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Different Thresholds for Detection and Discrimination of Odors in the Honey bee (*Apis mellifera*)

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Abstract

Naturally occurring odors used by animals for mate recognition, food identification and other purposes must be detected at concentrations that vary across several orders of magnitude. Olfactory systems must therefore have the capacity to represent odors over a large range of concentrations regardless of dramatic changes in the salience, or perceived intensity, of a stimulus. The stability of the representation of an odor relative to other odors across concentration has not been extensively evaluated. We tested the ability of honey bees to discriminate pure odorants across a range of concentrations at and above their detection threshold. Our study showed that pure odorant compounds became progressively easier for honey bees to discriminate with increasing concentration. Discrimination is, therefore, a function of odorant concentration. We hypothesize that the recruitment of sensory cell populations across a range of concentrations may be important for odor coding, perhaps by changing its perceptual qualities or by increasing its salience against background stimuli, and that this mechanism is a general property of olfactory systems.

Key words: coding, concentration, discrimination, honey bee, invariance, olfaction

Introduction

Animals must detect a vast array of odorant ligands using a few dozen to at most a few thousand olfactory receptors (Buck and Axel, 1991; Malnic *et al.*, 1999; Vosshall *et al.*, 1999; Ma and Shepherd, 2000; Xu *et al.*, 2000; Firestein, 2001). In both vertebrates and invertebrates, it is hypothesized that these receptors are used in a combinatorial manner in which one receptor participates in coding for several different odorant ligands. Each sensory receptor cell expresses a single molecular receptor type (Mombaerts, 1999; Ma and Shepherd, 2000) and is tuned to a narrow range of odorant ligands at low concentration (Mombaerts, 1999; Araneda *et al.*, 2000; Ma and Shepherd, 2000; Xu *et al.*, 2000). The rate of firing of each cell then increases as the concentration of a target ligand increases (Ma and Shepherd, 2000; Xu *et al.*, 2000; Getz and Lansky, 2001; Kajiya *et al.*, 2001). Another subset of cells responds to that ligand, but only at higher concentrations, because the receptors expressed by those cells are less efficient in binding to the ligand (Voigt and Atema, 1992; Malnic *et al.*, 1999; Araneda *et al.*, 2000; Kajiya *et al.*, 2001; Lansky and Getz, 2001). Furthermore, some sensory cells that respond to a ligand at low concentrations may become inhibited by it at higher concentrations (Getz and Akers, 1995). Thus, different but overlapping populations of sensory cells respond to a given ligand at high rather than at low concentrations (Malnic *et al.*, 1999; Ma and Shepherd, 2000; Xu *et al.*, 2000; Firestein, 2001).

The perceptual qualities of odors have been shown to correlate with specific molecular features of odor ligands. Several studies have demonstrated that changes in carbon chain length or shape, or changes in functional groups, affect generalization from conditioned to test odorants (Smith and Menzel, 1989; Laska and Teubner, 1999; Laska *et al.*, 1999; Daly and Smith, 2000; Laska, 2002; Linster *et al.*, 2002). For different types of chemicals, and for phylogenetically diverse animal species, generalization follows a gradient which relates directly to incremental changes in molecular features, such as carbon chain length (Laska and Tuebner, 1999; Laska *et al.*, 1999; Daly and Smith, 2000; Laska and Hubener, 2001; Sakura *et al.*, 2002). However, other important features of odor stimuli, such as their concentration, may also be encoded by the olfactory system. Some odorants have been observed to have different perceptual qualities across concentration (Gross-Isseroff and Lancet, 1988; Bhagavan and Smith, 1997; Pelz *et al.*, 1997; Sakura *et al.*, 2002), and it has been hypothesized that this may arise because of different combinations of receptors that become activated for the same odorant at different concentrations (Ma and Shepherd, 2000; Firestein, 2001). When more receptors are activated at high concentrations of

odor, the increase in neural activity would also potentially increase the salience of the odor stimulus. In either case, the ability of an animal to differentiate among odorants would be expected vary as a function of concentration.

We used the honey bee as a model to examine the effect of concentration on odor discrimination. Odors are important cues that honey bees use to distinguish among species of flowering plants (Wright *et al.*, 2002), and the honey bee's olfactory system is well adapted to detect and discriminate a diverse array of odors (Chittka *et al.*, 1999). The ability of honey bees to learn and discriminate among odorants based on their molecular features is well documented (Smith and Menzel, 1989; Bhagavan and Smith, 1997; Pelz *et al.*, 1997; Smith, 1998; Laska *et al.*, 1999). Using an associative conditioning protocol, we examined how the rate of learning and the ability of honey bees to discriminate monomolecular odorants were affected by the concentration of an olfactory stimulus. We show that generalization, which we interpret as an indication of discrimination, is a function not only of molecular similarity of odors but also of stimulus concentration. Our data suggest that recruitment of sensory cell populations at higher concentrations enhances the discriminability of olfactory stimuli, perhaps by increasing the salience of the stimulus relative to background stimuli.

Materials and methods

Honey bees

A total of 325 honey bees were used for this series of experiments. Worker honey bees (*Apis mellifera*) were placed individually in restraining harnesses and held for 5–6 h before training. Bees were trained in a 'forward pairing' conditioning procedure with a 0.4 µl droplet of 1.5 M sucrose (Bitterman *et al.*, 1983), during which a 4 s odor pulse was presented in an air stream (0.425 ml/min). During conditioning, the droplet of sucrose was applied to the antenna of an individual honey bee ~3 s after the beginning of the odor pulse, causing the delivery of the unconditioned stimulus (sucrose droplet) to overlap slightly with the presentation of the conditioning stimulus (odor pulse). This increase in the probability of response over trials arises from associative conditioning (Bitterman *et al.*, 1983). We trained individual honey bees to one odorant at a single concentration over 16 acquisition trials, after which each honey bee was presented with three test trials. During a test trial, a 4 s odor pulse was presented at the same concentration as the conditioned odor, but it was not followed with sucrose reinforcement. Three different odors were presented in a random sequence across individual honey bees.

Odorants

Odorants used in these experiments were obtained at 99.8% or better purity from Sigma-Aldrich (St Louis, MO). Odorants were grouped into 'similar' and 'dissimilar' categories based on molecular similarity and the probability that they elicited a response in animals conditioned to a different odorant (Smith and Menzel, 1989; Shepherd, 1991; Laska *et al.*, 1999). Each odorant was diluted in hexane to three different concentrations: 0.0002 M (low), 0.02 M (intermediate) and 2.0 M (high). These concentrations refer strictly to the concentration of odor in the solution used for conditioning, not to the concentration of the odorant present in the stimulus during its delivery. Odorant stimuli were delivered by placing 5 µl of one of the solutions onto a small strip of filter paper that was then placed in a modified, 1 ml tuberculin glass syringe attached to an air source. Air was shunted through the cartridge for 4 s by way of solenoid valve actuated by a computer (Smith, 1998). The distance between the end of the syringe and each subject's head during conditioning was 3.5 cm and was the same for each conditioning trial.

Standardization of odor delivery concentration

We evaluated several measures in order to confirm that all odor concentrations were detectable to honey bees and that changes in concentration translated into changes in sensory input. Electroantennogram (EAG) recordings of all five odors were made using the antennae from the isolated heads of individual honey bees (Bhagavan and Smith, 1997), where the end of the distal flagellomere of the antenna was cut off, and an electrode was placed into the tip of the antenna. The ground electrode was placed in a saline pool that was in contact with the isolated head. The odorants at each of the concentrations were delivered only once as discrete stimuli 2 s in duration, with a 2 min interval between stimulations. One antenna from the isolated head was tested with only one odorant, and the order of presentation of the concentrations was randomized. In addition, each antenna was tested for background EAG response to an air stimulus and to an air stimulus that contained hexane. To test whether the low concentration odor was detectable above the stimulus background, we compared the response of an antenna to an air stimulus, a hexane–air stimulus, and the latter stimulus containing low concentration of odor. Each is significantly different [repeated-measures analysis of variance (ANOVA), df = 1, 39, $P < 0.001$; Figure 1]. We also compared the response of an antenna to the high, intermediate, and low concentrations, and the response elicited by each concentration was significantly different (repeated-measures ANOVA, df = 1, 42, $P < 0.001$; Figure 1).

We examined the detectability of the odorant in the compound stimulus in two different experiments. In one experiment we conditioned our subjects only with the low concentration odorant, and then tested them after the first conditioning trial and the last conditioning trial with two extinction trials. The extinction trials were either the training stimulus (0.0002 M odorant) or a stimulus containing only hexane. The order of presentation each extinction trial was randomized. This experiment was done to evaluate whether

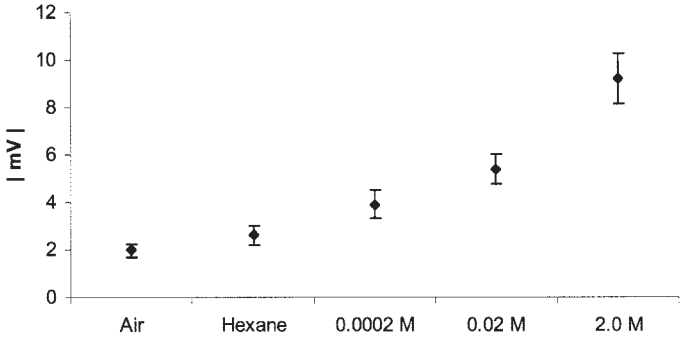

Figure 1 Electroantennogram response of the honey bee antenna to odorant at three concentration levels. The data represents the responses of individuals for five odorants (1-hexanol, 1-heptanol, 1-octanol, geraniol, and 2-octanone). As odorant concentration increased, the response of the olfactory receptor neurons in the antenna increased (Repeated-measures ANOVA, df = 4, 44, $P < 0.001$).

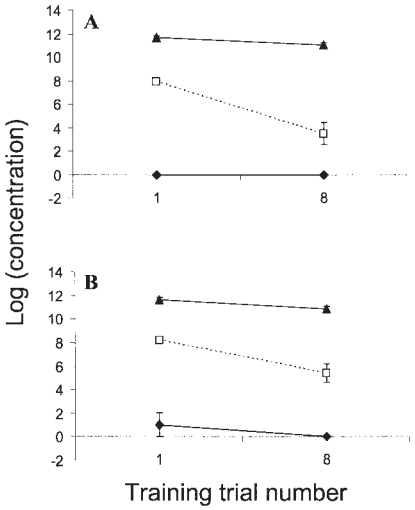

Figure 2 1-Hexanol volatiles collected from individual solid phase micro-extraction fibers (SPME) decreased with concentration (ANOVA, df = 2, 46, concentration by number of trials, $P < 0.001$). The rate of odorant depletion was greater for the low (circles) and intermediate (open squares) concentrations than for the high (triangles) concentration of odorant.

the odorant was detectable above the hexane solvent in which it was diluted.

In a second experiment, we examined whether the hexane produced conditioned responding and could potentially account for generalization during the test after 16 conditioning trials. We conditioned three different groups of subjects each with one of three types of stimuli: one of the odorants at the low concentration (0.0002 M); a stimulus containing only hexane; and a stimulus that contained no hexane and no odorant, which we termed the 'air' stimulus. The syringe for the air stimulus also contained a piece of filter paper. After training our subjects over 16 trials with one of these three treatments, they were presented with a 0.0002 M odorant stimulus, a hexane stimulus, and an air stimulus. The order of presentation of each of these test conditions was randomized between subjects.

To test whether our odorant stimulus would be different if we used mineral oil as a solvent, we conditioned honey bees using the same concentrations (0.0002, 0.02 and 2.0 M) of 1-hexanol in mineral oil. In contrast to the results for hexane as solvent (see Results), only the 2.0 M concentration in mineral oil yielded a response probability to odor that was significantly greater than to the blank (mineral oil) stimulus after the first conditioning trial (paired-samples $t = 7.21$, df = 16, $P < 0.001$) and the test after conditioning trial 16 ($t = 2.95$, df = 16, $P < 0.01$).

We further investigated the use of the mineral oil and hexane solvents by gas chromatographic analysis (Shimadzu GC-17A fitted with a J & W Scientific, DB-1, 30 m × 0.320 mm column) of the odor volatiles delivered through each solvent. Individual solid phase micro-extraction fibers (SPME) (Supelco, carboxen/polydimethylsiloxane, 75 µm 57344-U) were used to sample the odor plumes produced by the odor delivery system. The volatiles were sampled after the first and eighth trials. For sampling, SPME fibers were exposed to the odor stream for 4 s and then immediately desorbed in the injection port of the GC. We were unable to detect hexane, or contaminants from the hexane, as a part of the volatile component delivered to the SPME fiber in any of the samples. For both hexane and mineral oil, the amount of 1-hexanol collected by the SPME fiber decreased with concentration (ANOVA, df = 2, 46, concentration by number of trials, $P < 0.001$; Figure 2). The change from 2.0 M to 0.02 M in hexane produced a 40-fold decrease in captured odor. Furthermore, repeated use of odor cartridges depleted the odor that was delivered. The rate of odorant depletion was greater for the low concentrations than for the high concentrations of odorant. Because the odorant concentration decreased over trials, we replaced cartridges with fresh odor cartridges every 8 trials for all experiments. Furthermore, all test trials were performed with unused cartridges.

Experimental procedure

In our experiments we conditioned honey bees to extend their mouthparts (proboscis) to an odor by pairing that odor with a sucrose reward during 16 acquisition trials. We conditioned five odorants at three different concentrations (low, intermediate and high, as above) in different groups of honey bees. After training, we used a stimulus generalization test to evaluate discriminability of odors (Pearce, 1994). After honey bees had been conditioned to one concentration of an odorant they were tested without sucrose presentation in randomized order with three odorants at the same concentration as the conditioned odorant. One of the three test odorants was the conditioned odorant. The two remaining odorants differed from the conditioned odorant in terms of specific 'determinants' (Shepherd, 1991), which correspond to different molecular features of each odorant. For three

groups of honey bees the latter two odorants were structurally 'similar' to the test odorant. They contained the same terminal alcohol functional group but differed in regard to one or two methylene (-CH_2-) units in the straight carbon chain (1-hexanol, 1-heptanol and 1-octanol). The remaining three treatment groups were tested with the conditioned odorant and two odorants that are structurally 'dissimilar' to the conditioned odorant. Determinants of the latter two odorants differed in terms of functional group and elements of the carbon chain (1-hexanol, geraniol and 2-octanone). Previous studies with honey bees have shown that these determinants regulate perceptual similarity of these odorants (Smith and Menzel, 1989).

Animals show generalized responses because those stimuli are perceptually similar or because the stimuli have been cognitively grouped into the same class (Shepard, 1987). Using the conditioning and test protocol described above, we assume that stronger generalization reflects *reduced* ability to discriminate (Mackintosh, 1983). However, we do not mean to imply that equal generalization among stimuli means that it is impossible for honey bees to discriminate among them. Changing conditioning protocols, especially to include non-reinforcement or aversive reinforcement of generalization responses, can reveal that conditioned subjects have the ability to discriminate very similar stimuli (Smith *et al.*, 1991). Therefore, when treatment conditions produce stronger, even equivalent, generalization among test stimuli, we argue only that the stimuli are relatively less distinguishable in one condition versus another.

Results

Odorant detectability

The odor was a salient part of the stimulus even at the lowest concentration, because honey bees responded significantly more often to the odorant stimulus than to a background stimulus containing hexane (Figure 3). When tested after the first and after 16 acquisition trials, a higher percentage of honey bees responded to the odor than to the background stimulus (Paired-sample t, df = 46, trial 1: $P < 0.001$; trial 16: $P = 0.01$). We concluded that honey bees were able to distinguish the low concentration of odor stimulus from the hexane stimulus.

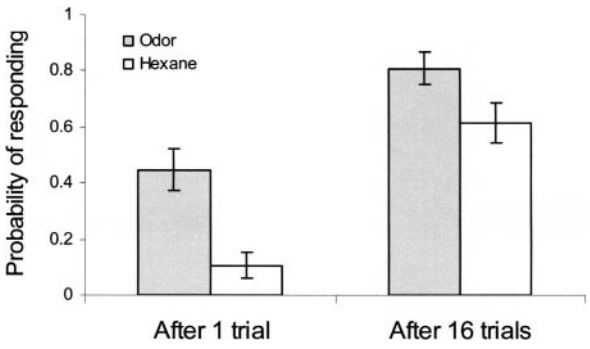

Figure 3 The low concentration of all 5 odor stimuli can be discriminated from the background stimulus. After training with a low concentration odorant, the response to the low concentration odor stimulus was significantly greater than response to the air + solvent after trials 1 and 16 (paired-samples t-tests: $n = 47$; trial 1: $P < 0.001$; trial 16: $P = 0.027$).

When our subjects were conditioned with the low concentration odorant, they learned the odorant at a faster rate (Figure 4a). The asymptotic level of conditioning was also higher for any stimulus that contained odorant than for either hexane or air after 16 trials (logistic regression: odor versus hexane, df = 1, $P < 0.001$; odor versus air, df = 1, $P < 0.001$). The levels reached by the hexane stimulus and the air stimulus were not significantly different (logistic regression; df = 1, $P = 0.235$).

If subjects had been conditioned with 0.0002 M odorant (Figure 4b), they responded to odorant with a greater probability than they responded to either the hexane or the air stimulus (logisitic regression: odor versus hexane: df = 1, $P = 0.007$). The response to hexane was not significantly different from the response to the air stimulus (logisitic regression: df = 1, $P = 0.263$). If subjects were conditioned to hexane (Figure 4c), during the test there was not a significant difference between any pairs among the three test conditions (logisitic regression, hexane versus air: df = 1, $P = 0.071$; odor versus air: df = 1, $P = 0.338$; odor versus hexane: df = 1, $P = 0.402$). If subjects were conditioned to an air stimulus (Figure 4d), the relative response to the stimuli during the test was never significantly different (logisitic regression, hexane versus air: df = 1, $P = 0.912$; odor versus air: df = 1, $P = 0.195$; odor versus hexane: df = 1, $P = 0.239$). If the responses for the hexane and air stimuli during the test are averaged for each of the conditioning stimuli, the mean response for each is not significantly different (logisitic regression, df = 2, P = 0.681). Thus, it appears that the response to the air–hexane compound was primarily driven by the mechanosensory stimulation provided by the air stream.

Rate of learning

The response to each concentration of each odor increased across the 16 acquisition trials (Figure 5a,b). The highest concentration of the conditioned odor produced the fastest rate of acquisition for all six odorants (logistic regression, df = 30, $P = 0.014$), and the response to all odor concentrations reached approximately the same peak level of responding in the latter half of the trials, although not at precisely the same rate (logistic regression, df = 8, $P < 0.001$). The percentage of subjects responding to odorants of different concentrations on trial 16 was not significantly different (logistic regression, df = 2, $P = 0.196$), and this was not affected by the conditioning odorant (logistic regression, df = 2, $P = 0.293$). The mean rate of generalization to the conditioned odor during the test was not significantly different compared across the concentrations of the conditioning odor (logistic regression, df = 1, $P = 0.773$) and was not different

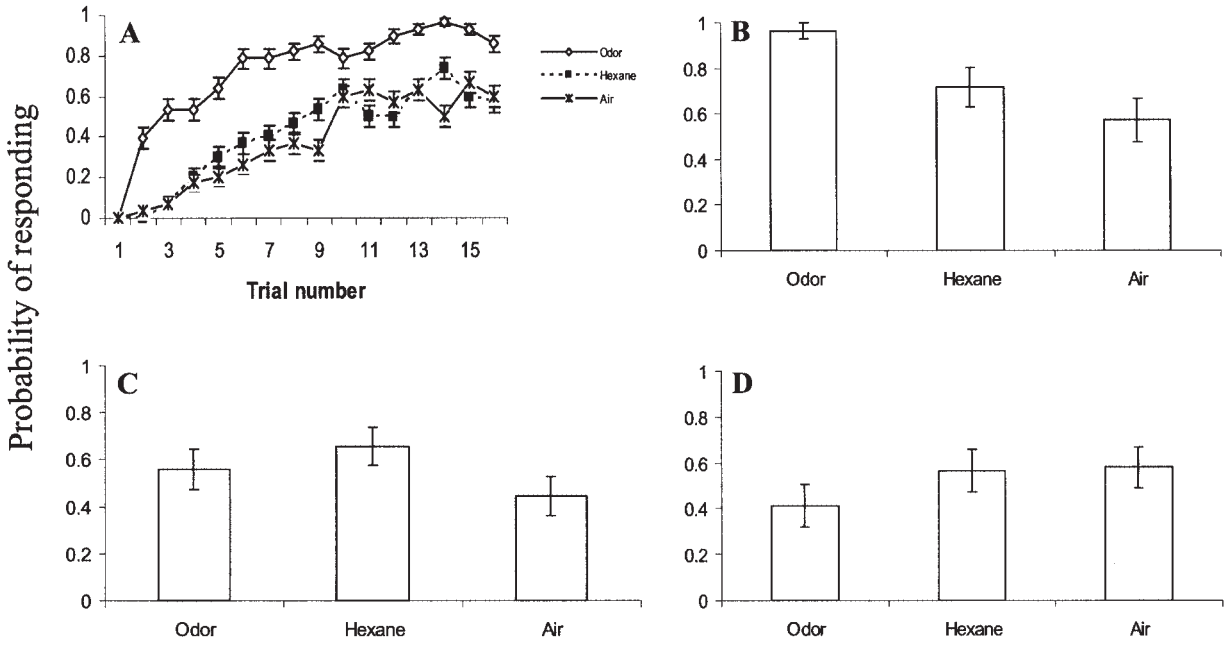

Figure 4 The low concentration odorant is a more salient stimulus than a stimulus containing only hexane or a stimulus with neither hexane nor odorant (air). **(a)** Individuals conditioned with the low concentration odorant over 16 trials reach a greater level of association than subjects conditioned only with hexane or air (for odorant: $n = 28$; for hexane: $n = 30$; for air: $n = 30$). **(b)** Individuals conditioned with odorant respond more to the odorant stimulus than to the hexane or air stimuli (logistic regression, df = 2, $P < 0.001$). **(c)** Individuals conditioned with hexane respond equally well to all stimuli (logistic regression, df = 1, $P = 0.263$). **(d)** Individuals conditioned with an air stimulus respond equally to all test stimuli (logistic regression, df = 2, $P < 0.361$).

compared among the conditioning odors (logistic regression, df = 1, $P = 0.132$).

Generalization among odorants

We found that generalization was a function of molecular structure and concentration. As expected for similar odorants, the response to conditioning and test odorants was consistently high over all of our test concentrations (Figure 6a). The slight trend toward less generalization at higher concentrations was not significant at the $\alpha = 0.05$ level (logistic regression, df = 2, $P = 0.08$).

Dissimilar odorants (Figure 6b) also elicited generalization responses. However, in this case generalization was significantly stronger at low concentration than at high (logistic regression, df = 2, $P = 0.003$). In all three groups, stronger generalization occurred at low concentration, indicating that the dissimilar odorants at low concentration were less distinguishable. At both the intermediate and high concentrations the response to the conditioned odorant was significantly higher than the response to the other test odorants (logistic regression, df = 4, $P = 0.03$). Furthermore, comparing only the intermediate with the high concentration showed that there was more generalization at the intermediate than at the high concentration (logistic regression, df = 1, $P = 0.04$). This latter test in particular indicates that generalization increases with concentration, because at both concentrations the conditioned odor is easily discriminable from the other test odors.

Discussion

Several behavioral studies have shown that both invertebrates and vertebrates generalize from one monomolecular odorant to another to an extent that is proportional to changes in the odorants' molecular features (Smith and Menzel, 1989; Laska and Teubner, 1999; Laska *et al.*, 1999; Daly and Smith, 2000; Laska, 2002; Sakura *et al.*, 2002). Others have also noted that the perceptual qualities of odorants change as a function of concentration, such that the same monomolecular odorant may be perceived differently depending on its concentration (Gross-Isseroff and Lancet, 1988; Marfaing *et al.*, 1989). However, the ability of animals to discriminate among different odorants across levels of concentration is rarely tested (Wise and Cain, 2000; Cleland and Narla, 2004).

In our experiments, monomolecular odorants that were perceptually very distinct at high concentrations were not as easily discriminated at low concentrations, even though the low concentration was above threshold for detection. All the odorants at low concentration were detectable above the solvent/air stream background. The threshold at which the dissimilar odorants became distinguishable appeared to occur at some point between 0.0002 and 0.02 M concentration, and the dissimilar odorants in our experiments became progressively easier to discriminate as odorant concentration increased. The same trend existed for similar odorants, although it was not statistically significant. Indeed, when the subjects were tested at the lowest concentration, they

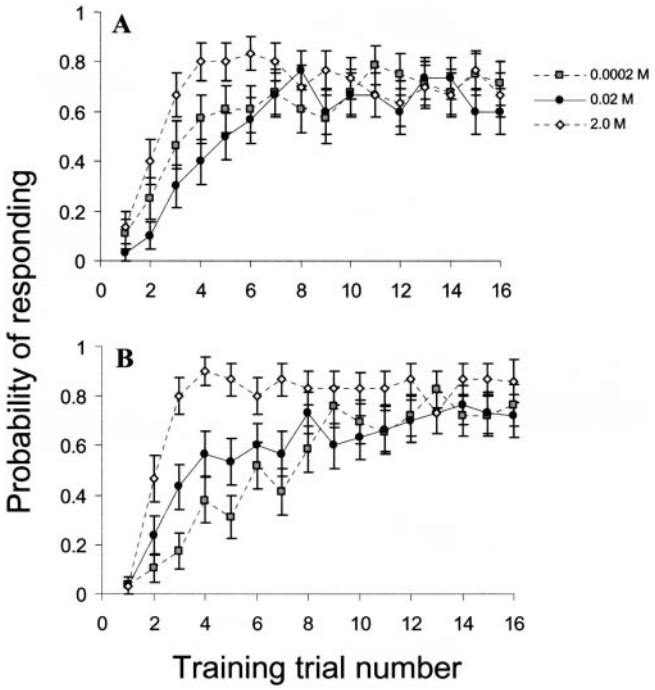

Figure 5 Odor concentration affected the rate of learning. **(a)** Individuals conditioned to one of the similar odorants (1-hexanol, 1-heptanol, 1-octanol) (n = 88). **(b)** Individuals conditioned to one of the dissimilar odorants (1-hexanol, 2-octanone, geraniol) (n = 89). For both sets of odorants, individuals trained with the high concentration (2.0 M) learned to associate odor with sucrose faster than those trained with either the intermediate (0.02 M) or the low (0.0002 M) concentration of odorant (logistic regression, n = 175, concentration × trial number, P = 0.0143). The level of response reached by trial 16 was not significantly different for each of the concentrations (logistic regression, df = 2, P = 0.196) or for each of the odorants (logistic regression, df = 2, P = 0.293).

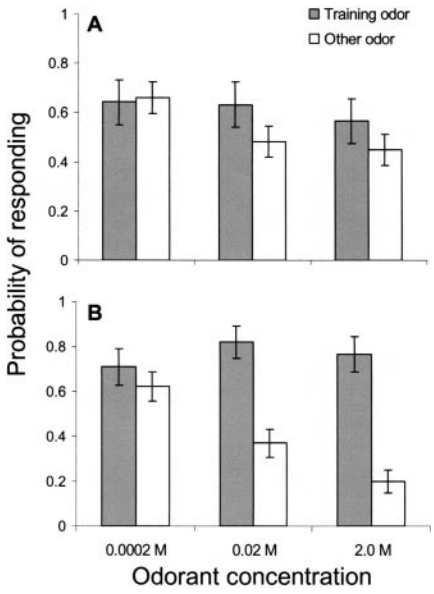

Figure 6 Discrimination increases with concentration for dissimilar odors but not for similar odors. Conditioning and testing were performed at the same concentration for the similar (**a**; n = 87 animals) or dissimilar (**b**; n = 88 animals) test odors. Generalization response levels to novel odors (open columns) are shown next to the response to the conditioned odor (shaded column) at a given training/testing concentration.

behaved as though they could detect an odor but were unable to determine its identity.

We propose that the increase in the ability of bees to distinguish among odorants as concentration increases is a function of the way that odors are coded by the olfactory system. We also suggest that at least two different thresholds exist for the perception of odors over a range of concentrations: a threshold for the detection of odor, and a threshold for the identification of the odor. One possible explanation for our data would be that our odorants do not have an invariant identity across the range of concentrations we tested, as our data show that the ability of subjects to discriminate among odorants continued to increase as a function of concentration beyond the 0.02 M level. For dissimilar odors there was a significant increase in discrimination from intermediate to high concentrations; however, its magnitude was not as great as that observed between the low and intermediate concentrations we tested. It is possible that odor identity, at least as a function of molecular features, increases and then remains stable after it crosses a specific threshold of concentration. Once a threshold for identification of an odor stimulus is reached, odor identity might then be effectively invariant over a range of concentrations encompassed by our intermediate and high concentrations. Testing with a more extensive series of concentrations would be necessary to evaluate whether there is a threshold above which discrimination remains stable.

Support for these hypotheses exists in the current literature on the mechanisms of olfactory coding. Coding for odorants is likely to involve the participation of multiple subtypes of olfactory receptor neurons (ORNs) (Hopfield, 1999; Malnic *et al.*, 1999; Ma and Shepherd, 2000; Getz and Lansky, 2001; Kajiya *et al.*, 2001). In *Drosophila*, all of the ORNs responding to a determinant directly map from the antenna to a specific glomerulus in the antennal lobe (AL) (Vosshall *et al.*, 1999). This arrangement potentially provides a topology that relates directly to odorant quality (Vosshall *et al.*, 1999; Korsching, 2002; Sachse and Galizia, 2002; Wong *et al.*, 2002). Imaging studies of activation of glomeruli in response to odorant over a range of concentrations indicate that recruitment of ORNs and their associated glomeruli may be vital to the coding for odorant quality (Rubin and Katz, 1999; Johnson and Leon, 2000; Xu *et al.*, 2000; Uchida *et al.*, 2000; Meister and Bonhoeffer, 2001). In the rat and the mouse, the qualitative pattern of response across glomeruli to odors, as well as the intensity of the response, was positively correlated with the intensity of the odorant stimulus (Rubin and Katz, 1999; Xu *et al.*, 2000; Fried *et al.*, 2002). In particular, patterns of glomerular activity in the olfactory bulb became increasingly specific to the odorant as the odorant concentration increased

(Duchamp-Viret and Duchamp, 1997; Carlsson and Hansson, 2003). This increase in activity suggests that more ORNs are responding at higher concentrations (Ma and Shepherd, 2000; Meister and Bonhoeffer, 2001). Absolute detectability may be facilitated by highly specific receptors (Araneda *et al.*, 2000) that have high binding affinity with an odorant. Once a concentration threshold is reached, the information added by new ORNs that only respond to higher concentrations of the odorant increases the ability to discriminate among odorants. Thus, the data from imaging studies implies that coding for odorant quality may not be uniform over the entire range of odor detection.

Electrophysiological recordings of neurons in the insect antennal lobe and mushroom bodies also suggest that the temporal response patterns of neurons are affected by odor concentration (Laurent, 1999; Stopfer *et al.*, 2003). Odor stimulation causes oscillations in the local field potential recorded from the antennal lobe that are a result of evolving, synchronized spike activity across several subsets of projection neurons (MacLeod and Laurent, 1996; MacLeod *et al.*, 1998; Laurent *et al.*, 2001). In honey bees, if synchronous spiking activity is disrupted, the ability of honey bees to make fine discriminations among odorants is diminished (Stopfer *et al.*, 1997). In the locust, low concentrations of odorant are less capable of evoking synchronization among projection neurons in the locust antennal lobe (Stopfer *et al.*, 2003); if this mechanism also occurs in honey bees, it might affect the ability of honey bees to identify an odorant's quality as we have observed in our experiments (Cleland and Linster, 2002).

Stopfer *et al.* (2003) have recently demonstrated that specific aspects of the firing of neurons in the antennal lobe also change with respect to concentration. In particular, the power and the duration of the local field potential in the locust antennal lobe increase with respect to the concentration of odorant during stimulation, and the slow temporal patterns of firing also change. When examining the population responses of all the neurons they recorded from 15 locusts, they showed that the responses of projection neurons to an odorant presented at different concentrations were more similar than the responses of the same projection neurons to different odorants at the same concentration. As there is likely to be a direct relationship between odor representation in the antennal lobe and an insect's ability to discriminate odorants, their results imply that odorants should be discriminated across concentration. However, this conclusion is not incompatible with our study as the range of concentrations they used was approximately equal to our intermediate and high concentration odorants; it was only at lower concentrations that discrimination no longer occurred.

These physiological mechanisms, especially the recruitment of new or different sensory cell populations at higher concentrations, could also increase the salience of odors. Salience of a conditioned stimulus is an important factor in an animal's ability to associate it with an unconditioned stimulus (Rescorla and Wagner, 1972; Mackintosh, 1983), and stimulus intensity is one determinant of salience (Shettleworth, 1998). Specifically, more intense stimuli should be more easily perceived against background stimuli. In any conditioning or testing procedure, background stimuli compete with conditioned stimuli for association with reinforcement; the associability of these various stimuli is a function of their relative salience. In our study, background stimuli were certainly a salient part of the context. The background stimuli included visual cues associated with the conditioning context which can influence expression of olfactory conditioning in the PER paradigm (Gerber and Smith, 1998). Other background stimuli included mechanosensory stimulation from the air used for delivery, as well as possible remnants of solvent used for odor dilution. Indeed, our control procedures seemed to reveal that the most important part of the background was the mechanosensory stimulation provided by the air stream in which odor was presented. Honey bees generalized equally between those stimuli in all test conditions, and we could not detect even trace amounts of the solvent in our chemical analyses.

One possible explanation for the increase in generalization at the low concentration in our study could be that the increase in salience of the background stimuli confounded discrimination, as our subjects were responding to the background present in all the low concentration stimuli, rather than the odorant signal present in the background. Since the background was common to all test stimuli, the test stimuli would have been relatively more similar at low odor concentration than at high. During conditioning with the low concentration of odorant, the response to the background stimuli increased across trials, which indicated that the background was also an important part of the conditioning stimulus. In the control experiment in which we condition subjects with the air and hexane stimuli, the asymptotic level of association was two-thirds of the level attained by conditioning subjects with the low concentration odorant, indicating that the salience of these stimuli was not equal to the salience of the odorant stimulus. Therefore, the salience of the background increased, but it was not equal to the salience of the low concentration odorant stimuli.

Our data indicate that the information contained in the olfactory component of the low concentration stimuli was both detectable and discriminated from the hexane and mechanosensory background of odorant delivery. When our subjects were conditioned to the low concentration odorants and tested with dissimilar low concentration odorants, we observed that our subjects generalized to each of the odorants equally well, regardless of the nature of the conditioning odorant. This indicates that they were using the presence of odorant to determine whether the stimulus was similar to the conditioning stimulus. Although similarity of the odorants at the low concentration may increase as a result of the background stimuli, the amount of

information about the identity of the odorant stimulus at the low concentration was not great enough for our subjects to discriminate among the odorant stimuli. We, therefore, hypothesize that at the lowest concentrations, the amount of odorant present was less able to support discrimination than at higher concentrations. The recruitment of additional sensory neurons at higher concentrations could increase the salience of the odor relative to that of the background stimuli arising from multiple sensory modalities. As discussed above, recruitment might also simultaneously cause the perceptual qualities of the odorant to change, as the addition of new subpopulations of neurons could produce a perceptual identity for an odorant that varied as a function of concentration.

Recruitment of sensory neurons as concentration increases is likely to play an important role in odor detection and discrimination (Hopfield, 1999; Firestein, 2001). An inability to discriminate a conditioned odorant from a dissimilar test odorant at low concentration implies that the first information available to an organism about an odorant at the lowest detectable concentration is simply that an odor is present. Increasing the concentration produces more information about the specific nature of the odorant relative to background stimuli which contributes critically to that odorant's encoding by the olfactory system. In future studies, we plan to examine the specific point at which odorants can be discriminated by honey bees. We also plan to test whether odorant identity is invariant with respect to concentration when odors reach concentrations beyond this point. Pharmacological manipulation of spatial and temporal patterns of activation in the AL, in combination with behavioral studies such as we present here, are now necessary for further evaluation of this hypothesis.

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