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SENSORY PRECONDITIONING IN HONEYBEES

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Summary

Sensory preconditioning means that reinforcement of stimulus *A* after unreinforced exposure to a compound *AB* also leads to responses to stimulus *B*. Here, we describe and analyze sensory preconditioning in an insect, the honeybee *Apis mellifera*. Using two-element odorant compounds in classical conditioning of the proboscis extension reflex, we found (i) that sensory preconditioning is not due to stimulus generalization, (ii) that paired, but not unpaired, presentation of elements supports sensory preconditioning, (iii) that simultaneous, but not sequential, exposure to the elements of the compound supports sensory preconditioning and (iv) that a single presentation of the compound yields maximal sensory preconditioning. The results are discussed with respect to configural and chain-like associative explanations for sensory preconditioning. We suggest an experience-dependent step of compound processing, establishing configural units, as an additional explanation for sensory preconditioning.

Key words: sensory preconditioning, within-compound association, olfaction, learning, configural learning, honeybee, *Apis mellifera*.

Introduction

All animals are confronted with stimuli that, in changing combinations, characterize relevant objects and predict important events; thus, it is a basic challenge for all animals to adjust their behaviour accordingly through learning. This involves at least two tasks, one is that animals must learn which elements of the sensory input belong together and constitute an object, and the other task is more of a Pavlovian problem in that animals must learn the predictive relationships among these objects. While such predictive learning is intensively studied, less emphasis has been given to issues of object recognition. Here, we use a sensory preconditioning paradigm to examine some aspects of object recognition.

In the first phase of a sensory preconditioning paradigm (Brogden, 1939), a compound stimulus is presented without reinforcement (compound pretraining: *AB*–); then the element *B* is tested (pre-test). In the second phase, the other element is reinforced (element training: *A*+) and then *B* is tested again (test). Sensory preconditioning is indicated by an increase in the response levels to *B* between pre-test and test. This effect was observed for compounds within (Barnet et al., 1991, 1997; Lavin, 1976; Lyn and Capaldi, 1994; Rescorla and Cunningham, 1978; Rizley and Rescorla, 1972; Ward-Robinson and Hall, 1996) and across (Brogden, 1939; Hall and Subowski, 1995; Hoffeld et al., 1960; Prewitt, 1967; Tait et al., 1972) sensory modalities. Thus, during *AB* pretraining, animals learn that *A* and *B* belong together, and responses to *B* are ultimately a result of that learning. Therefore, sensory preconditioning can be used as a model to analyze how perception of objects characterized by combined stimulus elements are formed in an experience-dependent way.

Several explanations for associative learning have been proposed specifying mechanisms that could underlie sensory preconditioning. First, combined stimuli might result in single units (Rescorla, 1980), configural cues (Rudy and Sutherland, 1992) or configural units (Pearce, 1994) as a result of experience, in which case at least one simultaneous occurrence of the stimuli is required to form such a unitary representation. We think it is important that the formation of this configural unit is itself envisaged to be an experience-dependent process. In the formalized model of Pearce (1994), a unit once formed does not change through further presentations. Thus, if sensory preconditioning came about through these configural units, it should not improve with additional compound presentations. Furthermore, simultaneous presentation of the stimuli forming the compound would be predicted to yield stronger sensory preconditioning than sequential presentation.

Second, it has been suggested that associations might be formed between the elements of a compound (Speers et al., 1980; Rescorla and Durlach, 1981). If such associations included the asymmetric, predictive nature of stimulus–reward associations, one would predict that these associations would be stronger for sequentially presented than for simultaneously presented stimuli (Rescorla, 1980). If sensory preconditioning is due to these associations (e.g. Rescorla and Freberg, 1978;

a model termed the 'standard' explanation of sensory preconditioning *via* an associative chain; for an overview, see Hall, 1996; Pearce, 1997), sensory preconditioning should correspondingly be stronger for sequential than for simultaneous presentations of the stimuli forming the compound; in particular, the exact sequence of the stimuli (forward *versus* backward presentation) should be a critical parameter. Furthermore, additional compound presentations should strengthen these associations and hence increase sensory preconditioning. In vertebrate studies, simultaneous presentations of *A* and *B* lead to the strongest sensory preconditioning (Lyn and Capaldi, 1994; Lavin, 1976; Rescorla, 1980; but see Hoffeld et al., 1958; Wynne and Brogden, 1962). Furthermore, only minor acquisition (Prewitt, 1967; Tait et al., 1972) was found. These findings cast some doubt on the chain-like associative nature of sensory preconditioning.

However, similar learning procedures have also been reported in some invertebrates: honeybees *Apis mellifera* (Couvillion and Bitterman, 1982; Müller et al., 1996) and pond snails *Lymnaea stagnalis* (Kojima et al., 1998). Interested in the mechanism involved, we have investigated sensory preconditioning in the honeybee, using a well-established laboratory paradigm, classical conditioning of the proboscis extension reflex (Kuwabara, 1957; Bitterman et al., 1983).

Because of their life style as generalist foragers in an uncertain and ever-changing environment, honeybees have evolved rapid and dynamic learning abilities (Menzel and Müller, 1996). These learning abilities can be analyzed under restrained laboratory conditions (Hammer, 1997; Hammer and Menzel, 1995; Menzel and Müller, 1996). In this study, we have used odorants and their binary compounds for classical conditioning of the proboscis extension reflex. In harnessed honeybees, odorants were used as conditioned stimuli and were paired with a presentation of sucrose solution to the antennae and mouthparts (including the proboscis) as the rewarding, unconditioned stimulus. Bees form associations within one to a few trials (fewer than four) so that they will extend their proboscis on presentation of the odorant alone.

Olfactory proboscis extension conditioning shows many of the characteristics of classical conditioning (Bitterman et al., 1983). More complex phenomena such as the Kamin effect of memory consolidation (Gerber et al., 1998; Hammer and Menzel, 1995; Menzel, 1990), overshadowing (Pelz et al., 1997; Smith, 1996), inhibitory learning (Hellstern et al., 1998) and second-order conditioning (Takeda, 1961; Menzel, 1990; Mosolff et al., 1998) have also been demonstrated. Together with recent reports of blocking (Smith and Cobey, 1994; Smith, 1996, 1997; Thorn and Smith, 1997), these findings suggest that proboscis extension conditioning of single odorants and of binary compounds could, to some extent, follow similar learning rules to those developed for vertebrates (Mackintosh, 1975; Pearce and Hall, 1980; Rescorla and Wagner, 1972; Sutton and Barto, 1981; Wagner, 1981). An advantage of this system is that the contributions of single, identified neurons can be analyzed during conditioning (Mauelshagen, 1993; Hammer, 1993; Rybak and Menzel, 1998; for a review, see Hammer, 1997) and the contribution of the whole neuropile can be examined using Ca^{2+} imaging (Faber et al., 1999). Furthermore, *in vivo* measurements of odorant conditioned stimulus processing can be obtained using optical imaging techniques (Joerges et al., 1997; Galizia et al., 1997, 1999) and from intracellular recordings (Homberg, 1984; Sun et al., 1993; Fonta et al., 1993).

Thus, demonstrating sensory preconditioning in honeybee proboscis extension conditioning will enable a physiological analysis of the relative contributions of variations in the conditioned and unconditioned stimulus processing that underlie sensory preconditioning.

Materials and methods

Experimental animals

Honeybees (*Apis mellifera* L.) were caught from hives maintained outdoors or in an indoor flight room (Praagh, 1972). They were harnessed in metal tubes that allow free movement of the antennae and mouthparts, including the proboscis (Bitterman et al., 1983), fed to satiation with a $2\,mol\,l^{-1}$ sucrose solution (a concentration used throughout the experiments) and then kept in the dark overnight at 20 °C and high humidity (relative humidity close to 100 %). On the following day, animals were tested for an intact proboscis extension reflex, i.e. complete extension of the proboscis immediately after the antennae had been touched with sucrose solution. A complete extension was scored if the proboscis crossed an imaginary line between the tips of the opened mandibles (Smith, 1997). Immediately after the reflex test, animals were placed in position on a motor-driven wheel (Vareschi, 1971), 0.5 m in diameter, upon which 10 bees could be arranged simultaneously. Movement of the wheel to move animals to the training site in succession was computer-controlled. At the training site, odorants were delivered by an olfactometer (described by Galizia et al., 1997; see also below); the timing of sucrose delivery was acoustically signalled by the computer. An exhaust system behind the animals removed odorants. Experiments started 30 min after the animals had been positioned on the wheel.

Stimulus application and training procedure

The sucrose reward was delivered with a precision syringe (Gilmont Instruments, Barrington, IL, USA). One antenna was briefly touched with a 2 µl droplet of sucrose solution, and bees were then allowed to feed on the droplet for approximately 2 s. Bees do not swallow more than a maximum of 0.5 µl during this period. Sucrose delivery lasted for a total of 3 s.

The odorant used were 1-hexanol, 1-octanol, geraniol and 1-hexanal (Sigma). We balanced the use of the odorants during the experiments such that all 24 possible combinations of odorants were used equally often. After treating one (experiment 4), two (experiments 1 and 2) or four (experiment 3) multiples of 24 animals in each group, we stopped the

experiment. We then selected for analysis only those animals that showed the unconditioned response after the experiments. The number of animals used for the analysis therefore differed from multiples of 24, and not all permutations were used in each group. For this reason, we continued the experiments until each group included the required number of permutations. Analyses (not shown) for odorant specifity revealed no difference between these totally counterbalanced experiments and the actual data presented.

The abbreviations *A, B* and *C* (and *D* in experiment 1) in the text and figures refer only to the experimental role of an odorant, and not to its chemical identity. Daily, 3 µl of each odorant was loaded onto a strip of filter paper and placed into a 1 ml tuberculin plastic syringe, which was loaded into the olfactometer (Galizia et al., 1997). Binary compounds were produced by two syringes with one filter paper each. An aquarium pump delivered a continuous flow of air through the olfactometer, which had its opening at the training site 3 cm in front of bees. This air flow provided a continuous stimulation to adapt out mechanosensory input (Pelz et al., 1997). Odorant pulses were applied by computer-controlled solenoid valves (Lee, Westbrook, CT, USA) programmed to shunt air through the respective odorant-loaded syringe.

All trials lasted for 1 min. At the beginning of a trial, the wheel was moved to place individual bees at the training site in front of the exhaust system. Bees were allowed a 25 s accommodation period before stimulus delivery. Depending on the experimental condition, odorant alone, sucrose alone or both odorant and sucrose were applied (see below). After stimulation, animals were left untreated until the full minute had passed (approximately 20 s), and the wheel was then moved to the next position. Since 10 bees were positioned on the wheel, the inter-trial interval was 10 min. Odorant stimulations usually lasted for 4 s (but see experiment 4), and all reward stimulations lasted for a total of 3 s. During conditioning trials, sucrose was applied 3 s after odorant onset (the onset-to-onset inter-stimulus interval was 3 s) leading to an overlap between odorant and sucrose stimulation of 1 s. In such a conditioning trial, the animal received the odorant alone for 3 s, followed by a combined presentation of the odorant and the reward and, finally, the reward was presented alone for 2 s. This procedure is well-established and has a strong conditioning effect. Increasing the interval between the conditioned stimulus and the unconditioned stimulus up to a few seconds does not change the amount of proboscis extension conditioning. Only backward conditioning with an interval of more then 6 s or trace conditioning of 10 s or more reduces the proboscis extension to some extent (Hellstern et al., 1998; Menzel et al., 1993). During pre-exposure and test trials, the reward was omitted; during reward-only trials, odorant stimulation was omitted. Pre-exposure to sequential odorant presentations was carried out with onset-to-onset inter-stimulus intervals of either 4 s or −4 s (see experiment 3); thus, the second odorant was applied at the offset of the first with zero programmed overlap.

Response measurements and statistical analyses

During rewarded trials, a response was scored if animals showed a proboscis extension after odorant onset but before reward onset; during pre-exposure trials and tests, responses during the 15 s after odorant onset were scored. Data are presented as the percentage of bees showing proboscis extension.

Chi-square (χ^2) tests were applied for between-group analyses of response frequencies during test trials (χ^2 values). The Freeman test (Freeman and Halton, 1951) was used to compare response frequencies during tests of more than two groups (*F* values). The McNemar test was applied for within-group analyses of response frequencies before *versus* after the conditioning phase (McNemar χ^2 values). Response levels during pre-exposure or during conditioning phases were compared between two groups using the Mann–Whitney *U*-test (*U* values) and between more than two groups using Kruskal–Wallis tests (*H* values). For within-group comparisons during the pre-exposure phase, Cochran's *Q*-test was used (*Q* values).

Experimental procedures

Experiment 1

The first experiment follows the classical test for sensory preconditioning introduced by Brogden (1939). Sensory preconditioning can be inferred if, after the presentation of a binary odorant compound (pre-exposure phase), conditioning of one of the elements also leads to increased response levels to the other element. Controls were introduced to exclude the possibility that conditioning of any odorant (generalization) or presentation of the rewarding stimulus alone (sensitization) might account for this increase in response levels. Moreover, to test whether these increased response levels are specifically directed towards the test odorant, we also ran test trials with a novel odorant.

The procedure used is shown in Table 1. All three groups received five pre-exposure trials with the compound stimulus (*AB*) followed by single test trials to the element *B* and to a novel odorant *C* (pre-test). The sequence of testing was balanced between animals. Groups differed with respect to treatment in the subsequent conditioning phase. The ELEMENT group (*N*=62) received four conditioning trials of

Table 1. *Summary of the design for experiment 1*

Group	*N*	Pre-exposure (five trials)	Pre-test	Conditioning (four trials)	Test
ELEMENT	62	*AB*	*B/C*	*A+*	*B/C*
GENERALIZATION	64	*AB*	*B/C*	*D+*	*B/C*
SENSITIZATION	63	*AB*	*B/C*	+	*B/C*

A, B, C and *D* indicate odorants. *AB* indicates a compound stimulus, and + indicates a sucrose reward.

Results are shown in Fig. 1.

B/C, animals were tested with both *B* and *C*.

Table 2. *Summary of the design for experiment 2*

Group	*N*	Pre-exposure	Pre-test	Conditioning (four trials)	Test
$COMPOUND_5$	62	5×*AB*	*B/C*	*A*+	*B/C*
$COMPOUND_{10}$	48	10×*AB*	*B/C*	*A*+	*B/C*
UNPAIRED	49	5×*A*, 5×*B*	*B/C*	*A*+	*B/C*

A, *B* and *C* indicate odorants. *AB* indicates a compound stimulus, and + indicates a sucrose reward.
Results are shown in Fig. 2.
B/C, animals were tested with both *B* and *C*.

the element *A*, the GENERALIZATION group (*N*=64) received four presentations of odorant *D*, and the SENSITIZATION group (*N*=63) received four presentations of the sucrose reward alone. The conditioning phase was followed by a second test of *B* and *C* (test) in a sequence balanced between animals. The occurrence of sensory preconditioning would be indicated if a specific increase in response levels to *B* was confined to the ELEMENT group.

Experiment 2

The classic explanation for sensory preconditioning is that an association is formed between *A* and *B* during pre-exposure (Rescorla and Freberg, 1978; for overviews, see Hall, 1996; Pearce, 1997). Thus, sensory preconditioning would be predicted not to occur with unpaired presentations of *A* and *B*. Following the learned equivalence argument of Honey (1990), however, unpaired presentation of *A* and *B* should also result in sensory preconditioning. We therefore designed an experiment to compare performance between groups that during pre-exposure either received *A* and *B* simultaneously as a compound or each singly.

The procedures followed are summarized in Table 2. Bees were trained in three groups. During pre-exposure, the UNPAIRED group (*N*=49) received five presentations of each of the stimuli *A* and *B* separately in a pseudo-randomized sequence (*ABBABAABAB*), whereas the $COMPOUND_5$ (*N*=62) group received five simultaneous presentations of *A* and *B* as a compound *AB*. Thus, the $COMPOUND_5$ group is identical to the ELEMENT group of experiment 1. Since both experiments were run largely in parallel, the ELEMENT group from experiment 1 is included and presented again to aid comparison as the $COMPOUND_5$ group. To control for a possible effect of the total number of pre-exposure trials (2×5 in the UNPAIRED and 1×5 in the $COMPOUND_5$ group) and for differences in the total duration of the experiment, we included a group with 10 compound presentations during pre-exposure (the $COMPOUND_{10}$ group; *N*=48).

After pre-exposure, all groups were treated identically: pre-test with *B* and *C*, four conditioning trials with *A* and a test with *B* and *C*.

Experiment 3

If the association of *A* with *B* is of the same sort as are 'regular' associations of stimuli with rewards, a prediction of an associative chain model is that simultaneous presentation of *A* and *B* during pre-exposure is not required for sensory preconditioning. A test presentation of *B* should elicit responses *via* the associations *B*-with-*A*-with-reward. In contrast, with the alternative model, which explains sensory preconditioning *via* the experience-dependent formation of a configural unit, simultaneous presentation of *A* and *B* should be necessary for sensory preconditioning.

Interestingly, a third explanation was recently proposed that predicts an entirely different result. Hall (1996) and Ward-Robinson and Hall (1996) suggested that a reward can be associated with an associatively activated representation (or 'image') of a stimulus (*B*, in our case) (see also Holland, 1990). In the backward condition (*A* precedes *B*), the pre-exposure trials establish associations that allow *A* to evoke a representation of *B*. The point is that this representation will also be evoked during conditioning of *A* and that this representation is eligible to be associated with a reward. Therefore, sensory preconditioning is predicted for backward pairings of *A* and *B* during pre-exposure. Importantly, test responses are proposed to be based on associations of *B* with reward. To test these different predictions, we performed an experiment to compare the effect of a sequential presentation of two odorants (either with forward or with backward pairing) with a simultaneous presentation of *A* and *B* during pre-exposure.

The four groups of this experiment, described in Table 3, differed only in their treatment during the pre-exposure phase. After pre-exposure, treatment was identical in all four groups and corresponds to that of the ELEMENT group of experiment 1. During the 10 trial pre-exposure phase, two of the groups received training with sequential presentations of the elements in which either stimulus *B* immediately preceded stimulus *A*

Table 3. *Summary of the design for experiment 3*

Pooled group	Group	*N*	Pre-exposure (10 trials)	Pre-test	Conditioning (4 trials)	Test
SIMULTANEOUS	$SIMULTANEOUS_{short}$	51	*AB* 4 s	*B/C*	*A*+	*B/C*
	$SIMULTANEOUS_{long}$	45	*AB* 8 s	*B/C*	*A*+	*B/C*
SEQUENTIAL	FORWARD	53	*B–A* (4 s+4 s)	*B/C*	*A*+	*B/C*
	BACKWARD	51	*A–B* (4 s+4 s)	*B/C*	*A*+	*B/C*

A, *B* and *C* indicate odorants. *AB* indicates a compound stimulus, and + indicates a sucrose reward.
Results are shown in Fig. 3.
B/C, animals were tested with both *B* and *C*.

(FORWARD; *N*=53) or *B* immediately followed *A* (BACKWARD; *N*=51). Stimulus duration for each stimulus was 4 s. The two other groups were pre-exposed to 10 simultaneous presentations of *A* and *B*. The two sequential groups received odorant stimulation for a total of 8 s per trial (4 s+4 s), so one of the groups with a compound presentation of *A* and *B* ($SIMULTANEOUS_{short}$; *N*=51) received a compound stimulus of 4 s duration and the other group a compound stimulus of 8 s ($SIMULTANEOUS_{long}$; *N*=45). Thus, the stimulus duration for the $SIMULTANEOUS_{short}$ group was the same as the duration of the stimulus elements *A* and *B* of the two sequential groups, whereas the stimulus duration of the $SIMULTANEOUS_{long}$ group matched the total duration of odorant stimulation.

Experiment 4

The final experiment investigated the effects of repetitive compound presentation on sensory preconditioning. Pearce (1994) proposed that elementary stimuli form a configural unit during their first joint presentation and that these units remain unaltered during subsequent repetitions. This model would therefore predict that sensory preconditioning does not increase in strength with increasing numbers of compound stimulations. The associative chain model, however, holds that associations of *A* and *B* are the basis of sensory preconditioning; therefore, one would predict that repetitive compound presentations would enhance these associations, leading to enhanced sensory preconditioning. To test these different predictions, we investigated the effect of increasing the number of *AB* compound pre-exposure trials on sensory preconditioning.

Treatment of the three groups of this experiment was identical to that of the ELEMENT group of experiment 1, except that one group received a single compound stimulation ($COMPOUND_1$; *N*=33), while a second group received five ($COMPOUND_5$; *N*=31) and a third group 10 ($COMPOUND_{10}$; *N*=30) compound stimulations during the pre-exposure phase (Table 4).

Table 4. *Summary of the design for experiment 4*

Group	*N*	Pre-exposure	Pre-test	Conditioning (four trials)	Test
$COMPOUND_1$	33	1×*AB*	*B/C*	*A*+	*B/C*
$COMPOUND_5$	31	5×*AB*	*B/C*	*A*+	*B/C*
$COMPOUND_{10}$	30	10×*AB*	*B/C*	*A*+	*B/C*

A, *B* and *C* indicate odorants. *AB* indicates a compound stimulus, and + indicates a sucrose reward.

Results are shown in Fig. 4.

B/C, animals were tested with both *B* and *C*.

Results

Experiment 1

As shown in Fig. 1, conditioning of stimulus *A* leads to an increase in the response level to stimulus *B* between pre-test and test for the ELEMENT group (Fig. 1A,B; McNemar χ^2=5.3, *P*<0.05). This effect is dependent on conditioning with one of the elements of *AB*, since conditioning of a novel stimulus *D* or presentation of the reinforcing stimulus alone did not change response levels to *B* between pre-test and test (Fig. 1A,B; GENERALIZATION group, McNemar χ^2=0.3, not significant; SENSITIZATION group, McNemar χ^2=0.3, not significant).

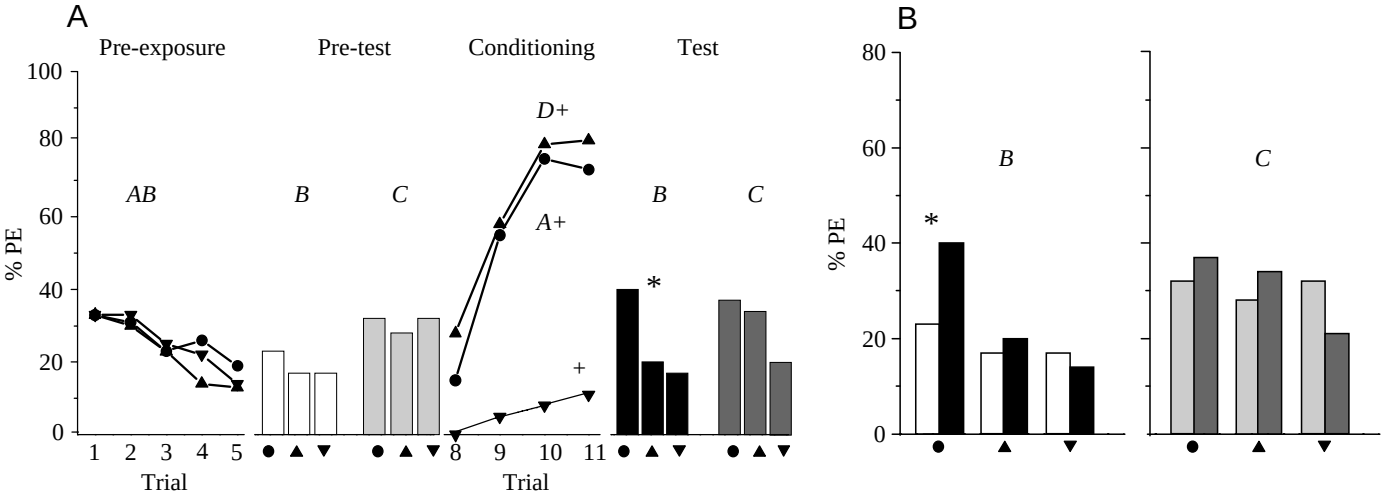

Fig. 1. Percentage of honeybees demonstrating proboscis extension (% PE) in experiment 1 (see Table 1 for the experimental design). (A) An overview of the complete experiment. Groups differ with respect to the conditioning phase: the ELEMENT group (●) (*N*=62) received training with an element of the pre-exposed compound, the GENERALIZATION group (▲) (*N*=64) with an odorant to which it had not been pre-exposed and the SENSITIZATION group (▼) (*N*=63) received trials with reward only. The responses to *B* and *C* were analyzed between groups for pre-test trials and test trials. (B) Same data as in A, but showing within-group comparisons of pre-test (lighter columns) *versus* test (darker columns). In all subsequent figures, only these within-group comparisons will be presented. *A*, *B*, *C* and *D* refer to odorant stimuli, with *C* testing the generalization level. + indicates a sucrose reward. An asterisk denotes a significant difference between the test and pre-test groups (A) or between test and pre-test results within a group (B) (*P*<0.05).

Between-group comparisons reveal a significant difference in the response level to *B* during the test (Fig. 1A,B; H=1180, d.f.=2, P<0.05); this difference cannot be attributed to spurious differences in group composition, because response levels during the pre-test do not differ (Fig. 1A,B; F=700, d.f.=2, not significant). A critical comparison of both olfactory conditioned groups, the ELEMENT and the GENERALIZATION groups, reveals a significant difference (χ^2=6.0, d.f.=1, P<0.05), which is in contradiction to the general effects of odorant learning to component *B*. Thus, an increase in response levels to *B* occurs only in the ELEMENT group and is, therefore, specific for conditioning to *A*.

In contrast to the effect on responses to element *B*, none of the three treatments during the conditioning phase changed the response levels to the novel stimulus *C* between pre-test and test (Fig. 1A,B; ELEMENT, McNemar χ^2=0.8, not significant; GENERALIZATION, McNemar χ^2=1.4, not significant; SENSITIZATION, McNemar χ^2=1.1, not significant). The response levels to stimulus *C* did not differ between groups either during the pre-test (Fig. 1A,B; F=1760, d.f.=2, not significant) or during the test (Fig. 1A,B; F=1770, d.f.=2, not significant) period.

Response levels during the pre-exposure phase did not differ between groups (Fig. 1A; H=0.41, d.f.=2, not significant), arguing against spurious differences in group composition. Since the three experimental groups received an identical pre-exposure treatment, we pooled the data from these groups to analyze whether repeated *AB* stimulation produced a reduction in response levels over the pre-exposure phase. This analysis showed that response levels to *AB* decreased (not shown; N=189, Q=40.04, d.f.=4, P<0.0001). This resulted in a difference between the response levels to *B* and to the novel odorant *C* during the pre-test (not shown; N=189, McNemar χ^2=8.2, P<0.05), but did not give rise to a retardation of acquisition of *A* compared with *D* during the conditioning phase (Fig. 1A; N=128, U=1710; not significant).

Experiment 2

As shown in Fig. 2, conditioning of stimulus *A* led to an increase in the response level to stimulus *B* between the pre-test and test for the $COMPOUND_{10}$ (McNemar χ^2=7.6, P<0.05) and the $COMPOUND_5$ groups (see experiment 1) but not for the UNPAIRED group (McNemar χ^2=0.8, not significant). Between-group comparisons of the response level to *B* yield a significant difference for the test (F=1710, d.f.=2, P<0.05); since neither performance during the pre-test with *B* (F=560, d.f.=2, not significant) nor performance during acquisition of *A* differs between groups (H=4.4, d.f.=2, not significant), spurious differences in group composition cannot account for this effect. In pairwise comparisons, test response levels to *B* in the UNPAIRED group are lower than in the $COMPOUND_5$ (χ^2=5.9, d.f.=1, P<0.05) and the $COMPOUND_{10}$ (χ^2=9.82, d.f.=1, P<0.05) groups, but there is neither a corresponding pre-test difference (UNPAIRED *versus* $COMPOUND_5$ group, χ^2=1.89, d.f.=1, not significant; UNPAIRED *versus* $COMPOUND_{10}$ group, χ^2=2.31, d.f.=1, not significant) nor acquisition difference (UNPAIRED *versus* $COMPOUND_5$ group, U=1181, d.f.=1, not significant; UNPAIRED *versus* $COMPOUND_{10}$ group, U=1132, d.f.=1, not significant). This shows that the absence of an increase in response levels to *B* after unpaired pre-exposure depends on the pairing of *A* and *B* and not on the total number of pre-exposure trials or on the total duration of the experiment.

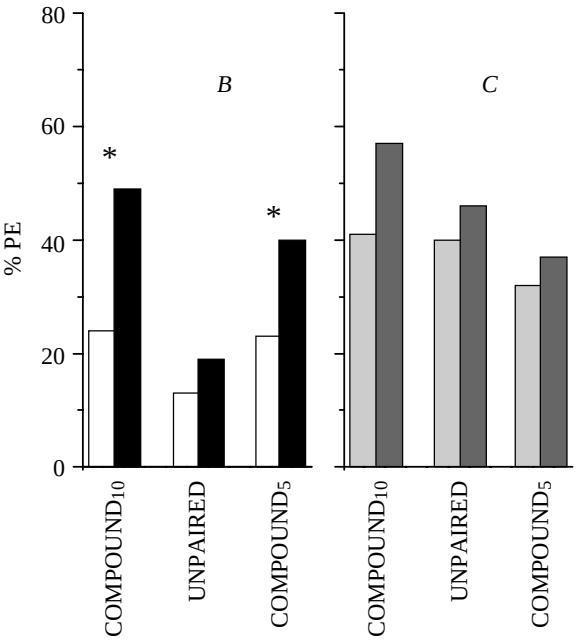

Fig. 2. Percentage of honeybees demonstrating proboscis extension (% PE) in experiment 2 (see Table 2 for the experimental design) showing within-group comparisons of pre-test (lighter columns) *versus* test (darker columns). Groups differ with respect to the pre-exposure phase: the UNPAIRED group (N=49) received unpaired presentations of *A* and *B*, whereas the COMPOUND groups received *A* and *B* as a simultaneous compound on five ($COMPOUND_5$, N=62) or 10 ($COMPOUND_{10}$, N=48) occasions. *B* and *C* refer to odorant stimuli, with *C* testing the level of generalization. An asterisk denotes a significant difference between test and pre-test results for the same group (P<0.05).

Between pre-test and test exposures, there was no change in the response levels to the novel stimulus *C* for any of the groups ($COMPOUND_5$, see experiment 1; $COMPOUND_{10}$, McNemar χ^2=3.5, not significant; UNPAIRED, McNemar χ^2=2.3, not significant). In addition, response levels to *C* did not differ between groups in either pre-test (F=1710, d.f.=2, not significant) or test (F=2090, d.f.=2, not significant). Thus, this experiment demonstrates sensory preconditioning for both COMPOUND groups but not for the UNPAIRED group.

Experiment 3

As shown in Fig. 3A, no increase in response levels for stimulus *B* between pre-test and test occurred for either group in which *A* and *B* were presented sequentially (FORWARD, McNemar χ^2=1.1, not significant; BACKWARD, McNemar χ^2=0.6, not significant). Moreover, the sequence of *A* and *B* did not yield a significant difference between the FORWARD

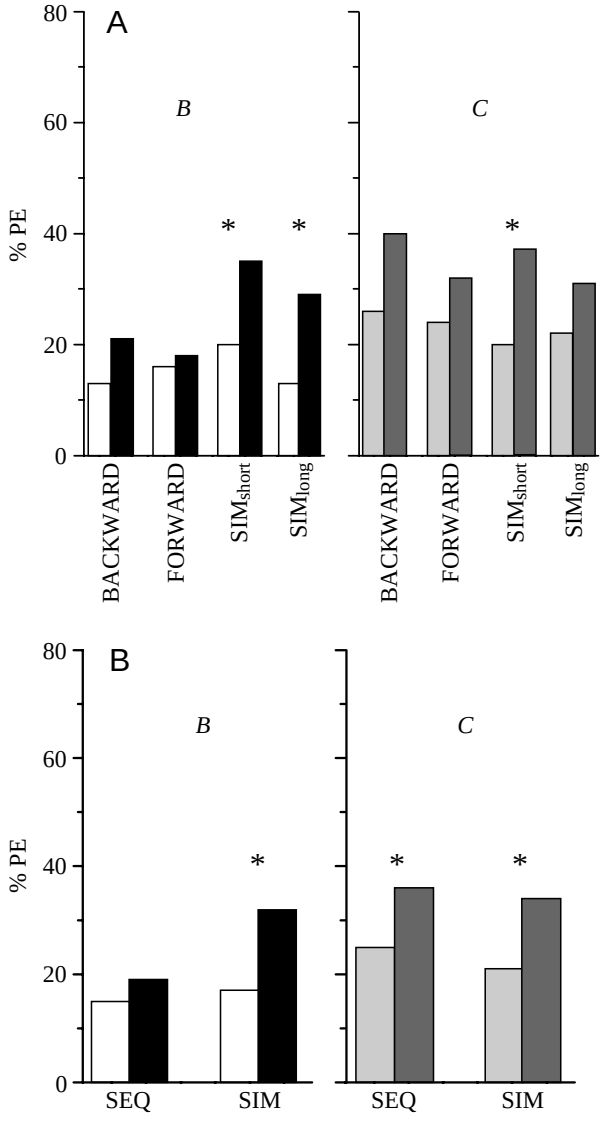

Fig. 3. Percentage of honeybees demonstrating proboscis extension (% PE) in experiment 3 (see Table 3 for the experimental design), showing within-group comparisons of pre-test (lighter columns) *versus* test (darker columns). (A) Groups differ with respect to the pre-exposure phase: the FORWARD (N=53) and BACKWARD (N=51) groups received sequential presentations of *A* and *B* (*B–A* and *A–B*, respectively), whereas the SIMULTANEOUS groups received *A* and *B* as a simultaneous compound (for 4 s, SIM_{short}, N=51, or 8 s, SIM_{long}, N=45). (B) Results pooled for both SIMULTANEOUS (SIM, N=96) and both SEQUENTIAL (SEQ, N=104) groups. *B* and *C* refer to odorant stimuli; *C* tests the level of generalization. An asterisk denotes a significant difference between test and pre-test results for the same group (P<0.05).

and BACKWARD groups for the responses during the pre-exposure phase (U=1114, d.f.=1, not significant), the pre-test with *B* (χ^2=0.2, d.f.=1, not significant) or with *C* (χ^2=0.4, d.f.=1, not significant) or the acquisition of *A* during conditioning (U=1151, d.f.=1, not significant). However, test responses to *B* (χ^2=0.1, d.f.=1, not significant) and *C* (χ^2=0.4, d.f.=1, not significant) were indistinguishable in the two groups. Since neither of these between-group comparisons yielded significant differences, data from the FORWARD and the BACKWARD groups were pooled and are subsequently referred to as the SEQUENTIAL group. Thus, the pattern of results shows that sequential presentation of two odorants does not result in sensory preconditioning.

In contrast, response levels to *B* did increase significantly for both groups that received a simultaneous presentation of *A* and *B* during pre-exposure ($SIMULTANEOUS_{short}$, McNemar χ^2=4.8, P<0.05; $SIMULTANEOUS_{long}$, McNemar χ^2=4.0, P<0.05). A between-group comparison of the $SIMULTANEOUS_{short}$ and the $SIMULTANEOUS_{long}$ groups does not reveal a significant difference during pre-exposure (U=1089, d.f.=1, not significant), pre-test for *B* (χ^2=0.68, d.f.=1, not significant), pre-test for *C* (χ^2=0.1, d.f.=1, not significant) or acquisition of *A* (U=1046, d.f.=1, not significant). In addition, test responses to *B* (χ^2=0.83, d.f.=1, not significant) and *C* (χ^2=0.4, d.f.=1, not significant) were indistinguishable in the two groups. The two groups were therefore pooled and are subsequently referred to as the SIMULTANEOUS group. Thus, the pattern of results shows that simultaneous presentation of two odorants does result in sensory preconditioning.

Comparing the pooled results, response levels to *B* increase in the SIMULTANEOUS group (Fig. 3B; McNemar χ^2=4.35, P<0.05) but not in the SEQUENTIAL group (McNemar χ^2=1.07, not significant). In a between-groups comparison, this leads to higher response levels to *B* in the SIMULTANEOUS group than in the SEQUENTIAL group during the test (χ^2=4.14, d.f.=1, P<0.05) that were not present during the pre-test (χ^2=0.03, d.f.=1, not significant). Correspondingly, acquisition of *A* did not differ between groups (U=4883, d.f.=1, not significant). To summarise, sensory preconditioning occurs for simultaneous but not for sequential presentations of *A* and *B*.

In the case of the novel odorant *C*, neither the SIMULTANEOUS nor the SEQUENTIAL groups differ in either the pre-test (χ^2=0.54, d.f.=1; not significant) or the test (χ^2=0.05, d.f.=1; not significant). Unlike experiments 1 and 2, however, we found increases in response levels to *C* in both groups (SIMULTANEOUS, McNemar χ^2=6.3, P<0.05; SEQUENTIAL, McNemar χ^2=4.35, P<0.05). Thus, generalization appears to contribute to the test response to the novel stimulus *C* irrespective of pre-exposure treatment and also might, partially, have influenced the responses to *B*. However, generalization is not sufficient to increase the response to *B* after sequential or unpaired (experiment 2) presentation of *A* and *B*, or after conditioning of the novel odorant *D* (experiment 1). Thus, generalization alone is not sufficient to account for sensory preconditioning.

Experiment 4

A single pre-exposure of the *AB* compound resulted in a significant increase in the response level to *B* between pre-test and test (Fig. 4; $COMPOUND_1$, McNemar χ^2=8.7, P<0.05). For the groups with multiple compound presentations, this trend did not reach significance ($COMPOUND_5$, McNemar χ^2=3.2, not significant; $COMPOUND_{10}$, McNemar χ^2=1.2). For none of

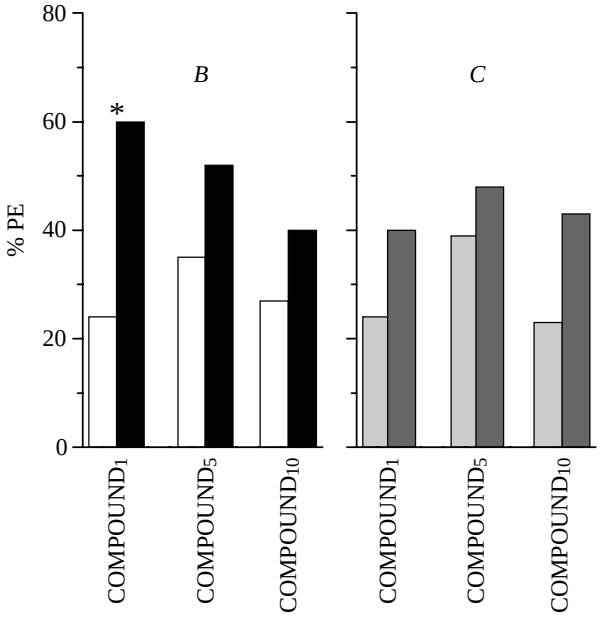

Fig. 4. Percentage of honeybees demonstrating proboscis extension (% PE) in experiment 4 (see Table 4 for the experimental design) showing within-group comparisons of pre-test (lighter columns) *versus* test (darker columns). Groups differ with respect to the pre-exposure phase: they received one, five or 10 presentations of *A* and *B* as a simultaneous compound ($COMPOUND_1$, N=33; $COMPOUND_5$, N=31; $COMPOUND_{10}$, N=30). *B* and *C* refer to odorant stimuli; *C* tests the level of generalization. An asterisk denotes a significant difference between test and pre-test results for the same group (P<0.05).

these groups did response levels to the novel stimulus *C* increase between pre-test and test ($COMPOUND_1$, McNemar χ^2=1.5, not significant; $COMPOUND_5$, McNemar χ^2=1.0, not significant; $COMPOUND_{10}$, McNemar χ^2=1.9, not significant), suggesting that the increase in response to *B* in the $COMPOUND_1$ group cannot be attributed to generalization alone.

The increase in test response levels to *B* in the $COMPOUND_1$ group is, however, insufficient to produce a significant difference between groups during the test (F=1081, d.f.=2, not significant), and the test response level to *C* also fails to show a between-group difference (F=1043, d.f.=2, not significant). Correspondingly, pre-test response levels to neither *B* (F=682, d.f.=2, not significant) nor *C* (F=653, d.f.=2, not significant) differ between groups. Finally, no between-group difference in the acquisition of *A* could be detected (H=0.2, d.f.=2, not significant).

Discussion

Experiment 1 clearly indicates that sensory preconditioning occurs: when honeybees are exposed to a binary odorant compound (*AB*) and are then trained to one of the elements (*A*), response levels increase for the other element (*B*). The absence of increased response levels to *B* in the GENERALIZATION and SENSITIZATION groups indicates that conditioning of element *A* of an *AB* compound is essential for sensory preconditioning. Similarly, conditioning of *A* increases responses only to the odorant (*B*) that has been presented with *A* during the pre-exposure phase, since no incremental effect was observed for a novel odorant.

One possible explanation for the increased response levels to the element *B* could be generalization from *A* to *B* (Smith and Menzel, 1989). To explain our results, however, such a generalization mechanism would have to assume that odorant *B* is more similar to *A* than to *D*, since only conditioning of *A*, but not of *D*, increased responses to *B*. This seems unlikely, given that the design of our experiment was completely balanced for the experimental role of the four odorants used. All 24 possible combinations of experimental roles as *A*, *B*, *C* or *D* for the four chemical substances were used and, therefore, asymmetries in generalization from *B* to *A versus B* to *D* should not have occurred.

A second explanation could be the occurrence of habituation during the pre-exposure phase and the fact that, during the test phase, the response level to *C* is as high as that to *B*. The increase in the response to *B* could reflect dishabituation. A non-specific dishabituation can be excluded for the same reasons as can generalization. Since only the conditioning of *A* would provoke such dishabituation, any association between *A* and *B* must contribute to such a specific effect. However, since, after the association between *A* and *B*, the conditioning of one component leads to dishabituation specific for the other component, this mechanism could explain sensory preconditioning. This could also be applied to many sensory preconditioning experiments reported previously, although no habituation effect was detected during the pre-exposure phase. Since such an explanation would not rule out learning between neutral stimuli, this hypothesis is almost identical to any other explanation for sensory preconditioning and would conform to the definition of sensory preconditioning. The findings reported here may not contribute essential aspects to an explanation of preconditioning, but they could explain why most sensory preconditioning effects are much weaker than conditioning effects.

Control groups in all four experiments have ruled out some effects of generalization or dishabituation as explanations for the observed sensory preconditioning effect. However, as in experiments 1 and 2, some generalization to stimulus *C* did occur in experiment 3. This is, by itself, not surprising, since some generalization is regularly observed after olfactory proboscis extension conditioning (Bitterman et al., 1983; Menzel, 1990; Smith and Menzel, 1989). Experiment 3 differs from experiments 1 and 2 in that generalization levels increased between the first and the second test and, thus, generalization may contribute to (but cannot by itself account completely for) sensory preconditioning in this experiment. Generalization and spontaneous response levels can depend on the olfactory experience of the bees prior to experiments, e.g. during foraging (Gerber et al., 1996), and changes in experience over the seasons may well lead to differences in generalization and spontaneous responses. One reason for the variable role of generalization in sensory preconditioning in this compared with our previous experiments could, therefore,

be different rearing conditions. Whereas the bees used for experiment 3 (and experiment 4) were kept in an indoor flight room, those used in experiments 1 and 2 were caught from a hive maintained outdoors. Regardless of these changing levels of generalization, taken together with the finding that the animals in the preconditioning groups increased their responses to component *B* significantly more strongly than to novel odorant *C*, three additional points suggest that the observed effects are stimulus-specific and that generalization alone cannot account for sensory preconditioning. First, sensory preconditioning occurred in experiments 1, 2 and 4 (see below) without any increase in generalization. Second, experiment 1 showed that training with a novel odorant *D* instead of odorant *A* did not result in sensory preconditioning. Third, in the SEQUENTIAL groups of experiment 3, training of *A* leads to response increments to *C* but not to *B*.

Thus, in the SEQUENTIAL groups, training of *A* leads to an increased response to 'every' odorant, but not to *B*. This suggests that in the SEQUENTIAL groups *B* had been habituated more strongly. Direct evidence for such differences in habituation in experiment 3 would be as follows: (i) if pre-test response levels to *B* were lower in the SEQUENTIAL than in the SIMULTANEOUS group and (ii) if acquisition of *A* (*A* and *B* should be habituated to the same extent during pre-exposure) were delayed relative to the SIMULTANEOUS groups. Neither comparison, however, was significant (see above). Similarly, experiment 2 does not provide direct evidence for stronger habituation for unpaired *versus* paired presentations of *A* and *B*. Response levels to *B* in the pre-test are statistically indistinguishable for the UNPAIRED *versus* the $COMPOUND_5$ or $COMPOUND_{10}$ groups, and acquisition of *A* also does not differ. Thus, there is no direct evidence for a habituation effect that would be stronger for unpaired or sequential presentations compared with simultaneous presentations. However, in all cases, sensory preconditioning is combined with a generalized increase in responses to untrained odours, and this is most clearly seen in experiment 3, in which there was a significant increase in the response between pre-test and test for the control stimulus *C*.

To evaluate the extent to which generalization contributes to the increased responses in the test or whether this procedure yields sensory preconditioning, it is necessary to compare the non-specific increase in the response to the novel odorant *C* directly with the increase in the response to component *B*. All animals in the study that received the compound in the preconditioning phase and an element *A* in the conditioning phase were pooled for an overall test (in experiments 1–4). These animals responded significantly more often specifically to component *B* (44 bees) than to the novel odorant *C* (25 bees) (McNemar $\chi^2=4.7$, $P<0.05$, $N=301$). The increase in response levels is specifically directed to *B* and does not occur to the same extent for any novel odorant, such as *C*. Thus, the sensory preconditioning effect is mediated by an experience-dependent step during compound processing.

One explanation for such an effect was proposed by Honey (1990). In the pre-exposure phase, both *A* and *B* signal the absence of reward. This redundancy could lead to learned equivalence (Hall, 1996), so that *A* and *B* would, so to speak, be actively taken for the same thing (i.e. extinction had occurred). Therefore, habituation that has occurred during *AB* pre-exposure would be countered by subsequent acquisition of *A* and, because of learned equivalence, this recovery from extinction would also be effective for *B*. Thus, response levels to *B* that have been lowered below generalization level in the pre-test would be restored in the test. Unlike the generalization hypothesis outlined above, this would incorporate an active learning process taking place during pre-exposure.

Interestingly, the model of Honey (1990) would predict that an unpaired presentation of *A* and *B* during pre-exposure would be sufficient to support sensory preconditioning: *A* and *B* are redundant irrespective of whether they occur in a paired or unpaired way. Experiment 2 was designed to address this issue. The major finding of experiment 2 is that sensory preconditioning occurs when the two stimuli *A* and *B* are presented in a compound stimulus *AB*, but not when they are presented in an unpaired way. An explanation of sensory preconditioning *via* learned equivalence, however, would predict that sensory preconditioning would also have occurred with unpaired presentations. Thus, learned equivalence does not seem to be an adequate explanation of sensory preconditioning, and sensory preconditioning appears to require that the elementary stimuli are experienced within a compound.

Thus, it is clear that sensory preconditioning does occur and is experience-dependent. Specifically, the occurrence of sensory preconditioning requires the joint presentation of *A* and *B* in a compound. The mechanisms underlying sensory preconditioning, however, have yet to be addressed. As noted in the Introduction, the joint presentation of the two odorants could result in an experience-dependent formation of a compound-specific representation (a single unit, Rescorla, 1980; a configural cue, Rudy and Sutherland, 1992, or a configural unit, Pearce, 1994) or, alternatively, sensory preconditioning could be mediated through a chain-like association.

The associative chain hypothesis

The 'standard' interpretation of sensory preconditioning involves an associative chain (Rescorla and Freberg, 1978; for overviews, see Hall, 1996; Pearce, 1997). It is suggested that two associations are involved: one between *A* and *B* established during compound pre-exposure and one between *A* and reward established during the second experimental phase. Test responses to *B* are thus likely to be based on chain-like associations of *B*-with-A-with-reward. This model stresses the importance of predictive relationship among stimuli, arguing that, during tests, *B*-predicts-A-predicts-reward. Therefore, *B–A* training should be optimal to equip *B* with the capacity to predict *A* (Rescorla, 1980) and should, therefore, support sensory preconditioning *via* an associative chain, assuming that the *B–A* association includes the typical predictive asymmetries known from stimulus–reward associations (which leads to excitatory learning for forward pairings and inhibitory

learning for backward pairings; Hellstern et al., 1998; Plotkin and Oakely, 1975).

Experiment 3 does not support an interpretation of sensory preconditioning *via* an associative chain. Sequential pre-exposure is not effective in supporting sensory preconditioning, and the sequence of presentation of *A* and *B* (either forward, *B* precedes A; or backward, *A* precedes B) does not influence sensory preconditioning (Fig. 3A). However, it is possible that either forward-sequential or backward-sequential presentations might also yield sensory preconditioning if shorter inter-stimulus intervals were used and, in particular, if there was some overlap of the odorants tested. That is, the stimulus traces of the odorants that are to be linked (into an association, an image or a configural unit) might dissipate so quickly that they are already gone upon onset of the second odorant. Since we used sequential odorants with presentation of the second odorant programmed to start immediately upon offset of the first, it would have to be assumed that odorant traces do not last much longer than 1 s after stimulation offset. This is unlikely because single-trial trace conditioning with a 3 s trace between odorant offset and reward is as effective as overlapping presentation with multiple trials. Even traces of 10 s (where the unconditioned stimulus starts 8 s after the conditioned stimulus offset) support response levels of approximately 50–60 % (Menzel et al., 1993). This clearly shows that an odorant trace can last up to 10 s and that this trace is sufficient for associations, at least for reward associations, to be formed. It should be mentioned that we did not use the overlap in the conditioning trials to optimise the conditioning. However, since most experiments with honeybees have used overlapping procedures for the conditioning trials, we also used it here to allow comparison of our results with other studies.

If sensory preconditioning in our case is mediated by an associative chain mechanism, this odorant trace could have supported sensory preconditioning in the forward sequential group. However, the stronger effect in the compound groups could be based on the temporal relationship between *A* and *B* and between *A* and the unconditioned stimulus (Cole et al., 1995). In that case, simultaneous *AB* training places *B* in a predictive relationship to the unconditioned stimulus and should, therefore, produce the best sensory preconditioning according to temporal coding hypothesis. In contrast, *A*-before-*B* training places *B* in a simultaneous relationship with the unconditioned stimulus; this should not support a strong conditioned response to *B*, whereas *B*-before-*A* training establishes a trace relationship, in our case a gap of 3 s, between the conditioned stimulus and the expected unconditioned stimulus. Even though trace conditioning with such a gap would be nearly as effective as overlap conditioning, in this advanced learning of a temporal relationship the procedure could fail to produce sensory preconditioning. This could be the reason why animals show the best sensory preconditioning after compound experience in the pre-exposure phase, even though they might connect *A* and *B* by an associative chain mechanism.

The pattern of results does not support an explanation of sensory preconditioning by associatively activated stimulus representations (Hall, 1996), which would have predicted sensory preconditioning also occurring for backward pairings of *A* and *B* during pre-exposure. Such backward pairings do not support sensory preconditioning in our case (Fig. 3A).

Although the vertebrate literature does offer evidence for backward sensory preconditioning (Ward-Robinson and Hall, 1996), the strongest effects were usually seen with simultaneously applied compounds (Lyn and Capaldi, 1994; Lavin, 1976; Rescorla, 1980). In any case, we argue that the critical point is whether sensory preconditioning is stronger in backward than in simultaneous or forward presentations. As far as we know, there is no evidence for such a pattern of results in the vertebrate literature. In addition, until now, the experiments are consistent with an associative chain account, including a precise learned timing of the stimuli *via* a temporal encoding chain (Cole et al., 1995).

The fourth experiment was designed to test the associative chain hypothesis against a hypothesis involving configural units; specifically, it investigated the effects of repeated compound exposures on sensory preconditioning. The results of experiment 4 show that a single presentation of a binary odorant compound is sufficient to produce sensory preconditioning. A trend towards sensory preconditioning was also observed for the five and 10 compound presentation groups; the fact that sensory preconditioning was not significant in those groups, however, suggests that, at the very least, sensory preconditioning does not strengthen with repeated compound presentations. Associations, however, typically strengthen with repetition. Thus, an association of *A* with *B* may not completely account for sensory preconditioning.

A longer pre-exposure phase could, however, lead to stronger habituation of the compound and the elements. If spontaneous response levels reflect the olfactory experience of the bees prior to experiments (Gerber et al., 1996), the pre-exposure phase could lead to extinction. An absence of sensory preconditioning with multiple compound presentations could be due to incomplete recovery from extinction or incomplete dishabituation. Two points indicate that, although such recovery from extinction or habituation may contribute to our results, it cannot completely account for the lack of the sensory preconditioning effect in those groups. First, response levels to *B* in the pre-test are not different between groups, arguing that the same amount of extinction accrued to *B* in all groups. Second, there is no retardation of acquisition during training of *A* (*A* and *B* should have equal associative strength after pre-exposure). Thus, there is no direct evidence for between-group differences in extinction.

An explanation for sensory preconditioning weakening with increasing compound presentations should not, therefore, include associative chain events and it should also avoid implementing different levels of extinction.

Our pattern of results is, in general, similar to that found in vertebrates because sensory preconditioning is usually stronger for simultaneous than for sequential presentations of the elements (Lyn and Capaldi, 1994; Lavin, 1976; Rescorla, 1980; but see Hoffeld et al., 1958; Wynne and Brogden, 1962).

Furthermore, no acquisition of the sensory preconditioning effect with an increasing number of compound presentations (Hall and Subowski, 1995; Hoffeld et al., 1960; for an early review, see Seidel, 1959) or only minor acquisition (Prewitt, 1967; Tait et al., 1972) was reported.

The configural unit hypothesis

It has become increasingly clear that the processing of compound stimuli might be considerably more complex than the processing of the individual elements of which it is formed (Holland, 1993; Pearce, 1994; Rescorla and Durlach, 1981; Rudy and Sutherland, 1992). To explain how compound stimuli may have properties that cannot be explained on the basis of the properties of each individual element, it has been suggested that exposure to a compound stimulus initiates a learning process leading to the establishment of a compound-specific single unit (Rescorla, 1980), a configural cue (Rudy and Sutherland, 1992) or a configural unit (Pearce, 1994). Here, we outline how such a model could explain sensory preconditioning in honeybee proboscis extension conditioning.

Exposure to a compound *AB* leads to a learning process that establishes a configural unit *AB*. The elements of this configural unit can, *via* their similarity with *AB*, also activate the memory of *AB* to some extent. Consequently, conditioning with one of the elements, e.g. *A*, would increase not only the associative strength of *A* but also that of the configural unit *AB*. Thus, the configural unit would also be associated with reinforcement. The important point is that, during the test, the other element *B* can, because of its similarity to *AB*, also activate the configural unit *AB*. Since *AB* is associated with reward, animals will respond. Such a model provides stimulus specificity for sensory preconditioning by proposing that *A* is more like *AB* than is a novel stimulus *D*. Therefore, *A* has a stronger capacity to elicit *AB* than has *D*.

In the model of Pearce (1994), the temporal requirements for *A* and *B* to form a configural unit are not specified. What is obvious, however, is that the model was devised for simultaneous exposures to compounds. Furthermore, the formation of the configural unit obviously does not involve an associative mechanism that includes the predictive asymmetries of stimulus–reward associations (see above). Thus, we propose that the formation of a configural unit within the model of Pearce (1994) follows a coincidence rule, so that stimuli that occur together are bound together. If their occurrence is offset in either temporal direction, no configural unit (or a weaker one) will be formed. With this added assumption, the model can explain why sensory preconditioning occurs with simultaneous but not with sequential (experiment 3) or with unpaired (experiment 2) presentations of *A* and *B*.

What remains to be explained is the counterintuitive fact that sensory preconditioning does not become stronger (acquisition) with repeated compound presentations (Fig. 4). Interestingly, within the vertebrate literature, most researchers have also found no acquisition (Hall and Subowski, 1995; Hoffeld et al., 1960; for an early review, see Seidel, 1959) or weak acquisition (Prewitt, 1967; Tait et al., 1972) for sensory preconditioning. According to Pearce (1994), configural units are formed during a single trial and the capacity of their elements to activate them then remains unaltered. Thus, this model can account perfectly for the general pattern of results.

An explanation of our results, however, is complicated by the fact that sensory preconditioning tended to be weaker with repeated presentations of the compound (Fig. 4), a result that cannot be explained by either of the models mentioned above. It is unlikely that different levels of extinction (or latent inhibition) established during pre-exposure can account for this effect. Instead, being able to detect the elements within a compound might require learning and repetition. However, this dissecting process would be in contrast to the finding that compound repetition decreases generalization to the elements (Bellingham and Gillette, 1981).

Sensory preconditioning bears some similarities to the perceptual ‘priming’ described in human experiments (Boller, 1997; Schachter, 1995). The general picture emerging for sensory preconditioning in honeybees is remarkably similar to what has been reported for vertebrates; sensory preconditioning is strongest for simultaneous compounds, it is a single-trial effect and it does not strengthen with repetition (Moscovitch, 1995).

Thus, the surprise expressed by Tulving (1995) about the absence of reports of perceptual priming in animal studies, despite the seemingly obvious fundamental biological significance of the effect, might reflect a terminological rather than a scientific problem.

Sensory preconditioning and olfactory processing

Our results suggest that the processing of binary odorant compounds involves the rapid, experience-dependent formation of a configural representation. This agrees with the standard hypothesis of olfactory compound processing which holds that odorant compounds are largely perceived in a unique configural way (Laurent, 1996; Laing and Francis, 1989). Our study demonstrates the importance of learning and, in particular, the role of single odorant exposures for the formation of these unique perceptions.

However, the idea of unique perceptions of compounds is not unchallenged. In particular, for proboscis extension conditioning in honeybees, ‘blocking’ (Kamin, 1968) was found for odorants in binary compounds (Smith and Cobey, 1994; Smith, 1996, 1997; Thorn and Smith, 1997) and, similarly, Couvillon et al. (1997) have also reported blocking for olfactory compounds in a dual-choice paradigm with freely flying honeybees. Furthermore, Sahley et al. (1981) have reported olfactory blocking in the terrestrial slug *Limax maximus*. Since standard interpretations of blocking commonly assume individual processing of the elements of a compound (Mackintosh, 1975; Pearce and Hall, 1980; Rescorla and Wagner; 1972; Sutton and Barto, 1981), a role for elementary processing of odorant compounds was proposed (Sahley et al., 1981; Smith and Cobey, 1994; Smith, 1996, 1997; Thorn and Smith, 1997). Configural theories (Pearce, 1994) also predict

'blocking', however, and 'blocking' cannot therefore be used as an argument to discriminate between configural *versus* elementary accounts of compound processing.

The question remains whether there are experimental results that can be explained only if compounds are processed as elements. As Rescorla (1997) has argued, one such result would be summation (see, however, Pearce, 1997), in which separate training to the elements *A* and *B* leads to higher response levels to the compound *AB* than in controls (for control procedures, see Rescorla, 1997). In proboscis extension conditioning, Hellstern et al. (1998) demonstrated summation of excitatory learning after forward pairings of odorant and reward with inhibitory learning after backward pairings. Thus, the idea that the elements of an odorant compound might to some extent be processed as elements seems reasonable in honeybees.

It is possible that elementary and configural compound processing are not mutually exclusive. In spiny lobsters (*Panulirus argus*), certain forms of training enhance the ability to recognize compound elements, although compounds are normally perceived configurally (Livermore et al., 1997). Whether animals are able to detect and process the elements of a compound or whether compounds are processed configurally might depend on the specific learning task. The issue at hand would, therefore, be to investigate how animals are able to switch between these two processing modes.

The quest for the neuronal substrate

Neuronal plasticity at the level of the antennal lobes, which are the functional analogues of the olfactory bulb in vertebrates, is likely to be involved in olfactory proboscis extension conditioning (Erber, 1981; Faber et al., 1999; Sigg et al., 1997) and might also contribute to learning processes such as sensory preconditioning. This would mean that the envisaged experience-dependent configuration process leading to sensory preconditioning affects stimulus perception and thus is acting at a rather peripheral processing step. This would correspond to priming in humans, in that priming is also regarded as a more perceptual phenomenon (e.g. Hamann and Squire, 1997). In the honeybee, however, Joerges et al. (1997) have suggested for the antennal lobes that binary compounds might be processed mainly as the arithmetic sum of the elementary patterns of activity, with some inhibitory and synergistic effects. Thus, for binary compounds such as we have used, the antennal lobe might primarily use an elementary code, while other parts of the brain might be involved in configural learning for such binary compounds.

From the antennal lobes, olfactory information is conveyed to the lateral protocerebrum, a premotor centre, and the mushroom bodies, a higher brain centre involved in olfactory learning in bees (for reviews, see Hammer, 1997; Menzel et al., 1994; Menzel and Müller, 1996) and fruitflies (*Drosophila melanogaster*) (DeBelle and Heisenberg, 1994). The mushroom bodies have repeatedly been suggested as sites for configural learning. Such a central site for sensory preconditioning would correspond to the hippocampus-dependence of configural learning in rats (Rudy and Sutherland, 1992, 1995). In addition, sensory preconditioning in invertebrates would also correspond to the specificity of learning for neutral stimuli in rats (Young et al., 1998), in particular to the occurrence of sensory preconditioning by impaired reward-processing systems (Nader and LeDoux, 1999).

Hypotheses about the contributions of the antennal lobes and the mushroom bodies to sensory preconditioning are readily testable in honeybees and are in progress. Intracellular recordings from antennal lobe interneurons and from olfactory projection neurons (Homberg, 1984; Sun et al., 1993; Fonta et al., 1993) can be obtained *in vivo* and, potentially, during sensory preconditioning. In addition, the activity of neuronal populations can be measured *in vivo* using optical imaging techniques (Galizia et al., 1999; Joerges et al., 1997). A technique to ablate the mushroom bodies during development (DeBelle and Heisenberg, 1994) has recently been applied to honeybees (Malun, 1998), and this will enable a study to be performed of whether honeybees lacking mushroom bodies show specific losses of configural learning ability.

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