Molecular Architecture of Smell and Taste in *Drosophila*

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Key Words

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Abstract

The chemical senses—smell and taste—allow animals to evaluate and distinguish valuable food resources from dangerous substances in the environment. The central mechanisms by which the brain recognizes and discriminates attractive and repulsive odorants and tastants, and makes behavioral decisions accordingly, are not well understood in any organism. Recent molecular and neuroanatomical advances in *Drosophila* have produced a nearly complete picture of the peripheral neuroanatomy and function of smell and taste in this insect. Neurophysiological experiments have begun to provide insight into the mechanisms by which these animals process chemosensory cues. Given the considerable anatomical and functional homology in smell and taste pathways in all higher animals, experimental approaches in *Drosophila* will likely provide broad insights into the problem of sensory coding. Here we provide a critical review of the recent literature in this field and comment on likely future directions.

Contents

INTRODUCTION

Chemosensory systems allow animals to orient themselves in their chemical environments. Given the diversity of chemicals involved and given their combinatorial and temporal variability, understanding how the brain handles this task is a real challenge. A major breakthrough in chemosensory research was achieved with the identification of odorant receptor (OR) genes in rodents (Buck & Axel 1991), in *Caenorhabditis elegans* (Sengupta et al. 1996), and in *Drosophila melanogaster* (Clyne et al. 1999b, Gao & Chess 1999, Vosshall et al. 1999). These studies revolutionized our understanding of olfactory coding, primarily at the olfactory receptor neuron (ORN) level. Moreover, investigating OR expression patterns made it possible to dissect the circuits underlying olfaction at a hitherto unknown level of resolution (Ressler et al. 1994, Vassar et al. 1994, Gao et al. 2000, Vosshall et al. 2000). The evidence obtained by these new tools confirmed previous concepts of a common design of mammalian and insect olfactory systems (Hildebrand & Shepherd 1997, Strausfeld & Hildebrand 1999). Remarkably, insect chemosensory systems, in particular in *Drosophila*, comprise only a fraction of the cell numbers of the vertebrate systems, providing an attractively simple option for investigating the chemical senses. An even simpler alternative is offered by the larval chemosensory system of *Drosophila* and other holometabolous insects. In this review, we highlight peripheral and central aspects of the chemical senses smell and taste in *Drosophila*, touching on the neuroanatomy, circuitry, and molecular biology that underlie these sensory modalities.

OR: odorant receptor

ORN: olfactory receptor neuron

Antennal lobe (AL): primary olfactory association center in the insect brain, equivalent to the mammalian olfactory bulb

Sensillum: typical sensory structure of arthropods, consisting of one or several sensory neurons, three accessory cells, and a common cuticular protrusion

SMELL AND TASTE: PERIPHERY

Olfactory Organs of Adult Flies

Like all other higher animals, flies sense odors with olfactory organs located exclusively on the head. All fly ORNs are housed in the third segment of the antenna and in the maxillary palp (**Figure 1*a–c***). Both of these olfactory appendages are covered with specialized hairs, called sensilla, which protect the ORNs from the insults of the external environment. Although fly "noses" look quite different from the mammalian nose, the underlying neurons are morphologically similar to vertebrate ORNs (**Figure 1*e***). Fly ORNs are bipolar and extend a single axon from the basal end of the neuron, terminating in an olfactory glomerulus in the antennal lobe (AL), the functional homologue of the mammalian olfactory bulb (**Figure 1*f***). From the apical side, each ORN extends a sensory dendrite ending in ciliated projections into the shaft of the sensillum. A given sensillum houses between one and four ORNs that are surrounded by support cells, which secrete sensillum lymph and keep each sensillum electrically insulated from its neighbor. This arrangement has made single-unit electrophysiology possible for a given

sensillum. A sharp electrode is inserted into the base of a sensillum, and extracellular activity of the ORNs in response to a panel of odorous stimuli can be measured. Each ORN in a sensillum has a characteristic spike amplitude, making it possible to infer the activity of one neuron, even in a sensillum that houses up to four ORNs (de Bruyne et al. 1999, 2001). This technique has been used to define the ligand receptive range for every maxillary palp ORN (de Bruyne et al. 1999, Goldman et al. 2005) and a majority of the antennal ORNs (de Bruyne et al. 2001, Hallem & Carlson 2006) (see below).

The antenna is covered with three different types of sensilla—basiconic, trichoid, and coeloconic—that differ in size and morphology (**Figure 1*C***) (Shanbhag et al. 1999) and the types of substances detected by the underlying neurons (**Figure 3**; see below). Sensilla are distributed in a stereotyped and bilaterally symmetric pattern, with large basiconic sensilla clustered at the medial-proximal side of the antenna and trichoid sensilla clustered at the lateral-distal edge. Small basiconic and coeloconic sensilla are interspersed in the middle region of the antenna. In total, there are between 1100–1250 ORNs in each antenna, with roughly 20% fewer large basiconic and 30% more trichoid sensilla in males than females (Stocker 2001). The functional consequences, if any, of this sexual dimorphism in sensillum number have not been documented. All ~1200 ORNs fasciculate into the antennal nerve and project along with auditory fibers from the second antennal segment and presumed hygro- and thermosensory neurons from the arista into the brain. The aristal neurons and ORNs both terminate in the AL (**Figure 1*f***; see also below).

The maxillary palp is a simpler structure than the antenna and contains only one class of basiconic sensilla. The distal tip of each palp is decorated with ~60 sensilla, each housing two ORNs (**Figure 1*b–c***). There is no documented sexual dimorphism in the structure or function of the palp. Functional analysis of the ligand receptive range of these 120 neurons revealed six different functional classes (de Bruyne et al. 1999), now known to represent the different combinations of ORs expressed in these ORNs (Couto et al. 2005, Goldman et al. 2005). Maxillary palp ORNs also terminate in the AL but fasciculate with gustatory neurons in the labial nerve and project through the suboesophageal ganglion (SOG) to reach the AL (**Figure 1*f***). The function of this olfactory organ is unknown at present, but its proximity to gustatory neurons on the proboscis suggests it may play an appetitive role in the fly.

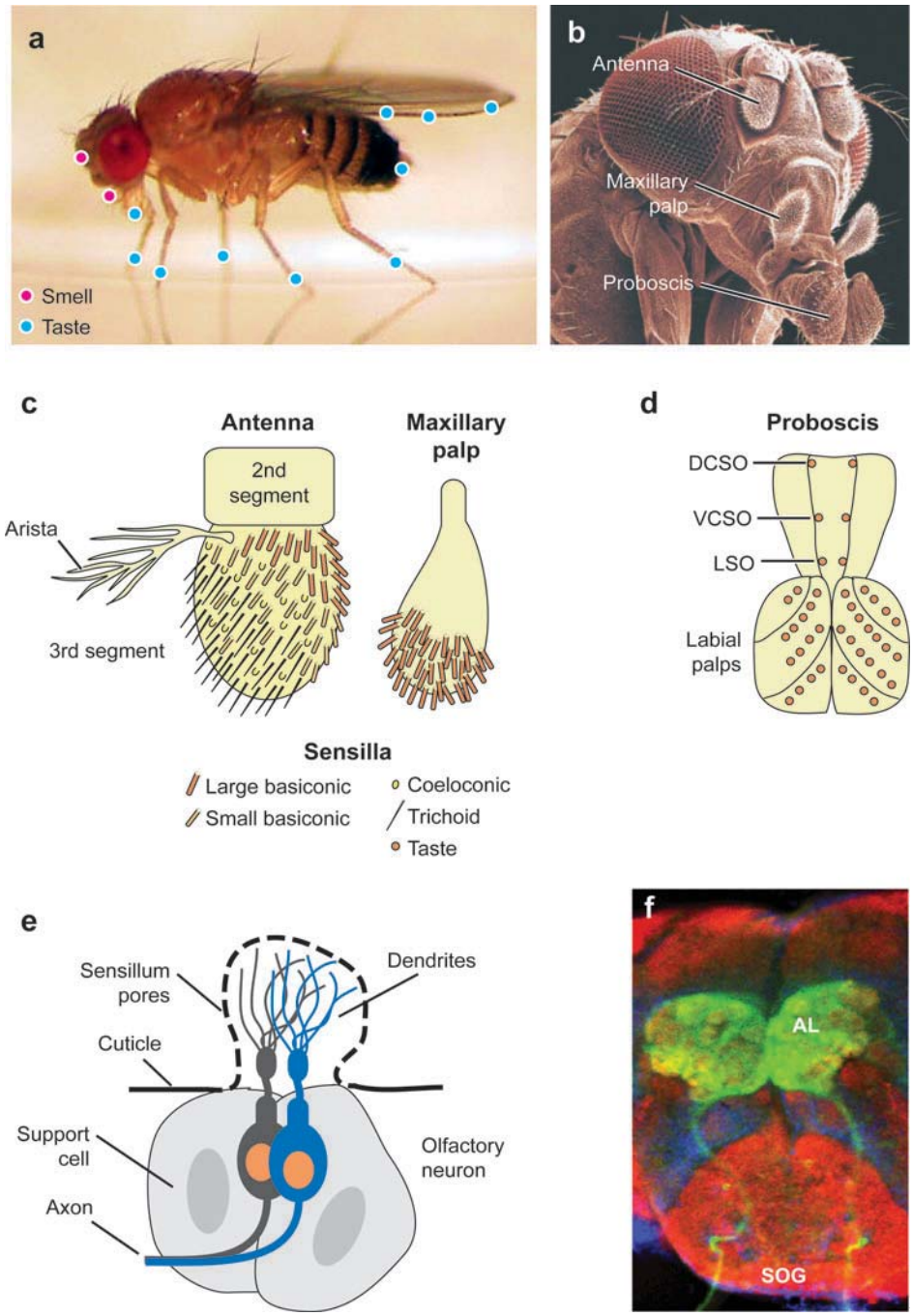
Suboesophageal ganglion (SOG): a region behind the brain proper in the insect central nervous system including the primary gustatory association area

GRN: gustatory receptor neuron

Gustatory Organs of Adult Flies

Although insects and mammals exhibit the same appetitive responses to sugars and aversive responses to bitter compounds, the organization of the gustatory system is completely different in these animals. Unlike vertebrate taste, which is restricted to a single gustatory organ in the head, insects distribute taste organs over their entire body surface. So although flies have a functional homologue of our tongue in their proboscis (**Figure 1*b*,*d***), they also taste with their legs and wings (**Figure 1*a***). Possibly the strangest sensory specialization is the gustatory capacity of vaginal plate sensilla at the tip of the female abdomen, which may be important for oviposition site selection (Stocker 1994). The proboscis houses a diversity of gustatory receptor neurons (GRNs), most of which reside on the labial palps (also known as labella) (**Figure 1*d***). Each labellum is decorated with 31 taste bristles, each housing between two and four GRNs. In addition, each labellum has ~30 taste pegs housing one GRN each. These are the primary gustatory neurons that allow flies to evaluate food quality before ingesting it. The pharynx of the fly is lined with three bilaterally symmetric internal taste organs, the labral sense organ (LSO) and the ventral and dorsal cibarial sense organs (VCSO, DCSO). The LSO contains nine sensilla, three of which are gustatory, for a total of ten GRNs (Gendre et al. 2004).

The VCSO comprises three sensilla with a total of probably eight GRNs, and the DCSO comprises two sensilla with three GRNs each (Gendre et al. 2004). These internal taste organs may permit the fly to evaluate food quality after it is ingested and before it transits to the digestive organs. Each side of the proboscis therefore has a total



of 69 taste sensilla. External taste sensilla are categorized by size, distribution, and number of GRNs into three classes: Small (s-type) and long (l-type) sensilla each have four GRNs, whereas intermediate (i-type) sensilla have two GRNs each (Rodrigues & Siddiqi 1981). Electrophysiological analysis of the tuning of s-type and l-type taste sensilla on the labellum suggests that each contains one neuron tuned to sugar (S cell), one neuron activated by water (W cell), one neuron activated by low salt (L1 cell), and one activated by high salt (L2 cell) (Rodrigues & Siddiqi 1981, Amrein & Thorne 2005). The i-type sensillum is missing the W cell and segregates both L1 cell and S cell activity into a single GRN; the second GRN has L2 cell properties (Hiroi et al. 2004). The relationship between the electrophysiological classification of gustatory sensilla and the molecular map of gustatory receptor (GR) expression in the underlying GRNs is still being clarified (Dunipace et al. 2001, Scott et al. 2001, Hiroi et al. 2004, Thorne et al. 2004, Wang et al. 2004b)(see below).

Taste perception by other body parts is mediated by large numbers of taste bristles (Nayak & Singh 1983, Stocker 1994). The first leg has ~50 taste sensilla in males and ~37 in females. This sexual dimorphism is due to the presence of specialized male-specific sensilla that detect female pheromones and are important for sexual behavior (Bray & Amrein 2003, Park et al. 2006). Each second and third leg has 30 and 32 taste sensilla, respectively, with no sexual dimorphism in number or function. Each leg taste sensillum houses between two and four GRNs. The wing margin is decorated with 40 taste bristles, each containing four GRNs. The vaginal plates on the female possess ~10 poorly characterized sensilla that have a chemosensory morphology. Although they may modulate egg-laying behavior, essentially nothing is known about the neurophysiology, molecular biology, or neuroanatomy of these unusual genital GRNs. In conclusion, much work remains to be done to characterize the biological function of the atypical body GRNs and how they contribute to the gustatory behavior of the fly.

GR: gustatory receptor

Larval Olfactory and Gustatory Organs

In insects that undergo complete metamorphosis, adults and larvae display very distinct lifestyles. For example, adult *Drosophila* flies must forage over considerable distances to locate nutrients, mates, and egg-laying sites. In contrast, fly larvae live directly on their food source and hence do not need long-range locomotion. These different lifestyles are expected to be paralleled by differences in chemosensory performance, as well as in the complexity of the underlying neural circuits. Although larvae respond to many volatiles (Rodrigues 1980, Cobb 1999, Heimbeck et al. 1999, Cobb & Domain 2000, Fishilevich et al. 2005), the larva's olfactory system is much

Figure 1

Neuroanatomy of the peripheral fly chemosensory system. (*a*) Schematic indicating the position of olfactory (*pink*) and gustatory (*blue*) neurons on the body of the fly. (*b*) Scanning electron micrograph of a fly head, indicating the major chemosensory organs. SEM image courtesy of J. Berger, MPI-Developmental Biology, Tübingen, Germany. (*c*) Schematic of the exterior surface of the olfactory organs. (*d*) Schematic of the proboscis. Abbreviations: LSO, labral sense organ; VCSO, ventral cibarial sense organ; DCSO, dorsal cibarial sense organ. (*e*) Schematic of a typical olfactory sensillum, housing two ORNs (*gray and blue*). (*f*) Immunofluorescence staining of fly brain whole mount with Or83b:GFP-labeled ORN axons and their terminals in green, brain neuropil in red, and nuclei in blue. Note prominent labeling of maxillary palp axons traversing the SOG en route to the AL. Panels (*b-c*) adapted from Benton et al. (2006), published by the Public Library of Science, which uses the Creative Commons Attribution License.

Glomeruli: well-delimited neuropil microareas comprising the synapses between first- and second-order olfactory neurons or second- and third-order olfactory neurons

Local interneuron (LN): inhibitory interneurons that modulate incoming odor signals in the antennal lobe

simpler than the adult system, at least in terms of cell numbers (Python & Stocker 2002a, Fishilevich et al. 2005, Kreher et al. 2005, Masuda-Nakagawa et al. 2005, Ramaekers et al. 2005). This simplicity is evident particularly at the sensory level, which also differs anatomically from its adult counterpart. However, the central olfactory circuit is surprisingly similar at the two stages, as shown below. For the gustatory system, differences between adults and larvae are less obvious.

The larval chemosensory apparatus includes three major sense organs on the head surface, dorsal organ, terminal organ, and ventral organ, as well as three pharyngeal organs (Gendre et al. 2004) (**Figure 2**). These sense organs consist of multiple sensilla, each comprising one to several sensory neurons and three accessory cells. The dorsal organ is composed of the central, multiporous "dome" and six peripheral sensilla. The dome is innervated by the dendritic arbors of 21 sensory neurons, which proved to be ORNs, using electrophysiological (Oppliger et al. 2000, Kreher et al. 2005) and ablation studies (Heimbeck et al. 1999). Larvae in which these cells were selectively blocked or ablated by toxin expression became anosmic, suggesting that these neurons are the sole larval ORNs (Larsson et al. 2004, Fishilevich et al. 2005). In analogy to *Musca* (Chu & Axtell 1971, Chu-Wang & Axtell 1972), the remaining sensilla of the dorsal organ as well as those of the terminal and ventral organs may be

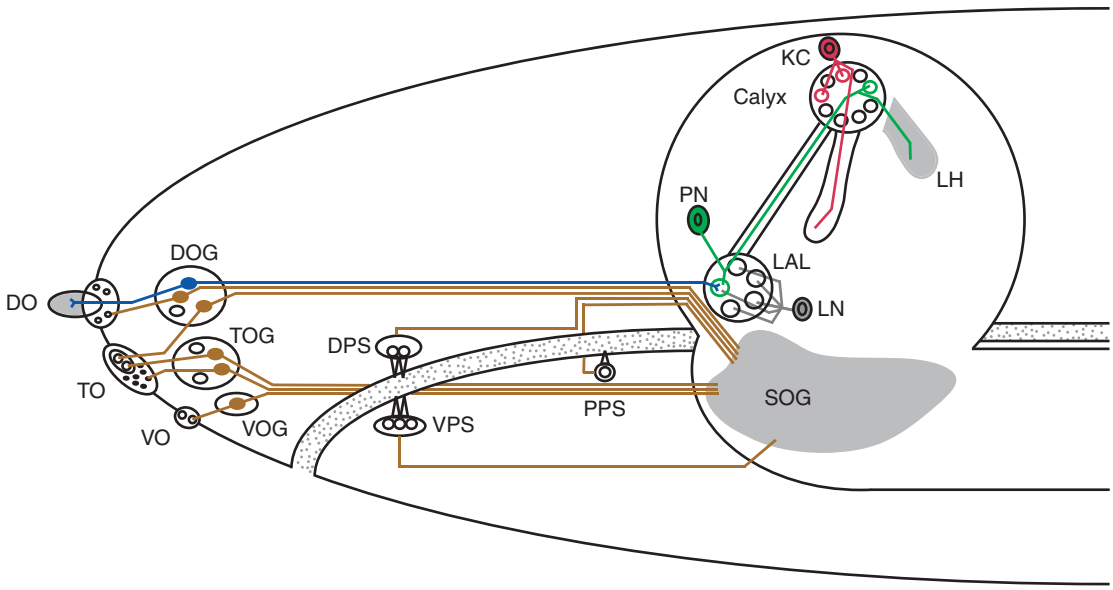


Figure 2

Neuroanatomy of the larval chemosensory system. From the three external chemosensory organs, the mixed dorsal organ (DO) comprises the olfactory "dome" (*gray*) and a few putative taste sensilla (*small circles*). The terminal organ (TO), the ventral organ (VO), and the dorsal, ventral, and posterior pharyngeal sense organs (DPS, VPS, PPS) include mainly taste sensilla. The sensory neurons' cell bodies are collected in ganglia below each sense organ (DOG, TOG, VOG). Some neurons innervating the TO are located in the DOG. ORNs (*blue*) project into individual glomeruli of the larval antennal lobe (LAL), which are interconnected by local interneurons (LN). Projection neurons (PNs, *green*) link the LAL with two higher olfactory centers, the mushroom body (MB) calyx and the lateral horn (LH). An intrinsic MB Kenyon cell (KC) is shown in red. GRN afferents (*brown*) extend via four different nerves to the SOG. The pharynx is shown stippled. Adapted from Stocker (2007) with permission from Eureka.com/Landes Biosciences.

mostly taste sensilla. However, these organs very likely include thermosensory (Liu et al. 2003b) and mechanosensory or hygrosensory neurons. Gustatory and mechanosensory function is also to be expected for the three pharyngeal sense organs.

The most striking difference when comparing peripheral chemosensation between larva and adult is the dramatic drop of ORN numbers from 1300 in the adult to a mere 21 in the larva. For GRNs, the difference is much less pronounced; an estimated 300 GRNs on the adult head (Stocker 1994, Matsunami & Amrein 2003) are facing perhaps 80 GRNs on the larval head (Python & Stocker 2002a). Thus, quite in contrast with the adult fly, GRNs outnumber ORNs in the larva, consistent with an expected predominant short-range chemical orientation. Another larval-specific feature is the mix of smell and taste functions at the sensory level, even within an individual sense organ. Distinction between smell and taste may be less important for a substrate feeder than for a flying insect, but whether this indicates that larval ORNs can also sense tastants and GRNs can sense volatile cues is not known. Nevertheless, at the primary target level in the CNS, the two functions become clearly segregated: Olfactory afferents project into a glomerular-type AL, whereas taste information is sent to multiple target areas in the SOG (**Figure 2**). Thus, adult and larval chemosensory systems are anatomically different at the peripheral level but share the same design of sensory projections and central pathways (see below).

ODORANT AND GUSTATORY RECEPTORS

Odorant Receptors

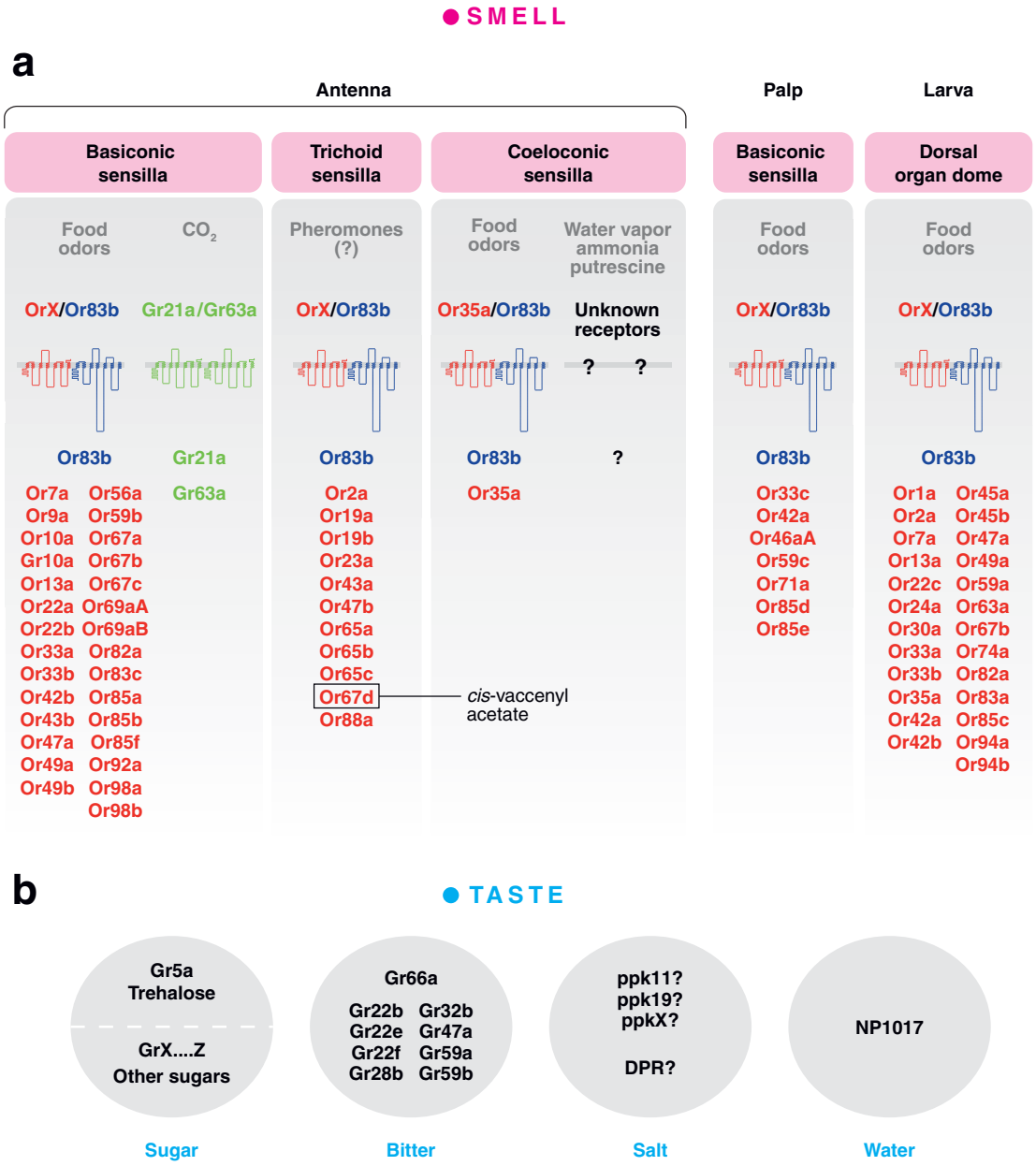
Drosophila ORs were identified in 1999 by combined difference cloning (Vosshall et al. 1999) and bioinformatic approaches (Clyne et al. 1999b, Gao & Chess 1999, Vosshall et al. 1999) that searched for a family of seven transmembrane domain proteins selectively expressed in ORNs. These indirect approaches were used because homology-based approaches that searched for fly genes resembling vertebrate or nematode ORs had failed in multiple laboratories. Initially the OR gene family comprised 57 receptors (Vosshall et al. 2000), but subsequent genome curation brought the final number to 62 ORs transcribed from 60 OR genes, two ORs being the product of alternative RNA splicing (Robertson et al. 2003) (**Figure 3**). Fly OR genes, like nematode and vertebrate OR genes, encode receptor proteins with seven membrane-spanning domains. However, unlike worm and vertebrate ORs (Buck & Axel 1991, Troemel et al. 1995), fly ORs have no homology to G protein–coupled receptors (GPCRs) and evolved independently of chemosensory receptors in these other animals (Vosshall et al. 1999). Recent experimental and bioinformatic analysis confirms that the fly ORs represent a novel family of membrane proteins with a membrane topology inverted relative to that of GPCRs (Benton et al. 2006, Wistrand et al. 2006). The functional meaning of this atypical topology, and what implications it might have for fly OR signal transduction, remains to be elucidated.

The fly OR family is relatively evenly distributed across all three major chromosomes, with only a few instances of OR genes found in clusters. Some of these clusters represent recent duplications, but most are only distantly related. Robertson et al. (2003) argue that the absence of large clusters of related ORs suggests a very ancient origin of the gene family. Further supporting this hypothesis, the overall amino acid homology across the family of OR genes is low, in the range of 20%. RNA in situ hybridization analysis of the fly ORs revealed that each gene is expressed in a spatially conserved subpopulation of ORNs either in the antenna, maxillary palp, or larval dorsal organ (Clyne et al. 1999b, Gao & Chess 1999, Vosshall et al. 2000, Couto et al. 2005, Fishilevich et al. 2005, Goldman et al. 2005, Kreher et al. 2005) (**Figure 3*a***). A single OR, *Or83b*, is expressed in virtually all ORNs in

Projection neuron (PN): second-order olfactory neurons, which connect the primary olfactory center—the AL—with higher olfactory centers

Mushroom body (MB): higher integrative brain center of insects involved in olfactory learning, other forms of learning, locomotor control, and sleep regulation

the fly (Vosshall et al. 1999) (see below). Of the 25 ORs detected in the larva, 14 are larval specific, whereas 11 ORs are expressed in both larval and adult olfactory organs (Fishilevich et al. 2005). There is no obvious molecular or evolutionary logic dictating which OR is expressed in a particular olfactory organ, or even a sensillum type (Couto et al. 2005, Hallem & Carlson 2006). Carlson and colleagues have used single sensillum electrophysiology to assign specific ORs to specific sensilla types and to identify odorous ligands for most of the fly ORs (Goldman et al. 2005, Kreher et al. 2005, Hallem & Carlson 2006). From this analysis



one can see that ORs expressed in trichoid ORNs tend to be weakly activated or strongly inhibited by general food odors and strongly activated by none. This observation suggests that the true ligands for these receptors might be pheromones, as recently confirmed for *Or67d*, which is strongly activated by the aggregation pheromone 11-*cis*-vaccenyl acetate (Ha & Smith 2006) (**Figure 3*a***). The 11-*cis*-vaccenyl acetate response requires both *Or67d* and an odorant binding protein called *lush* for full ligand activation of the ORN (Xu et al. 2005). ORs expressed in antennal and palp basiconic ORNs tend to be strongly activated by at least one of the general food odors tested (Goldman et al. 2005, Hallem & Carlson 2006) (**Figure 3*a***). Larval ORs are tuned to either aromatic or aliphatic food odor compounds (Kreher et al. 2005) (**Figure 3*a***). The most enigmatic aspect of the fly olfactory system that remains to be decoded is the molecular basis of chemoreception in the coeloconic sensilla. ORNs in these structures are tuned to special stimuli, including amines, ammonia, water vapor, and putrescine (Yao et al. 2005). Whereas a single class of coeloconic sensillum expresses *Or35a*, a broadly tuned member of the OR gene family, the nature of the receptor(s) in the other coeloconic sensilla remains a mystery and suggests that additional classes of ORs remain to be discovered in the fly genome.

In vertebrates, each ORN expresses only a single OR allele (Malnic et al. 1999), whereas in flies each ORN expresses one conventional ligand-binding OR and the atypical receptor, Or83b (Vosshall et al. 2000, Larsson et al. 2004, Benton et al. 2006). Or83b is an obligate coreceptor that associates with the conventional OR early in the endomembrane sorting pathway and targets the OR/Or83b heteromeric complex to the ciliated dendrite of the ORN. Or83b is necessary and sufficient to mediate both the ciliary targeting and functional expression of ORs in any ciliated neuron in vivo (Benton et al. 2006). Although some studies report OR/OR homodimerization and ORs functioning in heterologous cells without Or83b, the functional significance of these in vitro results is unclear (Wetzel et al. 2001, Neuhaus et al. 2004). In fact, although initial reports of *Or83b* gene expression suggested that it is expressed in only about two thirds of all ORNs (Larsson et al. 2004), more recent functional experiments suggest that *Or83b* is required for trafficking and functioning of all basiconic and trichoid ORs in the adult and all larval ORs (Benton et al. 2006) (**Figure 3*a***). The only class of ORN that neither expresses nor requires *Or83b* for function is the carbon dioxide (CO_2)-sensitive ORN expressing *Gr21a* and *Gr63a* (Suh et al. 2004, Benton et al. 2006, Faucher et al. 2006). Two recent

Figure 3

Distribution, composition, and tuning of *Drosophila* odorant and taste receptors. (*a*) A comprehensive list of ORs and gustatory receptors (GRs) expressed in each olfactory sensory organ, subdivided by functional class and sensillum. Note that *Or83b* is an obligate coreceptor for all olfactory neurons except the CO_2-sensitive neurons expressing the Gr21a/Gr63a CO_2 receptor (Jones et al. 2007, Kwon et al. 2007). The identity of the receptors expressed in antennal coeloconic neurons that sense water vapor, ammonia, and putrescine is unknown. Data are taken from Vosshall et al. (1999), Suh et al. (2004), Couto et al. (2005), Fishilevich et al. (2005), Fishilevich & Vosshall (2005), Goldman et al. (2005), Kreher et al. (2005), Yao et al. (2005), Benton et al. (2006), Hallem & Carlson (2006) Jones et al. (2007), Kwon et al. (2007). (*b*) The molecular neuroanatomy of taste. Sweet taste neurons express the trehalose receptor *Gr5a* and presumed additional GRs that detect other sugars (Dahanukar et al. 2001, Dunipace et al. 2001, Wang et al. 2004b). Bitter taste neurons express *Gr66a* and a combinatorial of additional GRs (Dunipace et al. 2001, Wang et al. 2004b). Salt taste may be mediated by members of the DEG/EnaC family (*ppk11*, *ppk19*) (Liu et al. 2003a) and the Ig-containing DPR gene (Nakamura et al. 2002). Water-sensitive gustatory neurons have no known receptor but are marked by the NP1017 Gal4 line (Inoshita & Tanimura 2006).

Table 1 Known instances of odorant receptor coexpression in *Drosophila*. Data from Couto et al. 2005 and Fishilevich & Vosshall 2005

Organ	Receptor 1	Receptor 2	Receptor 3
Antenna	Or10a	Gr10a	
Antenna	Or19a	Or19b	
Antenna	Or22a	Or22b	
Antenna	Or33a	Or56a	
Antenna[+]	Or33b	Or47a	
Antenna	Or33b	Or85a	
Antenna	Or49a	Or85f	
Antenna	Or65a	Or65b	Or65c
Antenna	Or69aA	Or69aB	
Antenna	Or85b*	Or98b*	
Maxillary palp	Or33c	Or85e	
Larval dorsal organ[+]	Or33b	Or47a	
Larval dorsal organ	Or94a	Or94b	

[+]Coexpressed both in the larva and the antenna (Fishilevich & Vosshall 2005, Fishilevich et al. 2005).

*Coexpression mentioned as possible but not validated in Couto et al. 2005.

studies have shown that Gr21a and Gr63a together are sufficient to induce CO_2 sensitivity when ectopically expressed in *Or22a*-expressing ORNs (Jones et al. 2007, Kwon et al. 2007). *Gr63a* mutant flies lose all electrophysiological and behavioral responses to CO_2, suggesting that Gr21a and Gr63a function together as a membrane-bound CO_2 receptor in *Drosophila* (Jones et al. 2007).

Beyond the coexpression of a single OR with the Or83b coreceptor, there are 13 known cases of a given ORN expressing two or even three ORs along with *Or83b* (**Table 1**). The functional significance of this receptor coexpression—if any—is unknown (Couto et al. 2005, Fishilevich & Vosshall 2005, Goldman et al. 2005), but it contrasts starkly with the strict one neuron to one receptor rule that has been established in vertebrates (Malnic et al. 1999, Serizawa et al. 2003).

Gustatory Receptors

Gustatory receptor (GR) genes were identified in *Drosophila* genome databases by the same bioinformatic algorithm used to identify the ORs (Clyne et al. 2000). GRs are distantly related to the ORs, and together these two families comprise a large superfamily of insect chemosensory receptor genes (Robertson et al. 2003). GRs as a class are even more divergent than the ORs; some share as little as 8% overall amino acid identity (Robertson et al. 2003). A small number of GR genes are expressed at levels sufficient to be detected in GRNs by RNA in situ hybridization (Scott et al. 2001). Most GR genes, however, could not be detected by such techniques, and their tissue distribution was subsequently determined with transgenic flies that express marker genes under control of GR promoter elements (Dunipace et al. 2001, Wang et al. 2004b). GR gene expression is complex, with two major genes *Gr5a* and *Gr66a* expressed in a very large number of nonoverlapping GRNs (**Figure 3*b***). Within the expression domain of the *Gr66a* gene, a larger number of other GR genes are found in partially overlapping subdomains (Dunipace et al. 2001, Wang et al. 2004b). No other GR genes coexpress with *Gr5a*, which was independently shown to encode a narrowly tuned trehalose receptor (Dahanukar et al. 2001, Ueno et al. 2001). *Gr5a* mutant GRNs lose responses to trehalose, but they retain normal responses to sucrose, suggesting that additional sucrose-tuned GRs must be expressed along with *Gr5a* in these GRNs (Dahanukar et al. 2001, Amrein & Thorne 2005).

Behavior genetic experiments in which *Gr5a* or *Gr66a* neurons were silenced or ablated strongly suggest that these GRNs correspond to the S cell and L2 cell, respectively. Animals lacking functional *Gr66a* cells fail to avoid bitter substances and high concentrations of salt, whereas animals lacking functional *Gr5a* GRNs are not attracted to sugar and low salt concentrations (Thorne et al. 2004, Wang et al. 2004b). The molecular identity of the receptor in the W cell that senses water is unknown, although a recent report identified a molecular marker (NP1017) for these neurons (Inoshita & Tanimura 2006) (**Figure 3*b***). Finally, the mechanisms of salt

taste in the fly remain to be fully characterized. Two members of the DEG/ENaC ion channel family, *ppk11* and *ppk19*, modulate the avoidance of high salt in behavioral assays (Liu et al. 2003a). A founding member of an Ig-containing gene family, DPR, also appears necessary for salt avoidance (Nakamura et al. 2002). More detailed gene knockout, gene expression, and heterologous expression of these candidate salt receptors will be necessary to confirm a role as the salt receptor in either the L1 or L2 salt-sensing GRNs.

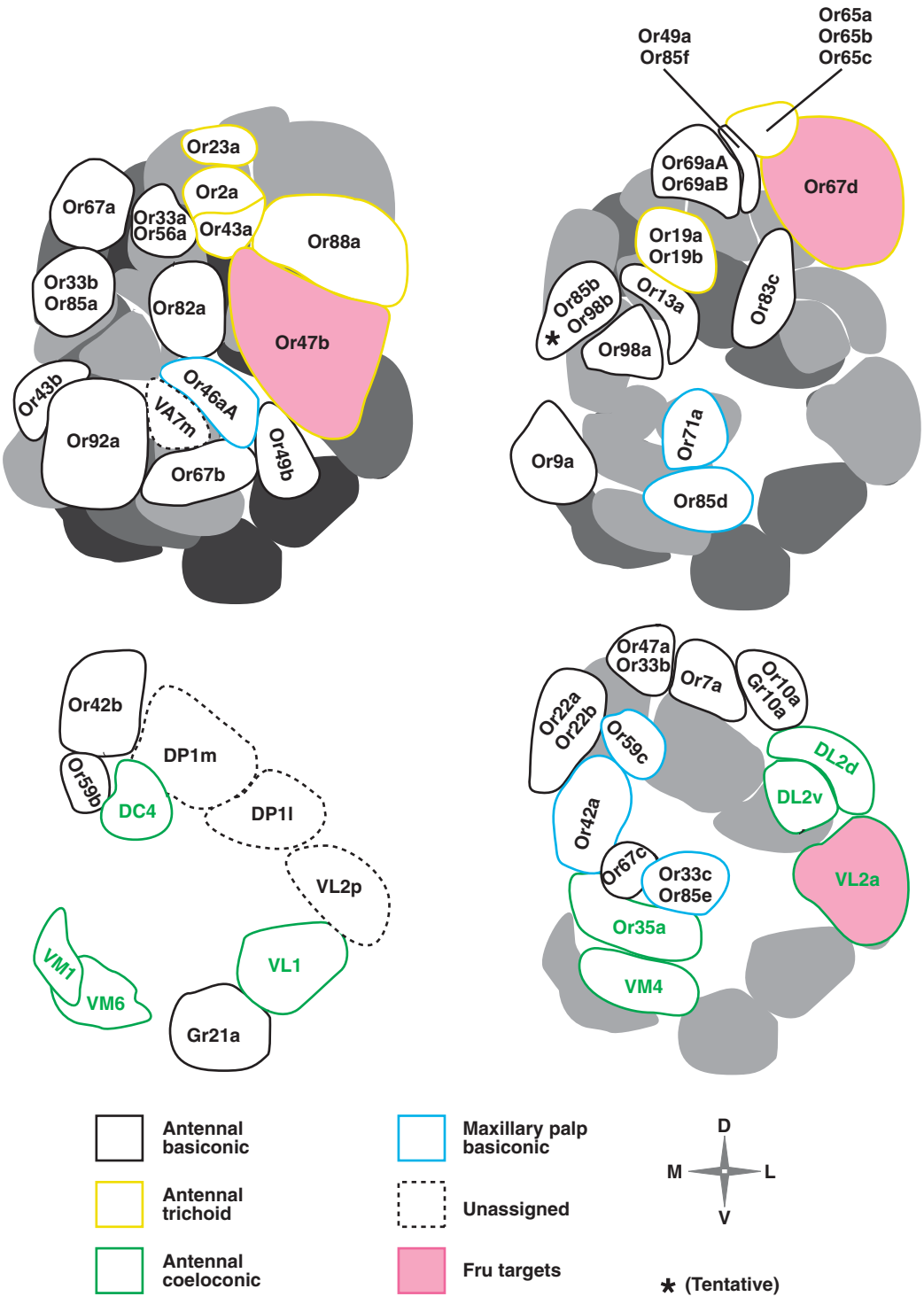
Some GRs may encode pheromone receptors that respond to cuticular hydrocarbons produced by males and/or females (Jallon 1984). A male-specific GR, *Gr68a*, is expressed in the first leg of male flies (Bray & Amrein 2003). Although investigators have not identified a candidate ligand for this GR, behavior genetic experiments demonstrated that the *Gr68a*-expressing GRNs and the *Gr68a* receptor are necessary for the normal progression of male courtship behavior. These manipulated males initiate early stages of courtship more rapidly than do control males but seem to stall in later stages of courtship, causing an overall reduction in normal courtship behavior. It will be interesting to define the full repertoire of GRs that respond to gustatory pheromones and study the circuitry that controls this complex social behavior.

CENTRAL OLFACTORY AND GUSTATORY CIRCUITRY

Adult Olfactory Pathway

In rodents, all ORNs expressing the same OR converge upon discrete glomeruli in the mouse olfactory bulb (Ressler et al. 1994, Vassar et al. 1994, Mombaerts et al. 1996). The availability of fly ORs made it possible to ask whether AL glomeruli were organized using the same OR-based logic. With 43 morphologically defined glomeruli in the AL (Laissue et al. 1999) and 39 ORs known to be expressed in the adult (Vosshall et al. 2000), it seemed plausible that the fly olfactory system also followed the 1 OR to 1 glomerulus rule. Early studies using transgenic flies in which all ORNs expressing a given OR were labeled with a fluorescent tracer supported the notion that ORNs expressing a given OR indeed target one or two glomeruli (Gao et al. 2000, Vosshall et al. 2000). Recent studies that have essentially completed the OR-to-glomerulus map confirmed and extended these initial findings (Couto et al. 2005, Fishilevich & Vosshall 2005) (**Figure 4**). There are some differences in nomenclature and numbers of glomeruli in the literature, and **Figure 4** attempts to present a consensus view of the AL (Laissue et al. 1999, Fishilevich & Vosshall 2005, Couto et al. 2005). Genetic tracing of 44 (Couto et al. 2005) and 30 (Fishilevich & Vosshall 2005) different OR-expressing populations of ORNs yielded a total of 46 different ORs definitively mapped to glomeruli. Another two (*Or85b*/*Or98b*) were inferred but not confirmed (**Figure 4**). In their remarkably complete study, Couto et al. (2005) assigned a glomerular identity for every antennal basiconic and trichoid ORN (black and yellow glomeruli in **Figure 4**) and every palp basiconic ORN (cyan glomeruli in **Figure 4**), and they used a genetic marker for the coeloconic ORNs to infer which eight glomeruli are targeted by these neurons (green glomeruli in **Figure 4**). Three glomeruli that are innervated by *fruitless*-expressing neurons (Manoli et al. 2005, Stockinger et al. 2005), and have been implicated in sexual courtship behavior, are indicated in pink in **Figure 4**. Four glomeruli (VA7m, DP1m, DP1l, VL2p) remain unassigned, and the type of sensory neuron that targets these structures is unknown.

Several salient conclusions can be drawn from this olfactory sensory map: (*a*) Projections from different sensilla types tend to cluster in the AL, with antennal basiconic neurons at the medial edge, antennal trichoid neurons at the lateral edge, palp basiconic neurons at the anterior middle portion, and coeloconic neurons at the ventral middle region (Couto et al. 2005). (*b*) Two large lateral glomeruli


Or23a
Or2a
Or67a
Or33a
Or56a
Or43a
Or88a
Or33b
Or85a
Or82a
Or47b
Or43b
Or46aA
VA7m
Or92a
Or49b
Or67b
Or49a
Or85f
Or65a
Or65b
Or65c
Or69aA
Or69aB
Or67d
Or19a
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Or9a
Or71a
Or85d
Or42b
Or59b
DP1m
DC4
DP1l
VL2p
VM1
VM6
VL1
Gr21a
Or47a
Or33b
Or7a
Or10a
Gr10a
Or22a
Or22b
Or59c
DL2d
DL2v
Or42a
Or67c
Or33c
Or85e
VL2a
Or35a
VM4
Antennal basiconic
Antennal trichoid
Antennal coeloconic
Maxillary palp basiconic
Unassigned
Fru targets
D
M
L
V
* (Tentative)

that are sexually dimorphic (Kondoh et al. 2003) and *fruitless*-positive (Manoli et al. 2005, Stockinger et al. 2005) are innervated by trichoid sensilla. These are thus good candidates to process pheromonal cues. In fact, ORNs expressing the *Or67d* 11-*cis*-vaccenyl acetate receptor project to one of these special glomeruli (Ha & Smith 2006). (*c*) Many additional cases of OR coexpression were uncovered in the course of making this map (see also **Table 1**). Thus there is a compression in the convergence of ORNs in the fly AL: ORNs expressing a combinatorial of 45 unique ORs converge on 36 glomeruli. The great mystery in the map is the identity of the receptors that innervate the 8 coeloconic glomeruli at the posterior face of the AL. (*d*) A possible chemotopic arrangement of glomeruli in the AL, such that glomeruli activated by similar odors cluster in the AL, remains a point of controversy (Couto et al. 2005, Fishilevich & Vosshall 2005, Hallem & Carlson 2006).

The convergent OR projections onto a common AL glomerulus (see above) define the input properties of the glomeruli: The odor information that each glomerulus receives is constrained to the ligands that activate its corresponding OR. However, the AL is a site of intricate synaptic complexity, implying that olfactory information is modified at this level. The two major target neurons of the ORNs are local interneurons (LNs), which provide "horizontal" connections among glomeruli, and cholinergic projection neurons (PNs) most of which link individual glomeruli "vertically" with two higher olfactory centers, the mushroom body (MB) and the lateral horn (Stocker 1994) (**Figure 5**).

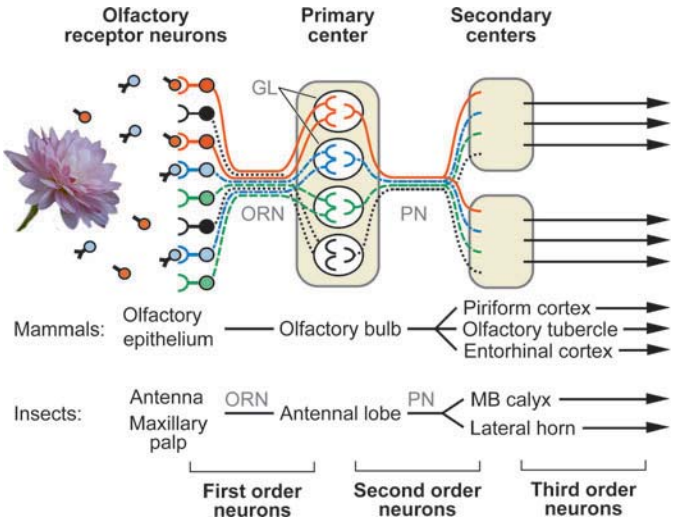

Figure 5

Parallels in olfactory processing between mammals and insects. Odorants emitted from a stimulus activate distinct subsets of ORNs, which converge on glomeruli in either the olfactory bulb or the AL. From here information is relayed to higher brain centers, which have functional and neuroanatomical parallels in mammals and insects. Adapted from Tanaka et al. (2004) with permission from Elsevier.

Whether odor information passes straight from ORN to glomerulus and to PN is currently under debate (Ng et al. 2002, Wang et al. 2003, Wilson et al. 2004). Imaging of odor-evoked activity in AL glomeruli revealed essentially identical responses in ORN terminals and PN dendrites (Ng et al. 2002, Wang et al. 2003). However, whether the genetically encoded sensors used faithfully reflect neuronal activity is unclear. Indeed, whole-cell patch recordings demonstrated that PNs are

Figure 4

Molecular neuroanatomy of the adult AL annotated with the molecular and functional identity of the glomeruli. Glomeruli receiving projections from the ORNs expressing a given OR or GR are indicated, with antennal basiconic projections indicated in black, antennal trichoid projections in yellow, and antennal coeloconic projections in green. Maxillary palp projections are in cyan, and unmapped glomeruli in black dotted line. Glomeruli innervated by *fruitless* positive neurons (Manoli et al. 2005, Stockinger et al. 2005) are indicated in pink. The AL model is adapted from Laissue et al. (1999) and Fishilevich & Vosshall (2005) using data from Couto et al. (2005) and Fishilevich & Vosshall (2005) with permission from Elsevier. AL sections are presented from anterior to posterior, clockwise from top left, with depth-coding of black for posterior, gray for intermediate, and white for anterior sections.

more broadly tuned and display more complex firing patterns in terms of temporal structure than do the ORNs (Wilson et al. 2004), suggesting that cross-talk may occur in the AL between the vertical pathways. Evidence that LNs provide the substrate of this task was obtained in bees (Sachse & Galizia 2002), moths (Christensen et al. 1998), and *Drosophila* (Ng et al. 2002). The mostly GABAergic LNs (Wilson & Laurent 2005) receive excitatory input from ORNs and PNs and establish inhibitory synapses with both afferents and PNs. Recently, a second class of cholinergic excitatory LN was described, which may underlie the observed interglomerular excitation of PNs (Shang et al. 2007). The major role that seems to be accomplished by this intricate LN network is to synchronize PN activity, either within a given glomerulus or between PNs innervating different glomeruli (Ng et al. 2002). The transformation of olfactory signals may encompass both quantitative and qualitative parameters, such as detection threshold and odor discrimination, respectively. By widening the range of odor sensitivities in PNs compared with their afferents (Ng et al. 2002, Wilson et al. 2004), the AL may generate some kind of glomerulus-specific "odor image" (Laurent 1996) that is then transferred to higher olfactory centers. Hence, odor images are reflected both by temporal and by spatial PN activity (Lei et al. 2004). Recent studies in moths and flies demonstrate that odor representation in the AL becomes modified after appetitive or aversive odor conditioning (Daly et al. 2004, Yu et al. 2004), suggesting that the AL may represent a second substrate for olfactory memory apart from the MB (see below).

Many of these anatomical and functional features are shared by the mammalian olfactory system, apart from the glomerular convergence principle of ORNs (Hildebrand & Shepherd 1997, Strausfeld & Hildebrand 1999, Ache & Young 2005) (**Figure 5**). Both the insect AL and the mammalian olfactory bulb are characterized by inhibitory LNs (Hayar et al. 2004) whose main task seems to be the extraction of behaviorally relevant information from the incoming signals by changing their temporal structure (Luo & Katz 2001). Given this surprising conservation, the advantage of the numerically much-reduced insect olfactory system as a model is evident.

The next question is how the PNs convey to higher centers—MB and lateral horn—the activation pattern generated in the AL glomeruli. The MBs are integrative centers controlling various functions such as olfactory learning, other forms of learning (Heisenberg 2003, Davis 2005), locomotor activity (Martin et al. 1998), male courtship behavior (Sakai & Kitamoto 2006), and sleep (Joiner et al. 2006, Pitman et al. 2006). In contrast, the lateral horn seems to be involved in experience-independent odor recognition (de Belle & Heisenberg 1994, Heimbeck et al. 2001, Tanaka et al. 2004). The major input region of the MBs is the calyx, in which the terminals of PNs synapse with ~2500 intrinsic MB neurons, called Kenyon cells (Ito et al. 1997, Crittenden et al. 1998, Lee et al. 1999, Yasuyama et al. 2002, Strausfeld et al. 2003). The calyx is composed of hundreds of glomeruli (Yasuyama et al. 2002), two to eleven of which are targeted by individual PNs (Wong et al. 2002). Consistent with optical imaging data (Fiala et al. 2002), concentric target zones could be defined in the calyx for PNs deriving from specific AL glomeruli (Tanaka et al. 2004). In the lateral horn, the terminals of the same types of PN map along the dorsoventral and anteroposterior axes rather than concentrically (Tanaka et al. 2004). Single cell clones demonstrated that PNs innervating a particular AL glomerulus exhibit overlapping but distinct projection patterns in the lateral horn (Marin et al. 2002, Wong et al. 2002) and that this map shows some tendency to preserve glomerular neighborhood relations. Together, these results demonstrate that a topographic map of olfactory information is retained in the two higher olfactory centers, but the character of the maps differs from the one in the AL. Both

convergent and divergent projections of PNs contribute to this transformation, providing an opportunity for integration of olfactory information. As an interesting parallel in mice, the PN equivalents, the mitral cells, receive input from a single glomerulus in the olfactory bulb and establish stereotypic but widespread connections in the olfactory cortex (Zou et al. 2001).

To appreciate the level of integration in these higher olfactory centers, the connectivity between PNs and third-order olfactory neurons was assessed (Tanaka et al. 2004). In the MB calyx, dendrites of most of the Kenyon cell subtypes defined by their output patterns collectively cover the concentric PN zones. These neurons may thus act as coincidence detectors for interpreting combined PN activity (Perez-Orive et al. 2002, Heisenberg 2003, Wang et al. 2004a). This suggests that MBs are capable of integrating a wide range of odor information, consistent with their proposed role in olfactory learning (Heisenberg 2003, Davis 2005). In contrast, dendritic arborizations of third-order neurons in the lateral horn are constrained within a particular PN target area. These different zones are linked with different brain areas, forming preferential associations between distinct subsets of glomeruli and particular brain regions that ultimately control odor-driven behavior (Tanaka et al. 2004). This suggests little integration between different subsets of the olfactory signal repertoire in the lateral horn, consistent with its proposed role for mediating direct behavioral responses to odors (de Belle & Heisenberg 1994, Heimbeck et al. 2001).

Larval Olfactory Pathway

The basic organization of the larval olfactory circuit is surprisingly similar to its adult counterpart and to the one in mammals but is numerically much simpler (**Figure 6**). As in the adult and consistent with moth larvae (Itagaki & Hildebrand 1990), larval ORNs target two types of interneurons in the larval AL: LNs, which establish lateral connections, and PNs, which link the AL with the MB calyx and the lateral horn (Python & Stocker 2002a, Marin et al. 2005). Moreover, some evidence demonstrates that, comparable to adults, larval ORNs and PNs are cholinergic, whereas LNs express GABA (Python & Stocker 2002b).

The expression patterns of different *Or-Gal4* driver lines and single-cell clones generated in the *Or83b-Gal4* line revealed the presence of 21 identifiable glomeruli in the larval AL (Fishilevich et al. 2005, Kreher et al. 2005, Ramaekers et al. 2005) (**Figure 6**). As shown above, each of these glomeruli is the target of a single ORN expressing its proper OR (Fishilevich et al. 2005, Kreher et al. 2005). Single-cell clones in the PN-specific GH146-*Gal4* driver (Stocker et al. 1997) showed that these glomeruli are also recognized by the dendritic arborizations of larval PNs. These arbors are usually restricted to one or, rarely, two glomeruli (Marin et al. 2005, Ramaekers et al. 2005). Single-cell labeling further demonstrated that each glomerulus is innervated by only one or a few PNs (Ramaekers et al. 2005), suggesting that their number may not be much higher than the number of glomeruli.

How do the PNs pass on the activation pattern of the larval AL glomeruli to higher brain centers? In contrast with the adult MB calyx with its hundreds of glomeruli (see above) (Yasuyama et al. 2002), the larval MB calyx comprises only a small number of well-defined glomeruli (Marin et al. 2005). By studying choline acetyltransferase immunoreactivity in PN terminals in the GH146-*Gal4* driver (Ramaekers et al. 2005) or by expressing GFP-actin under the control of the MB-specific line OK107, up to 34 stereotypic calyx glomeruli were identified (Masuda-Nakagawa et al. 2005). PNs choose mostly single or, exceptionally, two calyx glomeruli as targets (Marin et al. 2005, Ramaekers et al. 2005). Again, each calyx glomerulus seems to be innervated by only one or a few PNs (Ramaekers et al. 2005). Comparison of input and output sites revealed several types of PNs, which

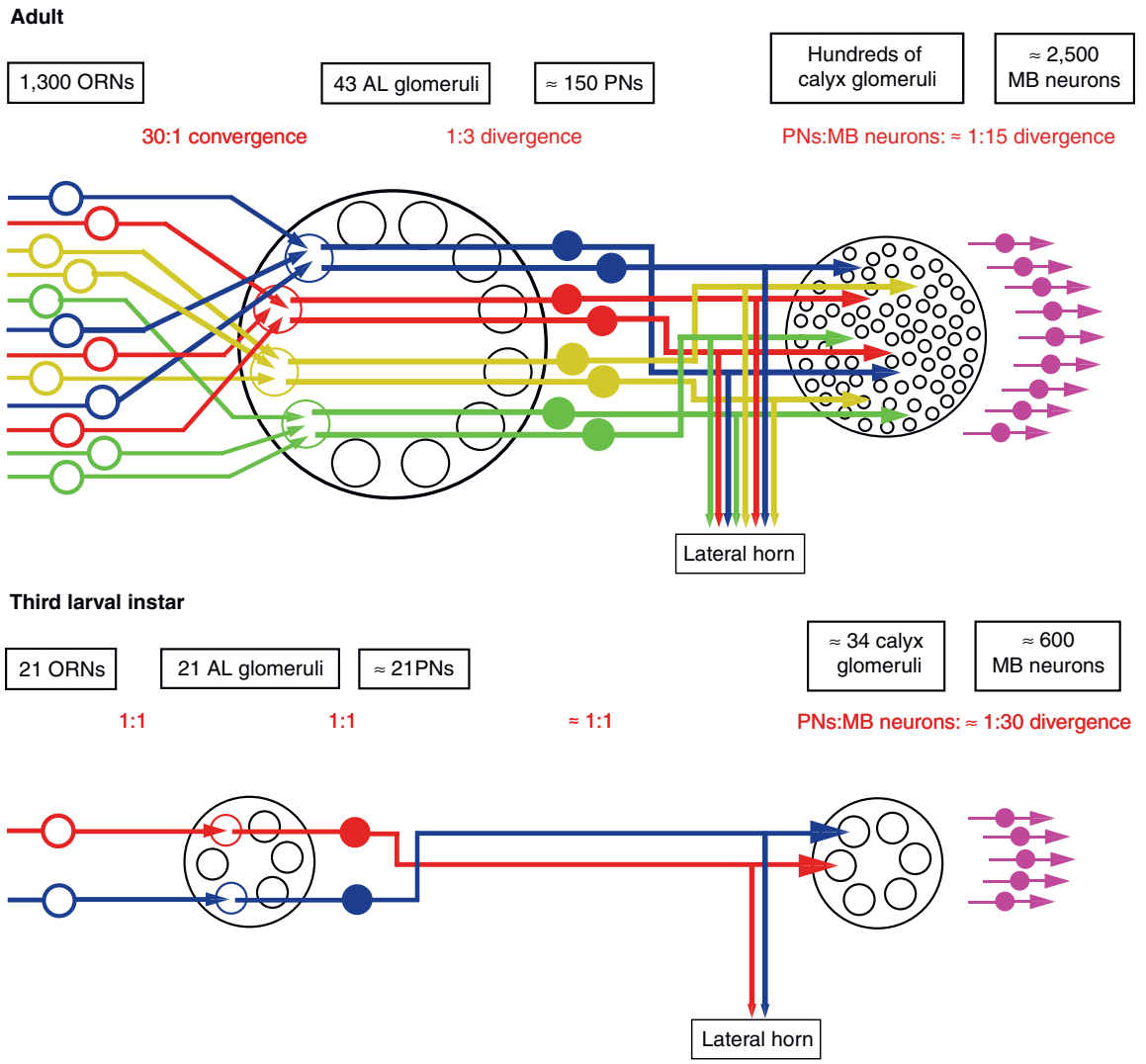

Figure 6

Comparative circuitry of adult and larval olfactory systems. Adult and larval olfactory pathways share the same design. However, adults comprise more primary olfactory dimensions, as shown by higher numbers of ORN types (*open circles*) and AL glomeruli. Moreover, in the adult, the different types of ORN and PN (*filled circles*) occur in multiple copies, whereas larval ORNs and PNs are unique. Thus, the adult olfactory pathway is characterized by converging and diverging connectivity in the AL (ratios indicated refer to the preceding line), whereas the larval pathway lacks cellular redundancy. Larval ORNs, AL glomeruli, PNs and calyx glomeruli are related essentially in a 1:1:1:1 fashion. Adapted from Ramaekers et al. (2005) with permission from Elsevier.

link a specific AL glomerulus with a specific calyx glomerulus (Masuda-Nakagawa et al. 2005, Ramaekers et al. 2005). Thus, the combinatorial activity pattern set up in larval AL glomeruli, as the result of ORN input and modulation by LNs, seems to be rather faithfully transferred to the calyx. Whether such strict relations between inputs and outputs apply to all PNs is unknown. The more straightforward connectivity of larval PNs

compared with their adult counterparts (see above) seems appropriate for analyzing calyx function.

Single-cell clone labeling in MB-specific *Gal4* lines allowed investigators to classify intrinsic MB neurons (Lee et al. 1999) according to their dendritic patterns. A minority of these neurons establish dendritic projections in a single calyx glomerulus (Ramaekers et al. 2005), but most of them have multiple arbors in up to six glomeruli (Masuda-Nakagawa et al. 2005, Ramaekers et al. 2005). These two subtypes may thus allow different modes of signal transfer, acting either in elementary odor coding or as coincidence detectors (Perez-Orive et al. 2002, Heisenberg 2003). In terms of cell numbers, 21 PNs (or a few more) may be confronted with an estimated 600 functional MB neurons (L. Luo, personal communication; Lee et al. 1999). Hence, the larval calyx, similar to its adult homologue, is a site of divergence (Masuda-Nakagawa et al. 2005, Ramaekers et al. 2005). In fact it is the only such site along the larval olfactory pathway (see below). Although both AL and calyx are glomerular, the logic of connectivity is different: AL glomeruli exhibit stereotypic connectivity between defined ORNs and PNs, whereas calyx glomeruli have stereotypic PN input but mostly combinatorial output.

Taken together, present evidence shows that the design of the larval olfactory pathway is similar to that of adults. A number of features, however, are larval-specific (**Figure 6**): (*a*) Every larval ORN and most (if not all) larval PNs are unique, leading to an almost complete lack of cellular redundancy in first- and second-order olfactory neurons (Ramaekers et al. 2005). Theoretically, any loss of these cells should affect olfactory function more severely than in the adult system. (*b*) The presence of only 21 ORNs and 21 AL glomeruli suggests that the number of primary olfactory dimensions is reduced in the larva compared with adult flies with ~43 glomeruli (Laissue et al. 1999). (*c*) Given the uniglomerular patterns of ORNs and PNs and the similar number of ORNs, AL glomeruli, PNs, and calyx glomeruli, the lower levels of the larval olfactory circuit lack convergent and divergent connectivity and are organized in a 1:1:1:1:1 fashion (Ramaekers et al. 2005). This differs from the adult olfactory pathway, in which 1300 ORNs express at least 47 ORs (Vosshall et al. 2000, Couto et al. 2005) and converge onto 43 glomeruli, which diverge again to ~150 PNs and hundreds of calyx glomeruli (Stocker 1994, Stocker 2001). The lack of cellular redundancy in the larva, the low number of input channels, and the absence of a convergent AL architecture likely reduce the signal-to-noise ratio and, consequently, olfactory sensitivity. However, its performance still seems adequate for a substrate feeder and is obviously enough to solve simple discrimination tasks. To sum up, the larval olfactory system, despite its reduced cell numbers, still possesses the essential design of the mammalian olfactory system, but in its simplest form. It has become an attractive elementary model for studying olfactory coding.

Adult Gustatory Pathway

GRNs located in different organs and body appendages all target the SOG, which is located slightly behind and ventral to the brain proper. Unlike the AL, the SOG contains no morphologically apparent structural divisions such as glomeruli. The availability of genetic reagents for tracing the projections from individual GR-expressing GRNs has begun to offer a glimpse into the neuroanatomical organization of the SOG (Thorne et al. 2004, Wang et al. 2004b).

Two approaches were recently followed to dissect the central pathways underlying gustatory coding. In the first, the effects of the peripheral origin versus receptor expression on the central taste projections were investigated. Consistent with previous tracing studies (Stocker & Schorderet 1981, Nayak & Singh 1985), gustatory afferents from the pharynx, labellum, and legs traveling through different nerves terminated in distinct areas of the SOG (Thorne et al. 2004, Wang et al.

2004b). Internal taste organs are represented in the anterior dorsal SOG, whereas proboscis GRNs and leg GRNs target the central and posterior SOG, respectively. Some of these spatially distinct afferents express the same receptor, suggesting that a given tastant may trigger different behaviors depending on the site of stimulation.

In a second approach, the projections of labellar neurons expressing *Gr5a* versus those expressing *Gr66a* were studied. It turned out that *Gr5a* projections remain on the ipsilateral side of the SOG, whereas *Gr66a* projections converge in a ring-like web in the medial SOG, slightly anterior to those of *Gr5a* (Thorne et al. 2004, Wang et al. 2004b). The projections of other putative bitter receptors, such as *Gr32a* or *Gr47a*, largely overlap with *Gr66a* projections (Wang et al. 2004b). Hence, sweet-responsive and bitter-responsive neurons establish partially overlapping but spatially segregated projections. A follow-up imaging study demonstrated that indeed the *Gr5a* region responds specifically to labellar stimulation with trehalose, whereas the *Gr66a* target region becomes activated upon stimulation with the bitter compounds caffeine and denatonium (Marella et al. 2006) (**Figure 7**). This directly illustrates the presence of a spatial map of taste activity in the SOG, which is probably the substrate of attractive versus repulsive responses. In support of this, projections of water-sensitive GRNs, whose stimulation induces feeding, at least partially overlap with *Gr5a* projections (Inoshita & Tanimura 2006).

Given the neuroanatomical, imaging, and behavioral studies in *Gr5a/Gr66a*, the terminals of the two sets of afferents very likely connect with two different subsets of gustatory interneurons mediating attractive and aversive responses, respectively. Indeed, intracellular recording in the blowfly SOG revealed two functionally distinct types of interneurons. Most of the impaled neurons, which were of the local type, responded to sucrose and water but not to the repellent KCl, whereas a minority responded to KCl but not to sucrose (Mitchell & Itagaki 1992). Thus, handling of attractive and repulsive inputs appears to remain separate through the first stage of central integration. Other candidate taste interneurons, reported from *Drosophila* (Nayak & Singh 1985, Sinakevitch & Strausfeld 2006) and bees (Hammer 1993, Schröter & Menzel 2003), link the SOG with the MBs. Taste input to the MBs is appealing given their proposed role as a site of convergence between olfactory and gustatory signals during olfactory conditioning (Heisenberg 2003, Davis 2005).

Although some taste information is sent to higher brain centers, simple reflexes, such

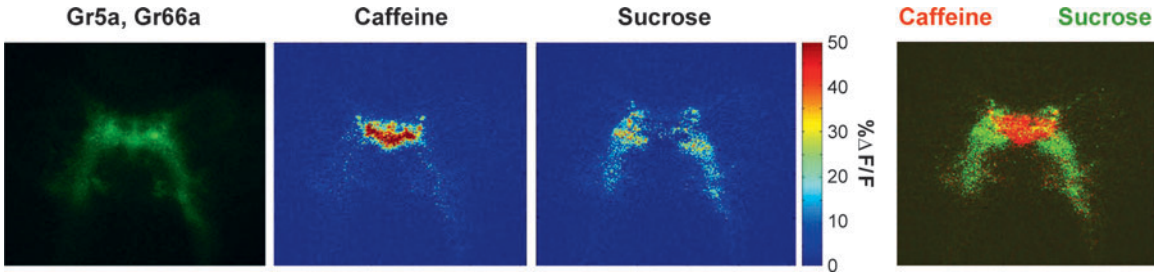

Figure 7

A map of taste quality in the fly SOG. Changes in G-CaMP fluorescence in the central projections of sweet-responding *Gr5a* neurons and bitter-responding *Gr66a* neurons reveal spatial segregation of the two responses. The first image shows initial G-CaMP fluorescence, the second and third images illustrate fluorescence intensity increase (% ΔF/F) after stimulation with 100 mM caffeine and 1 M sucrose, respectively, and the fourth is an overlay of caffeine-induced (*red*) and sucrose-induced fluorescence changes (*green*). Reproduced from Marella et al. (2006) with permission from Elsevier.

as proboscis extension or food ingestion, may rely on local circuitry with fairly limited processing. That is, some interneurons probably directly link taste input and motor output, or taste afferents may even establish monosynaptic connections onto motor neurons driving the reflex. A number of SOG motor neurons innervating various targets in the proboscis have been described (Rajashekhar & Singh 1994, Tissot et al. 1998), but their functional inputs remain to be investigated.

Gustatory and olfactory maps in the fly reveal clear differences, in accord with receptor expression and functional and behavioral studies, reflecting the different design of smell and taste. The two distinct target regions represented by *Gr5a* and *Gr66a* suggest that taste deals with a very limited set of categories: "attractive" versus "aversive." In contrast, olfactory maps in both primary and higher centers may permit analysis of a wide array of molecular qualities, concentrations, and blends that change dynamically over time. Also in contrast, the map of the different taste organs in the SOG provides positional information about a stimulus. This implies that not only taste quality but also the site of stimulation—leg, labellum or pharynx—is relevant for the fly's behavioral response.

Larval Gustatory Pathway

By single-cell labeling in various *Gal4* driver lines, four major target subregions were identified in the larval SOG (Colomb et al. 2007). They seem to be correlated primarily with the nerve through which the afferents travel and less with the *Gr* gene expressed. Consequently, as in the adult, neurons in different sense organs but expressing the same gene, such as *Gr2a*, may have different central targets (Scott et al. 2001). Gustatory afferents from external sense organs, such as those from the terminal organ labeled by *Gr66a-Gal4* (Scott et al. 2001), generally establish exclusive ipsilateral projections. This is striking, given that *Gr66a* projections in the adult are bilateral (see above). Circumstantial evidence suggests that afferents involved in attractive responses (Heimbeck et al. 1999) may project to a region slightly different from the four subregions mentioned (Colomb et al. 2007). Moreover, the neuron from the terminal organ expressing *Gr21a* (Faucher et al. 2006) appears to have its own, specific SOG target region apart from the four subregions (Colomb et al. 2007). Finally, *Or30a-Gal4*, *Or42a-Gal4*, and *Or49a-Gal4* lines that label neurons in the terminal organ, apart from ORNs, also label sensory terminals in the SOG (Fishilevich et al. 2005, Kreher et al. 2005).

Little information is available about potential target neurons of larval gustatory afferents. However, intriguing candidates are a set of 20 neurons in the SOG that express the *hugin* gene (Melcher & Pankratz 2005). They establish arborizations, very likely of dendritic nature, that partially overlap with the terminals of GRNs and send processes to the protocerebrum, the ventral nerve cord, the ring gland, and the pharyngeal apparatus. In adults, blocking synaptic output from *hugin*-expressing neurons increases feeding. Hence, these interneurons may integrate taste processing, the endocrine system, higher-order brain centers, and motor output to modify feeding.

In summary, the collected data suggest that the larval taste system, regardless of its distinct peripheral anatomy, is organized much like the adult taste system, although in numerically reduced form. Compatible with this notion, circumstantial evidence suggests that many of the central elements of the gustatory system persist through metamorphosis.

CHEMOSENSORY BEHAVIOR

In this review, we have discussed the major advances in our understanding of the molecular neuroanatomy of smell and taste in *Drosophila*. A major challenge for the future will be to relate the function of the underlying chemosensory circuit to behaviors elicited by particular smells and tastes.

Many simple and robust assays can measure olfactory responses in this insect. The olfactory t-maze is a simple assay, typically coupled with electric shock conditioning to measure odor learning (Quinn et al. 1974). This assay is most robust for measuring avoidance and has recently been adapted to studying behavioral avoidance of CO_2 (Suh et al. 2004). Another simple avoidance assay involves presenting an odor either on a filter paper (Keene et al. 2004) or a cotton swab (Anholt et al. 1996) and measuring the distance the fly maintains from the odor. The latter assay has been used to identify a large number of *smell impaired* (*smi*) mutants in a forward genetic screen (Anholt et al. 1996). The tendency for flies to exhibit a startle response when they encounter an odor has been exploited in the chemosensory jump assay, which was used successfully to isolate the *acj6* mutant and which disrupts a transcription factor necessary for the expression of a subset of the ORs (McKenna et al. 1989, Clyne et al. 1999a). Naïve attractive responses to odors are measured by trap assays, which require flies to be starved for a period before the assay to increase their motivation to respond to food-related odors (Woodard et al. 1989, Larsson et al. 2004). Chemotaxis behavior of larvae is measured in both population (Monte et al. 1989) and single-animal assays (Fishilevich et al. 2005) by quantifying the movement of larvae relative to a filter paper containing a drop of odor. The olfactory assays currently used have a number of key limitations. No assay yet devised can reliably test odor discrimination in the fly, a key experimental gap that will need to be addressed if the field is to understand odor coding. Assays typically use high concentrations of odors, which may recruit nonolfactory sensory neurons and complicate interpretation of the results (Keene et al. 2004). Finally, the underlying structural and functional redundancy of the *Drosophila* olfactory system has made it resistant to behavioral genetic analysis. For instance, no behavioral phenotype is discernable in flies lacking *Or43b* (Elmore et al. 2003) and deleting single larval ORNs produces only subtle phenotypes (Fishilevich et al. 2005). More sensitive and quantitative behavioral assays will be needed as the field moves forward toward relating the underlying circuit properties of the olfactory system to the relevant behavioral output.

Likewise, behavioral assays that measure taste are simple and robust. Feeding acceptance is assayed by providing starved flies with a choice between appetitive and aversive or appetitive and neutral stimuli, each dyed either blue or red. The amount of each stimulus ingested in the dark is later scored by examining the abdominal tint of the fly (Amrein & Thorne 2005). This assay has been used to demonstrate that *Gr5a* mutants show a selective trehalose taste defect (Dahanukar et al. 2001, Ueno et al. 2001). A second assay uses the proboscis extension reflex to measure the appetitive or aversive character of a stimulus. This assay exploits an unconditioned reflex in a hungry fly in which a taste substance presented to GRNs on the first leg will elicit the extension of the proboscis toward the tastant. This task has been used to demonstrate that *Gr5a* neurons and *Gr66a* neurons, respectively, mediate appetitive and aversive responses (Wang et al. 2004b). As for olfaction, currently no highly quantitative assays can measure discrimination between two bitter or sweet substances. Such an assay will be necessary to discern whether flies can discriminate among different bitter tastants, despite organizing their GRNs to express multiple GRs tuned to bitter substances.

THE *DROSOPHILA* CHEMOSENSORY SYSTEM AS A SIMPLE MODEL: LIMITS AND PROSPECTS

The usefulness of adult *Drosophila* as a model system in olfactory research is evident, given the genetic and molecular tools available in this species, the simplicity of its olfactory system in terms of cell number, and the striking similarities with the mammalian olfactory

system. These include the expression of one or a few OR types per neuron, the glomerular convergence of ORNs expressing a given OR, and consequently the coding principle in the primary olfactory center. Even the larval olfactory system shows the same basic design as the mammalian system but in almost the simplest conceivable form (Ramaekers et al. 2005). Larvae with a single pair of functional ORNs can be generated (Fishilevich et al. 2005). In such animals, OR expression and electrophysiological and behavioral responses can be directly correlated, allowing the analysis of olfactory behavior at the level of single, identified receptor cells. Thus, the larva is a particularly attractive model for olfactory studies from the cellular to systems level.

Concerning the taste system, the model character of *Drosophila* is less obvious, with respect to both adults and larvae. Anatomically, the taste systems of mammals and insects are different. Nevertheless, there are a number of striking parallels. In both insects and mammals, taste receptor cells are tuned to classify inputs as either attractive or aversive (Zhang et al. 2003, Thorne et al. 2004, Wang et al. 2004b, Marella et al. 2006). Moreover, many more of the taste receptors seem dedicated to repulsive ligands than to attractive ones. Also, in both phyla, cells responding to bitter substances express multiple receptors.

The parallels in the chemosensory systems of mammals and insects are not necessarily an argument in favor of their common ancestry. This is reflected, for example, by the nonhomology of the receptor gene families in the two phyla (Benton et al. 2006). Rather, the similarities may reflect common functional constraints. Analyzing these constraints in the simple smell and taste systems of the fly model, with its wealth of molecular tools, may be crucial to expanding our understanding of chemosensory function in general.

SUMMARY POINTS

1. The olfactory systems of *Drosophila* flies and larvae are useful models because of their reduced cell numbers and their strikingly similar design with the mammalian olfactory system, which may reflect common functional constraints. Studying the simple fly system may thus provide general insights into the logic of smell function in all animals.
2. Insect odorant receptors and gustatory receptors define a novel family of polytopic membrane proteins with no homology to vertebrate chemosensory receptors, which are G protein–coupled receptors.
3. Insect ORs are obligate heteromeric proteins composed of a conventional OR complexed with the universal coreceptor subunit, Or83b.
4. The olfactory sensory map in the AL is formed by convergent projections that segregate input from the antenna and maxillary palp according to the type of OR expressed, such that all ORNs expressing a given OR target a unique and stereotyped glomerulus.
5. Sweet-responsive and bitter-responsive brain maps are spatially distinct, providing substrates for attractive and aversive responses, respectively.
6. GRNs expressing the same GR but located on different body positions have different central targets, suggesting that a given tastant may trigger different responses depending on the stimulation site.

7. Olfactory and gustatory brain maps are different, possibly reflecting the different processing needs of these senses. Smell is designed to analyze a wide array of qualities, concentrations, and blends, whereas taste deals with a limited set of categories, essentially "attractive" versus "aversive."
8. Diverging connectivity suggests that MBs integrate a wide range of odor information, consistent with their proposed role in olfactory learning, whereas spatially constrained connectivity in the lateral horn is compatible with a role in mediating direct behavioral responses to odors.

FUTURE ISSUES

1. It will be of great interest to elucidate the axon guidance mechanisms that target functionally distinct ORNs to AL glomeruli with precision.
2. Greater information about the second- and third-order neurons that process gustatory information in insects and their links to motor output is required.
3. It will be necessary to clarify the coupling mechanisms by which insect ORs and GRs transduce ligand binding to neuronal signaling.
4. Future experiments are needed to provide the mechanisms by which the activation of combinatorials of ORNs is transformed into an odor percept in the fly brain to produce a given behavioral output.
5. More information is needed concerning whether insect host preference has co-evolved with odorant and gustatory receptor repertoires, particularly for blood-feeding mosquitoes and other pest insects (see sidebar on Insect Host Preference).

INSECT HOST PREFERENCE

Female mosquitoes transmit a host of dangerous infectious diseases, including malaria, yellow fever, West Nile encephalitis, and others. These insects are attracted to their human hosts largely by chemosensory cues, such as body odor and carbon dioxide exhaled in the breath (Takken & Knols 1999). The recent availability of the complete genome of the malaria mosquito, *Anopheles gambiae*, has opened up the possibility of studying the molecular basis of olfaction in this deadly insect (Fox et al. 2001, Hill et al. 2002). The genome of *Anopheles gambiae* contains 79 odorant receptors and 76 gustatory receptors, including receptors that are close homologues of those in *Drosophila* and a large number of receptors that appear to be mosquito specific. A more complete understanding of which receptors mediate the strong attraction of these insects to humans could be exploited to design novel mosquito repellents that target selected odorant and gustatory receptors (Justice et al. 2003, Hallem et al. 2004, van der Goes van Naters & Carlson 2006). Such compounds would be an important tool in the global war against insect-borne infectious disease.

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FlyBrain: An Online Atlas and Database of the *Drosophila* Nervous System. **http://web.neurobio.arizona.edu/Flybrain/html/index.html**

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