

Chemosensory processing in the fruit fly, *Drosophila melanogaster*: Generalization of a feeding response reveals overlapping odour representations

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Insects are capable of detecting, and discriminating between, a very large number of odours. The biological relevance of many of those odours, particularly those related to food, must first be learned. Given that the number of sensory receptors and antennal lobe (AL) glomeruli is limited relative to the number of odours that must be detectable, this ability implies that the olfactory system makes use of a combinatorial coding scheme whereby each sensory cell or AL projection neuron can participate in coding for several different odours. An important step in understanding this coding scheme is to behaviourally quantify the degree to which sets of odours are discriminable. Here we evaluate odour discriminability in the fruit fly, *Drosophila melanogaster*, by first conditioning individual flies to not respond to any of several odourants using a nonassociative conditioning protocol (habituation). We show that flies habituate unconditioned leg movement responses to both mechanosensory and olfactory stimulation over 25 unreinforced trials. Habituation is retained for at least 2 h and is subject to dishabituation. Finally, we test the degree to which the conditioned response generalizes to other odourants based on molecular features of the odourants (e.g. carbon chain length and the presence of a target functional group). These tests reveal predictable generalization gradients across these molecular features. These data substantiate the claim that these features are relevant coding dimensions in the fruit fly olfactory system, as has been shown for other insect and vertebrate species.

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1. Introduction

Olfactory systems of insects and mammals exhibit an array of similarities (Homberg *et al* 1989; Hildebrand 1995; Hildebrand and Shepherd 1997; Strausfeld and Hildebrand 1999). A large number of sensory cells, which transduce chemical information into electrical signals, converge onto several orders of magnitude fewer regions – termed glomeruli – in the insect antennal lobe (AL; Homberg *et al* 1989; Sandeman and Sandeman 1998) and in the mammalian olfactory bulb (OB; Shipley and Ennis 1996;

Kashiwadani *et al* 1999). Peripheral sensory cells exhibit a range of tuning properties, from narrowly tuned insect pheromone receptor cells (Boeckh *et al* 1965; Mustaparta 1997) to more broadly tuned cells characteristic of mammals and some insects (Boeckh *et al* 1965; Getchell 1974; Akers and Getz 1993; Bozza and Kauer 1998; Shields and Hildebrand 2001). The latter type of cell is excited to varying degrees by a small set of odourants or mixtures (Bozza and Kauer 1998). In the AL and OB, the incoming sensory information is further processed by way of local interneurons, the majority of which are GABAergic

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Abbreviations used: AL, Antennal lobe; CS, conditioned stimulus; OB, olfactory bulb.

(Homberg *et al* 1989; Shipley and Ennis 1996), as well as by descending modulatory inputs from other brain centers (Shipley and Ennis 1996).

These similarities in coding schemes have arisen through use of families of odourant receptors (Buck 1996, 2000; Mombaerts 1999). Odourants interact with a large array (less than 100 to approximately 1,000 depending on the species) of G-protein coupled receptors expressed by sensory cells (Buck and Axel 1991; Clyne *et al* 1999; Vosshall *et al* 1999). These receptors exhibit significant similarity in molecular sequences across vertebrate species from fish to mammals (reviewed in Mombaerts 1999), which implies that the receptors are evolutionarily homologous. Yet sequences differ significantly across insect orders and between insects and vertebrates (Strausfeld and Hildebrand 1999). This lack of homology indicates that the similarities in coding properties at the systems level may have arisen due to convergent evolution.

Convergence implies that there are important functional principles of olfactory systems in general that may be revealed by more in-depth comparative analyses (Strausfeld and Hildebrand 1999). One component of these analyses must involve behavioural investigations of how these convergent groups of animals (invertebrates and vertebrates as well as different insect orders) represent information about odours. Studies of sex pheromone processing, for example, have proven to be particularly useful for mapping the relationship between behaviour and physiology (Hildebrand 1995). Male moths identify and orient to conspecific females by means of a blend of odourants that is specific to that species (Mustaparta 1997). Sensory cells are relatively narrowly tuned to components of the blend (Hildebrand 1995), and they project to specialized processing centers in the brain (Homberg *et al* 1989). This scheme is generally referred to as labelled-line coding.

Insects also learn about a variety of nonpheromonal odourants that are usually associated with food (Smith and Getz 1994). Several studies have now begun to reveal how these odours are represented in the insect olfactory system (Boeckh *et al* 1965, 1984; Akers and Getz 1993; Stopfer *et al* 1997; Galizia *et al* 1999, 2000; Jansson and Anderson 1999; Shields and Hildebrand 2001). There is a large number of such odours relative to the number of sensory cells and receptor types. Therefore, broadly tuned cells contribute to encoding these odours such that a given cell most likely participates in encoding several different odours (Akers and Getz 1994; Shields and Hildebrand 2001). This cross-fiber sensory input is converted by processing in the AL into a code that has both spatial and temporal properties (Laurent 1996, 1999).

This 'combinatorial' coding would lead to greater overlap in neural representations for odourants across sensory cells and in the AL and OB (Cinelli *et al* 1995;

Bozza and Kauer 1998; Laurent 1999; Malnic *et al* 1999). To evaluate this type of processing, honey bees and moths have been associatively conditioned to a target odourant followed by generalization testing with an array of odourants that differ from the target odourant along one or more dimensions of molecular structure (Smith and Menzel 1989; Fan *et al* 1997; Stopfer *et al* 1997; Hosler *et al* 2000; Daly and Smith 2000; Daly *et al* 2001). After conditioning to a target alcohol, ketone or aldehyde, for example, honey bees and moths reveal smoothly decaying generalization gradients as a function of carbon chain length, shape and functional group (Daly *et al* 2001). This pattern of generalization would be consistent with a combinatorial coding scheme in which the neural representations for those same odourants overlap as a function of the same molecular structures, as has been reported for the rabbit OB (Imamura *et al* 1992; Katoh *et al* 1993; Mori *et al* 1999).

Several molecular and physiological techniques must now be employed to test this hypothesis in the AL. These types of investigations would be greatly augmented by use of genetic information and techniques that has been developed for the fruit fly, *Drosophila melanogaster*. Establishment of behavioural protocols would permit use of lines in which sensory receptors or more central biochemical pathways have been genetically identified (Vosshall 2001). Furthermore, behavioural analysis of olfactory dimensions in the fruit fly would provide valuable comparative information because of the somewhat more limited capacity for odour coding in this species, which possesses only 43 glomeruli as opposed to 60 in the sphinx moth or 160 in the honey bee, respectively (Flanagan and Mercer 1989; Rospars and Hildebrand 1992; Stocker 1994).

Here we report on development of an assay that makes use of nonassociative modification of behaviour to odours. This assay is relatively easy to use and should be adaptable to a wide variety of investigations that involve hypotheses about molecular genetic mechanisms of olfaction in this species. More importantly, this assay reveals that there is a correspondence between molecular characteristics of odourant molecules and how flies perceive odours. Smoothly decaying generalization gradients imply that the odour code for this species makes use a combinatorial mechanism.

2. Materials and methods

The subjects used were male and female *Drosophila melanogaster*. All flies were cultured on a standard cornmeal medium in a half-pint milk bottles and maintained on a 16 h : 8 h light/dark cycle at 25°C and 50% relative humidity. Groups of 20–25 flies were transferred everyday

to an empty vial, where they were anaesthetized by cooling in a freezer (-20°C) for approximately one minute. Each fly was mounted in a $200\ \mu\text{l}$ micropipette tip in such way that its head and forelegs protruded through the small hole at one end, which was widened to approximately of $0.1\ \text{mm}$ inner diameter (Vargo and Hirsch 1982). The wide end of the micropipette tip was closed with modelling clay, which prevented the fly from escaping and provided flexibility for positioning the flies in the training arena. Mounting of flies was done between 10:00 am and 1:00 pm. Experiments were started 30 min later.

Each fly was conditioned for movement of its first pair of legs using odour as the conditioned stimulus (CS). We used geraniol, 1-hexanol or blank as the CS depending on the experiment. A conditioning trial began after we placed a subject into a conditioning arena through which air was constantly drawn over the subject's antennae and into an exhaust vent. The exhaust ensured that odour was removed quickly from the arena. Odour delivery began approximately 30 s after a subject was placed into the arena and it lasted 4 s. Odourant delivery was accomplished by activation of a valve that was controlled by a parallel port on a computer. When it was activated, the valve carried air through a 1 cc glass syringe that contained $3\ \mu\text{l}$ of pure odourant applied to small strip of filter paper. Each trial lasted for 60 s, after which the next subject was placed into the conditioning arena and the procedure was repeated.

2.1 Response measures

The response of flies to odour was categorized into three levels based on their movement of their first pair of legs. Flies normally move their legs intermittently. Generally, the CS was presented when the fly was motionless. Most flies either did not respond or paused a response during the 4 s presentation of CS. Flies typically initiated leg movement after termination of the CS. We recorded the response as '0' if a fly failed to start moving its legs within 3 s after termination of the CS. We recorded the response as '1' if a fly started moving its legs within 3 s after termination of the CS and continued to move its legs up to but not exceeding 9 s. We recorded the response as '2' if the fly continued to move its legs for more than 9 s after the presentation of CS. The latter response is the most vigorous response, which is on average the response from flies when first presented with a novel odour.

2.2 Habituation to odours

The goal of this experiment was to demonstrate habituation of the leg movement response to odour. In this experiment, flies were exposed to 25 trials of odour presentation as described above. Three different treatment groups

were used, and each group corresponded to different odour treatments: 1-hexanol, geraniol and air alone. Odour treatments were presented in an air background. By comparison of the response to odour + air to air alone we could evaluate whether any observed habituation was to the odour, to the mechanosensory stimulation provided by air, or to a combination of both. The response of each fly was recorded on each trial as described above. We conditioned five flies per day and therefore maintained an inter-trial interval of 5 min. Approximately 20 animals were conditioned for each treatment group.

2.3 Dishabituation to odours

The purpose of this experiment was to find out whether a sensitizing stimulus could disrupt habituation to odourants, which is an important test criterion for habituation (Thompson and Spencer 1966). The previous experiment indicated that 25 presentations of odourant were sufficient to habituate flies to odour. We therefore chose 25 trials as a standard for this and all subsequent experiments. From this point onwards, we used an automated delivery apparatus to habituate flies to odours. A trial began after we placed 10 flies at a time into a conditioning arena through which air was constantly drawn into an exhaust vent. Automated odour delivery was programmed for every 5 min for 25 trials. Each day one of the three odours (geraniol, octanal and hexanal) was used as the CS. Because we used an automated odourant delivery apparatus to habituate flies, we did not record the habituation curve.

After the presentation of 25 trials of odour, flies were removed from the conditioned arena and held on a desk for approximately 10 min. We chose 5 flies at random that were then brought to the test arena. Each fly was first tested for a response to the conditioned odour that was used on that day, and its response was recorded as described above. Each trial lasted for 60 s. Then, each fly was fed with 1.5 M sucrose solution for approximately 2 s and tested again immediately afterwards. A total of 28 flies were used in this experiment.

2.4 Retention of habituation to odours

This experiment was designed to determine how long flies retain habituation to odours. Each day flies were conditioned to 25 trials with one odour (geraniol, octanal or hexanal). A group of 5 flies were then tested for their response to the conditioned odour they had been exposed to 10 min after the end of conditioning period. The second group of subjects was kept on a desk for 2 h. This second group was then identically tested. A total 25 animals were used in each treatment group.

2.5 Generalization to odours

The purpose of this experiment was to evaluate generalization to odourants that are of similar or dissimilar chemical structures. Moths (Daly *et al* 2001) and bees (Smith and Menzel 1989; Stopfer *et al* 1997; Hosler *et al* 2000) generalize among the odourants to a degree that relates to molecular similarities of conditioning and test odourants. The basic structure of our first series of generalization tests was to test flies with three odourants, which were presented in a randomized sequence across flies. One of the test odourants was the conditioned odourant, which had been presented over 25 trials as described above. A second test odourant was similar to the molecular structure of the conditioned odourant. The structure of the remaining test odourant was dissimilar to the conditioned odourant.

The first groups were conditioned either to 1-hexanol or to 1-octanol and then tested with 1-hexanol, 1-octanol and geraniol. The first two odourants are alcohols that differ in carbon chain length by two carbons. These two odourants are structurally dissimilar to geraniol, which is a terpene. In the second group, we used either hexanal or octanal as conditioned odours. Both are aldehydes that differ in chain length by 2 carbons. Again we used geraniol as the dissimilar odour. In the third group, we conditioned flies to the terpenes geraniol or linalool. Here we used hexanal as the dissimilar odour. In each group we tested 5 flies per day, which maintained a 5 min inter-trial interval for the test series.

2.6 Statistical analysis

For the habituation curve, we compared the distribution of responses to stimuli over 25 trials by Two-way ANOVA (Sokal and Rohlf 1997). Data were recorded as 0 for no response, 1 for a response and 2 for a normal/baseline response as described above. These data were used to create a habituation curve, which showed the average response across flies by trial for each stimulus. This analysis revealed the main effects of stimulus and trial as well as the interaction between the two main effects. For all other experiments, we calculated the accumulated number of flies that fell in each response category (0, 1, 2). Then we compared the distribution of responses between different treatment groups by a chi-squared analysis (Sokal and Rohlf 1997).

3. Results

3.1 Habituation to odours

Figure 1 illustrates the mean response levels of flies to geraniol, hexanal and air alone over 25 trials. Each fly had a response of 0 or 1 or 2 in each trial. Each point at

each trial number (from 1 to 25) shown in the figure represents an average response of 14 flies to one of the three stimuli (Hexanal or Geraniol or air). There was a significant effect of stimulus ($df = 2$; $F = 22.24$; $P < 0.001$). Flies showed more habituation to an air stimulus than to air that contained either of the two odour stimuli. This effect is most evident after trial number 17, when response levels of flies tested with air alone were consistently lower than either group exposed to air that contained odour. There was also a significant effect of trial ($df = 24$, $F = 9.01$, $P < 0.001$), which reveal habituation. In the first trials flies showed on average significantly higher levels of response to all stimuli than in later trials. This habituation across trials was evident to all three stimuli, because the interaction between stimulus and trial was not significant ($df = 48$; $F = 0.65$; ns).

The mean response levels shown in figure 1 reflect the average response probability across a population of 14 flies. But this mean population response level is reflected on average in the behaviour of individual flies (figure 2). However, we cannot say for sure exactly what fraction of flies behaves as in figure 2. This is simply because no two flies show exactly the same degree of habituation. Most flies initially show strong responses ('2') toward odour. After a few trials the probability of the less vigorous response levels (a '1' or a '0') increases. Thus the responses are predominantly vigorous ('2') early on, and across trials the frequency of a '2' decreases in favour of the less vigorous categories. Note that after 25 trials we do not completely habituate the response, which is evident in an occasional '2'. Furthermore, in any population of flies there are always a few flies that fail to show evidence of habituation (figure 2D).

3.2 Dishabituation to odours

After habituation treatment, sucrose stimulation caused dishabituation to odour. Figure 3 shows the response of flies to the conditioned odour before and 2 s after sucrose stimulation. Flies tested immediately after sucrose stimulation showed much higher level of responses to the conditioned odourant than they did before stimulation ($N = 28$, $X^2 = 20.01$, $P < 0.0001$).

3.3 Retention of habituation

Habituation to odours lasted at least for 2 h, which was the longest interval over which we tested for retention. Figure 4 shows the response of flies to the conditioned odour in two control and odour treatment groups, one tested 10 min (figure 4A) and the other 2 h (figure 4B) after habituation treatment. Ten min after habituation treatment, flies in the control group showed significantly

higher levels of response than that of the subjects in the treatment group ($N = 24$; $X^2 = 11.23$; $P < 0.003$). Two h after the habituation treatment, again flies in the control group showed significantly higher level of response than that of flies in the experimental group ($N = 24$; $X^2 = 12.21$; $P < 0.0001$).

3.4 Generalization to odours

Flies generalize habituation to odours to a degree that is correlated to molecular similarity. Figure 5 illustrates the response of three sets of flies to the conditioned, similar and dissimilar odours. When we conditioned flies either to 1-hexanol or to 1-octanol (figure 5A), and then tested their responses to the remaining alcohol and the dissimilar odour (geraniol), flies showed significantly higher level of responses to geraniol than to 1-hexanol and 1-octanol ($N = 26$; $X^2 = 12.21$; $P < 0.01$). When tested with the conditioned odour (either 1-octanol or 1-hexanol), most flies were scored as having responded at an intermediate ('1') level. The distribution of responses was similar for the similar odour, although there was a slight shift toward higher ('2') levels of responding. Finally, in

responding to the dissimilar odour most flies fell into the highest response ('2') category.

A similar pattern of generalization across the conditioned, similar and dissimilar odours was evident when we conditioned to the other two sets of three odours (figure 5B,C). When we conditioned flies either to hexanal or to octanal, then tested their responses to both aldehydes and to the dissimilar odour (geraniol), flies showed a significantly higher level of response to the dissimilar odour than to either hexanal or octanal (figure 5B; $N = 25$; $X^2 = 13.4$; $P < 0.01$). Note that in this set the distribution of responses to *S* was more intermediate in relation to the conditioned odour and the dissimilar than in the previous set of odourants (figure 5A). When we conditioned flies either to geraniol or to linalool (figure 5C), then tested their responses to a dissimilar odour (hexanal), flies showed a significantly higher level of responses to hexanal than to either geraniol or linalool ($N = 29$; $X^2 = 11.18$; $P < 0.02$).

In summary, in all three cases the habituated response to the conditioned odour generalized more to the similar odourant more than to the dissimilar odourant. Furthermore, the degree to which the distribution of responses

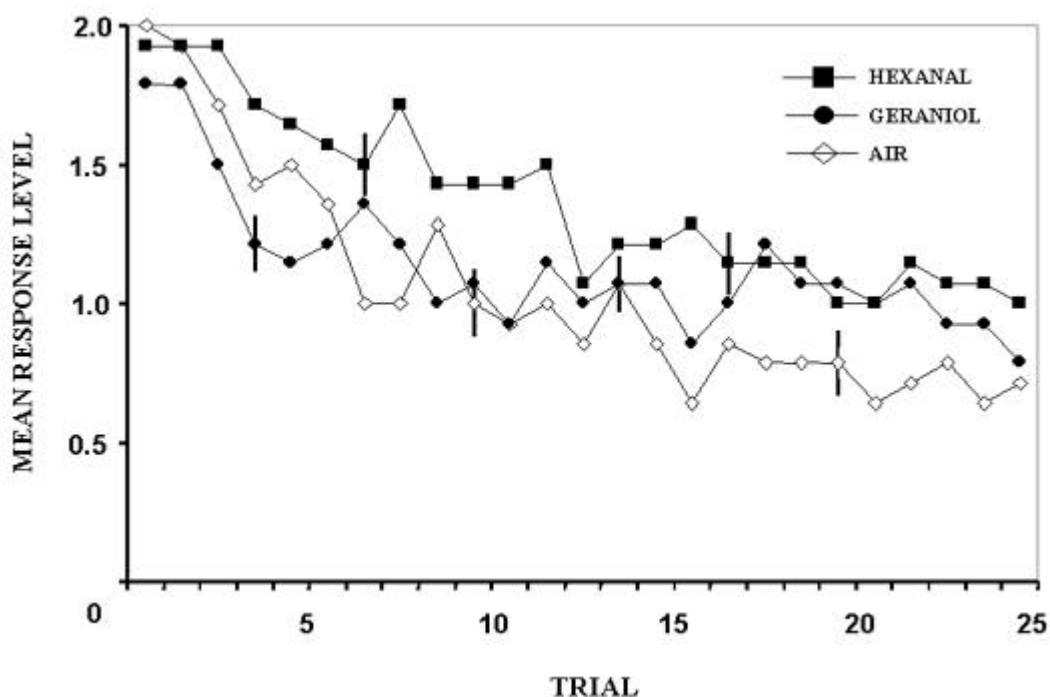


Figure 1. Habituation of fruit flies to air or to air containing geraniol or hexanal. Three groups of restrained flies (Vargo and Hirsch 1982) were exposed to 25 trials (x -axis) during which they were exposed to a stimulus for 4 s every 5 min (inter-stimulus interval). Leg movement behaviour was scored as 0, 1 or 2 based on criteria in the text, and the mean response level (y -axis) is shown as a function of trial. Vertical bars at trials 10 and 20 represent standard errors for the group exposed to air ($N = 14$). Vertical bars at trials 4 and 14 represent standard errors for the group exposed to geraniol ($N = 14$). Vertical bars at trials 7 and 17 represent standard errors for the group exposed to hexanal ($N = 14$).

changed from tests with the conditioned odour versus tests with the similar odour depended on the odours being tested.

4. Discussion

We have demonstrated generalization of habituation to odour in the fruit fly. We found that fruit flies show strong unconditioned responses to stimulation either with air or with air that carries odour. Given the means of restraint (Vargo and Hirsch 1982), these responses are manifested in a change in rates and patterns of leg movement. Typically, flies failed to move their forelegs with the onset of stimulation. Naïve flies responded to stimulus offset by a rapid and extended increase in the rates of leg movement. The latency and probability of initiation of movement change with repeated stimulation. After 4 or 5 trials the latency begins to increase and the duration, which is associated with very long latencies, decreases. Thus the distribution of response strength slowly shifts from 'strong' (short latency and long duration) responses to weak or nonexistent responses. This shift toward weak responses continues at least through trial 25. Without further training we cannot conclusively state at this point that the shift reaches an asymptote.

This decrease in response strength with repeated stimulation is consistent with habituation (Thompson and

Spencer 1966; Pearce 1997) rather than sensory adaptation. First, the decrement in responding lasted at least 2 h. In fact, we failed to observe any indication of recovery over this time. Second, we were able to demonstrate dishabituation, which is an important criterion in studies of habituation (Thompson and Spencer 1966). In our studies, sucrose presentation increased the response to a habituated odourant. Dishabituation refers to the reestablishment of a habituated response shortly after presentation of a sensitizing stimulus. Flies are sensitized by presentation of sucrose, which can be revealed by a transient increase in response to previously neutral stimuli such as water (Dethier *et al* 1965; Vargo and Hirsch 1982).

We have studied generalization of habituation as a means to investigate odour coding in the fruit fly olfactory system. Learned behaviours typically generalize to stimuli other than the CS based at least in part on the physical similarity of the stimuli (Mackintosh 1983; Pearce 1997). Once flies have undergone habituation treatment, they reveal a lowered responsiveness to stimuli other than the one used for habituation treatment. It is important that this generalization of habituation does not extend equally to all stimuli. Thus it does not represent a nonspecific decrement in responsiveness due to a change in, for example, motivational state or health. Furthermore, even though there is some decrease in response to air alone, the decrease is not attributable entirely to habi-

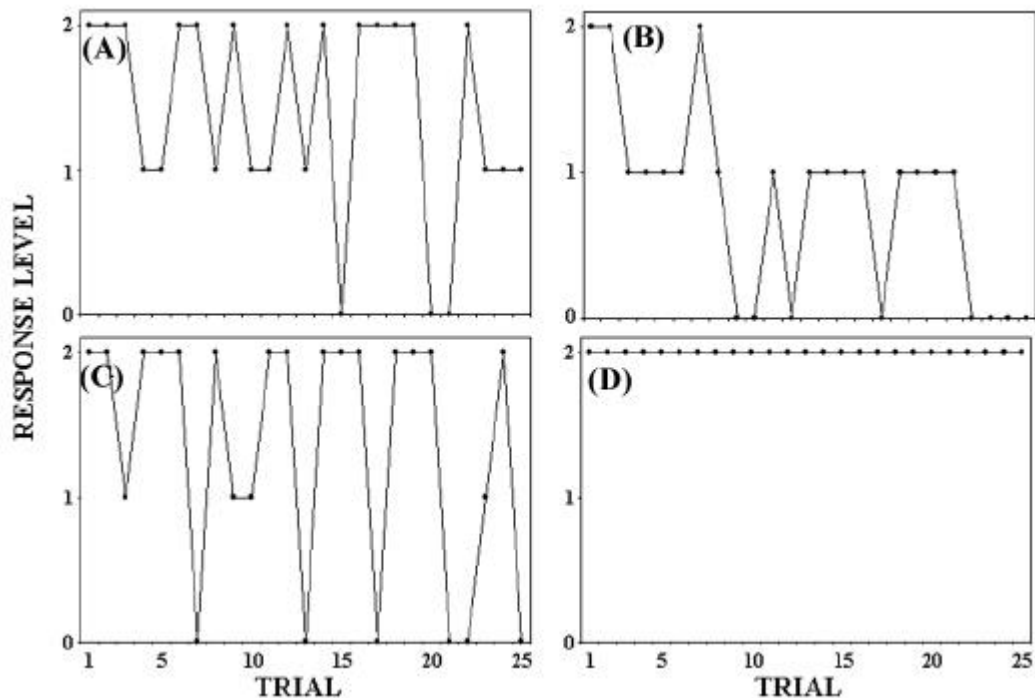


Figure 2. Responses of individual fruit flies across trials to air containing odour. (A–C) Flies that revealed differing degrees of habituation. (D) Example of a fly that failed to reveal habituation.

tuation to that component of the stimulus. Instead, generalization extends to other odourants as a function of similarity of molecular structure, which demonstrated that odourant is a salient part of the stimulus. Once conditioned to an aliphatic alcohol or aldehyde, such as 1-hexanol and hexanal, or to a terpene such as geraniol, there is an orderly increase in responding to odourants that are progressively more different in chain length or structure than to an odourant that represents a significantly different carbon chain structure. Furthermore, for a given carbon chain length generalization is stronger, that is, the response is lower, among compounds that contain the same functional group and the one to which flies were habituated.

We assume that the shapes of these gradients reveal information about the nature of olfactory coding at least for the range of odourants that we have tested. We hypothesize that there is a gradient of habituation that underlies the orderly generalization we observe across odourants. We have observed the same type of gradual generalization decrement in studies with honey bees (*Apis mellifera*; Smith and Menzel 1989; Stopfer *et al* 1997; Hosler *et al* 2000) and moths (*Manduca sexta*; Daly *et al* 2001) that used the same or similar sets of odourants. But for those species a different assay was used to evaluate the generalization gradients. Moths and honey bees typically fail to reveal strong appetitive response to odourants. Spontaneous response probability for any animal, or for a population of animals, to odourants

are typically on the order of 10% (Menzel 1990). Nevertheless, after excitatory conditioning (Menzel and Bitterman 1983; Menzel, 1990; Bitterman 1996; Daly and Smith 2000) the response rates usually lie between 50% and 100% of animals responding. Furthermore, when tested with similar or the same odourants used in this study, the excitatory response generalizes to odourants other than the one conditioned and, as reported here for habituation in fruit flies, generalization reveals an orderly decrement over carbon chain structure and functional group.

The fact that similar smoothly decaying generalization gradients occur across 3 widely dispersed insect species using 2 different conditioning protocols (associative conditioning and habituation) indicates that odour coding may occur by way of a common, perhaps convergent underlying process. Clearly these types of studies should be performed with a wider array of odourants and mixtures. And the systematic exploration of odour perception by way of generalization gradients should be performed with a wider array of animal species (Linster and Hasselmo 1999; Linster and Smith 1999). But in the meantime it is reasonable to propose that there exist perceptual dimensions in the olfactory systems of these animals that roughly, if not exactly, correspond to the molecular dimensions that we have systematically manipulated in our testing protocols. Discriminability of odourants along these dimensions should be a function of generalization (Mackintosh

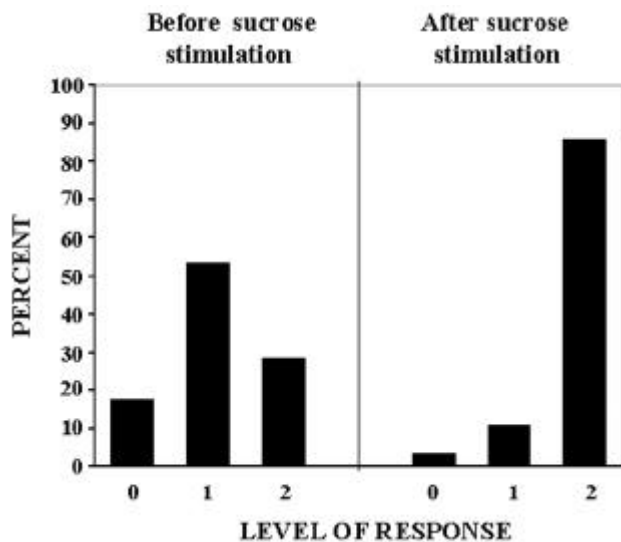


Figure 3. Dishabituation after presentation of sucrose. Distribution of leg movement scores after habituation treatment to air containing odour (left), and again in the same group of flies after sensitization treatment that involved sucrose presentation to tarsi (right). The y-axis represents the percentage of flies that fell into each of the response categories ($N = 28$ flies).

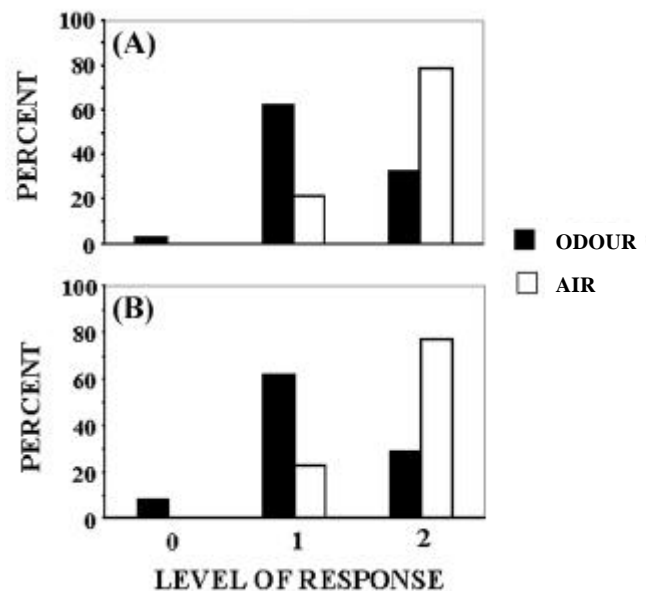


Figure 4. Retention of habituation 10 min (A) or 120 min (B) after habituation treatment. In each retention treatment one group of flies was habituated to air as a reference (open bars) and a second group was habituated to air containing odour (filled bars). The y-axis represents the percentage of flies in each treatment that fell into each of the response categories. Sample sizes are: (A) air = 23; air + odour = 24; (B) air = 22; air + odour = 24.

1983), such that stronger generalization should correlate to lowered discriminability.

These dimensions defined in behavioural studies should correspond to spatial and/or temporal codes for odours in sensory processing in the olfactory system. One would predict, for example, that odourants for which there is strong generalization, and hence lowered discriminability, should show greater similarity in the way that they are represented in early sensory processing (Linster and Hasselmo 1999). One way that this gradient of similarity might be implemented is in a combinatorial coding scheme in which any given sensory cell, or projection neuron in the AL, would participate in coding for a few to several different odourants (Bozza and Kauer 1998; Laurent 1999; Malnic *et al* 1999). Thus the neural code for an odour would be combinatorial in the sense that it would depend on the ensemble of neurons that are activated, and a similar odour would activate a slightly different ensemble that overlaps with the first in its spatial (Galizia *et al* 1999, 2000) and temporal (Laurent 1996; Stopfer *et al* 1997) properties. Dissimilar odours would activate

perhaps a neural ensemble that failed to overlap with the first. This would be consistent with the 'zonal' organization recently proposed for the mammalian main and accessory OBs (Mori *et al* 1999) and for the zebrafish OB (Friedrich and Korsching 1997).

Fruit flies are endowed with approximately 1,300 olfactory sensory neurons that collectively express on the order of 50 odourant receptors (Clyne *et al* 1999; Vosshall *et al* 1999; Vosshall 2001). Each sensory cell expresses one or a very small subset of odourant receptors (Vosshall 2001). As in mammals (Mombaerts 1999), sensory cells that express the same receptor project axons that converge onto one or two of the 43 glomeruli in the antennal lobe in a manner that is spatially invariant across individuals (Vosshall 2001). This type of spatial organization is conserved across other vertebrates and invertebrates in regard to patterns of glomerular activation upon stimulation with different odourants (Galizia *et al* 1999; Mori *et al* 2000). Sensory neurons in fruit flies reveal a combination of excitatory and inhibitory responses to different odourants (de Bruyne *et al* 1999). Neurons are

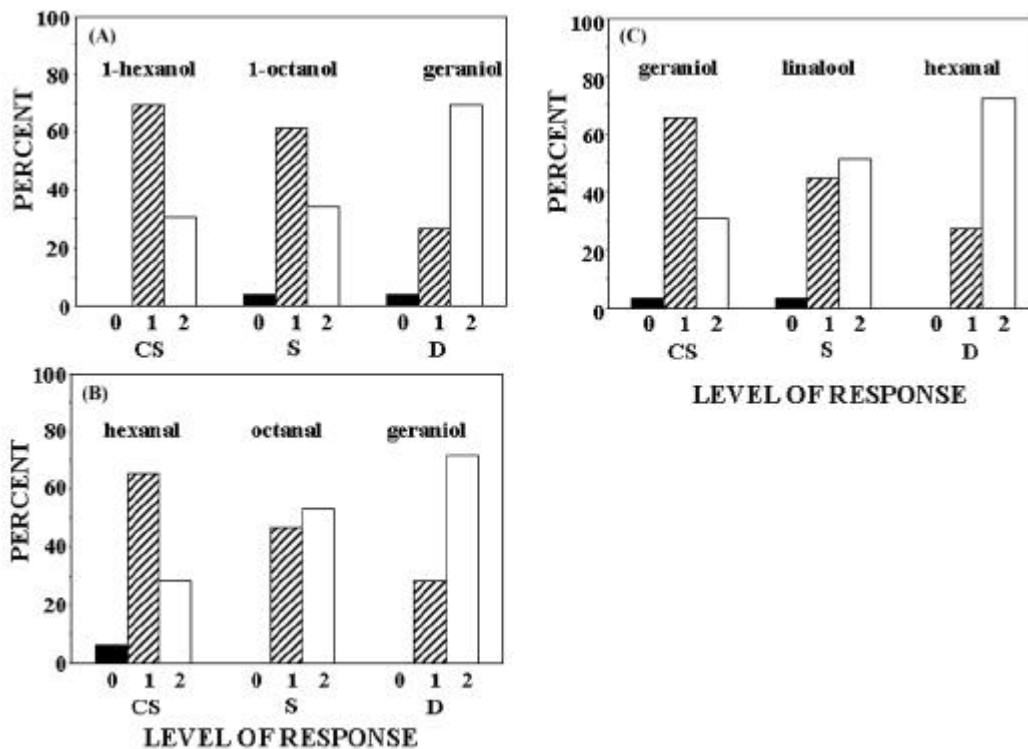


Figure 5. Generalization of habituation. Three different groups of flies were habituated over 25 trials to hexanal (A), 1-hexanol (B) or geraniol (C). After habituation treatment they were tested once with each of the three odourants listed in each graph presented in a randomized order across flies. In each case, the habituated odourant is referred to on the x-axis as the CS, whereas the structurally similar and dissimilar odourants are referred to as 'S' and 'D', respectively. The three bars for each test odourant represent the percentage of animals that fell into each of the three response categories. Samples sizes are: (A) $N = 26$; (B) $N = 32$; (C) $N = 29$.

tuned to respond to odourants from a restricted set or class of compounds. Within that class the neurons are somewhat broadly but nevertheless differentially tuned to respond to a more restricted range of odourants. It is possible that this coding scheme could give rise to the orderly generalization gradients we have observed. We would predict that the overlap of activation of different, related receptors by different odourants will correlate to the degree of generalization between those odourants. Given the possibilities that exist to now record from sensory cells in fruit flies and manipulate receptor expression it should be feasible to test this hypothesis.

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References

- Akers R P and Getz W M 1993 Response of olfactory receptor neurons in honeybees to odorants and their binary mixtures; *J. Comp. Physiol.* **A173** 169–185
- Bitterman M E 1996 Comparative analysis of learning in honeybees; *Anim. Learn Behav.* **24** 123–141
- Boeckh J, Ernst K D, Sass H and Waldow U 1984 Anatomical and physiological characteristics of individual neurons in the central antennal pathways of insects; *J. Insect Physiol.* **30** 15–26
- Boeckh J, Kaissling K E and Schneider D 1965 Insect olfactory receptors; *Cold Spring Harbor Symp. Quant. Biol.* **30** 263–280
- Bozza T C and Kauer J S 1998 Odorant response properties of convergent olfactory receptor neurons; *J. Neurosci.* **18** 4560–4569
- Buck L B 1996 Information coding in the vertebrate olfactory system; *Annu. Rev. Neurosci.* **19** 517–544
- Buck L B 2000 The molecular architecture of odor and pheromone sensing in animals; *Cell* **100** 611–618
- Buck L B and Axel R 1991 A novel multigene family may encode odorant receptors: a molecular basis for odor recognition; *Cell* **65** 175–187
- Cinelli A R, Hamilton K A and Kauer J S 1995 Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. III. Spatial and temporal properties of responses evoked by odorant stimulation; *J. Neurophysiol.* **73** 2053–2071
- Clyne P J, Warr C G, Freeman M R, Lessing D, Kim J and Carlson J R 1999 A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*; *Neuron* **22** 327–338
- Cohen J and Cohen P 1983 *Applied multiple regression/correlation analysis for the behavioral sciences* (Hillsdale, New Jersey: Lawrence Erlbaum Associates)
- Daly K C and Smith B H 2000 Associative Olfactory Learning in the moth *Manduca sexta*; *J. Exp. Biol.* **203** 2025–2038
- Daly K C, Durtschi M L and Smith B H 2001 Olfactory-based discrimination learning in the moth, *Manduca sexta*; *J. Insect Physiol.* **47** 375–384
- Daly K, Chandra S, Durtschi M L and Smith B H 2001 Generalization of olfactory-based conditioned response reveals unique but overlapping odour representations in the moth, *Manduca sexta*; *J. Exp. Biol.* **204** 3085–3095
- De Bruyne M, Clyne P J and Carlson J R 1999 Odor coding in a model olfactory organ: the *Drosophila* maxillary palp; *J. Neurosci.* **19** 4520–4532
- Dethier V G, Solomon R L and Turner L H 1965 Sensory input and central excitation and inhibition in the blowfly; *J. Comp. Physiol. Psychol.* **60** 303–313
- Fan R, Anderson P and Hansson B S 1997 Behavioral analysis of olfactory conditioning in the moth *Spodoptera littoralis* (boisd.) (Lepidoptera: Noctuidae); *J. Exp. Biol.* **200** 2969–2976
- Flanagan D and Mercer A 1989 An atlas and 3-D reconstruction of the antennal lobes in the worker honey bee, *Apis mellifera* L. (Hymenoptera: Apidae); *Int. J. Insect Morphol. Embryol.* **18** 145–159
- Friedrich R W and Korsching S I 1997 Combinatorial and chemotopic odorant coding in the zebra fish olfactory bulb visualized by optical imaging; *Neuron* **18** 737–752
- Galizia C G, Küttner A, Georges J and Menzel R 2000 Odour representation in honeybee olfactory glomeruli shows slow temporal dynamics: an optical recording study using a voltage sensitive dye; *J. Insect Physiol.* **46** 877–886
- Galizia C G, Sachse S, Rappert A and Menzel R 1999 The glomerular code for odour representation is species specific in the honeybee *Apis mellifera*; *Nat. Neurosci.* **2** 473–487
- Getchell T V 1974 Unitary responses in frog olfactory epithelium to sterically related molecules at low concentrations; *J. Gen. Physiol.* **64** 241–261
- Hildebrand J G 1995 Analysis of chemical signals by nervous systems; *Proc. Natl. Acad. Sci. USA* **92** 67–74
- Hildebrand J G and Shepherd G M 1997 Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla; *Annu. Rev. Neurosci.* **20** 595–631
- Homberg U, Christensen T A and Hildebrand J G 1989 Structure and function of the deutocerebrum in insects; *Annu. Rev. Entomol.* **34** 477–501
- Hosler J S, Buxton K L and Smith B H 2000 Impairment of olfactory discrimination by blockade of GABA and nitric oxide activity in the honey bee antennal lobes; *Behav. Neurosci.* **114** 514–525
- Imamura K, Mataga N and Mori K 1992 Coding of odour molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds; *J. Neurophysiol.* **68** 1986–2002
- Jonsson M and Anderson P 1999 Electrophysiological responses to herbivore-induced hostplant volatiles in the moth *Spodoptera littoralis*; *Physiol. Entomol.* **24** 377–385
- Kashiwadani H, Sasaki Y F, Uchida N and Mori K 1999 Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb; *J. Neurophysiol.* **82** 1786–1792
- Katoh K, Koshimoto H, Tani A and Mori K 1993 Coding of odour molecules by mitral/tufted cells in rabbit olfactory bulb. II. Aromatic compounds; *J. Neurophysiol.* **70** 2161–2175
- Laurent G 1996 Dynamical representation of odors by oscillating and evolving neural assemblies; *Trends Neurosci.* **19** 489–496
- Laurent G 1999 A systems perspective on early olfactory coding; *Science* **286** 723–728
- Linster C and Hasselmo M E 1999 Behavioral responses to aliphatic aldehydes can be predicted from known electro-

- physiological responses of mitral cells in the olfactory bulb; *Physiol. Behav.* **66** 497–502
- Linster C and Smith B H 1999 Generalization between binary odor mixtures and their components in the rat; *Physiol. Behav.* **66** 701–707
- Mackintosh N J 1983 *Conditioning and associative learning* (Oxford, UK: Oxford University Press)
- Malnic B, Hirono J, Sato T and Buck L B 1999 Combinatorial receptor codes for odors; *Cell* **96** 713–723
- Menzel R 1990 Learning, memory, and 'cognition' in honey bees; in *Neurobiology of comparative cognition* (eds) R P Kesner and D S Olten (Hillsdale, New Jersey, Lawrence Erlbaum) pp 237–292
- Menzel R and Bitterman M E 1983 Learning by honeybees in an unnatural situation; in *Neuroethology and behavioral physiology* (eds) F Huber and H Markl (New York: Springer-Verlag) pp 206–215
- Mombaerts P 1999 Seven-transmembrane proteins as odorant and chemosensory receptors; *Science* **286** 707–711
- Mori K, Nagao H and Yoshihara Y 1999 The olfactory bulb: coding and processing of odor molecule information; *Science* **286** 711–715
- Mustaparta H 1997 Olfactory coding mechanisms for pheromone and interspecific signal information in related moth species; in *Insect pheromone research: New directions* (eds) R T Cardé and A K Minks (New York: New York Press) pp 144–163
- Rospars J P and Hildebrand J G 1992 Anatomical identification of glomeruli in the antennal lobes of the male sphinx moth, *Manduca sexta*; *Cell Tissue Res.* **270** 205–227
- Sandeman D C and Sandeman R E 1998 Orthodromically and antidromically evoked local field potentials in the crayfish olfactory lobe; *J. Exp. Biol.* **201** 1331–1344
- Shields V and Hildebrand J G 2001 Responses of a population of antennal receptor cells in the female moth *Manduca sexta* to plant associate volatile organic compounds; *J. Comp. Physiol.* **A186** 1153–1151
- Shipley M T and Ennis M 1996 Functional organization of the olfactory system; *J. Neurobiol.* **30** 123–176
- Smith B H and Getz W M 1994 Non-pheromonal olfactory processing in insects; *Annu. Rev. Entomol.* **39** 351–375
- Smith B H and Menzel R 1989 The use of electromyogram recordings to quantify odorant discrimination in the honey bee, *Apis mellifera*; *J. Insect Physiol.* **35** 369–375
- Sokal R R and Rohlf J F 1997 *Biometry* 3rd edition (New York: W H Freeman Press)
- Stocker R F 1994 The organization of the chemosensory system in *Drosophila melanogaster*: a review; *Cell Tissue Res.* **275** 3–26
- Stopfer M, Bhagavan S, Smith B H and Laurent G 1997 Impaired odour discrimination on desynchronization of odour-encoding neural assemblies; *Nature (London)* **390** 70–74
- Strausfeld N J and Hildebrand J G 1999 Olfactory systems: common design, uncommon origins?; *Curr. Opin. Neurobiol.* **9** 634–640
- Thompson R F and Spencer W A 1966 Habituation: A model phenomenon for the study of substrates of behavior; *Psychol. Rev.* **73** 16–43
- Vareschi E 1971 Duftunterscheidung bei der Honigbiene-Einzelzell-Ableitungen und Verhaltensreaktionen; *Z. Vgl. Physiol.* **75** 143–173
- Vargo M and Hirsch J 1982 Central excitation in the fruit fly (*Drosophila melanogaster*); *J. Comp. Physiol. Psychol.* **96** 452–459
- Vosshall L 2001 The molecular logic of olfaction in *Drosophila*; *Chem. Senses* **26** 207–213
- Vosshall L B, Amrein H, Morozov P S, Rzhetsky A and Axel R 1999 A spatial map of olfactory receptor expression in the *Drosophila* antenna; *Cell* **96** 725–736

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