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Translation of sensory input into behavioral output via an olfactory system

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Summary

We investigate the logic by which sensory input is translated into behavioral output. First we provide a functional analysis of the entire odor receptor repertoire of an olfactory system. We construct tuning curves for the 21 functional odor receptors of the *Drosophila* larva, and show that they sharpen at lower odor doses. We construct a 21-dimensional odor space from the responses of the receptors and find that the distance between two odors correlates with the extent to which one odor masks the other. Mutational analysis shows that different receptors mediate the responses to different concentrations of an odorant. The summed response of the entire receptor repertoire correlates with the strength of the behavioral response. The activity of a small number of receptors is a surprisingly powerful predictor of behavior. Odors that inhibit more receptors are more likely to be repellents. Odor space is largely conserved between two dissimilar olfactory systems.

Keywords

Olfaction; *Drosophila*; odor receptor; larva; behavior

Introduction

One of the great challenges of neuroscience is to understand the rules by which sensory input is translated into behavioral output. Enormous progress has been made in elucidating principles by which sensory information is encoded by receptors and cells of the peripheral nervous system. Less is known, however, about the internal representations of sensory stimuli at each successive level of circuitry. Anatomical studies have traced synaptic connections in early stages of some circuits, and electrophysiological or imaging studies have revealed how representations of stimuli are transformed at some of these stages. However, the overall logic by which sensory information dictates a particular behavioral response in an organism remains largely unknown. An important long-term goal is the ability to predict the behavior of an organism from the activity of its sensory receptors.

In the case of olfaction, a major impediment to understanding how sensory input is translated into behavior is the numerical complexity of olfactory systems. A full molecular description

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of the sensory input into the system requires a functional analysis of the entire receptor repertoire. Olfactory systems of mammals and of *C. elegans* contain hundreds of different receptors (Ache and Young, 2005), and a functional analysis of an entire repertoire has not been possible with the available technology. In *Drosophila*, a large-scale analysis recently described the coding of odors by 24 antennal receptors (Hallem and Carlson, 2006; Hallem et al., 2004b); however, the analysis did not include eight other antennal receptors or the seven receptors of the maxillary palp, the other organ of the fly olfactory system.

In this study we present an analysis of the full receptor repertoire of an olfactory system and of the behavior that it generates. The analysis concerns the olfactory system of the *Drosophila* larva, which is morphologically and developmentally distinct from that of the adult. Odors are sensed by the larval dorsal organ, a dome of cuticle at the anterior end of the larva that is perforated by pores through which odors can pass (Hertweck, 1931; Louis et al., 2008; Oppliger et al., 2000; Singh and Singh, 1984; Stocker, 1994). The dome is innervated by the dendrites of 21 olfactory receptor neurons (ORNs). As in the adult fly and in vertebrates, each ORN expresses one or a small number of receptors (Fishilevich et al., 2005; Kreher et al., 2005). Each ORN projects to a discrete region of the larval antennal lobe that is referred to as a glomerulus (Python and Stocker, 2002a). Each glomerulus appears to be innervated by one projection neuron, which in turn sends an axon to one or two glomeruli in the mushroom body (Ramaekers et al., 2005). The glomeruli of the antennal lobe are interconnected by local interneurons that arborize throughout the antennal lobe.

Of the 60 *Or (Odor receptor)* genes of *Drosophila*, a subset was found to be expressed in the larva, and 11 of these were demonstrated to encode functional odor receptors (Kreher et al., 2005). The odor response spectra of these receptors were examined using the “empty neuron” system, an *in vivo* expression system based on a mutant ORN of the fly that lacks an endogenous receptor. Individual larval *Or* genes were expressed in this ORN and the odor responses that they conferred were determined by electrophysiological recordings. These 11 genes represent less than half of the total larval odor receptor repertoire, however. Expression studies (Couto et al., 2005; Kreher et al., 2005), and most notably a systematic *in situ* hybridization analysis (Fishilevich et al., 2005), have shown that 25 of the *Or* genes are expressed in the larval olfactory system. Approximately half of these larval *Or* genes are also expressed in the adult olfactory organs.

Olfactory responses of fly larvae have inspired investigation since the 17th century (Cobb, 1999). *Drosophila* larvae respond behaviorally to a wide variety of odors (Aceves-Pina and Quinn, 1979; Ayyub et al., 1990; Cobb, 1999; Fishilevich et al., 2005; Larsson et al., 2004; Monte et al., 1989; Oppliger et al., 2000). Some odors elicit strong attractive responses whereas others are repellent. A recent study showed that when ORNs expressing the *Or1a*, *Or42a*, or *Or49a* genes were genetically ablated, larval responses to subsets of odorants were defective (Fishilevich et al., 2005). Conversely, larvae containing only an Or42a-functional ORN, i.e. larvae in which the only functional ORN is one that expresses Or42a, were able to respond to a subset of stimuli. This study further showed that although larvae containing only Or1a- or Or49a-functional ORNs did not chemotax toward any tested odors, larvae containing both an Or42a-functional ORN and either an Or1a-functional ORN or an Or49a-functional ORN responded to a somewhat different subset of odorants than did larvae containing only an Or42a-functional ORN. These data supported a model in which the larval behavioral response depends on the combined input from multiple odor receptors. The results illustrate the value of a systematic analysis of the role of the entire larval receptor repertoire in generating behavior.

To investigate the logic by which sensory input is converted to behavioral output, we first define odor response spectra for the full larval odor receptor repertoire. We measure behavioral responses to all odorants of the panel and find that the responses show a smooth distribution

from strong attractive responses to repellent responses. We then examine the perceptual relationship among odors. A 21-dimensional odor space is constructed from the responses of the 21 receptors, and the distance between two odors in the space correlates with the likelihood that one odor will mask the other. We then examine how the responses of multiple receptors are integrated to generate behavior. Using mutants of receptor genes, we show that responses to different concentrations of an odorant depend on different receptors. Or42b, which in electrophysiological analysis has a low threshold for ethyl acetate, is required for behavioral response to low concentrations of ethyl acetate; Or42a, which has a high threshold, is required for behavioral response to high concentrations. We next consider responses to all odors of the panel and find that the summed receptor input across the entire receptor repertoire shows a modest correlation with the behavioral response index. We then find that the behavioral response can be predicted with surprising accuracy from the responses of a small subset of receptors. Moreover, odors that inhibit many receptors are more likely to be repellents, and a discrimination function is able to distinguish repellents from non-repellents on the basis of receptor responses. Finally, we provide evidence that odor space is largely conserved between two dissimilar olfactory systems, those of the larva and the adult.

Results

Response spectra of the larval odor receptor repertoire

A consensus set of 25 larval Or receptors was identified on the basis of three studies of *Or* gene expression (Couto et al., 2005; Kreher et al., 2005). The expression of these 25 genes in larval ORNs has been documented either by in situ hybridization (Fishilevich et al., 2005) or, in the case of *Or49a*, by reporter gene expression driven by an *Or* promoter via the *GAL4/UAS* system (Fishilevich et al., 2005; Kreher et al., 2005). We have expressed all 25 genes in the empty neuron system and tested them with a diverse panel of odorants. A previous work found that the response spectrum, response mode, i.e. excitation or inhibition, and temporal dynamics of antennal receptors were the same in the empty neuron as in the ORN in which they were normally expressed (Hallem et al., 2004b). The present analysis includes 14 receptors that were not tested in our earlier study of larval odor receptors (Kreher et al., 2005), and it extends the analysis of the other 11 receptors.

Of the 25 *Or* genes, 21 conferred odor responses. Alleles of the remaining four genes *(Or1a, Or33a, Or63a*, and *Or83a)* in our laboratory Canton-S strain appeared to be non-functional. They did not confer a regular pattern of spontaneous action potentials, and they did not confer any odor-evoked responses when expressed from genomic constructs in the empty neuron system. Or1a and Or33a each contain an amino acid polymorphism, in comparison to another wild-type strain, Oregon-R. The Oregon-R alleles conferred a regular pattern of spontaneous action potentials; the Oregon-R allele of Or33a did not confer strong responses (≥100 spikes/s) to any odorant of our panel or to mixtures representing 100 odorants (Figure S1A and data not shown). The Oregon-R allele of Or1a yielded a response >50 spikes/s only to a mixture containing methyl benzoate, which activates an Or1a-expressing ORN in that strain (Fishilevich et al., 2005; Louis et al., 2008). Not only did Or63a not function when expressed from Canton-S genomic DNA, but it did not function when expressed from Canton-S cDNA, or from Oregon-R genomic DNA. We recovered a second allele of *Or83a* from Canton-S that contains a premature stop codon. In summary, the simplest interpretation of our results is that in our Canton-S laboratory strain, 21 *Or* genes encode functional odor receptors.

The 21 receptors were systematically tested with 26 diverse odorants, each examined at two concentrations, and CO_2; thus 1113 receptor-odorant combinations were tested (Figure 1). The odorants were selected to include ketones, aromatics, alcohols, esters, aldehydes, a terpene, and an organic acid, and they include compounds of varying chain lengths. We initially tested the odorants as "10^{-2}" dilutions (see Experimental Procedures); this dosage has been used in

many previous studies (De Bruyne et al., 2001;Hallem et al., 2004b;Kreher et al., 2005;Larsson et al., 2004;Wilson et al., 2004) and allows a convenient assessment of the response spectra of receptors. Lower concentrations were administered as 10^{-4} dilutions (Hallem and Carlson, 2006). At the higher concentration tested, 19% of the receptor-odorant combinations yielded an excitatory response of ≥50 spikes/s, 12% yielded a strong response, defined here as ≥100 spikes/s, 8% yielded a response of ≥150 spikes/s, and 4% yielded a response of ≥200 spikes/s (Figure 1A and Figure S2A).

Receptors varied a great deal in the number of odorants that excited them strongly. At one extreme, Or67b was strongly excited by eight odorants (30% of the panel) and Or42a and Or85c were strongly excited by seven at the higher concentration. At the other extreme, four of the 21 receptors were not strongly excited by any odorants. The receptors showed a smooth distribution between these two extremes (Figure S2A).

Odorants varied according to the number of receptors that they strongly excited. 1-hexanol elicited a strong excitatory response from six receptors at the higher concentration, whereas four compounds did not strongly excite any receptor (Figure 1A).

Inhibitory responses, defined as a reduction in the firing rate to ≤50% of the spontaneous level, were also observed (Figure 1, Figure S2A). At the higher doses, most odorants inhibited at least one receptor, and most receptors were inhibited by at least one odorant. Our estimate of the extent of inhibition should be regarded as an approximation. For example, it is difficult to observe inhibition in the receptors with low spontaneous firing rates. We also note that we have adopted a conservative definition of inhibition.

A small fraction of the odorant-receptor combinations tested here were also evaluated in a previous study of antennal receptors (Hallem and Carlson, 2006). The results are generally in good agreement (see Experimental Procedures).

At lower odorant concentrations, strong excitatory responses are much more sparse. When the concentration was reduced by two orders of magnitude, the percentage of combinations producing ≥50 spikes/s fell from 19% to 4%, the percentage producing ≥100 spikes/s fell from 12% to 2%, and the percentage producing ≥200 spikes/s fell from 4% to 1% (Figure 1B). Of the nine receptors that responded strongly to at least one odorant at low concentrations, six responded strongly to one odorant only. No odorant elicited a strong response from more than one receptor.

We have established tuning curves for each receptor of the larval repertoire, at each concentration tested (Figure 2). When tested with 10^{-2} dilutions, the tuning curves showed a range of tuning breadths, with some receptors appearing very broadly tuned. By contrast, when tested with 10^{-4} dilutions, none of the receptors appeared broadly tuned, and many appeared more narrowly tuned.

Behavioral responses

Having thus examined the physiological responses generated by each odor of the panel, we then measured the behavioral responses of the larva to each odor. We used a simple behavioral paradigm in which larvae migrate across a Petri plate toward a source of odorant (Figure 3A). A response index (RI) was calculated at the end of a 5 min test period, as in earlier studies (Monte et al., 1989; Rodrigues and Siddiqi, 1978), by counting the number of animals, S, on the half of the plate containing the odorant, subtracting the number, C, on the control half, and dividing by the total; thus RI=(S-C)/(S+C). The RI ranges from 1 (complete attraction) to 0 (no response) to -1 (complete repulsion).

The odors of the panel elicited a broad range of responses (Figure 3B). Most odors were attractive at the test concentration, with E2-hexenal eliciting the strongest response: RI= 0.74 ±0.07 (n=10 trials). Among repellents, the strongest was geranyl acetate: RI= -0.40±0.07 (n=10 trials; for statistical analysis of repellency, see Experimental Procedures). Odorants of diverse chemical classes elicited strong responses of the same response mode, *i.e.* the strongest attractants (E2-hexenal, propyl acetate, 2-heptanone) are an aldehyde, ester, and ketone, respectively. Likewise, the repellents included a terpene, a ketone, an alcohol, and aromatics.

Predicting perceptual quality from odor space: odor masking

We used the responses of the receptor repertoire to examine perceptual relationships among odorants. We constructed a 21-dimensional odor space in which each dimension represents the response of one odor receptor in spikes/s. Odorants were plotted in this space based on the responses they elicited at a 10^{-2} dilution. We then calculated distances pairwise between all odorants. Two kinds of distances were determined. First, Euclidean distances were calculated pairwise between all odorants. Second, angular distances were calculated, based on the angle between the two vectors that extend from the origin of the 21-dimensional space to each of the two odorants (see Experimental Procedures).

From the matrix of distances we constructed two separate three-dimensional projections of odor space, one based on the Euclidean distances and one based on the angular distances, using Multi-Dimensional Scaling (MDS; Figure 4A). The closest and most distant pairs of odorants in terms of Euclidean distance are shown in Figure 4B. Members of the closest pairs are structurally related. (We exclude from consideration three odorants that fail to elicit a response of >50 spikes/s from any receptor and that map near each other for this reason.) The most distant relationships, by contrast, are in each case between an aliphatic and an aromatic odorant. In the case of angular distance, the most closely related pairs are again structurally related, and the distant pairs are again structurally dissimilar (Figure 4C). The two closest pairs in angular distance are also two of the closest in Euclidean distance. Overall, the Euclidean and angular distances were generally concordant for pairs of odors that were close in odor space, but showed less agreement for pairs of odors that were more distant (Figure S3).

We note that in general the Euclidean distance is more sensitive to the magnitudes of receptor responses, whereas the angular distance is more sensitive to the "pattern" of receptor firing, i.e. to the identity of the responding receptors. (Consider, for example, two vectors in a three-dimensional space whose directions are very similar; increasing the magnitude of one vector will have an effect on the Euclidean distance but not on the angular distance.) The Euclidean distances among odors were used as the basis of a hierarchical cluster analysis (Figure S4), which shows that in a number of cases structurally similar odors clustered together, although in no case did all odorants of a particular structural class cluster together.

We then asked whether two odorants that are close in odor space are close in perceptual qualities. There are several paradigms for examining perceptual relationships between two odorants. We have examined odor masking, a phenomenon that is convenient to measure and may be of direct importance to animals in their natural environment by affecting what odors they perceive.

The odor masking paradigm is based on the behavioral paradigm shown in Figure 3A. Responses to a point source of odorant A are measured in the presence of a background of odorant B; odorant B is uniformly distributed across a filter that covers nearly the entire lid of the Petri dish in which the assay is conducted. We then determine whether the background of odorant B decreases the response of the animals to odorant A. This paradigm was used in a previous study of *Drosophila* larvae (Rodrigues, 1980), and a very similar paradigm has been used in *C. elegans* (L'Etoile and Bargmann, 2000; Wes and Bargmann, 2001): if the worms

responded to odor A in a background of B, then they were inferred to be able to discriminate A from B; if they cannot discriminate A from B, then a background of A was expected to block the response to B. We will interpret the results not in terms of discrimination but in terms of masking. The ability to identify odors in a background of other odors has also been described as odor segmentation (Wilson and Mainen, 2006).

As an initial test we asked whether the response to a point source of odorant A was decreased by the presence of a background of odorant A. We found that the response to a 10^{-4} dilution of ethyl acetate was in fact decreased by a background of ethyl acetate (Figure 5A). The mean response to the ethyl acetate source decreased progressively as the dose of the background odor was increased, over six orders of magnitude: a severe decrease was observed when the dilution of the masking odorant reached 10^{-4}, and the response was abolished when the masking odorant reached a 10^{-3} dilution.

We then systematically tested the odorants of our panel at a 10^{-2} dilution for their ability to block the response to ethyl acetate. The background odorants varied across a broad range in their effects, from complete masking to no masking (Figure 5B). In addition to ethyl acetate, a background of propyl acetate, ethyl butyrate, or 1-hexanol reduced the RI to below 0.2.

The analysis was then extended to examine masking of responses to five additional odorants that elicited strong behavioral responses: 2-heptanone, 3-octanol, E2-hexenal, ethyl butyrate, and 2,3-butanedione. We tested the ability of ethyl acetate and other odorants to mask the responses to these five odorants (Figures 5C-G).

We found that at least one odor masked the response to a point source of each of the five tested odorants. When ethyl butyrate was used as the point source, ethyl acetate masked the response as potently as did ethyl butyrate itself. Reciprocally, among the five odorants used as a point source, ethyl butyrate most potently masked response to ethyl acetate (Figure 5B). When all the masking data (Figures 5B-G) were pooled, yielding 48 odorant pairs, there were 13 cases (27%) in which the RI to point source odorants was reduced by ≥50% compared to the control value observed when paraffin oil was used as the masking odor.

We then asked whether there was a correlation between the extent to which odor A masked odor B and the distance between them in odor space. We defined a masking index, MI, as a ratio of the RI in the absence and presence of the masking odorant (see Experimental Procedures). Thus, a high MI value indicates a high degree of masking, and a low MI value indicates a low degree of masking.

We found that odor masking correlated with distance in odor space. When angular distances were used to measure distance in odor space, we found an R^2 value of 44.0% by regression analysis ($p<0.0009$; Figure 6, black line). Moreover, for most of the point source odors in this analysis, the masking odors do not provide a broad sampling of odor space; when we limit the analysis to the point source odor ethyl acetate, in which all odors of the panel were sampled as masking odors, $R^2=55.9\%$ ($p<0.0009$; Figure 6, orange line). For 2-heptanone, which was also tested with a broader sampling of masking odors, R^2 was even higher: 58.4% ($p<0.0009$; Figure 6, red line).

When Euclidean distances were used to analyze the entire data set, a predictive relationship was still clearly observed, but the variation explained was lower than when angular distances were used: $R^2=35.1\%$, as opposed to $R^2=44.0\%$ for angular distances.

Behavioral response to one odorant across a concentration range depends on two receptors

Having observed a relationship between perception and the responses of the entire receptor repertoire, we next addressed how the responses of individual receptors are integrated to engender behavioral responses. We began by examining the response to ethyl acetate, which elicits behavioral responses over doses spanning more than six orders of magnitude (Figure 7A), but which elicits strong responses from only two receptors, Or42a and Or42b (Figure 1A).

Or42a and Or42b show a striking difference in their sensitivity to ethyl acetate, as measured physiologically (Figure 7B). The half-maximal response of Or42b occurs at a dose that is below the threshold of Or42a, suggesting that Or42b is a high-affinity receptor and that Or42a is a low-affinity receptor. Thus Or42b is most informative at low ethyl acetate concentrations and Or42a is most informative at high concentrations.

We next examined the roles of these receptors in driving behavioral response to ethyl acetate at concentrations ranging over three orders of magnitude, a range in which the behavioral response is relatively uniform (Figure 7C). To determine how the behavioral response depends on the activities of the two receptors, we examined mutants of each. Mutants of *Or42b*, i.e. $Or42a^{+}Or42b^{-}$, which lack the receptor that is sensitive to low concentrations, show a reduced response at low concentrations, but an approximately normal response at high concentrations (Figure 7C). Mutants of *Or42a*, i.e. $Or42a^{-}Or42b^{+}$, give a response index that is approximately normal at low concentrations, but reduced at high concentrations. These results suggest that the behavioral response to low concentrations is driven primarily by Or42b, and the response at high concentrations is dependent on Or42a.

Interestingly, the response of $Or42a^{-}Or42b^{+}$ mutants declines and becomes repellent as the concentration increases. One possible interpretation, among others, is that hyperactivation of Or42b, which is very sensitive to ethyl acetate, triggers a repulsion circuit, but that in wild type this circuit is overridden or suppressed by activation of Or42a. In any case, this result is consistent with the existence of non-linearity in the olfactory circuitry and the possibility of interactions among ORNs in the larval antennal lobe via lateral connections, which have been documented in the more complex olfactory system of the adult fly (Bhandawat et al., 2007; Kazama and Wilson, 2008; Olsen et al., 2007; Olsen and Wilson, 2008; Shang et al., 2007).

As a control for these experiments, we have shown that mutations of *Or42a* and *Or42b* do not have a general effect on odor response: both respond normally to 2-heptanone and propyl acetate (Figure 7D).

In summary, the receptors that have low and high thresholds, respectively, for physiological response to ethyl acetate are the receptors that are required at low and high concentrations, respectively, for behavioral response. Thus two receptors are required in order for the animal to respond strongly to a broad range of ethyl acetate concentrations. We note that the consistency between the results of the physiological analysis and the behavioral genetic analysis provides additional validation of the empty neuron system.

Predicting the behavioral response index from receptor responses

Having shown how the response of two receptors can be integrated to engender the behavioral response to a single odor, we next sought to expand our focus and consider how the responses of the entire receptor repertoire are integrated. As an initial step, we summed the total number of action potentials elicited by each odorant from all 21 receptors of the receptor repertoire, and plotted each sum against the response index obtained for that odorant. Although we had not expected to find a simple relationship, we found a modest but clear correlation between the total spike input and the behavioral response: greater spike input correlated with greater RI_L (Figure S5A,B). (RI_L is used for statistical rigor; the RI was expressed as a log-odds ratio,

i.e. logit transformed to yield RI_L, as described in Experimental Procedures, to best satisfy the normality assumptions of the analysis. A plot of RI v. total spike input is nearly superimposable.) The correlation was observed when we plotted the total number of spikes elicited by a 10^{-2} dilution of each odorant (R^2=0.33, p=0.002) or by a 10^{-4} dilution (R^2=0.28, p=0.006). We found comparable correlation coefficients for attractants and repellents when considered separately, in the case of both concentrations (not shown).

This relationship encouraged us to ask whether a more refined function of receptor response might provide a more powerful prediction of behavior. We asked whether there exists a set of coefficients {a, b, c….,} which when multiplied by the physiological responses of receptors in spikes/s {R_1, R_2, R_3…,} would yield products that sum to equal the behavioral response, i.e. $RI_L = aR_1 + bR_2 + cR_3 +$…. In other words, we asked whether the behavioral response can be predicted as a weighted integration of input from the respective receptors.

We tested all possible subsets of receptors to identify subsets whose activities together predicted behavior. A stepwise linear regression model was used, along with several criteria for model selection. It should be noted that the modeling approach used here attempts to predict the behavioral outcome dictated by the olfactory circuitry, and does not presume to necessarily reveal the form of the circuitry. In particular, the number of receptors in the model arises from statistical procedures to minimize overfitting the data (see Experimental Procedures).

We were surprised to find that the responses of small subsets of receptors were powerful predictors of behavioral response. Remarkably, 81% of the behavioral variation could be explained by the activity of only five receptors (Or42a, Or45a, Or74a, Or82a, and Or85c), as follows:

$$RI_L = 0.0509 + 0.0061\ Or42a + 0.0022\ Or45a - 0.0039\ Or74a - 0.0113\ Or82a + 0.0050\ Or85c$$

We note that while the model above was ranked most highly in our analysis, we identified other models that explained similar levels of variation. However, of the ten models that explained the greatest degree of variation, while controlling for overfitting, all contain Or42a, Or82a and Or85c, and each model is based on six or fewer receptors. The models were selected using the criteria described in Experimental Procedures.

How well does the five-receptor model defined above predict the behavioral response to the odors of the panel? We assessed the predictive power of the model in two ways: first with a drop-out analysis and second with a novel set of odorants. For the drop-out analysis we systematically withdrew the data for each of the 26 odorants, fitted the regression using the responses of the five receptors to the remaining 25 odorants, and then predicted the behavioral response for the withdrawn odorant. A comparison of the predicted and observed behavior is shown for each odorant in Figure 8. Overall, the model predicted 74% of the behavioral variation when each odorant was withdrawn in turn.

We then tested the model with an entirely different set of odorants. We chose a set of 14 odorants that were not used in the original regression model. They were selected to have a similar representation of functional groups and chain lengths as the original set. We measured the activities they elicited from the five receptors of the model and the behavioral responses they elicited from the larva (Fig. S6). The model again predicted the behavioral responses well. Specifically, the model predicted 55% of the total behavioral variation, and a single odorant, 1-pentanol, accounted for 46% of the prediction error. If this odorant had been excluded, then the model would have predicted 71% of the behavioral variation among the remaining 13 odorants.

Predicting repellency from receptor responses

Perhaps the most fundamental classification of odor responses is the division into attractive and repellent behavioral responses. Little is known about the mechanistic basis by which an individual odor stimulus elicits attraction v. repulsion. In addition to the intrinsic scientific interest of this problem, the basis of repellency is of great practical interest: the ability to predict repellency could be of value, for example, in the development of new insect repellents.

Among the initial set of 26 odorants examined, six had negative RI values and can be considered as repellents (for statistical analysis, see Experimental Procedures). We wondered whether there were any common features among the physiological responses that these odorants evoked.

We noticed a striking relationship between repellency and inhibitory responses across the repertoire: of the three odors that elicit the greatest number of inhibitory responses from the receptors of the repertoire, all are repellents. The 26 odors of our panel elicited between 0 and 8 inhibitory responses from the 21 receptors when tested at a 10^{-2} dilution (Figures 1A and S2A). The two odors that elicited 8 inhibitory responses were 2-methyl phenol and fenchone, which are among the strongest repellents; the one odor that elicited 7 inhibitory responses was 1-nonanol, also a repellent (Figure 3B and Cobb et al., 1992). The one odor that elicited 6 inhibitory responses, moreover, was 4-methyl phenol, one of the weakest attractants. Overall, the mean number of receptors inhibited by the repellents was 5.8, whereas only 2.9 inhibitory responses were elicited by non-repellents ($p<0.01$, Mann-Whitney test).

Heretofore we have used a stringent definition of inhibition: a reduction in response to <50% of the spontaneous rate. As another test of the relationship between repellency and inhibition, we adopted a less stringent criterion: that the mean response rate is less than the mean spontaneous rate. Using this criterion we found that repellents inhibited 10.0 receptors v. 5.3 receptors for non-repellents ($p<0.01$, Mann-Whitney test).

While there is a clear difference in the mean number of receptors inhibited by repellents v. non-repellents, there is also overlap in the distributions. We therefore asked whether repellents could be predicted from the responses of the entire receptor repertoire using a multivariate classification procedure. We first used a linear discriminant function that provided a clear separation of repellents from non-repellents (Figure S7), but with some cross-validation error. We then found that perfect discrimination of the six repellents from the 20 non-repellents could be achieved using a non-parametric kernel density discrimination function (SAS, Proc DISCRIM), with zero cross-validation error. The number of repellents in the data set is low, and thus the results must be interpreted with caution. Moreover, the form of the discriminant function does not necessarily reflect the form of the circuitry driving the repellent response. However, this analysis suggests that a useful statistical prediction of repellent behavior can be developed from the physiological responses of the 21-receptor repertoire.

Odor space is largely conserved between two olfactory systems

Our analysis of the larval odor receptor repertoire allowed us to address the conservation of odor space among olfactory systems. Do two odorants that elicit similar patterns of activity among the receptors of one receptor repertoire elicit similar patterns among the receptors of another?

We examined the 16 odorants that have been tested against both the larval receptors and against adult antennal receptors {Hallem, 2006 #1491}. We computed both Euclidean distances and angular distances between each pair of odorants within each olfactory system and then compared the distances between the two olfactory systems by matrix correlations. The matrix correlation using angular distances was 0.728 and for Euclidean distances it was 0.559; both

correlations were significant at $p < 0.0001$ by a matrix permutation test (Mantel test) (Manly, 1991); this test shows that the elements of two matrices containing corresponding distances are significantly correlated). Thus, even though only 6 receptors overlap between the two sets of tested receptors (n=21 larval receptors; n=24 adult receptors), the overall distance relationships between the 16 tested odors, as measured by electrophysiological activity patterns, remain largely consistent between the two receptor repertoires.

To investigate further the similarity between larval and adult odor space, we performed the analysis again after excluding the six receptors that are common to both sets of receptors (*i.e.* we considered 15 larval receptors and 18 adult receptors). For the angular distance the matrix correlation was 0.675 ($p < 0.0001$) and for the Euclidean distance the matrix correlation was 0.606 ($p < 0.0001$). Thus the overall distance relationships between the 16 tested odors, based on these larval receptors and these adult receptors, are also largely consistent.

Discussion

Here we report a functional analysis of a complete odor receptor repertoire. This analysis has allowed us to measure the sensory input elicited by each of a panel of diverse odorants across an entire chemosensory system. We have also measured the behavioral responses generated by each odorant. By comparing the sensory physiology with the behavioral responses we have been able to address fundamental questions about how sensory input is translated into behavioral output.

The larval odor receptor repertoire

By systematically testing each receptor with a panel of odorants we were able to construct tuning curves for each receptor. At neither higher nor lower odor concentrations did we find evidence for a dichotomy between “specialist” and “generalist” receptors, as might have been expected from classic electrophysiological studies of insect ORNs (Boeckh et al., 1965; de Brito Sanchez and Kaissling, 2005; Schneider and Steinbrecht, 1968). At the higher concentration the receptors showed a smooth distribution in terms of the number of odorants that excited them strongly (Figure S2A). When tested at the lower concentration, no receptors appeared broadly tuned. Rather, many receptors showed strong excitation to only one odorant, or two closely related odorants, and none showed strong excitation to more than two of the tested odorants.

The functional diversity of the receptors may reflect the striking economy of this olfactory system. In a system that contains only 21 ORNs and 21 functional receptors, the receptors may be under strong selective pressure to diversify their odor response spectra. In addition to the ability to detect odors, the ability to discriminate odors may be a major source of selection on the receptor repertoire. The 26 odorants elicited highly diverse patterns of responses among the receptor repertoire. The results support a model in which odor quality can be identified based on the combination of receptors that respond (Hallem and Carlson, 2006; Malnic et al., 1999). A recent study of larval odor receptors in the mosquito *Anopheles gambiae* lends further support to this model (Xia et al., 2008).

Receptor activity and perception

We have demonstrated and quantitated a relationship between receptor activity and odor masking. Specifically, we constructed an odor space based on the response of the 21 receptors and found that the distance between odors correlates with the extent to which one odor masks the other. The correlation was highest when the distance between odors was measured in terms of angular distance.

One interpretation of these results is that odors that are close in odor space elicit similar activity patterns that are difficult for the system to resolve. Thus the signal of the point source is lost in the background of the masking odorant because they elicit similar firing patterns. Another explanation is cross-adaptation: if the point source odor and the background odor activate the same receptors, then adaptation to the background odorant might prevent these receptors from signaling the presence of the point source (Cobb and Domain, 2000; De Bruyne et al., 1999; L'Etoile et al., 2002; Stortkuhl et al., 1999).

There were exceptions to this relationship: ethyl acetate and 2,3 butanedione, for example, are close in both Euclidean and angular odor space, but neither masks the other. It is possible that the representations of these odorants, while similar among the receptor repertoire, become dissimilar following transformations that occur in the CNS (Bhandawat et al., 2007; Daly et al., 2004; Olsen et al., 2007; Olsen and Wilson, 2008; Root et al., 2007; Shang et al., 2007; Wilson and Mainen, 2006). It is also possible that these two odors elicit markedly distinct temporal patterns of activity among the receptor repertoire that were not revealed in our count of spikes in the first 0.5 s of stimulation. Studies of other insects have provided evidence that similar odorants can be discriminated in behavioral paradigms by virtue of differences in the temporal dynamics of the responses they elicit (Stopfer et al., 1997; Stopfer et al., 2003). Accordingly, other kinds of odor spaces that incorporate temporal data, physiological responses at other odorant concentrations, or other parameters may provide refined predictions of odor masking.

The odor masking paradigm we have used here provides one behavioral measure of relationships among odorants. It will be interesting to determine whether distances in odor space predict other measures of perceptual distance, including measures based on odor learning. If odorants A and B are close in odor space, and an animal is trained to avoid odorant A, will it then avoid odorant B? However, such an analysis must be interpreted carefully -- for example, odor representations can be changed by prior experience (Faber et al., 1999; Yu et al., 2004).

Compounds that effectively masked each other, interestingly, were in some cases structurally dissimilar: cyclohexanone, for example, masked the response to E2-hexenal, and 1-hexanol masked the response to ethyl acetate, defying a simple chemical logic to masking. We note that of 48 pairs of odorants examined, in 73% of the cases the point source elicited an RI >50% that of the unmasked control value, despite the presence of the masking odor. These results illustrate the rich coding capacity of this olfactory system and suggest a robust ability to distinguish among odorants.

Intensity coding

In addition to the identification of odor quality, assessment of odor quantity is a critical function of olfactory systems. We have found evidence from genetic analysis that the evaluation of quantity depends on multiple receptors. We examined the case of ethyl acetate, which is present at low concentrations in some fruits and very high concentrations in others: It constitutes as much as 33% of the volatiles in pineapple (TNO, 2004; Umana et al., 1992).

The larva expresses two receptors, Or42a and Or42b, that respond strongly to ethyl acetate and that differ by more than two orders of magnitude in the concentrations at which they show a half-maximal physiological response. In behavioral tests, the more sensitive receptor is essential for response at low concentrations and the less sensitive receptor is required at high concentrations. These results suggest that the animal has extended the dynamic range of its ethyl acetate response by expressing two receptors of differing sensitivity.

These results offer one possible explanation for why the receptor repertoires of some species are so large (Ache and Young, 2005). By integrating the responses of multiple receptors the animal can both extend the dynamic range of its response and acquire a more precise assessment of concentration. The ability to detect concentration differences at high resolution may be selected for strongly in an animal that must move up an odor gradient towards a food source or a mating partner.

Behavioral response can be predicted from the responses of a small number of receptors

The behavioral responses to odorants lie along a continuum, ranging from strong attraction to repulsion. Odorants of diverse chemical classes were capable of eliciting attractive responses, and of eliciting repellent responses. We note, however, that among seven aromatic odorants tested, none elicited an RI > 0.4 and three elicited mean RIs that were negative.

We were surprised to find that 81% of the variation in behavioral response could be explained and 74% of the variation could be predicted with a model based on the activity of only five receptors, using the test set of 26 structurally diverse odors. We had not expected such high predictive power from a linear model for a variety of reasons. First, we had imagined that the extent of olfactory processing would be so great, and that the transformations would be sufficiently non-linear, as to preclude a useful linear model. Second, our evaluation of sensory input was based solely on a count of action potentials during a 0.5 s stimulation period and did not take into account the temporal dynamics of the responses within this period. Third, our measure of behavior is also simple, based solely on a single binary choice per animal. Fourth, the physiological and behavioral paradigms are markedly different in mode of odor presentation and in duration, and the concentrations used in the two paradigms are difficult to compare.

The predictive power of a linear model is consistent with the simplicity of some features of larval olfactory system anatomy (Masuda-Nakagawa et al., 2005; Ramaekers et al., 2005). Each ORN projects to a single region of the antennal lobe, where it appears to form a synapse with a single projection neuron. Each projection neuron in turn sends an axon to only one or two glomeruli of the mushroom body calyx. Thus these first stages of the olfactory circuit do not show the convergence or divergence characteristic of the adult antennal lobe. However, there are interneurons that connect diverse regions of the larval antennal lobe (Python and Stocker, 2002a; Python and Stocker, 2002b), and the anatomy of the circuitry beyond the mushroom body has not been elucidated.

We do not suggest that linear models can explain all aspects of odor perception. For the original odorant panel, 26% of behavioral variation was not predictable by the model. This variance may be due in part to nonlinearities in ORN-PN synapses and in the properties of PNs, which have been elegantly described in the adult (Bhandawat et al., 2007; Kazama and Wilson, 2008). Some of the variation may also be due to processing at synapses other than those between the ORNs and PNs, including synapses that connect glomeruli, which have been well documented in the more complex adult olfactory system (Olsen et al., 2007; Olsen and Wilson, 2008; Shang et al., 2007). Receptors not included in the model may also explain much of the variation, especially in the case of 1-pentanol and other odors that are not well described by the model. In the case of 1-pentanol, we know from earlier work (Hallem and Carlson, 2006) that at least two other larval receptors, Or7a and Or35a, respond strongly to it and could possibly activate a circuit that would be better described by another model. Finally, some of the behavioral variation can be explained by experimental error and noise.

We were nonetheless surprised not only that a linear model was so powerful, but also that such predictive power can be achieved with so few receptors. Two of the receptors in the model, Or42a and Or85c, are broadly tuned, and respond most strongly to subsets of odorants that are

largely complementary. These odorants are attractants, and the coefficients of Or42a and Or85c in the model are, correspondingly, positive. By contrast, Or82a is excited only by geranyl acetate, and Or82a is the only receptor in the repertoire that is excited by geranyl acetate, presumably explaining its value in the model. Consistent with this explanation, its coefficient in the model is negative, indicating that sensory input via this receptor contributes to the repellent response elicited by geranyl acetate. Likewise, of all 21 receptors, Or74a is the receptor that responds most strongly to the repellent 1-nonanol, and its coefficient in the model is negative as well. Thus it is possible that the Or82a and Or74a receptors activate a repulsion circuit.

Odors vary in their volatility. In an earlier study of larval olfactory behavior that used a large number of odorants, no clear correlation was found between vapor pressure and behavioral efficacy (Fishilevich et al., 2005); nonetheless it is formally possible that had we normalized the intensity of the odor stimuli, a five-receptor model would be less powerful.

We note that in other sensory systems a small number of receptors is capable of driving behavioral responses to a great diversity of stimuli. Behavioral and physiological responses to a wide variety of sugars are mediated by two members of the Gr family of taste receptors, Gr5a and Gr64a: one subset of sugars depends on Gr5a and a complementary subset depends on Gr64a (Dahanukar et al., 2007).

If a model containing five receptors explains most of the behavioral variation we have observed, why does the animal contain 21 functional receptors? First, other receptors may report the presence of other odorants not included in our study. In a previous study of receptors expressed in the adult antenna, several did not respond to any of a panel of odorants present in food sources (Hallem and Carlson, 2006). A subsequent study showed that they responded to fly odors (van der Goes van Naters and Carlson, 2007). Likewise, some of the larval receptors may be tuned to specific compounds of biological importance that were not included in our study. Second, the multiplicity of receptors may aid in the coding of odor intensity, as considered above. The model is based on physiological data from a single odor concentration, and behavioral data from a single concentration. We have shown in the case of ethyl acetate that different receptors are essential for response at different concentrations. Third, the other receptors may contribute to other functions, such as olfactory discrimination or learning. Finally, two larval ORNs have been shown to coexpress two receptors (one expresses Or33b and Or47a; the other expresses Or94a and Or94b)(Fishilevich et al., 2005). It is possible that receptor coexpression may allow an ORN to perform simple logical operations, such as an “and” function that signals the coincidence of two odorants.

We note that the predictive power of our linear model is reminiscent of the recent success of a simple linear model in relating the physicochemical properties of an odorant to its human perceptual qualities (Khan et al., 2007). In this study, the pleasantness of odor molecules could be predicted from their structure alone, with a linear model accounting for ~30% of the variance in the data.

Repellent odors and neuronal inhibition

Remarkably little is known about the molecular and cellular mechanisms underlying repellency. The problem is of interest in part because of its importance in understanding the principles of odor coding, and in part because of its practical importance. Insects transmit disease to hundreds of millions of people each year. Compounds that repel insects are in great demand, and there is a pressing need for new repellents that are effective, safe, and inexpensive. Efforts to identify new repellents, however, have been severely hampered by the inefficiency of the available behavioral screening methods. Such methods often require the rearing of large numbers of insect pests or entail difficult field studies; such behavioral studies are often

complicated by variables that are difficult to control. A simple logic to the identification of insect repellents from a rapid physiological screen of receptor activity could thus be of value in the control of insect pests (Hallem et al., 2004a; van der Goes van Naters and Carlson, 2006).

We found a correlation between repellency and neuronal inhibition. A priori, this correlation is consistent with two mutually compatible interpretations. First, inhibitory responses may contribute to a repulsion response by virtue of a general reduction in overall activity of the receptor repertoire. Second, a repulsion circuit may be triggered by the specific patterns of receptor activity. For example, all odors that are repellents at a 10^{-2} dilution inhibited Or13a and Or42b, suggesting the possibility that one or both receptors are elements of a repulsion circuit.

When the activities of the entire receptor repertoire were analyzed with a discrimination function, repellents could be distinguished from non-repellents. These results suggest the possibility of a new means of identifying candidate repellents of *Anopheles* mosquitoes, for example, whose receptor repertoire has begun to be functionally analyzed in the empty neuron system (Hallem et al., 2004a), and which transmits malaria to hundreds of millions of people each year.

Conservation of odor space

We have provided a quantitative comparison of the odor spaces of two highly distinct olfactory organs. The larval olfactory organ and the adult antenna have different developmental origins and markedly different morphology. They operate primarily in different milieux: larvae burrow in food sources, whereas adults fly to food sources and walk upon them. Most of the larval receptors are larval-specific, and most of the antennal receptors are adult-specific. Despite these differences, and although our description of adult odor space is less complete than that of larval odor space, we have found evidence that the distance relationships among odors as measured by receptor activity patterns remain largely consistent between the two olfactory organs.

It will be interesting to determine after another odor receptor repertoire from another system, invertebrate or vertebrate, has been extensively characterized, whether odor space is conserved across species. It seems plausible that two species that live in the same habitat may tend to have similar odor spaces; however, it also seems likely that odors of particular relevance to one species may occupy an expanded region of its odor space. It will be of particular interest, for example, to determine whether human odors map to a large and distinct domain of mosquito odor space.

Concluding remarks

We were surprised by the degree to which several aspects of an animal's behavior could be predicted from the activities of its odor receptors. We found that the strength of the animal's olfactory response (response index), the mode of its response (attraction v. repulsion), and the response to the integrated input from two odorants (odor masking) could all be described to a large extent by relatively simple functions of the activities of its odor receptors.

We were surprised in part because of the simplicity of our behavioral and physiological measurements, and in part because we had imagined that the complexity of the neural processing underlying these behaviors would defy a simple, linear analysis. Our results do not, however, imply a lack of complexity in the processing of olfactory information. Our predictions of behavior, while much more accurate than we had expected, are still limited. For example, the five-receptor model predicted 81%, not 100%, of the variation in RI among the initial,

larger odor panel; for the masking of 2-heptanone and ethyl acetate, the point source odors for which we have the most extensive data, the R^2 values were 58% and 56%. While we suspect these values could be increased by expanding our analysis of the receptor responses, in particular as a function of time and concentration, there are almost certainly limits imposed by the nature of the circuitry and the logic of the processing, even in the simpler larval system. An observation that may arise from more complicated processing is the switch from a positive to a negative RI with increasing ethyl acetate concentration in an *Or42a*$^{-}$*Or42b*$^{+}$ mutant (Figure 7C). This decline in RI may arise from non-linearities in the processing and by interactions between inputs in the larval antennal lobe or at higher levels.

Finally, in this study we have found that different aspects of olfactory behavior can be explained by different aspects of the receptor code. Overall strength of an attractive response correlated with the summed magnitude of the receptor input, and RI could be predicted by the response magnitudes of small subsets of receptors. However, odor masking appeared more sensitive to the identity of the responding receptors than to the magnitudes of their responses: angular distances in odor space explained the results better than Euclidean distances. It will be interesting to determine how these different aspects of the receptor code are decoded by higher regions of the larval brain, and whether there are anatomically distinct circuits that decode them.

Experimental Procedures

Drosophila stocks and transgenes

UAS-Or genes were constructed as described previously (Kreher, et al. 2005), using Canton-S (CS) genomic DNA as a template, unless otherwise specified. *UAS-Or13a* and *UAS-Or42b* were constructed from CS cDNA; a *UAS-Or42b* construct was also synthesized from genomic DNA and gave similar results. *UAS-Or22c* was constructed from P1 DNA. The transgenes were injected into w^{1118} or w^{1118}*; Δhalo* flies (Dobritsa et al., 2003).

The mutant alleles of *Or42a* and *Or42b* ($Or42a^{f04305}$ and $Or42b^{EY14886}$) were obtained from the Bloomington stock center. The *Or42a* allele is a piggyBac transposon insertion in the second intron of the gene. The *Or42b* allele is a P-element insertion in the second exon. The location of insertions was verified by inverse PCR, and each line was backcrossed to a *w* CS line for 10 generations. The *w* CS line was used as a wild-type control in behavioral experiments.

Electrophysiology

All recordings were conducted as previously described (Dobritsa et al., 2003; Kreher et al., 2005). All electrophysiological data were analyzed as described previously, except that mean spontaneous activity and mean solvent response of each receptor was subtracted from each odor response for that receptor.

Of the 1113 odorant-receptor combinations reported here (Figure 1, Table S1), 156 have been tested previously in a study of antennal receptors (Hallem and Carlson, 2006). Among these, 144 (92%) showed agreement within 50 spikes/s in the two studies. Of the 12 discrepancies, the measured frequencies were in every case higher in Hallem and Carlson (2006), which may reflect differences in the airflow rates in the delivery systems used in that study (5.9 ml/s for the odor stream and 24 ml/s for the airstream, as opposed to 3.75 ml/s for the odor stream and 37.5 ml/s for the airstream in the present study). Only one measured odorant-receptor combination, the response of Or82a to geranyl acetate, showed a difference of more than 90 spikes/s, and further analysis revealed that this response exhibited slow onset kinetics, such that a minor change in the airflow could produce a major reduction in measured spike rate.

Statistical analysis

Regression analysis was performed using the SAS system (SAS Institute, Cary, NC) with the REG procedure and multiple regression model selection option, using minimum R^2 improvement (MINR). The resulting top ten models were also evaluated for Mallow's C, Bayesian Information Criterion (BIC), and Predicted Residuals (PRESS) (Mallows, 1973; Schwarz, 1978). The final model presented in the main text was selected based on the ratio of full model residual error, PRESS error, and percent variation explained. Data were taken from receptor responses to 10^{-2} dilutions.

Euclidean distance for odors *x* and *y* were defined as $d_{\mathrm{euc}}(x,y)=\sqrt{\sum_{i=1}^{21}(x_i-y_i)^2}$ where x_i and y_i denote the spike rate of each odorant for the *i*th receptor. Angular distances are defined by $d_{\mathrm{ang}}(x,y)=\cos^{-1}\left(\sum_{i=1}^{21}\tilde{x}_i\tilde{y}_i/\sqrt{\sum_{i=1}^{21}\tilde{x}_i^2\sum_{i=1}^{21}\tilde{y}_i^2}\right)$ where $\tilde{x}_i$, $\tilde{y}_i$ denote the unit length normalized spike values for the *i*th receptor. Regression distances are defined as the absolute value of the differences in the regression predicted values of the odorants.

Euclidean distances, regression model distances, and angular distances were computed by a custom Perl program. To calculate RI_L, responses were first expressed as the probability of migration to the odor half of the plate (P = (RI+1)/2), and then the probability was logit transformed to yield RI_L.

The masking index was defined as MI=log((RI+1)/(RI′+1)), where RI and RI′ are the response indices in the absence and presence, respectively, of masking odor.

To identify repellents, a multiple test-corrected Bayesian 95% confidence interval around RI=0 of (-0.14, 0.14) was constructed based on the number of trials (~500 individuals), and batch variability was estimated using a control dataset (not shown) with a beta distribution prior. By this criterion, 2-methylphenol, 1-nonanol, benzaldehyde, fenchone, geranyl acetate, and methyl salicylate all produced an RI significantly below 0 and can be considered repellents.

Behavioral assays

Behavioral assays were conducted essentially as described previously (Monte et al., 1989). Briefly, two filter paper discs were placed diametrically opposed to each other on a thin layer of 1.1% agarose in a 10 cm Petri dish. Approximately 50 third-instar larvae were placed in the center of the dish and allowed 5 min to migrate, after which the Response Index (RI) was calculated. Often a small number of larvae remained in a clump at the center of the plate; these animals were excluded from the analysis. Masking assays were conducted similarly, except that 750 microliters of a diluted odorant were spread on a 90 mm filter paper disc that was placed on the inner surface of the lid of the Petri dish. This volume was chosen following pilot experiments in which larger volumes were found to drip from the filter. The background (mask) odor was a 10^{-2} dilution in all cases except for in Figure 5A, in which it was varied over six orders of magnitude. As negative controls in the masking studies, responses to point source odorants were measured in the presence of 750 microliters of paraffin oil added to the disc placed on the inner surface of the lid.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Aceves-Pina EO, Quinn WG. Learning in normal and mutant *Drosophila* larvae. Science 1979;206:93–95. [PubMed: 17812455]

Ache BW, Young JM. Olfaction: diverse species, conserved principles. Neuron 2005;48:417–430. [PubMed: 16269360]

Ayyub C, Paranjape J, Rodrigues V, Siddiqi O. Genetics of olfactory behavior in *Drosophila melanogaster*. J Neurogenetics 1990;6:243–262. [PubMed: 2121923]

Bhandawat V, Olsen SR, Gouwens NW, Schlief ML, Wilson RI. Sensory processing in the Drosophila antennal lobe increases reliability and separability of ensemble odor representations. Nat Neurosci 2007;10:1474–1482. [PubMed: 17922008]

Boeckh J, Kaissling K, Schneider D. Insect Olfactory Receptors. Cold Spr Har Symp Quant Biol 1965;30:263–280.

Cobb M. What and how do maggots smell? Biological Reviews of the Cambridge Philosophical Society 1999;74:425–459.

Cobb M, Domain I. Olfactory coding in a simple system: adaptation in Drosophila larvae. Proc Biol Sci 2000;267:2119–2125. [PubMed: 11416918]

Couto A, Alenius M, Dickson BJ. Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr Biol 2005;15:1535–1547. [PubMed: 16139208]

Dahanukar A, Lei YT, Kwon JY, Carlson JR. Two Gr genes underlie sugar reception in Drosophila. Neuron 2007;56:503–516. [PubMed: 17988633]

Daly KC, Christensen TA, Lei H, Smith BH, Hildebrand JG. Learning modulates the ensemble representations for odors in primary olfactory networks. Proc Natl Acad Sci U S A 2004;101:10476–10481. [PubMed: 15232007]

de Brito Sanchez MG, Kaissling KE. The antennal benzoic acid receptor cell of the female silk moth Bombyx mori L.: structure-activity relationship studies with halogen substitutes. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 2005;191:189–196. [PubMed: 15614531]

De Bruyne M, Clyne PJ, Carlson JR. Odor coding in a model olfactory organ: the Drosophila maxillary palp. J Neurosci 1999;19:4520–4532. [PubMed: 10341252]

De Bruyne M, Foster K, Carlson JR. Odor coding in the *Drosophila* antenna. Neuron 2001;30:537–552. [PubMed: 11395013]

Dobritsa AA, van der Goes van Naters WM, Warr CG, Steinbrecht RA, Carlson JR. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 2003;37:827–841. [PubMed: 12628173]

Faber T, Joerges J, Menzel R. Associative learning modifies neural representations of odors in the insect brain. Nat Neurosci 1999;2:74–78. [PubMed: 10195183]

Fishilevich E, Domingos AI, Asahina K, Naef F, Vosshall LB, Louis M. Chemotaxis behavior mediated by single larval olfactory neurons in Drosophila. Curr Biol 2005;15:2086–2096. [PubMed: 16332533]

Hallem EA, Carlson JR. Coding of odors by a receptor repertoire. Cell 2006;125:143–160. [PubMed: 16615896]

Hallem EA, Fox AN, Zwiebel LJ, Carlson JR. Olfaction: mosquito receptor for human-sweat component. Nature 2004a;427:212–213. [PubMed: 14724626]

Hallem EA, Ho MG, Carlson JR. The molecular basis of odor coding in the Drosophila antenna. Cell 2004b;117:965–979. [PubMed: 15210116]

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

Kazama H, Wilson RI. Homeostatic matching and nonlinear amplification at identified central synapses. Neuron 2008;58:401–413. [PubMed: 18466750]

Khan RM, Luk CH, Flinker A, Aggarwal A, Lapid H, Haddad R, Sobel N. Predicting odor pleasantness from odorant structure: pleasantness as a reflection of the physical world. J Neurosci 2007;27:10015–10023. [PubMed: 17855616]

Kreher SA, Kwon JY, Carlson JR. The molecular basis of odor coding in the Drosophila larva. Neuron 2005;46:445–456. [PubMed: 15882644]

L'Etoile ND, Bargmann CI. Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron 2000;25:575–586. [PubMed: 10774726]

L'Etoile ND, Coburn CM, Eastham J, Kistler A, Gallegos G, Bargmann CI. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in C. elegans. Neuron 2002;36:1079–1089. [PubMed: 12495623]

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 2004;43:703–714. [PubMed: 15339651]

Louis M, Huber T, Benton R, Sakmar TP, Vosshall LB. Bilateral olfactory sensory input enhances chemotaxis behavior. Nat Neurosci 2008;11:187–199. [PubMed: 18157126]

Mallows CL. Some comments on Cp. Technometrics 1973;15:661–675.

Malnic B, Hirono J, Sato T, Buck LB. Combinatorial receptor codes for odors. Cell 1999;96:713–723. [PubMed: 10089886]

Manly BJF. Randomization and Monte Carlo Methods in Biology (London). 1991

Masuda-Nakagawa LM, Tanaka NK, O'Kane CJ. Stereotypic and random patterns of connectivity in the larval mushroom body calyx of Drosophila. Proc Natl Acad Sci U S A 2005;102:19027–19032. [PubMed: 16357192]

Monte P, Woodard C, Ayer R, Lilly M, Sun H, Carlson J. Characterization of the larval olfactory response in Drosophila and its genetic basis. Behav Genet 1989;19:267–283. [PubMed: 2497723]

Olsen SR, Bhandawat V, Wilson RI. Excitatory interactions between olfactory processing channels in the Drosophila antennal lobe. Neuron 2007;54:89–103. [PubMed: 17408580]

Olsen SR, Wilson RI. Lateral presynaptic inhibition mediates gain control in an olfactory circuit. Nature 2008;452:956–960. [PubMed: 18344978]

Oppliger FY, Guerin PM, Vlimant M. Neurophysiological and behavioral evidence for an olfactory function for the dorsal organ and a gustatory one for the terminal organ in Drosophila melanogaster larvae. Journal of Insect Physiology 2000;46:135–144. [PubMed: 12770245]

Python F, Stocker R. Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. The Journal of Comparative Neurology 2002a; 445:374–387. [PubMed: 11920714]

Python F, Stocker RF. Immunoreactivity against choline acetyltransferase, gamma-aminobutyric acid, histamine, octopamine, and serotonin in the larval chemosensory system of Dosophila melanogaster. J Comp Neurol 2002b;453:157–167. [PubMed: 12373781]

Ramaekers A, Magnenat E, Marin EC, Gendre N, Jefferis GS, Luo L, Stocker RF. Glomerular maps without cellular redundancy at successive levels of the Drosophila larval olfactory circuit. Curr Biol 2005;15:982–992. [PubMed: 15936268]

Rodrigues, V. Olfactory behavior of Drosophila melanogaster. New York: Plenum; 1980.

Rodrigues V, Siddiqi O. Genetic Analysis of Chemosensory Pathway. Proceedings of the Indian Academy of Sciences Section B 1978;87:147–160.

Root CM, Semmelhack JL, Wong AM, Flores J, Wang JW. Propagation of olfactory information in Drosophila. Proc Natl Acad Sci U S A 2007;104:11826–11831. [PubMed: 17596338]

Schneider D, Steinbrecht RA. Checklist of insect olfactory sensilla. Symp Zool Soc Lond 1968;23:279–297.

Schwarz G. Estimating the dimension of a model. Annals of Statistics 1978;6:461–464.

Shang Y, Claridge-Chang A, Sjulson L, Pypaert M, Miesenbock G. Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. Cell 2007;128:601–612. [PubMed: 17289577]

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

Stocker RF. The organization of the chemosensory system in *Drosophila melanogaster*: a review. Cell Tissue Res 1994;275:3–26. [PubMed: 8118845]

Stopfer M, Bhagavan S, Smith BH, Laurent G. Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. Nature 1997;390:70–74. [PubMed: 9363891]

Stopfer M, Jayaraman V, Laurent G. Intensity versus identity coding in an olfactory system. Neuron 2003;39:991–1004. [PubMed: 12971898]

Stortkuhl KF, Hovemann BT, Carlson JR. Olfactory adaptation depends on the Trp Ca2+ channel in Drosophila. J Neurosci 1999;19:4839–4846. [PubMed: 10366618]

TNO. Volatile compounds in food: qualitative and quantitative data. 2004

Umana K, Hagi Y, Nakahara K, Shoji A, Shibamoto T. Volatile constituents of green and ripened pineapple (Ananas comosus [L.] Merr.). J Agric Food Chem 1992;40:599–603.

van der Goes van Naters W, Carlson JR. Insects as chemosensors of humans and crops. Nature 2006;444:302–307. [PubMed: 17108954]

van der Goes van Naters W, Carlson JR. Receptors and neurons for fly odors in Drosophila. Curr Biol 2007;17:606–612. [PubMed: 17363256]

Wes PD, Bargmann CI. C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature 2001;410:698–701. [PubMed: 11287957]

Wilson RI, Mainen ZF. Early events in olfactory processing. Annu Rev Neurosci 2006;29:163–201. [PubMed: 16776583]

Wilson RI, Turner GC, Laurent G. Transformation of olfactory representations in the *Drosophila* antennal lobe. Science 2004;303:366–370. [PubMed: 14684826]

Xia Y, Wang G, Buscariollo D, Pitts RJ, Wenger H, Zwiebel LJ. The molecular and cellular basis of olfactory-driven behavior in Anopheles gambiae larvae. Proc Natl Acad Sci U S A 2008;105:6433–6438. [PubMed: 18427108]

Yu D, Ponomarev A, Davis RL. Altered representation of the spatial code for odors after olfactory classical conditioning; memory trace formation by synaptic recruitment. Neuron 2004;42:437–449. [PubMed: 15134640]

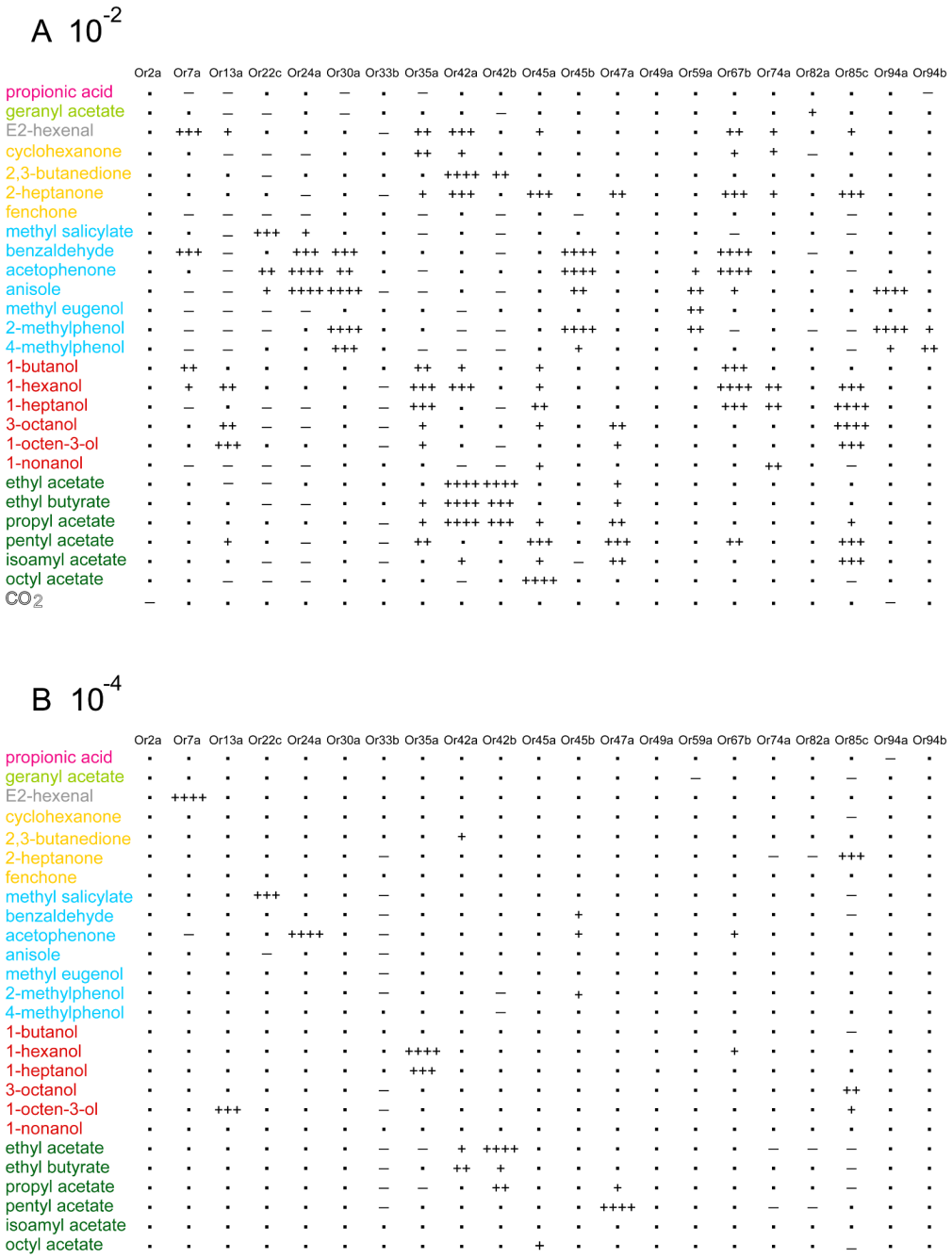


Figure 1. Odor responses

(A) Responses to odorants at a 10^{-2} dilution. ".", n<50 spikes/s; "+", 50≤n<100 spikes/s; "++", 100≤n<150 spikes/s; "+++", 150≤n<200 spikes/s; "++++", n≥200 spikes/s. "-" denotes inhibition to ≤50% of the spontaneous firing rate. Inhibition was not calculated for Or47a; its low spontaneous firing rate (<4 spikes/s) made it difficult to quantitate inhibition. Each value represents the mean activity during a 0.5 s odor stimulation period. Spontaneous activity and response to solvent alone have been subtracted from response values. Numerical values are provided in Table S1. n≥6. Responses of Or30a, Or42a, Or45, Or45a, Or49a, Or59a, Or67b, Or74, Or85c, Or94, and Or94b were taken from (Kreher et al., 2005). Odorants are color coded by functional group: pink=organic acid; light green=terpene; gray=aldehyde; yellow=ketone;

light blue=aromatic; red=alcohol; dark green=ester. (B) Responses to odorants at a 10^{-4} dilution. n≥6.

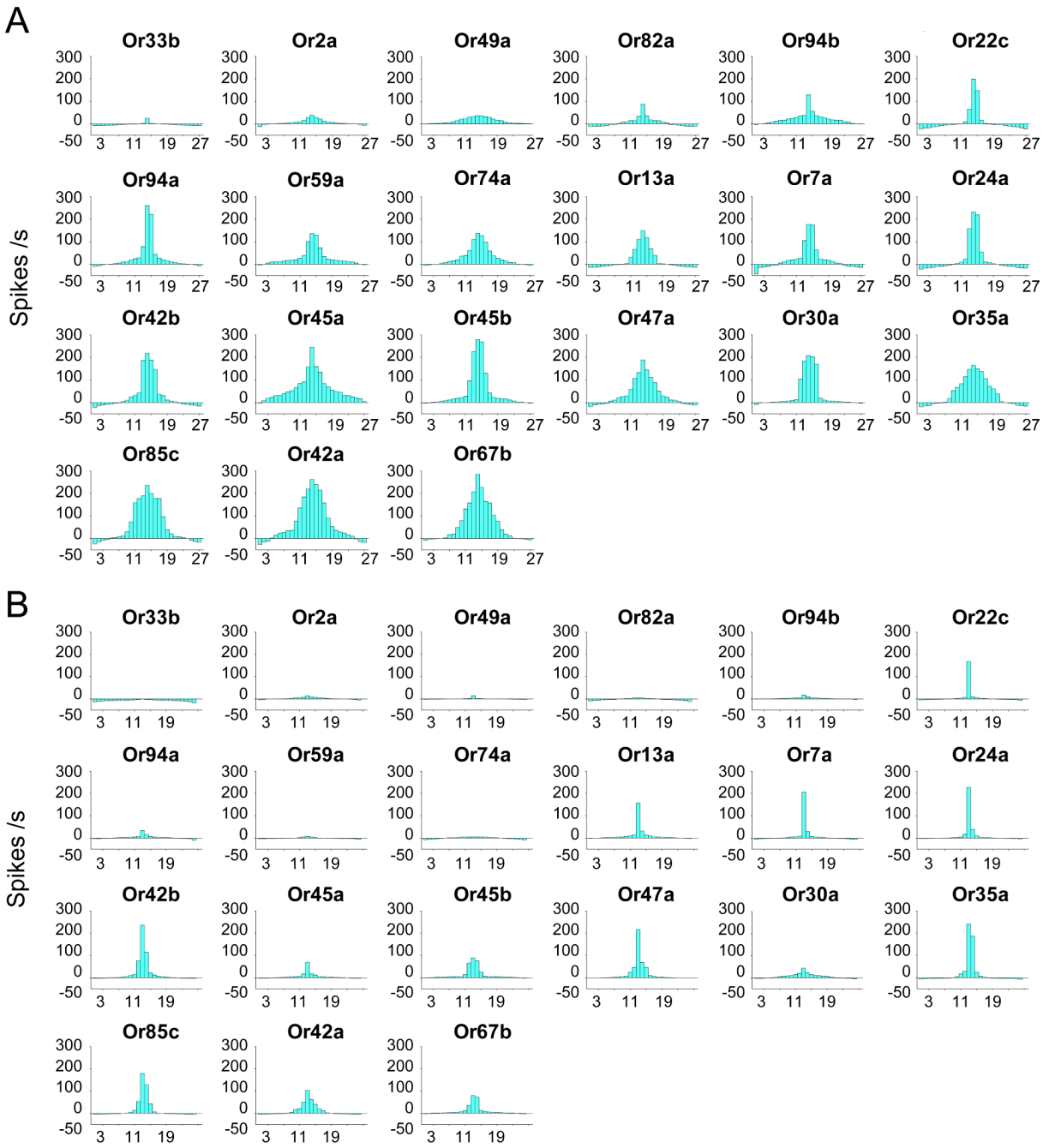
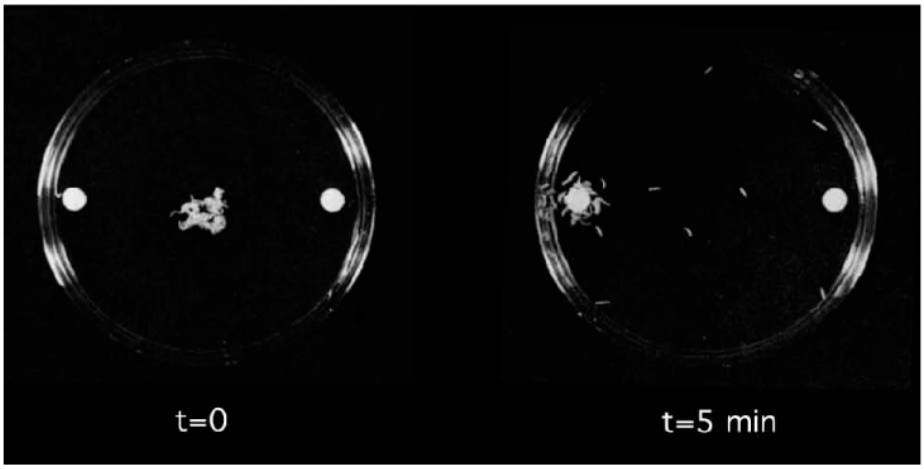


Figure 2. Tuning breadths of larval odorant receptors

(A) Tuning curves for odorants at a 10^{-2} dilution. Odorants are arranged to give the smoothest curve, with order of odorants differently arranged for each receptor; odorants that elicit the strongest responses are near the center of the distribution, while odorants that elicit weak responses are near the edges. Negative values indicate inhibitory responses. The graphs are ordered according to the number of odorants that elicit responses >100 spikes/s. The first four graphs are ordered according to the strongest response of each receptor. (B) Tuning curves of receptors to odorants at a 10^{-4} dilution.

A



B

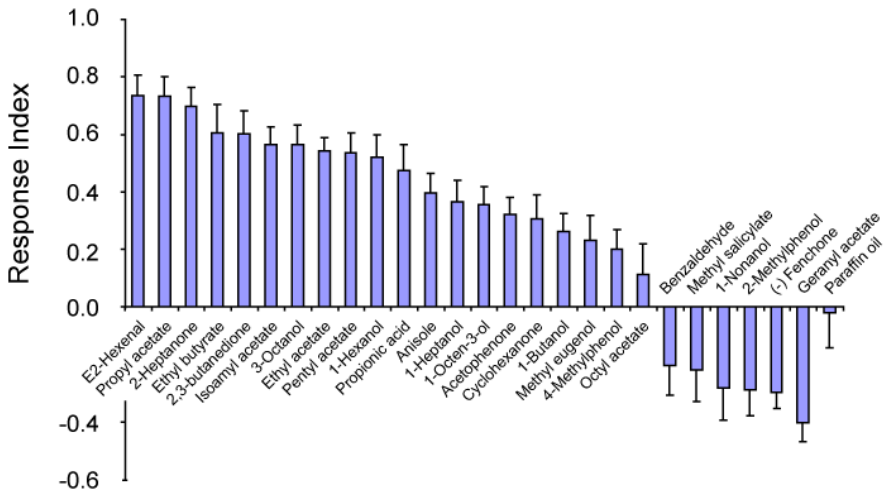


Figure 3. Behavioral responses to odorants

(A) Olfactory behavioral assay. Left, initial conditions; right, response to the odor after five minutes. Odorant was loaded on left filter paper disk. Taken from Monte et al. (1989). (B) Behavioral responses to 10^{-2} odorant dilutions. Each bar represents RI±SEM; n=10.

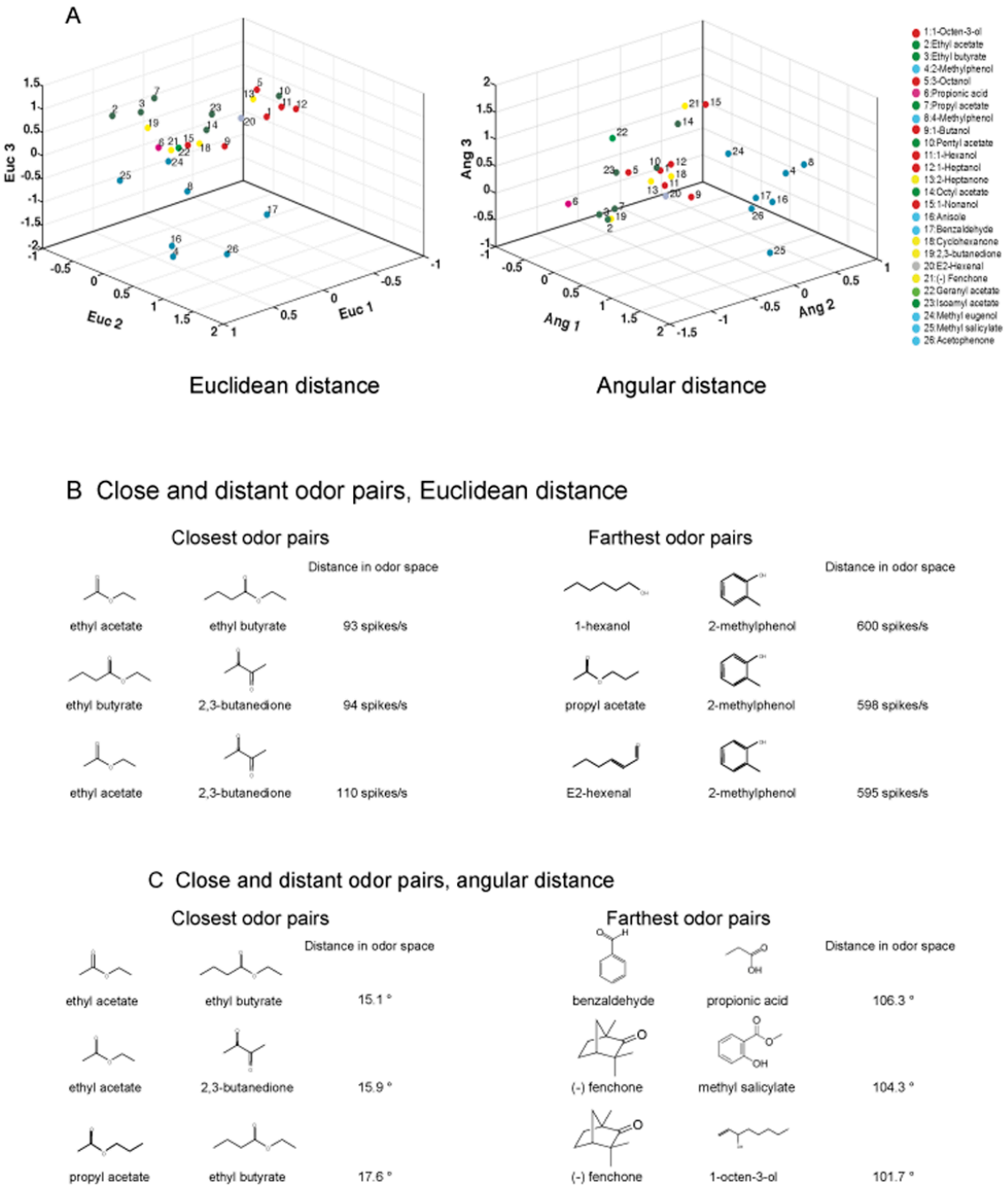


Figure 4. Odor space

(A) Three-dimensional projections of odor space based on Euclidean distances (left) and angular distances (right), constructed using Multi-Dimensional Scaling. Data are from odorants tested at 10^{-2} dilutions. Odorants are color coded by functional group as in Figure 1. (B) Closest and most distant odorant pairs as measured in Euclidean distance. (C) Closest and most distant odorant pairs as measured in angular distance.

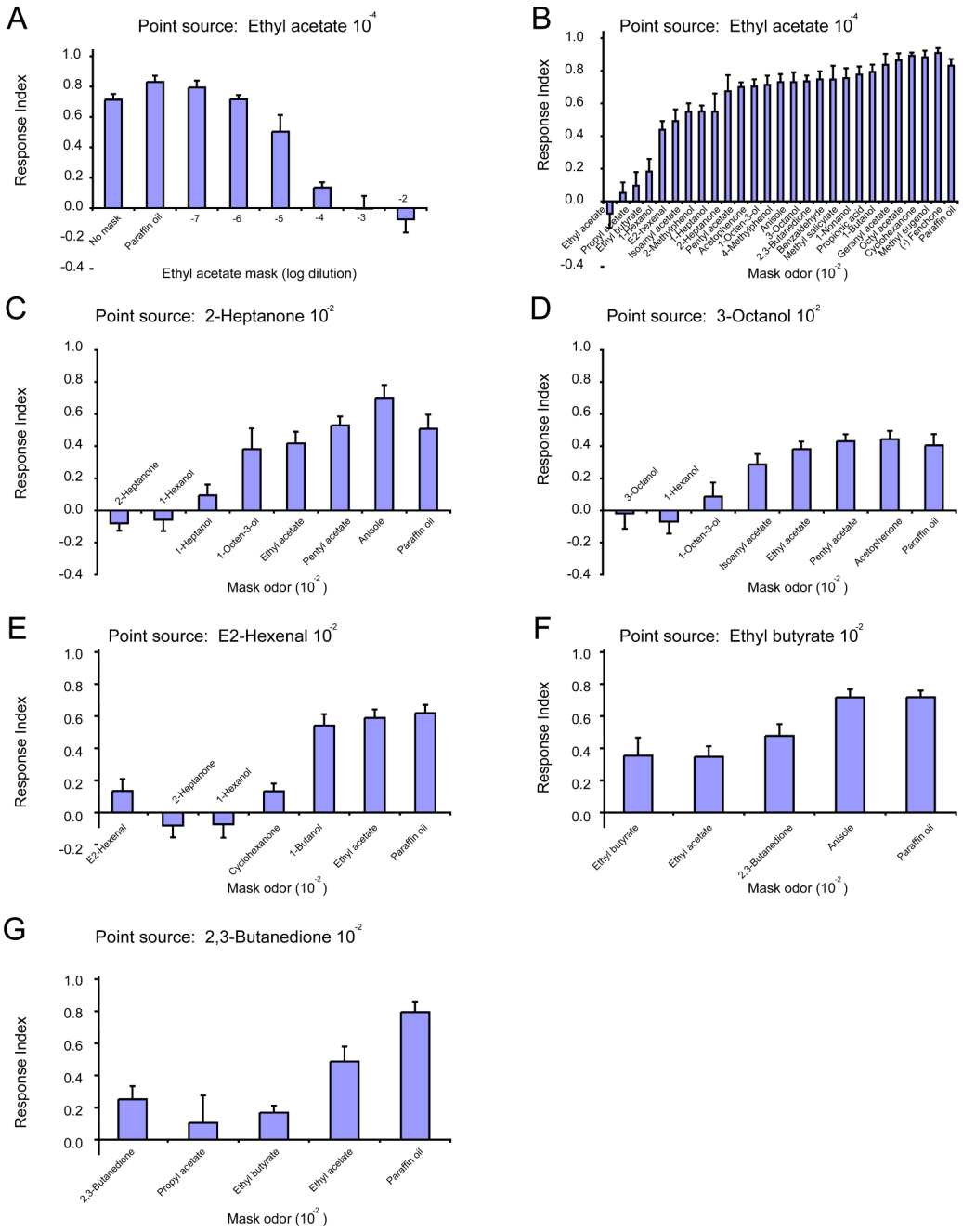


Figure 5. Behavioral responses to point source odors are reduced by the presence of masking odors

(A) Masking of a point source of ethyl acetate (diluted 10^{-4}) with dilutions of ethyl acetate. (B) Masking of a point source of ethyl acetate (10^{-4}) with other odorants. Masking of a point source of (C) 2-heptanone (10^{-2}); (D) 3-octanol (10^{-2}); (E) E2-hexenal (10^{-2}); (F) ethyl butyrate (10^{-2}); (G) 2,3-butanedione (10^{-2}). Each bar represents RI±SEM, n=6, except that in the case in which 2,3-butanedione was masked with propyl acetate, n=4.

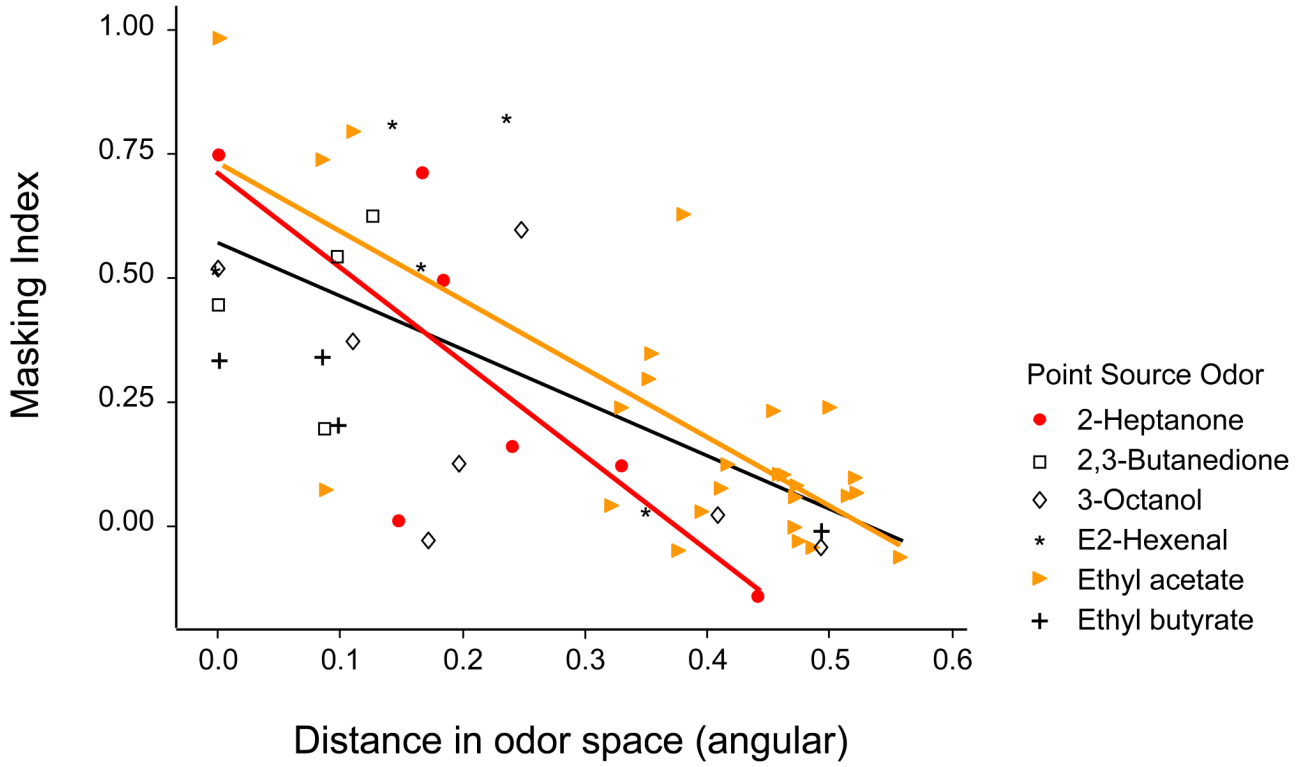


Figure 6.
Relationship between odor masking and angular distance in a 21-receptor odor space. A high masking index value indicates that fewer larvae were attracted to the point source odor in the presence of the masking odor than in the presence of the solvent alone.

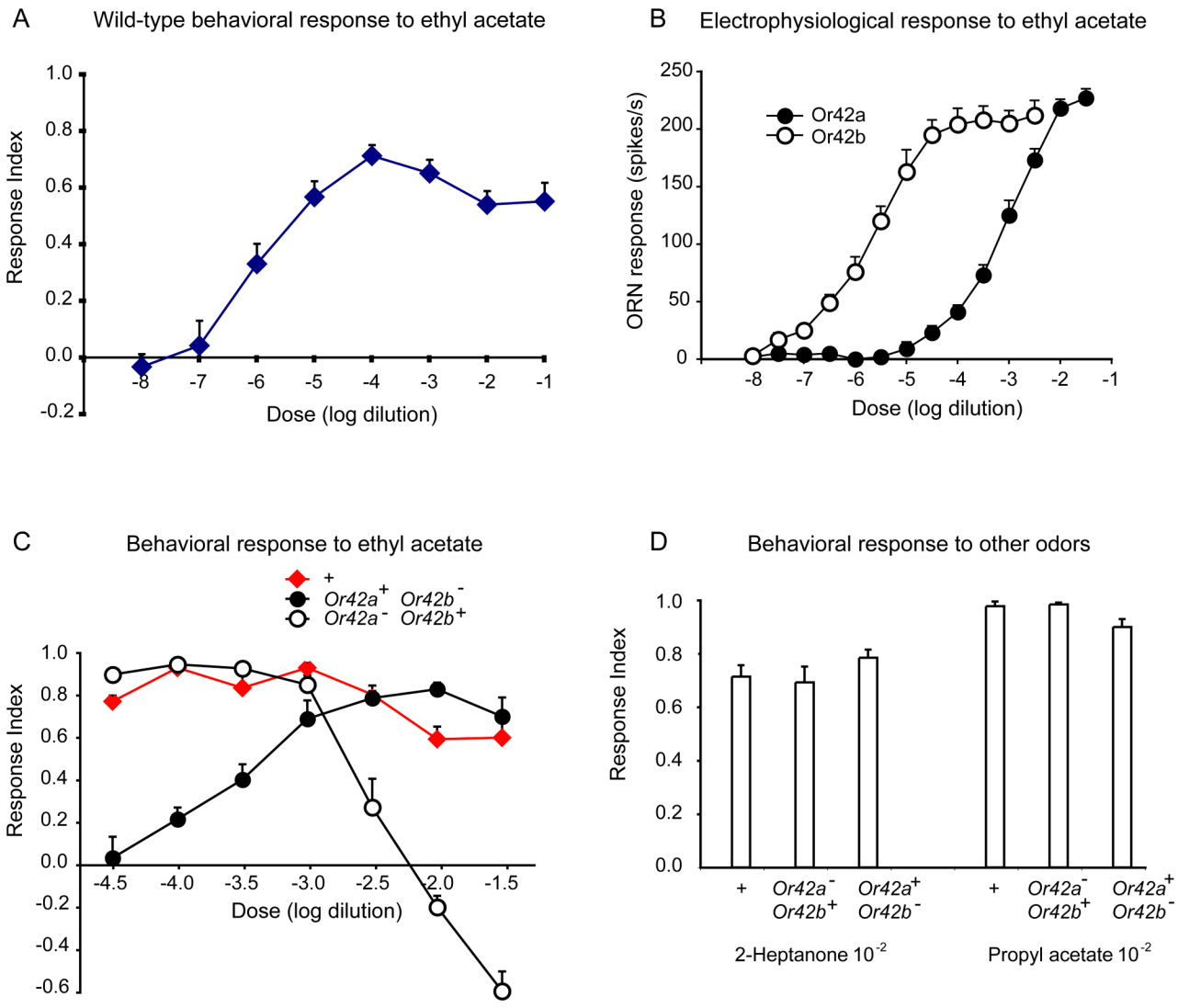


Figure 7. The response to one odorant depends on two receptors

(A) Behavioral response of wild-type to ethyl acetate. For entire figure, error bars=SEM; n=10. (B) Electrophysiological responses to ethyl acetate conferred by Or42a and Or42b in the empty neuron system; n=6 (C) Behavioral responses of larvae with mutant alleles of *Or42a* or *Or42b* to ethyl acetate; 6≤n≤10. (D) Behavioral responses to 2-heptanone and propyl acetate, at 10^{-2} dilutions; n=6. Responses are not statistically different from wild-type controls ($p>0.05$).

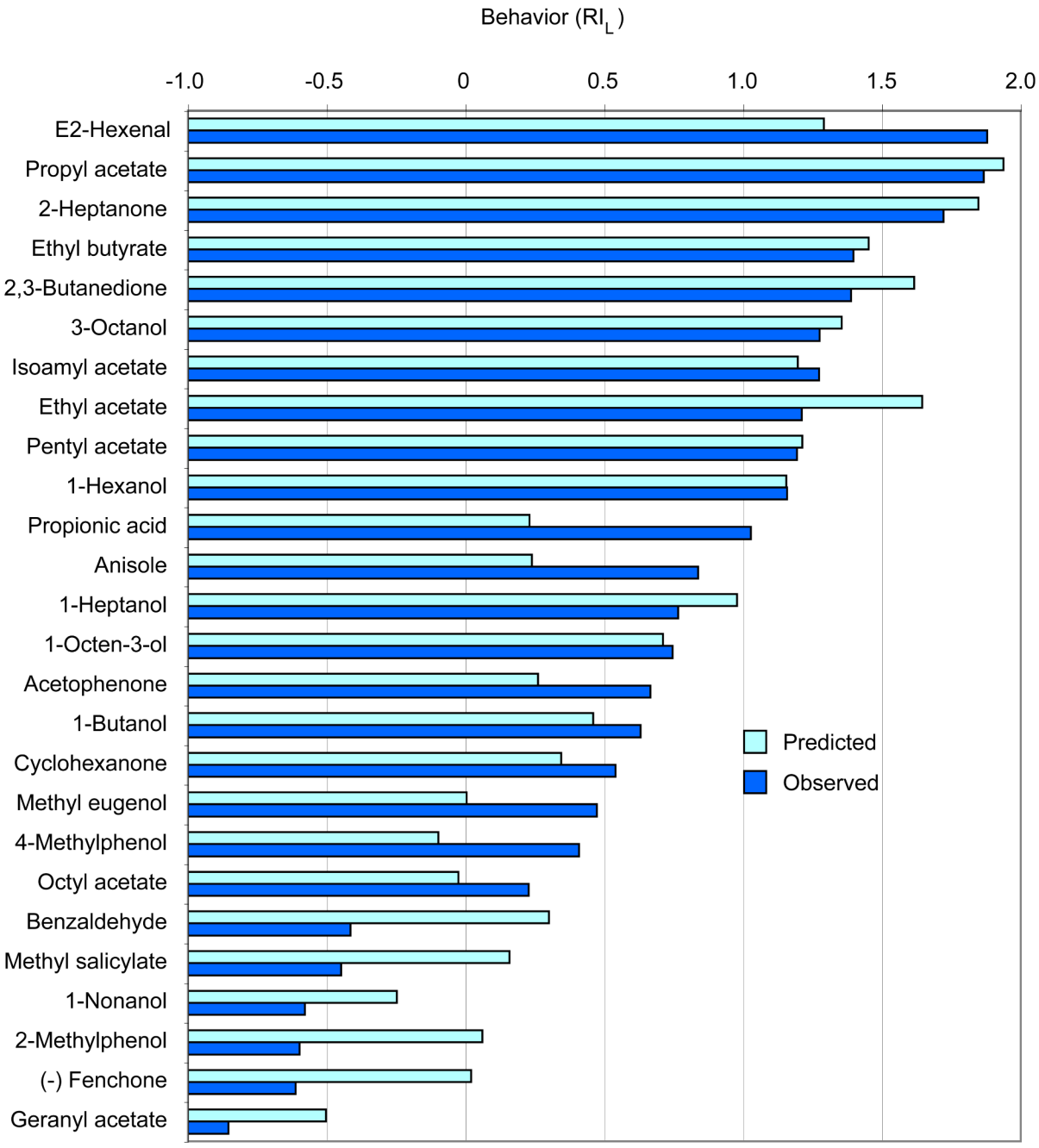


Figure 8.
Comparison of observed behavior and behavior predicted by the model based on five receptors: Or42a, Or45a, Or74a, Or82a, and Or85c. RI values are logit transformed for statistical rigor.