Chem. Senses 28: 781–789, 2003 DOI: 10.1093/chemse/bjg070

Odor Similarity Does Not Influence the Time Needed for Odor Processing

Mathias Ditzen[1], Jan-Felix Evers[1] and C. Giovanni Galizia[1,2]

[1]Institut für Biologie—Neurobiologie, Freie Universität Berlin, Königin Luise Str. 28–30, 14195 Berlin, Germany
[2]Current address: Department of Entomology, University of California Riverside, 383, Riverside, CA 92521, USA

Correspondence to be sent to: Mathias Ditzen, Institut für Biologie, Neurobiologie, Freie Universität Berlin, Königin-Luise-Str. 28–30, 14195 Berlin, Germany. e-mail: mathiasd@zedat.fu-berlin.de

Abstract

The brain's link between perception and action involves several steps, which include stimulus transduction, neuronal coding of the stimulus, comparison to a memory template and choice of an appropriate behavioral response. All of these need time, and many studies report that the time needed to compare two stimuli correlates inversely with the perceived distance between them. We developed a behavioral assay in which we tested the time that a honeybee needs to discriminate between odors consisting of mixtures of two components, and included both very similar and very different stimuli spanning four log-concentration ranges. Bees learned to discriminate all odors, including very similar odors and the same odor at different concentrations. Even though discriminating two very similar odors appears to be a more difficult task than discriminating two very distinct substances, we found that the time needed to make a choice for or against an odor was independent of odor similarity. Our data suggest that, irrespective of the nature of the olfactory code, the bee olfactory system evaluates odor quality after a constant interval. This may ensure that odors are only assessed after the olfactory network has optimized its representation.

Key words: honeybee, odor concentrations, odor mixtures, olfaction, response time

Introduction

Olfactory senses enable animals to perceive a seemingly infinite number of different odors. The primary olfactory centers of mammals (olfactory bulbs) and insects (antennal lobes) share many characteristics (Hildebrand and Shepherd, 1997). Stimulating with an odor evokes characteristic spatio-temporal activity patterns of their functional units, the olfactory glomeruli (Galizia and Menzel, 2001; Kauer, 2002). Each characteristic spatial activation pattern contains sufficient information to deduce the experienced odor (Galizia *et al.*, 1999). Similar odors, such as different concentrations of the same substance or chemicals differing only slightly in chain length, evoke overlapping patterns (Galizia and Menzel, 2001). This information is relayed to higher order brain centers by mitral/tufted cells in mammals and projection neurons (PNs) in insects. The PNs response profiles are determined by the innervated glomerulus (Vickers *et al.*, 1998; Sachse and Galizia, 2002) so that the antennal lobe's spatial pattern is transformed into an identity pattern across the PNs axons. This means that similar activity patterns in the AL lead to similar identity patterns in the PNs. The question arises: can animals still differentiate between these patterns and if so, do they need more time to do so? In most examples studied so far, response time and accuracy were inversely related to each other (Ratcliff and Rouder, 2000; Schall, 2001; Roitman and Shadlen, 2002). Under given conditions of reward and motivation, the brain appears to accumulate the evidence against or in favor of a certain choice until a determined threshold is reached (Usher and McClelland, 2001; Gold and Shadlen, 2002). This leads to longer reaction times when the stimulus alternatives are more ambiguous (Roitman and Shadlen, 2002), which suggested the idea of using reaction times rather than choice performance for quantifying odor similarity of close odors (Wise and Cain, 2000).

Response latencies may even be more interesting in another context. The physiological responses of PNs have been investigated in detail in several species (Wehr and Laurent, 1996; Christensen *et al.*, 1998; Lei and Hansson, 1999; King *et al.*, 2000; Müller *et al.*, 2002; Sachse and Galizia, 2002). The PN and mitral cell responses show slow rate-intensity developments (at the scale of tens of milliseconds; Friedrich and Laurent, 2001). Some PNs have specific delays in activity onset that can be as late as 400 ms (Müller *et al.*, 2002). The partially synchronized activity of

olfactory neurons leads to oscillations in the range of 30–40 Hz (Laurent *et al.*, 2001). Based on these observations, at least three different proposals about the nature of the olfactory code have been put forward: in the 'combinatorial code' the identity of active glomeruli delivers all information, and timing is used to code temporal properties of the stimulus (Christensen *et al.*, 2000). 'Winnerless competition' was proposed for a code based on the slow development of PN activity, in which the sequence of events contains the information, rather than the activity at any given point in time (Laurent *et al.*, 2001). In the third scheme (referred to here as 'synchrony code') different subsets of PNs are at least partially coincidentally active at different cycles of a global oscillatory response, and the odor is coded in the sequence of active neuronal ensembles (Laurent *et al.*, 1996). These views are not mutually exclusive, and any combination of them may be true. However, in all models, more time should lead to a more accurate odor representation, and consequently give better discrimination in behavior. In a pure 'combinatorial code', similar odors evoke similar spatial patterns with overlapping sets of active glomeruli (Sachse *et al.*, 1999; Uchida *et al.*, 2000; Meister and Bonhoeffer, 2001). This might suggest that such odors need more time to be correctly discriminated than more dissimilar stimuli. Similarly, for the 'synchrony code' and 'winnerless competition', the code for two dissimilar odors may differ earlier in the sequence than in a case where two similar odors are compared, giving the animal the possibility to react more quickly. Alternatively, an odor may be assessed by judging the activity reached after a set time-window, where the information resides either in the state reached, or in the sequence of activity passed along the way toward that point. In these cases, odor discrimination would require equal time irrespective of odor similarity. Therefore, observing behavioral latencies can yield information about olfactory processing.

We addressed these questions using the honeybee as the experimental animal. Bees are ideal animals for studying olfactory processing and memory (Hammer and Menzel, 1995). Odor responses in their antennal lobes show both the spatial arrangement of activated glomeruli (Galizia and Menzel, 2001) as well as characteristic odor-evoked oscillations (Stopfer *et al.*, 1997). We trained individual free-flying honeybees to collect artificial nectar (sugar water) in an artificial meadow, consisting of 48 optically identical odor sources. In order to span a wide range of similar and dissimilar stimuli with a quantifiable similarity metric, we created a systematic mixture matrix of two components, linalool and 1-nonanol, at four log-concentration levels. In an operant conditioning paradigm we trained each bee to a reward associated with one specific odor, while all other odors were not rewarded. Once the bee had learned the task, we recorded the bee's behavior on 10 subsequent foraging trips when bees cruised over the matrix of odors and had to compare the experienced odor with the learned odor. In this way, we specifically addressed olfactory memory retrieval.

Material and methods

Setup and odorants

The experimental setup has been described before (Laska *et al.*, 1999; Laska and Galizia, 2001). Briefly, it consisted of an 80×70 cm wooden rack containing 48 opaque glass bottles in six rows of eight bottles each. Only the bottle apertures were visible (Figure 1A). Four microliters of odorant were placed onto strips of filter paper into each bottle. A 3 cm plastic tube with a wire mesh closing off its inner end was then placed into the bottlenecks, in order to prevent the bees from entering too deep into the bottles. A suction pump was placed behind the bottles in order to limit odor diffusion to the area immediately at each bottle opening.

The tested odorants consisted of two components, 1-nonanol (vapor pressure: 0.02 mmHg) and linalool (0.16 mmHg), diluted in mineral oil and mixed at different concentrations. The two odors were chosen on the basis of their neural representation in the honeybee brain: in optical imaging experiments, linalool and nonanol elicit clearly different glomerular activity patterns, but share one common active glomerulus (Galizia and Menzel, 2001). The 23 mixtures used are shown in Figure 2. Odor similarity was calculated with both city block and Euclidean metric. All odors were subject to double blind encoding. The stimuli also included a blank odor (0% linalool, 0% nonanol) that was equally encoded as a testing odor to the experimenter. In addition, an 'open' blank, 'X', was used, that consisted of bottles with just the solvent on filter paper and which, in contrast to the blank odor, was never used as CS+. Thus, each bee was differentially trained to one odor against 22 others (including the blank) plus the 'X' control.

For the calculation of odor-similarity in the binary mixture we followed results conceived from a similar experiment conducted with humans (Olsson, 1994). We used two distance measures between the trained odor and all other odors: the Euclidean distances, where the distance between point 1 (x_1,y_1) and point 2 (x_2,y_2) is $((x_2 - x_1)^2 + (y_2 - y_1)^2)^{1/2}$; and the city-block distances (distance is $|x_2 - x_1| + |y_2 - y_1|$). The Euclidean distance between two points corresponds to the length of the line connecting the two points; the city-block distance (also referred to as Manhattan distance) is the sum of the individual distances of the components. The odors used are presented in Figure 2.

Training and experimentation

Foraging worker honeybees were lured from the apiary to a feeder filled with 20% (w/v) sucrose solution that was placed in the windowsill of the room where the experiment was conducted. Single bees were collected in a glass vial, marked and progressively trained to enter the room, approach the experimental setup and find the bottles containing the

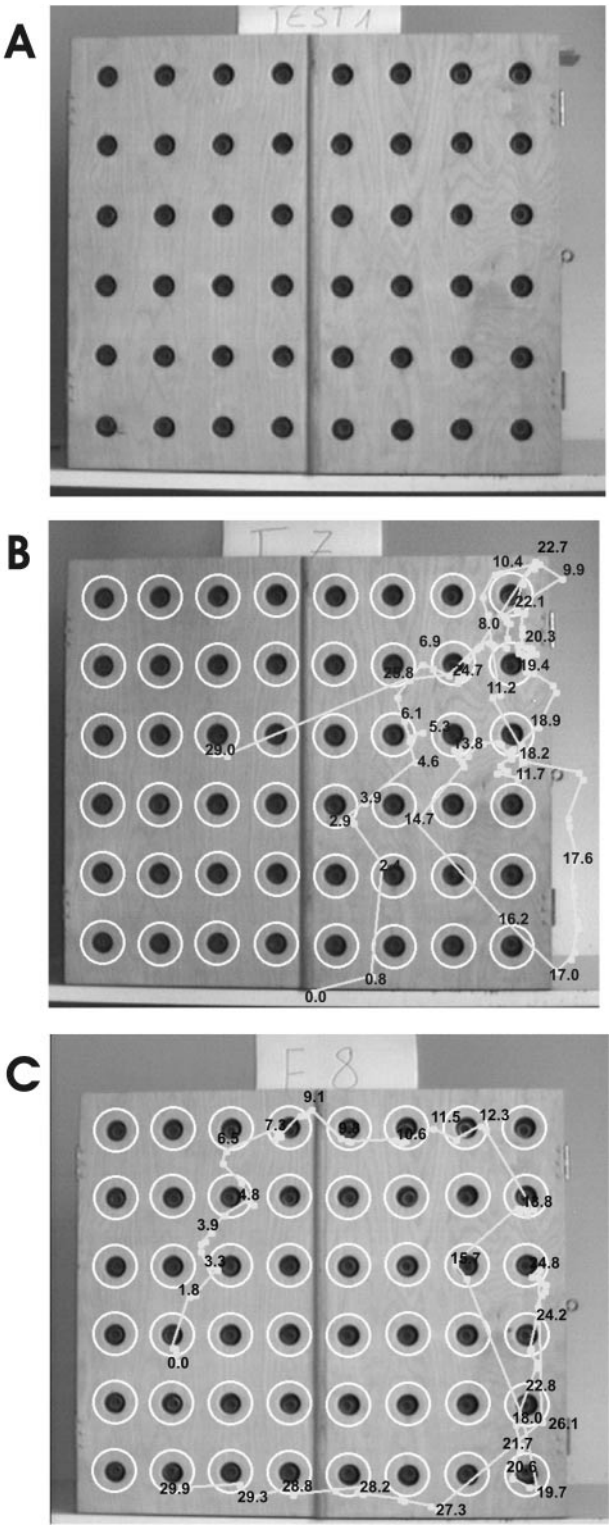

Figure 1 **(A)** Experimental setup containing 48 bottles with 23 different odors. **(B, C)** Exemplary 30 s flight tracks of two different bees. White circles are centered on the odor sources and depict the regions within which the bees were considered to be able to perceive the specific odor. Numbers indicate flight time (s). Both tracks are available as movies under http://www.neurobiologie.fu-berlin.de/galizia/BeeFlightTrack. (B) This bee showed a relatively inefficient foraging behavior, with repeated visits to the same bottle. (C) This bee's track was more systematic.

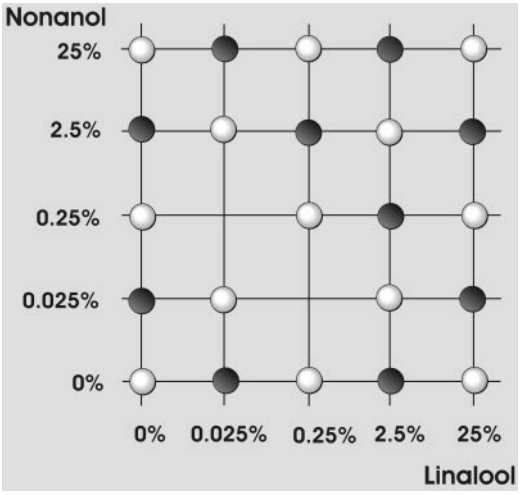

Figure 2 Mixture scheme of the relative concentrations of linalool and nonanol in each tested stimulus. The balls represent the different odors present in the experimental rack. Light balls are those used as CS+. Odors along the axes and the diagonal differ only in concentration.

conditioning stimulus (CS+). This was done by repeatedly releasing them at increasing distances from the bottle containing the CS+ and 60 µl of a 30% (w/v) sucrose solution. This 'initial training' was completed when the bee had repeatedly entered the room, approached the rack, searched and found the CS+, ingested the sucrose reward and left for its hive without experimenter interaction. Recording started with the next return to the apparatus. Only one bee was tested at a time.

The recorded data consist of 10 three-minute test trials in which the conditioning odor was presented without sugar reward. The reward was ommitted during test trials in order to insure that the bee would only respond to the odor, and not respond to the presence of the reward, and in order to avoid a confusion between odor-choice and training situations. During the test trials one bottle contained the unrewarded CS+ and three bottles contained mineral oil ('X'). All other odors were present in two bottles each. In order to keep the bees motivated, each test trial was immediately followed by a training trial in which the CS+ was rewarded. During training trials three bottles contained the CS+, to enhance the probability of successful foraging. The three CS+ bottles were treated with 10 µl of the 30% (w/v) sucrose reward (a honeybee's full crop load is ~60 µl). In order to force animals to collect the reward from different positions CS+ bottles that had already been visited were only baited again when the animal had landed on one of the other two CS+ bottles. Training trials lasted as long as the bee needed to fill its crop and leave the experimental setup to return to the hive. After each trial the positions of the bottles were changed in a pseudo-randomized way and the bottlenecks were cleaned with a moist cloth.

Bees were sacrificed at the end of the 10 trial sessions in order to insure that only experimentally naive bees were used.

Recording and evaluation

The test trials were recorded with a CCD camera (Hitachi KP-C551, Hitachi Ltd, Tokyo, Japan) equipped with a 20/80 mm objective. The camera was connected to a computer via a PCI-TV tuner card (Hauppauge! WinTV PCI/FM, Hauppauge Computer Works, Hauppauge, NY). The bee's movement in front of the rack was tracked with ~25 frames/s using custom written software. The time spent within 3.6 cm of the bottle center was taken as visit duration. Changing the size of the region (2.88 or 4.32 cm around the bottle center) did not change the conclusions, but led to shifting the overall distribution of visit durations by ~10–20 ms without changing its shape (data not shown).

Statistical analyses and figures were done in SPSS (http://www.SPSS.com) and R (http://www.r-project.org). To test learning performance visits were attributed to landings or passes on the basis of visit duration, using 1580 ms ($10^{3.2}$ ms) as cut-off value (Figure 3). This value was chosen because it marks the point where visit duration became equally probable to a rewarded and a non-rewarded odor. We assessed differences in visit number and significant discrimination for learned odors with a one-tailed binomial test (hypothesized probability value $P = 0.5$; see Laska *et al.*, 1999; Laska and Galizia, 2001) (Figure 4). Analysis of visit duration was done on log-transformed data. This yielded data with a normal distribution, allowing parametric statistics (General linear model).

This paper is based on 39 042 recorded odor-visits by 50 bees.

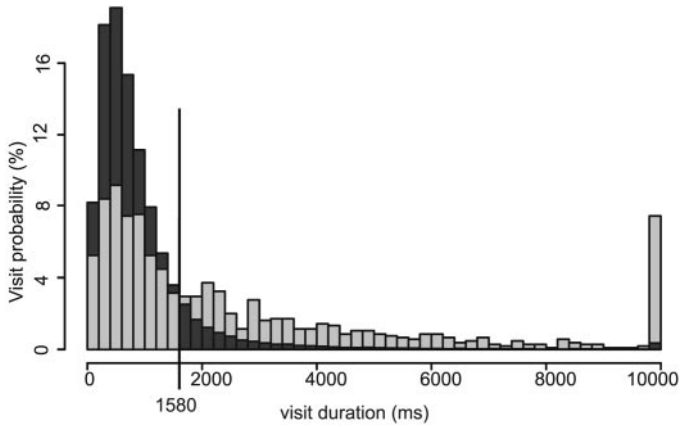

Figure 3 Histogram of visit duration pooled for all rewarded odors (light histogram) and unrewarded odors (dark histogram). The thin black line depicts the intersection of the two histograms at a visit duration of 1580 ms, i.e. a visit of this duration is equally probable a visit to the trained or any untrained odor.

Results

Bees learned to fly back from the hive and search for the rewarded bottle in the setup. As compared with previous experiments with the same apparatus (Laska *et al.*, 1999; Laska and Galizia, 2001), the necessary initial training time was much longer (~12 initial training sessions in this work compared with five training sessions in the previous experiments; see Laska *et al.*, 1999). Bees that were not sufficiently trained prior to data recording lost motivation and failed to come back to the apparatus after flying back to the hive. This longer training time is most likely due to the quite difficult task of identifying a rewarded odor within an array of many very similar but unrewarded odors, which leads to a sequence of negative reinforcers during the initial training phase of this differential conditioning paradigm.

The most common initial strategy of bees returning from the hive was to search at the same place where the rewarded bottle had been in the previous round, indicating that a spatial cue strategy was used as default. Odors could only be sensed in close proximity to the bottle. Bees differed in their strategy of how to scan the array of odor bottles, with some bees flying in a very systematic way, and others employing a more chaotic strategy (compare Figure 1B with Figure 1C, and the additional movies). These behavioral aspects were not further analyzed in this study.

For each bee, we measured the hovering time in front of each odor source at each visit. Median visit duration for the rewarded odor was 1527 ms, for unrewarded odors the median was 690 ms. Comparing the two distributions (Figure 3) showed that the two distributions have a strong overlap, but are clearly distinct. The distribution for rewarded odors is strongly skewed towards longer visits, because the bees stayed with the odor-baited bottle to intensively search for the sugar reward (there was no sugar water in these test trials). The difference in time spent in front of the rewarded and the unrewarded odor was significantly different ($P << 0.01$, paired t-test on log-transformed data; $n = 50$ bees). This difference reflects that the successful identification of the CS+ elicited landing behavior and the search for the sucrose solution, and consequently long permanence times, while the identification of a CS– made the bees move on to look for the CS+ elsewhere, resulting in short permanence times.

Discrimination performance

Over the 10 experimental bouts the mean (± SD) number of visits per animal was 781 ± 262, resulting in a mean visit number of 32 ± 11 per odor. The control 'X' was visited more often (mean of 47 ± 17, which can be fully attributed to the fact that there were 3 bottles containing 'X' in comparison to only two bottles containing the other odors. However, there was a significant preference for the bottles containing the CS+. Here on average 24 ± 13 visits were recorded per animal, even though the CS+ was only present in one bottle (binomial test, $P = 0.007$), suggesting that bees

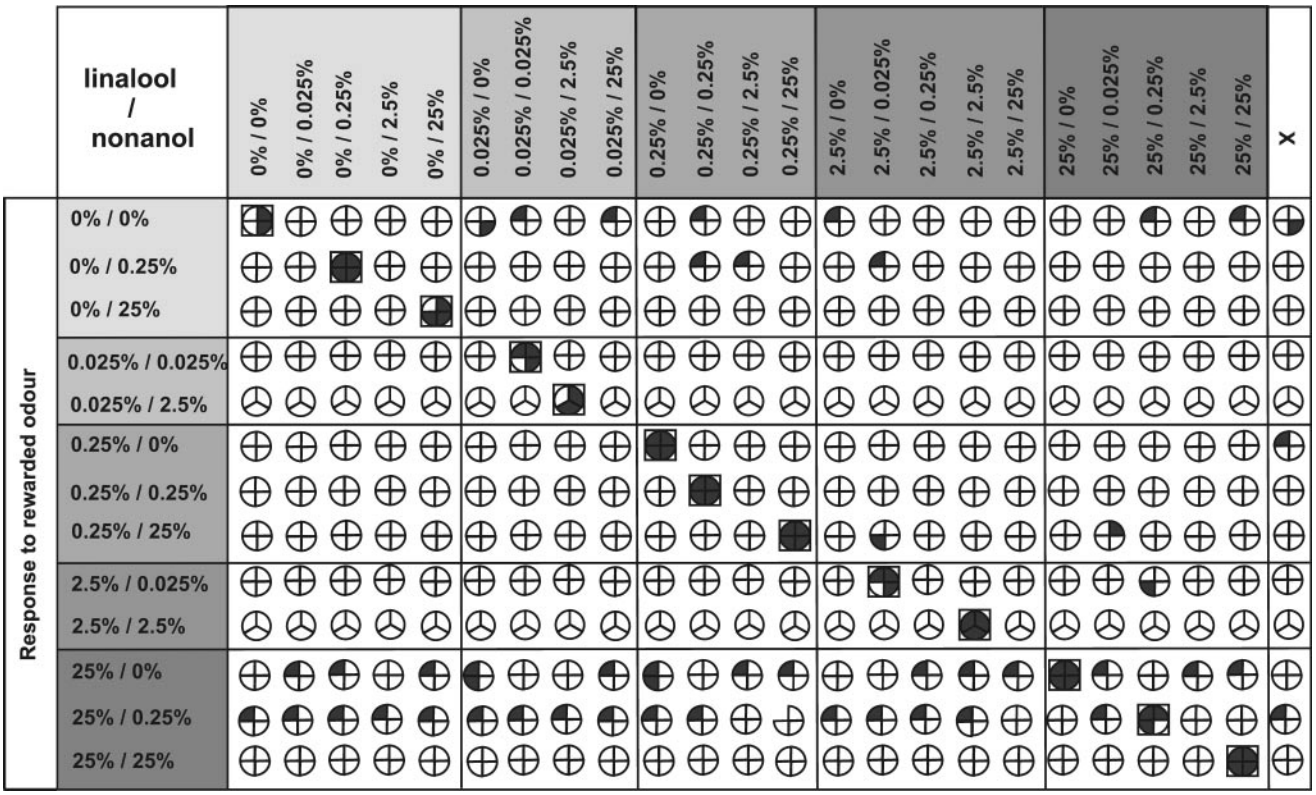

Figure 4 Olfactory discrimination performance. Each row shows the 13 odors the bees have been trained to (with the relative content of linalool and nonanol). Each column shows the 23 tested odors as well as the control (X, mineral oil). Each quadrant within a circle represents the response of one trained bee to a given odor. For most odors, four trained bees (represented by the four quadrants within a circle) were used, but two odors (0.25%/0.25% and 2.5%/2.5%) had only three bees. The diagonal of gray circles surrounded by boxes shows the responses of the trained bees to the rewarded odors. White quadrants indicate significant 'passes' (bees did not land); gray ones indicate significant 'landings' (binomial test on classified landings/passes events, $P < 0.05$). Bees with perfect performance have a shaded quadrant for the rewarded odor (gray entry in the diagonal) and white quadrants in all other cases (for example, all bees trained to 0.25%/0.25%). One of the bees trained to 25% linalool/0.25% nonanol failed to visit 0.25% linalool/25% nonanol: the quadrant has been ommitted. There was no relationship between odor similarity and lack of discrimination.

repeatedly returned to the position of the CS+ within the trials.

To further test whether a bee could correctly discriminate the learned odor from the alternatives, visits were categorized into 'landings' and 'passes' (Laska and Galizia, 2001). A 'pass' was an event where the bee was in front of an odor source but did not land on it, whereas a 'landing' was a bee entering the bottle in search of the sugar water reward. Based on the difference between the distributions of visit durations to trained and untrained odors, we categorized visits below 1580 ms as passes and longer visits as landings (see Materials and methods and Figure 3). In 95.4% of the cases [1145 of 1200 (50 × 24) bee–odor pairings], the bee correctly addressed the odor (i.e. she 'passed' on a non-rewarded odor or she 'landed' on a rewarded one) at a statistical significance of 5%. The only odor that bees failed to learn was the control, showing that bees are able to learn discriminating any of the presented stimuli from all others (Figure 4). Some bees performed less than others (e.g. one of the 25% linalool/0.25% nonanol bees was a poor learner). A closer analysis revealed that most errors derived from bees with rare visits. Bees with at least one non-learned odor had an average of 25 visits per odor over all 10 trials [these are all bees with at least one filled entry in a non-diagonal position in Figure 4 ($n = 15$)], while bees that learned all odors had an average of 36 visits [these are all bees with all non-diagonal entries being white and all diagonal entries filled in Figure 4 ($n = 35$, $P < 0.01$, t-test)], suggesting that their apparent failure to discriminate was due to limited experimental sampling. Considering all but the two worst bees, the error rate drops to 2.0%.

There was no generalization to similar odors. Of 121 bee–odor cases with city-block distance (CBD) of 1 to the rewarded odor, only four were erroneously not rejected to a significant level. These four compare to a total of 48 erroneous bee–odor cases for all CBDs. Thus, odor similarity was not a factor leading to wrong choices: once an odor had been stably learned in this discriminatory task, discrimination was equal with respect to all non-reinforced odors present in the training phase. In particular, bees could also discrim-

inate an odor from the same odor at higher or lower concentrations, and that was true for the pure substances linalool and nonanol, as well as for the 1:1 mixture linalool + nonanol.

Choice-time was constant irrespective of odor similarity

Did the time span vary during which a bee hovered in front of an odor source before making the decision either to fly to the next source, or enter the feeder and look for the nectar? In order to answer this question we analyzed visiting durations.

Since visit duration in front of the rewarded odor consisted in both odor-recognition and food-search behavior, we limited our analysis to unrewarded odors. We analyzed the visit duration to all these odors as a function of odor-similarity to the rewarded odor. Irrespective of the metric used for odor similarity, there was no significant difference between visit durations of all non-rewarded odors (General linear model: $P = 0.188$ and $P = 0.309$ for city block and Euclidean metric, respectively; $n = 50$ bees). In particular, there was no increase in visit duration with increasing difficulty of the discriminatory task, i.e. with increasing similarity to the rewarded odor (Figure 5A).

Possibly, odor similarity could be reflected in the shape of the distribution of visit times. We therefore compared these distributions, and found no difference. Figure 5B shows the distribution of visit duration for odors with CBDs of 1, 3 and 5, as well as the overall distribution of visit duration. The distribution did not differ, again corroborating that visiting times are not influenced by odor similarity.

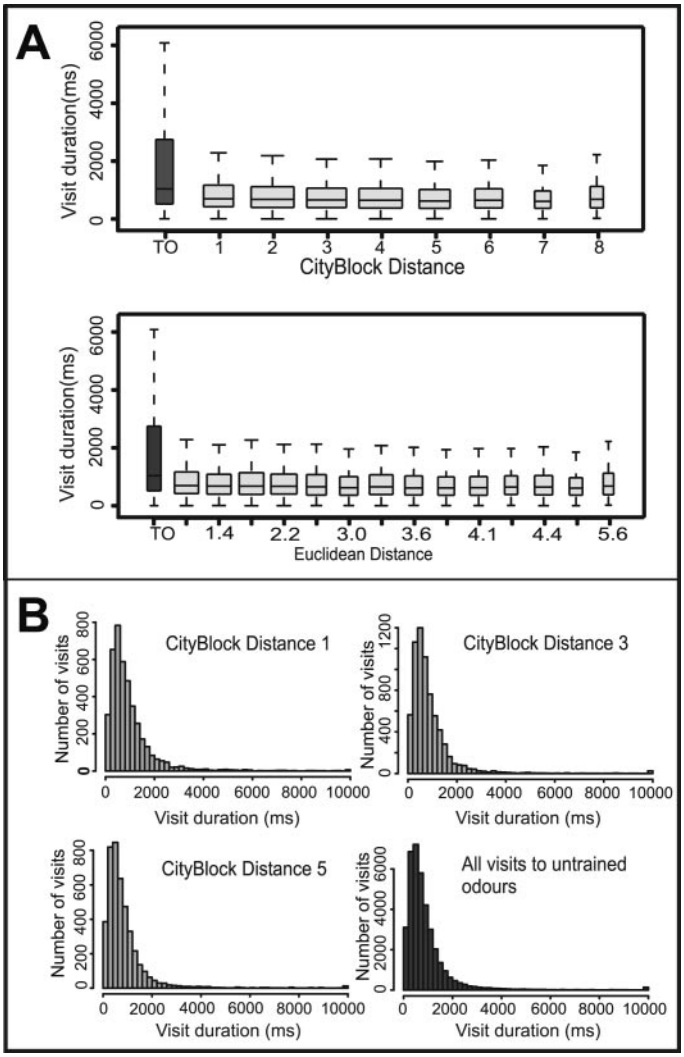

Figure 5 **(A)** Distributions of visit durations to odors having a specific city-block (upper panel) or Euclidean (lower panel) distance from the trained odor (TO). Box plots indicate median, 25% and 75% quartiles, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Differences between visit durations for non-rewarded odors were not significant. **(B)** From top to bottom: frequency plots of visit duration for three different CBD measurements (CBD 1, 3 and 5 to the rewarded odor) and of visit duration for all unrewarded odors. Note that there is no visible difference between them, indicating an identical distribution irrespective of odor dissimilarity.

Discussion

By training free-flying bees to an array of different odors, we have shown that (i) bees can learn to discriminate very similar odors; (ii) bees can learn to discriminate an odor at a given concentration from the same odor at higher or lower concentrations. These results strengthen and extend our knowledge about the honeybee olfactory system, and confirm its quality as an experimental model system for chemosensory research. Furthermore, we found that (iii) choice time was independent of the difficulty in the discriminatory task, in that bees did not need more time to choose the appropriate behavior for similar odors than for dissimilar odors. Rather, decision time was constant across all odor pairs. This finding has important implications for models of olfactory processing.

Bees can discriminate very similar odors

Due to our lack of knowledge about the primaries in the olfactory code, measuring whether an odor is similar or dissimilar from another odor can only be done behaviorally. Perceived qualities of binary mixtures fall between the qualities of their components (Laing *et al.*, 1984; Wise and Cain, 2000). We therefore used an array of differential mixtures of two components (linalool and nonanol) that, by changing the relative proportions of the components, created an array of many odors with differing similarity. While it is not possible to directly quantify the similarity on the basis of the chemical mixtures, it appears plausible that, say, the 25% nonanol/0.25% linalool mixture is more similar to the 25% nonanol/0.025% linalool than to the 0.25% nonanol/25% linalool (Figure 2).

In a similar experimental approach, generalization between odors had previously been shown, for example, for some enantiomer pairs, or aliphatic alcohols differing in just one carbon atom chainlength (Laska *et al.*, 1999; Laska and

Galizia, 2001). However, in those experiments bees were monitored during the acquisition phase, while in our experiments data recordings started only after the task had been stably learned. It should be noted, also, that during the learning phase bees were differentially trained, i.e. the bees were negatively reinforced (no sugar water reward) to all odors in the rack with exception to the one rewarded stimulus. Linster *et al.* (2002) have shown that rats can be differentially trainined to discriminate enantiomer pairs which they generalize in a different protocol. Interestingly, the odor pairs that need differential training to be recognized as different chemicals are those that elicit almost identical activity patterns in the bulb (Linster *et al.*, 2002). This suggests that the border between a generalized odor and a discriminated odor can be modified by training, i.e. that the neural olfactory space is plastic.

It may be argued that our good learning results may derive from scent marks left by the bees on the bottles. Indeed, bees and bumblebees mark visited flowers with both attractant and repellent scents (Giurfa and Nunez, 1992; Gilbert *et al.*, 2001). These marks may not have been completely removed by cleaning the bottles with a moist cloth between trials. There are two possible ways in which scent marks could have contributed to the good discrimination results: either repellent markings left on unrewarded bottles, or attractive marks on the rewarded ones. However, bees leave repellent scent marks after succesful foraging and not on unrewarded feeders (Giurfa and Nunez, 1992). Furthermore, these scents persist only for a short period (Giurfa and Nunez, 1992). Alternatively, bees may enhance the discrimination result by leaving attractant scents. We have experimentally addressed this point by including a control (solvent only), and having one of the trained odors also as a control (which, due to the double-blind labeling of the odors, was unknown to the experimenter). With this odor (0% linalool / 0% nonanol, first row in Figure 4), tagging the rewarded bottle with attractants should lead to a preference of these over the unrewarded control (last column, depicted as X). However, 3/4 bees did not make a difference between rewarded and unrewarded bottles. Two bees were even unable to recognize the trained odor in a significant way, suggesting that possible markings on these bottles were not effective in promoting the bees' choices.

Bees can discriminate different concentrations of the same odor

We confirm previous reports that bees are able to learn a particular odor concentration as the rewarded stimulus (Vareschi, 1971; Kramer, 1976). In other experiments, however, bees generalized from low to high concentrations (Bhagavan and Smith, 1997; Pelz *et al.*, 1997). The discrepancy may result from differences in the experimental procedure. In the first study (Bhagavan and Smith, 1997) bees were not differentially conditioned as in our study, and were tested for different concentrations only after training, showing a generalization that may be comparable to naive animals. In the latter study (Pelz *et al.*, 1997) restrained bees were tested after 6 training pairs, while our experiment was done with free-flying animals that could sample all odors without time and trial limitations during acquisition. Again, these different results show that the olfactory space is plastic, and generalization and discrimination is influenced by experience. They also confirm that the experimental design strongly influences the olfactory response space within a particular task (Linster *et al.*, 2002).

Odor processing occurs in a fixed time unit

We show here that choice-speed in this olfactory assay did not depend on the difficulty of the task (odor similarity). The measured latency of 690 ms comprises the time needed to successfully identify the odor in addition to the odor-independent time required for the overall behavior, including higher order processing, motor control, etc. Due to the very large sampling size the second part is likely to be normally distributed, to be equal for all tested odors and to behave additively to the time needed to identify the odor. Indeed, the data do not show a requirement for exactly 690 ms (the distribution of choice-times is fairly broad; see Figure 5A,B), but they show a highly significant lack of relationship between choice time and odor similarity (Figure 5A). These long processing times are not limited to free-flying bees: restrained honeybees, whose motor behavior is limited to proboscis extension, also need several hundred milliseconds to recognize an odor (T. Dekker and R. Cardé, personal communication). The time that rats need to perform an olfactory discrimination appears also to be influenced only to a very limited degree by odor similarity (Uchida *et al.*, 2001). Also, since we only tested two odors, and their mixtures, we cannot exclude that other odors may impose different constraints. Indeed, while well in the range of honeybee nectar collecting behavior, 690 ms will most likely be too long for some specialized olfactory tasks, such as sexual pheromone detection. For example, upwind flight reaction time is very fast in moths (up to 150 ms; see Todd and Baker, 1999), and their projection neurons can follow individual pulses up to 10 Hz frequency (Hansson and Christensen, 1999). Thus, as has often been suggested, the pheromone and the non-pheromone system may indeed code odors in different ways (for a different view, see Christensen and Hildebrand, 2002). Humans need several seconds to make a same–different discrimination of odor quality (Wise and Cain, 2000). Interestingly, odor representation in the mitral cells of zebra fish is optimized within the first 800 ms, after which it improves only slightly (Friedrich and Laurent, 2001). Optical imaging recordings from honeybees also showed that the representation of odors slowly evolves during a 2 s stimulus time (Galizia *et al.*, 2000).

A task-independent choice time implies that quality is assessed after a predetermined time-span, and not as soon as the neural codes for distinct odors differ to a sufficient

degree in order to justify a decision. This finding has important implications for olfactory coding since it implies that information about the odor is only available at a predefined time-point after stimulus onset. Assuming a 'combinatorial code', we would have to postulate that the across-glomeruli activity patterns are only read out after a given delay. To that end, an internal 'clock' would be necessary, a task which could be performed by the evoked oscillations. A time-span of 690 ms gives a high-end estimate of 21 cycles at 30 Hz, from which the time not involved in odor detection, such as further processing in the brain and motor commands, would have to be subtracted. Both the 'synchrony code' and 'winnerless competition' rely on the sequence of activity patterns. The sequences of two very different odors may differ earlier than the sequences of two very similar odors. Under these conditions, one would expect that the brain would continuously monitor the evidence in favor of each alternative, and make a choice as soon as the evidence suffices for one, as has been shown in other experimental paradigms (Schall, 2001; Gold and Shadlen, 2002). However, our results suggests that odor evaluation is not incremental, but rather occurs in single information units, consisting in the entire sequence of neural activity following an odor sample. Possibly, an interim storage of the sequence would be needed in order to explain the time-constancy found in our experiments. Whichever is the correct model for the olfactory system, our results show that in all of these models there is a missing component: a mechanism that would allow for a constant readout time of olfactory quality. This constant time requirement for odor choice may guarantee that odor quality is assessed when its coding is optimal.

Acknowledgements

Thanks to the Volkswagenstiftung and HFSP for financial support, to Doreen Burrmann and Kawe Toutounian-Mashhad for helping with the experiments. Thanks to Nico Schmidt and Florian Evers for assistance in computer programming. Special thanks to Ring Cardé, Teun Dekker, Zachary Mainen and Randolf Menzel for comments on the paper.

References

Bhagavan, S. and **Smith, B.H.** (1997) *Olfactory conditioning in the honey bee,* Apis mellifera*: effects of odor intensity*. Physiol. Behav., 61, 107–117.

Christensen, T.A. and **Hildebrand, J.G.** (2002) *Pheromonal and host-odor processing in the insect antennal lobe: how different?* Curr. Opin. Neurobiol., 12, 393–399.

Christensen, T.A., Waldrop, B.R. and **Hildebrand, J.G.** (1998) *GABA-ergic mechanisms that shape the temporal response to odors in moth olfactory projection neurons*. Ann. N. Y. Acad. Sci., 855, 475–481.

Christensen, T.A., Pawlowski, V.M., Lei, H. and **Hildebrand, J.G.** (2000) *Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles*. Nat. Neurosci., 3, 927–931.

Friedrich, R.W. and **Laurent, G.** (2001) *Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity*. Science, 291, 889–894.

Galizia, C.G. and **Menzel, R.** (2001) *The role of glomeruli in the neural representation of odours: results from optical recording studies*. J. Insect Physiol., 47, 115–130.

Galizia, C.G., Sachse, S., Rappert, A. and **Menzel, R.** (1999) *The glomerular code for odor representation is species-specific in the honeybee* Apis mellifera. Nat. Neurosci., 2, 473–478.

Galizia, C.G., Küttner, A., Joerges, 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.

Gilbert, F., Azmeh, S., Barnard, C., Behnke, J., Collins, S.A., Hurst, J. and **Shuker, D.** (2001) *Individually recognizable scent marks on flowers made by a solitary bee*. Anim. Behav., 61, 217–229.

Giurfa, M. and **Nunez, J.A.** (1992) *Honeybees mark with scent and reject recently visited flowers*. Oecologia, 89, 113–117.

Gold, J.I. and **Shadlen, M.N.** (2002) *Banburismus and the brain: decoding the relationship between sensory stimuli, decisions, and reward*. Neuron, 36, 299–308.

Hammer, M. and **Menzel, R.** (1995) *Learning and memory in the honeybee*. J. Neurosci., 15, 1617–1630.

Hansson, B.S. and **Christensen, T.A.** (1999) *Functional characteristics of the antennal lobe.* In Hansson, B.S. (ed.), Insect Olfaction. Springer, Berlin, pp. 125–161.

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.

Kauer, J.S. (2002) *On the scents of smell in the salamander*. Nature, 417, 336–342.

King, J.R., Christensen, T.A. and **Hildebrand, J.G.** (2000) *Response characteristics of an identified, sexually dimorphic olfactory glomerulus*. J. Neurosci., 20, 2391–2399.

Kramer, E. (1976) *The orientation of walking honeybees in odour fields with small concentration gradients*. Physiol. Entomol., 1, 27–37.

Laing, D.G., Panhuber, H., Willcox, M.E. and **Pittman, E.A.** (1984) *Quality and intensity of binary odor mixtures*. Physiol. Behav., 33, 309–319.

Laska, M. and **Galizia, C.G.** (2001) *Enantioselectivity of odor perception in honeybees (*Apis mellifera carnica*)*. Behav. Neurosci., 115, 632–639.

Laska, M., Galizia, C.G., Giurfa, M. and **Menzel, R.** (1999) *Olfactory discrimination ability and odor structure–activity relationships in honeybees*. Chem. Senses, 24, 429–438.

Laurent, G., Wehr, M., MacLeod, K., Stopfer, M., Leitch, B. and **Davidowitz, H.** (1996) *Dynamic encoding of odors with oscillating neuronal assemblies in the locust brain*. Biol. Bull., 191, 70–75.

Laurent, G., Stopfer, M., Friedrich, R.W., Rabinovich, M.I., Volkovskii, A. and **Abarbanel, H.D.** (2001) *Odor encoding as an active, dynamical process: experiments, computation, and theory*. Annu. Rev. Neurosci., 24, 263–297.

Lei, H. and **Hansson, B.S.** (1999) *Central processing of pulsed pheromone signals by antennal lobe neurons in the male moth* Agrotis segetum. J. Neurophysiol., 81, 1113–1122.

Linster, C., Johnson, B.A., Morse, A., Yue, E. and **Leon, M.** (2002) *Spontaneous versus reinforced olfactory discriminations*. J. Neurosci., 22, 6842–6845.

Meister, M. and **Bonhoeffer, T.** (2001) *Tuning and topography in an odor map on the rat olfactory bulb*. J. Neurosci., 21, 1351–1360.

Müller, D., Abel, R., Brandt, R., Zockler, M. and **Menzel, R.** (2002) *Differential parallel processing of olfactory information in the honeybee,* Apis mellifera *L*. J. Comp. Physiol. A, 188, 359–370.

Olsson, M.J. (1994) *An interaction model for odor quality and intensity*. Percept. Psychophys., 55, 363–372.

Pelz, C., Gerber, B. and Menzel, R. (1997) *Odorant intensity as a determinant for olfactory conditioning in honeybees: roles in discrimination, overshadowing and memory consolidation*. J. Exp. Biol., 200, 837–847.

Ratcliff, R. and **Rouder, J.N.** (2000) *A diffusion model account of masking in two-choice letter identification*. J. Exp. Psychol.: Hum. Percept. Perform., 26, 127–140.

Roitman, J.D. and **Shadlen, M.N.** (2002) *Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task*. J. Neurosci., 22, 9475–9489.

Sachse, S. and **Galizia, C.G.** (2002) *Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study*. J. Neurophysiol., 87, 1106–1117.

Sachse, S., Rappert, A. and **Galizia, C.G.** (1999) *The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code*. Eur. J. Neurosci., 11, 3970–3982.

Schall, J.D. (2001) *Neural basis of deciding, choosing and acting*. Nat. Rev. Neurosci., 2, 33–42.

Stopfer, M., Bhagavan, S., Smith, B.H. and **Laurent, G.** (1997) *Impaired odour discrimination on desynchronization of odour-encoding neural assemblies*. Nature, 390, 70–74.

Todd, J.L. and **Baker, T.C.** (1999) *Function of Peripheral Olfactory Organs.* In Hansson, B.S. (ed.), Insect Olfaction. Springer, Berlin, pp. 125–162.

Uchida, N., Takahashi, Y.K., Tanifuji, M. and **Mori, K.** (2000) *Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features*. Nat. Neurosci., 3, 1035–1043.

Uchida, N., Macknik, S.L. and **Mainen, Z.F.** (2001) *Psychophysical measurement of odor quality perception in rodents using two-alternative choice task with odor mixtures*. Soc. Neurosci. Abstr., 27, 458.11.

Usher, M. and **McClelland, J.L.** (2001) *The time course of perceptual choice: the leaky, competing accumulator model*. Psychol. Rev., 108, 550–592.

Vareschi, E. (1971) *Duftunterscheidung bei der Honigbiene. Einzelzellableitungen und Verhaltensreaktionen*. Z. Vergl. Physiol., 75, 143–173.

Vickers, D. (1980) *Discrimination.* In Welford, A.T. (ed.), Reaction Times. Academic Press, London, pp. 25–72.

Vickers, N.J., Christensen, T.A. and **Hildebrand, J.G.** (1998) *Combinatorial odor discrimination in the brain: attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli*. J. Comp. Neurol., 400, 35–56.

Wehr, M. and **Laurent, G.** (1996) *Odour encoding by temporal sequences of firing in oscillating neural assemblies*. Nature, 384, 162–166.

Wise, P.M. and **Cain, W.S.** (2000) *Latency and accuracy of discriminations of odor quality between binary mixtures and their components*. Chem. Senses, 25, 247–265.

Accepted October 10, 2003