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The Molecular Basis of Odor Coding in the *Drosophila* Larva

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Summary

We have analyzed the molecular basis of odor coding in the *Drosophila* larva. A subset of *Or* genes is found to be expressed in larval olfactory receptor neurons (ORNs). Using an in vivo expression system and electrophysiology, we demonstrate that these genes encode functional odor receptors and determine their response spectra with 27 odors. The receptors vary in their breadth of tuning, exhibit both excitation and inhibition, and show different onset and termination kinetics. An individual receptor appears to transmit signals via a single ORN to a single glomerulus in the larval antennal lobe. We provide a spatial map of odor information in the larval brain and find that ORNs with related functional specificity map to related spatial positions. The results show how one family of receptors underlies odor coding in two markedly different olfactory systems; they also provide a molecular mechanism to explain longstanding observations of larval odor discrimination.

Introduction

The numerical simplicity of the larval olfactory system makes it a convenient model system for the study of sensory reception and processing. The system has also attracted great interest because insect larvae, which recognize their food sources through chemosensory cues, are a major source of global agricultural loss. However, despite the rapid progress made in the understanding of odor coding in adult *Drosophila* in recent years (Hallem and Carlson, 2004), remarkably little is known about the molecular basis of olfaction in the larva. In particular, larval odor receptors have not been identified.

The principal larval olfactory organ in *D. melanogaster* is the dorsal organ, a cuticular dome pierced by pore channels through which odorants can pass (Hertweck, 1931; Singh and Singh, 1984; Stocker, 1994; Oppliger et al., 2000). Underlying the dome are the dendrites of 21 neurons, bundled in seven groups of three. The neurons send axons to the larval antennal lobe (LAL), which consists of small glomeruli, the spheroidal modules that constitute the adult antennal lobe as well as the olfactory bulb of vertebrates (Heimbeck et al., 1999; Python and Stocker, 2002).

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The larval olfactory system is sensitive to a wide diversity of odorants (Aceves-Pina and Quinn, 1979; Rodrigues, 1980; Monte et al., 1989), which can elicit either attractive or repellent responses. Among a large number of odorants tested in *D. melanogaster*, 87% were found to induce a response of some kind (Cobb, 1999). Moreover, behavioral analysis has provided evidence that larvae are capable of odor discrimination (Aceves-Pina and Quinn, 1979; Rodrigues, 1980). Thus, despite the numerical simplicity of the system, the 21 ORNs of the larval olfactory system support diverse olfactory-mediated behaviors. Furthermore, the larval system operates primarily in the presence of high odorant concentrations: the larva hatches from an egg laid directly in a food source and generally remains immersed in the food source for most of the larval stage. The striking capabilities of this system, its cellular economy, and its operation in the midst of exceptionally high odor concentrations invite investigation into the molecular mechanisms by which it encodes odorants.

In order to understand the molecular basis of odor coding in the larva, it is necessary to identify larval odor receptors and then to characterize their responses to odorants. Odor coding in the adult depends on the activation of seven-transmembrane domain receptors encoded by a family of 60 *Or* genes (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999; Robertson et al., 2003). Subsets of *Or* genes have been shown to be expressed in the two principal adult olfactory organs, the antenna and the maxillary palp, but no *Or* genes were found to be expressed in the larva in a sensitive in situ hybridization analysis (Vosshall et al., 2000).

Genetic analysis has identified a number of mutants defective in larval olfaction, but none has been shown to affect an odor receptor (Carlson, 1996; Cobb, 1996; Park et al., 1997; Cobb, 1999), with the exception that larval olfaction is impaired by mutations affecting Or83b, an atypical Or protein that is broadly expressed in olfactory receptor neurons (ORNs) and that forms heterodimeric complexes with other Or proteins (Larsson et al., 2004; Neuhaus et al., 2005), but which does not confer odor responses by itself (Elmore et al., 2003).

A deletion mutation of an adult odor receptor gene, *Or22a*, produces a loss of odorant response in a particular functional class of ORN, ab3A, in the adult antenna (Dobritsa et al., 2003). The *Or22a* mutant provides an in vivo expression system that has been used to characterize other odor receptors of the *Drosophila* antenna (Hallem et al., 2004b) and maxillary palp (Goldman et al., 2005), and even of another species, the mosquito *Anopheles gambiae* (Hallem et al., 2004a). Specifically, expression of another odorant receptor in the mutant, "empty" neuron is driven by an *Or22a* promoter via the *GAL4/UAS* system. Such expression of receptors confers odor responses upon the mutant neuron, responses that can be measured physiologically in single-unit recordings. In most cases, the responses conferred by an individual receptor match in great detail the odor

responses of one of the ORN classes previously defined in electrophysiological studies (de Bruyne et al., 1999; de Bruyne et al., 2001). Thus, the empty neuron system has provided a means of characterizing the response properties of many odor receptors.

Here we identify a subset of *Or* genes as larval odor receptor genes, and we characterize physiologically the response properties of the receptors they encode. We provide evidence that the expression of individual *Or* genes is restricted to only one of the 21 larval ORNs in each dorsal organ. We define the odor response spectra of the receptors by physiological analysis in the empty neuron system. Some receptors respond only to a small fraction of tested odors, whereas others are broadly tuned. Larval receptors exhibit different response modes and different termination kinetics, including a slow-onset response that to our knowledge has not previously been described in *Drosophila*. We provide a spatial map of the projections of ORNs expressing receptors of different specificities. We find that ORNs with related specificities map to targets that are related in spatial position. The results of this analysis provide a molecular explanation for how sensitivity and discrimination can be achieved, using members of the same receptor family, in an olfactory system that differs dramatically from that of the adult fly. The data support a simple but unprecedented model of functional organization in which a single odor receptor transmits signals via a single neuron to a single glomerulus in the brain.

Results

Expression of a Subset of *Or* Genes in Larval ORNs

As a first step in identifying larval odor receptors, we tested the possibility of *Or* gene expression in larval ORNs with two approaches: RT-PCR amplification from larval RNA and *Or* promoter-*GAL4* analysis. We initially carried out RT-PCR analysis with primers representing all 60 *Or* genes, using larval RNA from a Canton-S strain as template. In addition to *Or83b*, a gene that is broadly expressed among ORNs and that is functionally distinct from other *Or* genes (Larsson et al., 2004; Neuhaus et al., 2005), we found amplification products of 23 *Or* genes in multiple, independent reactions, and for all of these genes at least one amplification product was verified by DNA sequencing. Among these genes, 13 (*Or2a*, *Or7a*, *Or10a*, *Or13a*, *Or19a*, *Or33a*, *Or33b*, *Or35a*, *Or42a*, *Or42b*, *Or43b*, *Or67b*, and *Or88a)* had previously been shown to be expressed in the adult antenna (Clyne et al., 1999; Vosshall et al., 2000; Vosshall, 2001) or, in the case of *Or42a*, the maxillary palp (Goldman et al., 2005), and most of these have been analyzed in a detailed functional study of antennal receptors (Hallem et al., 2004b). Another ten genes (*Or30a*, *Or45a*, *Or45b*, *Or49a*, *Or59a*, *Or63a*, *Or74a*, *Or85c*, *Or94a*, and *Or94b*) had not been detected in adult olfactory organs in a sensitive in situ hybridization analysis (Vosshall et al., 2000) and were not analyzed by Hallem et al. (2004b). We chose to focus our attention on this latter class of ten *Or* genes detected in larvae and attempted to confirm and extend our analysis of their expression by an independent means: *Or-GAL4* analysis.

We constructed *Or-GAL4* drivers for all but one of the *Or* genes that appear to be larval specific (*Or30a*, *Or45b*, *Or49a*, *Or59a*, *Or63a*, *Or74a*, *Or85c*, *Or94a*, and *Or94b*) and for *Or67b*, which is also expressed in the antenna; we also analyzed an available *Or42a-GAL4* driver (Goldman et al., 2005), which shows expression in the maxillary palp. Expression in larval ORNs was clearly observed for 10 of the 11 *Or-GAL4* drivers (Figure 1). *Or94b-GAL4* drove GFP reporter expression in a single symmetric pair of larval ORNs, one in each dorsal organ (Figure 1C). Likewise, expression of *Or59a-GAL4*, *Or67b-GAL4*, and *Or45b-GAL4* was detected in exactly one ORN of each dorsal organ. *Or85c-GAL4* consistently drove reporter gene expression in at least one neuron of the dorsal organ, and in some cases additional neurons were observed in either the dorsal organ or terminal organ, a neighboring organ implicated in taste (Stocker, 1994; Oppliger et al., 2000). *Or30a-GAL4*, *Or49a-GAL4*, *Or42a-GAL4*, and *Or63a-GAL4* drove expression in a single ORN of each dorsal organ and a single neuron of the terminal organ. In the case of *Or30a-GAL4* and *Or49a-GAL4*, the neuron of the terminal organ was faint, and *Or30a-GAL4*, *Or49a-GAL4*, and *Or63a-GAL4* also showed labeling of some additional cells. *Or74a-GAL4* was expressed in a single ORN of each dorsal organ in addition to an unidentified structure adjacent to the terminal organ. The *Or94a-GAL4* construct did not drive expression in either larval or adult chemosensory organs, which could indicate either that the *Or* gene is not expressed in the larval olfactory system or that the reporter construct lacks regulatory sequences necessary for faithful expression, as has been found for a number of constructs made from adult *Or* genes (Goldman et al., 2005) (A. Ray and J.R.C., unpublished data), or that expression depends on epigenetic factors. In summary, ten of the *Or-GAL4* drivers showed expression in larval ORNs. These results confirm that some *Or* genes are expressed in larval ORNs, including some genes that are also expressed in either the adult antenna or the maxillary palp.

Different Receptors Map to Different ORNs and to Different Regions of the Antennal Lobe

We next investigated the projections of the larval ORNs in the LAL of the brain. We found that each *Or-GAL4* driver labeled a small, discrete subregion of the antennal lobe (Figure 2A). For example, *Or94b-GAL4*, which labels one ORN in each dorsal organ, labels a single subregion in the lateral portion of the lobe. *Or59a-GAL4*, which also labels a single ORN in each dorsal organ, also shows labeling in a subregion of the LAL, but the label is more anterior than that of *Or94b-GAL4. Or67b-GAL4*, *Or45b-GAL4*, *Or85c-GAL*, and *Or74a-GAL4* label distinct regions of the LAL. *Or30a-GAL4*, *Or49a-GAL4*, *Or42a-GAL*, and *Or63a-GAL4* also label subregions of the LAL, but their staining patterns are somewhat more complex, which is likely due to additional expression in neurons of the terminal organ and their targets in the suboesophageal ganglion.

We then investigated the distribution of *Or* gene expression among ORNs. Are each of these different *Or*

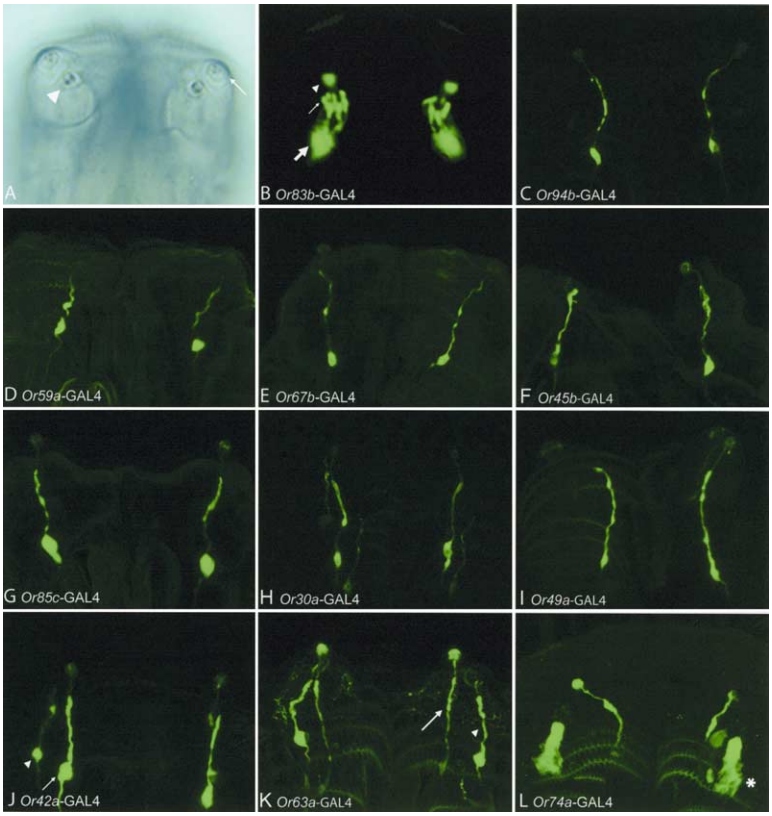

Figure 1. *Or-GAL4* Expression in Larval ORNs

The reporter gene is *UAS-GFP* in each case. Anterior is at top. (A) Bright-field micrograph of *Drosophila* larva showing chemosensory organs. Arrowhead indicates the dorsal organ; arrow indicates the terminal organ. (B) *Or83b-GAL4* labels seven bundles, each containing three ORNs. The arrowhead indicates the dome of the dorsal organ, which shows autofluorescence. The small arrow indicates the dendrites of ORNs. The large arrow indicates the cell bodies of the ORNs. (C–I) The indicated *Or-GAL4* drivers label one neuron innervating each dorsal organ, i.e., a symmetric pair of ORNs is visible. (J) *Or42a-GAL4; UAS-GFP.* The arrow indicates an ORN; the arrowhead indicates a neuron of the terminal organ. (K) *Or63a-GAL4;UAS-GFP.* The arrow indicates an ORN; the arrowhead indicates a neuron of the terminal organ. (L) *Or74a-GAL4; UAS-GFP.* One neuron innervating each dorsal organ is labeled. Another unidentified structure is also labeled (asterisk).

genes expressed in different ORNs, or, at the other extreme, are all of these *Or* genes coexpressed in a single ORN? We constructed fly strains containing pairs of *Or-GAL4* drivers and asked whether the patterns of reporter gene expression were additive. For example, the *Or94b-GAL4* and *Or59a-GAL4* constructs each drive expression in a single ORN of each dorsal organ when tested singly, but when tested together in the same animal, two ORNs are labeled (Figure 2B), indicating that they are expressed in different ORNs. In all, we tested 21 pairs of drivers—all pairwise combinations of seven *Or-GAL4* drivers—and found additivity in all cases. In the case of three pairs (*Or42a* and *Or59a*; *Or67b* and *Or85c*; and *Or74a* and *Or94b*), the two ORNs were more difficult to resolve than in the other cases and appear to reside in the same bundle of three ORNs. These results indicate that *Or-GAL4* expression is distributed among different ORNs and support a model in which each ORN expresses only one or a small number of *Or* genes.

To determine whether the projections of different ORN classes in the LAL are distinct, we examined the strains containing combinations of the seven *Or-GAL4* drivers. We found that in all 21 pairwise combinations, the patterns were additive (Figure 2B). These results indicate that different ORNs project to different glomeruli. Hence, we have found no evidence for convergence of larval ORNs.

ORNs of the Dorsal Organ Respond to Multiple Odors, with Different Dynamics

As an initial means of addressing odor coding in the larva, we recorded extracellular physiological responses from the dorsal organ. We found that a wide variety of odors elicited increased firing frequencies (Figure 3), consistent with a previous report (Oppliger et al., 2000). We also observed differences in the dynamics of the responses. For example, the response to acetophenone was initiated shortly after the onset of the odor stimulus. By contrast, the response to methyl eugenol showed a slower onset. We were unable to assign action potentials to individual ORNs on the basis of amplitude in these recordings because of the large number of ORNs, 21, in the dorsal organ. Therefore, in order to analyze the basis of odor coding at high resolution, we have used an expression system, as described below.

Odor Response Spectra of Larval Odor Receptors

We have provided evidence above that a subset of *Or* genes is expressed in larval ORNs. To test directly whether these genes encode functional odor receptors, we have used an in vivo expression system, the empty neuron system (Dobritsa et al., 2003), to decode them. This approach was successful for the characterization of the antennal repertoire of odor receptors (Hallem et al., 2004b) and odor receptors of the mosquito *Anopheles gambiae* (Hallem et al., 2004a).

We generated *UAS-Or* constructs for 12 of the candidate larval odor receptors, including all 10 whose expression in larval ORNs was confirmed by *Or-GAL4* analysis, and functionally tested them in the empty neuron system. We tested a panel of 27 olfactory stimuli that represent a wide variety of chemical classes,

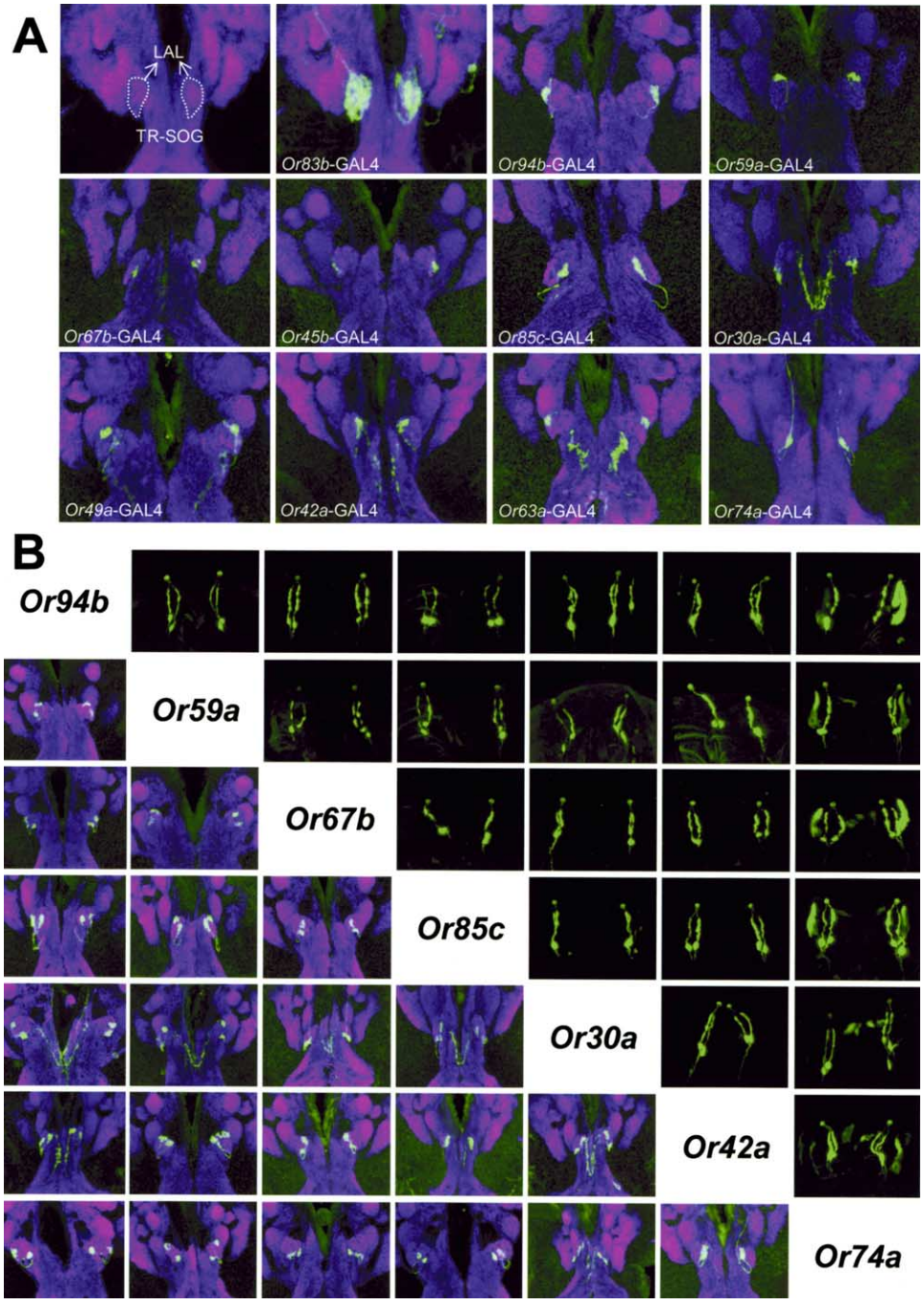

Figure 2. Larval Odorant Receptors Are Expressed in Single ORNs and Project to Nonoverlapping Regions in the Larval Brain

(A) Larval antennal lobes, showing GFP reporter expression generated by *Or-GAL4* drivers. LAL, larval antennal lobes; TR-SOG, tritocerebrum-suboesophageal ganglion. The *Or83b-GAL4* driver shows labeling of all or almost all of the LAL, whereas other drivers label only a single glomerulus. (B) Double-driver analysis, showing GFP reporter expression in larval ORNs (above, right) and antennal lobe (below, left) driven by pairs of *Or-GAL4* drivers. The brain neuropil is counterstained with monoclonal antibody nc82 (magenta).

including acetate esters, alcohols, aldehydes, aromatics, ketones, an organic acid, and CO_2. The panel includes all of the odors used in recent physiological studies of the antenna and maxillary palp (Hallem et al., 2004b; Goldman et al., 2005) as well as a number of odorants used in studies of larval olfaction (Monte et al., 1989; Cobb, 1996; Heimbeck et al., 1999). We found that 11 of the 12 receptors conferred odor responses to the mutant ab3A neuron (Figure 4). Only one tested gene, *Or63a*, did not confer an odor response, either when expressed as a cDNA or as a genomic construct. The odor response spectrum is different for each function-

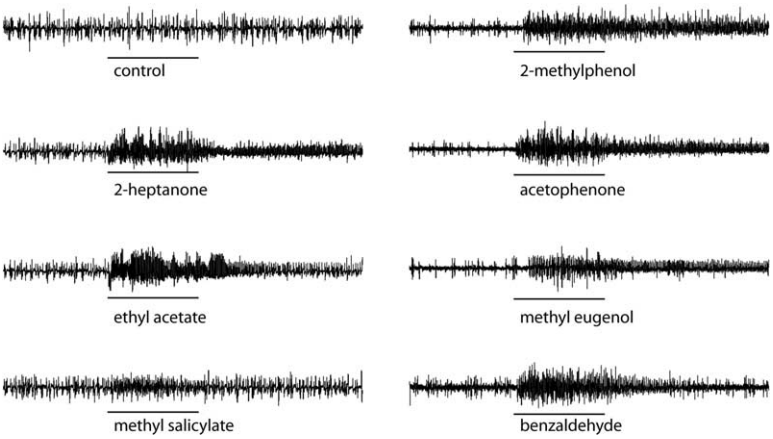

Figure 3. Extracellular Recordings from the Dorsal Organ

The horizontal bar indicates a 0.5 s stimulus. The control stimulus was the paraffin oil diluent.

ally expressed receptor. These results indicate that these genes do in fact encode functional odor receptors.

The odor response spectra of these receptors vary greatly. If we define a strong response as ≥100 spikes/s (Hallem et al., 2004b), then Or94b responds strongly to a single odorant, 4-methylphenol, among 27 tested stimuli. By contrast, Or42a and Or85c each responds strongly to nine odorants. Only one receptor, Or49a, gave no strong responses to any of the 27 stimuli or to several natural odor mixtures, suggesting that it may be narrowly tuned to an untested odorant of particular biological significance, such as a pheromone. Most of these receptors respond strongly to multiple odorants, and among the odorants that elicit any strong responses, most elicit strong responses from multiple receptors (Figure 5).

We note that a number of receptors, such as Or42a, Or74a, and Or85c, which we term class 1 receptors, respond most strongly to linear aliphatic compounds, including acetate esters, alcohols, and ketones, whereas others, such as Or30a, Or45b, Or59a, Or94a, and Or94b, termed class 2 receptors, seem tuned to aromatic compounds containing a benzene ring. Or67b is distinct in that it responds strongly to compounds of both categories.

Larval Odor Receptors Exhibit Multiple Response Modes

Our physiological analysis revealed both excitatory and inhibitory responses among larval odor receptors. Most of the responses we observed were excitatory, but an example of an inhibitory response is shown in Figure 6A. The traces are from an ab3 sensillum, which contains two paired ORNs, ab3A and ab3B. In this strain, the ab3A ORN expresses Or67b, instead of the endogenous receptor, via the *GAL4/UAS* system and is referred to as the Δab3A: Or67b neuron. Physiological recordings from this sensillum show the activity of both the Δab3A: Or67b neuron, which produces the large action potentials in the trace, and the neighboring neuron, ab3B, which produces the small action potentials (Dobritsa et al., 2003). The frequency of the large action potentials decreases markedly below the spontaneous rate of firing following stimulation with 2-methylphenol, indicating that the Δab3A: Or67b neuron is inhibited by this odorant. (The ab3B neuron is weakly excited by 2-methylphenol, as shown by the modest increase in the frequency of small spikes.) The inhibition of Δab3A: Or67b by 2-methylphenol is strong: the spontaneous firing frequency is 12 ± 2 spikes/s (SEM; n = 12), and the frequency decreases by 9 ± 2 spikes/s (n = 12) following odor stimulation (Figure 6B). While Δab3A: Or67b is strongly inhibited by 2-methylphenol, it is strongly excited by acetophenone (Figures 6A and 6B), indicating that a single larval odor receptor can be excited by one odorant and inhibited by another, structurally related odorant.

Diverse Response Dynamics of Larval Odor Receptors

One larval receptor, Or59a, responds strongly to both anisole and methyl eugenol; the two odorants elicited comparable numbers of action potentials in the 0.5 s period following odorant stimulation (Figure 4). However, the dynamics of the two responses were markedly different in two respects (Figure 6C). Anisole elicited a response with fast onset kinetics, reaching peak firing frequency shortly after the onset of odorant delivery. Moreover, the response to anisole terminated quickly after the termination of the odor stimulus. By contrast, the response to methyl eugenol did not reach peak firing frequency until near the end of the odorant delivery period. Furthermore, the response continued for a sustained period of time after the end of odor delivery. We have quantitated these response dynamics by counting the numbers of spikes in 200 ms bins (Figure 6D). Quickly terminating responses, such as those to anisole, and slowly terminating responses, like those to methyl eugenol, have been observed for adult receptors (de Bruyne et al., 1999; de Bruyne et al., 2001), but responses with such slow onset kinetics as those for methyl eugenol have not been documented previously in studies of *Drosophila* odor receptors.

Different Response Thresholds of Larval Odor Receptors

To examine in more detail the sensitivities of different receptors to individual odorants, we chose four recep-

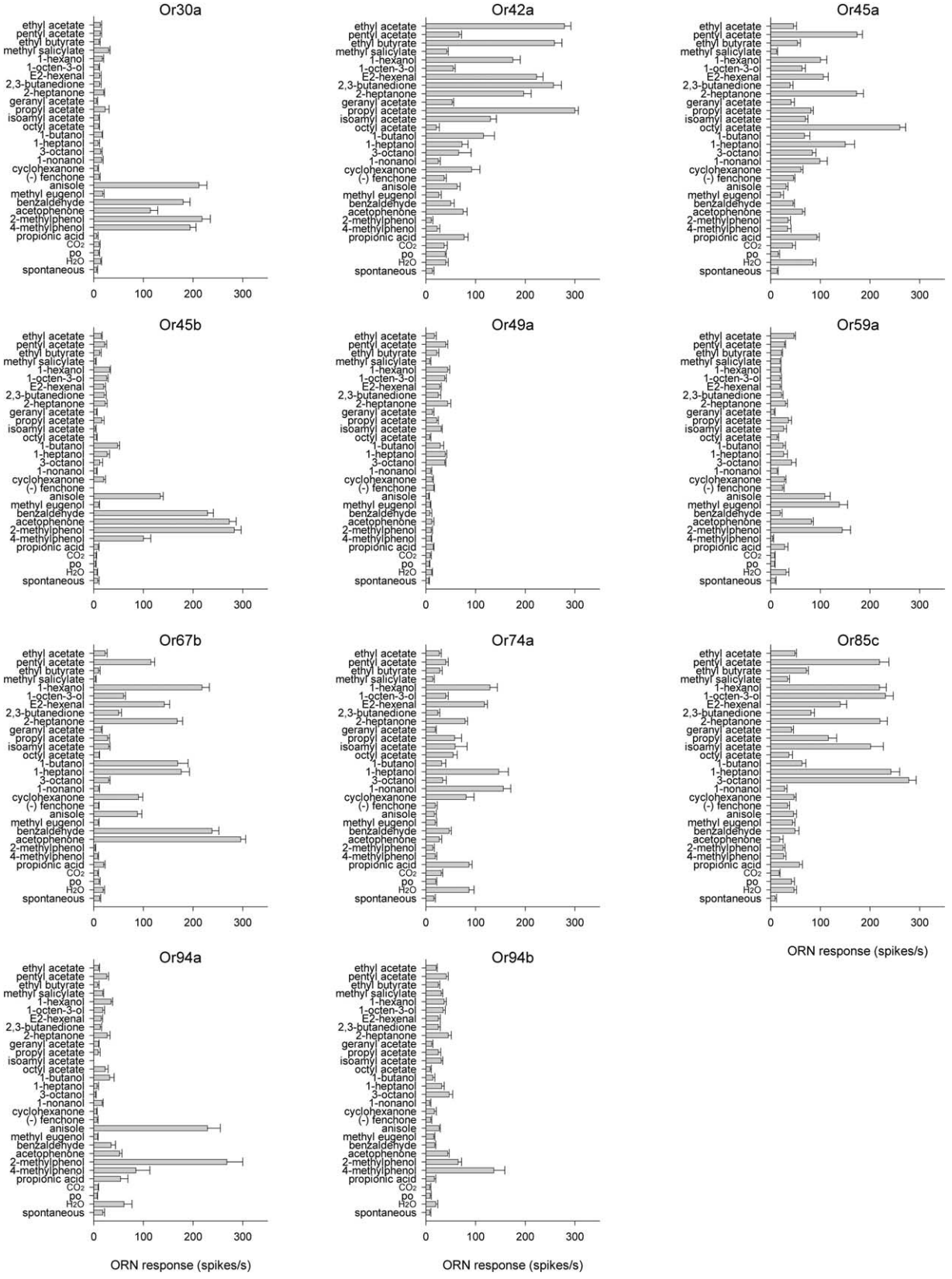

Figure 4. Odor Response Spectra of Larval Odor Receptors

Odorants were applied to testing cartridges at a dilution of 10^{-2}, but were diluted further in an airstream before reaching the fly (Hallem et al., 2004b). Spontaneous activity and response to diluent are indicated for each receptor and were not subtracted from odor responses; hence inhibitory responses are null or have a positive valence. n = 6–12. Error bars indicate SEM.

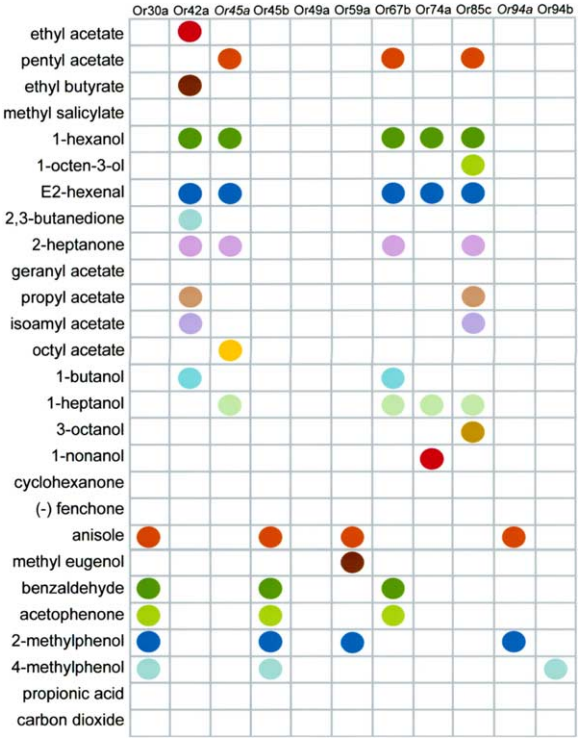

Figure 5. Combinatorial Coding of Odors

Colored dots indicate strong odor responses (≥100 spikes/s to a "10^{-2} dilution"; see note about dosage in Figure 4 legend). Italics indicate *Or* genes whose expression in larval ORNs has not been confirmed by *Or-Gal4* analysis: *Or45a* and *Or94a*.

tors that respond strongly to 2-heptanone and measured their responses to this odorant across a range of dilutions (Figure 6E). We found that Or85c exhibited a sigmoid dose-response relationship, with saturation occurring at a dilution of approximately 10^{-4}. The response increased markedly between 10^{-6} and 10^{-5} dilutions. By contrast, the other receptors showed no increase in this interval; for Or67b and Or45a, the response threshold appeared to be between 10^{-5} and 10^{-4}, and the responses continued to increase between 10^{-2} and 10^{-1} dilutions. Thus, the different receptors are sensitive to changes in 2-heptanone concentrations over different ranges.

A Spatial Map of Odor Representation in the Larval Antennal Lobe

Based on our analysis of the projections of larval ORNs (Figure 2), we have constructed a map of their glomerular targets (Figure 7). The map shows that different ORNs project to discrete, nonoverlapping regions of the LAL. We find no evidence for convergence of different ORNs to a common target.

Having identified targets of ORNs and having identified ligands for many of the receptors that they express (Figures 4 and 5), we are able to integrate the functional and anatomical data to provide a spatial map of odor representation in the LAL (Figure 7). The map suggests a relationship between glomerular position and function. The two receptors that are most similar in response spectrum, i.e., that respond strongly to the same odors, are Or30a and Or45b. Their target glomeruli are adjacent to each other. We have noted above that a number of receptors, the class 1 receptors, respond most strongly to linear aliphatic compounds, whereas the class 2 receptors respond most strongly to aromatic compounds containing a benzene ring. Or67b was distinct in that it responds strongly to compounds of both categories. It is striking that among the receptors we have mapped, all the class 1 receptors (Or42a, Or85c, and Or74a) map to glomeruli that are clustered in the center of the LAL, whereas all the class 2 receptors (Or30a, Or45b, Or94b, and Or59a) map to the lateral periphery of the LAL, and the receptor that responds to both classes, Or67b, maps to an intermediate target.

Thus, the results support a model in which receptors with related odor specificities send projections to spatially related portions of the antennal lobe to create a distributive spatial map of olfactory information in the larval brain.

Discussion

One Family of Receptors, Two Olfactory Systems

We have characterized the molecular basis of odor coding in the *Drosophila* larva. We have presented evidence that a subset of *Or* genes encodes larval odor receptors expressed in larval ORNs, and we have characterized the response spectra of these receptors in detail.

In the case of 10 *Or* genes, we have provided *Or-GAL4* data to confirm the original RT-PCR evidence for larval expression. Another 13 *Or* genes were identified as candidate larval odor receptor genes in our original RT-PCR analysis, but their identification as larval odor receptors is tentative pending further expression analysis. If expression of all 13 of these genes is confirmed, then the results would suggest a close numerical relationship between the number of ORNs in the dorsal organ, 21, and the number of larval *Or* genes, ~23, exclusive of *Or83b*, which is broadly expressed (Larsson et al., 2004; Neuhaus et al., 2005).

It is striking that the same gene family serves both larval and adult olfactory systems. These systems differ markedly in anatomy and developmental origins, and they operate largely in different chemical environments (Hertweck, 1931; Singh and Singh, 1984; Stocker, 1994; Oppliger et al., 2000). The larva burrows in rich food sources where odorant concentrations are high and sustained. A navigating adult, by contrast, must make olfactory computations while flying through the air, where odor stimuli are intermittent and at much lower concentrations. In these two contexts the detection and discrimination of a particular olfactory signal occur at remarkably different noise levels. Despite the different anatomical, developmental, and environmental contexts, olfactory responses in both larvae and adults are mediated by *Or* genes. We note that there is also evidence for expression of mosquito orthologs, *AgOr* genes, in both the larval and adult stages (Fox et al., 2002).

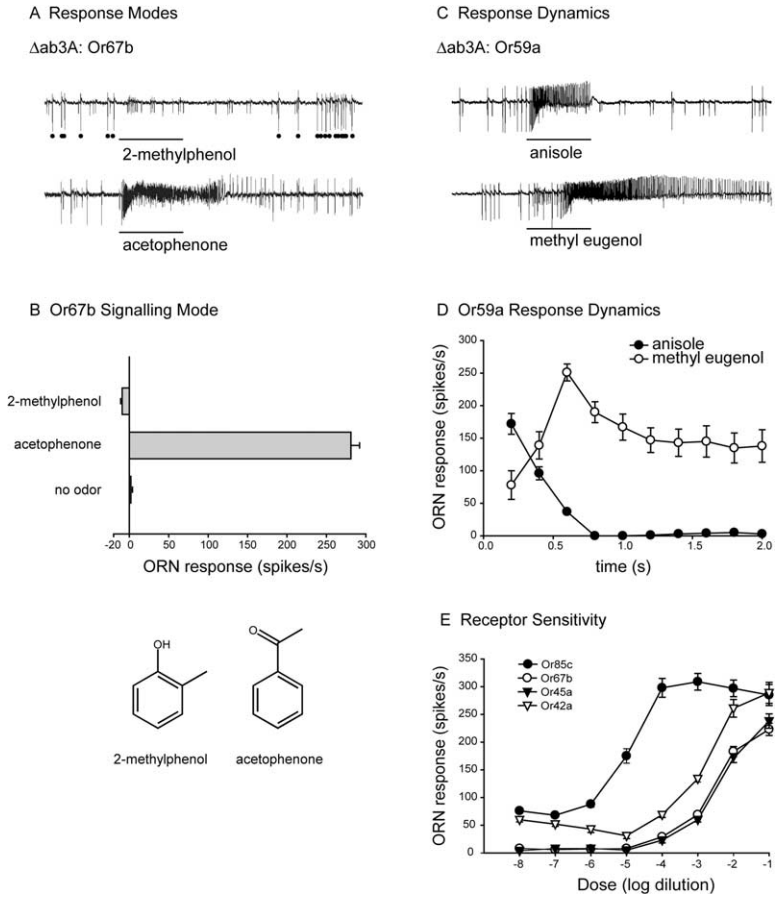

Figure 6. Larval Odorant Receptors Display Diverse Response Properties

(A) Response modes. Top trace shows inhibitory response of Δab3A: Or67b (large spikes, indicated by dots) to 2-methylphenol. The weak excitatory response of the neighboring ab3B ORN (small spikes) can also be seen. Bottom trace shows excitatory response of Δab3A: Or67b to acetophenone. Bars represents 0.5 s odor stimulus period. (B) Quantitation of inhibitory and excitatory responses of Or67b to 2-methylphenol and acetophenone (structures below). The number of action potentials during the 0.5 s before odor stimulation was subtracted from the number of action potentials during the odor stimulation period. n = 12. The data were taken from Figure 4. (C) Different response dynamics of Δab3A: Or59a to different odors. The bar represents the 0.5 s stimulation period. (D) Temporal dynamics of response of Or59a to anisole and methyl eugenol. Action potential frequency was counted in 0.2 s bins for 2 s after odor stimulus. 0 represents the initiation of odor stimulus. The odor stimulus ended at 0.5 s. n = 12. (E) Different sensitivities of receptors to 2-heptanone. Or85c has a high rate of response to the paraffin oil diluent, which has not been subtracted. n = 6. Error bars are SEM.

The larval *Or* genes are distributed widely throughout the phylogenetic tree of *Or* genes; they do not represent a single branch of the *Or* gene family (Robertson et al., 2003). In fact, some individual *Or* genes are shared by both the larva and adult. These data suggest that as the *Or* gene family has expanded, new members

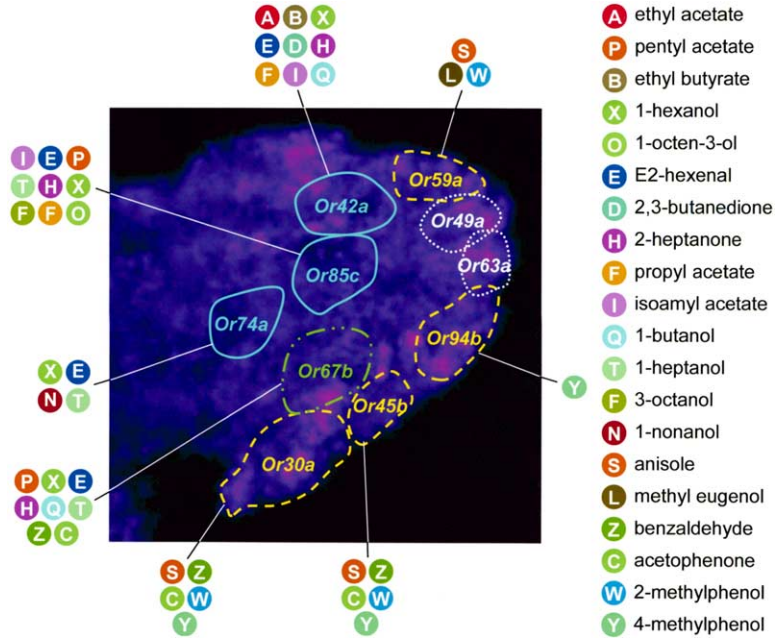

Figure 7. Spatial Map of Olfactory Information in the LAL

The photograph indicates the regions of the LAL that receive projections from each of the indicated *Or-GAL4* drivers. Also indicated are the odorants to which each Or responds strongly. Anterior is at top; medial is to left. Although there appears to be some overlap in the figure between the target of Or63a and neighboring targets, they are at different depths. Blue, solid outlines represent targets of ORNs expressing class 1 receptors, e.g., Or74a; yellow, dashed outlines correspond to class 2 receptors, e.g., Or30a; the green outline consisting of alternating dashes and dots corresponds to Or67b, whose sensitivity overlaps with those of both class 1 and class 2 receptors. Targets of receptors whose sensitivity is unknown are outlined with white dots.

have been recruited for use in either or both systems. The results also suggest the existence of regulatory elements that specify expression in the larval ORNs, and separate elements that specify expression in adult ORNs. It is possible that these elements act additively in the case of *Or* genes expressed in both systems.

The Response Spectra of Larval Odor Receptors and the Mechanism of Odor Coding

Physiological measurements of larval odor receptors have revealed a wide range of odor response spectra. At one extreme is Or49a, which responds strongly to none of the 27 tested stimuli, and Or94b, which responds strongly only to 4-methylphenol. At the other extreme are receptors such as Or42a and Or85c, which respond strongly to nine of the tested odors.

How are odors discriminated by the larval olfactory system? Our results demonstrate that different odorants activate different subsets of larval receptors (Figure 5), which in turn transmit information to different glomeruli in the LAL (Figure 7). The number of receptors that are strongly activated varies a great deal: methyl eugenol strongly excites only one of these receptors, Or59a, whereas other odorants strongly activate multiple receptors. For example, E2-hexenal activates a substantial fraction of receptors: Or42a, Or45a, Or67b, Or74a, and Or85c. These results are consistent with a model of combinatorial coding in which the identification of an odorant is based on the differential activation of the population of odor receptors (Malnic et al., 1999; Kajiya et al., 2001; Hallem et al., 2004b).

We have found that most of the larval odor receptors we have characterized may be divided into two classes. The first class, including Or42a, Or74a, and Or85c, responded strongly to aliphatic odors such as ethyl acetate and 1-hexanol, but not to benzene derivatives. The second class, consisting of receptors such as Or30a, Or45b, Or59a, and Or94b, responded most strongly to compounds of the panel that contain a benzene ring, such as benzaldehyde, 2-methylphenol, 4-methylphenol, acetophenone, anisole, and methyl eugenol, but did not respond strongly to aliphatic compounds. Only one receptor, Or67b, fell into a different category, marked by strong responses to both kinds of odorants.

This categorization of receptors into two broad classes suggests a molecular mechanism to explain results of a classical study of larval odor discrimination (Rodrigues, 1980; Siddiqi, 1983). Specifically, larvae responded to benzene derivatives such as benzaldehyde in a background of aliphatic compounds or cyclohexanone, but the responses were inhibited in a background of other benzene derivatives. This behavioral phenomenon can be explained by the distinct receptor sensitivities we have documented: larvae respond to benzene derivatives via the activity of class 2 receptors, which are insensitive to the presence of aliphatic compounds. A high concentration of one aromatic compound, however, competes with other aromatics for activation of class 2 receptors, and thus a background of one benzene derivative inhibits the response to another.

We note that an additional degree of freedom for larval odor coding may be provided by the existence of different modes of response: excitation and inhibition. We found that at least three larval receptors, Or67b, Or45b, and Or94a, are excited by some odors and inhibited by others (Figures 4 and 6) and thus may be thought of as bidirectional chemical detectors (Friedrich, 2004). We have found earlier that some adult antennal receptors are also capable of both excitation and inhibition and have proposed a model in which the receptor exists in two conformations, an active conformation that leads to activation of a G protein-mediated signal transduction cascade and an inactive conformation that does not (Hallem et al., 2004b). The binding of an excitatory odorant would stabilize the active conformation of the receptor, leading to an increase in the firing rate. The binding of an inhibitory odorant would stabilize the inactive conformation, leading to a decrease in the firing rate below that of the spontaneous rate. It will be interesting to determine how these differing responses are processed in the antennal lobes of both larvae and adults.

The coding capacity of this minimalist olfactory system may also be expanded by another means. We have documented differences in the dynamics of odor responses generated by larval receptors. Responses vary not only in duration, but also in onset kinetics. Odors may therefore be identified in part by the time course of the spike trains they elicit. More particularly, two odors that activate the same larval ORN might in principle be discriminated by virtue of the different response dynamics they elicit. The temporal structure of olfactory information has been shown to be essential to odor coding in other systems (Laurent et al., 2001), and it is now clear that the information transmitted by larval odor receptors contains a variable temporal structure that can be transformed further in subsequent processing events.

Most of the larval period is spent in an environment containing very high odorant concentrations, but the animal may experience lower concentrations if it becomes separated from its food source or when it seeks a pupation site. In addition, food sources are heterogeneous, and the concentration of individual odorants varies within and between sources. Thus, there may be selective pressure to maintain a wide dynamic range in the larval olfactory system. We have shown that different larval odor receptors exhibit differing sensitivities to an individual odorant, providing a mechanism for expanding the dynamic range of the system. We note that the larval receptor repertoire confers a high degree of sensitivity to 2-heptanone that is comparable to the high sensitivity to this odorant observed in the adult (Hallem et al., 2004b), suggesting a role for this odorant in larval migration or selection of food sources. It will be interesting to investigate olfactory adaptation in this system, given the possibility that the system may operate under conditions that vary enormously in ambient odor levels.

The distinction between olfaction and taste deserves special consideration in an animal that burrows in a semisolid medium. Following the isolation of the *Or* genes, a second large family of seven-transmembrane domain genes, the *Gr* genes, was identified and proposed to encode taste receptors (Clyne et al., 2000).

One *Gr* gene, *Gr5a*, was subsequently demonstrated to be a taste receptor for the dissacharide trehalose (Dahanukar et al., 2001; Ueno et al., 2001; Chyb et al., 2003). However, the *Or* genes and the *Gr* genes can be considered as members of a single large superfamily (Scott et al., 2001; Robertson et al., 2003), and some *Gr* genes have been shown to be expressed in the antenna, an olfactory organ (Scott et al., 2001). Moreover, one *Gr-GAL4* line drove reporter gene expression in the larval olfactory organ (Scott et al., 2001), and some of the *Or-GAL4* lines we describe here show expression in neurons of both the dorsal organ and the terminal organ. In our functional study of larval *Or* genes, all genes tested have conferred odor responses upon the empty neuron, with only one exception. These results support the identity of the larval *Or* genes as odor receptor genes. If further expression analysis confirms the expression of some *Or* genes in taste cells, then a functional analysis of their role in taste would be of great interest. Likewise, it is possible that some *Gr* genes may function in larval olfaction.

We note that the responses we have measured in our functional expression system seem likely to represent the responses of these receptors in their native larval ORNs. The expression system we have used is an in vivo system, in which receptors are expressed in another ORN, in an intact animal of the same species. Previous work has shown that receptors that are normally expressed in all three morphological classes of antennal sensilla, and in maxillary palp sensilla, respond faithfully in the empty neuron system (Hallem et al., 2004b; Goldman et al., 2005). Moreover, some of the larval receptors we have identified are normally expressed in both larval and adult ORNs, suggesting that they are compatible with either signaling context. We note finally that a number of odors that elicit strong responses in our in vivo expression system also elicit strong responses in the larval dorsal organ (Figures 3 and 4) and that the delayed onset of methyl eugenol response is observed in both the dorsal organ (Figure 3) and the empty neuron (Figure 6).

Odor Receptors and the Functional Organization of the Larval Olfactory System

Among each of the ten *Or-GAL4* drivers that produced reporter gene expression in this study, consistent labeling was observed in only one of the dorsal organ ORNs. These data support a model in which each odor receptor is expressed in only one of the ORNs of the dorsal organ. Pairs of drivers labeled pairs of ORNs in all cases; these data support a model in which each ORN expresses one or a small number of *Or* genes. Thus, the 21 ORNs of the larval olfactory system could each express a single, distinct odor receptor.

The ORNs of the dorsal organ are organized in seven bundles of three (Singh and Singh, 1984; Stocker, 1994). Or42a and Or59a, which are expressed in ORNs of a common bundle, are most sensitive to nonoverlapping subsets of the tested odorants; they are members of class 1 and 2, respectively. Likewise, Or74a and Or94b, also in a common bundle, are of class 1 and 2, respectively. These results indicate that ORNs in a bundle may be functionally diverse. Moreover, they may project to glomeruli that are either close to each other (Or42a and Or59a) or far apart (Or74a and Or94b). Two receptors expressed in a common bundle may be highly divergent in sequence (Robertson et al., 2003) and located at widely dispersed cytogenetic positions.

ORNs expressing different receptors map to different regions of the antennal lobe. The LAL has previously been shown to be structurally heterogeneous (Heimbeck et al., 1999; Python and Stocker, 2002), and our results provide evidence for functional heterogeneity as well. The differential activation of receptors in the dorsal organ is thus transformed into a spatial representation of an olfactory stimulus in the LAL, as it is in the adult antennal lobe (Rodrigues, 1988; Fiala et al., 2002; Ng et al., 2002; Wang et al., 2003).

We have found that ORNs expressing receptors that are related in functional specificity map to glomeruli that are related in spatial position (Figure 7). Receptors responding to linear aliphatic compounds transmit signals to a cluster of glomeruli that are spatially distinct from glomeruli receiving signals from receptors responding to aromatic compounds. If testing of additional receptors confirms this relationship, then it has implications for the mechanism of information processing in the LAL and for the transmission of olfactory information to higher centers in the larval brain. Such a relationship would also suggest a connection between the process of receptor gene choice and axon targeting. Odor mapping in the antennal lobe of adult insects and the olfactory bulb of mammals, accomplished via optical imaging of intrinsic signals in the olfactory bulb, calcium-sensitive imaging, or multielectrode recording from the antennal lobe, has also provided evidence that representations of different classes of odorants are spatially distinct (Uchida et al., 2000; Hansson et al., 2003; Lei et al., 2004).

The functional organization of the larval olfactory system is unprecedented, to our knowledge. Although the olfactory system of *C. elegans* functions in an environmental context similar to that of the *Drosophila* larva, its organization is markedly different. In particular, individual ORNs in *C. elegans* express a large number of odor receptors (Troemel et al., 1995). The olfactory systems of the *Drosophila* adult and of vertebrates differ from that of the larva in their cellular redundancy. In the adult fly and in vertebrates, an individual odor receptor is expressed by many ORNs that converge upon a small number of common glomeruli (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Gao et al., 2000; Vosshall et al., 2000). By contrast, the number of ORNs in the *Drosophila* larva is small, each receptor appears expressed in a single ORN, and we have found no evidence for convergence.

Although cellular reiteration is not used as a mechanism for increasing the sensitivity of the larval system, molecular redundancy is likely used as a means of heightening sensitivity: many tested odorants elicit a strong response from several receptors. In addition, the multiplicity of receptors for a given odorant, with distinct dynamic ranges, provides a mechanism for extending the dynamic range over which the concentration of an odor can be

evaluated. The functional organization of the system thereby provides sensitivity via overlapping response spectra, as well as discriminatory power via combinatorial coding, with remarkable molecular and cellular economy.

Experimental Procedures

Drosophila Stocks and Transgenes

All transgenic constructs were injected into w^{1118} flies. *GAL4* constructs were created using primers to amplify DNA sequence immediately upstream of the translation initiation codon of odorant receptors. *Or42a-GAL4* was described previously (Goldman et al., 2005); *Or83b-GAL4* contained a 7.7 kb segment of upstream DNA whose 3′ end lies 0.57 kb upstream of the translation initiation codon. In this study, additional *GAL4* lines were constructed, initially with upstream sequence lengths as follows: *Or30a*, 8.5 kb; *Or45b*, 8.8 kb; *Or49a*, 1.6 kb; *Or59a*, 2.9 kb; *Or63a*, 2.9 kb; *Or67b*, 2.5 kb, *Or74a*, 2.5 kb; *Or85c*, 2.7 kb. For *Or94a-GAL4* and *Or94b-GAL4*, which are separated by 300 bp in the genome, upstream and downstream fragments were cloned into the *GAL4* construct. For *Or94a-GAL4*, 2.4 kb of upstream sequences and 4.5 kb of downstream sequences were used. For *Or94b*, 4.4 kb upstream and 2.4 kb of downstream sequences were used. Reporter gene expression was not observed for three of these *GAL4* lines, *Or45b*, *Or49a*, and *Or63a*, and additional lines were therefore constructed for these genes using upstream and downstream sequences as follows: *Or45b*, 4.8 kb upstream and 0.5 kb downstream; *Or49a*, 4.9 kb upstream and 1.2 kb downstream; *Or63a*, 2.9 kb upstream and 2.9 kb downstream. At least two independent *GAL4* lines were examined for each *Or* gene. Where possible, larvae bearing two copies each of *UAS-GFP* and the *Or-GAL4* driver were examined.

UAS-Or constructs were created as previously described (Hallem et al., 2004b) from Canton-S genomic DNA inserted into a *UAS* expression vector. Coding sequence was taken from the second codon to the stop codon and fused in-frame with an encoded N-terminal *myc* tag. Clones were examined from three independent PCR reactions, in order to distinguish PCR errors from polymorphisms. Polymorphisms of some *Or* genes, representing variations from sequences in the *Drosophila* genome database, were observed consistently among the PCR reactions. At least two lines were tested physiologically for each *UAS* construct. *UAS-Or63a* was not able to rescue the *halo* physiological phenotype, which includes bursting of action potentials upon 2-heptanone application and/or little if any spontaneous activity (Dobritsa et al., 2003). An *Nmyc* tagged cDNA version of this receptor also failed to rescue the *halo* phenotype.

Immunohistochemistry

At least ten larval brains of each genotype were dissected and immunostained as previously described (Python and Stocker, 2002) using rabbit anti-GFP (Clontech) and nc82 monoclonal antibody (a gift of Dr. Alois Hofbauer, University of Regensburg). All images were collected on a Bio-Rad 1024 laser-scanning confocal microscope.

Electrophysiology

Odor stimuli were presented, action potentials of ORNs were recorded extracellularly via an electrode placed through the sensillum wall, and responses were quantified as described previously (Dobritsa et al., 2003), except where noted otherwise. Odors were obtained from Sigma at the highest available purity (>99%) and were dissolved in paraffin oil, with the exception of propionic acid, which was dissolved in water. Liquid odorants were diluted 10^{-2} and 4-methylphenol was dissolved at 10 mg/ml. Fifty microliters of diluted odorant was placed in a Pasteur pipette, and the headspace of this pipette was used as the odor stimulus; odorants were further diluted in the airstream directed at the fly, as discussed elsewhere (Hallem et al., 2004b). Each odor cartridge was used no more than three times. The odor was presented for 0.5 s. All adult flies were 5–15 days old; no more than three sensilla were recorded per fly. For dose-response curves, only one sensillum was taken from each fly. For quantitation of inhibition, the number of action potentials during the 0.5 s preceding the odor stimulus was subtracted from the number of action potentials during the 0.5 s odor stimulus. Error bars indicate SEM.

Recordings were made from dome sensilla of third instar larvae by placing the larva on a toothpick that had been soaked in water to provide moisture. Parafilm was wrapped around the body and the toothpick to immobilize the larva, leaving only the anterior part, including the dome sensilla, exposed. A reference electrode was inserted through the Parafilm into the body. Electrical activity of the neurons was recorded extracellularly by inserting a second electrode into the lumen of the dome sensillum. Odors were presented as described above.

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References

Aceves-Pina, E.O., and Quinn, W.G. (1979). Learning in normal and mutant *Drosophila* larvae. Science *206*, 93–95.

Carlson, J. (1996). Olfaction in *Drosophila:* from odor to behavior. Trends Genet. *12*, 175–180.

Chyb, S., Dahanukar, A., Wickens, A., and Carlson, J.R. (2003). *Drosophila Gr5a* encodes a taste receptor tuned to trehalose. Proc. Natl. Acad. Sci. USA *100*, 14526–14530.

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

Clyne, P.J., Warr, C.G., and Carlson, J.R. (2000). Candidate taste receptors in *Drosophila*. Science *287*, 1830–1834.

Cobb, M. (1996). Genotypic and phenotypic characterization of the *Drosophila melanogaster* olfactory mutation *Indifferent*. Genetics *144*, 1577–1587.

Cobb, M. (1999). What and how do maggots smell? Biol. Rev. Camb. Philos. Soc. *74*, 425–459.

Dahanukar, A., Foster, K., Van der Goes van Naters, W.M., and Carlson, J.R (2001). A Gr receptor is required for response to the sugar trehalose in taste neurons of Drosophila. Nat. Neurosci. *4*, 1182–1186.

de Bruyne, M., Clyne, P.J., and Carlson, J.R. (1999). Odor coding in a model olfactory organ: the Drosophila maxillary palp. J. Neurosci. *19*, 4520–4532.

de Bruyne, M., Foster, K., and Carlson, J.R. (2001). Odor coding in the *Drosophila* antenna. Neuron *30*, 537–552.

Dobritsa, A.A., Van der Goes van Naters, W.M., Warr, C.G., Steinbrecht, R.A., and Carlson, J.R. (2003). Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron *30* (37), 827–841.

Elmore, T., Ignell, R., Carlson, J.R., and Smith, D.P. (2003). Targeted mutation of a Drosophila odor receptor defines receptor requirement in a novel class of sensillum. J. Neurosci. *23*, 9906–9912.

Fiala, A., Spall, T., Diegelmann, S., Eisermann, B., Sachse, S., Devaud, J.-M., Buchner, E., and Galizia, C.G. (2002). Genetically expressed cameleon in *Drosophila melanogaster* is used to visualize olfactory information in projection neurons. Curr. Biol. *12*, 1877–1884.

Fox, A.N., Pitts, R.J., and Zwiebel, L.J. (2002). A cluster of candidate odorant receptors from the malaria vector mosquito, Anopheles gambiae. Chem. Senses *27*, 453–459.

Friedrich, R.W. (2004). Neurobiology: odorant receptors make scents. Nature *430*, 511–512.

Gao, Q., and Chess, A. (1999). Identification of candidate Drosophila olfactory receptors from genomic DNA sequence. Genomics *60*, 31–39.

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

Goldman, A.L.,, Van der Goes van Naters, W., Lessing, D., Warr, C.G., and Carlson, J.R. (2005). Coexpression of two functional odor receptors in one neuron. Neuron *45*, 661–666.

Hallem, E.A., and Carlson, J. (2004). The odor coding system of Drosophila. Trends Genet. *20*, 453–459.

Hallem, E.A., Fox, A.N., Zwiebel, L.J., and Carlson, J.R. (2004a). Mosquito receptor for human-sweat odorant. Nature *427*, 212–213.

Hallem, E.A., Ho, M., and Carlson, J.R. (2004b). The molecular basis of odor coding in the *Drosophila* antenna. Cell *117*, 965–980.

Hansson, B.S., Carlsson, M.A., and Kalinova, B. (2003). Olfactory activation patterns in the antennal lobe of the sphinx moth, Manduca sexta. J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol. *189*, 301–308.

Heimbeck, G., Bugnon, V., Gendre, N., Haberlin, C., and Stocker, R.F. (1999). Smell and taste perception in Drosophila melanogaster larva: toxin expression studies in chemosensory neurons. J. Neurosci. *19*, 6599–6609.

Hertweck, H. (1931). Anatomie und Variabilitat des Nervensystems und der Sinnesorgane von *Drosophila melanogaster* (Meigen). Z. Wiss. Zool. *139*, 559–663.

Kajiya, K., Inaki, K., Tanaka, M., Haga, T., Kataoka, H., and Touhara, K. (2001). Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. J. Neurosci. *21*, 6018–6025.

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

Laurent, G., Stopfer, M., Friedrich, R.W., Rabinovich, M.I., Volkovskii, A., and Abarbanel, H.D. (2001). Odor encoding as an active, dynamical process: experiments, computation, and theory. Annu. Rev. Neurosci. *24*, 263–297.

Lei, H., Christensen, T.A., and Hildebrand, J.G. (2004). Spatial and temporal organization of ensemble representations for different odor classes in the moth antennal lobe. J. Neurosci. *24*, 11108–11119.

Malnic, B., Hirono, J., Sato, T., and Buck, L.B. (1999). Combinatorial receptor codes for odors. Cell *96*, 713–723.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Visualizing an olfactory sensory map. Cell *87*, 675–686.

Monte, P., Woodard, C., Ayer, R., Lilly, M., Sun, H., and Carlson, J. (1989). Characterization of the larval olfactory response in Drosophila and its genetic basis. Behav. Genet. *19*, 267–283.

Neuhaus, E.M., Gisselmann, G., Zhang, W., Dooley, R., Stortkuhl, K., and Hatt, H. (2005). Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. Nat. Neurosci. *8*, 15–17.

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

Oppliger, F.Y., Guerin, P.M., and Vlimant, M. (2000). Neurophysiological and behavioral evidence for an olfactory function for the dorsal organ and a gustatory one for the terminal organ in Drosophila melanogaster larvae. J. Insect Physiol. *46*, 135–144.

Park, Y., Caldwell, M.C., and Datta, S. (1997). Mutation of the central nervous system neuroblast proliferation repressor *ana* leads to defects in larval olfactory behavior. J. Neurobiol. *33*, 199–211.

Python, F., and Stocker, R. (2002). Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. J. Comp. Neurol. *445*, 374–387.

Ressler, K.J., Sullivan, S.L., and Buck, L.B. (1994). Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell *79*, 1245–1255.

Robertson, H.M., Warr, C.G., and Carlson, J.R. (2003). Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *100*, 14537–14542.

Rodrigues, V. (1980). Olfactory Behavior of Drosophila melanogaster (New York: Plenum).

Rodrigues, V. (1988). Spatial coding of olfactory information in the antennal lobe of Drosophila melanogaster. Brain Res. *453*, 299–307.

Scott, K., Brady, R., Jr., Cravchik, A., Morosov, P.S., Rzhetsky, A., Zuker, C., and Axel, R. (2001). A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell *104*, 661–673.

Siddiqi, O. (1983). Olfactory Neurogenetics of Drosophila (New Delhi, Bombay, Calcutta: Oxford and IBH).

Singh, R.N., and Singh, K. (1984). Fine structure of the sensory organs of *Drosophila melanogaster* meigen larva (Diptera: Drosophilidae). Int. J. Insect Morphol. Embryol. *13*, 255–273.

Stocker, R. (1994). The organization of the chemosensory system in Drosophila melanogaster: a review. Cell Tissue Res. *275*, 3–26.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A., and Bargmann, C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell *83*, 207–218.

Uchida, N., Takahashi, Y.K., Tanifuji, M., and Mori, K. (2000). Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. Nat. Neurosci. *3*, 1035–1043.

Ueno, K., Ohta, M., Morita, H., Mikuni, Y., Nakajima, S., Yamamoto, K., and Isono, K. (2001). Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene *Gr5a*. Curr. Biol. *11*, 1451–1455.

Vassar, R., Chao, S.K., Sitcheran, R., Nunez, J.M., Vosshall, L.B., and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. Cell *79*, 981–991.

Vosshall, L.B. (2001). The molecular logic of olfaction in *Drosophila*. Chem. Senses *26*, 207–213.

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

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

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