3		DM2	l	+	Or85e	pb2		VC1		+
Or22c		+				Or85f	ab10		DL4		+
Or23a	at2		DA3	ad	+	Or88a	at4		VA1d	ad	+
Or24a		+				Or92a	ab1		VA2	ad	+
Or30a		+				Or94a		+			
Or33a	ab4	+	DA2		+	Or94b		+			
Or33b	ab5	+	DM3	ad	+	Or98a	ab7		VM5v*		-
Or33b	ab2		DM5	l	+	Or98b	ab6		VM5d		+
Or33c	pb2		VC1		+	Gr10a	ab1		DL1	ad	+
Or35a	ac3	+	VC3*		-	Gr21a	ab1	+	V		-
Or42a	pb1	+	VM7	ad	+	Gr63a	ab1	+	V		-
Or42b	ab1	+	DM1	l	+	Ir31a	ac1				
Or43a	at3		DA4l		+	Ir75a	ac2, 3				
Or43b	ab8		VM2	ad	+	Ir75b	ac3				
Or45a		+				Ir75c	ac3				
Or45b		+				Ir75d	ac1, 2, 4				
Or46aA	pb2		VA7l	ad	+	Ir76a	ac4		VM4*	ad	+
Or47a	ab5	+	DM3	ad	+	Ir76b	ac1, 2, 3, 4				
Or47b	at4		VA1lm	ad, v	+	Ir84a	ac4				
Or49a	ab10	+	DL4		+	Ir92a	ac1				
Or49b	ab6		VA5		+		ac		DC4		-
Or56a	ab4		DA2		+		ac		DL2d*		+
Or59a		+					ac		DL2v		+
Or59b	ab2		DM4		+				DL6	ad	+
Or59c	pb3		l	ad	+				DP1l*		-
Or63a		+							DP1m	ad	+
Or65a	at4		DL3	l	+				VA7m	l	+
Or65b	at4		DL3	l	+		ac		VL1	v	+
Or65c	at4		DL3	l	+		ac		VL2a		+
Or67a	ab10		DM6	ad	+				VL2p	l	+
Or67b	ab9	+	VA3	ad	+				VL2p+	ad	+
Or67c	ab7	+	VC4*		-		ac		VM1	l	+
Or67d	at1		DA1	l,v	+		ac		VM6		-
Or69aA	ab9		D	ad	+				VP1		-
Or69aB	ab9		D	ad	+				VP2		-
Or71a	pb1		VC2	l	+				VP3		-

a) The general OR classes are according to references [9,10,11,30]; The OR→Sensillum correspondence is according to references [8,30]; The OR presence in larva is according to references [8,11]; The glomerular classes are according to references [8,27]; The OR→Glomerulus correspondence is according to references [8,11,30]; The Glomerulus→PN classes (organized according to neuroblast lineages) is according to references [37]; GH146-Gal4 expression pattern in PNs is according to the analysis by M. Spletter, C. J. Potter and Y.-H. Chou. The unfilled space and lack of correspondence between Ir-expressing ORNs and the glomeruli at the bottom of the right column represent incomplete knowledge. *, Acj6-Gal4 positive but GH146-Gal4 negative PNs in the anterodorsal lineage [38]. Abbreviations: ab, antennal basiconic; at, antennal trichoid; ai, antennal intermediate; ac, antennal coeloconic; pb, maxillary palp basiconic; ad, anterodorsal lineage; l, lateral lineage; v, ventral lineage.

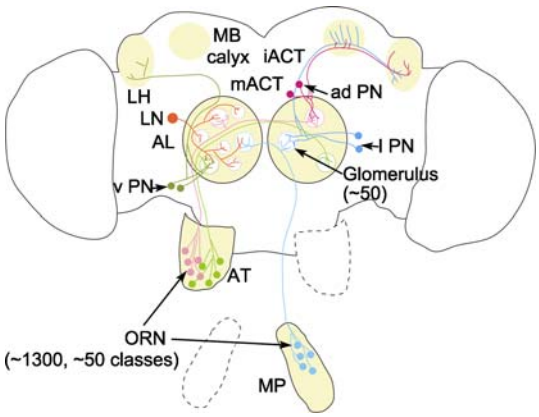

Figure 2 Anatomical organization of the adult fly olfactory circuit. Each color represents ORNs expressing the same odorant receptor, and their corresponding postsynaptic PNs (with a darker shade). ORNs expressing the same olfactory receptor converge onto a single pair of glomeruli (one on each antennal lobe), where they provide input to PNs and LNs. LN processes usually ramify broadly in the antennal lobe. PNs send axons to the MB and LH through the iACT (for adPNs and lPNs) or mACT (for vPNs). For clarity, neurons and their processes are shown on only one side of the fly brain. Each module present in this figure has a symmetric counterpart in the opposite hemisphere of the brain. Abbreviations: MP, maxillary palp; AT, 3rd antennal segment; AL, antennal lobe; LH, lateral horn; MB, mushroom body; ORN, olfactory receptor neuron; PN, projection neuron. LN, local interneuron; adPN, anterodorsal PN; lPN, lateral PN; vPN, ventral PN; iACT, inner antennocerebral track; mACT, middle antennocerebral track. Modified from reference [114].

Thus, for the most part, there is a duplication of odorant information entering each antennal lobe.

2.2 Antennal lobe projection neurons (PNs)

Within the antennal lobe glomeruli, ORN axons synapse with dendrites of projection neurons (PNs) and local interneurons (LNs), which were originally characterized with Golgi staining and electron microscopy [32]. A large subset of PNs (~90 out of an estimated total of 150 per hemisphere) have been the subject of intense study owing to their specific expression in an enhancer trap line GH146-Gal4 [33]. Studies using systematic mosaic analysis with a repressible cell mark (MARCM) methods have revealed that all GH146+ PNs (with the exception of the pan-antennal lobe ventral PNs) are uniglomerular PNs originating from three separate neuroblast lineages [34]. Anterodorsal and lateral PNs innervate non-overlapping glomeruli; their axons travel through the inner antennocerebral track (iACT), form collaterals in the mushroom body calyx, and terminate at the lateral horn of the protocerebrum with stereotyped branching patterns. Axons of ventral PNs travel through the middle antennocerebral tract (mACT) and terminate at the lateral horn, except for the pan-antennal lobe ventral PNs, which extend their axons beyond the lateral horn to a currently uncharacterized structure [35,36]. Anterodorsal and lateral PNs are excitatory cholinergic PNs, whereas ventral PNs are GABAergic [37] (Figure 2).

Much less is known about GH146-negative PNs, and even their total number is unclear at present. The anterodorsal neuroblast contains ~24 GH146-negative "classic uniglomerular PNs" that can be labeled using the enhancer trap line Acj6-Gal4. These project to six glomeruli that are not innervated by GH146-positive PNs (Table 1) [38]. The ventral neuroblast contains ~45 additional GH146-negative PNs that either innervate single (DA1, VA1lm, VL1) or multiple glomeruli; their axons travel through the mACT to the lateral horn similarly to GH146-positive ventral PNs [38]. The lateral neuroblast produces additional "atypical" PNs, some of which are Acj6-positive, that innervate multiple glomeruli [38] (M. Spletter, Y. Chou, unpublished observations). In addition to PNs that connect the antennal lobe to the mushroom body or the lateral horn (the classical higher olfactory centers), there are additional PNs connecting the antennal lobe to other parts of the brain. These neurons have only been partially described by Golgi staining [32], MARCM clones of various Gal4 lines [38] (M. Spletter, Y. Chou, unpublished observations) or screening through Gal4 line expression patterns [39]. The functional significance of these additional projections is entirely unknown, but their existence points to the sobering fact that there are likely more "higher olfactory centers" than just the mushroom body and lateral horn.

2.3 Antennal lobe local interneurons (LNs)

In addition to PNs that presumably send olfactory information to other parts of the brain, LNs are an important class of neurons that restrict their projections within the antennal lobe. Early Golgi staining studies have described these LNs as having projections that cover the entire antennal lobe [32]. Recent studies have revealed increasing diversity of LNs [38,40–44], culminating in a comprehensive analysis of >1500 singly labeled LNs [45].

Most LNs are GABAergic, acting on fast ionotropic $GABA_A$ and slow G-protein coupled $GABA_B$ receptors that can be inhibited by picrotoxin and CGP54626. Application of these pharmacological agents disinhibits PN spontaneous firing and responses to odorants [40]. LNs themselves appear to only have picrotoxin-sensitive $GABA_A$ receptors [40]. Recent studies (see below) indicate that at least part of the effect of GABA on PN firing is indirectly caused by presynaptic inhibition of ORN neurotransmitter release [46,47]. In addition to GABAergic LNs, some LNs are cholinergic and excitatory [41], and some are neither GABAergic nor cholinergic, a subset of which are glutamatergic [45].

2.4 PN terminals at the mushroom body (MB) and lateral horn (LH)

At the MB calyx, each classical PN (from anterodorsal and lateral lineages) forms 4-10 collaterals, terminating in large

boutons enriched for synaptotagmin-HA [37]. Each bouton likely corresponds to the center of a "calycal glomerulus" as viewed with electron microscopy (EM), surrounded by numerous small profiles that are presumably dendritic branches of MB neurons [48]. At the LH, PNs form en passant synapses along their entire axonal paths [37,49]. Single-cell labeling experiments have revealed striking stereotypy of PN axon terminal arborization patterns in the lateral horn according to dendritic glomerular innervation [35,36]. Image registration techniques have permitted the generation of quantitative PN presynaptic terminal maps, supporting strong stereotypy in the lateral horn and revealing a limited degree of organization in the mushroom body calyx [37]. The stereotypy at the MB calyx has been emphasized in studies comparing PN axon projections and dendrites of different MB neurons [50,51].

2.5 Neurons associated with the mushroom bodies

One adult MB in *Drosophila* is composed of approximately 2500 MB neurons — also called Kenyon Cells (KCs) — which are derived from four neuroblasts [52]. Each neuroblast sequentially gives birth to γ, α'/β' and α/β (α/β can be further divided into pioneer, early and late α/β) neurons [53,54] (Figure 3). The MB calyx can be segregated into as many as 17 distinct domains according to the dendritic projections of MB neurons originating from different neuroblasts and different birth orders [51]. MB axons form a peduncle that extends into five distinct axonal lobes in the adult: α and α' form two dorsal (vertical) lobes; γ, β, β' form three medial (horizontal) lobes [55]. The earliest born

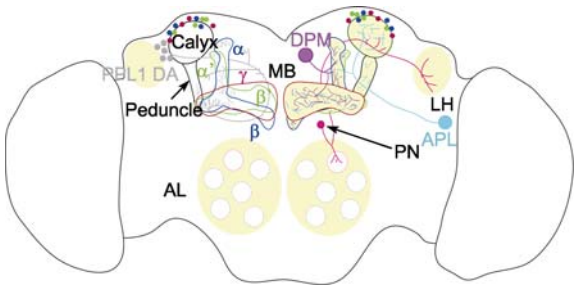

Figure 3 The organization of the adult mushroom body (MB). MB neuron dendrites innervate the calyces and their axons project through the peduncle and extend into dorsal (α/α') and medial (β/β'/γ) lobes. MB neurons receive input from PNs at the calyx. The MB is also innervated by other neurons, for example, DPM, APL, and DA neurons. The projection of one of the PPL1 DA (out of 12) neurons is shown here. Notably, other PPL1 DA neurons may have different innervation patterns. MB neurons are subdivided into three types (α/β, α'/β' and γ) whose somata are labeled with distinct colors. For clarity, neurons and their processes are drawn on only one side of the fly brain. Each module present in this figure has a symmetric counterpart in the opposite hemisphere of the brain. Abbreviations: AL, antennal lobe; LH, lateral horn; MB, mushroom body; PN, projection neuron; DPM, dorsal paired medial neuron; APL, anterior paired lateral neuron; DA, dopaminergic neurons; PPL1 DA, PPL1 cluster of dopaminergic neurons. The schematic incorporates information from references [53,60,94–96,112].

γ neurons initially branch into both the medial and dorsal lobes in larvae. These branches are pruned through local degeneration in early pupa and afterwards γ neurons only extend the medial branches that form the adult γ lobe [53,56]. Presynaptic terminals of MB neurons are restricted to peduncles and axonal lobes and are absent from the calyx [57], suggesting that the calyx is purely dendritic and only receives information. By inference, the peduncles and lobes should send information, although they may receive information as well.

Neurons that receive input from MB neurons, presumably by intersecting their dendrites with MB peduncles and lobes, have been described by Ito using Golgi staining [58] and more recently by an analysis of Gal4 enhancer trap lines [39]. A complex picture emerges from these descriptions. These neurons are genetically heterogeneous, with small subsets labeled by different Gal4 lines. They also differ in their cell body positions, their contacts with different parts of the MB neuropil (the calyx, peduncle, and different parts of the lobes), and their projections elsewhere (many parts of the brain known only by anatomic locations, such as the "middle inferior lateral protocerebrum"). In addition, the direction of information flow from these neurons is unclear. They may receive input from the MB and send output to other brain regions, in which case they would be MB output neurons (fourth-order neurons with regard to the olfactory system). Alternatively, they may receive input from other areas and send output to MB — such input could come from other sensory modalities such as the visual or taste systems. Finally, they could also be sending information bi-directionally, as seems to be the case for some of the lateral horn neurons (see below). Beyond these MB extrinsic neurons, there also exist neurons whose projections appear to be restricted within the MB calyx and lobes, but whose cell bodies are far away and distinct from MB neurons [39]. Two of the better characterized neurons are neuropeptidergic (amnesic) DPMs [59] and GABAergic APLs [60], which appear to regulate MB activity during learning and memory (Figure 3; see Section 4 below).

2.6 Neurons associated with the lateral horn

Lateral horn neurons, which send a subset of their processes into the lateral horn and the rest to different parts of the brain, were first identified using six Gal4 enhancer trap lines (from 4000) [50]. Three of these lines were analyzed at the single-cell level using MARCM clones. Colabeling with synaptotagmin-HA revealed that these are indeed third-order olfactory neurons that receive information at the LH and send elsewhere where syt-HA is enriched. However, syt-HA is also present in projections in the LH neuropils, suggesting that these third-order neurons can send information bidirectionally. Image registration techniques have allowed the construction of a putative connection matrix between PNs and lateral horn neurons. These studies suggest

that lateral horn neurons that preferentially connect to PNs representing fruity odorants project to different areas of the brain (dorsal) from those preferentially connecting with PNs representing pheromones (ventral) [37]. GH146-Gal4 also labels a few lateral horn local interneurons that have processes confined within the LH (L. Luo, unpublished data). We screened over 1000 Gal4 enhancer trap lines (from U. Heberlein) for additional lateral horn neurons, but found that Gal4 lines that label processes projecting into the LH also tend to label many neurons in other parts of the brain, making it difficult to tease apart the LH network (E. Marin & L. Luo, unpublished data). Lateral horn neurons are thus genetically heterogeneous, much like the MB output neurons described above. It seems that new methods are needed to understand the principles of information propagation, integration and spread at these higher-order centers that mediate olfactory perception and behavior.

In summary, neuronal elements become increasingly complex from the periphery to deeper brain areas. At the level of primary sensory neurons (ORNs), discrete channels deliver specific odorant information to discrete glomerular units at the antennal lobe. Most second-order neurons (PNs) are relatively well organized by their clustered cell bodies around the antennal lobe, by sharing a small number of neuroblasts, and by sharing a rather clean enhancer trap line GH146-Gal4. However, there is already heterogeneity and complexity for those PNs not labeled by GH146-Gal4, including multi-glomerular PNs and PNs that project to brain structures other than the mushroom body and lateral horn. In addition, the local interneuron network in the antennal lobe is complex, composed of diverse types of LNs with distinct and variable morphological and physiological properties. The MB neurons of the mushroom body are exceptionally well organized in the CNS. They are numerous, and vary greatly in different insect species, with this variability hypothesized to correspond to "intelligence" [61]. This organization is likely to reflect a useful circuit design for processing olfactory and other sensory information, and for participating in learning and memory (see Section 4). However, the output pathways of the MB and the output of PNs at the LH are heterogeneous genetically, anatomically and probably functionally. Moreover, it is unclear how many synapses away from the MB or LH olfactory information would start to impinge on the regulation of motor output, and in what form such regulation would take. A deeper understanding will require new approaches, such as the complete reconstruction of the *Drosophila* brain at the EM level with corresponding light microscopic identification of cell types and their gross projection patterns.

3 Information processing by the antennal lobe circuit

3.1 Transmission from ORNs to PNs

At ORN terminals in the glomeruli, action potentials are converted to the release of excitatory neurotransmitter acetylcholine [62] that presumably depolarizes their cognate PNs directly. The uniglomerular targeting of both ORN axons and PN dendrites (classic type) thus defines three discrete olfactory processing channels: an ORN class, a glomerulus, and a PN class (Table 1).

The biophysical basis for ORN-PN synaptic transmission has been examined further by studying PN response to minimal stimulation of the antennal nerve which excites a single presynaptic ORN axon [63]. It has been found that the ORN-PN synapse is extremely robust, contains multiple release sites that scale with glomerular size, and exhibits short-term depression to produce the non-linearity of the ORN-PN response. Additional properties that help PNs reliably transmit information to high olfactory centers include gap junction and mutual chemical synapses among PNs of the same class that help synchronize spontaneous and odor-evoked PN responses [64].

3.2 Transformation of ORN code to PN code

In addition to exciting PNs that are their direct postsynaptic partners, ORNs also excite a diverse array of LNs that could transmit excitatory or inhibitory information to other olfactory processing channels. It is assumed that the local circuits at the antennal lobe process incoming information from ORNs and deliver it to higher olfactory centers via PNs, akin to the well-studied vertebrate retinal circuit that transforms photoreceptor input to ganglion cell output.

How olfactory information carried by ORNs (ORN code) is transformed at the antennal lobe and transmitted by PNs (PN code) to the higher olfactory center is a contentious issue. Optical imaging studies using genetically encoded calcium dye suggested that odor tuning properties of PNs essentially mimic those of their cognate ORNs [42,65]. However, a study using whole-cell electrophysiological recording of PNs has suggested significant broadening of odor response of PNs compared with their cognate ORNs [66]. These initial polarized views have been somewhat reconciled by more recent studies, for instance by taking into account that optical imaging is not as sensitive as electrophysiology in detecting weak signals [67]. Despite this, a consensus has by no means been reached.

A direct test of whether PNs receive significant indirect input from non-cognate ORN classes is to record PN activities when their presynaptic ORNs are silenced, removed acutely (cutting the antenna, maxillary palp or both) or removed chronically (with genetic mutations in genes encoding odorant receptors). Three independent research groups have conducted such experiments [41,68,69]. The results of these studies are largely consistent (despite differences in the authors' interpretations): responses from cognate PNs have been found to be significantly reduced but not eliminated. In addition, one group also examined the converse situation, revealing that activation of a single ORN channel

causes excitation of PN channels other than its direct postsynaptic partner, and that such lateral spread appears glomerular-specific and stereotyped across animals [69]. Another group identified a potential mechanism for the lateral spread: activation of excitatory LNs that spread the excitation from one glomerulus to others. This general "noise" has been proposed to enhance the reliability of signal propagation through "stochastic resonance" [41]. This general and non-specific excitation model appears to be at odds with the non-random and stereotyped spread of lateral excitation.

Wilson and colleagues have presented more extensive data comparing the odor responses of ORN/PN pairs using electrophysiological methods. One study examined two narrowly tuned olfactory channels, Or67d and Or82a. Or67d is the receptor for the mating pheromone cVA and Or82a is specifically activated by a fruity odorant geranyl acetate. ORNs expressing these receptors project their axons to the DA1 and VA6 glomerulus, respectively. Both odors can elicit attractive behavior when properly tested, and such attraction can be abolished by killing these ORNs. The ORN→PN code transformation differs significantly between these two olfactory processing channels. DA1 PNs are as narrowly turned as their cognate Or67d ORNs, responding only to cVA. VA6 PNs are much more broadly tuned than Or82a ORNs, suggesting a different processing logic for pheromone and general odorants [70]. In a second study [71], seven additional ORN/PN pairs were compared for their odor responses. In addition to the general broadening of odor response, several other properties of PNs were uncovered. PNs transmit the rising phase of ORN responses, and do so in a highly non-linear manner for the 6/7 pairs examined (weak ORN inputs are selectively amplified, strong ORN inputs saturate). Odor responses are more reliable in individual PNs than ORNs (less variance), and are more "efficiently coded in PN space than ORN space" (more separable in multi-dimensional space). While certain rules may appear obvious (individual PN responses are less variable than ORN responses due to ORN convergence), others are more speculative. For instance, the speculation of more efficient coding in PNs is derived from an information theory perspective. Whether downstream neurons are capable of extracting information from such "efficient and complex" coding for the benefit of odor recognition and discrimination is an open question.

3.3 Lateral inhibition

Lateral inhibition is an additional mechanism regulating ORN-PN synaptic transmission and code transformation. After all, the vast majority of LNs are GABAergic [45]. Two studies have characterized presynaptic inhibition of ORNs by GABAergic LNs as a general mechanism for gain control [46,47]. Lateral inhibition dominates lateral excitation when ORNs are intact, and scales with total ORN input [46]. This result seemingly contradicts the demonstration of lateral excitation when recording from PNs postsynaptic to ORNs that are removed genetically or acutely, but can be reconciled by the finding that the site of action of lateral inhibition is the presynaptic terminals of ORNs, which expresses $GABA_B$ receptors [46,47] and potentially also $GABA_A$ receptors [46]. Interestingly, different classes of ORNs express different levels of $GABA_B$ receptors, accounting for the channel-specific gain control between ORN→PN transmission [47]. Notably, CO_2 channels express no detectable $GABA_B$ receptors and ORN→PN transmission is not affected by GABA inhibition. In contrast, VA1lm and DA1 channels (mating pheromones) have high levels of $GABA_B$ receptors and ORN→PN transmission is markedly affected by GABA inhibition. Knocking down $GABA_B$ receptor expression in the VA1lm channel is sufficient to cause a mating defect [47], suggesting a physiological function of such presynaptic inhibition.

The channel-specific processing of ORN→PN transmission may also be caused by the arborization patterns of LNs. A recent comprehensive analysis of LNs revealed a high degree of heterogeneity of LNs with regard to their glomerular innervation patterns [45]. For example, some GABAergic LNs selectively avoid pheromonal glomeruli. These neurons also tend to fire a higher percentage of their spikes in the first 100 msec of odor exposure, suggesting that pheromonal glomeruli would be preferentially excluded from this transient pulse of inhibition [45].

In general, lateral inhibition would be expected to sharpen odor-tuning curves of PNs as compared to ORNs, rather than broadening them. How lateral inhibition and excitation work together in transforming ORN input to PN output remains to be characterized in more detail. Ultimately, these questions should be settled by examining the functional responses of downstream neurons or animal behavior, which we will address in the following section.

3.4 Using behavior to test olfactory coding

The ultimate purpose of olfactory information processing is to direct animal behavior in response to odor stimuli. As such, behavioral assays provide the final test for our understanding of the system. Below we discuss how behavior can be used to examine information processing in the antennal lobe.

The case of fruit flies under stress provides an interesting first example. When stressed, flies produce odors that naïve flies typically avoid. CO_2 is a major component of this stress odor [72]. Evidence has suggested the existence of a dedicated olfactory channel for CO_2 processing, with a specific receptor pair (Gr21a/Gr63a) expressed in a specific ORN class whose axons project to the V glomerulus [72]. Remarkably, artificial activation of channelrhodopsin-2 ex-

pressed specifically in Gr21a-ORNs with light is sufficient for eliciting avoidance behavior [73]. To determine whether and how the antennal lobe circuit transforms this ORN code, it would be informative to test whether inactivation or activation of PNs that connect to the V glomerulus is necessary or sufficient for the CO_2 avoidance behavior.

The second example concerns a mating pheromone. *Drosophila* use mating pheromones to communicate with each other about their gender and mating status. A male-specific pheromone, cVA, has been identified to serve multiple purposes. This pheromone inhibits the courtship of males towards other males, or to mated females that contain cVA transferred from a previous male partner [74–76]. A single class of ORNs that express the Or67d receptor and project to the DA1 glomerulus has been identified as a mediator of this behavior. Artificial activation of these neurons with a moth pheromone ligand is sufficient to mediate courtship inhibition [21]. cVA has also been identified as a male-male aggression pheromone. Again, Or67d and Or67d-expressing ORNs are necessary, and activation of these ORNs is sufficient, for the aggressive behavior to occur. Whereas a lower concentration of cVA can induce aggression, a higher concentration is required for male-male courtship to be inhibited [77]. These experiments indicate that a single ORN class responsive to the same pheromone can encode information about two specific behavioral responses.

A third example concerns a recent study using an odor preference test to tease apart mechanisms of odor attraction and avoidance [78]. Fruit flies are typically attracted to vinegar, but if the concentration is too high even vinegar can become repulsive. Although in the attractive range vinegar activates a relatively large number of classes of ORNs, activation of just one specific class (DM1) accounts for most of the observed attraction response. In addition, the recruitment of an additional ORN class (DM5) accounts for the repulsive action towards higher concentrations of vinegar [78]. If this model proves to be generally applicable to other odorants, it would indicate that attraction and repulsion are encoded by distinct processing channels in the first olfaction layer, the ORNs.

In all the three examples described above, behavioral experiments have suggested that specific ORN classes appear to encode information about behavioral output in response to specific stimuli, whether to avoid a stress odor, to avoid a previously mated partner, or to be attracted to food. If so, why not deliver such information directly to motor programs using dedicated channels (so called "labeled lines")? Why do flies appear to require antennal lobe LNs to transform the ORN code? Given the special biological values of pheromone sensation, and perhaps the stress odor, flies may well also use labeled lines. Indeed, physiological experiments support the notion that the cVA channel undergoes minimal transformation from ORNs to PNs [70]. However it is puzzling that attraction and repulsion of general odorants such as vinegar also operate in an analogous manner. In principle, if one can activate or inactivate the postsynaptic partner PNs while animals are sensing these odorants, the behavioral consequences of such manipulations should provide a direct test of whether there is significant transformation of the PN code from the ORN code, and whether this differs across these different processing channels. Such experiments are crucial to settle the debate about information processing principles in the olfactory circuit.

4 Information processing by the mushroom body circuit

4.1 Mushroom body and olfactory learning and memory

Fruit flies can associate odorants with reward and punishment [79-81]. In classical conditioning paradigms, a reward (sugar solution) or punishment (electric shock) is paired with an odor A during training. When choosing odor A versus a naïve odor B in a T-maze, flies prefer or avoid odor A depending on whether it has previously been paired with reward or punishment. The amount of time that individual flies can remember this pairing depends on the training regime. These paradigms, particularly the shock-odor pairing [81], have been used extensively in the past 25 years to study learning and memory in flies. We discuss them here as an example of olfactory circuit analysis, because a range of experiments varying from lesion studies, molecular genetic analyses of learning and memory mutants, genetic silencing and functional imaging experiments have shown that the MB plays an essential role in olfactory associative learning and memory [82-84].

Classical conditioning requires pairing of a conditioned stimulus (CS, odor) with an unconditional stimulus (US, sugar or electric shock). The CS and US pathways thus must converge at some point, such that the US can modulate the synaptic connection strength of the CS. MB neurons are a prime candidate for the convergence of US and CS. We will begin by describing the CS pathway input from PNs.

4.2 PN input to MB neurons

PNs connect with MB neurons at the MB calyx and constitute a major source of excitatory input to the MB through their release of the neurotransmitter acetylcholine [48,85]. The potential connectivity between different PN classes and MB neuron subtypes was previously predicted based on morphology by aligning the PN axonal arborization with the MB neuron dendrite fields [51]. It has been estimated that each MB neuron receives input from an average of 10 PNs in *Drosophila* [86].

MB neurons have a very low spontaneous firing rate and

their responses to odors are highly selective and sparse. One electrophysiological study reported that a given odor evoked a spiking response in only (6 ± 5)% of the MB neurons, compared with (59 ± 14)% in PNs. These responses were typically comprised of only a few spikes. Three mechanisms might contribute to this sparse coding: the rapid decay of PN excitatory synaptic potentials; the low convergence of PNs onto individual MB neurons; or the high firing thresholds of MB neurons. Interestingly, α'/β' neurons are more broadly tuned than γ and α/β neurons. In addition, α'/β' neurons have a higher spontaneous firing rate and stronger responses to odors [86]. The sparse odor responses in MB neurons were suggested to decrease overlap between odor representations and increase the ability for the fly to discriminate different inputs.

Functional imaging with G-CaMP in response to odor stimulation suggests stereotyped odor-evoked activity in the MB calyx and cell bodies [87]. The MB neurons displaying fluorescence transients evoked by cineole (three or four MB neurons responded in total) or 4-methyl cyclohexanol (one or two MB neurons responded in total) in different individuals were mapped to similar positions with a spatial precision on the order of the size of one or two MB neuron somata. Broader responses were observed from the MB neurons and calyx in response to other odors that also have stereotyped areas.

However, the stereotypy of MB neuron odor responses was questioned in a recent electrophysiological study using a more sensitive recording procedure [88]. Whole-cell patch-clamp recordings were performed on a small subset of MB neurons, ~23 late born α/β neurons in the left lateral posterior clonal unit (L-LP) labeled by the Gal4 line NP7175 [50]. MB neurons randomly sampled from L-LP+ neurons in 27 different flies failed to reveal obvious functional repeats in either spiking or subthreshold odor response profiles. The responses from L-LP+ neurons across animals were as diverse as the responses across the entire MB neuron population [88]. The authors suggest that these data indicate a lack of connection stereotypy from PN to MB, in the form of connection pattern or synaptic weights or both. The PN→MB connection variability could be established during initial circuit development or affected by an individual fly's experience, reflecting the role of MB neurons in learning and memory.

4.3 Monoaminergic input to MB neurons

In addition to receiving cholinergic input from PNs in the calyx, MB neurons also express dopamine (DA) and octopamine (OA) receptors [89–91]. Genetic silencing of neural activity and mutant analysis suggested that DA neurons are necessary for aversive associative conditioning while appetitive conditioning is likely mediated by the OA neurons [92]. In *Drosophila* larvae, it was further demonstrated that activation of DA or OA neurons is sufficient to enforce the aversive or attractive stimuli respectively [93].

The adult MB is innervated by several clusters of DA neurons. Among them, the PPL1, a cluster of 12 DA neurons in each hemisphere, appears to represent information from aversive stimuli. Calcium imaging experiments have indicated that PPL1 neuronal processes innervating the tip of the α lobe and the lower stalk/junction show preferential responses to electric shock [94]. Furthermore, utilizing an optical method to activate genetically restricted DA neuron subtypes, PPL1 neurons have been identified as sufficient for directing aversive reinforcement to form aversive olfactory memory in behaving flies [95].

Interestingly, three PPL1 DA neurons per hemisphere express receptors for neuropeptide F (dNPF), which signals food deprivation. When flies are well fed (and dNPF activity decreases), their performance in appetitive learning decreases. Stimulating dNPF expressing neurons can promote learning in well-fed files. Furthermore, blocking the activity of these dNPF receptor expressing PPL1 DA neurons improves memory performance of well-fed flies whereas stimulating these neurons suppresses memory performance of hungry flies [96]. These data suggest the three PPL1 DA neurons, which project to the MB heel and peduncle occupied by α/β but not α'/β' neurons, carry satiety information that modulates MB-mediated appetitive learning.

Octopaminergic (OA) neurons ramify in many regions of the fly brain, including the MB [97]. It remains to be demonstrated in the adult fruit fly whether specific OA neurons convey appetitive US to the MB. However, findings from honeybee VUMmx1 neurons may shed light on this neural circuit [98]. VUMmx1 neurons arborize profusely in the brain, including in the MB calyces. Sugar application has been found to excite VUMmx1, while pairing odor stimuli with subsequent VUMmx1 depolarization showed associative effects on the response to the training odor. Octopamine immunostaining in *Drosophila* revealed VUMs with similar projection patterns [97]. The challenge is to obtain genetic access to specific subpopulations of OA neurons to test their role in the appetitive conditioning.

4.4 Integrating the US and CS in the MB

The anatomical and behavioral data we have presented so far support an attractive model, that MB neurons are the site of convergence of CS (odors) and US (sugar as reward or electric shock as punishment) for olfactory associative learning and memory. This model is further strengthened by genetic rescue experiments, which have shown that adult expression of an adenylyl cyclase encoded by *rutabaga* only in MB neurons is sufficient to rescue the olfactory learning defect of *rutabaga* mutant animals [99,100]. It is well known that cAMP, a product of adenylyl cyclase, serves as a regulator of synaptic plasticity in well known learning paradigms such as *Aplysia* siphon withdrawal [101]. By analogy, the pairing of CS and US in MB neurons may regulate the

synaptic strength of the CS pathway. Indeed, recent imaging experiments have suggested that PN activation (mimicked by application of acetylcholine in MB calyx) and dopaminergic activation (by applying dopamine to the MB lobe) result in synergistic increases of the level of cAMP in MB neurons, and that such cAMP elevation in turn facilitates calcium transients in MB neurons [102]. *In vivo* imaging of the cAMP-dependent protein kinase A (PKA) activity has further indicated this synergy between the dopaminergic and cholinergic inputs occurs subcellularly in the α lobe and is Rut dependent [103]. However, it remains to be determined which plastic synapses are important in this associative learning, and which postsynaptic target neurons interpret such olfactory associations.

4.5 Functional subdivisions of different MB lobes

Lesion studies, molecular genetic analysis of learning and memory mutants, and functional and behavior studies have all suggested that different MB lobes may be associated with different phases of memory. MB γ neurons are critical for olfactory associative short-term memory, while the vertical α/α' lobes play a role in long-term memory formation [99,104,105]. The outputs from α'/β' neurons are required during memory acquisition and consolidation, whereas the outputs from α/β are required during memory retrieval [106–108]. DPM neurons, which use the Amnesiac neuropeptide as well as acetylcholine as neurotransmitters [59,109,110], are required during memory consolidation but not for acquisition or retrieval [110,111]. The DPM neuron has a large cell body residing in the dorsal protocerebrum of each brain hemisphere, and extends a single neurite that branches into all the MB lobes and the base of the peduncle (Figure 3). It has been proposed that MB α'/β' neurons and DPM neurons form a reciprocal loop to stabilize memory [107].

Functional imaging of MB neurons and DPM neurons provides further evidence regarding when and where olfactory memory is formed in the MB. An increase of calcium transients in response to electric shock-paired odor in DPM neurons (readout of 'memory trace') is observed after 30 min and lasts for at least 1 h following classical conditioning. This increase is specific for the DPM neuropil innervating the MB dorsal lobes [112]. When detected at ~ 1 h after conditioning, such memory traces have been observed in α'/β' lobes in response to a conditioned olfactory stimulus, compared to when it is not conditioned [113]. After five cycles of spaced training which would be expected to induce long-term memory, memory traces were observed in the α/β lobe of the mushroom body when tested at 9 or 24 h after training, but not after 3 h. These findings suggest that memory is formed and stored in different locations at different phases and can be transferred sequentially (e.g., from the DPM neuropil, to the α'/β' lobes, to the α/β lobes). However, it remains to be determined whether these different memory traces represent sequential or parallel information processing events, how these traces interact with each other, and whether these traces are causally related to memory performance.

5 Future perspectives

The last decade has seen a dramatic increase in our knowledge of the mechanisms underlying olfaction, and particularly the olfactory circuit in the fruit fly. Despite this, as discussed in each section above, there is still much to be learned before we have a complete picture of how odorant information is processed in the olfactory circuit, how such information directs innate odor-mediated behavior, and how experience modifies the circuits that underlie odor-associated learning and memory. We suggest three general directions for the future that will be helpful in answering these questions.

First, it is currently unclear how olfactory information is processed beyond the mushroom body and lateral horn, or in other "higher olfactory centers". To elucidate this issue it is necessary to identify the target neurons, and map their connectivity with known neuronal elements. In addition, a thorough understanding of the antennal lobe and mushroom body circuits will require knowledge of detailed connectivity among already identified neurons. Perhaps the most effective strategy will be to solve the fly connectome, producing an EM reconstruction of the wiring diagram of the entire fly brain. Although a complete wiring diagram will not be sufficient to decipher the information processing principle of a neural circuit, it would lay a solid foundation towards achieving a complete understanding.

Second, we need to systematically measure the responses of neurons at different stages of the olfactory circuit to decipher the way in which information flows across the olfactory circuit. To ensure that such measurements are reproducible across different animals, it would be ideal to have genetic access to all types of neurons involved in the olfactory information processing. We can then use electrophysiology and optical imaging to measure the activity of these identifiable neurons.

Third, to test the causal relationship between activities of specific populations of neurons and their physiological or behavioral consequences, we need to systematically perturb the activities of specific populations of neurons, while measuring circuit output in downstream neurons, or in behavior. A number of tools capable of silencing or activating neuronal activity using genetically encoded effectors are being developed; the limiting factor is obtaining genetic access to specific subpopulations of neurons, and establishing specific physiological or behavioral paradigms in which

output of the circuit can be quantitatively measured.

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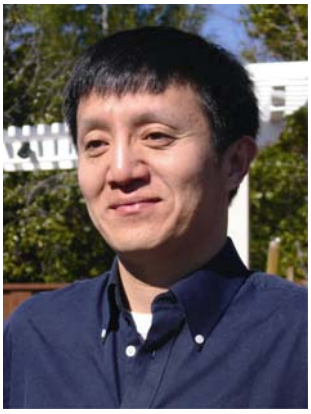
Biographical Sketch



Dr. Luo Liqun is a Professor in the Department of Biology at Stanford University, and an Investigator of the Howard Hughes Medical Institute. Dr. Luo grew up in Shanghai, China, and earned his bachelor's degree in molecular biology from the University of Science and Technology of China. He was a CUSBEA fellow of Class VI (1987). After obtaining his Ph.D. in Brandeis University, and postdoctoral training at the University of California, San Francisco, Dr. Luo started his own lab in Stanford in December, 1996. Together with his postdoctoral fellows and graduate students, Dr. Luo studies the logic of organization and assembly of neuronal circuits using genetic tools. They have developed mosaic marking systems and used it to study how signals are transduced from cell surface receptors to the cytoskeleton, how neuronal processes are pruned, and how neuronal circuits are organized and built. Dr. Luo teaches to Stanford undergraduate and graduate students "Molecular and Cellular Neurobiology" and "Exploring Neural Circuits". He is the recipient of several awards, including the Society for Neuroscience Young Investigator Award and the McKnight Technological Innovation in Neuroscience Award.