

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

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Summary

We have undertaken a functional analysis of the odorant receptor repertoire in the *Drosophila* antenna. Each receptor was expressed in a mutant olfactory receptor neuron (ORN) used as a “decoder,” and the odor response spectrum conferred by the receptor was determined *in vivo* by electrophysiological recordings. The spectra of these receptors were then matched to those of defined ORNs to establish a receptor-to-neuron map. In addition to the odor response spectrum, the receptors dictate the signaling mode, *i.e.*, excitation or inhibition, and the response dynamics of the neuron. An individual receptor can mediate both excitatory and inhibitory responses to different odorants in the same cell, suggesting a model of odorant receptor transduction. Receptors vary widely in their breadth of tuning, and odorants vary widely in the number of receptors they activate. Together, these properties provide a molecular basis for odor coding by the receptor repertoire of an olfactory organ.

Introduction

Olfactory systems of animals from insects to humans are able to detect and distinguish among a diverse array of odors (Hildebrand and Shepherd, 1997). This ability is crucial for the identification of food, mates, and predators. An understanding of the basic principles of odor coding is necessary for an understanding of olfactory-mediated behavior.

Odor information is first received by ORNs, located in the peripheral olfactory organs. ORNs respond to odors with a sequence of action potentials that reflects the quality, intensity, and temporal structure of the odor stimulus (Heinbockel and Kaissling, 1996; de Bruyne et al., 1999, 2001; Duchamp-Viret et al., 1999; Nikonov and Leal, 2002). The generation of action potentials by ORNs leads to the activation of second-order neurons in the brain (Hildebrand, 1996; Galizia et al., 1999; Mori et al., 1999; Vickers et al., 2001; Ng et al., 2002; Wang et al., 2003; Wilson et al., 2004).

Odorant receptors are predicted seven transmembrane domain G protein-coupled receptors encoded by large and diverse gene families. In both mammals and insects, different odorant receptor genes are expressed in different subsets of ORNs, and the axons of ORNs expressing the same gene converge onto one or a few

spheroidal modules, called glomeruli, in the brain (Resler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Gao et al., 2000; Vosshall et al., 2000). While there have been a number of descriptive studies of odorant receptor gene expression, there have been few functional studies of the receptors, either individually or collectively. The first odorant receptor to be functionally characterized was the *C. elegans* receptor *odr-10*, for which diacetyl was identified as a ligand (Sengupta et al., 1996). Odor response spectra of only a limited number of receptors have been characterized subsequently, in large part because expression in heterologous systems has proved difficult.

The fruit fly *Drosophila melanogaster* has two olfactory organs, the antenna and maxillary palp, which contain ~1200 and ~120 ORNs, respectively (Stocker, 1994; Shanbhag et al., 1999). ORNs are compartmentalized into sensilla, which can be subdivided into three major morphological types: basiconic, coeloconic, and trichoid. Each sensillum contains the dendrites of up to four ORNs.

Drosophila ORNs can be subdivided into distinct functional classes on the basis of their odor response spectra. Extensive electrophysiological characterization of the antennal basiconic sensilla identified 18 functional classes of ORNs, which are found in stereotyped combinations within eight types of sensilla (de Bruyne et al., 2001; Elmore et al., 2003). These ORNs show diverse responses to odorants: not only do they exhibit distinct odor response spectra but they can show excitatory or inhibitory responses, and they show different response dynamics (de Bruyne et al., 2001). A critical problem in sensory physiology is to elucidate the molecular basis of this diversity of response.

The odorant receptor (*Or*) genes in *Drosophila* are a highly diverse family of ~60 genes (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999; Robertson et al., 2003). Two of them, *Or22a* and *Or22b*, were recently characterized in detail and were shown to be coexpressed specifically in the ab3A antennal neuron (Dobritsa et al., 2003). A deletion mutant called $\Delta halo$ that lacks these receptor genes suffers loss of odorant response in the ab3A neuron (Dobritsa et al., 2003). This mutant ab3A neuron was then used to characterize another odorant receptor, *Or47a*, by introducing it into the mutant neuron and recording the electrophysiological response to odorants.

Here we provide a systematic functional analysis of the antennal repertoire of *Or* genes, with the goal of elucidating the molecular basis of odor coding in an entire olfactory organ. We use the mutant ab3A neuron as a decoder to characterize the odor response spectrum of each receptor. The results establish a receptor-to-neuron map that underlies sensory coding in the *Drosophila* antenna, the first such olfactory map of its kind. We find that the receptor dictates many of the diverse ORN properties that serve as the foundation of the odor code, suggesting a model for odorant receptor transduction. Finally, we consider the coding of odorants across the antennal repertoire of *Or* receptors. We find

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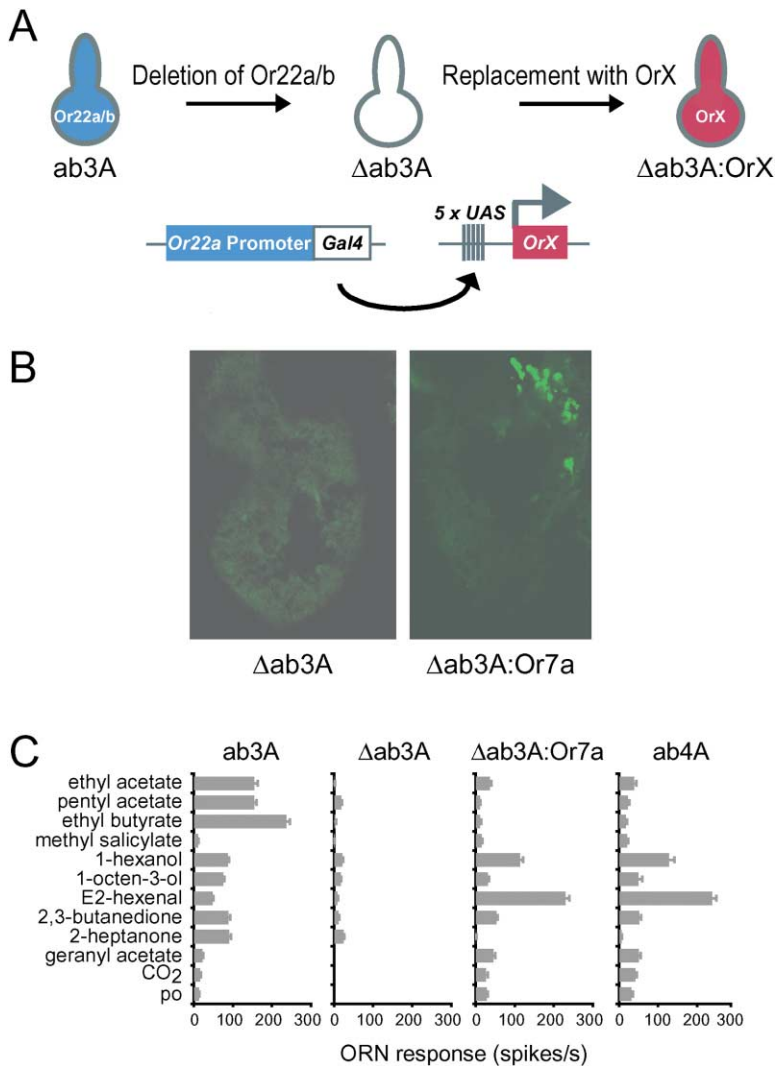


Figure 1. Analysis of Odor Response Spectra of Individual Odorant Receptors

(A) A mutant ab3A antennal neuron (Δ ab3A) lacks odor response due to the deletion of its endogenous receptor genes, *Or22a* and *Or22b*. Odorant receptors are introduced specifically into Δ ab3A using the *GAL4/UAS* system. *Or22a-GAL4* is used to drive expression from a *UAS-Or* construct. The odorant response of the neuron (Δ ab3A:OrX) is assayed electrophysiologically.

(B) Expression of Or7a in Δ ab3A. Fluorescent immunolabeling of antennal sections with an anti-myc antibody labels a subset of dorsomedial sensilla where ab3A dendrites are located. The antenna on the left contains Δ ab3A neurons (*w*; Δ halo). The antenna on the right contains Δ ab3A:Or7a neurons (*w*; Δ halo; *Or22a-GAL4/UAS-Or7a*).

(C) Odor response spectrum of control ab3A (first panel, *w*), Δ ab3A (second panel, *w*; Δ halo), Δ ab3A:Or7a (third panel, *w*; Δ halo; *Or22a-GAL4/UAS-Or7a*), and ab4A (fourth panel, *Canton-S*) neurons. For all graphs, *n* = 12.

that receptors vary markedly in their tuning breadth with respect to a panel of diverse odorants and that odorants vary markedly in the number of receptors that they activate strongly. More receptors are activated at higher odorant concentrations, providing a molecular basis for intensity coding.

Results

A Receptor-to-Neuron Map of the *Drosophila* Antenna

We examined the odor response spectra of individual odorant receptors using the *in vivo* expression system based on the Δ halo mutant. Individual odorant receptors were expressed specifically in the ab3A neuron of the Δ halo mutant, designated as Δ ab3A, by using an *Or22a-GAL4* construct to drive expression of *UAS-Or* constructs (Figure 1A). The *UAS-Or* constructs contained an N-terminal myc tag, allowing expression to be verified (Figure 1B). The odor response spectrum of the Δ ab3A neuron expressing each receptor, designated as Δ ab3A:OrX, was analyzed by single-unit electrophysiology using a panel of 11 diagnostic odorants that we have used in previous

studies (de Bruyne et al., 2001; Dobritsa et al., 2003) and that was selected from a larger panel of ~50 odorants based on its ability to identify and distinguish among neuronal classes (de Bruyne et al., 2001). We then compared the odor response spectra of Δ ab3A:OrX neurons to the odor response spectra of defined wild-type ORNs to determine whether any of the spectra matched (example shown in Figure 1C).

Of the 32 *Or* genes that were reported to be expressed in the antenna in an *in situ* hybridization study (Vosshall et al., 2000), a total of 31 odorant receptor genes have been expressed in this manner (including three, *Or22a*, *Or22b*, and *Or47a*, that were analyzed previously [Dobritsa et al., 2003]). Of these, 24 receptor genes engendered odorant responses, each with a distinct response spectrum. Of these 24, the profiles of 13 closely resembled the profile of an identified ORN (Figure 2). The simplest interpretation of these results is that, in each of these cases, the receptor derives from the matching ORN and that each of these receptors accounts for the full response spectrum of the corresponding ORN. To test this interpretation more stringently, we expanded the odorant panel to include additional odorants that

elicit a response from the wild-type ORNs. We found that the response of $\Delta ab3A:OrX$ was in each case similar to that of the corresponding wild-type ORN. These results confirm the fidelity of the mapping and are consistent with a model in which a single receptor defines the response profile of an ORN.

To further confirm the apparent matches between receptors and ORNs, we represented the tuning of the 24 receptors and the 13 ORNs as vectors in 10-dimensional space, with the dimensions of the space corresponding to the response magnitudes for the 10 volatile test odors. We then compared the vectors with an angular similarity measure and performed a cluster analysis. In each case, the receptor-ORN pairs shown in Figure 2 formed distinct clusters, with the one exception of the *ab2B* neuron, which formed a cluster with both *Or85a* and *Or67c* (data not shown). However, a match between *ab2B* and *Or85a* was unambiguously established using additional odors—for example, both *ab2B* and *Or85a* responded very strongly to a 10^{-4} dilution of ethyl-3-hydroxybutyrate, while *Or67c* responded only weakly to a 10^{-2} dilution of the same odorant. Thus we were able to establish a map between the 13 receptors and corresponding ORNs shown in Figure 2A.

The spectra of the other receptors are shown in Figure 3. These receptors are likely to map to ORNs that have not yet been characterized. While an extensive survey of trichoid and coeloconic ORNs is not yet available, limited recordings identified at least two functional classes each of coeloconic and trichoid ORNs (Clyne et al., 1997). Among the unmapped receptors are several—constituting $\sim 20\%$ of the total number of receptors in Figures 2 and 3—that showed little response to any tested odorant. This fraction corresponds to the fraction of projection neurons (PNs), the second-order neurons with which ORNs form synapses in the antennal lobe, that were unresponsive to any tested odorant in a physiological study and that were hypothesized to respond to specific compounds such as pheromones (Wilson et al., 2004).

These results establish a receptor-to-neuron map for the *Drosophila* antenna (Figure 2B) and support the hypothesis that the odorant receptor is the primary determinant of one ORN response property, the odor response spectrum. We next examined the contribution of the odorant receptor to other functional properties of ORNs.

Spontaneous Firing Rate of ORNs Is Determined by the Odorant Receptor

ORNs fire spontaneous action potentials in the absence of odorant stimulation. *Drosophila* ORNs vary in their rates of spontaneous activity, with previously reported rates ranging from ~ 1 spike/s to ~ 30 spikes/s (de Bruyne et al., 1999, 2001). Are the spontaneous firing rates of ORNs determined by the receptors they express or by other factors, such as the population of ion channels they express?

Recordings from $\Delta ab3A:OrX$ neurons revealed that spontaneous firing rates differed when different receptors were expressed and that these rates corresponded well with the spontaneous firing rates of the wild-type ORNs from which the receptors were derived. This cor-

respondence was then examined in detail for four different $\Delta ab3A:OrX$ neurons and their wild-type ORN counterparts (Figure 4A). In each case, we found that the spontaneous firing rates of $\Delta ab3A:OrX$ neurons and corresponding wild-type neurons were similar. For example, expression of *Or47b* in the $\Delta ab3A$ neuron increased the spontaneous firing rate to a high level comparable to that of a wild-type *atXA* neuron (“*atXA*” and “*acXB*” designations are tentative pending publication of a formalized nomenclature for trichoid and coeloconic neurons by W. van der Goes van Naters and J.C., and by R. Ignell and J.C., respectively [unpublished data]), from which *Or47b* derives, while expression of *Or7a* produced a lower level of spontaneous firing comparable to that of the wild-type *ab4A* neuron, from which *Or7a* derives. These results demonstrate that the spontaneous firing rate of ORNs is determined by the odorant receptor.

Signaling Mode of ORNs Is Determined by the Odorant Receptor

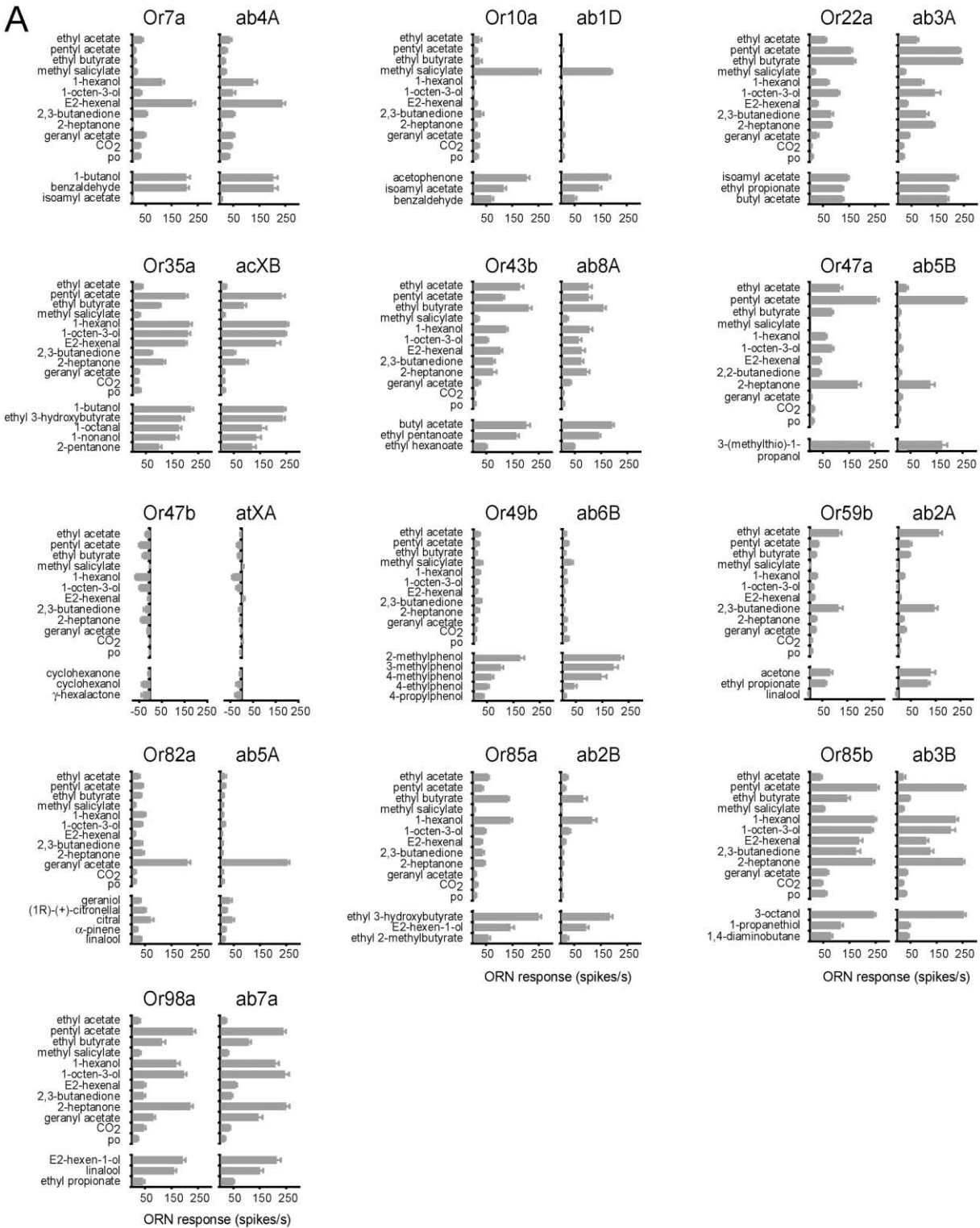
Two modes of olfactory signaling, excitation and inhibition, are used by ORNs in the coding of olfactory information (Duchamp-Viret et al., 1999, 2000; de Bruyne et al., 1999, 2001; Nikonov and Leal, 2002; Shields and Hildebrand, 2001). Most ORNs are capable of generating excitatory responses in which the action potential frequency increases following the onset of odorant stimulation. Some ORNs generate inhibitory responses in which the action potential frequency decreases below the level of spontaneous firing following the onset of odorant stimulation. The *ab3A* ORN falls into the former category in that it shows excitation to a wide variety of odors, but inhibition has not been observed (de Bruyne et al., 2001).

The mechanism specifying signaling mode in antennal ORNs is unknown. One possibility is that signaling mode is an inherent property of the ORN that is determined by the signal transduction machinery within the ORN. Another possibility is that ORNs are capable of supporting multiple signaling modes and that signaling mode is determined by the receptor.

We found that, although the wild-type *ab3A* neuron has been observed only to generate excitatory responses, expression of another receptor in the $\Delta ab3A$ neuron can lead to inhibitory responses. For example, the $\Delta ab3A:Or47b$ neuron is inhibited by 1-hexanol, and the $\Delta ab3A:Or59b$ neuron is inhibited by linalool (Figure 4B). The finding that other receptors confer excitatory responses to these odors in the $\Delta ab3A$ cell (Figure 4C) confirms that the inhibitory responses are specific to these receptors and do not represent a nonspecific toxic effect of the odors on *ab3A*. The simplest interpretation of these results is that ORN signaling mode is determined by the receptor and that an ORN that normally generates only excitatory responses can generate inhibitory responses when ectopically expressing a different receptor.

A Single Odorant Receptor Can Mediate Both Excitatory and Inhibitory Responses

The ability of an odorant receptor to determine the signaling mode of an ORN raises a fundamental question regarding the molecular basis of odor coding. Certain



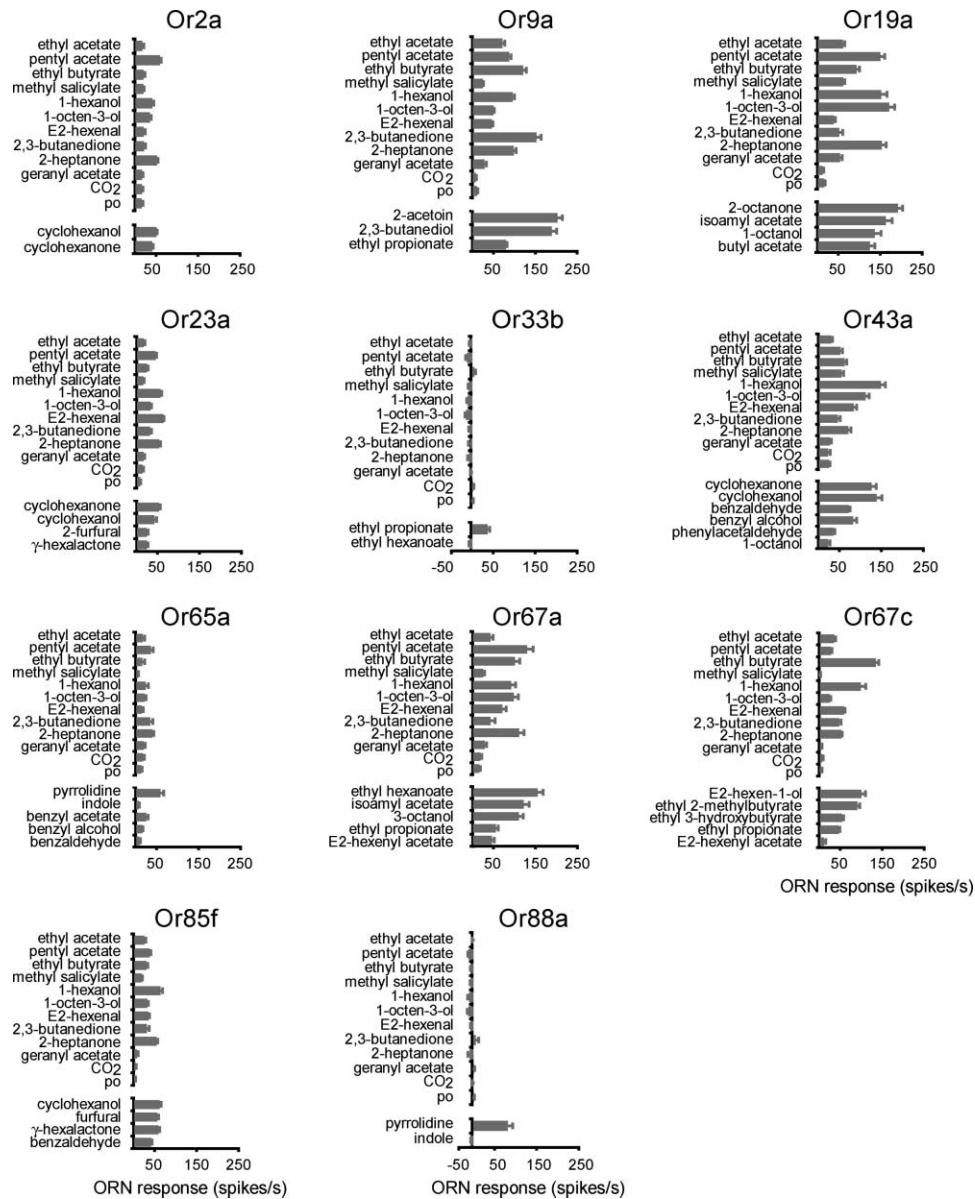


Figure 3. Odor Response Spectra of Receptors for which Corresponding Wild-Type ORNs Have Not Been Identified

Odorants indicated at the bottom of each panel were included to demonstrate receptor specificity or as examples of additional ligands. For flies expressing *Or2a* and *Or33b*, some variability in response magnitudes was observed between independent transgenic lines. For all graphs, $n = 12$.

ORNs have been observed to exhibit excitatory responses to some odorants and inhibitory responses to others (de Bruyne et al., 1999, 2001; Duchamp-Viret et al., 1999, 2000; Nikonov and Leal, 2002; Shields and Hildebrand, 2001). Do these ORNs express two recep-

tors, one excitatory and the other inhibitory, or a single receptor that is capable of mediating responses of differing modes to different odorants?

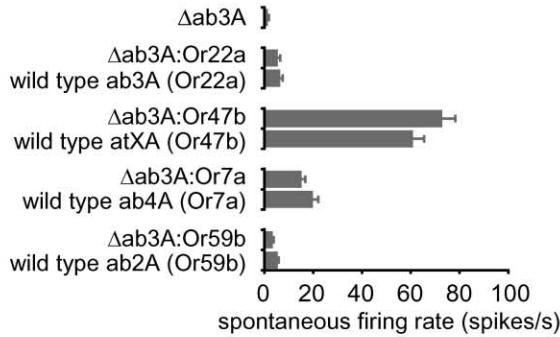
We found cases in which an individual receptor confers excitatory responses to some odorants and inhibi-

Figure 2. Establishment of a Receptor-to-Neuron Map for the *Drosophila* Antenna

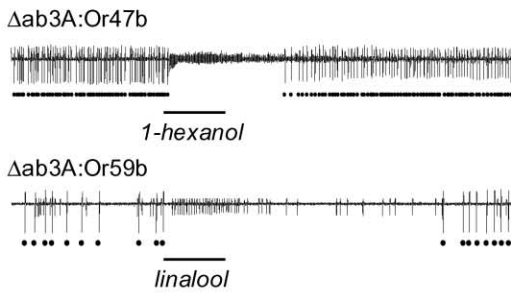
(A) Odor response spectra conferred by individual odorant receptors (left panels, *w; Δhalo; Or22a-GAL4/UAS-OrX*) and response spectra of corresponding wild-type ORNs (right panels, *Canton-S*). In each case, an initial correspondence was established with a panel of odorants (12 stimuli); mappings were then confirmed with additional odorants, indicated at the bottom of each panel. Odorants were diluted "10⁻²ⁿ" (see text), with the exception of ethyl-3-hydroxybutyrate in the Or85a and ab2B graphs, which was diluted 10⁻⁴ due to difficulty in quantifying the high response obtained at 10⁻². $n = 12$ for all graphs except the atXA panel, where $n = 7$.

(B) A receptor-to-neuron map.

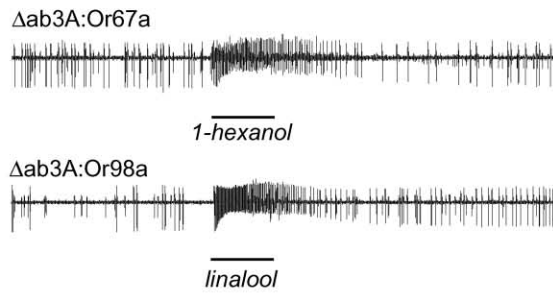
A Spontaneous Activity



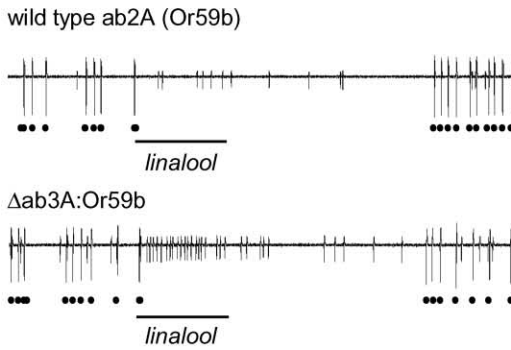
B Inhibition



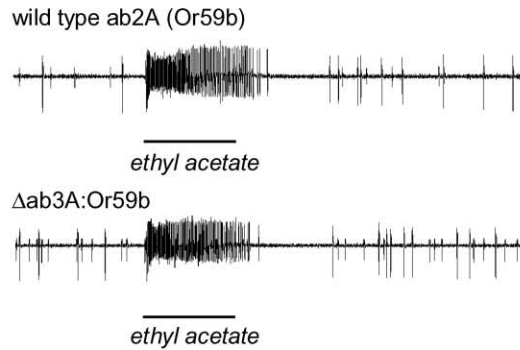
C Excitation



D Inhibition



Excitation



E Coexpression of Inhibitory and Excitatory Receptors

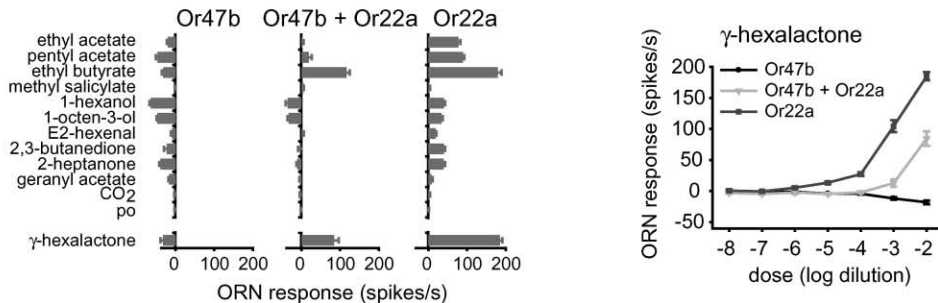


Figure 4. Spontaneous Firing Rate and Signaling Mode of ORNs Are Determined by the Odorant Receptor

(A) Spontaneous firing rates of $\Delta ab3A:OrX$ neurons (*w*; $\Delta halo$; *Or22a-GAL4/UAS-Or*) and corresponding wild-type ORNs (*Canton-S*). Rates were quantified from the number of spikes in one second of spontaneous activity. *n* = 12. (B) Inhibitory responses of $\Delta ab3A:OrX$ neurons (large spikes; positions indicated by dots). The excitatory response of the ab3B neuron, which resides in the same sensillum, is also visible (small spikes). (C) Excitatory responses of $\Delta ab3A:OrY$ neurons (large spikes) stimulated with the same odorants that evoked inhibition in different, $\Delta ab3A:OrX$ neurons in (B). (D) A single odorant receptor confers inhibitory responses to some odorants and excitatory responses

tory responses to others. For example, we have mapped Or59b to ab2A, a neuron that was previously shown to generate inhibitory responses to linalool and excitatory responses to ethyl acetate (de Bruyne et al., 2001; Figure 4D). When expressed in Δ ab3A, Or59b confers inhibition by linalool and excitation by ethyl acetate, thus accounting for both the inhibitory and excitatory responses of ab2A (Figure 4D). These results support a model in which responses of both signaling modes are conferred in a single neuron by a single odorant receptor.

We also asked whether an excitatory receptor and an inhibitory receptor could function together in the same cell to generate excitatory responses to some odorants and inhibitory responses to others. We compared the odor response spectra of three cells: Δ ab3A:Or47b, in which Or47b confers an inhibitory response to the mutant Δ ab3A cell; ab3A, the wild-type ab3A neuron, which shows an excitatory response to certain odorants due to endogenous expression of the Or22a receptor; and ab3A:Or47b, in which the inhibitory Or47b receptor is expressed in an ab3A cell that also expresses the endogenous excitatory Or22a (Figure 4E). We found that the response of the ab3A:Or47b neuron (Or47b + Or22a) is intermediate between that of the wild-type ab3A neuron (Or22a alone) and that of Δ ab3A:Or47b (Or47b alone) in the case of all odorants that are common ligands for the two receptors (Figure 4E, left). The finding of an intermediate response was further supported by dose-response curves generated for γ -hexalactone (Figure 4E, right), which generated strong responses from Or22a and Or47b when tested individually. Thus, two receptors with different signaling modes can function simultaneously in the ab3A neuron to generate both excitatory and inhibitory responses, and the cell is capable of integrating their responses.

Response Termination Rate Is Determined by the Odorant Receptor

The rate of response termination varies for different ORNs and different odorants: a given odorant can elicit a long-lasting response from some ORNs and an abruptly terminating response from others, while the same ORN can generate a long-lasting response to some odorants and an abruptly terminating response to others (Kaissling et al., 1989; Heinbockel and Kaissling, 1996; de Bruyne et al., 1999, 2001; Duchamp-Viret et al., 2000; Shields and Hildebrand, 2001). The mechanisms underlying differences in the dynamics of response termination are unknown. These differences could be attributable to differences among odorant receptors, or they could be attributable to differences in the populations of molecules that interact with receptors, such as arrestins or odorant binding proteins (OBPs).

We observed that the poststimulus firing rate of the Δ ab3A:OrX neuron was similar to that of the wild-type ORN from which OrX is derived (Figure 5A). We quantitated the termination dynamics in detail in the cases of six receptors by measuring the poststimulus firing rate 1 s after the beginning of the 0.5 s odorant stimulus. All responses were examined using multiple odorants. We found that, for each receptor, the poststimulus firing rate, as well as the initial firing rate, of the Δ ab3A:OrX neuron was similar to that of the wild-type neuron expressing OrX (Figure 5B).

In the case of two receptors, we extended the analysis by measuring the decline in firing rate throughout the 2 s period following the onset of the odorant stimulus for each of three odorants. We found that the rate of decline of the response was similar in Δ ab3A:OrX neurons and corresponding wild-type neurons (Figure 5C). These results suggest that response termination is determined primarily by the odorant receptor rather than the cellular environment in which the receptor operates.

Extracellular Spike Amplitude of ORNs Is Independent of the Odorant Receptor

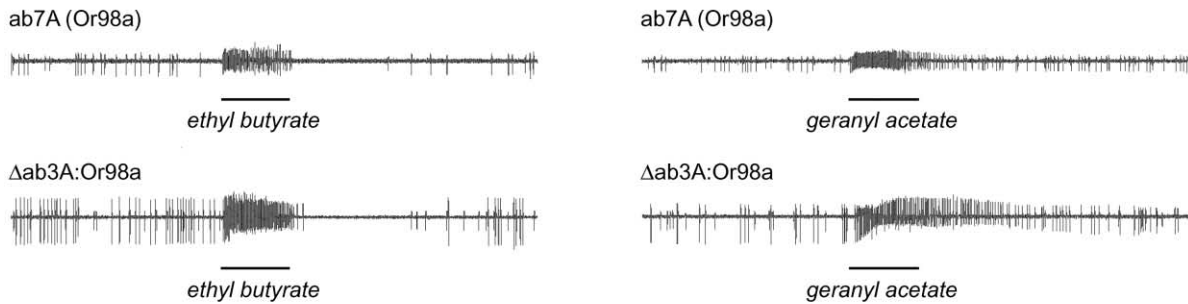
Different functional classes of ORNs have different action potential amplitudes as measured in extracellular single-unit recordings. These amplitudes are consistent among neurons of the same functional class and do not depend on the location of the recording electrode within the sensillum (de Bruyne et al., 2001). We observed in this study that, regardless of which receptor we expressed in the Δ ab3A neuron, the extracellular spike amplitude appeared invariant. To substantiate this observation quantitatively, we measured spike amplitudes of the Δ ab3A:OrX neuron expressing each of four different receptors as well as spike amplitudes of the neurons from which these receptors are derived. We found that spike amplitudes of Δ ab3A:OrX neurons were similar for all OrX, even in cases where OrX was derived from a neuron with an amplitude smaller than that of ab3A (Figure 5D). Moreover, there was no difference between ab3A and Δ ab3A in spike amplitude. These results show that extracellular spike amplitude is a property of the neuron that is independent of the odorant receptor it expresses, suggesting that it may depend on factors such as ion channel composition of the cell membrane or cell morphology.

Antennal Odorant Receptors Can Function in the Maxillary Palp

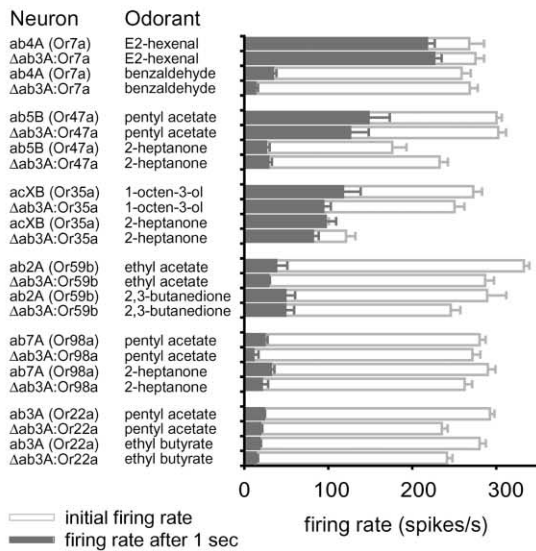
We have shown above (e.g., Figures 2 and 3) that multiple receptors are capable of functioning normally in an individual ORN, ab3A. Are individual receptors capable of functioning normally in multiple neurons? We asked

to others. Both the wild-type ab2A neuron (upper traces, *Canton-S*), which expresses Or59b, and the Δ ab3A:Or59b neuron (lower traces, *w*; Δ halo; Or22a-GAL4/UAS-Or59b) show inhibition to linalool (large spikes in left traces; positions indicated by dots) and excitation to ethyl acetate (right traces). (E) A receptor that confers excitation and a receptor that confers inhibition can function simultaneously in the same neuron. Odorant responses of the ab3A neuron expressing both Or22a and Or47b (center graph of left panel, *w*; +/+; Or22a-GAL4/UAS-Or47b) are intermediate between those of the Δ ab3A:Or47b neuron (left graph, *w*; Δ halo; Or22a-GAL4/UAS-Or47b) and those of the wild-type ab3A neuron, which expresses Or22a (right graph, *w*). Responses to γ -hexalactone across a range of odorant concentrations show intermediate responses of neurons expressing both receptors (right panel). Data in the leftmost bar graph (Or47b) were taken from Figure 2. For all graphs, $n = 12$.

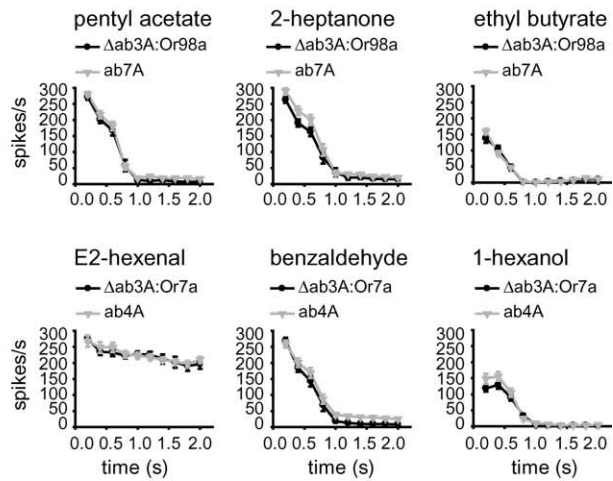
A Response Dynamics



B Response Termination



C Response Dynamics



D Extracellular Spike Amplitude

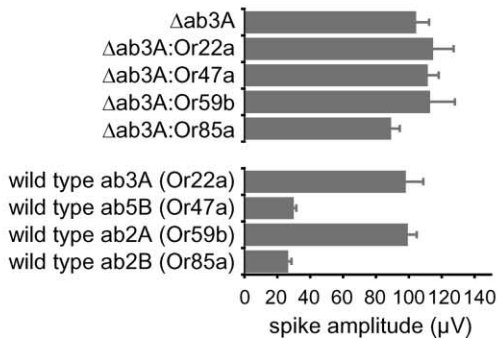


Figure 5. The Receptor Determines Response Dynamics but Not Extracellular Spike Amplitude

(A) Recordings from wild-type ab7A (large spikes in top traces, *Canton-S*), which expresses Or98a, and $\Delta ab3A:Or98a$ (large spikes in lower traces) showing similar response dynamics. Both ab7A and $\Delta ab3A:Or98a$ show abruptly terminating responses to ethyl butyrate that are followed by a quiescent period (left traces) but prolonged responses to geranyl acetate (right traces).

(B) Response termination in $\Delta ab3A:OrX$ neurons and corresponding wild-type ORNs. Response termination was assayed as the firing rate during the 200 ms bin that begins 1 s after the onset of the odorant stimulus (dark gray bars). Initial firing rates, representing the 200 ms bin at the onset of the odorant stimulus, are shown for comparison (light gray outlines). $n = 12$.

(C) Response dynamics of $\Delta ab3A:OrX$ neurons and corresponding wild-type ORNs. Response dynamics were assayed as firing rates in consecutive 200 ms bins, beginning at the onset of the odorant stimulus and continuing for 2 s. The end of each 200 ms bin is indicated by the time points on the x axis. “0.0” indicates the onset of the odor stimulus. For all graphs, $n = 12$.

(D) Spike amplitudes of $\Delta ab3A$ and $\Delta ab3A:OrX$ neurons were similar to that of the wild-type ab3A neuron. Spike amplitudes were quantified by measuring the amplitude of 10 spikes from each of 12 recordings of spontaneous activity. $n = 12$; each of the 12 values was the mean of 10 measurements.

whether two antennal receptors, Or7a and Or47b, were able to function in neurons of the other olfactory organ of the fly, the maxillary palp. Expression was driven by *Or83b-GAL4*, which drives expression in the majority of ORNs on the antenna and maxillary palp (Vosshall et al., 1999; Ng et al., 2002). As an initial means of investigating whether these antennal receptors could be functionally expressed in the maxillary palp, we recorded electropalpograms (EPGs), a simple means of recording from multiple cell types simultaneously: the EPG is believed to reflect the summed receptor potentials of all ORNs in the vicinity of the recording electrode (Ayer and Carlson, 1992). We then measured the responses of individual ORNs with single-unit electrophysiology.

Expression of Or7a in the maxillary palp yielded a large and specific increase in EPG response to E2-hexenal (Figures 6A and 6B), consistent with our identification of E2-hexenal as a ligand for this receptor in the antenna. Likewise, expression of Or47b resulted in decreased EPG responses to most odorants but most strongly to 1-hexanol and 1-octen-3-ol, where the polarity of response frequently reversed (Figures 6A and 6B). Differences in EPG responses were apparent across a wide range of concentrations (Figure 6C) and were consistent with odor response spectra obtained by single-unit analysis (Figure 2).

Single-unit recordings confirmed the functional expression of both receptors in all six functional classes of maxillary palp ORNs (de Bruyne et al., 1999): pb1A, pb1B, pb2A, pb2B, pb3A, and pb3B. The results of ectopic expression in one neuronal class, pb3A, are shown in Figure 6D. We note that functional expression in the pb1B, pb2B, and pb3B ORNs was observed in some but not all sensilla, perhaps due to variability in expression driven by the *Or83b-GAL4* driver (data not shown). Similar overexpression phenotypes were observed in the antenna by EAG analysis as well as by single-unit analysis of at least four different neuron classes in limited recordings (data not shown). Taken together, these results show that antennal odorant receptors are capable of functioning when expressed ectopically in multiple neurons, including those of a different olfactory organ.

Odor Coding by the Antennal Repertoire of Odorant Receptors

Of 31 antennal odorant receptors, 24 engendered odorant responses when expressed in the Δ ab3A neuron (Figure 7A). These 24 receptors comprise most of the odorant receptors of an entire olfactory organ, the *Drosophila* antenna, and thus allowed us to consider the molecular basis of odor coding in terms of the system at large.

We examined the functional diversity of this large receptor repertoire and observed three underlying principles of receptor coding in this system. First, the odor response spectra of all 24 odorant receptors are distinct (Figures 2 and 3). Second, different receptors vary in their breadth of tuning with respect to the odorant panel. Some receptors responded strongly to only one test odorant, e.g., Or82a to geranyl acetate, while others, e.g., Or85b, responded strongly to many of the odorants (Figure 2). Specifically, if a strong response is defined for convenience as >100 spikes/s, which corresponds

to $\sim 40\%$ of the maximum odorant response observed in this study, then 16 of the 24 functional antennal receptors responded strongly to at least one of the diagnostic volatile odorants (Figures 7A and 7B). Eight of these 16 receptors responded strongly to only one or two of these odorants, while eight responded strongly to between three and seven odorants (Figures 7A and 7B). Third, many odorant receptors responded strongly to common ligands, demonstrating that substantial functional overlap exists among the entire population of antennal odorant receptors (Figures 7A and 7B).

We next considered principles of receptor coding from the point of view of the odors that are encoded. First, we considered the coding of odorant identity. We found that different odorants strongly activate different numbers of receptors. Some odorants strongly activated a small number of receptors. For example, geranyl acetate and methyl salicylate strongly activated only one receptor in this repertoire (Figure 7B, right). By contrast, other odorants elicited strong responses from $\sim 1/3$ of the antennal odorant receptors: ethyl butyrate strongly activated nine receptors, while pentyl acetate strongly activated eight. For those odorants that are sensed by multiple receptors, the receptors are distributed widely through the genome (Figure 7A; receptors are named according to cytogenetic map position).

To determine how odorant concentration might be encoded in the activity of the entire receptor repertoire, we then compared responses to 10^{-2} , 10^{-4} , 10^{-6} , and 10^{-8} dilutions of odorant, choosing for this analysis all diagnostic odorants that elicited strong responses from five or more receptors at a 10^{-2} dilution (Figure 7C). We found that, for some odorants, the number of strongly responding receptors decreased sharply at lower odorant concentrations (Figure 7C). For example, of the six receptors that were strongly activated by 1-octen-3-ol at a 10^{-2} dilution, none were strongly activated by 1-octen-3-ol at a 10^{-4} dilution. By contrast, for pentyl acetate, three of the eight receptors that responded strongly at a 10^{-2} dilution still responded strongly at a 10^{-4} dilution. Thus, the number of strongly responding receptors decreased as a function of decreasing odorant concentration but by differing degrees.

Discussion

A Receptor-to-Neuron Map for the *Drosophila* Antenna

We have mapped odorant receptors to the ORNs from which they derive using the Δ ab3A neuron as an *in vivo* expression system (Figure 2). For the ORNs to which a receptor has been mapped, a single odorant receptor appears sufficient to account for the complete odor response spectrum of the ORN. The simplest interpretation of these data is that these *Drosophila* ORNs express a single functional odorant receptor. Thus, this study provides functional data consistent with the "one receptor-one ORN" model proposed for mammalian ORNs on the basis of molecular expression studies (Ressler et al., 1993; Vassar et al., 1993; Malnic et al., 1999). Moreover, the ability of these odorant receptors to confer the odor response spectrum of a "donor" ORN upon the recipient Δ ab3A neuron indicates that the receptors are

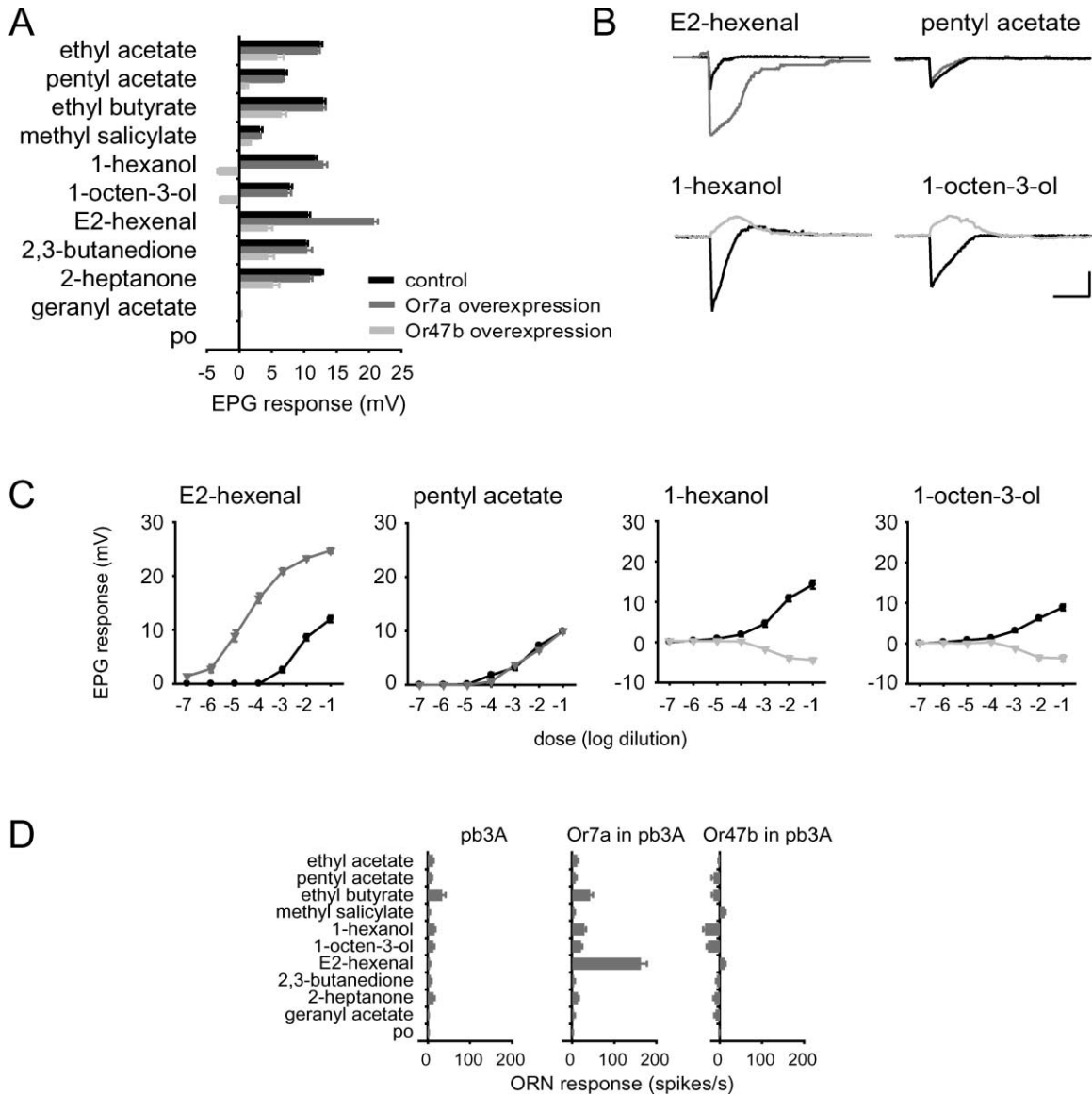


Figure 6. Overexpression of Antennal Receptors in the Maxillary Palp

(A) EPG responses of control (black bars, w), Or7a-overexpressing (dark gray bars, w; *UAS-Or7a/+; Or83b-GAL4/+*), and Or47b-overexpressing (light gray bars, w; *+/+; UAS-Or47b/Or83b-GAL4*) flies. n = 10. Bar shadings also apply to graphs in (B) and (C).

(B) Sample EPG traces of control flies and of flies overexpressing either Or7a or Or47b. For these traces, 1-hexanol and 1-octen-3-ol were diluted 10^{-1} in paraffin oil. Vertical scale bar represents 5 mV; horizontal scale bar represents 2 s.

(C) EPG dose-response curves. n = 10.

(D) Odor response spectra of pb3A neurons from control, Or7a-overexpressing, and Or47b-overexpressing flies. n = 12.

the primary determinants of the odor response spectrum. These receptors do not appear to require neuron-specific or sensillum-specific perireceptor molecules in order to confer the odor response spectrum.

Eleven of the receptors we expressed conferred odor response spectra that did not match those of identified ORNs. Not all antennal ORNs have been characterized, e.g., only a limited survey of ORNs in trichoid sensilla is currently available (Clyne et al., 1997). It seems likely that most of these 11 unmapped receptors derive from ORNs that have not yet been defined. At the same time, a number of defined ORN classes, such as ab1A, have not been matched to a receptor. One possibility is that

these ORNs express *Or* genes whose expression was not initially detected in the antenna and that have not been tested in our study. Alternatively, some could express *Gr* genes (Clyne et al., 2000), many of which are expressed in taste organs, where some have been functionally implicated in taste (Chyb et al., 2003; Dahanukar et al., 2001) or pheromone perception (Bray and Amrein, 2003) but at least three of which are expressed in the antenna (Scott et al., 2001). Another formal possibility is that some of the unmapped receptors in fact derive from unmatched ORNs but act in pairs in these ORNs or in conjunction with perireceptor molecules that are not available to receptors expressed in ab3A. Several

Or genes did not confer detectable odorant sensitivity upon the $\Delta ab3A$ neuron. These receptors could be non-functional in vivo, or they could respond specifically to a ligand not present in our odorant panel, such as a pheromone. Finally, two of the receptors we analyzed, Or43a and Or43b, have also been functionally characterized by others using different approaches. Our results are similar to those reported previously, with only a few exceptions (Storkuhl and Kettler, 2001; Wetzel et al., 2001; Elmore et al., 2003; Wang et al., 2003).

The receptor-to-neuron map we have provided in this study does not reveal a simple logic relating the ORN and the receptor that it expresses. For example, adjacent ORNs do not consistently express receptors encoded by adjacent genes (Figure 2B). Although some pairs of neurons (such as ab5A, ab5B and ab2A, ab2B) express receptors that are relatively closely related by sequence similarity, this relationship does not hold universally: the receptors of ab3A and ab3B are distantly related (Figures 2B and 7B). Thus, our studies define a problem whose solution is likely to be complex: the evolution of the receptor-to-neuron map.

The Odorant Receptor Is the Primary Determinant of Multiple ORN Response Properties

We have shown that the odorant receptor dictates the odor response spectrum of the ORN in which it is expressed in many and perhaps all cases. The results of this study also indicate that the receptor is the primary determinant of three other ORN response properties: spontaneous firing rate, signaling mode, and response dynamics (Figures 4 and 5). All four of these properties are likely to play critical roles in odor coding, and some are closely related. For example, the level of spontaneous activity affects the capacity of inhibitory signaling as a mode of information transmission: a high level of spontaneous activity provides a wide operating range in which inhibition can act. A high spontaneous firing level could also affect the sensitivity of the ORN or could have effects on the state of postsynaptic neurons in the antennal lobe. Thus, our results demonstrate a critical role for the odorant receptor in multiple aspects of odor coding. The complexity of the odor code transmitted from the peripheral olfactory organs to the brain depends primarily on the functional properties of odorant receptors.

The Receptor Dictates Signaling Mode and Spontaneous Firing Rate: A Model for Odorant Receptor Transduction

We found that the signaling mode of an ORN is determined by its odorant receptor. Different receptors, when expressed in the same ORN and given the same odorant stimulus, can confer responses that differ in signaling mode (Figures 4B and 4C). A second finding is that a single receptor can mediate both excitatory and inhibitory responses (Figure 4D).

A simple model could explain how the receptor determines both the signaling mode and spontaneous firing rate of the ORN. According to this model, in the absence of odorants, receptors exist in an equilibrium between an “active” conformation that leads to activation of the G protein-mediated signal transduction cascade and an

“inactive” conformation that does not (Figure 8). The equilibrium constant differs for different receptors, thus explaining differences in spontaneous firing rate among ORNs. The binding of an excitatory odorant stabilizes the active conformation of the receptor, leading to an increase in firing rate. The binding of an inhibitory odorant stabilizes the inactive conformation, leading to a decrease in firing rate. A particular odorant, such as 1-hexanol, might stabilize the active conformation of some receptors, such as Or67a, but stabilize the inactive conformation of other receptors, such as Or47b. Similar models have been proposed for other GPCRs (Strange, 2002).

A Receptor-Dependent Mechanism of Response Termination

We have found that response termination kinetics, like spontaneous firing rate, signaling mode, and odor response spectrum, is determined by the odorant receptor (Figures 5A–5C). By what mechanism does termination kinetics depend on the receptor but not on the cellular context in which the receptor is expressed? One possibility is that termination kinetics depends primarily on the dissociation constant of the receptor for its odorant ligand. This possibility could explain why an individual receptor can show differences in the termination rate for two odorants, as we have observed with Or7a for E2-hexenal and benzaldehyde. It could also explain why two receptors can show differences in the termination rate for the same odorant, as observed with Or47a and Or98a for pentyl acetate (Figure 5B).

A Broad Compatibility between Odorant Receptors and ORNs

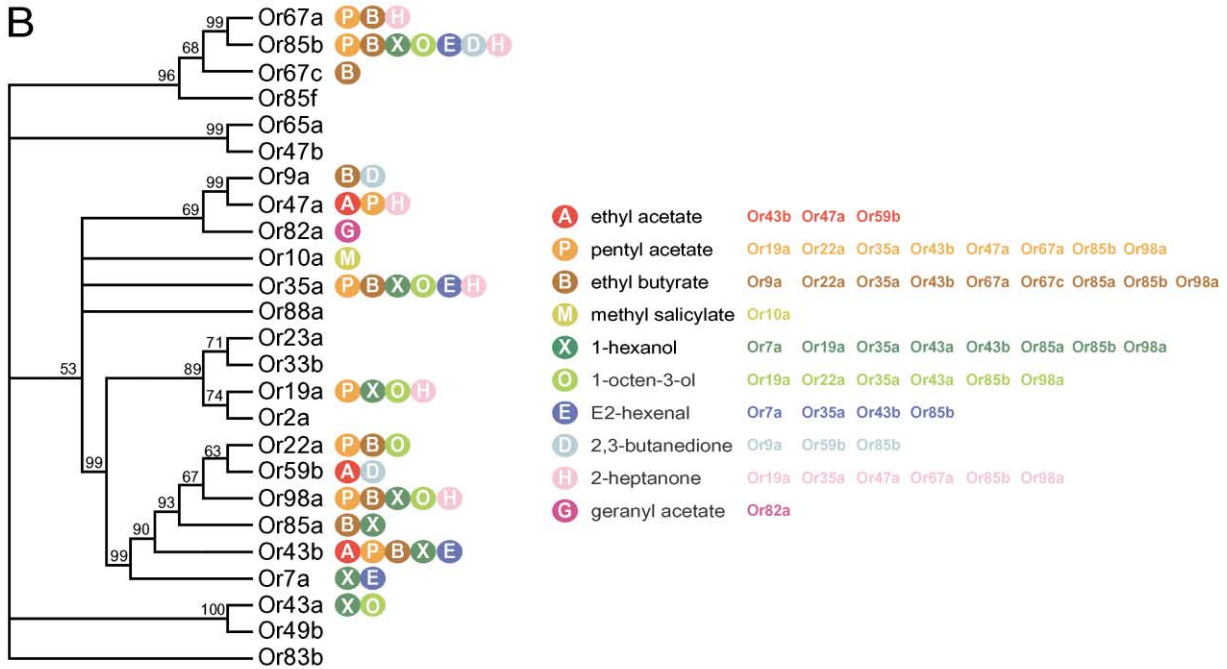
Drosophila ORNs operate in different environments. They reside in different olfactory organs, in sensilla of radically different morphology, and in different molecular contexts, e.g., in proximity to different OBPs. Moreover, the receptors are themselves remarkably divergent in sequence (Robertson et al., 2003). Given this heterogeneity, one might have expected severe limitations on the ability of receptors to function normally when expressed ectopically in different ORNs.

We found that many odorant receptors function normally with respect to a variety of parameters when expressed in the ab3A neuron, and we found that at least some receptors can function in a number of diverse neurons (Figure 6). We found that receptors normally expressed in ORNs of trichoid and coeloconic sensilla (Figure 2, atxA and acXB, respectively) can function in a basiconic sensillum (ab3), despite differences in morphology and OBP content, and antennal receptors can function in the maxillary palp, a developmentally and morphologically distinct organ. We have also recently shown that odorant receptors from the malaria vector mosquito *Anopheles gambiae* can function in a *Drosophila* ORN (Hallem et al., 2004). While it is certainly possible that some receptors, such as those specialized for pheromone detection, might function normally only in their native contexts, our results suggest a broad compatibility between most receptors and ORNs.

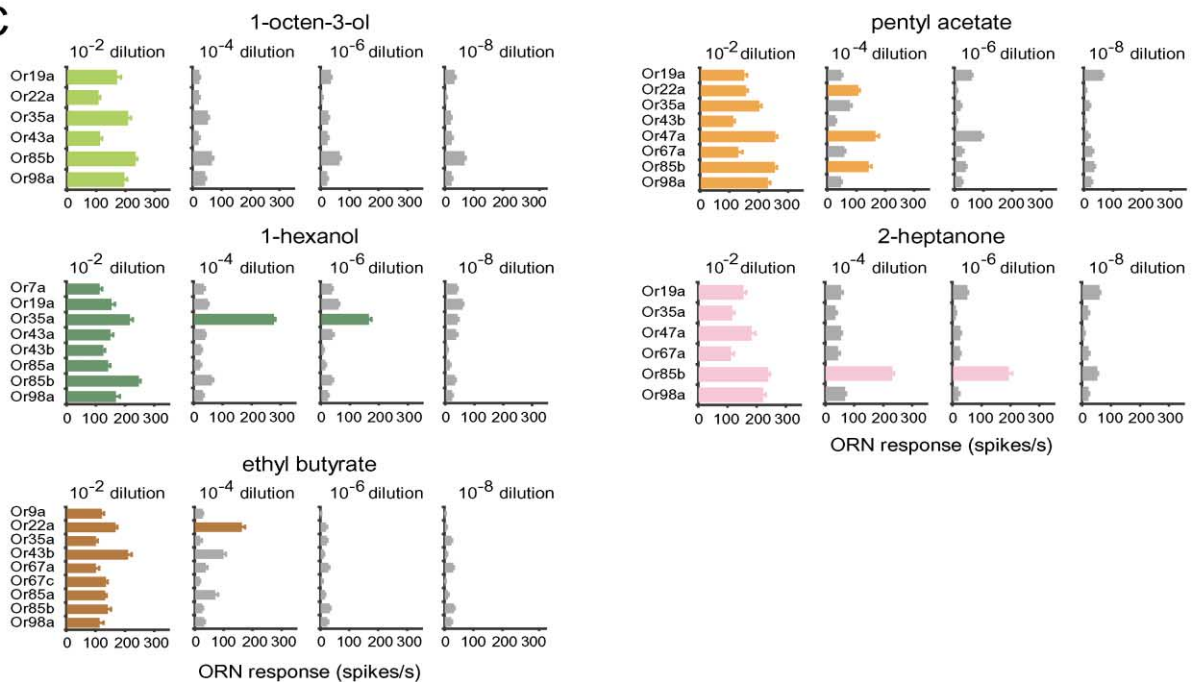
A



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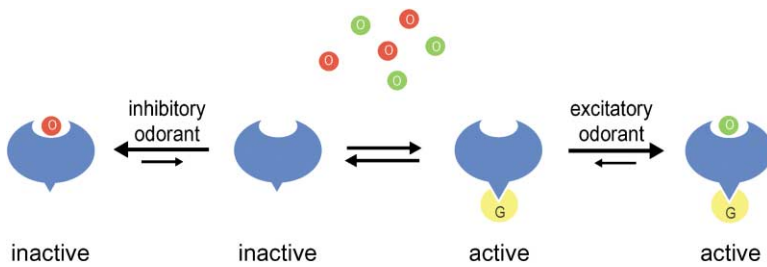


Figure 8. A Model for Signal Transduction by Odorant Receptors

Excitatory and inhibitory odorants are indicated in green and red, respectively. The diagram does not depict active intermediates that are unbound to G protein, and other conformations are likely to exist. A single odorant binding site is depicted, but the receptor could contain one binding site for excitatory odorants and a different site for inhibitory odorants.

An Integrated View of the Molecular Basis of Coding by a Receptor Repertoire

We have examined nearly the entire repertoire of Or receptors in a highly sensitive olfactory organ, the *Drosophila* antenna. This analysis has allowed us to consider the molecular basis of odor coding across an entire olfactory organ, with respect both to the mechanisms of coding and to the functional organization of the system.

We have analyzed the odor response spectra of these receptors with an odorant panel that is both chemically diverse and ecologically relevant. The odorants include acetate esters, organic acids, alcohols, an aldehyde, ketones, and a monoterpene ester. All of these odorants can be found in either bananas, apples, oranges, pineapples, or black currants (TNO, 2004). Ethyl acetate, for example, constitutes 33% of the volatiles in pineapple (Umano et al., 1992).

In their natural environment, flies encounter not only a vast array of odorants but also a vast range of odorant concentrations, ranging from low concentrations for a fly in flight to high concentrations for a fly immersed in rotting fruit. In this study, we directed 500 ms pulses of air over odorant solutions that varied in dilution from 10^{-8} to 10^{-2} . Although we refer to these doses in terms of the dilutions of odorant in the solvent, i.e., " 10^{-2} ," these air pulses then undergo a large dilution in another air stream before reaching the fly. We do not know how many molecules of odorant are thereby carried from their hydrophobic solvent to the antenna at room temperature or how this exposure compares to that of a fly standing on a fermenting fruit at higher temperatures. However, virtually all of the firing rates we have measured in this study are below the maximum firing rates observed for *Drosophila* ORNs (Dobritsa et al., 2003) and are thus within the dynamic ranges of ORNs. Moreover, the responses we have observed are comparable in magnitude to those produced by exposure to natural food sources such as banana, orange, pineapple, mango, and grape, all of which we have found to yield responses of ~ 80 – 270 spikes/s from ab2A and ab3A neurons (de Bruyne et al., 2001; and data not shown).

It will be of interest to extend our sampling of odor space to include not only a broader panel of odorants at a wide range of concentrations (as in Figure 7C) but, perhaps most important, mixtures of odorants, as flies in the wild rarely encounter a pure odorant.

All receptors we have characterized are distinct. Odor response spectra differ between receptors that are encoded by tightly linked genes, receptors that map to neighboring neurons in the same sensillum, and receptors that are more closely related in sequence. At the same time, there is overlap among response spectra. Some odorants elicited strong responses from $\sim 1/3$ of the tested receptors. Different receptors vary in their breadth of tuning with respect to the odorant panel: some respond strongly to a single odorant and others to as many as $\sim 70\%$ of the volatile odorants selected for inclusion in the panel.

The functional overlap among receptors expands the coding capacity of the system by allowing for combinatorial coding, which has been documented previously in other systems (Malnic et al., 1999; Kajiya et al., 2001). We have found that coding capacity is further expanded, however, by additional diversity in receptor function: we have shown that receptors confer not only the odor response spectrum but also the response mode and the response dynamics upon the ORNs that express them, as well as the level of spontaneous activity. Thus, there are several degrees of freedom available to each receptor, and the response of the system is multidimensional not only by virtue of its multiplicity of receptors but also by virtue of the multiplicity of response characteristics exhibited by each receptor.

The olfactory system encodes not only odorant quality, i.e., the identity of an odorant stimulus, but also its intensity. Analysis of a large population of receptors revealed that different odorants are encoded differently across different concentrations. Some odorants elicit strong responses from multiple receptors even at low concentrations, whereas others do not. These results show that differential receptor activation provides a rich coding space in which to register odor intensity.

Figure 7. Odor Coding by the Antennal Odorant Receptor Repertoire

(A) Table listing receptors by cytogenetic location. Colored dots indicate strong responses (as defined by a rate of >100 spikes/s following stimulation with a 10^{-2} dilution).

(B) Functional and phylogenetic relationships among antennal receptors. Colored dots with letters indicate odorants to which each receptor responds strongly, as defined above. Phylogenetic tree was constructed using the neighbor-joining method, Or83b was used as an outgroup, and numerical values indicate bootstrap support for each node. Receptors that give strong responses to each odorant are listed to the right of the odorant in the key.

(C) Responses of receptors as a function of concentration. Colored bars depict strong responses as defined above. Data for 10^{-2} dilutions were taken from Figures 2 and 3. For all graphs, $n = 12$.

Our results provide an underlying molecular basis for odor coding, whose cellular basis has been the focus of several recent studies. Optical imaging (Wang et al., 2003; Ng et al., 2002; Fiala et al., 2002) and electrophysiological studies (Wilson et al., 2004) showed that different odorants activate distinct but overlapping subsets of glomeruli in the antennal lobe of *Drosophila* and that higher odorant concentrations elicit stronger responses and activate larger numbers of glomeruli (Ng et al., 2002; Wang et al., 2003). Extensive electrophysiological recordings from PNs revealed that they differ in breadth of tuning, signaling mode, and response dynamics (Wilson et al., 2004), and it will be of interest to determine how the diverse odorant receptor responses described here are ultimately transformed into those of the PNs. Of particular interest in the representation of odors in the antennal lobe is the role of local interneurons, which form widespread connections among glomeruli and which could register the simultaneous activation of receptors that recognize different features of an odor stimulus.

The patterns of receptor activation described here may not provide all the information necessary for odorant discrimination. For example, the temporal structure of olfactory information has been shown to be critical for odor coding in several systems, and there are other ways of analyzing the temporal dynamics of neuronal activity (Laurent et al., 2001). However, all of the parameters we have measured in this study are likely to be essential to odor coding. Olfactory responses are based on the activities of the first-order neurons of the system, the ORNs, and the activities of these neurons are in turn based on the activities of the receptors that we have characterized here.

Odor coding depends on the existence of multiple ORN classes, each with different response characteristics. This organization depends ultimately on the regulated expression of individual receptors in defined subsets of ORNs. Little is known about the mechanisms by which ORNs select, from among a large repertoire, which genes to express. The receptor-to-neuron map we have established in this study provides a foundation for exploring the developmental mechanisms by which the molecular basis of odor coding in this system is established.

Experimental Procedures

Drosophila Stocks and Transgenes

ab3A mutant flies, and *Or22a-GAL4* and *UAS-Or47a* constructs, were described previously (Dobritsa et al., 2003). To generate *UAS-Or* constructs, the predicted *Or* coding region beginning with the second codon was PCR amplified from *Canton-S* genomic DNA, with the exceptions that *Or33b* and *Or43b* were amplified from P1 DNA (*y¹; cn bw sp*), and *Or35a* and *Or69a* were amplified from *Canton-S* cDNA. (The "*Or19a*" gene amplified from *Canton-S* genomic DNA contained some nucleotide variations consistent with *Or19a* P1 DNA and some consistent with the closely related *Or19b* P1 DNA.) PCR fragments were cloned into the pNmyc-UAST vector (C. Warr, personal communication) in frame with the initiation codon and three copies of the myc tag coding sequence. The resulting protein had an N-terminal myc tag. Immunolabeling was performed as described previously (Dobritsa et al., 2003). At least two independent lines were tested for each transgene.

Electrophysiology

Extracellular single-unit recordings were performed essentially as described previously (de Bruyne et al., 2001). Odorant stimuli and CO₂ stimuli were prepared in Pasteur pipettes as described previously (Dobritsa et al., 2003). Chemicals were >99% pure or of the highest purity available (Fluka, Sigma, and Aldrich) and were racemic mixtures with the exception of (+)-citronellal. Acetoin and 1-propanethiol were diluted 10⁻² in H₂O. All other liquid odorants were diluted 10⁻², unless otherwise noted, in paraffin oil (Fluka). Solid odorants were dissolved 0.1 g in 5 ml paraffin oil. Stimuli were presented by placing the tip of the pipette through a hole in a tube carrying a purified air stream (24 ml/s) directed at the fly and administering a 0.5 s pulse of charcoal-filtered air (5.9 ml/s) through the pipette containing the odorant. CO₂ stimuli were used once. All other stimuli were used for a maximum of three presentations. In graphs of odor response spectra, responses in graphs showing only excitation were quantified from a count of the number of impulses during the 0.5 s stimulus period. Responses in graphs showing inhibition (the *Or47b*, *atxA*, *Or59b*, and *ab2A* panels in Figure 2; the *Or33b* and *Or88a* panels in Figure 3; Figures 4E and 6D) were quantified by subtracting the number of impulses in the 1 s prior to odorant stimulation from the number of impulses in the 1 s following odorant stimulation. Each recording was from a separate sensillum, with no more than three sensilla analyzed per fly. EPGs were obtained as described previously (Ayer and Carlson, 1992). Odor stimuli were prepared as for single-unit recordings, except that the pipette was connected by ~2.5 cm of plastic tubing to a 5 ml syringe. Stimuli were presented by placing the tip of the pipette through a hole in a tube carrying a charcoal-filtered air stream (~2 l/min) over the fly and rapidly depressing the plunger of the syringe so as to pass 3 ml of air through the pipette and into the air stream. Recordings were obtained from flies aged <3 weeks. Error bars represent SEM.

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