

Chapter III

The influence of training length on visual categorization in honeybees

Abstract

Generalization and categorization are fundamental abilities which allow treating similar stimuli as equivalents, and thus responding to them in the same manner. Typically a categorization experiment involves a discrimination in which reward is not signalled by a single stimulus, but rather by a variety of stimuli that share a common feature. Recently it was shown that honeybees could categorize visual patterns on the basis of a common configuration of orientations ('configural categorization') after training to a randomised sequence pattern pairs, each group containing a different spatial arrangement of four oriented edges (Stach & Giurfa, 2003, Chapter II). In this kind of procedure, animals are explicitly trained to perform in a given way, defined *a priori* by the experimenter. Here we asked whether configural stimulus representations are a specific consequence of a given training method or can also arise under simpler training forms. In asking this question we analysed the role of the cumulative experience in the visual recognition strategies used by the bees. Contrarily to training with a changing sequence of patterns sharing a common feature, which has to be extracted to solve the task, training with a single, constant pair of patterns does not impose explicitly the extraction of a specific feature as any available cue can be used to discriminate between patterns. Here we show, that after training to a single pair of patterns categorization of novel stimuli can also be based on the extraction of four different edge orientations arranged in a specific spatial relationship. In a differential procedure, bees do not only learn the orientation configuration of the rewarded but also the one of the non rewarded stimulus. Furthermore we demonstrate that different levels of experience with one pair of training stimuli determine important changes in the discrimination strategies employed by the bees towards a higher level of generalization with increasing training length.

Introduction

Honeybees foragers *Apis mellifera* are ‘floral constant’ (Grant, 1951), i. e. they visit repeatedly flowers belonging to the same species as long as this species provides profitable reward of nectar and/or pollen (Chittka et al., 1999; Menzel, 1985; Waser, 1986). Recognition of the flower species currently exploited is therefore crucial for a bee forager in order to implement an efficient foraging behaviour. Recognition implies comparison between the actual perception of a flower and a memorized representation. Such an exercise is possible in bees as they exhibit remarkable learning and memory capabilities (Giurfa, 2003; Menzel & Giurfa, 2001; Menzel, 1999; 2001), which allow them to remember the specific properties of flowers belonging to the same species, such as location, odor, colour, shape and pattern (Menzel, 1985; Menzel et al., 1993). Recognition operates when the bee is approaching a flower during its foraging flight. In such an approach flight, and depending on the visual range to the target, different cues may activate different representations and trigger different responses (Giurfa & Menzel, 1997; Giurfa & Lehrer, 2001). Depending on the similarity between the perceived and the stored information the bee will classify a flower as similar or not.

Visual input is crucial in assessing flower similarity as visual cues drive the choice behaviour of bees throughout a large range of distances (Giurfa & Menzel, 1997; Giurfa & Lehrer, 2001). They are responsible for the very first detection of a distant flower and guide the bee until it lands on the corolla (Giurfa & Lehrer, 2001). At each point of the approach flight, recognition has to be flexible enough to resist eventual modifications of the image. In other words, a bee should be able to recognize the flower exploited despite its different orientation in space, or despite distortions in shape introduced by wind, approach direction, occlusion by leaves, etc. Thus although flower constancy requires improved discrimination abilities, it also requires the capability of generalizing stored information to

similar one. The basic principle of generalization is that animals having learned to respond to the presence of one stimulus will also respond in the presence of similar stimuli (Spence, 1937; Ghirlanda & Enquist, 2003). Generalization implies the evaluation of present perceptual input with respect to its similarity to past experience. Similarity can be measured along one or multiple dimensions (Shephard, 1987; Ghirlanda & Enquist, 2003). Honeybees can generalize their choices of trained visual stimuli to similar, non-trained ones (Stach & Giurfa, 2001). Thus, they can cope with eventual variations of the images searched while foraging and ensure an efficient foraging behaviour. A further instance in stimulus generalization is categorization. Categorization is a fundamental cognitive ability that allows treating similar stimuli as equivalent, and thus responding to them in the same manner. In categorization, similarly to generalization, stimuli are evaluated with respect to their similarity to one or multiple stimuli, but additionally, they are explicitly classified as members or non-members of a given group defined on the basis of previous experience. Thus, categorization assumes grouping of stimuli based on certain criteria, a task that is not necessarily at the core of basic generalization phenomena in which response to similar stimuli may not imply ordering them as members of a given group. Typically, a categorization experiment involves a discrimination in which reward is not signalled by a single stimulus, but rather by a variety of stimuli that share some common feature. Animals should be able to extract such a feature and to transfer their choice to novel instances in which the critical feature is present. These conditions are fulfilled by recent experiments performed on visual discrimination learning in honeybees. In these experiments, bees were shown to categorize visual stimuli on the exclusive basis of bilateral (Giurfa et al., 1996) and radial (Horridge, 2000; Horridge & Zhang, 1995) symmetry, edge orientation (Srinivasan et al., 1993; 1994), and pattern disruption (Hertz, 1933; Horridge, 1997a). In other words, bees trained with sets of stimuli sharing a specific feature (i. e. bilateral

symmetric vs. asymmetric, or vertical vs. horizontal edge orientation) preferred the corresponding novel stimuli in transfer tests.

Such a single-feature categorization can be based on relatively simple neural mechanisms. For example, Srinivasan et al. (1994) developed a functional model for the detection of edge orientation to account for different experiments on orientation discrimination in bees. The model relied on the existence of three basic orientation detectors in the bee brain. The neuronal correlates for these orientation detectors were found by Yang and Maddess (1997) in the visual neuropiles of the bee brain. Such detectors encode the global orientation of a pattern by means of their neural activity. Recognition of a given edge orientation in a trained or in a novel pattern simply depends, therefore, on the appropriate activation of these detectors. In a categorization task, bees will classify the novel stimuli according to the activation pattern of orientation detectors they generate: whenever global neural activity will be comparable to that elicited by the training stimuli, the novel stimuli will be classified as belonging to the trained category.

Experiments showing visual categorization on the basis of a single feature at a time could be interpreted as evidence for a limited brain capacity constraining in consequence the use of visual information. This view could be supported by the reduced size of the insect brains (1 mm^3 in the case of the honeybee). Recently, however, insect plastic behaviour has been shown to attain sophistication levels that were previously attributed to some vertebrates exclusively (Giurfa, 2003; Menzel & Giurfa, 2001). In particular, experiments on visual recognition by bees have shown that visual cognition in bees is far from being explained by a reduced or constrained brain capacity (Stach & Giurfa, 2003). The idea that single-feature categorization is the governing mechanism in bee visual categorization should be abandoned as it was demonstrated that honeybees could categorize visual patterns on the basis of configurations of various visual features ('configural categorization'). In

vertebrates, strategies of object recognition imply the use of spatial relations between features amongst others. Human faces for example can be perceived as an arrangement of features (two eyes above a nose above a mouth) establishing a specific configuration. If these first order relations are changed, recognition is impaired (Maurer et al., 2002). Accordingly, bees trained with a series of complex patterns sharing a common configuration made from four edge orientations, extracted all four orientations and integrated them in a configural representation, which allowed responding to novel stimuli preserving the trained configuration. Thus, bees can categorize visual stimuli on the basis of configural information that allows generating a large set of object descriptions and categories from a finite set of elements.

In this study (Stach & Giurfa, 2003), bees were trained with a sequence of six different pairs of patterns, which succeeded each other in a pseudorandom sequence. One group of the pattern pairs termed was ‘positive’ and was always rewarded by the experimenter, and the other was termed ‘negative’ and was never rewarded. Positive and negative patterns were all different and had different common configurations. With such training, bees could only rely on the positive and negative common configurations to solve the task as patterns were otherwise randomised with respect to all other parameters. This procedure, which is the very basis of the learning sets (Harlow, 1949), prevents the forming of an association between any of the variable features and the reward, leaving the bee with only the common set of features as the useful information, namely those preserved in all training stimuli. Therefore, the basic principle of a learning set is that animals are explicitly trained to perform in a given way, defined *a priori* by the experimenter. This procedural aspect raises a basic question on the nature of the categorization task emerging from our experiments: do such categorization abilities correspond to the natural strategies employed by the bees, or do they simply reflect a latent capacity of the nervous system that is, otherwise,

unemployed? In other words, does the extraction of a common feature or a set of features from a series of different stimuli occur under training conditions different from those of a learning set?

Contrarily to training with a changing sequence of patterns sharing a common feature, which has to be extracted to solve the task, training with a single, constant pair of patterns does not impose explicitly the extraction of a specific feature as any available cue can be used to discriminate between patterns. In the present study, we examine whether or not configural categorization based on the extraction of four different edge orientations arranged in a specific spatial relationship (Stach & Giurfa, 2003) also occurs if honeybees are trained with only a single pair of stimuli and, if yes, whether or not different levels of experience determine changes in the discrimination strategies employed by the insects.

Material and Methods

Individually marked honeybees (*Apis mellifera*) from a hive distant ca. 50m from the experimental site were trained to collect 50 % sucrose solution (weight/weight) on black and white patterns presented vertically on the back walls of a wooden y-maze (Fig. 1). In our training procedure, we allowed always one bee at a time in the maze. Thus, training as well as testing was always performed on single, individual bees. Experiments were conducted during the summer, when floral competition is low and the bees' motivation to collect sucrose solution is highest.

Apparatus

The y-maze (see Fig. 1) was covered with UV-transparent Plexiglas to ensure natural daylight illumination within the maze. The bee entered the maze by flying through an aperture, 5 cm in diameter, in the frontal panel of the maze. A sliding door situated in

front of the entrance aperture was closed to impede the eventual access of other bees into the maze. Bees entering the decision chamber of the maze (see Fig. 1) could see simultaneously both back walls of the two arms of the maze. Each back wall (20 x 20cm) presented a circular black and white pattern; only one of them, termed ‘positive’, provided a reward of sucrose solution, while the other one, termed ‘negative’, was not rewarded. Back walls (and thus patterns) were placed at a distance of 15 cm from the entrance to the decision chamber. Sucrose solution was provided in the centre of the positive pattern, in a transparent plastic pipette tip, 4 mm in diameter. Due to the poor optical resolution of the insect eye and to the small size of the pipette tip, bees flying within the decision chamber could not detect the presence of the reward on the positive pattern. The sides of positive and negative patterns were interchanged in a pseudorandom way to avoid the use of positional information. For the tests, new back walls with fresh background and patterns were inserted in front of the training stimuli.

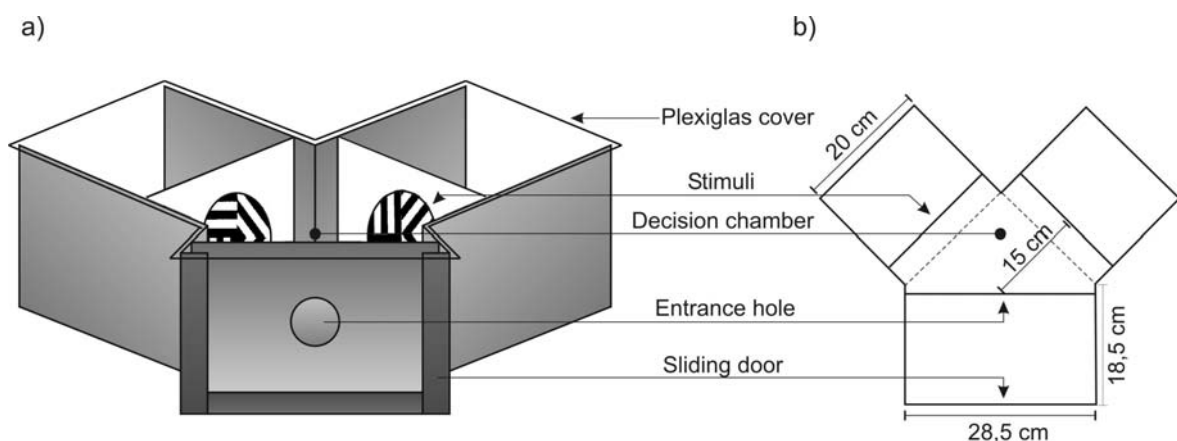


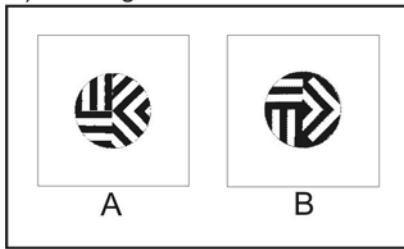
Figure 1. a) Front view of the Y-maze apparatus. Bees entered the maze through a sliding door and faced a wall with a central hole, 5 cm in diameter, through which they gained access to the decision chamber. There they faced two black and white patterns presented vertically on the back walls of the arms of the maze. One of the patterns was rewarded with sucrose solution and the other was non-rewarded. The whole apparatus was covered with an ultraviolet transmitting Plexiglas ceiling. b) Overall view and dimensions of the set-up.

Stimuli

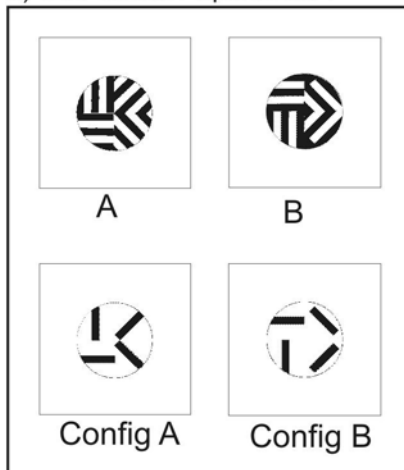
The stimuli (Fig. 2) were black and white circular patterns, 10 cm in diameter, which were printed on a high quality laser printer on white copying paper of constant quality. Seen from the entrance hole of the y-maze, the patterns subtended a visual angle of 37° to the bee's eye. At the decision point (imaginary point in the middle of the decision chamber) the patterns subtended a visual angle of 44° . In both cases, the values of visual angle ensured that bees could detect and resolve well the patterns presented on the back walls of the maze.

Each training pattern was divided into four quadrants, each of which containing a regular grating of a different orientation (0° , 45° , 90° , 135° with respect to the vertical). These orientations were chosen as they can be discriminated from each other by honeybees (Giger & Srinivasan, 1996; Srinivasan et al., 1993; 1994; Zhang & Horridge, 1995). Gratings in the quadrants had a period of 2 cm, which corresponds to a visual angle of 8° as seen from the entrance hole. This value ensured that stripes were always well resolvable for the bees' eyes (Srinivasan & Lehrer, 1988).

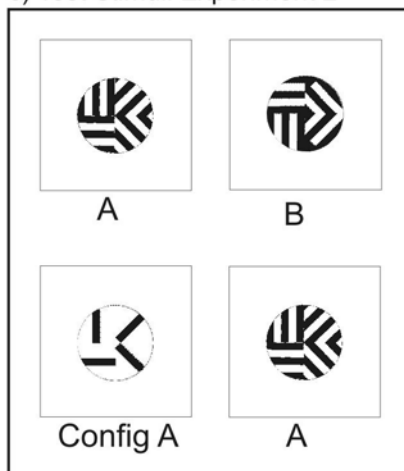
a) Training Stimuli



b) Test Stimuli Experiment 1



c) Test Stimuli Experiment 2



d) Test Stimuli Experiment 3 & 4

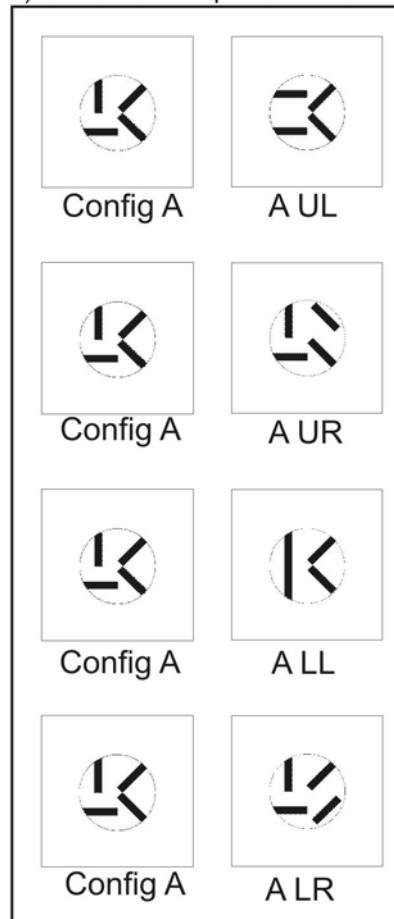


Figure 2. The training and test stimuli. a) Training stimuli as used for the long as well as for the short training. b) Test stimuli used in Experiment 1 a and b. c) Test stimuli used in Experiment 2 a and b. c) Test stimuli used in Experiments 3 and 4 (Config: configuration of orientations, UL: upper left bar rotated, UR: upper right bar rotated, LL: lower left bar rotated, LR: lower right bar rotated). Experiment 3. Test stimuli for bees rewarded on training pattern A are given as example. Experiment 4: Test stimuli for bees rewarded on training pattern B are given as example.

Procedure

Training followed the principle of a differential conditioning procedure (Giurfa et al., 1999), i.e. bees had to learn to choose the positive pattern and to avoid the negative one. Under such a procedure bees learn the global visual information of the patterns and not only local cues (Giurfa et al., 1999). Whenever the bee chose the correct pattern, it was rewarded with sucrose solution (see above). The bee drank the solution during ca. 1min (which corresponds to a full crop load; see Núñez, 1966) and flew then back to the hive. Bees needed usually 2 to 5 minutes to come back to the experimental site. If the bee chose the wrong pattern, it was subjected to a correction procedure in which it was gently removed from the maze by hand such that it had to re-enter through the entrance hole and make a new decision. This procedure was repeated until the bee chose the correct pattern. In this way, wrong decisions were penalized and performance was improved. A decision was judged as right or wrong when the bee passed the imaginary line that separated the decision chamber from the two side arms. Only the first choice of each trial was taken into account in order to obtain the acquisition curves.

In all experiments, a single pair of patterns was used as training stimuli (Fig. 2a). Depending on the experiment, training was either 21 or 42 trials long. In a previous study on configural categorization, bees were trained along 42 training trials with six different pairs of patterns presented seven times in a pseudorandom sequence, a procedure that was conceived to promote extraction of a configuration of four orientations (Stach & Giurfa 2003, see introduction). Here we ask whether training with a single pair of patterns can also result in configural categorization, depending on the amount of experience with the patterns. Fixing a number of trials (21 or 42) allowed to control the individual experience of each bee and to determine the effect of cumulative experience on its visual discriminations strategies. In each experiment, two groups of bees were trained and tested.

The stimulus, that was positive for one group was negative for the other and vice versa. By performing such a reciprocal, balanced training possible preferences for one of the stimuli can be ruled out.

After the last acquisition trial (21st or 42nd depending on training length), several tests were performed under extinction conditions (no reward was available on the patterns). During each test, the bee was presented with fresh patterns on exchangeable walls for 2min. Each test was performed twice changing the sides of the patterns from one test to the next. In this way, it was possible to control for possible position biases in the choice of the bee. During the tests, we recorded the number of touches (flights towards a patterns that ended with a contact of the pattern surface, usually with the antennae of the bee). After 2min, the bee under study was removed from the maze and the test patterns were replaced by the training patterns. The sliding door of the maze was then opened and the bee, which stayed around searching for sucrose solution, re-entered the maze immediately and received a reward if it chose the positive training pattern. Because during the tests no reward was provided on the patterns, refreshing trials were performed between the tests. Three refreshing trials were given between consecutive tests presenting the same patterns at interchanged sides, and eight trials were given between consecutive tests presenting different patterns. With this kind of protocol, it was possible to train 2 bees in parallel: while one bee was present in the maze, the other was on its return trip to the hive. The whole experiment (training and tests) was finished within one day.

Four basic experiments were performed:

Experiment 1

In this experiment we asked, whether or not bees trained with a single pair of pattern could generalize their choice to simplified versions of the training patterns. Bees were trained with the patterns shown in Fig. 2a. After 21 or 42 trials, bees were first tested

with fresh versions of the patterns used during the training in order to verify that they had learned to discriminate them (control test). In the second test, bees were tested with the patterns shown in Fig. 2b. These patterns presented just one oriented bar of 1 cm width per quadrant but preserved nevertheless the trained positive and negative configurations. They thus constituted simplified versions of the training patterns.

Experiment 2

In this experiment we confronted the positive training stimulus and its simplified, non-trained version in the tests. In this competition experiment, we thus tested whether the memorized pattern representation resulting from training drives the bee choices towards the stimulus effectively rewarded during the training or is flexible enough to generalize the bee choices to the novel simplified version of the training stimulus.

Bees were trained with the same single pair of patterns shown in Fig. 2a. After 21 or 42 training trials, they were tested with the patterns presented in Fig. 2c. The first test consisted in presenting the patterns used for training in order to verify that bees had learned to discriminate them (control test). In the second test, the bees had to choose between the positive stimulus effectively rewarded during training and the corresponding positive simplified configuration.

Experiment 3

In this experiment, we tested whether training with a single pair of stimuli results in the building of a configural pattern representation including all four orientations (Stach & Giurfa 2003), even in the absence of training with a randomised succession of different pairs of patterns. Bees were trained during 42 training trials with the same single pair of patterns shown in Fig. 2a. Tests were performed using simplified patterns with a single

oriented bar per quadrant (Fig. 2d). One of the test stimuli preserved always the configuration previously rewarded (positive configuration) whilst the other differed from the positive configuration in a single orientation. To this end, the orientation of one of the four bars was rotated by 90° with respect to the positive configuration. Four different alternatives were thus possible and each one was confronted against the simplified positive configuration in a separate test (Fig. 2d, UL = upper left bar rotated, UR = upper right bar rotated, LL = lower left bar rotated, LR = lower right bar rotated). These tests allowed deciding whether or not bees extracted the four orientation configuration after being trained with a single pair of patterns, in which case they should always prefer the complete positive configuration to any stimulus in which one of the four bars was rotated. Moreover, they allowed detecting possible differences in the weight accorded to the relative position of the bars within the visual field of the bees' eyes (Giurfa et al., 1999; Wehner, 1972).

Experiment 4

In this experiment, we studied how the bees learn and process the negative (non-rewarded) stimulus of the training pair. In a differential training procedure, bees learn to choose the positive and to avoid explicitly the negative pattern (Giurfa et al., 1999). Here we asked whether learning the non-rewarded stimulus also results in the building of a configural representation based on the spatial relationship of the four orientations present in the negative pattern. This would demonstrate that differential training not only promotes the building of a configural representation of the positive but also of the negative pattern. Bees were again trained with the single pair of patterns shown in Fig. 2a. Training was 42 trials long. After the last acquisition trial, four tests were performed. These followed the same rationale of the test patterns used in Experiment 3, but this time, the simplified *negative configuration* was presented against four alternatives, which differed in a single

oriented edge (Fig. 2d, UL, UR, LL, LR). Four different alternatives were thus possible and each one was confronted against the simplified negative configuration in a separate test. As in Experiment 3, the bees had to decide between two patterns differing in only one orientation.

Statistics

In order to obtain acquisition curves, the decisions from the training trials were divided in blocks of seven trials. The odd number of trials per block is justified by the training length of both groups of bees studied (21 and 42 trials). For the long training (42 trials) performance was thus presented in terms of 6 blocks while for the short training (21 trials) it was presented in terms of 3 blocks. From the number of correct choices (choice of the rewarded pattern) per block, choice proportions were calculated (number of correct choices per block/ total number of choices per block). With this procedure, 7 correct choices in one block correspond to a proportion value of 1.

ANOVAs were used for between-group as well as for within-group comparisons. Where necessary, Newman-Keuls-Tests were used for post hoc comparisons. From the tests, the number of touches during the test period of two minutes were recorded and the choice proportion was calculated. A one-way ANOVA was performed to determine whether the two groups of bees reciprocally trained and tested in each experiment differed in their performance. For the choice performance during the tests within groups Wilcoxon matched pairs test was used for comparison on the absolute data. For comparison between the preference levels of subsequent tests in one experiment, also Wilcoxon matched pairs tests were used. To compare between choice levels in tests of different experiments, the Mann-Whitney U-test was used. The alpha-level was set to 0.05 for all analyses.

Results

In all four experiments, two groups of bees were trained with a single pair of patterns A and B (Fig. 2a); one group was trained with A rewarded and B not (A+ vs. B-) while the other had the reversed contingency (A- vs. B+). In all experiments, there were no differences in the acquisition between both groups both for the long (ANOVA for repeated measures: Experiment 1: $F_{1,10} = 3.53$, NS; Experiment 2: $F_{1,12} = 0.052$, NS; Experiment 3: $F_{1,14} = 2.2$, NS; Experiment 4: $F_{1,22} = 0.52$, NS) and the short training (Experiment 1: $F_{1,10} = 0.06$, NS; Experiment 2: $F_{1,12} = 0.26$, NS). We therefore pooled the results of both groups and show a single acquisition curve per experiment.

Experiment 1

In Fig. 3a the acquisition of the bees trained with 21 (short training) and with 42 (long training) is depicted. Despite its increasing trend, the acquisition observed in the case of the short training was marginally not significant ($F_{2,20} = 3.25$, $P = 0.059$). In the third block, bees reached nevertheless a proportion of 0.7 correct choices thus indicating that learning to discriminate the positive and the negative patterning was possible even after a short training of 21 trials. This conclusion was supported by the results of the tests (see below). The acquisition obtained in the case of the long training increased significantly along blocks (ANOVA for repeated measures: $F_{5,50} = 5.89$, $p < 0.005$), thus showing that 42 training trials also resulted in discriminative learning. For comparison between short and long training a Group x Blocks (2 x 3) ANOVA for the first three blocks was computed. The main effect Group was significant ($F_{1,22} = 7.56$, $p < 0.05$) and shows that in the three blocks of the short training, bees had a better performance than bees in the first three blocks of the long training. This result is intriguing because in both cases, the task

was identical. The main effect Blocks ($F_{2,44} = 2.11$, NS) and the interaction Group x Blocks ($F_{2,44} = 0.26$, NS) were not significant.

After the last acquisition trial, bees were tested with the training patterns A and B (control test) and with simplified versions of these patterns (Config A vs. Config B) that preserved the corresponding configuration of edge orientations (Fig. 2b). There were no differences in the test performance of the groups trained with A+ vs. B- and with A- vs. B+ both for both the short (one-factor ANOVA: $F_{1,9} = 1.49$, NS) and the long training ($F_{1,10} = 3.61$, NS). Fig. 3 shows therefore a pooled test performance for this experiment discriminated according to training length (control test: S+ vs. S-; test with simplified patterns: Config S+ vs. Config S-).

After both the short and the long training, the bees significantly preferred S+, the pattern that was rewarded during training in the first test (Wilcoxon test, short training: $N = 12$, $p < 0.005$; long training: $N = 12$, $p < 0.005$). This shows that in both cases bees learned the task. Moreover, there were no significant differences between the two training lengths in the choice of positive and negative patterns (Mann-Whitney U test: $N = 23$, $p = 0.1$). In the second test, the performance of bees varied significantly depending on training length (Mann-Whitney U test: $N = 23$, $p < 0.005$). Bees trained with 21 trials could not discriminate the simplified patterns (Wilcoxon test: $N = 11$, $p = 0.12$) whilst bees trained with 42 trials significantly preferred the simplified pattern whose orientation configuration corresponded to that of the positive training pattern (Wilcoxon test: $N = 12$, $p < 0.005$). Thus, depending on training length, bees could generalize or not from a trained pair of patterns to a novel pair of simplified patterns. Our results indicate that more learning trials (here 42) are required to transfer the choice of the known patterns to the simplified configuration. However, this experiment does not demonstrate that after a longer training with a single pair of patterns bees extracted the positive and negative configurations of

orientations of the training stimuli. Discrimination in the second test can occur if the bees just focus on a single quadrant and thus use the information provided by a single oriented edge. Experiment 1 shows, nevertheless, that increasing experience with a single pair of patterns promotes a higher level of generalization.

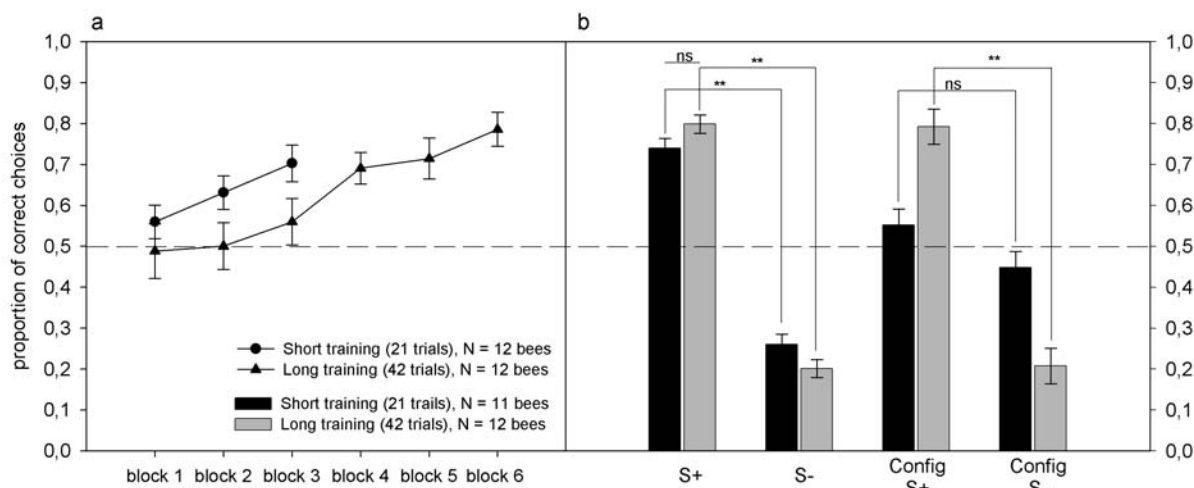


Figure 3. Results of Experiment 1 (S+ and S-: training pattern previously rewarded and non rewarded; ConfigS+ and ConfigS-: configurations of the training pattern previously rewarded and non-rewarded, respectively). a) Acquisition in the first experiment during training. b) Proportion of correct choices in the tests of the first experiment. **: $p < 0.005$.

Experiment 2

In Fig. 4a the acquisition of the bees trained with 21 (short training) and with 42 (long training) trials is depicted. In both cases, the acquisition curves significantly increased along blocks of training (long training: $F_{5,60} = 12.07$, $p < 0.005$; short training: $F_{2,24} = 5.99$, $p < 0.05$), thus showing that the two kinds of training resulted in discriminative learning. For comparison between short and long training a Group x Blocks (2 x 3) ANOVA for the first three blocks was computed. The main effect Blocks ($F_{2,52} = 10.49$, $p < 0.005$) was significant. The main effect Group ($F_{1,26} = 1.89$, NS) and the interaction Group x Blocks ($F_{2,52} = 0.08$, NS) were not significant. In this experiment, therefore, bees in both groups learned to discriminate the patterns similarly along the first three blocks of training trials.

After the last acquisition trial, bees were tested with the training A and B patterns (control test) and with the positive pattern vs. the positive simplified pattern (positive configuration) (Fig. 2c). In both tests, there were no differences between the group trained with A+ vs. B- and that trained with A- vs. B+ both for the short (one-factor ANOVA: $F_{1,10} = 1.74$, NS) and the long training ($F_{1,11} = 0.51$, NS). Fig. 3 shows therefore a pooled test performance for this experiment, discriminated according to training length.

In the control test, the bees significantly preferred S+, the pattern that was previously rewarded during training, to S-, the pattern that was non-rewarded both after the short and the long training (Wilcoxon test: short training: $N = 12$, $p < 0.005$; long training: $N = 12$, $p < 0.005$). This shows that in both cases bees learned to discriminate between the positive and the negative pattern. Moreover, there were no significant differences between the two training lengths in the choice of these patterns (Mann-Whitney U test: $N = 24$, $p = 0.01$).

In the second test, the performance of the bees varied significantly depending on the training length (Mann-Whitney U test: $N = 24$, $p < 0.005$). Bees trained with 21 learning trials significantly preferred the positive pattern S+ to its simplified version Config S+ (Wilcoxon test: $N = 12$, $p < 0.005$). However, after 42 learning trials bees chose randomly between both patterns (Wilcoxon test, $N = 12$, $p = 0.44$). This non-significant performance was not due to an inability to discriminate the test stimuli because bees were able to differentiate the patterns after the short training. The lack of discrimination after a long training suggests that increasing experience promotes a higher level of generalization such that bees treated the positive pattern and its simplified configuration as equivalent. Contrarily, a shorter training does not promote generalization and bees preferred the known pattern to the novel one. Our results thus indicate a change in recognition strategy depending on training length. As for Experiment 1, we do not claim that bees extracted the

four-orientation configuration of the stimuli after a long training; again, performance can be based on the orientation available in a single quadrant.

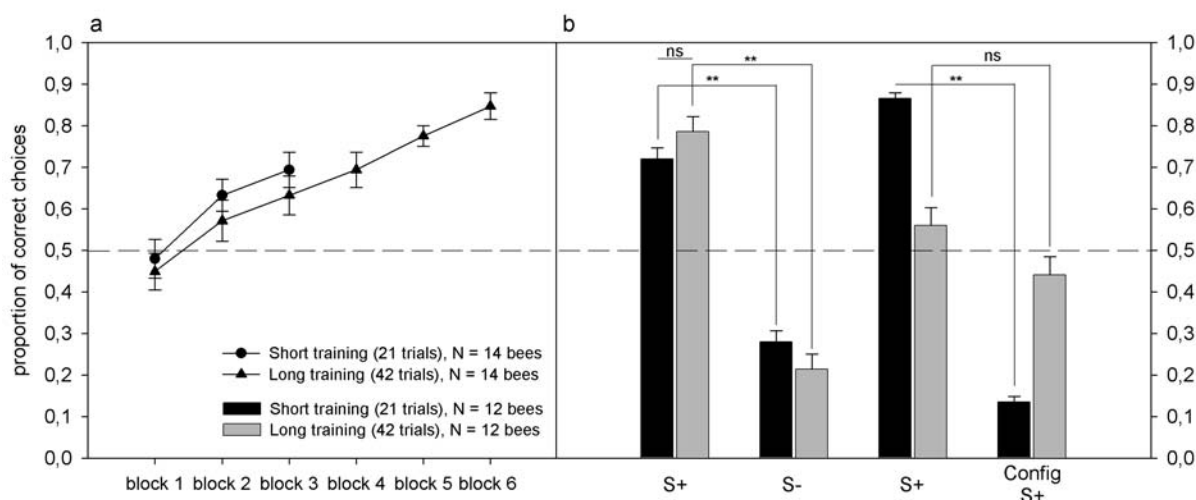


Figure 4. Results of Experiment 2 (S+ and S-: training pattern previously rewarded and non rewarded; ConfigS+: configurations of the training pattern previously rewarded). a) Acquisition in the first experiment during training. b) Proportion of correct choices in the tests of the first experiment. **: $p < 0.005$.

Experiment 3

In this experiment, bees were only subjected to the long training (42 trials). The acquisition curve significantly increased along blocks of training (Fig. 5a, ANOVA for repeated measures: $F_{5,70} = 15.99$, $p < 0.005$), thus showing that bees learned to discriminate the positive from the negative training pattern.

After the last acquisition trial, bees were tested with the positive, simplified configuration Config S+ against four alternatives differing from it in a single orientation (Fig. 2d). These tests allow deciding whether or not bees can extract the configuration of four orientations, in which case they should always prefer the complete positive configuration to any stimulus in which one of the four bars was rotated. There was no difference between the group trained with A+ vs. B- and that trained with A- vs. B+ (one-

factor ANOVA: $F_{1,14} = 1.66$, NS). Fig. 5b shows therefore a pooled test performance for this experiment.

In all four tests, bees significantly preferred the novel stimulus having the configuration of orientations corresponding to the positive training stimulus (Wilcoxon test: $N = 16$, $p < 0.005$ in all cases), independently of the quadrant in which the orientation of the bar was rotated. The level of preference did not differ between tests (Wilcoxon test: $N = 16$, $p > 0.1$ in all cases).

These results indicate that after a long training with a single pair of patterns, bees built a simplified pattern representation that allows transfer to novel stimuli preserving the spatial relationship between orientations. In such a representation all four quadrants had the same weight. Experiment 3 therefore shows the building of a configural representation corresponding to the positive pattern. As bees were trained with a differential conditioning, Experiment 4 studies whether an equivalent representation is also established for the negative pattern.

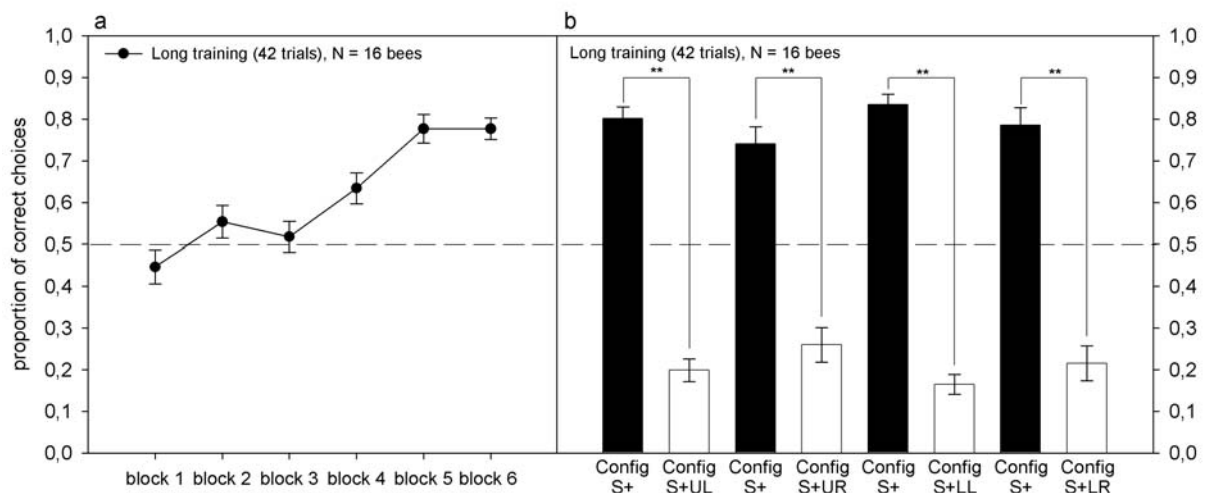


Figure 5. Results of Experiment 3 (S+ and S-: training pattern previously rewarded and non rewarded; ConfigS+: configurations of the training pattern previously rewarded; UL = upper left bar rotated, UR = upper right bar rotated, LL = lower left bar rotated, LR = lower right bar rotated). a) Acquisition in the first experiment during training. b) Proportion of correct choices in the tests of the first experiment. **: $p < 0.005$.

Experiment 4

Figure 6 shows the performance of bees in this experiment. As in experiment 3, bees were only subjected to the long training (42 trials). Here we ask, whether the long training also results in the building of a generic, simplified representation of the *negative pattern*.

The acquisition curve increased significantly along blocks of training (Fig. 6a, ANOVA for repeated measures: $F_{5,110} = 11.8$, $p < 0.005$), thus showing that bees learned again to discriminate the positive from the negative training pattern. The tests confronted the simplified version of the negative pattern (Config S-) against four alternatives, each having one of the four bars rotated by 90° with respect to the corresponding quadrant of the negative configuration (Fig. 2d). There was no difference between the group trained with A+ vs. B- and that trained with A- vs. B+ (one-factor ANOVA: $F_{1,17} = 0.91$, NS). Fig. 5b shows therefore a pooled test performance for this experiment. In all four tests, the bees significantly preferred the novel stimulus having one rotated bar to the complete negative configuration (Wilcoxon test: $N = 19$, $p < 0.05$ in all cases). The level of preference did not differ between tests (Wilcoxon test: $N = 19$, $p > 0.1$ in all cases).

These results indicate that after a long training with a single pair of stimuli, bees are able to build not only a simplified pattern representation of the *positive* but also of the *negative* pattern. The former had an excitatory strength while the latter had an inhibitory strength and bees explicitly avoided it. Since the alternatives in which one bar was rotated had one coincident orientation with the positive configuration (while the negative configuration had no coincident orientation), the bees always preferred them. To this end, with our data we are not able to tell, whether bees avoid the complete negative configuration due to its inhibitory strength or prefer the negative configuration with one rotated bar due to its coincidence with the positive configuration.

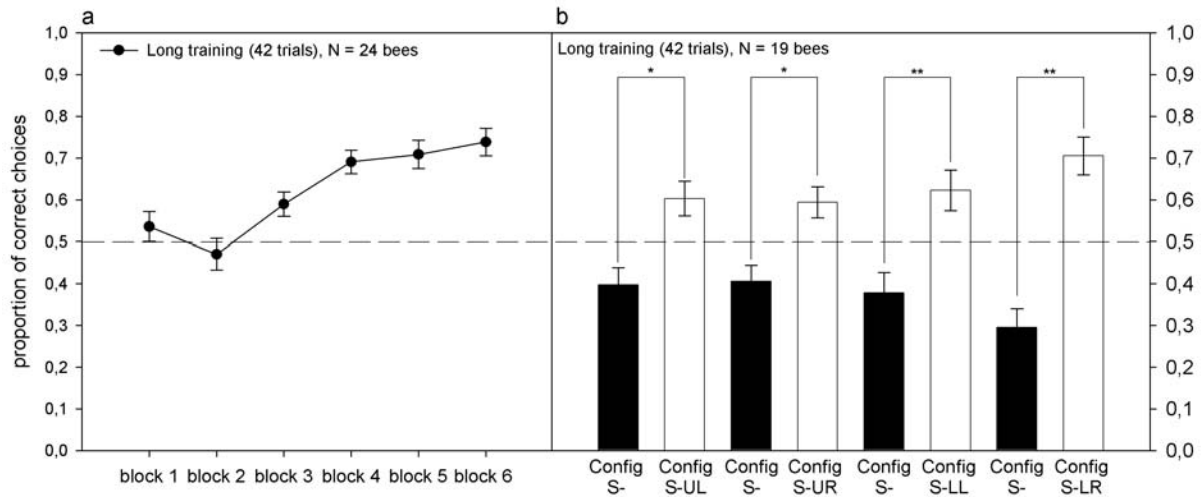


Figure 6. Results of Experiment 4 (S+ and S-: training pattern previously rewarded and non rewarded; ConfigS-: configurations of the training pattern previously non rewarded; UL = upper left bar rotated, UR = upper right bar rotated, LL = lower left bar rotated, LR = lower right bar rotated). a) Acquisition in the first experiment during training. b) Proportion of correct choices in the tests of the first experiment. *: $p < 0.05$; **: $p < 0.005$.

Comparison between Experiments 3 and 4

The level of choices for the positive configuration in Experiment 3 and for the modified negative configurations with one bar rotated in Experiment 4 was similar. No significant differences were found (Mann-Whitney U-test: $N = 35$, $p > 0.1$ in all cases), thus indicating that bees learned the orientation configuration of the positive and the negative pattern equally well.

Discussion

The results of Experiments 1 and 2 show that increasing experience with a single pair of patterns results in higher generalization levels reflected in significant responding to novel stimuli. Moreover, Experiments 3 and 4 show that in the case of the complex patterns used in our study, increasing experience with them resulted in the building of a configural representation both for the positive and the negative stimulus. Such

representations preserved the basic details of the patterns, i.e. the spatial relationship between edge orientations in the four pattern quadrants.

Generalization of the discrimination performance to the simplified versions of the trained patterns was possible after a long training but not after a short one. It could be therefore hypothesized that during the initial learning trials, the bees learn the training stimuli (or part of it) taking into account spatial details (for instance, in terms of a retinotopically fixed template). With ongoing training, redundant information is eliminated and the representation is reduced to the amount of information that is necessary and sufficient to solve the task.

There are important parallels between our results and findings on object recognition and categorization in humans. Experiments on this subject with humans have shown, that the processing of object recognition changes with increasing experience. One example for the development of this kind of expertise is provided by a work of Gauthier et al. (1998), where humans are trained to categorize so-called greebles. These greebles are computer-generated figures that can be varied along certain pre-defined dimensions. Humans that followed an extensive categorization training with the greebles, thus being “greeble experts”, showed a higher sensitivity to changes in the configuration of greeble features compared to a control group that did not follow the initial training procedure, thus being “greeble novices”. The study revealed that novices relied more on certain characteristic features (e. g. the shape of certain greeble body parts), whereas experts used the whole appearance of the greebles. Moreover, experts show a high degree of generalization to novel greebles in a categorization task, which is not the case in novices. In Experiment 1, bees discriminated the training patterns but not their reduced versions after short training, whereas after the long training they readily discriminated them (Fig. 3a). Generalization to novel stimuli was higher after the long training. This fact is supported by the results of

experiment 2. This is the first important parallel between our data and the results of the study about expertise in human object recognition described above. The second parallel is, that in both cases, with a higher level of experience the cues that are used for recognition are different with respect to the cues used in the beginning. Bees with less experience cannot generalize to a version with reduced orientation information, whereas bees with a higher level of experience rely on relevant features that can be identified in a flexible manner in novel stimuli thus determining responding. Giurfa et al. (2003) have shown recently that increasing the level of experience from 6 to 40 conditioning trials with visual stimuli (one or two adjacent colored disks) results in a change in the way bees associate the stimuli with the reward. Few trials determine elemental associations (Rescorla & Wagner, 1972), i.e. bees presented with a two-disk pattern associate each of the two disks with the reward; more trials, on the other hand, determine a ‘configural’ association (*sensu* Pearce; Pearce, 1987), i.e., bees treat the two-disk pattern as a global entity different from the sum of its parts that becomes associate with the reward. Our experiments do not allow concluding about the kind of associations established between the four-edge representation and the reward. Bees may respond to any stimulus in which each of the four orientations appears in the appropriate retinal field without necessity of a linking algorithm. This possibility refers to elemental theories of compound processing in which a compound is treated as the mere sum of its elements (Rescorla & Wagner, 1972; see above). On the other hand, bees could treat a stimulus presenting the four orientations as an entity, different from the simple sum of the individual orientations (Pearce, 1987). Non-elemental processing and learning of olfactory (Deisig et al., 2001; 2002; 2003) and visual (Schubert et al., 2002) compounds has been shown in bees, thus suggesting that such a non-linear strategy may underlie visual configural categorization.

The results from experiments 1 and 2 do not allow conclusions about the strategies that are used by the bees to discriminate patterns. Theoretically, it would be sufficient to rely on one single orientation appearing in a specific region of the visual field to solve the discrimination task. In another study (Stach & Giurfa, 2003) we could show, that bees trained with a randomized sequence of patterns sharing a specific configuration made from four edges are able to extract this configuration and categorize visual patterns on this basis. The possibility that bees also use this configural representation of orientations when trained with a single pair of patterns was investigated in experiments 3 and 4. The results show that training with a single pair of patterns also leads to the building of a configural representation in which the four orientations are integrated into a specific spatial relationship. This is true not only for the positive but also for the negative configuration. Thus, after extensive training with a pair of patterns, the bees' choice becomes guided by excitatory and inhibitory simplified pattern representations resulting from the differential conditioning to which they were subjected. Our study clearly shows that configural processing of four orientations is also possible when bees are not explicitly trained in a way that the only possibility to solve the task is the extraction of a configuration of orientations, as it is the case with a training to a sequence of different pattern pairs sharing a common orientation configuration (Stach & Giurfa, 2003). Some of the recent results of experiments on honeybee pattern recognition are not consistent with our findings and have to be discussed. It has been argued recently that bees are not able to perceive patterns but only cues (e. g. Horridge, 1997; 1999; 2000; 2003). From this perspective, bees do not link features belonging to a pattern but rely on isolated parameters such as edge orientation, bilateral symmetry, or radially and ignore the actual shape or pattern. This would be due to the existence of feature specific perceptual filters in the bees' visual system that become activated by relevant cues (Horridge, 2000). Moreover, it is assumed, that cues activating

the same filter cannot be re-assembled (Horridge, 1996; 1997). The combination of several cues in one representation would only be possible, if these activate different filters (Horridge, 2000). Furthermore, Horridge (e. g. Horridge, 1996; 1997; 2003) claims that cues can only be evaluated if they appear in the same regions of the visual field relative to the point of choice. This last point is consistent with our findings, because orientations are consistently presented in a specific configuration (see also Stach & Giurfa, 2001; 2003) and are therefore appearing in the same parts of the visual field. A change in this configuration is readily detected (Experiments 3 and 4) and a stimulus is judged as being different from the original configuration. However, contrarily to Horridge's results, in our case a combination of several cues of the same kind (four orientations) occurs. The use of a single parameter of the kind as proposed by the model of visual filters as suggested by Horridge (2000) can not explain our results. This might be due to some essential differences in the training procedure. First, in most of his studies, Horridge uses 'baffles', i.e. transparent walls with a hole in the middle mounted at a fixed distance in front of his stimuli (for detailed description of the baffles, see Horridge, 1997a). These baffles have the effect, that bees are forced to fixate the patterns at a certain distance before they decide. A decision is recorded when bees pass the baffles. This difference in procedure may lead to a slightly different perception of the presented patterns and the impact of the baffles on configural pattern perception has to be clarified. Moreover, in all of his studies (Horridge, 1996 – 2003) bees are not trained individually and the number of visits per bee is not controlled, a fact which certainly has an impact on the results.

Altogether, this study shows that internal representations change with increasing number of training trials and that bees build a configuration of orientations even though this is not implemented by the training procedure. In experiments on human object recognition stimuli are often used, which combine stimuli features across different

modalities. Such intermodal features can be easily integrated in a configural representation (Tarr, 1994). Flowers represent composite stimuli that differ in different sensory dimensions such as colour, shape, odour and texture, among others. This raises the question of whether bees can build multimodal configurations to perform intermodal object categorization. Answering this question requires careful experimental design as different sensory cues may have different perceptual saliencies to honeybees (Menzel, 1985). If one element of a stimulus compound is more salient than the others it may simply overshadow the others such that the learning of configurations could be impaired. Depending on the visual range, bees prioritise different visual cues while approaching a floral target (Giurfa & Lehrer, 2001, Menzel & Giurfa, 2001). Configural representations of floral stimuli could therefore take such a temporal component into account and link features that appear sequentially during the approach flight of a bee towards a flower. Linking of sequences of different sensory stimuli has been shown in bees flying towards a food source (Zhang et al., 1999) but the possibility of categorizing new sequences on the basis of learned ones remains an open question.

Many experiments on honeybee pattern recognition are carried out by training bees in groups and without individual control over the exact number of visits. It is common to read in papers on visual recognition by bees that training lasted “two or three hours” or “20 trials or so”, thus neglecting a precise control of individual experience. As our results show that there is an important change in performance after two different training lengths, it becomes evident that bees with different levels of experience use different cues for pattern recognition and their performances cannot be directly compared. If training is not individually controlled, either by giving exactly the same number of trials to each bee or by training them until a certain criterion is reached, interpretation of the results may be biased due to different individual experience. By pooling data from bees with different levels of

experience one not only risks wrong interpretation of the results but also an important loss of valuable information. All in all, our results should motivate researchers working in visual recognition to achieve an exact control of the individual experience of their experimental subjects. Not taking into account this essential factor may lead to fundamental errors about the nature of the representations and the strategies used for visual object recognition.

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