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THE OLFACTORY MEMORY OF THE HONEYBEE *APIS MELLIFERA*

I. ODORANT MODULATION OF SHORT- AND INTERMEDIATE-TERM MEMORY AFTER SINGLE-TRIAL CONDITIONING

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Summary

In the first 15 min after a single learning trial the olfactory memory of the honeybee, *Apis mellifera*, proceeds through different processing phases during which time the memory is differentially sensitive to a cooling treatment that causes amnesia. During learning about floral odours in a natural situation, several decisions would normally be made about floral choice within that period. In order to study these phenomena in more detail, single-trial proboscis extension conditioning to different odorants was used. Several stimulus-specific effects on memory consolidation in the honeybee are shown. From previous experiments it was predicted that certain odorants would be more salient conditioning stimuli. This result is confirmed. Second, generalization from the conditioned odorant to a different odorant depends on the conditioned odorant and the time post-conditioning. In some combinations, responses to a novel odorant are significantly stronger than responses to the conditioning odorant after memory consolidation. These data indicate that memory recall in the honeybee, as it is evidenced by proboscis extension, is sensitive to several aspects of stimulus identity and presentation. The acquisition and recall processes are therefore much more dynamic processes than realized previously.

Introduction

The structure of an animal's environment can modulate memory storage and retrieval of associations among stimuli relevant for survival and reproduction. Reliable positive correlations among stimuli over evolutionary time may lead to the evolution of predispositions to learn specific associations of cues with motor patterns (Gould and Marler, 1984). In the honeybee, for example, floral odours *in general* serve to indicate resources such as pollen and nectar (von Frisch, 1967), but the reliability of any *specific* odour with floral resources may change rapidly

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within a bee's lifetime. Therefore, the learning task for a foraging bee is to track quickly which odours indicate presence or absence of resources at any given time.

This ecological background can help to interpret olfactory memory consolidation after proboscis extension conditioning of restrained honeybees (Kuwabara, 1957; see review in Menzel, 1990). After a single conditioning trial during which presentation of an odorant precedes the sucrose unconditioned stimulus (US) by 1–5 s, 30–50 % of restrained worker bees will extend their proboscides upon further presentation of the odorant alone (Menzel *et al.* 1974). Asymptotic responses to olfactory conditioning are typically attained after only two or three such trials. The evolution of such rapid acquisition, as well as temporal phases associated with olfactory memory processing (see below), can be understood in terms of the importance of olfactory cues for the identification of floral resources, the average flight times between flowers, and trips between flower patches and the colony (Menzel, 1983, 1985). Further work has shown that the conditioned response (CR) to an odorant depends on the odorant used for conditioning. Certain odorants elicit much stronger appetitive responses than others after the same conditioning procedure (Smith and Menzel, 1989*a*). Thus, the olfactory memory of the honeybee is biased, probably through experience and innate mechanisms, towards making an association of certain odorants with a sucrose reward.

In addition to the odorant itself, other sources of variation in the conditioned response to an odorant in restrained subjects have been identified (Erber *et al.* 1980). The expression of learning performance within 10–15 min of a learning trial reflects at least two physiological phases as the memory is being consolidated. Each phase is characterized by a specific treatment, or lack of one, that causes retrograde amnesia (Erber *et al.* 1980). Thus, the consolidation of olfactory memory from a more labile phase to a more permanent one can be established (Menzel, 1979, 1983). The initial phase lasts 3–5 min, and is characterized by the sensitivity of the memory to cooling of specific brain regions. After 5–7 min a less labile phase is formed, which cannot be interrupted by cooling.

The adaptations of this consolidation process must reflect alterations of the physiological states of neurones in a neural network through time as the network processes olfactory information and compares stimulus input with memory templates. However, little is known in the honeybee on how information processing in the 10–15 min after a conditioning trial, a period during which decisions based on this information must be made in a natural foraging context (Menzel, 1985), is modulated by different odorants that elicit differing strengths of appetitive responses (Smith and Menzel, 1989*a*). Such differences may reflect more reliable associations of certain kinds of odours with floral resources in areas in which honeybee populations have evolved (Koltermann, 1973). One question specifically addressed by the research reported below is how recall level, which is affiliated to the build-up of associative strength between neural representations of the conditioned stimulus (CS) and US (Wagner, 1981), at various times post conditioning, is affected by different odorants. A second, related question regards

generalization of the response to odorants other than the conditioning odorant at various post-conditioning times. Differing levels of generalization at different post-conditioning times might indicate changing specificity of the memory processes that give rise to the behavioural response. The answers to these questions are crucial for the evaluation of the specific capability of the honeybee olfactory system to perceive single odorants and mixtures of odorants (Getz and Chapman, 1988), and may have more general implications for olfactory systems in a wide variety of animals.

Materials and methods

Worker honeybees were captured as they departed from their colony. After immobilization by cooling to 8–12 °C, they were fixed in small metal harnesses with a tape strip placed between head and thorax (Menzel and Bitterman, 1983). The antennae and proboscis could be moved freely. After warming to room temperature, subjects were fed a 1.5 mol l^{-1} sucrose–water solution until satiated, i.e. the proboscides were retracted and extension could no longer be reliably elicited by application of sucrose to the antennae. Such feeding ensured that subjects were all in approximately the same motivational state on the following day. Subjects were then stored on a cool, dark shelf until 08:00 h the next morning, when they were fed again briefly, but not to satiation. Testing was then begun between 10:00 and 11:00 h.

All odorants were delivered by placing 1 μl of a substance onto a square mat of filter paper pinned to the plunger of a 20 ml syringe. 15 ml of air laden with the odorant was then delivered over 5 s by injecting it into a slowly moving air stream being drawn across the bee's antennae. Two pairs of odorants were chosen based on chemical structure (Smith and Menzel, 1989*a*). Hexanal and 2-hexanol are unbranched aliphatic hydrocarbons each containing one oxygen molecule in an aldehyde or an alcohol moiety, respectively. Neither substance is a known component of honeybee pheromones. Citral and geraniol are monoterpenoid compounds that differ with respect to the oxygen moiety in the same manner as the first pair. Citral is a 60:40 mixture of its isomers neral and geranial. Both substances are components of the honeybee Nasonov pheromone (Pickett *et al.* 1980), which is used during orientation and in marking unscented feeding dishes. Citral and hexanal were chosen because they had elicited strong appetitive responses in a previous experiment (Smith and Menzel, 1989*a*). Within each pair, the constants needed to calculate vapour pressure are very similar. Thus, for each odorant in a pair the concentrations of odorant (i.e. the number of molecules ultimately delivered to the antennae) in the delivery syringes would be similar. Statistical analyses (see below) are therefore designed to compare responses to the two odours within each group; that is, citral is compared to geraniol, and 2-hexanol to hexanal.

Odorant-specific effects on recall

The first experimental procedure explored the effects of different odorants on

recall. Each subject received one conditioning trial (i.e. odorant–sucrose pairing) in a trace-conditioning procedure (Byrne 1987). At one of 11 post-conditioning times, ranging from 0.5 to 14 min later, each subject received an extinction trial with the same odorant; that is, sucrose was not presented. Thus, subjects received a total of two trials with the same odorant, the first rewarded and the second unrewarded. During both trials the response in terms of extension of the proboscis to the odorant alone was categorized as either extended or not (if extension occurred on the first trial prior to US stimulation then the subject was scored as a spontaneous responder and tested normally in the second trial). After two trials the subject was never used in another test.

Through a comparison of the responses at the various post-conditioning times, the second trial provides a measure of the ability of memory to release behaviour over time.

The four odorants were tested in different groups of subjects. Within each odorant treatment, 11 independent treatments corresponding to the different post-conditioning intervals between the first and second trials were required. The time periods encompassed by these 11 treatments spanned those found for olfactory memory consolidation (Erber *et al.* 1980). Thus, a total of 44 independent treatment groups was tested. In order to factor out effects due to time of day as well as test day, one bee per group per day was conditioned (i.e. 44 subjects per day), and the order of the 44 treatments was randomized throughout the 3–4 h of daily testing.

Odorant generalization

A second experiment with different subjects tested the generalization among a range of olfactory stimuli at two different post-conditioning times and attempted to replicate recall levels from the previous experiment. Each bee experienced one conditioning trial, as above, and then received a second (*intermediate*) trial either 0.5 min or 15 min later, during which time no reward was presented. The intermediate trial was performed either with the conditioning odorant or with one of the three other odorants. Thirty seconds after the second trial each bee received a third (*final*) trial, again unrewarded, but this time always with the conditioning odorant. The latter test indicated whether the intermediate test had had an effect on the response to the conditioning odorant. The two times at which tests occurred (0.5 and 15 min) spanned the memory consolidation time frame reported in Erber *et al.* (1980).

A total of 32 independent treatment groups was employed. That is, four conditioning odorants and four test odorants (one of which was the conditioning odorant) at each of two post-conditioning times. Thirty-two subjects were tested each day, corresponding to one bee per treatment per day. The order of testing was randomized as above.

Statistical analysis

All statistical comparisons were performed by comparing the number of

responders *versus* non-responders in different treatment groups. To do so the categorical data modelling option of SAS-PC™ was employed. The program returns a chi-square value for the different treatment effects (e.g. odorant and test time post-conditioning) partitioned according to treatments in a way analogous to a two-way analysis of variance. For two or more treatments, interaction terms, indicating non-additive interactions between the treatment groups, are also calculated. A significant interaction term indicates that the relative ranks of means (referred to below as the 'profile' of means) has altered across-treatment conditions. For example, a significant interaction term would result if, under condition 1, the mean for treatment A is greater than that for treatment B, but, under condition 2, the mean for B is greater than that for A.

For all data presented below, the number of subjects that responded to the stimulus by extension of their proboscis is expressed as a percentage of the total number of subjects tested.

Results

2315 subjects were conditioned in the experiments outlined below. Of those, a small percentage responded spontaneously to odorant presentation, i.e. a response to the odorant was recorded on the first trial, before it had been associated with the sucrose US. The spontaneous responses to citral (N=584 subjects conditioned) and to geraniol (N=573) were 6.2% and 2.4%, respectively. The spontaneous responses to hexanal (N=584) and to 2-hexanol (N=574) were 5.0% and 2.8%, respectively.

Odorant modulation of recall

A single conditioning trial with any of the odorants significantly increased response at most, and for some odorants all, of the post-conditioning test times (Fig. 1). Odorants differed, however, in the magnitude of this increase and in the degree to which this increase reliably occurred. Within any given odorant treatment, response levels generally tended to decrease with time, but this decrease was significant only for 2-hexanol.

In the case of citral, differences in response levels among different groups of subjects at different time periods are not significant (Fig. 1, top left). Fifty percent of the subjects responded when tested 0.5 min or 1 min after the conditioning trial. Response decreased slightly thereafter to a low of about 25% at 4 min post-conditioning. Response increased at two test times afterwards to a high of 45% and then dropped again through the last test at 14 min. These transient increases could indicate statistical fluctuation away from a mean response among groups.

Response levels to geraniol were significantly lower than those to citral (Fig. 1, top right). Response levels were high (approximately 35%) after 0.5 min, but thereafter decreased to between 5 and 10%, which was only slightly higher than the initial spontaneous response to this odorant. The response remained constant

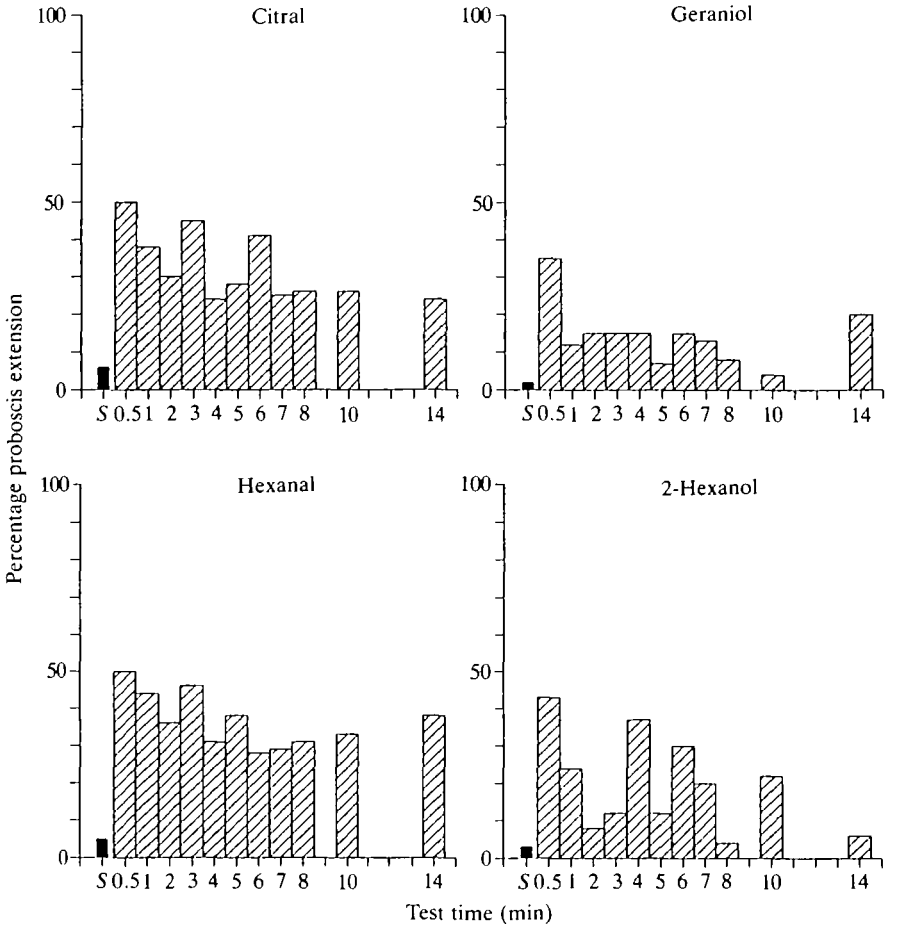

Fig. 1. Histograms showing the frequency of the proboscis extension response to odorant presentation across 11 different post-conditioning times (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 14 min). The solid columns indicate the levels of spontaneous (*S*) response to the odorants calculated from groups across all time periods. For columns that are not solid, two conventions are adopted in this and subsequent figures. First, each column indicates an independent group of subjects. Second, hatched columns indicate that subjects were tested with the conditioning odorant, while open columns indicate tests with novel odorants. Four different odorants were conditioned in parallel in independent treatments. Response on the ordinate indicates the percentage of bees that responded by extension of their proboscis upon presentation of the conditioning odorant at the time indicated on the abscissa. (An unlabelled tickmark on the abscissa indicates that no tests were performed at that time.) Sample sizes per group are approximately equal and range from a minimum of 27 to a maximum of 30. The chi-squared and probability values indicating differences within the two pairs of odorants are: citral–geraniol 25.85 ($P<0.001$, 1 d.f.); hexanal–2-hexanol 20.24 ($P<0.001$, 1 d.f.). The chi-squared and probability values indicating differences within each odorant across times are: citral 9.99 ($P>0.05$; 10 d.f.); geraniol 13.209 ($P>0.05$; 10 d.f.); hexanal 6.153 ($P>0.05$; 10 d.f.); 2-hexanol 26.17 ($P<0.01$; 10 d.f.).

at this level in groups tested at later times. However, these differences in response across time are not significant. Thus, it *appeared* that learned information from a single trial with geraniol did not control behaviour over time as well as did information from a trial with citral, the odorant to which it is physically the most similar. (However, see the generalization experiments below.)

After single-trial conditioning to hexanal, response increased to approximately 50 % (Fig. 1, bottom left). The slight tendency to decrease is not significant.

Response to 2-hexanol (Fig. 1, bottom right) was significantly lower than to hexanal. After 0.5 min the response level was above 40 %. The average response level changed significantly over the next several minutes. This decrease was not as consistent as it was for geraniol; in some groups (e.g. 4- and 6-min test groups) higher levels of recall were registered.

With regard to the odorants used, two conclusions can be drawn from the data in Fig. 1. (1) The level of response (recall) at any given time depends on the odorant. For similar stimulus concentrations, memory recall for certain odorants (aldehydes) is enhanced when compared to that for other odorants (alcohols) that have similar chemical structures and vapour pressures. (2) The level of recall after a single conditioning trial, at least with some odorants, also depends on the post-conditioning time at which the test takes place.

Odorant generalization

In this experiment, responses to the conditioning odorants after 0.5 min did not differ statistically among the odorants (Fig. 2, hatched columns from 0.5 min treatment) and were similar to the response levels at 0.5 min in the previous experiment. The response to the conditioning odorants across the time treatments (Fig. 2, hatched columns, compare 0.5 and 15 min columns within each graph) decreased significantly for all odorants except citral, which decreased only slightly from 0.5 min to 15 min post-conditioning. These results were similar, albeit statistically more significant, to the decrease in recall over 14 min from the previous experiment (Fig. 1).

Shortly after conditioning, generalization responses to odorants other than the conditioning odorant occurred for all conditioning odorants. In every case, these generalization responses were significantly higher than the original spontaneous responses to the odorants (approximately 3–5 %), indicating that the generalization response resulted from some aspect of the conditioning procedure. Three conclusions from this experiment will be discussed. (1) The response to an odorant after conditioning was not always specific to the odorant conditioned, and the strongest responses were, in some cases, to an odorant that was not experienced during conditioning. (2) There was no generalization profile common to all odorants. Generalization from conditioning to novel odorants was instead dependent on which odorant was conditioned and, in one case, the time at which the generalization test was made. (3) On some occasions the intermediate unrewarded test potentiated the response to the conditioned odorant on the final

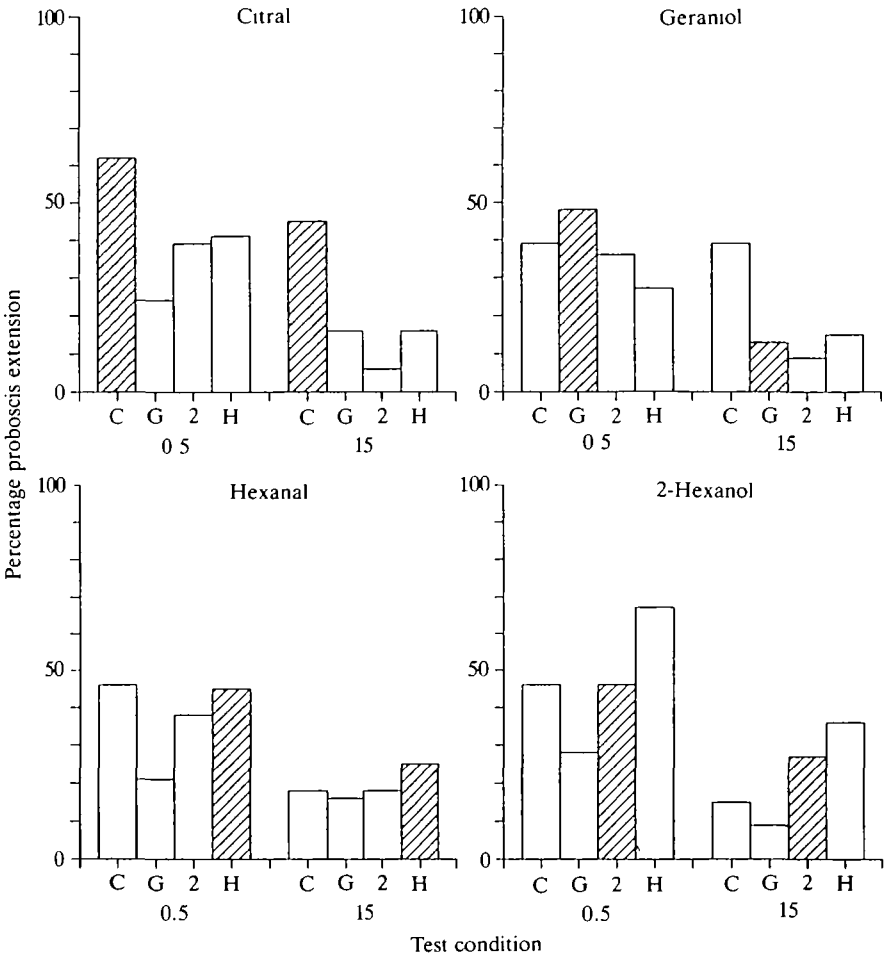

Fig. 2. Histograms showing the frequency of the proboscis extension response to either the conditioned stimulus (hatched columns) or one of three novel odorants (open columns) after conditioning to citral (top left), geraniol (top right), hexanal (bottom left) or 2-hexanol (bottom right). Letters or numbers on the abscissa are the abbreviations used for the test odorant (C, G, H, 2, for the four odorants, respectively) corresponding to each column in the figure. These data correspond to the first unrewarded test, i.e. the intermediate odorant. Numbers on the abscissa indicate when unrewarded tests took place, either 0.5 min or 15 min after the conditioning trial. Response on the ordinate indicates the percentage of bees that responded by extension of their proboscis upon presentation of the odorant listed on the abscissa. Samples sizes range from a minimum of 25 to a maximum of 30 bees per column. Chi-squared values and their associated probabilities indicate differences in response due to test odorant (left-hand values below; with 3 d.f.), time post-conditioning that the test occurred (middle values; 1 d.f.) and interaction between test odorants and time (right-hand values; 3 d.f.). Listed by conditioning odorant, these values are: citral 29.0 ($P<0.01$); 17.3 ($P<0.001$); 3.5 ($P>0.05$); geraniol 6.5 ($P>0.05$); 13.7 ($P<0.001$); 6.9 ($P<0.05$); hexanal 6.7 ($P>0.05$); 12.4 ($P<0.001$); 2.4 ($P>0.05$); and 2-hexanol 20.1 ($P<0.001$); 22.9 ($P<0.001$); 1.2 ($P>0.05$).

test, while on other occasions the latter response was interfered with. Owing to the complexity of the results, they will be discussed for each conditioning odorant individually.

After a single conditioning trial with citral, subjects responded to other odorants, albeit significantly less than they did to citral (Fig. 2, top left). There was no significant tendency to respond more to one novel odorant than to others. The response profile, i.e. the relative magnitudes of the responses, remained the same between the 0.5 and 15 min treatment groups, as shown by the non-significant interaction term, although the levels of response decreased significantly across the two times.

The response to citral on the final test was unaffected by the odorant tested during the intermediate test (Fig. 3, top left). The response to citral after an intermediate test with citral decreased slightly, though not significantly, when both tests took place within a minute of the conditioning trial. Thus, regardless of the odorant tested, an unrewarded exposure to any odour neither interfered with nor enhanced the response to citral at either post-conditioning time.

A remarkably different set of results was obtained after conditioning with geraniol, the odorant most similar to citral in structure and vapour pressure (Fig. 2, top right). After 0.5 min, although the response to geraniol was the highest, there were no significant differences in the responses among any of the odorants, including geraniol. Note the asymmetry between citral and geraniol: 0.5 min after conditioning to geraniol subjects responded equally strongly to citral and geraniol, whereas, 0.5 min after conditioning to citral subjects responded much more strongly to citral than to geraniol (Fig. 2, top left). Fifteen minutes after conditioning to geraniol there was a difference in the responses among the odorants, but the strongest response was to citral, an odorant that the subjects had not experienced during the conditioning trial. This response to citral was significantly stronger than the response to geraniol itself, and there were no significant differences between geraniol, 2-hexanol and hexanal. Therefore, unlike the results with citral, after geraniol conditioning the generalization profile changes as the memory consolidates with time, as is reflected in the significant interaction term.

Thirty seconds after the intermediate test (i.e. 1 min after conditioning to geraniol), the responses to geraniol were heterogeneous (Fig. 3, top right). At both test times, the second unrewarded test took place at a point when the response to geraniol would be expected to be low (Fig. 1, top right). However, when the intervening trial was with citral, the response to geraniol (the conditioning odorant) was potentiated significantly above that after an intermediate trial with any other odorant, with the exception of 2-hexanol in the earlier test groups. At 15 min post-conditioning, the response to geraniol was potentiated only by intermediate exposure to citral.

No significant differences in the responses to test odorants occurred either 0.5 min or 15 min after conditioning with hexanal (Fig. 2, bottom left). Subjects responded equally to all odorants, which generated a flat profile. The decrease in

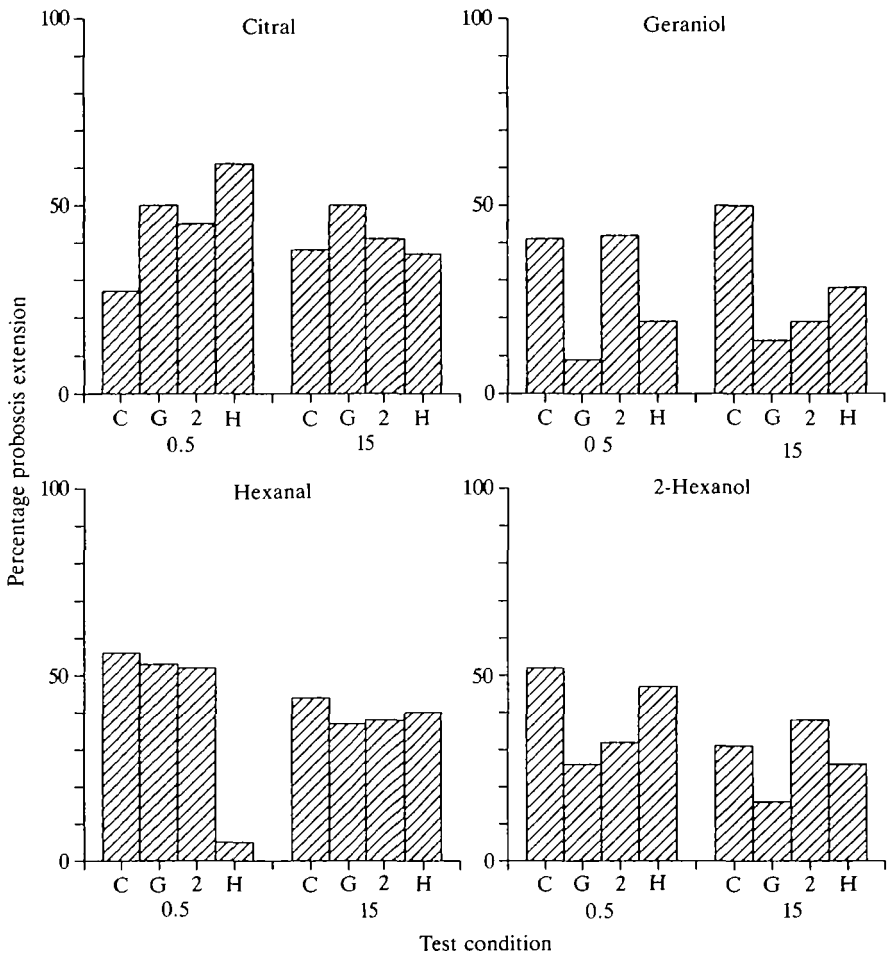

Fig. 3. Histograms showing the frequency of the proboscis extension response to the conditioning odorant 0.5 min after an intermediate unrewarded trial with either the conditioning odorant or one of three novel odorants indicated on the abscissa as C (citral), G (geraniol), 2 (2-hexanol) or H (hexanal). These data correspond to the second (final) unrewarded test. All else as in Fig. 2. Sample sizes range from a minimum of 25 to a maximum of 30 bees per group. Chi-squared values and their associated probabilities indicate differences in response due to intermediate test odorant C, G, 2, H (left-hand values below; 3 d.f.), time post-conditioning that the test occurred (middle values; 1 d.f.) and interaction between intermediate test odorants and time (right-hand values; 3 d.f.). Listed by conditioning odorant, these values are: citral 1.6 ($P>0.05$); 0.1 ($P>0.05$); 1.4 ($P>0.05$); geraniol 10.2 ($P<0.05$); 0.1 ($P>0.05$); 3.0 ($P>0.05$); hexanal 7.7 ($P<0.05$); 0.1 ($P>0.05$); 7.9 ($P<0.05$); 2-hexanol 4.1 ($P>0.05$); 1.7 ($P>0.05$); 1.2 ($P>0.05$).

response between 0.5 and 15 min was significant and affected all odorants equally, i.e. the interaction term was not significant.

Responses to hexanal on the third trial were, however, heterogeneous (Fig. 3,

bottom left). In the 0.5 min treatment, an intermediate trial with hexanal dramatically inhibited the response to hexanal on the final trial, whereas an intermediate trial with another odorant, to which the subjects strongly generalized, had no such effect. However, the response profile changed in the 15 min treatment group. In this group, there was no effect of an intermediate trial with any odorant, including hexanal. Because of the shift in hexanal response profile in Fig. 3, the interaction term was significant.

After conditioning with 2-hexanol (Fig. 2, bottom right), the strongest response was elicited with hexanal 0.5 min later, as indicated by significant differences among responses to odorants. The response levels in general decreased in the 15 min treatment, when the responses to hexanal and 2-hexanol were not significantly different, although both were greater than the responses to the remaining two odorants. The profile did not change significantly from 0.5 to 15 min, i.e. the interaction term was not significant.

There were no significant differences in the responses to 2-hexanol on the final test, either across test odorants or across the time treatment (Fig. 3, bottom right). Therefore, an intermediate test with an odorant had no detectable effect on response to the conditioning odorant.

Finally, in comparing the generalization responses to geraniol and 2-hexanol in Fig. 2 a trend may emerge. Bees tended to generalize from an alcohol to an aldehyde of comparable chemical structure somewhat more readily than from the aldehyde to the alcohol.

Discussion

The primary motivation for these studies was to assess how recall level and specificity is affected by different processing stages through which olfactory memory in the honeybee progresses (Erber *et al.* 1980; Menzel, 1983). To assess these parameters, a conditioning assay (single-trial conditioning to an odorant) relevant to natural foraging situations was used. There are two primary conclusions that can be drawn from these experiments. First, levels of recall after a single associative pairing of an odorant with a sucrose reward are affected by the identity of the odorant. Therefore the identity of the conditioning stimulus *modulates* some aspect of memory consolidation. Second, generalization among odorants is a complex phenomenon. Responses to novel odorants depend both on the time post-conditioning at which the tests are performed and on the conditioning odorant.

Stimulus generalization is an important component of memory processing (Shepard, 1987). Natural stimuli vary in time and space such that the likelihood of occurrence of exactly the same stimulus as the conditioning stimulus would be low. For example, floral odours can be complex mixtures of dozens of odours. A honeybee that does not generalize to similar floral odours may pass over several flowers of the same species that contain a nectar reward. That is, a generalization gradient helps to minimize mistakes. A bee that responds only to the exact odour

stimulus that it learned might in the extreme only revisit the flower that it had just depleted of nectar. In the other extreme, a bee that responds to every odour after a conditioning experience may waste time with unrewarding stimuli. Thus, any adequate description of the honeybee's olfactory system must contain a description and explanation of generalization rules that establish what *class* of stimuli, rather than what stimulus, predicts the occurrence of floral rewards.

This control of recall and propensity to generalize can be related to the 'associative strength' developed between neural representations of odorant and sucrose in the bee's central nervous system (Rescorla and Wagner 1972; Wagner 1981). In its basic form, this theory specifies that every time the sucrose US follows an odorant CS within a limited time the linkage *via* neural pathways between the representations of the two stimuli increases. This theory has led to verifiable predictions in a variety of animals regarding the relationship of stimulus strength (concentration) to rate of acquisition and asymptotic levels of conditioned responding.

In the studies above, using two different odorants at similar molar concentrations delivered to the antennae led to significantly different response probabilities. This result for citral and geraniol and for hexanal and 2-hexanol implies that different associative strengths were built up in spite of similar physical concentrations of the odorants. However, the data can be explained in terms of associative theories if we assume that a neural representation for each odorant exists and that the bee's peripheral and central nervous system biases these representations. That is, the neural representations of odorants do not map one-to-one onto physical dimensions of odorant stimuli such as molar concentration. For a given odorant, the strength of representation would be expected to change with changing molar concentrations. But for a given molar concentration of two different odorants, representation strength might be different.

Generalization after a single conditioning trial may also reflect a build-up of associative strength. In the insect peripheral olfactory system there is an overlap in the degree to which sensory neurones respond to different non-pheromonal stimuli. For the honeybee, Vareschi (1971) defined several sets of non-pheromonal sensory neurones based on the types of odorants to which they responded. Using a conditioning procedure involving eight conditioning trials, Smith and Menzel (1989*a*) showed that behavioural generalization is stronger to odorants that are chemically more similar to conditioning odorants. Thus, the associative strength that is developed in response to a given conditioning odorant appears to spread to the representations of odorants of similar chemical structure much more so than to odorants of different structure, although some generalization exists to all odorants.

Testable predictions can be made as to how the bee's olfactory system might bias the representation of an odorant. For example, larger numbers of peripheral olfactory sensory receptors may exist for a given odorant than for others. Complex cross-fibre coding schemes (Maes, 1984) might easily lead to this result. Evidence from electroantennogram studies in the honeybee indicates significantly different

parameters of stimulus–response functions for different odorants (Patte *et al.* 1989). Internal representations of odorants might also be biased either in response properties to sensory input or towards faster integration with motor areas that control the mouthparts. Specialization of central nervous system (CNS) processing neurones exists for connection of pheromonal coding to adaptive flight motor output in insects (Homberg *et al.* 1989). Similar processing specialization may exist to a lesser extent for adaptive feeding behaviours as well. With the behavioural data base reported above, these ideas can now be tested by physiological and biochemical methods.

Stimulus processing and generalization can be affected by at least four variables. First, differences observed in odorant responses in different studies may reflect genetic variation in biasing the representation. Koltermann (1973) showed significant racial variation for odorant preferences in freely flying honeybees. He tested genetic lines of bees from several colonies that had been collected in different geographic regions. These lines had presumably adapted to local variations in floral availability. There were consistent differences among these lines in responses to a wide variety of odorants. That is, subjects showed preferences for certain odours, but those preferences differed among lines. Subjects used in the present study did not derive from a pure race of bees, but these bees may differ from the carniolan race common throughout the European continent. For example, in contrast to results from geraniol conditioning reported above, workers from carniolan colonies obtained in Germany, consistently responded to geraniol up to 20 min post-conditioning (B. H. Smith, unpublished results); however, generalization to citral was not tested in that study.

Second, after a single conditioning trial, generalization responses appear to be less focused than with multiple trials. In a companion paper, B. H. Smith and F. A. Ganje (in preparation) show that, with six conditioning trials to citral or to hexanal, generalization responses remain at approximately 20–30 %; that is, comparable to generalization levels in the single conditioning trial experiments reported above. But the response to the conditioning odorant increases to 80–90 %. Therefore, with multiple trials the response becomes more focused on the conditioning odorant. This result would indicate that the increment of associative strength to a novel odorant by generalization asymptotes much more quickly than does the strength to the conditioning odorant.

Third, differences in level of recall and generalization between test times closer to the conditioning event and later times may also reflect different contributions of associative and non-associative processes. Brandes *et al.* (1988) have shown that sensitization produced by sucrose feeding enhances the response to an odorant for up to 0.5 min without associative pairing. The 0.5 min recall and generalization responses reported above may contain a much stronger sensitization component than later responses. For single trial conditioning, P. W. Hamlet, A. E. Menteir, B. H. Smith, H. K. Lehman and T. R. Tobin (in preparation) have used three treatments involving a single forward pairing, backward pairing, or sucrose feeding followed by a single extinction trial 45 min after the initial experience.

Responses to odorant during the latter test were significantly higher in the forward pairing treatment group than in the other two groups. Therefore, responses reported in the above experiments certainly have an associative component, even though those responses immediately after the conditioning event may result largely from sensitization.

Fourth, the response measure also affects the estimate of generalization. Smith and Menzel (1989*b*) used electromyogram recordings from a muscle that drives feeding movements of the honeybee's proboscis (Rehder, 1987). By testing subjects with the conditioning odorant or with a novel odorant they then analyzed responses of only those subjects that showed responses to the conditioning and novel odorants. Thus, these populations of subjects would show equal response probabilities (100%) to both types of odorants. However, response to the novel odorant was not as strong or consistent as response to the conditioning odorant. Therefore, response probabilities might overestimate generalization but can still be used as a measure of relative generalization to compare two or more odorants, as has been done above.

Generalization to salient stimuli can also uncover a build-up of associative strength that is subthreshold for releasing behaviour. The response to citral appeared to be most focused in that the response to it as the conditioning odorant was significantly stronger than were any generalization responses to novel odorants. This result may reflect the pheromonal nature of citral. It is a component of the Nasonov pheromone, which elicits strong orientation responses from freely flying bees in situations of confusion or for location of an unscented feeding dish (von Frisch 1967). However, the responses to geraniol, which is the major component of that pheromone (Pickett *et al.* 1980), were less focused in the short term, but focused instead on citral in the long term. In other words, what initially looked like a forgetting of the geraniol–sucrose association is actually recall of a more salient odorant that is perceptually similar to the conditioning odorant. Additionally, an intermediate trial with citral can enhance responses to geraniol when it was the conditioning odorant (Fig. 3).

Any study of memory must carefully separate the effect of storage and recall mechanisms (Spear *et al.* 1990). The results reported here indicate a complex relationship between olfactory storage and recall mechanisms in the honeybee. That is, what is recalled after memory consolidation (Erber *et al.* 1980) can be slightly different from the conditioning event, and a lack of response does not necessarily imply forgetting (Spear *et al.* 1990). As noted above, such biases in recall of olfactory information may reflect processing at either peripheral (Vareschi, 1971) or central (Mobbs, 1985) processing centres in the honeybee CNS. In addition to predictions made above, this system can now be used to ask from a behavioural standpoint what types of interconnections can be made between odorants, either sequentially or in mixtures, and what behavioural manifestations of memory result from acquisition *versus* recall failures. Such work will be invaluable for describing the nature of the honeybee olfactory network in its nervous system. Further work, through modelling (Getz and Chapman, 1987)

or through physiological analyses of predictions from these behavioural studies, should lead to insights into olfaction and learning mechanisms and provide access to information processing in a biological system.

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