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Olfactory information transfer in the honeybee: compared efficiency of classical conditioning and early exposure

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We investigated the ability of honeybees, *Apis mellifera*, to use olfactory information gained in a given experimental context, in other contexts. First, restrained bees were subjected to a Pavlovian associative learning procedure, based on the conditioning of the proboscis extension response (PER), where a floral odour was paired with a sugar reward. We observed the orientation behaviour of conditioned and naïve bees in a four-armed olfactometer with four contiguous fields either scented with the conditioning odour or unscented. Information transfer was clearly shown, conditioned bees orienting towards the conditioning odour, whilst naïve bees shunned it. Second, the effect of passive olfactory exposures during the bees' development was assessed in two behavioural contexts: either orientation in the olfactometer or a PER conditioning procedure. Two exposure periods were applied: (1) the pupal stage (9 days before emergence); (2) the early adult stage (8 days after emergence). No effect of preimaginal exposure was recorded, but exposure during the early adult stage induced a higher choice frequency of the odour field in the olfactometer, and lower learning performance in the PER conditioning assay. These observations show that olfactory information gained during development can modify bees' later behaviour in different contexts: this is another instance of olfactory information transfer in bees. These results also suggest that nonassociative learning phenomena, taking place at a critical period during development, might be involved in the maturation of the bees' olfactory system, and in the organization of odour-mediated behaviours.

Performance during learning and retrieval of established memories depends strongly upon context, namely the range of stimuli an animal experiences in its environment in addition to relevant stimuli involved in conditioning (contextual cues: Balsam 1985; Rescorla et al. 1985). The ability to transfer information learnt in one context to other contexts can be considered a key feature of behavioural plasticity. Indeed, this capacity may have an adaptive importance for animals, providing an optimized use of stored memories. The wide range of individual and social behaviours of honeybees, *Apis mellifera*, is of particular interest in this respect. The ability of bees to forage using information gained inside the hive from a returning forager (e.g. spatial cues: von Frisch 1967; olfactory cues: Wenner et al. 1969) is but one instance of information transfer in this species. Since olfaction plays a major role in foraging behaviour (Menzel et al. 1993), we aimed to address olfactory information transfer in the honeybee, either after an associative learning experience, or after a passive olfactory exposure.

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Associative learning has a strong effect on the odour-mediated behaviours of bees and is thus a type of experience that is likely to induce information transfer. The food search behaviour of honeybees outside the hive is based on two main types of associative learning processes: (1) classical (Pavlovian) conditioning, building a contingency between conditioned stimuli (e.g. floral cues) and unconditioned stimuli (e.g. nectar reward); and (2) instrumental (operant) conditioning, building a contingency between the insect's response (flying or walking towards a discriminative stimulus) and the outcome of such a response (e.g. flying towards a flower and obtaining a reward for it), and between the discriminative stimuli (e.g. floral odours, colours or patterns) and the insect's response.

In honeybees, these two types of associative learning have been studied using different experimental approaches. Classical olfactory learning has been investigated by conditioning the proboscis extension reflex (PER; Kuwabara 1957; Takeda 1961; Bitterman et al. 1983; Hammer & Menzel 1995). In such conditioning, an odour (conditioned stimulus, CS) gains control over the reflexive proboscis extension of bees when antennal, tarsal or

proboscis chemoreceptors are stimulated with a sucrose solution (unconditioned stimulus, US) as a consequence of a previous contingent presentation of CS and US. Since the animals are restrained in glass tubes, their overt behaviour does not contribute to the establishment of this association. In contrast, instrumental olfactory learning has been studied by quantifying the orientation either of bees walking in an olfactometer (Getz & Smith 1990; Bakchine et al. 1992) or of free-flying bees foraging on scented artificial feeders (Couvillon & Bitterman 1980; Greggers & Menzel 1993; Pham-Delègue et al. 1993; Greggers & Mauelshagen 1997). These two types of olfactory learning are thus related to the same general context of appetitive food search, but involve different kinds of associations and may be controlled by different neural pathways.

Despite intensive investigation of these two types of learning (for a comparative review, see Mauelshagen & Greggers 1993), few studies have explored information transfer from one paradigm to the other. Gerber et al. (1996) studied the proboscis extension responses of bees that had the opportunity to forage on basswood trees, *Tilia* sp. They showed initial responses to the basswood tree odour as high as 60% in this group compared with control bees which had low spontaneous response levels. This indicated a possible transfer of information learnt in a foraging situation (instrumental context) to the reflex response of proboscis extension (Pavlovian context). In the reverse situation, Bakchine et al. (1992) conditioned bees to geraniol (pheromonal/floral odour) in the PER conditioning assay and showed an orientation response of conditioned bees towards this odour in an olfactometer. They thus provided evidence that information could be transferred from a Pavlovian conditioning situation to an instrumental response. Nevertheless, they failed to reproduce this effect with a floral compound (limonene). One aim of our study was to show that bees can transfer olfactory information from a Pavlovian conditioning experience (conditioning of the proboscis extension) to an instrumental situation (orientation response to an odour in an olfactometer), using floral odorants.

Passive olfactory exposure could also affect later odour-mediated behaviours. Indeed, in numerous insect species, an exposure to environmental cues during development affects different behavioural tasks of the adult such as habitat selection (*Drosophila*: Thorpe 1939; apple maggot fly, *Rhagoletis pomonella*: Papaj & Prokopy 1988; parasitoid hymenoptera: Kester & Barbosa 1991), host location and selection (parasitoid hymenoptera: Thorpe & Jones 1937; Vet 1983; Wardle & Borden 1985), food preference (solitary bees: Dobson 1987; *Drosophila*: Jaenike 1988), nestmate recognition (ants: Jaisson 1972; Isingrini et al. 1985; Carlin & Schwartz 1989) and nest site selection (ants: Jaisson 1980; Djieto-Lordon & Dejean 1999). In most cases, the critical period for exposure is the early adult stage, but some studies also point out probable preimaginal learning phenomena (Isingrini et al. 1985; Dobson 1987; Carlin & Schwartz 1989). In the honeybee, an exposure to chemical signals can induce changes in food choices (Kunze 1933), in nestmate recognition (Breed & Stiller 1992) and in flower selection during foraging (Wenner et al. 1969). Other studies have investigated the effect of adding scents to the hive on foraging behaviour (Free 1969; Jakobsen et al. 1995) and on conditioning of proboscis extension (Gerber et al. 1996). Only Jakobsen et al. (1995) obtained a significant effect, free-flying bees being attracted to the odour added in the hive, but in their study the duration of olfactory exposures was not controlled. Using caged bees kept in incubators, other authors have applied olfactory exposures under more controlled conditions. Pham-Delègue et al. (1990) showed that an exposure to geraniol during the first 8 days of adult life increased the orientation response to this odour in an olfactometer. However, after exposing bees to a range of volatiles during the first 7 days after emergence, Getz & Smith (1991) did not find any significant effect on the performance of bees in the PER conditioning assay, except for citral, a pheromonal compound, to which exposed bees responded at lower levels than control individuals.

No study has sought to evaluate the effect of preimaginal olfactory exposures in the honeybee. Indeed, studies on the ontogeny of the olfactory nervous system of honeybees have suggested the existence of a critical period from 3 days before to 4–8 days after emergence during which the olfactory system appears very flexible in response to environmental changes (Masson et al. 1993). Olfactory deprivation experiments during this period have resulted in a decrease in antennal responsiveness to different odours (Masson & Arnold 1984) and in synapse frequency in the glomeruli (Gascuel & Masson 1987). Therefore, our second aim was to analyse information transfer after a passive olfactory exposure to floral odorants during development. Based on neurophysiological and behavioural data, two exposure periods were applied. The first period was the 9 days of the pupal stage (i.e. 9 days before emergence), since olfactory signals can diffuse through the wax cap (e.g. brood pheromones: Free 1987). The second was the first 8 days of the adult stage, as young workers may experience odours in the hive during this crucial period for olfactory system maturation. We evaluated the effects of the exposures on adult bees in two different contexts: in an olfactometer or with a PER conditioning procedure.

METHODS

In experiment 1, we subjected bees to a training procedure in the PER conditioning assay, and then observed their orientation behaviour in an airflow olfactometer. In the other two experiments, bees received an olfactory exposure either during the preimaginal stage (experiment 2), or during the early adult stage (experiment 3). We tested the olfactory learning performance of such individuals in a PER conditioning procedure or observed their orientation behaviour in an airflow olfactometer.

Olfactory Stimuli

We duplicated all experiments, using two olfactory stimuli. Linalool (95–97%, Sigma, Saint Quentin

Fallavier, France) and phenylacetaldehyde (95%, Sigma) were chosen as they are widely spread among natural flower blends (Knudsen et al. 1993). Moreover, they are both behaviourally relevant as they are key compounds in the recognition of oilseed rape mixture by honeybees (Blight et al. 1997).

Experiment 1: Transfer After Classical Conditioning

Emerging worker bees collected from outdoor hives were caged in groups of about 50 individuals and fed with sugar, water and pollen ad libitum. They were kept in an incubator (33°C, 55% relative humidity, darkness) until 14–16 days old when they were used in the conditioning and test procedures.

PER conditioning

The experimental procedure for conditioning the proboscis extension was the standard one used in previous studies on olfactory learning in honeybees (Bitterman et al. 1983; Pham-Delègue et al. 1993; Sandoz et al. 1995). The odour stimulation device created a constant flow of 52.5 ml/s, either scented or unscented, which was delivered to the bees through a 1-cm glass tube. This flow consisted of a main vector airflow (50 ml/s) and a secondary one (2.5 ml/s) used for odour stimulation and injected into the main airflow. The odour source was a piece of filter paper, 40 × 3 mm, soaked with 10 µl of pure odorant, which was inserted in a disposable Pasteur pipette. The secondary flow was injected by the experimenter either through the pipette containing the odour source or an identical empty pipette. A fan placed opposite the delivery tube extracted the released odours from the experimental room.

Bees were mounted individually in glass holders leaving their antennae and mouthparts free, and were deprived of food for 3–4 h. They were subjected to three conditioning trials with 15-min intertrial intervals. Before every trial, bees were positioned for 15 s in the airflow to familiarize them with the mechanical stimulation. We presented the odour stimulus for 6 s and then, 3 s after the onset of the odour, placed the antennae in contact with a 30% sucrose solution. The subsequent proboscis extension was rewarded with a drop of the same solution. Individuals showing spontaneous responses at the first presentation of the odour were not used in the experiment since later responses of such individuals could not be interpreted as purely associative. Furthermore, only bees that learned after the first odour–reward association (i.e. responding since the second odour presentation) were kept for further observation. In parallel to the conditioned bees, we subjected a control group to the same procedure but without odour delivery.

Orientation in a four-armed airflow olfactometer

We observed olfactory cued orientation with a four-armed olfactometer (Fig. 1) adapted to honeybees after Vet et al. (1983), and described by Bakchine et al. (1990). In this olfactometer, individual insects can walk around freely and explore four contiguous fields. The observation chamber of the olfactometer was star shaped, limited by four crescents made of Plexiglas (95° arc, radius 245 mm, thickness 20 mm). Compressed air entering the chamber through inlets at its four arms was sucked through a central hole, creating four contiguous but distinct fields (150 ml/min in each field). The four airflows were equalized with flowmeters, and the occurrence of four distinct fields was controlled by smoke as described in Pham-Delègue et al. (1990). The device was positioned on a light table providing red light of 160 lx, in a dark room, which prevented bees visually orienting and trying to fly. Each arm was connected to a glass vial either containing a piece of filter paper soaked with 10 µl of pure odorant (scented field), or empty (unscented fields). The position of the scented field was rotated, and the observation chamber was thoroughly cleaned with ethanol after each tested bee.

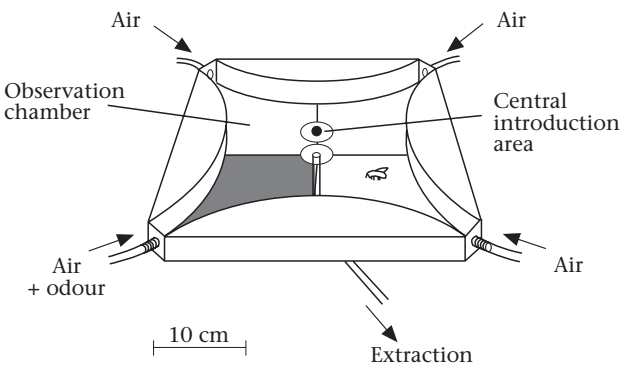

Figure 1. Four-armed olfactometer used for studying the orientation behaviour of bees. Four identical airflows (150 ml/min), entering the crescent-shaped chamber of the olfactometer at its four arms, were sucked through a central extraction hole which created four contiguous but distinct fields. One field was scented with an odour source and three fields were left unscented. During observations, an individual honeybee was placed at the centre of the chamber, and its orientation behaviour recorded for 5 min.

After the PER conditioning procedure, we allowed bees to rest for 3–5 h (same resting time in both conditioned and control groups), then tested them in the olfactometer with one field scented with the conditioning odour and three unscented fields. Bees were individually introduced into the central area of the chamber, and their walking behaviour (walking/motionless) and location in the different fields were recorded on a computer for 300 s. In parallel, possible proboscis extensions in the olfactometer were recorded according to the field visited.

Experiment 2: Transfer After Preimaginal Olfactory Exposure

We obtained two homogeneous brood combs by introducing two small combs (22 × 16 cm) of fresh wax in a compartmented outdoor hive. The queen was allowed to lay eggs on these combs for 2 days and was then placed in another compartment. We removed the combs when most of the brood was freshly capped (about 9 days before emergence), ready for exposure.

Olfactory exposure

We carried out olfactory exposures using air-tight exposure boxes made of Plexiglas and adapted to the size of small combs (internal dimensions 25 × 18 × 5 cm). The two lateral sides of the boxes were transparent, which allowed us to monitor the emergence of the bees. The boxes were kept in an incubator (33°C, darkness) and were connected to a constant airflow (150 ml/min in each box, 33°C). One comb (exposed group) was exposed to the odour continuously for 9 days. The odour source was provided by a glass vial (1.5 cm^2 evaporation surface) connected to the airflow and containing 1 ml of pure odorant, this amount being sufficient to ensure exposure during the 9-day period. To avoid odour contamination, all vapours were released outdoors after flowing through the exposure boxes. When we observed the first emergences, we removed the comb and brushed off bees that had already emerged. We then placed the comb in an unscented box for the following emergences, thus only individuals emerging after this change (i.e. without adult exposure) were kept for further tests. The second comb (control group) was subjected to the same procedure, but kept in an unscented box for both pupal period and emergence.

Test procedures

After emergence, exposed and control bees were caged and kept in an incubator until they were tested in either the PER conditioning or the olfactometer experiments. In the PER conditioning experiment, bees were used when 14–18 days old. They were subjected to three conditioning trials (acquisition phase) followed by five test trials (extinction phase) with 15-min intertrial intervals. We conducted conditioning trials as described in experiment 1, bees being stimulated with the odour stimulus for 6 s, and rewarded with a drop of sucrose solution for the last 3 s. Test trials consisted of a 6-s presentation of the odour stimulus alone. In the olfactometer procedure, bees were used at an age of 9–23 days. The experimental conditions were the same as those described in experiment 1.

Experiment 3: Transfer After Early Adult Olfactory Exposure

One standard comb of emerging brood was taken from the same hive as in the pre-emergence exposure experiment, and was divided so as to fit into two small comb frames, 22 × 16 cm. These combs were then ready for exposure.

Olfactory exposure

We carried out postemergence olfactory exposures using the same experimental device as in the experiment on pre-emergence exposure (experiment 2). One small brood comb (exposed group) was placed in a scented exposure box and bees were allowed to emerge over 2 days. After that time, young adults were kept in the scented box but the brood comb was replaced by a new comb of fresh wax filled with 40 ml of a commercial sugar solution. This amount of sugar solution was sufficient to feed the bees ad libitum for the whole of the exposure. This procedure allowed us to control the age of the experimental bees. The olfactory exposure lasted until bees were 8 days old on average. The second comb (control group) was subjected to the same procedure in an unscented box.

Test procedures

After the 8-day exposure period, exposed and control bees were caged in groups of at least 100 individuals and kept in an incubator until they were tested in either the PER conditioning or the olfactometer experiments. The test procedures were carried out as described in experiment 2. We conducted PER conditioning and olfactometer experiments when bees were 13–17 and 10–15 days old, respectively.

Statistical Analysis

Data recorded from the observation of individuals in the olfactometer were the time spent in the four fields, the central introduction area being excluded from the analysis. As a few individuals spent a lot of time motionless, only the data from bees walking for more than 200 out of 300 s were kept for further analysis: this included more than 90% of the bees tested. For comparisons between conditioned/exposed and control groups, Kolmogorov–Smirnov two-sample tests were applied on the time spent in the odour field. To assess within a given group the preference of bees for either scented or unscented fields, we compared the relative proportions of bees choosing (i.e. spending most of their time in) the odour field or one of the unscented fields to a random 1:3 distribution, using a log-likelihood ratio test. In experiments on pre- and postemergence exposure, we tested the performance of bees in a PER conditioning procedure. Bees were assigned a value corresponding to the total number of trials during which they exhibited a proboscis extension across the eight trials of the procedure. This value, which ranged from zero to eight, was used in a Mann–Whitney test to compare the performance levels between groups. Since the experiments were performed at different times, we did not carry out any between-experiment comparisons. All statistical tests were two tailed.

RESULTS

Experiment 1: Transfer After Classical Conditioning

The two odorants were efficiently learnt by honeybees in the PER conditioning assay. In the three-conditioning-trial procedure, 78% of honeybees responded to linalool and 69% to phenylacetaldehyde at the end of the procedure. Figure 2a shows the percentage of time spent in the odour field compared to the average time spent in unscented fields, for conditioned and control bees. For linalool and phenylacetaldehyde, conditioned bees spent, respectively, 34.0 (N=30) and 37.2% (N=23) of the time in the odour field, whilst control bees

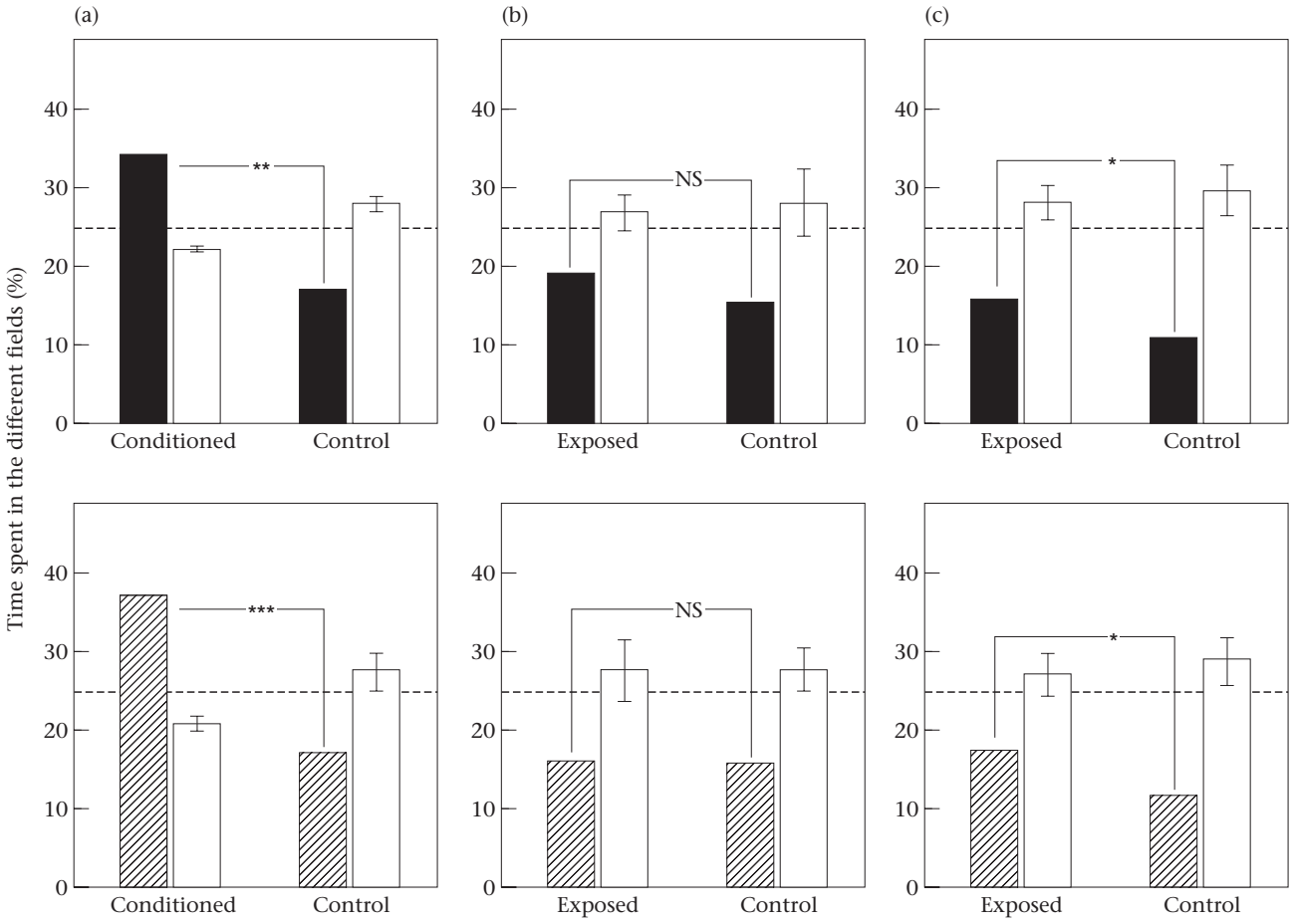

Figure 2. Orientation behaviour of bees in a four-armed olfactometer after different types of experience: (a) a classical conditioning procedure; (b) olfactory exposure during the preimaginal stage; (c) olfactory exposure during the early adult stage. The time spent in the field scented with the conditioning/exposure odour (■, ▨) and the average time spent in the three unscented fields (□) ±SE by bees of the different groups are shown for linalool (■) and phenylacetaldehyde (▨). Asterisks indicate significant differences (Kolmogorov–Smirnov two-sample test: **P*<0.05; ***P*<0.01; ****P*<0.001).

spent only 16.8 (*N*=30) and 17.3% (*N*=22). Conditioned bees spent significantly longer in the odour field than control bees (Kolmogorov–Smirnov test: linalool: D=0.50, *P*<0.01; phenylacetaldehyde: D=0.68, *P*<0.001). The preference of bees for scented or unscented fields was reversed by conditioning: conditioned bees chose the scented field (log-likelihood ratio test, linalool: G_1=4.47, *P*<0.05; phenylacetaldehyde: G_1=13.2, *P*<0.001) whilst control bees preferred unscented fields (G_1>6.71, *P*<0.01 in both cases). Inside the olfactometer, 80 and 79% of bees conditioned to linalool and phenylacetaldehyde, respectively, showed proboscis extensions in the odour field, compared to 3 and 0% in the other fields. In the control groups less than 10% of bees showed proboscis extensions in any field.

Experiment 2: Transfer After Preimaginal Olfactory Exposure

Figure 2b shows the time spent in the different fields of the olfactometer after pre-emergence exposure for exposed and control bees. Bees exposed to linalool and phenylacetaldehyde spent, respectively, 19.2 (*N*=15) and 16.1 (*N*=21) of the time in the odour field, whilst control bees spent 15.3 (*N*=14) and 15.9% (*N*=19). There was no significant effect of exposure on olfactory orientation (Kolmogorov–Smirnov test: linalool: *D*=0.37, *P*=0.28; phenylacetaldehyde: *D*=0.16, *P*=1). In exposed as well as in control bees, unscented fields were proportionally chosen more often than the scented one, this tendency being significant for phenylacetaldehyde (log-likelihood ratio test; G_1>5.29, *P*<0.05) but not for linalool (G_1<3.10, NS). We did not record any proboscis extension in exposed or control bees walking in the olfactometer.

Figure 3a shows responses in the PER conditioning procedure for exposed and control bees. Conditioning to linalool induced more than 80% conditioned responses after the first odour–reward association for both exposed (*N*=39) and control bees (*N*=37). This level was maintained during the two following conditioning trials (acquisition), then decreased when presentations of the odorant were unrewarded (extinction) to ca. 5–20% at the fifth testing trial. A Mann–Whitney test

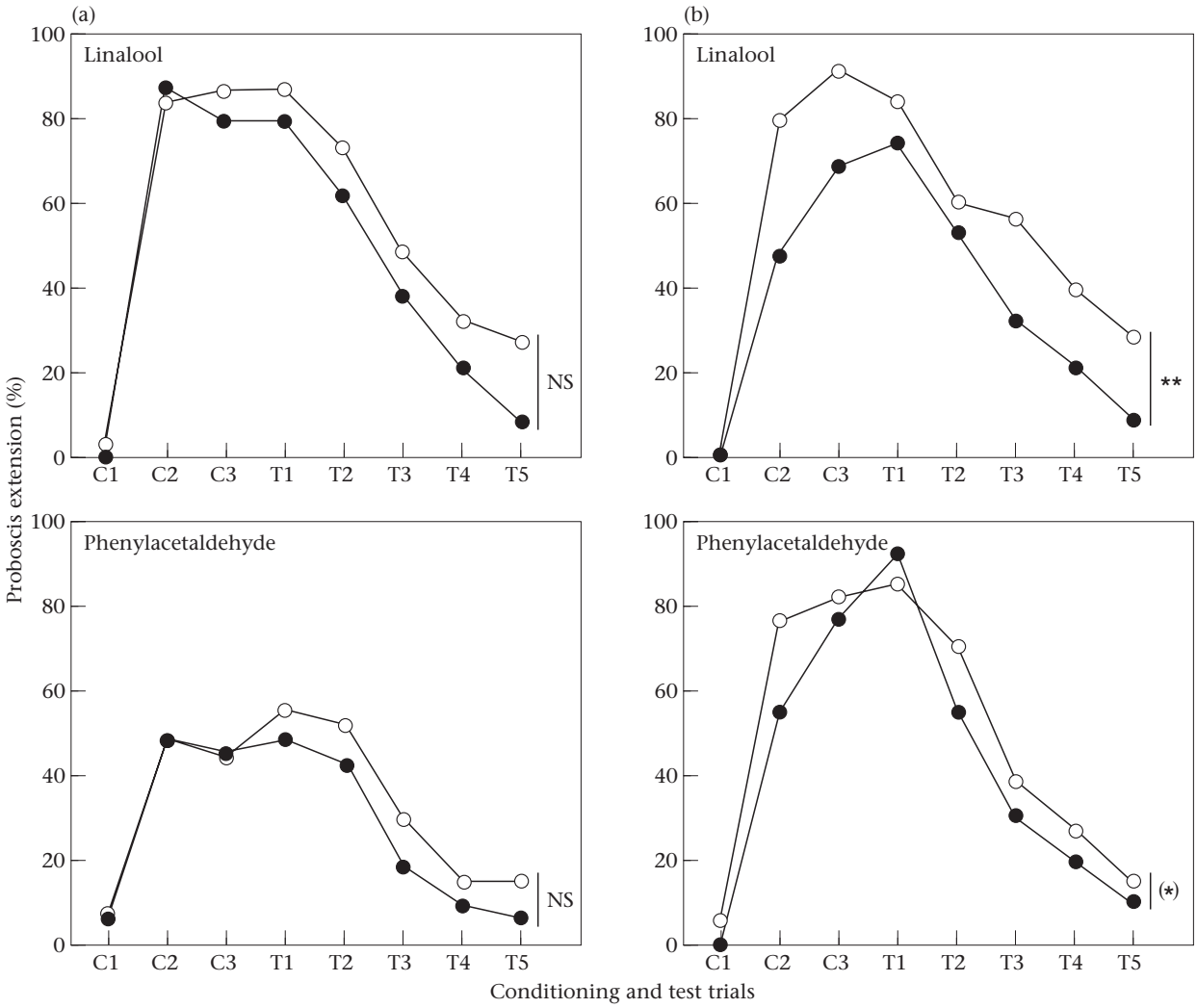

Figure 3. Learning performance of bees in a PER conditioning procedure after olfactory exposure during (a) the preimaginal stage and (b) the early adult stage. Percentages of proboscis extension responses to the exposure odour are shown for linalool and phenylacetaldehyde. ●: Exposed; ○: control. Asterisks indicate significant or near significant differences across the eight trials of the procedure (Mann–Whitney test: (*)P=0.063; **P<0.01).

comparing the number of proboscis extensions of exposed and control bees throughout the procedure did not indicate any significant difference (Z=1.44, P=0.15). Conditioning to phenylacetaldehyde produced lower acquisition than in the previous experiment. A maximum of 60% conditioned responses was reached after three conditioning trials for both exposed (N=33) and control bees (N=27), but no difference appeared between groups (Mann–Whitney test: Z=1.15, P=0.25).

Experiment 3: Transfer After Early Adult Olfactory Exposure

Figure 2c shows the orientation in the olfactometer after a postemergence olfactory exposure for exposed and control bees. Bees exposed to linalool and phenylacetaldehyde spent, respectively, 15.6 (N=23) and 17.7% (N=29) of the time in the odour field, whilst control bees spent only 10.9 (N=24) and 11.6% (N=29). The exposed groups spent significantly longer in the odour field than control groups (Kolmogorov–Smirnov test: linalool: D=0.40, P=0.049; phenylacetaldehyde: D=0.41, P=0.014), but there was no reversed preference after olfactory exposure as obtained after classical conditioning (experiment 1), unscented fields remaining preferred to the scented one (log-likelihood ratio test: G_1>4.7, P<0.05). We did not record proboscis extensions in exposed or control bees walking in the olfactometer.

Figure 3b shows PER conditioning performance for exposed and control bees. For linalool, bees from the control group (N=43) showed very quick acquisition of the conditioned response reaching 79% after the first odour–reward association. In contrast, exposed bees (N=38) acquired the conditioned response more slowly, reaching only 47% after the first association and 74% after three conditioning trials. During the extinction phase, the level of responses decreased in both groups with values for exposed bees remaining below those of the control group. A comparison of the proboscis extensions throughout the whole procedure indicated a

significant difference (Mann-Whitney test: $Z=2.86$, $P<0.01$). For phenylacetaldehyde, even though acquisition was qualitatively slower for exposed ($N=40$) than for control bees ($N=34$), the same comparison yielded only a near significant difference ($Z=1.85$, $P=0.063$).

DISCUSSION

We have shown that information gained in classical olfactory conditioning can increase significantly the orientation response of bees towards the learnt odours in an olfactometer device. In a similar experimental situation, Bakchine et al. (1992) tested the orientation of bees conditioned to geraniol (pheromonal/floral compound) or limonene (floral compound) in an olfactometer delivering both compounds simultaneously. Their results suggested that only conditioning to a compound with a pheromonal value could produce information transfer to an orientation response context. Our data indicate that the same effect is observed with floral compounds. In Bakchine et al.'s (1992) study, this phenomenon was probably masked by the spontaneous preference of bees for geraniol over limonene. This kind of information transfer from a Pavlovian associative learning experience to an instrumental orientation response takes place in a general context of food search, since conditioned bees showed proboscis extension responses in the field of the olfactometer scented with the conditioning odour. Whereas external contextual cues (e.g. experimental setup, lighting conditions, etc.) were very different in the two experimental situations, we may assume that the common alimentary motivation of bees (internal state) facilitated the retrieval of learnt information and thus information transfer.

Our results complement those of Gerber et al. (1996) who showed information transfer from an instrumental context (foraging experience on basswood trees) to a Pavlovian context (proboscis extension responses to the basswood odour). Taken together, these studies suggest that information transfer can be observed in both directions. This is of particular interest for the understanding of the associative mechanisms underlying instrumental and Pavlovian conditioning processes. In vertebrates, it is usually assumed that in an instrumental conditioning context, associations build up between the stimulus and the response produced by the animal, and between the response and the reinforcement obtained (Colwill & Rescorla 1986). Nevertheless, in instrumental conditioning paradigms, associations between stimulus and reinforcement, as in Pavlovian conditioning, may occur (Colwill & Rescorla 1988). In honeybees, instrumental and Pavlovian conditioning experiments have often shown very similar results (Mauelshagen & Greggers 1993; Pham-Delègue et al. 1993), and the observation of transfers from one context to the other suggests that the respective underlying stimulus–reward associations may be of the same type.

Besides the possibility of olfactory information transfer after associative conditioning, a passive olfactory exposure may also induce such a transfer. In the olfactometer device, we found an increased orientation of bees towards the odours used for passive exposure, although there was no attraction to these odours. Other studies have shown an increase in orientation towards a prior exposure odour, either in bees walking in an olfactometer (Pham-Delègue et al. 1990) or in free-flying bees visiting an artificial feeder (Jakobsen et al. 1995). In contrast, in the PER conditioning procedure, exposed bees tended to learn the exposure odour less efficiently than naïve bees. Gerber et al. (1996), after exposing a hive to odours (limonene and peppermint), did not detect any significant effect on learning performance. However, they hypothesized that if an effect of the exposure were to appear, it would be 'inhibitory rather than excitatory'. In another study (Getz & Smith 1991), bees exposed to pure odorants in cages did not show significant differences in their ability to learn these odours, except in the case of citral which induced lower learning rates in exposed individuals. From these studies and ours, it appears that exposed bees would tend to orient more towards an exposure odour (or avoid it less) and to learn it less efficiently than naïve bees.

In the hive, bees exposed to an odour during the early adult period have food readily available, which could result in the formation of food–odour associations. Nevertheless, olfactory associative learning relies on the discrete and forward pairing of odour and reward. Indeed, the permanent odour background prevents the establishment of such a predictive relationship between odour and food, making it possibly more difficult to learn because it is not a 'surprising' stimulus anymore (Kamin 1969). The pre-exposure to olfactory cues could also produce a 'learned irrelevance' where animals learn that the stimuli, here the exposure odour and food, are not correlated (Dickinson 1980). Nonassociative mechanisms can also be proposed for the observed behavioural effects of olfactory exposures. First, the prolonged exposure to the odour could have induced some kind of sensory adaptation of the bees' olfactory system. In this case, the exposure odour would later be less easily detected by bees. This would decrease a spontaneous avoidance by bees of the pure compound in the olfactometer, and make it a less salient compound to be learnt in a PER conditioning procedure. As sensory neurons continue to mature until 8 days after emergence (Masson & Arnold 1984; Allan et al. 1987), such exposure could affect olfactory sensitivity at the peripheral level. Further work should test modulations in the peripheral sensitivity after olfactory exposures, using electrophysiological techniques.

Another hypothesis refers to an imprinting process tuned to a social context. In bees, kin recognition is based mainly on olfaction (Smith & Breed 1995). Individual bees acquire their odour by means of their own cuticular hydrocarbons, and of compounds adsorbed in the comb wax, mainly cuticular hydrocarbons from other individuals and the odour of food stored in the hive (Breed et al. 1995, 1998). Food odours, and thus floral odours, play a role in colony recognition by honeybees. Breed & Stiller (1992) showed that early learning, taking place at a short critical period (the first hour after emergence), is involved in the recognition of the nest odour. Such imprinting-like

phenomena are also known in other hymenoptera for brood care (ants: Jaisson & Fresneau 1978; solitary bees: Kukuk et al. 1977) or nest site selection (ants: Djieto-Lordon & Dejean 1999). The behavioural effects we observed after olfactory exposure might be derived from such social phenomena. The increase in orientation of bees towards the exposure odour in the olfactometer could be related to nest orientation behaviour. Information would then be transferred from one social context to another. This is consistent with the fact that no proboscis extensions were observed for exposed bees in the olfactometer, an appetitive food search thus being unlikely. Moreover, imprinting by an odour in a social context might produce resistance to learning this odour in an alimentary context, which would thus hinder the performance of exposed bees in the PER conditioning procedure. Following this hypothesis, our observation of negative effects of olfactory exposure on learning performance suggests that information transfer from very different contexts (here from a social to an alimentary context) may produce inhibitory effects.

Neurobiological data suggest that the olfactory nervous system of honeybees is most sensitive to environmental odours during a critical period from 3 days before to 8 days after emergence (Masson & Arnold 1984; Gascuel & Masson 1987; Masson et al. 1993). However, our results indicate that only olfactory exposures during the post-emergence period would induce behavioural changes. In other insects, mostly postemergence critical periods have been found (reviews: Alloway 1972a; Papaj & Prokopy 1989). This could be considered an evolutionary advantage: since metamorphosis induces largescale neural replacements in the brain of insects (Nordlander & Edwards 1970; Technau & Heisenberg 1982; Malun 1998), retention of an exposure odour throughout this stage would have to rely on the relatively low number of conserved larval neurons (Alloway 1972b). Such phenomena have been shown in a few insect species, but seem to correspond to evolutionary answers to specific problems such as kin-related brood care in ants (Isingrini et al. 1985; Carlin & Schwartz 1989). In the honeybee, workers usually spend the first 2 weeks of adult life inside the hive performing various duties (Winston 1987). They may thus be exposed to hive odours until late in their development, which would not induce any adaptive advantage of pre-emergence over postemergence critical periods in this species. The exposure periods we used were rather long (8–9 days), mimicking natural situations. Nevertheless, the critical period for behavioural effects of olfactory exposures may well be restricted to a very short period after emergence, as was found for the recognition of the nest odour (Breed & Stiller 1992). Current work is therefore focusing on determining the moment and duration of this critical period.

In conclusion, we have shown two types of information transfer in bees. Classical olfactory conditioning strongly altered the orientation behaviour of bees, whilst passive olfactory exposure during development had a moderate impact on their orientation behaviour and on their learning performance. This indicates that even if the olfactory-mediated behaviour of bees can be significantly altered by the environment in which they have developed, through nonassociative phenomena, associative learning is still the main experience determining the control of later behaviour. As bees are known to bear 'innate search images' for flowers, which develop through experience into 'learnt search images' (Menzel 1985; Giurfa et al. 1995), studies on the respective effects of early passive or later associative olfactory experience may help to clarify the concept of 'innate search images'. Therefore, the continuation of such studies will lead to a better understanding of the extent to which olfactory-mediated behaviours of bees are determined genetically, on the one hand, and environmentally on the other.

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