

CHAPTER 7

The Olfactory Sensory Map in *Drosophila*

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Abstract

The fruit fly (*Drosophila melanogaster*) exhibits robust odor-evoked behaviors in response to cues from diverse host plants and pheromonal cues from other flies. Understanding how the adult olfactory system supports the perception of these odorous chemicals and translates them into appropriate attraction or avoidance behaviors is an important goal in contemporary sensory neuroscience. Recent advances in genomics and molecular neurobiology have provided an unprecedented level of detail into how the adult *Drosophila* olfactory system is organized. Volatile odorants are sensed by two bilaterally symmetric olfactory sensory appendages, the third segment of the antenna and the maxillary palps, which respectively contain approximately 1200 and 120 olfactory sensory neurons (OSNs) each. These OSNs express a divergent family of seven transmembrane domain odorant receptors (ORs) with no homology to vertebrate ORs, which determine the odor specificity of a given OSN. *Drosophila* was the first animal for which all OR genes were cloned, their patterns of gene expression determined and axonal projections of most OSNs elucidated. In vivo electrophysiology has been used to decode the ligand response profiles of most of the ORs, providing insight into the initial logic of olfactory coding in the fly. This chapter will review the molecular biology, neuroanatomy and function of the peripheral olfactory system of *Drosophila*.

Introduction

Sensory systems—touch, hearing, vision, taste, smell—map features of the external world into internal representations in the brain that ultimately allow all animals to navigate their environments. The physical senses of touch and vision use topographic mapping approaches to represent discrete dimensions of the external world. For example, the visual system uses retinotopic mapping to organize the field of view in the lateral geniculate nucleus, such that there is an orderly representation of the visual field in the brain.¹ The somatosensory system uses somatotopic mapping to project not the external world but the body plan onto the somatosensory cortex.^{2,3} Thus it is not the environment per se that is mapped, but the various parts of the body, allowing an animal to determine with precision where it is being touched by a physical stimulus. The auditory system maps sound frequencies along a tonotopic axis in the cochlea and the auditory cortex, allowing sound to be broken into its component parts and later synthesized into a coherent representation of what was heard.^{4,5} An important feature of the auditory system is the precision by which it permits animals to localize sound in space. This is accomplished by central brain comparisons of input into the left and right ears. These mapping approaches allow visual, somatosensory and auditory cortex to represent important features of visual, mechanical and auditory stimuli and relate them to physical space in the external world.

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The chemical senses—taste and smell—are less well understood than the physical senses but appear to use a different strategy to represent gustatory and olfactory cues encountered in the environment. Instead of mapping primarily the position of the external stimulus and its relationship to the individual, the gustatory and olfactory systems categorize the identity and quality of the stimulus. The tongue can detect at least five different taste qualities—bitter, sweet, sour, salty and umami, the taste of monosodium glutamate. Insects appear to have all of these taste qualities, with the possible exception of umami and the addition of a “water” sense.^{6,7} Each of these taste qualities is perceived by structurally and functionally discrete gustatory neurons in the tongue of vertebrates⁸ and labial palps of insects.^{6,9} It is still unclear in the field whether these taste qualities remain segregated into stimulus-specific labeled lines from the periphery to higher brain centers,^{8,10,11} or whether distributed coding across groups of sensory and central brain neurons allows animals to distinguish tastes of different modalities such as bitter and sweet.^{12,13} There is clear evidence in *Drosophila* that pathways for bitter and sweet tastes are anatomically and functionally separate senses that elicit innate aversive and appetitive responses, respectively.^{9,11}

The olfactory system is capable of detecting an extremely large number of volatile chemical stimuli, possibly exceeding tens of thousands, although the total olfactory coding capacity of any animal has never been exhaustively catalogued.¹⁴ The ability to recognize such a vast number of odorous ligands is thought to be due to the special properties of the ORs, the large family of membrane proteins that is selectively expressed in OSNs in the olfactory epithelium of vertebrates and antennae of insects. ORs have selective but broad ligand-binding properties, such that a given OR is activated by multiple odors and a given odor activates multiple ORs.^{15–18} This combinatorial coding strategy based on a large family of ORs with broad but selective ligand pharmacology in part accounts for the ability of animals to detect and discriminate a number of odors that far exceeds the number of ORs they possess.

In all arthropods and vertebrates studied to date, the early olfactory system is organized into a large number of spherical neuropil elements, called glomeruli.^{19,20} Olfactory glomeruli represent points of convergence where OSNs expressing the same OR synapse with inhibitory local interneurons and secondary neurons that relay olfactory information to higher brain centers.^{21–25} There is some evidence in mammals that the olfactory system maps odor stimuli along a chemotopic axis in the vertebrate olfactory bulb.^{26–28} Thus neurons responsive to odors sharing an alcohol functional group will tend to innervate adjacent regions in the bulb and these regions appear to be organized by carbon chain length.^{26,27} This type of chemotopy is less apparent in insect systems.^{29–32}

This chapter will review recent progress in our understanding of the organization and function of the adult *Drosophila* olfactory system. The accompanying chapter by Veronica Rodrigues and Thomas Hummel will address the development and early patterning of the olfactory system. The accompanying chapter by Reinhard Stocker concerns the unique organization of the larval olfactory system.

Olfactory Organs and Olfactory Sensory Neurons of *Drosophila*

Fruit flies detect odors through two olfactory sensory organs on the head, the antenna and maxillary palp (Fig. 1). These olfactory appendages are covered with a large number of sensory hairs, called sensilla, which house and protect the underlying OSNs that are specialized to detect odors. Olfactory sensilla can be distinguished morphologically from thermo- and hygro-sensitive sensilla by the presence of a large number of small pores that perforate the shaft of the sensillum and which are believed to allow access to odors (reviewed in ref. 33). A total of about 410 olfactory sensilla cover the antenna, while the maxillary palp has about 60 olfactory sensilla. These hairs can be divided into three distinct morphological and functional classes: Club-shaped basiconic sensilla, long and pointed trichoid sensilla and short, peg-shaped coeloconic sensilla (Fig. 1).

Further morphological and functional distinctions subdivide both basiconic and trichoid sensilla into additional subclasses, which differ by the size and density of odor pores, the number of neurons housed in each sensillum and their distribution on the antenna (Fig. 1)^{29,33–36} The different sensilla types are distributed in a highly stereotyped fashion over the surface of the antenna. Large basiconic sensilla are clustered at the medial face of the antenna, while the three types of trichoid sensilla are arranged in diagonal bands across the lateral face of the antenna (Fig. 1).

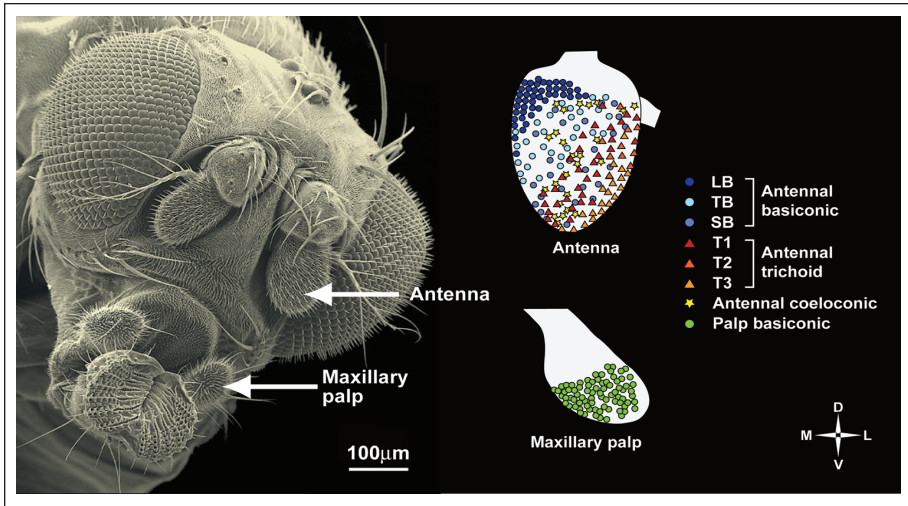


Figure 1. Peripheral organization of the *Drosophila* olfactory system. Scanning electron micrograph of a *Drosophila* head indicating the two major sensory organs, the third segment of the antenna and the maxillary palp. At right is a schematic of sensilla types and relative locations on both organs. Abbreviations: LB, large basiconic sensilla; TB, thin basiconic sensilla; SB, small basiconic sensilla; T1-T3, three different types of trichoid sensilla. SEM image by J.Scott and R.Bhatnagar, AMF, Biological Sciences, University of Alberta. Reprinted with permission from J. Scott ©2006 Biological Sciences. Cartoons adapted with permission from: Couto A, Alenius M, Dickson B. *Curr Biol* 2005; 15:1535-1547. ©2005 Elsevier Press.

Coeloconic sensilla are interspersed with other sensilla types, but are concentrated at the central face of the antenna. The relative position of these sensilla is well conserved as are the number of neurons innervating a given sensillum. Trichoid sensilla are named T1, T2 and T3 and contain one, two, or three OSNs, respectively. Most basiconic sensilla house two neurons, although there are several cases of four neurons per basiconic sensillum.^{29,33,36} Coeloconic sensilla typically have two or three neurons. Thus the third segment of the antenna is marked by a reproducibly ordered array of olfactory sensilla that house defined and stereotyped numbers of OSNs. This patterning arises through the interplay of a cascade of patterning genes that act early in development and is discussed in the accompanying chapter by Hummel and Rodrigues.³⁷⁻⁴⁰

The maxillary palp is a simpler olfactory organ, containing fewer OSNs housed in a smaller number of basiconic sensilla. Approximately sixty basiconic sensilla each housing two OSNs can be found in this organ. Although these sensilla are externally similar, Shanbhag et al used electron microscopic analysis of OSN terminal dendrite branching in the maxillary palp to further subdivide palp sensilla into three subtypes, PB-I, PB-II and PB-III.³⁶ PB-I OSNs contain highly branched terminal dendrites, while PB-II OSNs are characterized by ribbon-shaped dendrites. PB-III OSNs are rarer on the palp and have an unusual thick, hollow dendritic segment. The extent to which these ultrastructural differences in antennal and maxillary palp sensilla and OSNs have functional implications will be discussed below.

Odorant Receptor Gene Expression

In vertebrates, ORs were first identified in 1991 as a very large family of related genes encoding members of the G protein-coupled receptor (GPCR) superfamily, which couples ligand binding to production of cAMP second messenger signaling.⁴¹ During the 1990s, efforts by multiple investigators to find homologues of vertebrate ORs in insect genomes failed. In 1999 three groups used a combination of difference cloning⁴² and mining of genome databases for multi-transmembrane

domain proteins⁴²⁻⁴⁴ to identify candidate *Drosophila* ORs. There are a total of 62 ORs, encoded by a family of 60 genes through alternative splicing.⁴⁵ The fly OR genes encode a highly divergent family of membrane-associated proteins that are selectively expressed in *Drosophila* OSNs.⁴²⁻⁴⁴ These proteins are predicted to contain seven transmembrane domains, but contain no obvious homology to vertebrate ORs or the GPCR superfamily.^{42,46,47} Two recent reports that looked at the membrane topology of the fly OR gene family suggested that these proteins adopt an orientation in the membrane that is inverted relative to GPCRs, such that the N-terminus faces the cytosol.^{46,47} Benton et al⁴⁶ provided experimental evidence to support this atypical topology, calling into question the general assumption that fly ORs are classic GPCRs. Furthermore, different members of the fly OR family show considerably less homology to each other than most vertebrate ORs, leading to the hypothesis that this is a rapidly evolving gene family.⁴⁵

Detailed information about the expression of each *Drosophila* OR gene is now available. Initially, RNA in situ hybridization was used to examine in which tissue and in which OSNs a given OR is expressed.⁴²⁻⁴⁴ In these early papers, it was already obvious that there is a segregation of gene expression between the two major appendages: ORs expressed in the antenna are not expressed in the maxillary palp and vice versa. A later study that examined a group of 57 fly ORs confirmed this initial impression of segregation in OR repertoire between antenna (Table 1) and palp (Table 2). These appendages express non-overlapping subsets of 32 and seven OR genes, respectively (Fig. 2).²⁵ Two recent papers^{29,30} that monitored OR gene expression with transgenic reporter techniques bring the total number of antennal-specific genes to 40 and maxillary palp-specific genes to seven. The remaining OR genes are not detectably expressed in the adult and are now known to encode the larval ORs, as discussed in the accompanying chapter by Reinhard Stocker.^{48,49}

Each OR gene is expressed in a small subset of the OSNs in either olfactory organ, which varies from two to 50 OSNs per OR. The relative position and number of OR-expressing OSNs is bilaterally symmetric in the two appendages and highly stereotyped between individual flies. Early reports discussed the existence of “zones” of OR gene expression, reminiscent of the zones of OR gene expression on the olfactory turbinates of the rodent.^{50,51} Careful examination of the relationship between OR gene expression and sensilla type has revealed that there is a nearly perfect correlation between the expression of OR genes and subsets of morphologically distinct basiconic, trichoid and coeloconic sensilla (Table 1).^{29,52} Thus the same developmental pathways that specify the morphology of the sensilla must also dictate the numbers and functional properties of the OSNs and the specific ORs they express.

There are two unusual features of OR gene expression in *Drosophila* that set this system apart from the vertebrate paradigm, in which each OSN expresses only a single OR gene.^{16,53} First, each *Drosophila* OSN expresses a broadly expressed member of the OR gene family called *Or83b*, which associates with ORs and is necessary for the proper ciliary targeting and function of all OR genes.^{46,54,55} Second, a given OSN can co-express up to three conventional ORs mediating ligand selectivity along with the *Or83b* co-receptor.^{29,30,56} Thus mechanisms of OR gene choice are likely to be different in the fly compared to the mouse and the feedback system that limits vertebrate OSNs to express only a single OR allele does not operate in *Drosophila*.

Ligand Tuning Profiles

What types of odors activate fly ORs and OSNs? Extracellular electrophysiological recordings that take advantage of the electrical isolation of neurons housed in a given sensillum have been a powerful tool to answer this question. Such single sensillum recordings were used to define the complete olfactory profile of the maxillary palp⁵⁷ and the majority of basiconic⁵⁸ and coeloconic⁵⁹ sensilla on the antenna. The tuning of trichoid sensilla is less well studied, but T1 sensilla are thought to respond to the aggregation pheromone *cis*-vacccenyl acetate.^{34,60}

From these initial electrophysiological experiments, it became clear that the morphological differences in the olfactory sensilla are reflected in functional differences of the OSNs that are housed in the sensilla (Fig. 2). There is now excellent evidence that basiconic sensilla are specialized to detect food odors, both in the antenna and maxillary palp (Fig. 2, Tables 1 and 2). Trichoid

Table 1. Molecular and functional organization of the *Drosophila* antenna

Antenna				
OR	Neuron	Glomerulus	Odors Evoking Responses (of 110 Tested)^	
			+(-)	Strongest Ligand
<i>Or2a</i>	at3	DA4m	0 (5)	no strong ligand
<i>Or7a</i>	ab4A	DL5	19 (30)	E2-hexenal
<i>Or9a</i>	ab8	VM3	21 (0)	2-pentanol
<i>Gr10a</i>	ab1D	DL1		
<i>Or10a</i>	ab1D	DL1	9 (27)	ethyl benzoate
<i>Or13a</i>	ai1	DC2		
<i>Or19a</i>	at3	DC1	6 (26)	1-octen-3-ol
<i>Or19b</i>	at3	DC1		
<i>Gr21a</i>	ab1C	V		carbon dioxide
<i>Or22a</i>	ab3A	DM2	29 (0)	methyl hexanoate
<i>Or22b</i>	ab3A	DM2		
<i>Or23a</i>	at2	DA3	0 (22)	no strong ligand
<i>Or33a</i>		DA2		
<i>Or33b</i>	ab5B+ab2B	DM3+DM5	0 (6)	no strong ligand
<i>Or35a</i>	ac1	VC3l	28 (14)	1-hexanol
<i>Or42b</i>	ab1	DM1		
<i>Or43a</i>	at3	DA4l	1 (34)	1-hexanol
<i>Or43b</i>	ab8A	VM2	14 (0)	ethyl butyrate
<i>Or47a</i>	ab5B	DM3	11 (0)	propyl acetate
<i>Or47b</i>	at4	VA1m+l	0 (37)	no strong ligand
<i>Or49a</i>	ab10	DL4		
<i>Or49b</i>	ab6B	VA5	3 (19)	2-methylphenol
<i>Or56a</i>	ab4B	DA2		
<i>Or59b</i>	ab2A	DM4	6 (0)	methyl acetate
<i>Or65a</i>	at4	DL3	0 (3)	no strong ligand
<i>Or65b</i>	at4	DL3		
<i>Or65c</i>	at4	DL3		
<i>Or67a</i>	ab10	DM6	31 (6)	phenylethyl alcohol
<i>Or67b</i>	ab9	VA3		
<i>Or67c</i>	ab7	VC3m	8 (9)	ethyl lactate
<i>Or67d</i>	at1	DA1		
<i>Or69aA</i>	ab9	D		
<i>Or69aB</i>	ab9	D		
<i>Or82a</i>	ab5A	VA6	1 (5)	geranyl acetate
<i>Or83c</i>	at2	DC3		
<i>Or85a</i>	ab2B	DM5	4 (31)	ethyl 3-hydroxybutyrate
<i>Or85b</i>	ab3B	VM5d*	22 (1)	6-methyl-5-hepten-2-one
<i>Or85f</i>	ab10	DL4	0 (4)	no strong ligand
<i>Or88a</i>	at4	VA1d	0 (11)	no strong ligand
<i>Or92a</i>	ab1	VA2		
<i>Or98a</i>	ab7A	VM5v	21 (8)	ethyl benzoate
<i>Or98b</i>	ab6B*	VM5d*		

*tentative; ^from Hallem and Carlson 2006; + =# odors eliciting activation of > 100 spikes/second of 110 tested; - =# odors eliciting inhibition of > -10 spikes/second of 110 tested. Data from references 29, 30, 32, 58.

Table 2. Molecular and functional organization of the *Drosophila* maxillary palp

Maxillary Palp			
OR	Neuron	Glomerulus	Odors Evoking Strong Responses (of 10 odors)
<i>Or33c</i>	pb2A	VC1	ethyl acetate, cyclohexanone, (–) fenchone
<i>Or42a</i>	pb1A	VM7	ethyl acetate, isoamyl acetate, E2-hexenal, cyclohexanone, 2-heptanone
<i>Or46aA</i>	pb2B	VA7I	4-methyl phenol
<i>Or59c</i>	pb3A	1	<none>
<i>Or71a</i>	pb1B	VC2	4-methyl phenol
<i>Or85d</i>	pb3B	VA4	isoamyl acetate, 2-heptanone
<i>Or85e</i>	pb2A	VC1	ethyl acetate, cyclohexanone, (–) fenchone

Data from references 29, 30, 56.

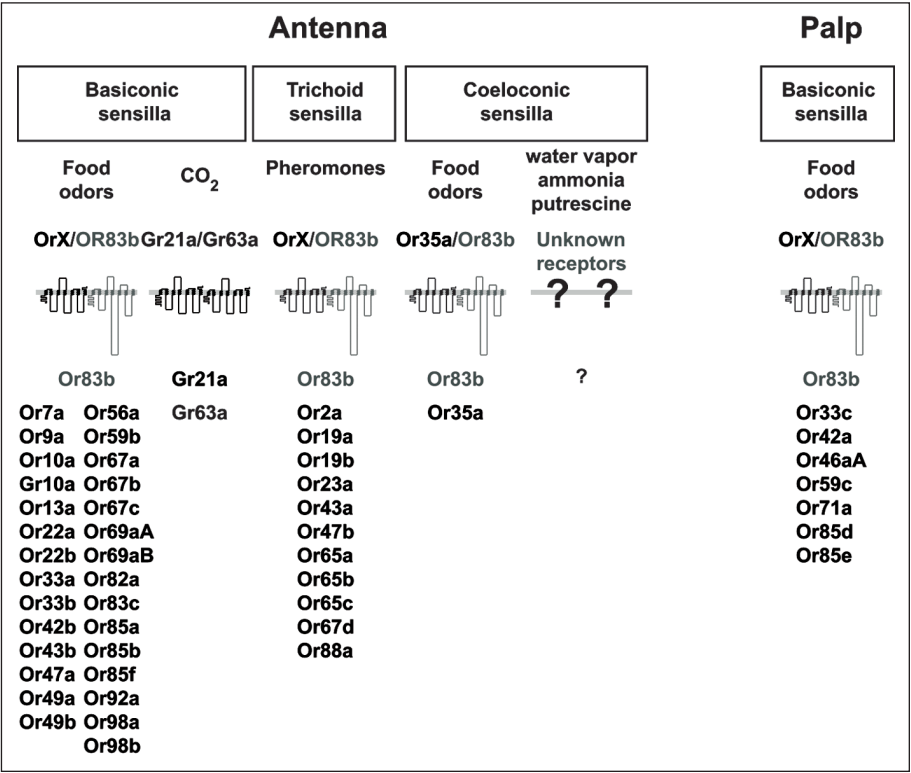


Figure 2. Molecular organization of the *Drosophila* olfactory system. Gene expression of chemosensory receptors responding to different classes of ligands is indicated. Co-receptors are listed in gray. *Gr21a* and *Gr63a* comprise the CO₂ receptor; it is not clear if either or both serve a co-receptor function. With the exception of *Or35a/Or83b* the coeloconic chemosensory receptors are still unknown. Data from references 30, 54, 56, 58-60 and 65-67.

sensilla, as observed for other insects, appear to be specialized for detecting pheromones (Fig. 2, Table 1).^{34, 60-63} The coeloconic sensilla appear to detect special chemical ligands, including water vapor, ammonia and putrescine (Fig. 2, Table 2).⁵⁹ Thus the morphological differences between these sensilla types catalogued by neuroanatomists relate directly to the ligands that the underlying OSNs detect.

To determine the explicit relationship between an OR and the ligands that activate it, Carlson and co-workers developed an *in vivo* preparation that allows them to screen large number of ORs for their ligand response properties.^{17,32,56,64} This preparation involves the Δ halo mutant, which lacks *Or22a/b* but retains expression of the *Or83b* co-receptor.⁶⁴ Different ORs can be expressed by transgenic techniques in this “empty neuron” and the OR response profile measured directly without interference from the resident OR. This technique has been used successfully to deorphanize all six classes of maxillary palp OSNs and assign specific ORs to functionally identified OSNs (Table 2).^{56,57} Twenty four antennal ORs were similarly examined for their ligand specificity and most were linked to identified sensilla types.^{17,29,32} A diversity of different response types for different ORs was uncovered in this work. First, some ORs are very narrowly tuned to a small number of odors, while others are broadly tuned and respond to a large number of the odorants tested (Tables 1 and 2). Second, ORs can show both excitatory and inhibitory responses to a panel of odors. Third, trichoid sensilla tend to show strong inhibitory responses and negligible excitatory responses to a large panel of general odors (Table 1),^{17,32} perhaps because the native ligands for these ORs are unidentified *Drosophila* pheromones. In support of this hypothesis, *Or67d* expressed in T1 sensilla has recently been proposed as a candidate *cis*-vaccenyl acetate receptor.⁶⁰

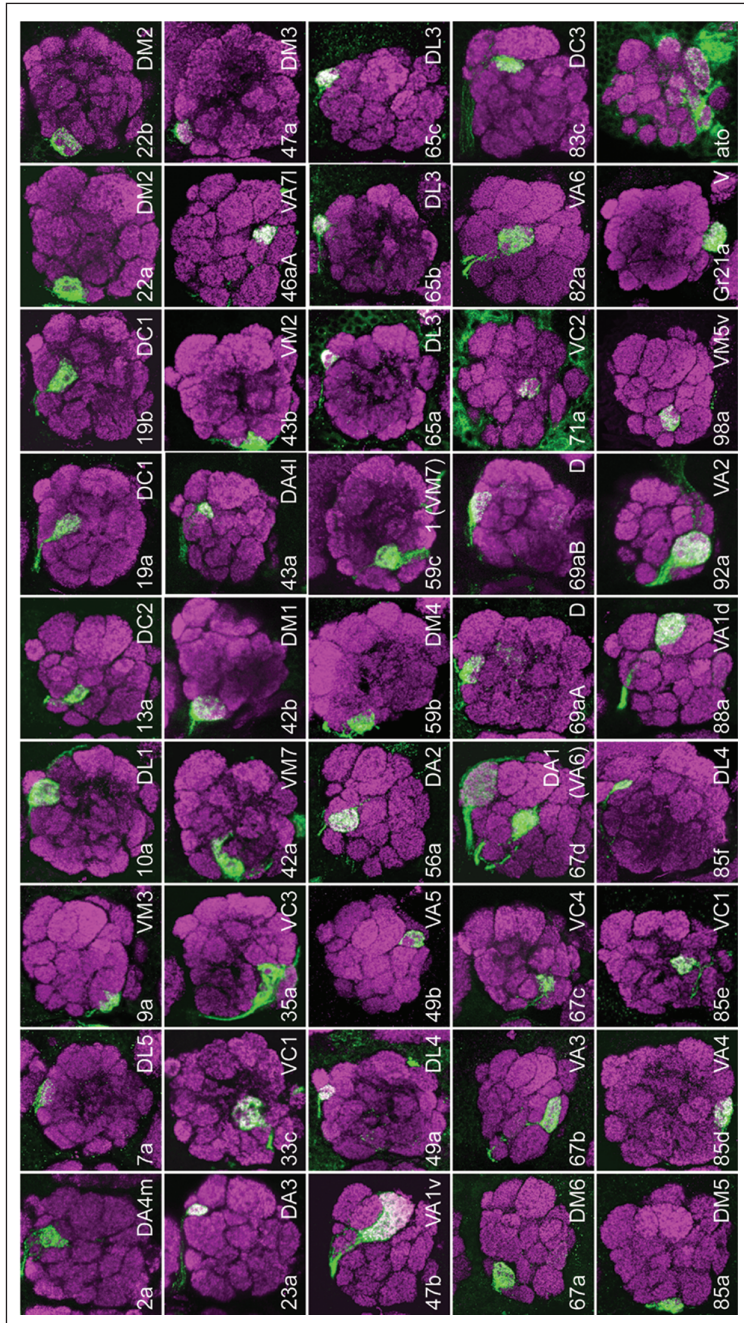
There is one conspicuous case in the antenna of a very narrowly tuned neuron, defined as ab1C. This OSN is activated selectively by and is extremely sensitive to carbon dioxide (CO₂).^{58,65} These CO₂-responsive neurons co-express *Gr21a* and *Gr63a*, two of three gustatory receptor (GR) genes expressed in the antenna that may subserve an olfactory instead of a gustatory function^{66,67} (Fig. 2). In fact, these two chemosensory receptors have recently been shown to mediate CO₂ detection in *Drosophila*.⁶⁷

These deorphanization efforts have lead to the conclusion that *Drosophila* ORs mediate all aspects of the odor responses in a given OSN. They determine the ligand specificity, the level of spontaneous firing of the OSN, whether an odorant will elicit excitatory or inhibitory firing patterns and the odor-evoked response dynamics.

A Receptor-Based Map of Glomerular Projections

How are axonal projections from thousands of OSNs expressing combinatorials of 47 ORs and 2 GRs organized in the antennal lobe, the insect homologue of the vertebrate olfactory bulb? The *Drosophila* antennal lobe is composed of well over 40 morphologically identifiable glomeruli whose sizes, shapes and positions are strongly conserved between different animals.⁶⁸ Genetic tools in *Drosophila* have permitted the elucidation of a nearly complete map of projections from peripheral olfactory organs to these glomeruli (Figs. 3 and 4; Tables 1 and 2).^{21,25,29,30} This was achieved by expression of the OR genes to mark distinct subpopulations of OSNs with green fluorescent protein, which could be followed from the peripheral sensory appendages to the first olfactory synapse in the antennal lobe (Fig. 3).

A number of important conclusions concerning this olfactory sensory map were reached in these studies. All OSNs expressing a unique combinatorial of ORs target a single antennal lobe glomerulus. This innervation pattern is bilaterally symmetric and invariant between different animals. There is broad agreement on the assignment of OR-expressing OSNs to glomeruli named solely by neuroanatomical criteria in an earlier study.⁶⁸ A few exceptions are worth noting. Couto et al²⁹ referred to the glomerulus receiving projections from *Or47b* neurons as VA1v, while Fishilevich and Vosshall³⁰ referred to the original name for this compartmentalized glomerulus, VA1m+I,⁶⁸ which we also use in this chapter. *Or67c* was previously mapped to VC4, which we suggest is more correctly mapped to VC3m. Fishilevich and Vosshall were unable to assign the *Or46a* glomerulus,³⁰ while Couto et al assigned this as VA7I.²⁹ Finally, *Or59c* was assigned to a glomerulus named “1”²⁹ which appears to be a new glomerulus that was never formally named (Fig. 4).⁶⁸



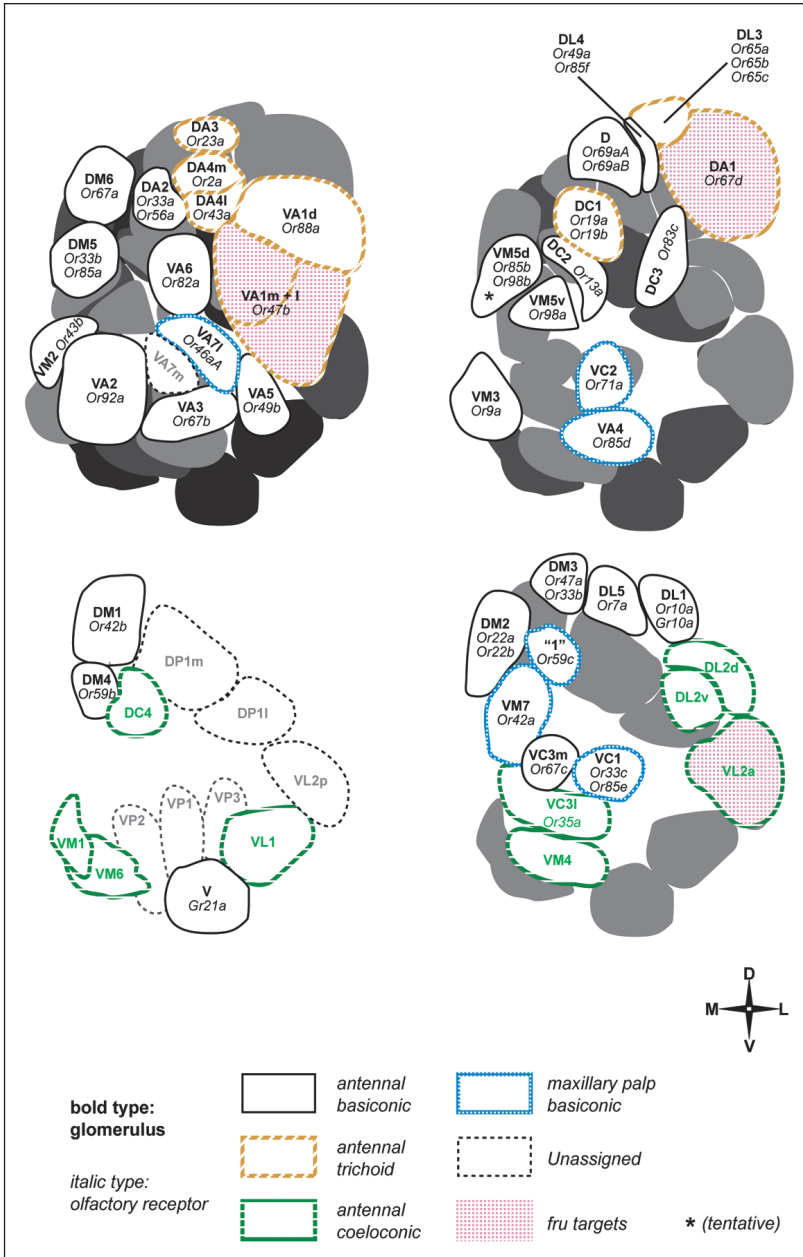


Figure 4. Molecular and anatomical map of the *Drosophila* antennal lobe. Schematic of the antennal lobe presented as frontal sections from anterior to posterior, organized clockwise from top left. Glomeruli are depth-coded with black for deep, gray for intermediate and white for superficial sections. Glomeruli are coded according to sensillum type, chemosensory organ and whether or not they are innervated by *fruitless*-positive neurons. Data from references 29, 30, 63, 66 and 68. Adapted with permission from: Fishilevich E, Vosshall LB. Curr Biol 2005; 15:1548-1553. ©2005 Elsevier Press.

We synthesize the conclusions reached in these disparate studies and present a complete map of the antennal lobe, indicating both the neuroanatomical and molecular name for each glomerulus in Figure 4. The antennal lobe comprises a total of 42 glomeruli, of which seven are subdivided into two compartments and one into three. While compartments were discovered on a purely morphological basis,⁶⁸ many have since been shown to express a single OR and due to these findings, compartments have been revealed in formerly undivided glomeruli. On a functional level, the antennal lobe can thus be said to have a total of 51 glomeruli. By including glomeruli VP1-3, the total number of glomeruli in the AL of *Drosophila* amounts to 54 glomeruli. While VP1-3 are visible in staining using synaptotagmin antibody,⁶¹ they are not discernible with the monoclonal antibody nc82.^{25,29,30,68} This may be due at least partly to their posterior-most location deep into the brain, where also the other glomeruli unassigned to ORs lie. Glomeruli in the antennal lobe are clustered into arrays, reflecting the bundling of sensilla types on the antenna and the maxillary palp. The glomeruli being connected with the maxillary palp are found predominantly in central positions, distinct from the glomeruli connected to the antenna. Antennal coeloconic OSNs project mainly to the posterior face of the antennal lobe, while antennal basiconic OSNs project to medial anterior regions of the antennal lobe. Trichoid OSNs project to the group of large glomeruli that lie at the extreme lateral regions of the antennal lobe. While these arrays have a fairly fixed design, there is no evidence for a topographic point-to-point mapping from the antenna to the antennal lobe. A direct correlation exists though between the size of a glomerulus and the number of OSNs projecting to it. For instance, *Or47b* is expressed in approximately 50 OSNs and marks a large glomerulus, VA1m+I, while *Or22a* is expressed in approximately 25 OSNs and marks a small glomerulus, DM2.

How accurate is this olfactory sensory map? Because these maps were generated with genetic reagents, it is important that the transgenic marker expression recapitulates the expression patterns of the endogenous genes. In most cases, this has been verified. Expression of the transgene, closely matching RNA in situ hybridization of the endogenous OR has been demonstrated for most published OR-Gal4 transgenes.^{21,25,29,30} Nevertheless, the transgenic approach has led to some variability in glomerular mapping that almost certainly reflects artefacts of the transgenic lines themselves. For instance, both early reports of *Or23a*-expressing OSNs showed that these target two glomeruli in the antennal lobe.^{21,25} Subsequent analysis of these same transgenes showed that OSNs expressing *Or23a* innervate only one of the two original glomeruli.^{29,30} Ectopic expression of both *Or59c* and *Or67d* was observed, such that both transgenic reagents label one authentic and one ectopic glomerulus.^{21,25} Sporadic cases of these transgenic reagents labeling multiple glomeruli have also been reported and these are almost certainly due to ectopic expression of the transgenes induced by position and other genetic background effects.⁵² Another possible explanation for variation in the olfactory map is that despite the highly conserved anatomy of the antennal lobe, additional or missing glomeruli and compartments are observed between individual flies, suggesting a moderate plasticity of the olfactory system on the individual level.^{29,68} Despite this inherent limitation of the genetic reagents, they have proven to be powerful tools that allowed investigators to describe the molecular neuroanatomy of the antennal lobe in unprecedented detail.

Sexual Dimorphism in the *Drosophila* Olfactory System

One further outcome of the molecular mapping of the antennal lobe was that it allowed the identification of putative pheromone receptors. Previous reports that examined sexual dimorphism in the antennal lobe of Hawaiian *Drosophila* species identified several prominent lateral glomeruli that are larger in male than female flies.⁶¹ Both DL3 and DA1 are considerably larger in male Hawaiian species than females. The same analysis in *Drosophila melanogaster* indicates that compared to the female, the male DA1 and VA1m+I are 62% and 33% larger, respectively, while DL3 and VA1d are isomorphic in both sexes.⁶¹ These glomeruli receive input from OSNs expressing *Or67d* (DA1) and *Or47b* (VA1m+I),^{29,30} both of which are housed in trichoid sensilla.²⁹ The basis for this size increase in males is unknown, but earlier investigators noticed that there is also a sexual dimorphism in sensilla number.^{33,36} Males have more trichoid sensilla

and fewer basiconic sensilla than females.^{33,36} Finally, neurons expressing *fruitless*, the master transcriptional regulator of sex-specific development and behavior project to these large lateral, sexually dimorphic glomeruli (Fig. 4, pink hatched glomeruli).^{62,69} Thus the hypothesis that male antennae are more sensitive to pheromones, as has been shown for a large number of other insects and that this sensitivity is mediated by specialized pheromone-sensing OSNs housed in trichoid sensilla is well supported by the available data.

Concluding Remarks

The advanced state of knowledge concerning gene expression and synaptic organization of the early olfactory system of the fly makes this a compelling system to address questions in odor coding. For instance, it is not yet clear in any species how and where odor concentration is encoded; how the brain solves odor mixture problems, by far the most likely physiological stimulus an animal will encounter; and how discrimination between perceptually similar odors is achieved.⁷⁰ Functional calcium imaging⁷¹⁻⁷³ and electrophysiology^{74,75} will be important tools in future research that seeks to answer these important questions at the cellular level. Finally, little is known about how the olfactory system processes odors to produce stereotyped behavioral outputs. The small size, genetic manipulability and availability of robust olfactory behavior paradigms for *Drosophila* olfaction strengthen the role of this little insect as a powerful genetic model system for the foreseeable future.

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