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Coding of Odors by a Receptor Repertoire

Elissa A. Hallem[1] and John R. Carlson[1,*]
[1] Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA
*Contact: john.carlson@yale.edu
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SUMMARY

We provide a systematic analysis of how odor quality, quantity, and duration are encoded by the odorant receptor repertoire of the *Drosophila* antenna. We test the receptors with a panel of over 100 odors and find that strong responses are sparse, with response density dependent on chemical class. Individual receptors range along a continuum from narrowly tuned to broadly tuned. Broadly tuned receptors are most sensitive to structurally similar odorants. Strikingly, inhibitory responses are widespread among receptors. The temporal dynamics of the receptor repertoire provide a rich representation of odor quality, quantity, and duration. Receptors with similar odor sensitivity often map to widely dispersed glomeruli in the antennal lobe. We construct a multidimensional "odor space" based on the responses of each individual receptor and find that the positions of odors depend on their chemical class, concentration, and molecular complexity. The space provides a basis for predicting behavioral responses to odors.

INTRODUCTION

Sensory systems produce internal representations of external stimuli. A fundamental problem in neurobiology is how the defining aspects of a stimulus, such as its quality, quantity, and temporal structure, are encoded by the activity of sensory receptors. The problem is particularly intriguing in the case of olfactory stimuli, which are not related according to a single, continuous function such as wavelength or frequency. Odorants, rather, have discrete molecular structures and differ with respect to a variety of physicochemical properties. Moreover, the natural odors that an olfactory system must encode are generally complex mixtures of varying concentration and duration. Previous studies of the molecular basis of odor coding have focused, with few exceptions, on one or a small number of receptors and odors. However, a clear understanding of odor coding requires a more global perspective in which the receptor repertoire of an entire olfactory organ is considered as a whole and analyzed with olfactory stimuli that vary widely in their features.

The fruit fly *Drosophila melanogaster* provides an excellent model system for the study of odor coding. Its olfactory system is similar in organization to that of other insects and vertebrates yet is small in size and easily amenable to molecular, genetic, and electrophysiological analysis (Hallem and Carlson, 2004). The *Drosophila* adult has two olfactory organs, the antenna and the maxillary palp, which contain only ~1200 and ~120 olfactory receptor neurons (ORNs), respectively (Shanbhag et al., 1999). The ORNs are found within sensory hairs called sensilla, of which there are three major morphological types: basiconic, coeloconic, and trichoid. Each sensillum contains the dendrites of up to four ORNs.

In both insects and mammals, activated ORNs produce sequences of action potentials that reflect the features of the odors that activate them (Bichao et al., 2003; de Bruyne et al., 1999, 2001; Duchamp-Viret et al., 1999; Heinbockel and Kaissling, 1996; Nikonov and Leal, 2002; Shields and Hildebrand, 2000; Stensmyr et al., 2003). The ORNs activate second-order neurons in the brain (Hildebrand and Shepherd, 1997). In *Drosophila*, extensive electrophysiological analysis of the antennal basiconic sensilla identified 18 functional classes of ORNs, which are found in stereotyped combinations within eight functional types of sensilla (de Bruyne et al., 2001; Elmore et al., 2003).

Odorant receptors are seven-transmembrane-domain proteins encoded by large gene families. *Drosophila* has a highly diverse family of 60 odorant receptor (*Or*) genes, of which individual members are expressed in different subsets of ORNs (Clyne et al., 1999; Gao and Chess, 1999; Robertson et al., 2003; Vosshall et al., 1999, 2000). ORNs that express the same odorant receptor project to the same glomerulus, or functional processing unit, in the antennal lobes (ALs) (Gao et al., 2000; Vosshall et al., 2000).

We recently examined the antennal odorant receptors of *Drosophila* (Hallem et al., 2004b) using a mutant antennal neuron that lacks endogenous odorant receptors, "the empty neuron," as an in vivo expression system (Dobritsa et al., 2003). Receptors were expressed in the empty neuron, and odorant responses were assayed electrophysiologically (see Figure S1 in the Supplemental Data available with this article online). Thirty-one of thirty-two receptors that were shown to be expressed in the antenna in an in situ hybridization study (Vosshall et al., 2000) were tested in the empty neuron. Twenty-four of these

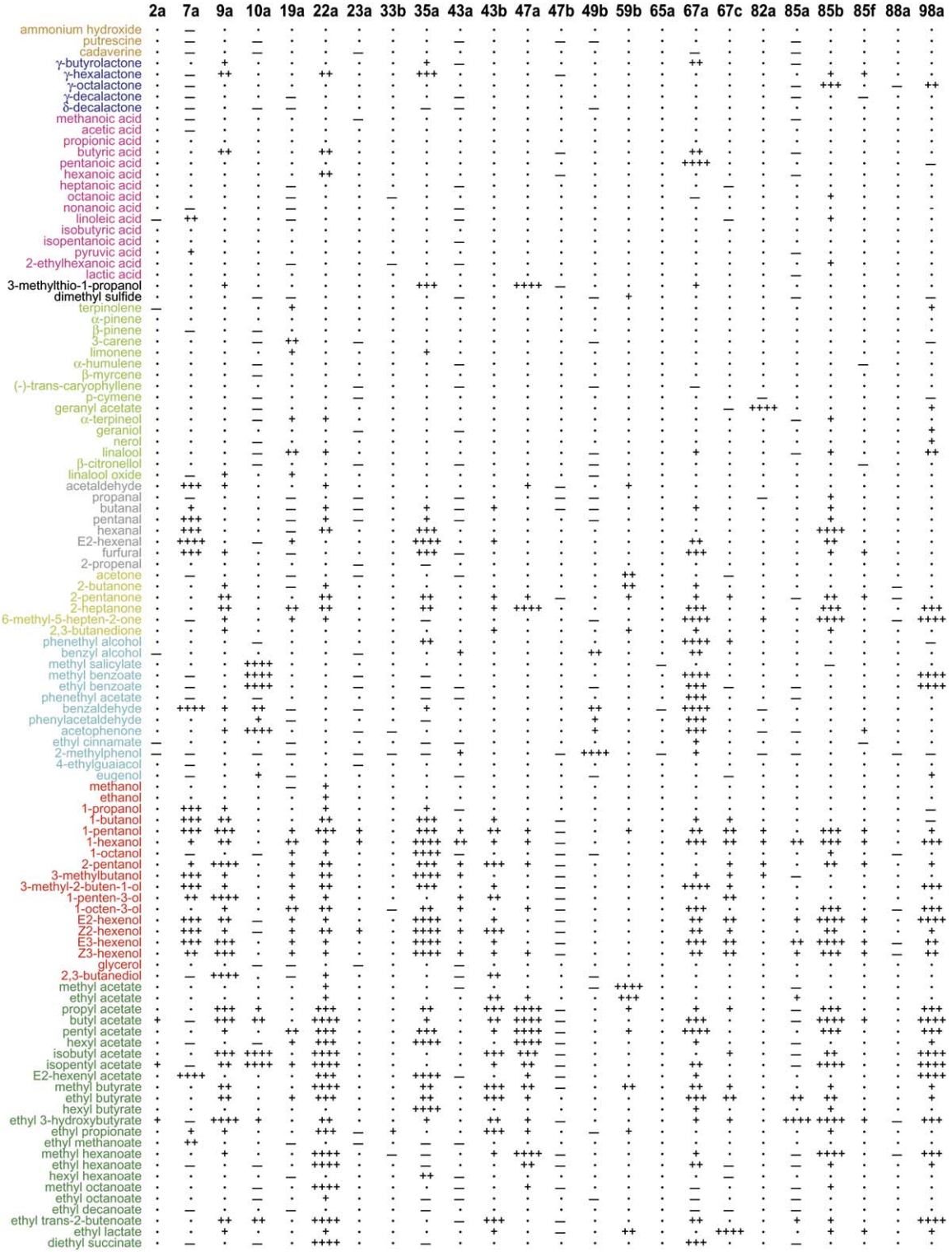


Figure 1. Odor Responses

"•," n < 50 spikes/s; "+," 50 ≤ n < 100 spikes/s; "++," 100 ≤ n < 150 spikes/s; "+++," 150 ≤ n < 200 spikes/s; "++++," n ≥ 200 spikes/s; "−" indicates inhibition to a level ≤ 50% of the spontaneous firing rate (Yao et al., 2005). Odorants are color coded by functional group (gold = amines, dark blue = lactones, pink = acids, black = sulfur compounds, light green = terpenes, gray = aldehydes, yellow = ketones, light blue = aromatics, red = alcohols, dark green = esters). In the case of odorants containing multiple functional groups, all odorants containing a phenol ring, a sulfur

receptors conferred odor responses and were therefore confirmed to be functional. This analysis revealed that the odorant receptor determines not only the odor response spectrum of the ORN in which it is expressed but also its spontaneous firing rate, response dynamics, and signaling mode (whether the response is excitatory or inhibitory) (Hallem et al., 2004b).

Here we provide a systematic analysis of how the antennal receptor repertoire encodes a large collection of odor stimuli that vary widely in identity, intensity, and duration. We examine the response of each receptor to a diverse panel of over 100 odorants, yielding 2640 odorant-receptor combinations. We find that nearly all odorants elicit a response from at least one receptor. Some classes of chemicals elicit many more strong responses than others. Individual receptors range along a continuum from narrowly tuned to broadly tuned. The broadly tuned receptors show strong excitatory responses to $\geq$20% of the tested odorants but are most sensitive to odorants that are structurally similar. Most receptors exhibit inhibitory as well as excitatory responses. Most receptors respond to complex natural odor mixtures, but one class of receptors responded to no tested odors and may include pheromone receptors. The temporal dynamics of the receptor repertoire are rich in information about odor identity, intensity, and duration. We construct a multidimensional "odor space" based on the responses of individual receptors and find that the map position of an odor in the space depends on chemical class, concentration, and molecular complexity. Finally, we investigate the functional organization of the glomeruli to which the receptors map in the AL of the brain; glomeruli have been found to exhibit ordered chemotopy in other organisms. We find that receptors with similar response properties often map to broadly distributed glomeruli. Our results provide understanding of the molecular logic by which the quality, quantity, and temporal structure of an odor are encoded across a receptor repertoire.

RESULTS

Excitatory and Inhibitory Responses of a Receptor Repertoire to a Large Odorant Panel Vary with Chemical Class

We systematically analyzed the responses of each of the 24 antennal receptors to a large and diverse panel of odorants using the empty neuron system. Previous studies of antennal odorant receptors with defined odor panels have used very small numbers of odorants (Dobritsa et al., 2003; Elmore et al., 2003; Goldman et al., 2005; Hallem et al., 2004b; Stensmyr et al., 2003; Stortkuhl and Kettler, 2001; Wetzel et al., 2001). In particular, our previous study of the antennal receptor repertoire used a panel of 10 odorants (Hallem et al., 2004b). However, some of the most fundamental principles of odor coding—for example, the tuning breadth of individual receptors and the extent of combinatorial coding—can only be incisively investigated with a large number of chemically diverse odorants. For the current study, we therefore expanded the dimension of the odorant panel by an order of magnitude: We selected 100 additional odorants. Our reasoning was that those conclusions derived from a study of 10 odors that withstood further scrutiny with a set of 100 odors are, by induction, the conclusions that are most likely to apply to odor sets of order 10^n.

Accordingly, from the immense number of odors a fly encounters in nature, we selected 100 odorants and added them to the original 10 to generate a panel of 110 odorants. The odorants were chosen to represent a broad sampling of ecologically relevant odors and include esters, alcohols, ketones, lactones, aldehydes, terpenes, organic acids, amines, sulfur compounds, and aromatics (Figure 1). The panel includes compounds of widely ranging chain lengths and also small odorants that may be of particular significance in the chemical ecology of *Drosophila*, such as ethanol, ammonia, and dimethyl sulfide. Most of the odorants are found in fruits, and some are products of fermentation (TNO, 2004).

We tested the 2640 odorant-receptor combinations individually by single-unit electrophysiology (Figure 1; Table S1; see Figure S2 for an alternative representation). Of these combinations, 72% yielded little if any response (<50 spikes/s) and are indicated as "•" in Figure 1, even though the odorant concentrations were deliberately set relatively high for this initial analysis (see Experimental Procedures). Only 445 odorant-receptor combinations, or 17%, resulted in responses of $\geq$50 spikes/s; only 234 combinations, or 9%, produced a strong response of $\geq$100 spikes/s; and only 69 of the combinations, or 3%, resulted in very strong responses of $\geq$200 spikes/s (Figure 1). Thus, when examined at a system-wide level, strong or even modest odor responses across the receptor repertoire were sparse, even at high odorant concentrations.

Inhibitory responses were strikingly prevalent: 300 odorant-receptor combinations, or 11%, resulted in inhibitory responses (Figure 1). Most receptors are inhibited by at least one odorant, and most odorants inhibit at least one receptor.

The distribution of responses varied among chemical classes. The density of strong responses ($\geq$100 spikes/s) was higher among esters (16%) and alcohols (18%) than terpenes (1%) or organic acids (2%), while the

moiety, or a terpene/terpenoid structure were grouped into the "aromatic," "sulfur compounds," or "terpenes and terpenoids" categories. Data for the set of odorants tested across concentrations are from Figure 4, and n = 6; for all other odorants, n = 6, except that n = 4 for responses of <50 spikes/s. Responses of each receptor to the diluent were subtracted from each odorant response. Inhibition was not quantified for receptors with spontaneous firing rates of <5 spikes/s (Or9a, Or22a, Or43b, Or47a, and Or59b) due to the difficulty in quantifying inhibition in the presence of very low background firing. Numerical values for each entry are given in Table S1.

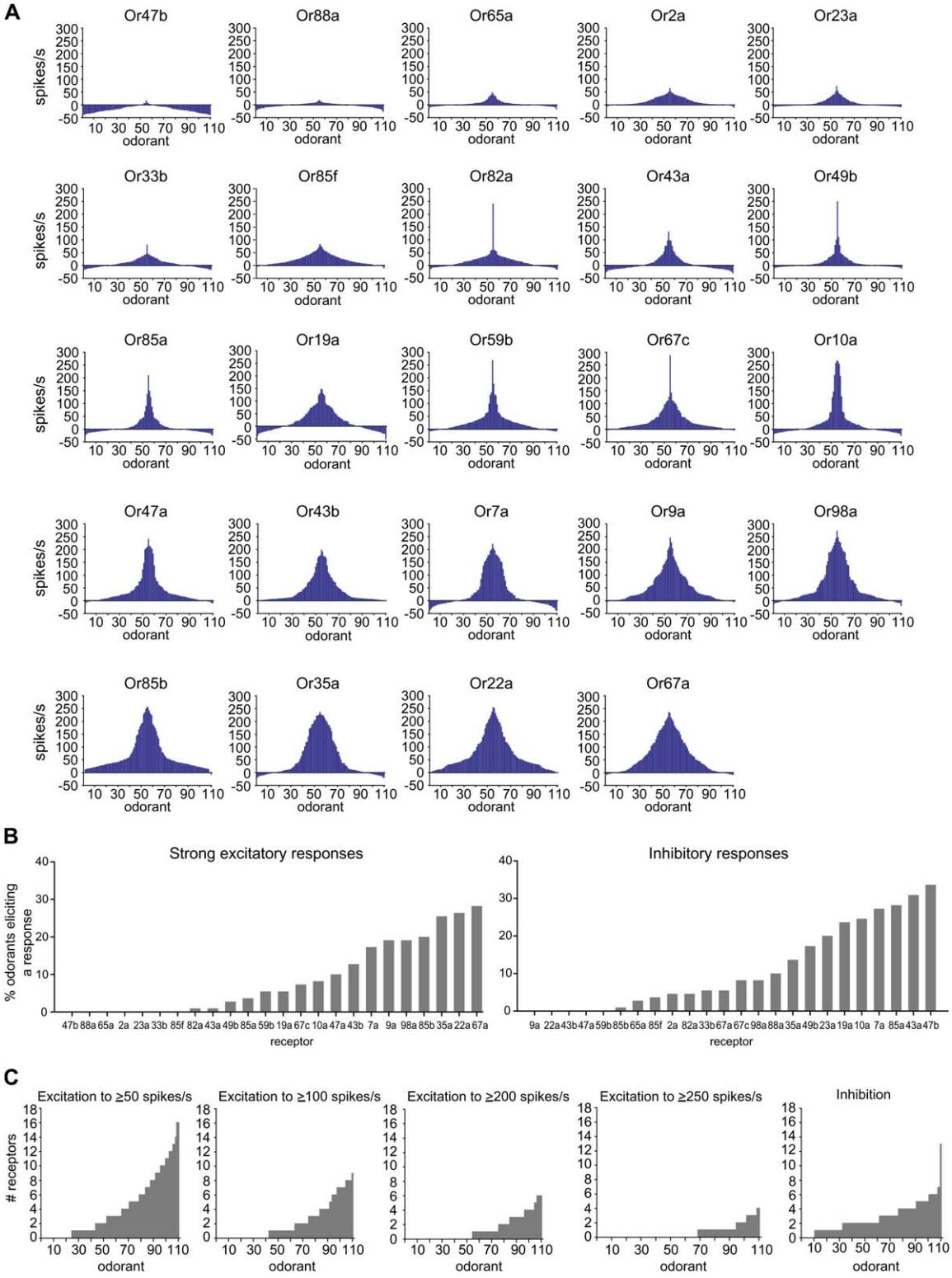


Figure 2. Tuning Breadths of Odorant Receptors

(A) Tuning curves for odorant receptors. The 110 odorants are displayed along the x axis according to the strengths of the responses they elicit from each receptor. The odors that elicit the strongest responses are placed near the center of the distribution; those that elicit the weakest responses are placed near the edges. The order of odorants is thus different for different receptors. Negative values indicate inhibitory responses. The 24

density of inhibitory responses was higher among aromatics (18%) than any other chemical class (Figure 1; Figure S2). These results suggest that the receptor repertoire may have an especially high degree of discriminatory power with which to resolve alcohols, esters, and aromatics by means of combinatorial coding.

Receptors Range along a Continuum from Narrowly Tuned to Broadly Tuned

We generated tuning curves for each receptor (Figure 2A). There is great variability in the number of odorants that elicit a strong excitatory response ($\geq$100 spikes/s) from each receptor. The first seven receptors shown in Figure 2A (Or47b through Or85f) were not strongly excited by any odorant. Or82a was strongly excited by 1 of the 110 tested odorants, Or10a by 9 odorants, and Or67a by 31 odorants. The results do not reveal a bimodal distribution of narrowly tuned and broadly tuned receptors, but rather a continuum of tuning breadths (Figure 2B).

A number of the more broadly tuned receptors responded strongly to odorants with diverse chemical structures (Figure 1). For example, Or67a was strongly excited by a lactone, organic acids, aldehydes, ketones, aromatics, alcohols, and esters. Of the 16 receptors that were strongly excited by more than one odorant, almost all were strongly excited by odorants of more than one chemical class. Or49b was exceptional in that the three odorants that elicited strong excitatory responses all contain a benzene ring.

Receptors also varied continuously in the number of odorants that inhibit them (Figure 2B). Some receptors were inhibited by as many as ~30% of all tested odorants.

How many receptors are activated by each odor? Fifteen odorants elicited $\geq$50 spikes/s from more than ten receptors, but only one odorant (1-hexanol) elicited $\geq$100 spikes/s from nine receptors, and only five odorants elicited $\geq$100 spikes/s from each of eight receptors (Figure 2C). Of 17 odorants that activated six or more receptors to $\geq$100 spikes/s, 7 are alcohols and 9 are esters. Inhibitory responses were elicited from multiple receptors by most odorants (Figure 2C). The odorant that elicited the most inhibitory responses was 2-methylphenol, an aromatic.

Molecular Determinants of Binding Specificity

Some receptors show a strong response to one odorant but no response to a structurally similar odorant. For example, Or49b, which shows excitatory responses only to aromatic compounds (Figure 1), is selective among aromatic compounds. It responds to 2-methylphenol and a number of other small aromatics, including benzyl alcohol, benzaldehyde, phenylacetaldehyde, and acetophenone, but the larger aromatics phenethyl acetate, ethyl cinnamate, and 4-ethylguaiacol do not elicit responses (Figure S3A). For this receptor, the presence of an aromatic ring, as well as the size of the molecule, appears to be a factor in determining binding specificity.

Another receptor, Or10a, responds strongly to the aromatic compounds ethyl benzoate, methyl benzoate, methyl salicylate, acetophenone, and benzaldehyde (Figure S3B). However, the structurally similar aromatics 4-ethylguaiacol, benzyl alcohol, and ethyl cinnamate elicit little if any response. Thus, all of the strong aromatic ligands for Or10a contain not only a benzene ring but also a carbonyl group on the primary carbon atom. The presence of both an aromatic ring and a carbonyl group and their close proximity may be determinants of odorant binding to Or10a. However, while these features may be sufficient to elicit a strong response from Or10a, not all are necessary: Or10 also responds very strongly to two aliphatic compounds, isobutyl acetate and isopentyl acetate (Figure 1). One possible implication of these results, taken together, is that the response of some Or proteins to particular odorants may be difficult to predict.

The Broadly Tuned Receptors Are Most Sensitive to Structurally Similar Odorants

Some individual receptors responded strongly to $\geq$20% of the tested odorants (Figures 2A and 2B). In this analysis, odorants were administered as "10^{-2}" dilutions (see Experimental Procedures), as in previous studies (de Bruyne et al., 2001; Dobritsa et al., 2003; Goldman et al., 2005; Hallem et al., 2004b; Kreher et al., 2005; Larsson et al., 2004; Wilson et al., 2004). We wished to know whether these receptors retained their promiscuity or appeared more selective when tested with odorants at lower concentrations. Accordingly, we examined the odor response specificities of the four most broadly tuned receptors at odor concentrations spanning seven orders of magnitude.

We found that at lower concentrations, fewer odorants elicited strong responses from these receptors (Figure 3). For example, of the ten odorants that elicited the strongest responses from Or35a at a 10^{-2} dilution, only four elicited strong responses ($\geq$100 spikes/s) from Or35a when tested at 10^{-4} dilutions, and only one elicited a strong response when tested at a 10^{-6} dilution.

The four odorants that continued to elicit strong responses from Or35a at 10^{-4} dilutions are structurally related. All four are six-carbon chains containing a terminal hydroxyl group (Figure 3). Likewise, fewer odorants

tuning-curve graphs are ordered based on the number of odorants eliciting strong responses of $\geq$100 spikes/s from each receptor; the first seven receptors did not respond strongly to any odorants and are listed in order of the strongest response of each receptor. Data are from Figure 1.

(B) Left, the percentage of the 110 odorants eliciting $\geq$100 spikes/s from each receptor; right, the percentage of odorants eliciting an inhibitory response from each receptor.

(C) The number of receptors activated to each indicated firing rate by each odorant. Odorants are ordered along the x axis according to the number of receptors they activate.

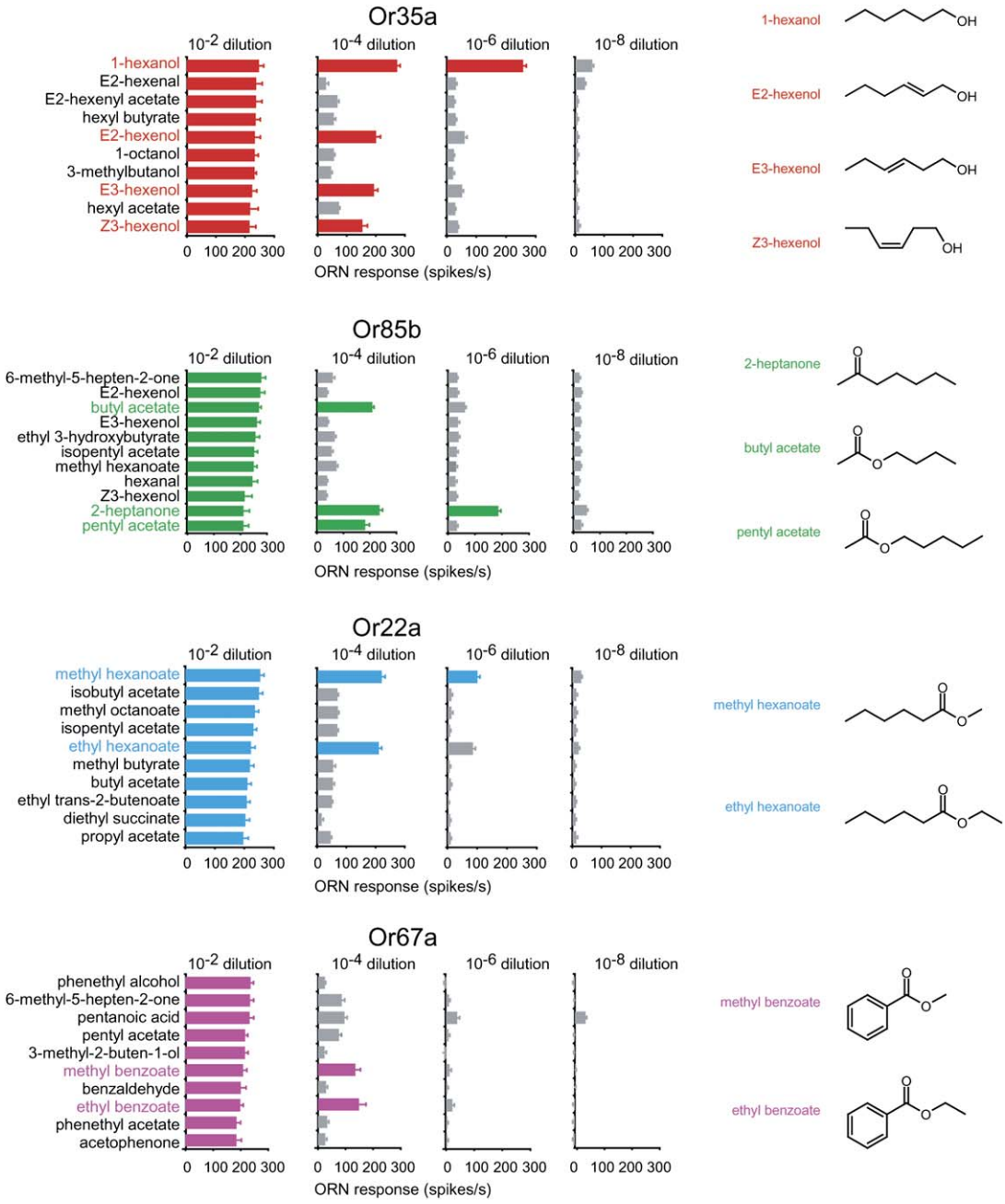


Figure 3. Specificities of Broadly Tuned Receptors across Concentrations

For each receptor, the ten odorants that elicited the strongest responses at a 10^{-2} dilution were tested at lower concentrations. Colored bars indicate strong responses (≥100 spikes/s). Structures of odorants eliciting strong responses at low concentrations are indicated. Eleven odorants were tested in the case of Or85b because 10^{-2} dilutions of 2-heptanone and pentyl acetate elicited responses of equal magnitude. Data for 1-hexanol, E2-hexenal, 2-heptanone, and pentyl acetate are from Figure 4. n = 6. Error bars = SEM.

elicited strong responses from the other receptors when tested at lower concentrations, and the most effective odorants for each receptor are closely related. Thus, even the most broadly tuned receptors are narrowly tuned at low concentrations.

Odor Coding across Concentrations by the Receptor Repertoire

We expanded the analysis of dose dependence to include all 24 receptors, which we tested with a diverse subset of odorants. We found that at high concentrations, most

odorants activated multiple receptors (Figure 4A). However, at lower concentrations of odorants, strong responses were sparse: Each of the five odorants that elicited responses of $\geq$50 spikes/s at a 10^{-6} dilution activated only a single receptor (Figure 4A; Table S2). Such odor representations are likely to be produced in a flying insect, for example, which encounters relatively low odorant concentrations.

Combinatorial Coding of Complex Odor Mixtures

Animals in nature rarely encounter pure odorants, but rather encounter complex mixtures of hundreds of compounds. We examined the responses of the antennal receptor repertoire to nine complex mixtures of odors—natural extracts of apple, apricot, banana, cherry, mango, peach, pineapple, raspberry, and strawberry—by systematically testing these fruit odors at a range of concentrations spanning seven orders of magnitude.

We found that most receptors are responsive to fruit odors (Figure 4B; Table S2). At the highest concentration tested, 16 receptors (67%) responded strongly ($\geq$100 spikes/s) to the odor of at least one fruit, 13 to at least two fruits, and 9 to at least three fruits (Table S2). Thus, most receptors respond strongly to fruit odors, and many fruit odors strongly activate multiple receptors.

A Distinct Class of Receptors that Respond to No Tested Odors

A small subset of receptors showed virtually no excitatory responses to any of the fruit odors tested. Three receptors—Or47b, Or65a, and Or88a—did not respond to any fruit odors or pure odorants above a rate of 50 spikes/s. These results suggest that while most receptors have evolved to detect or evaluate food sources, these receptors may have evolved to sense other classes of chemical information, such as pheromones. This possibility is supported by the expression of these receptors in trichoid sensilla, which are known to detect pheromones in other insects (Christensen and Hildebrand, 2002). Or47b in particular is expressed in sensilla that also express the male-specific isoform of the transcription factor *fruitless* (Couto et al., 2005; Fishilevich and Vosshall, 2005; Manoli et al., 2005; Stockinger et al., 2005). Interestingly, Or47b showed inhibitory responses to 34% of all tested odorants, more than any other tested receptor (Figure 2B). These results suggest that inhibition may be particularly important as a means of noise reduction in channels that transmit specific signals of particular biological significance.

The Temporal Dynamics of a Receptor Repertoire Are Rich in Information about Odor Identity, Intensity, and Duration

There is an expanding body of evidence that the temporal structure of olfactory information is critical to odor coding (Laurent et al., 2001). Since the initial representation of an odor lies in the differential activities of the receptors, the dynamics of receptor activity provide a temporal structure on which all subsequent representations are based. However, there has been remarkably little analysis of the temporal structure of this initial representation.

We demonstrated earlier, using several receptors and several odors, that the response kinetics of an ORN are determined by the odorant receptor that it expresses (Hallem et al., 2004b). We have now systematically investigated the temporal structure of the primary odor representation by examining the firing frequencies of all 24 receptors as a function of time. Firing frequencies were quantified over the course of a 2 s period beginning at the onset of odor stimulation, and responses were plotted to generate a "temporal surface" for each of a panel of odors.

We found that some odorants, such as methyl salicylate at a 10^{-2} dilution, elicited a pattern of receptor activation that was relatively constant throughout the 2 s time interval (Figure 5A; Tables S3 and S4). By contrast, other odorants elicited patterns of receptor activation that were highly dynamic over the time interval. For example, of the receptors that initially responded to a 10^{-2} dilution of pentyl acetate, some stopped responding during this time interval, while others continued to respond at nearly the same rate throughout the interval. The temporal surfaces for these odors contain a number of deep folds: Among the receptors that respond at the highest initial rates, some showed much more rapid declines in firing rates than others. At lower concentrations, many odorants elicited a prolonged response from only one receptor or from no receptors (Figure 5B, Tables S3 and S4). Thus, odors differ in the temporal dynamics they elicit across the receptor repertoire, and the temporal structure of receptor activity is rich in information. This temporal structure thereby provides a representation of an odor stimulus that may be transformed into central representations of odors.

Animals in nature experience a dynamic olfactory environment in which odors are encountered for varying lengths of time. Successful navigation toward an odor source may depend on the ability to encode temporal features of an odor stimulus, such as its duration. We therefore also examined responses to varying pulse lengths of odors. We compared the responses of four pentyl-acetate-sensitive receptors (Or22a, Or35a, Or85b, and Or98a) to a 10^{-4} dilution of pentyl acetate and four apple-sensitive receptors (Or22a, Or35a, Or67a, and Or98a) to a 10^{-3} dilution of apple extract. These relatively low odor concentrations were chosen to more closely approximate the odor environment that a fly might encounter while in flight at a distance from a food source. Odors were administered as either 100 ms, 500 ms, 1 s, or 5 s pulses.

We found that more prolonged pulses evoked more prolonged responses (Figure S4). Receptors are thus able to encode information about the duration of an odor stimulus and, by extension, the temporal dynamics of an odor plume, an ability that is likely to be necessary for localization of an odor source.

A Representation of Odor Space

We have found that different odors activate different combinations of receptors. Which odors elicit similar patterns

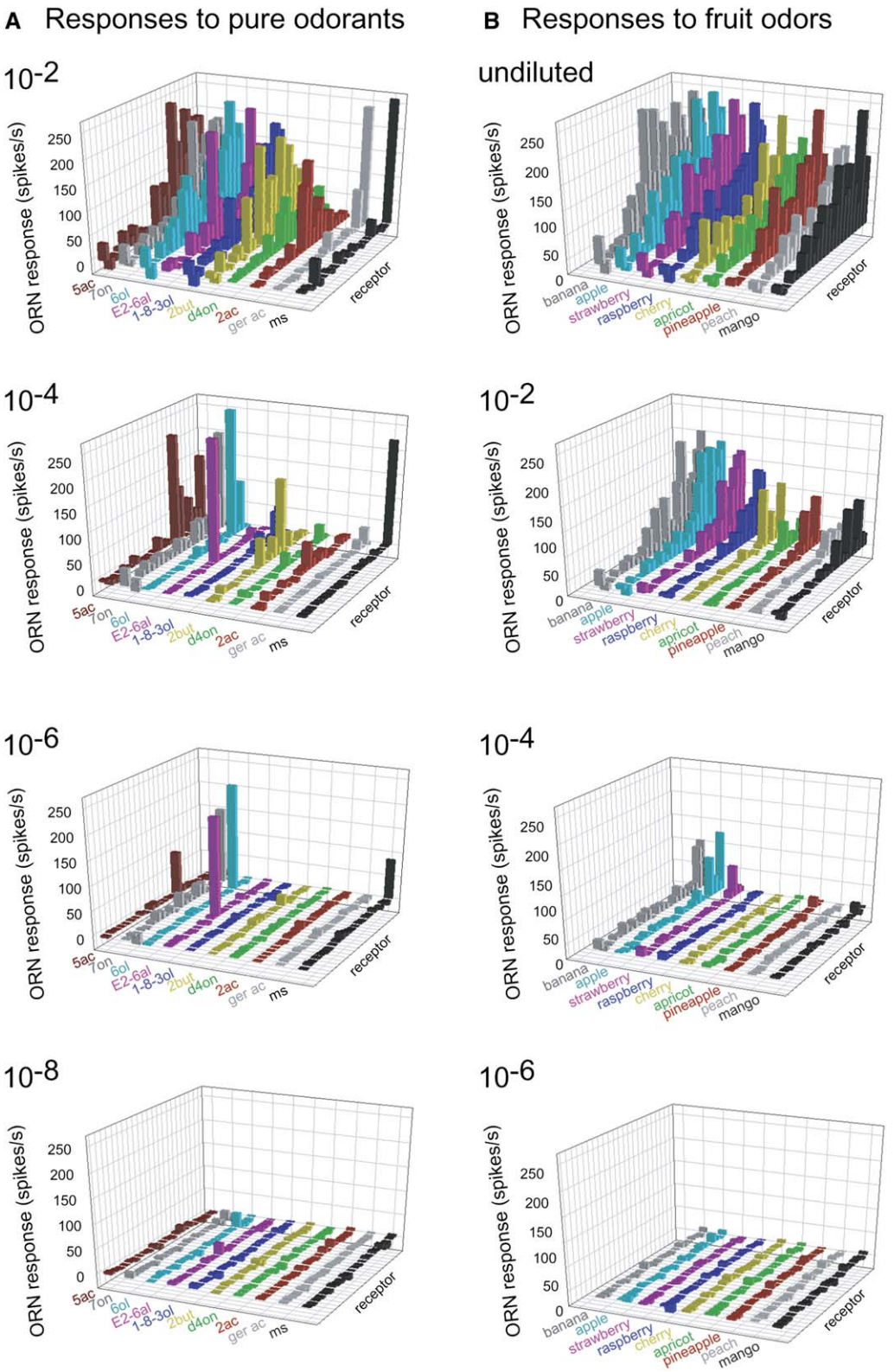


Figure 4. Odor Coding across Concentrations

(A) Responses of receptors to pure odorants. 5ac = pentyl acetate, 7on = 2-heptanone, 6ol = 1-hexanol, E2-6al = E2-hexenal, 1-8-3ol = 1-octen-3-ol, 2but = ethyl butyrate, d4on = 2,3-butanedione, 2ac = ethyl acetate, ger ac = geranyl acetate, ms = methyl salicylate.

of receptor activation, and which elicit very different patterns? What features are shared by odor stimuli that elicit similar patterns? To address these questions, we examined the spatial relationships among odorants in an odor space created by the responses of each of the 24 antennal receptors to each tested odorant.

We constructed a 24-dimensional space in which each axis represents the response of one of the 24 receptors in spikes/s, and we then mapped each odorant to a particular position in the space. To illustrate the concept, Figure 6A shows a three-dimensional space in which each axis represents the response to one of three receptors: Or85b, Or10a, and Or47a. Three odorants have been mapped to three distinct points in this space: Pentyl acetate and 2-heptanone map close together; methyl salicylate maps to a position distant from them. The odor space shows that pentyl acetate and 2-heptanone elicit similar patterns of activation among these three receptors, while methyl salicylate elicits a very different pattern of activation.

We performed a similar analysis of the 24-dimensional odor space constructed from the entire receptor repertoire. To quantitate relationships among odorants in this 24-dimensional space, we measured the Euclidean distances in spikes/s between all possible pairs of the 110 tested odorants.

Of the 5995 pairs, the three pairs whose members were closest were γ-decalactone and δ-decalactone (mean distance of 26 spikes/s), methanol and ethanol (28 spikes/s), and cadaverine and putrescine (29 spikes/s) (Figure 6B). The odorants of each pair differ by only a single carbon atom, and their proximity in odor space raises the possibility that the fly may not easily discriminate between them.

The odorant pairs that were farthest apart in odor space were 2-methylphenol and isobutyl acetate (585 spikes/s), 2-methylphenol and isopentyl acetate (582 spikes/s), E2-hexenol and methyl salicylate (580 spikes/s), and pentyl acetate and methyl salicylate (580 spikes/s). Interestingly, pentyl acetate and methyl salicylate are both esters, indicating that odorants that share a common functional group can be widely separated in odor space.

To visualize relationships among odorants in the space, we performed a hierarchical cluster analysis (Figure 6C). We found that odorants of the same chemical class often clustered together, although in no case did a cluster include all members of a class. Moreover, inspection of Figure 6C reveals many examples of structurally similar molecules that are tightly clustered (e.g., octanoic acid, heptanoic acid, and nonanoic acid).

We also mapped the fruit odors within the odor space and examined the Euclidean distances among all possible pairs. We found that peach and apricot mapped most closely in odor space, whereas cherry and banana mapped farthest apart. It is striking that the two fruits that show the most similar patterns of receptor activation, peach and apricot, belong to the same genus, as does the fruit that clusters most tightly with them, cherry (Figure 6D).

As a second means of analyzing the relationships among odors, we used principle components analysis (PCA) to represent the 24-dimensional odor space in a three-dimensional odor space. Consistent with the hierarchical cluster analysis, we found that odors of a particular chemical class were often clustered (Figure 7A). Odors of some classes, such as the acids, appeared more tightly clustered than those of other classes, such as the esters. Although odors of a particular class are clustered, there is intermingling in odor space among odors of different classes. Thus, the chemical class is one feature, but not the only feature, that determines the pattern of activation among the receptor repertoire.

A second feature we examined, the chain length of an odorant, did not reveal a simple relationship with position in odor space. Odorants of the same chain length mapped broadly across odor space; we did not observe striking patterns of clustering of odorants of the same chain length (data not shown).

A comparison of fruit odors and pure odorants revealed that these two classes of stimuli occupied different portions of odor space (Figure 7B). Thus, the molecular complexity of an olfactory stimulus may be a feature that influences its map position in odor space.

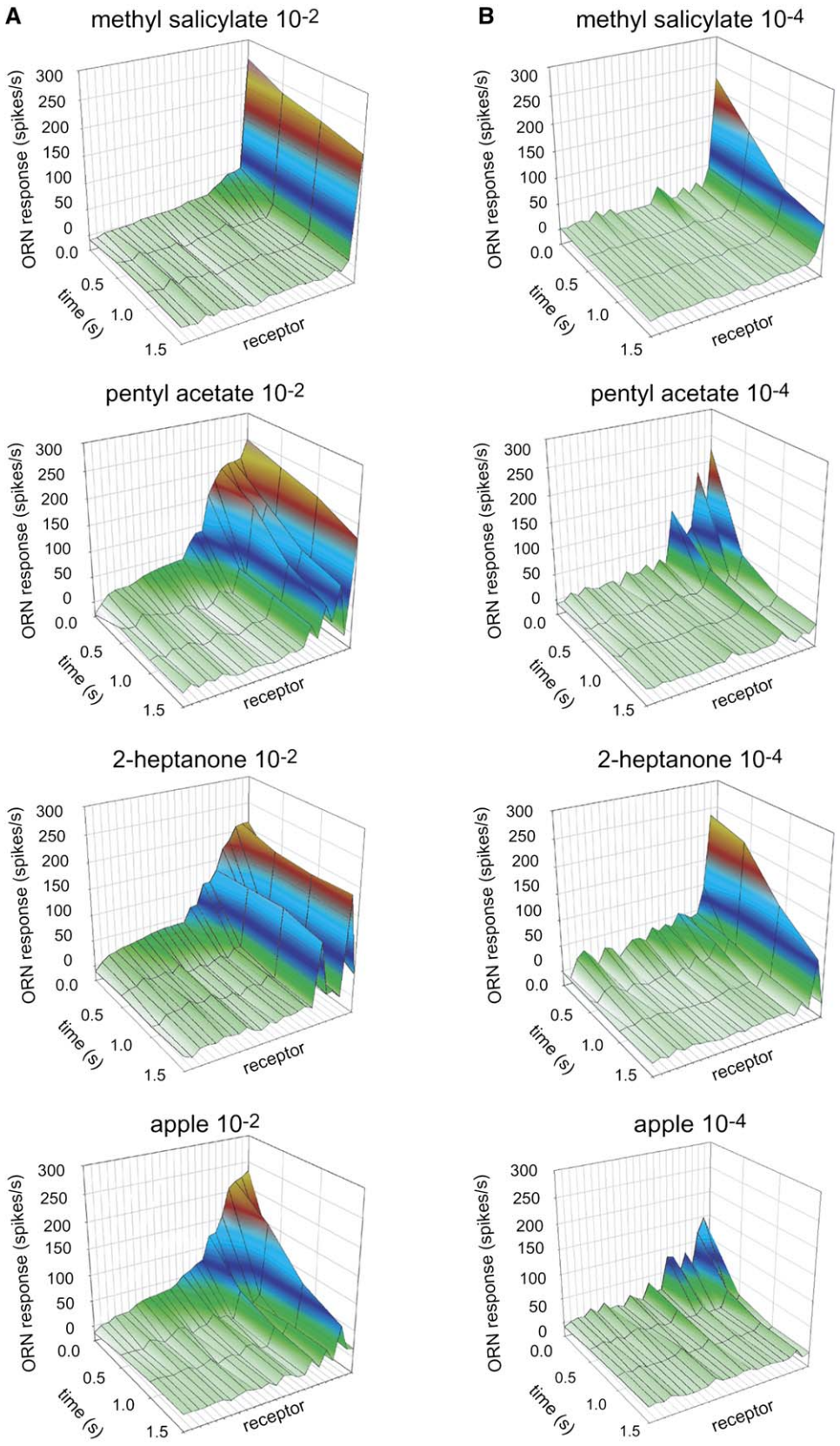
Finally, our analysis shows that the position of an odorant in odor space depends on its concentration. Different odorants elicit more similar patterns of receptor activity when tested at lower concentrations (Figures 7C and 7D). These results suggest that odors may be more difficult for the fly to discriminate between at low concentrations.

Odorant Representations in the Antennal Lobe

A central question in olfaction is how the responses of the receptor repertoire are represented in the AL or its vertebrate analog, the olfactory bulb. Having now systematically characterized receptor response properties in detail, we are in a position to integrate our results with a recently established receptor-to-glomerulus map (Couto et al., 2005; Fishilevich and Vosshall, 2005). Previous efforts to integrate functional data with this map were hampered by the paucity of available functional data and did not reach a clear consensus (Couto et al., 2005; Fishilevich and Vosshall, 2005).

We first investigated the extent of chemotopy in the glomerular map in a general sense: We asked whether receptors with similar odor specificity mapped to neighboring glomeruli in the AL. To quantitate the functional similarity of receptors, we constructed a 110-dimensional receptor space in which each dimension represents the response in spikes/s to one of the 110 odorants used in this study.

(B) Responses to complex mixtures. Responses of each receptor to the diluent were subtracted from each odorant response. Inhibitory responses are apparent as bars extending below the x-y plane. The order of receptors along the y axis in (A) and (B) is indicated in the Supplemental Data. n = 6.


A
methyl salicylate 10-2
B
methyl salicylate 10-4
pentyl acetate 10-2
pentyl acetate 10-4
2-heptanone 10-2
2-heptanone 10-4
apple 10-2
apple 10-4
ORN response (spikes/s)
300
250
200
150
100
50
0
0.0
0.5
1.0
1.5
time (s)
receptor

Each receptor was mapped to a particular position in this space, and we calculated the Euclidean distance between all possible pairs of receptors. For each pair, this distance was compared to the distance between the glomeruli to which they map. We did not find a correlation between functional distance and glomerular distance (Figure S5; r = 0.15, p = 0.18; Mantel test, two-tailed).

We then investigated chemotopy by examining a number of specific parameters. We considered first the class of odorant that elicited the strongest excitatory response from each receptor and found that receptors that respond most strongly to a particular chemical class—e.g., alcohols—map to glomeruli that are widely separated in some cases (Figure 8A); the same conclusion emerged for inhibitory responses (Figure 8B). We found that glomeruli whose receptors respond most strongly to aromatic odors are interspersed among their aliphatic counterparts (Figure 8C). Glomeruli whose receptors respond most strongly to aliphatic odors of a particular chain length are widely distributed (Figure 8D), and, when the analysis was restricted to esters, alcohols, ketones, or aldehydes, the same result was obtained: Glomeruli whose receptors respond most strongly to a particular chain length are broadly dispersed (Figures 8E–8H). When responses to each member of a homologous series of alcohols or esters were mapped onto glomeruli, strong responses were likewise observed to be widely dispersed and did not follow an obvious spatial progression with increasing chain length (Figure S6).

We then mapped the spontaneous firing rate of each receptor to its corresponding glomerulus and found that ORNs expressing receptors with lower spontaneous firing rates tend to project more medially (Figure 8I). We found that glomeruli whose receptors are broadly tuned to excitatory odorants, as quantitated in each of two ways (Figures 8J and 8K), tend to map more medially than those whose receptors are narrowly tuned, consistent with a previous study (Fishilevich and Vosshall, 2005). We did not observe a relationship between map position and the fraction of odors that elicited inhibitory responses from each receptor (Figure 8L).

DISCUSSION

We have investigated how diverse and complex odor stimuli are encoded by the odorant receptor repertoire of the *Drosophila* antenna. The activity of the receptor repertoire is the basis of all subsequent neural computation. Odorant receptors dictate the response spectrum, response dynamics, and signaling mode of the ORNs that express them (Hallem et al., 2004b). The activities of ORNs, in turn, underlie the activity of the rest of the neural network that identifies odors. Thus, an understanding of the molecular basis of odor coding by the receptor repertoire is essential to an understanding of odor perception.

The responses of individual odorant receptors were measured using the empty neuron system (Dobritsa et al., 2003; Goldman et al., 2005; Hallem et al., 2004a, b; Kreher et al., 2005). A major virtue of this system is that activities are measured in terms of action potential frequency, which provides a clear and direct indication of response magnitude and response dynamics. Some aspects of odor coding that we have analyzed, such as the temporal structure of odor responses, would be difficult to analyze by other means.

Responses of the Receptor Repertoire to Odors Are Pervasive and Anisotropic

We examined the responses of the antennal receptor repertoire to a diverse panel of 110 odorants. Only 17% of odorant-receptor combinations yielded even modest excitation at relatively high odorant concentrations, suggesting that strong responses are sparse (Figure 1). However, coverage of the odorants is pervasive in the sense that all but three of the 110 odorants tested, or 97%, elicit either an excitatory response of $\geq$50 spikes/s or an inhibitory response from at least one receptor; 78% elicit an excitatory response and 91% elicit an inhibitory response. The coverage of odorants is anisotropic in that some chemical classes elicited more dense patterns of excitation or inhibition than others (Figure 1). The high density of excitatory responses to alcohols and esters may reflect strong selective pressure for the ability to discriminate among these odorants, of which a broad diversity are present in fruits (TNO, 2004).

The extent of inhibition among the receptor repertoire is striking. The widespread existence of two response modes, excitation and inhibition, among most receptors substantially expands the coding space available to the receptor repertoire by adding an additional degree of freedom. Inhibition may also function in suppressing noise in certain channels that signal the presence of specific odorants of biological importance. Inhibitory responses may account, at least in part, for the inhibitory epochs that have been observed in recordings from projection neurons in the ALs (Wilson and Laurent, 2005; Wilson et al., 2004).

Narrowly and Broadly Tuned Receptors Represent Extremes on a Continuum of Tuning Breadths

The ability of an animal to detect and discriminate among an immense number of odors depends on the number of its odorant receptors and their breadth of tuning. Functional studies of a number of odorant receptors have

Figure 5. Temporal Dynamics of the Receptor Repertoire

Each graph shows a temporal surface constructed from the responses of each receptor over the course of 2 s to a 0.5 s pulse of odorant. Responses were assayed in 500 ms bins, beginning at the onset of odor stimulation. Odors were administered as 10^{-2} (A) or 10^{-4} (B) dilutions. For each odor, receptors are arrayed in order of ascending response of the receptor to the 10^{-2} dilution at t_0. The order in (A) and (B) is the same for each odor, but the orders differ for different odors, as specified in the Supplemental Data. n = 6.

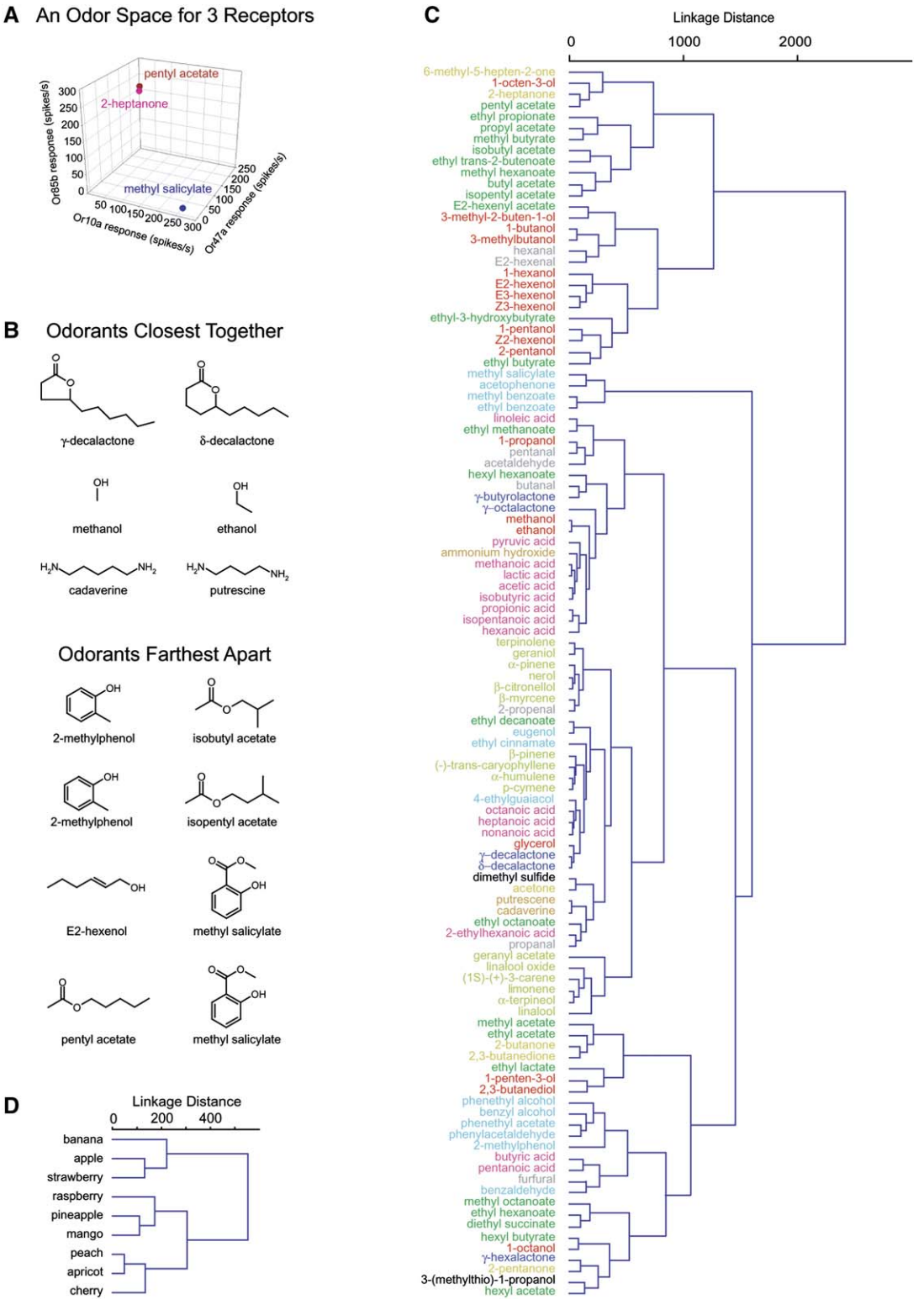


Figure 6. A Representation of Odor Space

(A) An odor space for three receptors. Each axis represents the response of one receptor in spikes/s to a given odorant. Each odorant maps to a particular position in this three-dimensional space.

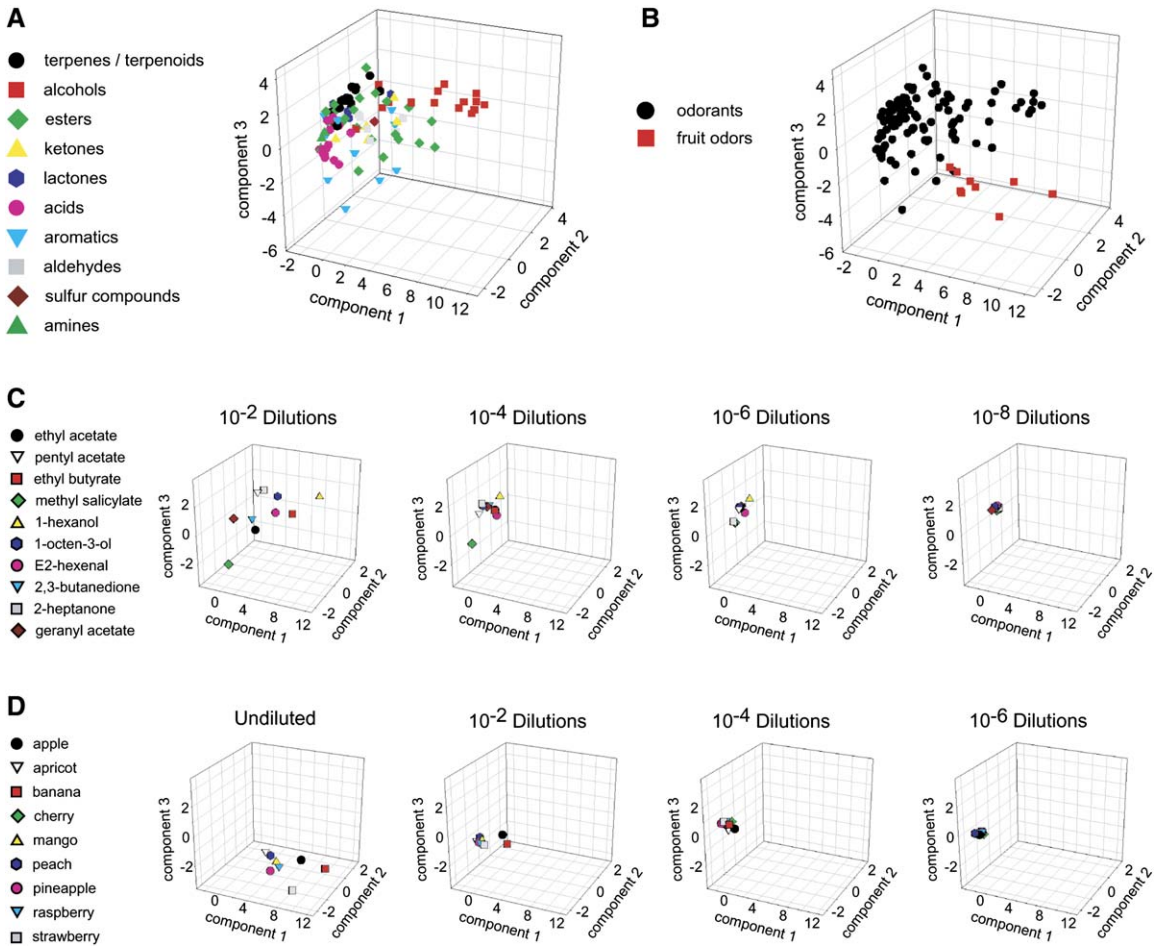


Figure 7. Odor Space as Visualized by PCA

(A) Relationships among pure odorants of the indicated chemical classes at 10^{-2} dilutions.

(B) Relationships among pure odorants (10^{-2} dilutions) and fruit odors (undiluted).

(C–D) Relationships among pure odorants (C) and fruit odors (D) across concentrations. In all of these panels, vectors quantifying the responses of the 24 antennal receptors to each tested odor were projected onto a three-dimensional region. Each axis represents the normalized action potential responses of the receptors in a new coordinate system determined by PCA. This three-dimensional representation captures ~58% of the variation in the original 24-dimensional data set. PCA was performed using the responses of each receptor to each of the pure odorants and fruit odors tested at every concentration tested. Thus, although each graph contains only a subset of the total data points used in the analysis, the same principal components have been used to plot each graph, and, thus, direct comparisons can be made between the different graphs. However, we note that only a limited number of data points for low odor concentrations were included in the analysis, and the graphs may not display the best possible view of each subgroup considered separately.

shown that some receptors appear more narrowly tuned than others (Araneda et al., 2000; Goldman et al., 2005; Hallem et al., 2004b; Kajiya et al., 2001; Krautwurst et al., 1998; Kreher et al., 2005; Malnic et al., 1999; Saito et al., 2004; Stortkuhl and Kettler, 2001; Wetzel et al., 2001), yet the response specificities of very few receptors have been examined in detail in any organism.

We found that receptors vary greatly in their breadth of tuning: Some receptors responded to many tested odorants, while others responded strongly to one or none (Figures 2A and 2B). Insect ORNs have been proposed to fall into two classes, specialists and generalists, based on classical electrophysiological studies (Boeckh et al., 1965; de Brito Sanchez and Kaissling, 2005; Schneider

(B) Odorant pairs that are closest together and farthest apart in the 24-dimensional odor space constructed from the responses of each of the 24 antennal receptors.

(C and D) Hierarchical cluster analysis for pure odorants (C) and fruit odors (D) based on the Euclidean distances between odors. Odorants are color coded by chemical class as in Figure 1.

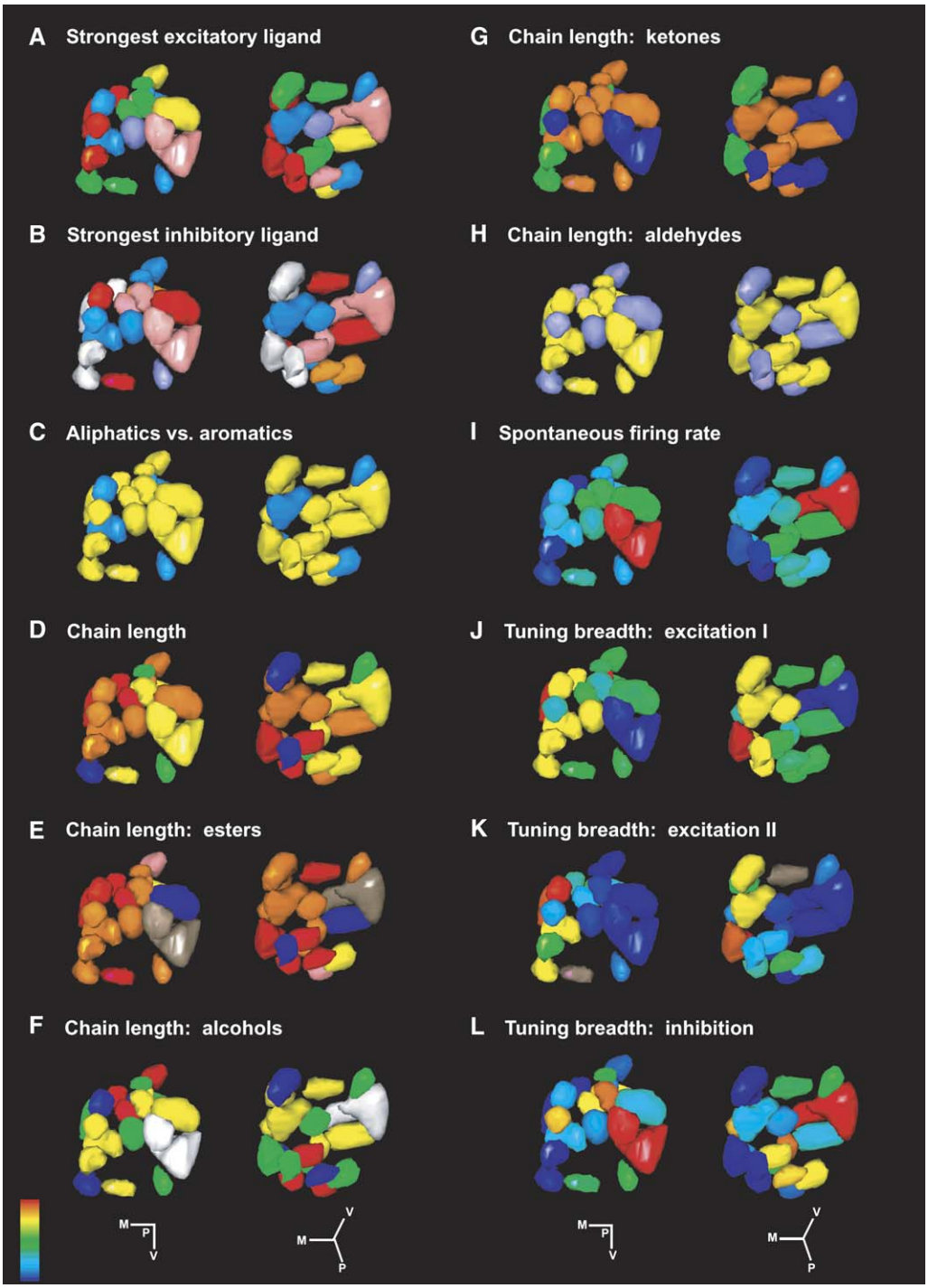


Figure 8. Mapping Receptor Response Properties onto Glomeruli in the AL

Each glomerulus is color coded based on the response properties of the receptor expressed in its presynaptic ORN population. In each panel, the AL is shown from two different perspectives, as indicated at the bottom of each column. In the perspective on the left of each panel, posterior is below the plane of the page; in the right perspective, all three axes point up from the page.

(A) Strongest excitatory ligand for each receptor based on its chemical class. Red = ester, pink = aldehyde, green = alcohol, purple = terpene, blue = aromatic, yellow = ketone.

(B) Strongest inhibitory ligand for each receptor based on its chemical class. Colors are as in (A), with the following additions: orange = amine, gray = no inhibitory responses.

and Steinbrecht, 1968). According to this dichotomy, specialists respond to one or a small number of odors of particular biological importance, such as a pheromone, whereas generalists respond to a wide variety of odors. Our identification of narrowly and broadly tuned odorant receptors provides a molecular explanation for the narrow and broad response spectra of specialist and generalist ORNs. However, our results do not yield a neat dichotomy. Rather, we find a continuum of tuning breadths (Figure 2B).

A Receptor Code for Odor Intensity

Animals in their natural environment encounter a wide range of odor concentrations. The ability to encode odor intensity is essential for successful navigation toward odor sources. Several previous studies have found that higher odor concentrations activate more neurons than lower concentrations (Bozza et al., 2004; Fried et al., 2002; Ma and Shepherd, 2000; Ng et al., 2002; Wang et al., 2003); however, intensity coding at the level of an entire olfactory organ has not been examined by direct analysis of the receptor repertoire.

We found that at higher concentrations, many odorants activate multiple receptors, while at lower concentrations, many odorants activate fewer receptors (Figure 4). These results provide a mechanism by which the receptor repertoire can extend the dynamic range of the olfactory system. Intensity coding would depend on both the strength of activation of individual receptors as well as the total number of receptors activated. The multiplicity of receptors that are activated by an individual odorant would allow a more precise assessment of the concentration of that odorant, especially if different receptors have different activation thresholds for the odorant.

Temporal Representations of Odor Identity, Intensity, and Duration

We have found that the temporal dynamics of activity across the receptor repertoire differ for odor stimuli of different quality and quantity. We have illustrated these patterns of activity as temporal surfaces and have found that some odors, but not all, evoke highly dynamic patterns (Figure 5). In the more dynamic patterns, receptors show striking differences in their response kinetics to the same odor. Among the receptors that give the strongest initial responses, some yield phasic responses that terminate quickly, while others show tonic responses that continue well beyond the end of the odor stimulus.

Thus, the receptor repertoire produces a complex temporal representation of an odor stimulus. Elegant experiments in other insects have provided evidence that temporal coding enhances the ability to discriminate between similar odors (Friedrich and Laurent, 2001; Laurent et al., 2001; Lei et al., 2004; Stopfer et al., 1997). It will be interesting to determine how the temporal representations we have documented in *Drosophila* are transformed into central representations and whether the rich temporal information we have documented in *Drosophila* contributes to odor discrimination.

We have also found that each of five tested receptors is capable of encoding information about the duration of an odor pulse (Figure S4). Since the spatiotemporal structure of an odor plume depends on its distance from the odor source, this mechanism for coding of pulse duration is likely to be essential for navigation toward an odor source.

The Odor Space of the Fly

We constructed a 24-dimensional space in which each dimension corresponds to the response of a single antennal receptor. The closest odorants in the space were structurally similar (Figure 6B), and the chemical class—defined by the functional group of its members—is one determinant of an odorant's map position in the space. The molecular complexity of an odor stimulus also appears to be a prominent determinant of position in odor space (Figure 7B).

Another determinant of position in odor space is concentration. As the concentrations of various odorants decrease, their positions in odor space change and in fact converge (Figures 7C and 7D). These findings are reminiscent of the results of psychophysical studies of human odor perception. In humans, odor quality often varies greatly as a function of odorant intensity, detection of small differences in odorant intensity is often possible,

(C) Aliphatic (yellow) versus aromatic (blue) responsive receptors. Glomeruli are colored according to the strongest ligand for the corresponding receptor.

(D) Chain length of the strongest excitatory aliphatic ligand for each receptor. Chain length of 8 = red, 7 = orange, 6 = yellow, 5 = green, 4 = blue.

(E–H) Chain length of the strongest aliphatic odorant of each chemical class for each receptor. Color coding is as in (D), with the following additions: chain length of 2 = purple, 9 = pink; 13 = brown, gray = no excitatory responses to odorants of the indicated chemical class.

(I) Spontaneous firing rates for each receptor. Glomeruli are color coded using a normalized color gradient (lower left); highest = red, lowest = dark blue.

(J) Breadth of tuning to excitatory ligands for each receptor. Breadth of tuning was calculated for each receptor by listing odorants in descending order of mean spike frequency and determining the number of odorants whose spike frequencies sum to >95% of the summed total frequencies for all excitatory odorants. Color coding is as in (I), with highest (i.e., most broadly tuned) = red and lowest (i.e., most narrowly tuned) = dark blue.

(K) Breadth of tuning to excitatory ligands, as determined by the percentage of odorants that elicit strong responses of ≥100 spikes/s. Color coding is as in (I).

(L) Breadth of tuning to inhibitory ligands for each receptor, as determined by the percentage of odorants that elicit inhibitory responses. Color coding is as in (I). Glomerular models were obtained from FlyBrain (http://flybrain.neurobio.arizona.edu/) (Laissue et al., 1999). The DM3 and DM5 glomeruli were shaded according to the responses of Or47a and Or85a, respectively, rather than the coexpressed but virtually unresponsive receptor Or33b (Couto et al., 2005; Fishilevich and Vosshall, 2005; Hallem et al., 2004b). Or2a, Or67c, and Or85b were not included in the analysis either because the receptor has not been conclusively mapped to a glomerulus or because the corresponding glomerular coordinates were not available (Laissue et al., 1999).

and low concentrations of odors rarely contain a qualitative component (Cain, 1977; Doty, 1992; Gross-Isseroff and Lancet, 1988). In *Drosophila*, it is clear that changes in odor concentration affect behavioral response: Many odorants are repellents at high concentrations but attractants at low concentrations (Rodrigues and Siddiqi, 1978; Stensmyr et al., 2003). However, the ability of *Drosophila* to distinguish between different concentrations of the same odorant has not been systematically tested.

The odor space we have constructed lays a foundation for a wide variety of behavioral studies. In particular, the space provides a rational basis for predicting the ability of an animal to discriminate between odors: It is possible that odors that map close together in the space are more difficult for the fly to distinguish than odors that map far apart. To assess the predictive power of the space, the relationship between Euclidean distance in the space and the ability to discriminate may be investigated by determining the following. (1) If odors A and B map close to each other but far from C in odor space, is the pair (A,B) less discriminable than (A,C) and (B,C)? (2) If A and C map far apart when tested at the same concentration (A(x) and C(x)) and can be discriminated, but A(x) and C(y) map close together, are A(x) and C(y) discriminable? (3) If the odor of binary mixture (A+B) maps close to odor C, are (A+B) and C discriminable?

We note that our analysis of odor space is a working model: It does not include every receptor in the adult olfactory system (Couto et al., 2005; Fishilevich and Vosshall, 2005; Vosshall et al., 2000; Yao et al., 2005), and it is not possible to test every odorant that the fly encounters in nature. To ascertain the extent to which the odor space would be likely to change with the inclusion of additional receptors, we performed four tests in which we repeated our analysis after the random removal of 5 of the 24 receptors (Figure S7). In each case, we found that, while the positions of individual odorants differed, the overall conclusion did not change: Odorants cluster by functional group. In addition, it is instructive to compare the results of the current analysis with those of our previous analysis (Hallem et al., 2004b). While that study did not systematically analyze inhibition, temporal dynamics, or responses to complex odor mixtures, it documented excitatory responses to a selected panel of ten odorants, and two of its conclusions have been further substantiated here: that receptors vary widely in their breadth of tuning, and that odorants vary widely in the number of receptors they activate.

Representations of Odorant Receptor Activity in the Antennal Lobe

In mammals and other insects, nearby glomeruli are often activated by similar odorants (Belluscio and Katz, 2001; Lei et al., 2004; Meister and Bonhoeffer, 2001; Sachse et al., 1999; Uchida et al., 2000). We have observed a general tendency for receptors that are more broadly tuned and those that have lower spontaneous firing rates to map more medially than receptors that are more narrowly tuned and those that have higher spontaneous firing rates. However, we have not found evidence in *Drosophila* of the ordered chemotopy that has been observed in mammals and other insects. Our analysis has shown that receptors with similar response properties often project to glomeruli located throughout the AL, and receptors with very different response properties often project to nearby glomeruli (Figure 8; Figure S6). Electrophysiological studies of AL neurons in *Drosophila* and other insects have found that odorant representations are transformed in the ALs (Stopfer et al., 2003; Wilson et al., 2004), and molecular and cellular analysis (Wilson and Laurent, 2005) should provide insight into the mechanisms of transformation in the *Drosophila* AL.

In summary, we have provided a systematic, quantitative analysis of the primary representation of an odor as registered in the differential activities of the receptor repertoire. This analysis provides a foundation for investigating how the primary odorant representation is transformed to subsequent representations and ultimately to the behavioral output of an olfactory system.

EXPERIMENTAL PROCEDURES

Drosophila Stocks and Transgenes

The ab3A mutant flies and *Or22a-GAL4* and *UAS-Or* constructs were described previously (Dobritsa et al., 2003; Hallem et al., 2004b). Electrophysiological recordings were obtained from flies of genotype *w;Δhalo/Δhalo;Or22a-GAL4/UAS-Or*, unless otherwise indicated.

Electrophysiology

Extracellular single-unit recordings were performed as described previously (Hallem et al., 2004b). Odorant stimuli were prepared in Pasteur pipettes (Dobritsa et al., 2003; Hallem et al., 2004b), with the exception that stimuli for the experiment described in Figure S4 were prepared in 25 ml pipettes to allow a sufficient volume of equilibrated headspace for the prolonged pulses. Chemicals were >99% pure or of the highest purity available (Sigma-Aldrich) and were racemic mixtures with the exception of (1S)-(+)-3-carene and (−)-trans-caryophyllene. The following odorants were diluted in H_2O: methanol, ethanol, acetaldehyde, acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, isobutyric acid, isovaleric acid, pyruvic acid, lactic acid, formic acid, ammonium hydroxide, putrescine, and cadaverine; all other odorants were diluted in paraffin oil. Fruit odors were from natural ethanol extracts of fruits (Polarome) diluted in H_2O.

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 pulse of charcoal-filtered air (5.9 ml/s) through the pipette containing the odorant. Pulse duration was 500 ms unless otherwise indicated. Stimuli were used for a maximum of four presentations. Responses were quantified by subtracting the number of impulses in 500 ms of unstimulated activity from the number of impulses in the 500 ms following odorant stimulation, unless otherwise indicated. For each odorant, each recording was from a separate sensillum, with no more than three sensilla analyzed per fly. Recordings were obtained from flies aged <4 weeks.

Data Analysis

Principle component analysis (PCA) and hierarchical cluster analysis were performed using PAST, a statistics program (http://folk.uio.no/ohammer/past/). PCA was performed using the correlation matrix, and Euclidean distances and Ward's classification method were used for the hierarchical cluster analysis.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, four tables, and seven figures and can be found with this article online at http://www.cell.com/cgi/content/full/125/1/143/DC1/.

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