Pattern learning by honeybees: conditioning procedure and recognition strategy

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In recognizing a pattern, honeybees, *Apis mellifera*, may focus either on its ventral frontal part, or on the whole frontal image. We asked whether the conditioning procedure used to train the bees to a pattern determines the recognition strategy employed. Bees were trained with the same patterns presented vertically on the back walls of a Y maze. Conditioning was either absolute, that is, bees should learn to choose a rewarded pattern when there is no alternative, or differential, that is, bees should learn to choose a rewarded pattern that is paired with a different, nonrewarded one. Bees used different pattern recognition strategies depending on the conditioning procedure: absolute conditioning restricted recognition to the lower half whilst differential conditioning extended it to the whole pattern. Bees trained with absolute conditioning saw and learned the features of the upper part of the trained patterns, but assigned more weight to the lower part. Bees trained with differential conditioning learned not only the features of the reinforced stimulus in an excitatory way, but also those of the nonreinforced one in an inhibitory way. Thus, conditioning tasks that involve not only excitatory acquisition of the conditioned stimulus per se, but also discrimination of nonreinforced stimuli, result in an increase in the visual field assigned to the recognition task. Conditioning tasks that involve only excitatory acquisition of the rewarded stimulus result in a higher weighting of the lower pattern half and thus in a more reduced field assigned to the recognition task. This difference may reflect that existing between a conditioned and an incidental behavioural modification.

Most of what we know about pattern recognition in insects derives from the analysis of how they learn and identify shapes in the context of appetitive learning (for reviews see Wehner 1981; Srinivasan 1994; Heisenberg 1995; Giurfa & Menzel 1997). Since the pioneer work on pattern recognition in honeybees by von Frisch (1915) and Hertz (1929, 1933, 1935), they have become a classical model in this field. They have well-established learning and memory capacities (Menzel et al. 1993; Hammer & Menzel 1995; Menzel & Müller 1996; Hammer 1997) which have allowed researchers to train them with particular shapes and then ‘ask’ them for perceptual differences and similarities in the appropriate discrimination and generalization tests.

Honeybees have been trained and tested with a great variety of patterns including simple shapes such as squares, circles, triangles or stripes and more complicated pattern configurations (Wehner 1981; Srinivasan 1994; Giurfa & Menzel 1997). The main result emerging from these experiments is that pattern recognition and discrimination by insects are based on two main strategies: the template strategy and the feature strategy. The former suggests that patterns are perceived, learned and evaluated as retinotopically fixed, whole images (Wehner 1972; Collett & Cartwright 1983; Gould 1985, 1986; Dill et al. 1993; Giurfa et al. 1995; Ronacher & Duft 1996), that is, as some kind of neural template that is stored in the insect brain. According to this idea, choice would be determined by how closely the memorized image matches the observed shape. In contrast, the feature strategy postulates that insects focus on particular, relevant features of a pattern and ignore other available cues (Hertz 1929; Wolf 1933, 1934; Wolf & Zerrahn-Wolf 1935; van Hateren et al. 1990; Giger & Srinivasan 1995; Giurfa et al. 1996a). In this case, insects may be liberated...
from the constraints of retinotopic matching between the perceived and the memorized image by sequentially experiencing the same image at different locations on the retina. Consecutive learning of different shapes sharing a common, rewarded feature may also lead to the extraction of such a feature from a set of randomly varying, alternative features (van Hateren et al. 1990; Giurfa et al. 1996a).

Training procedures are likely to play a critical role in determining which strategy is used. However, the effect of different training procedures has been practically ignored in studies on insect vision. With the exception of analyses on operant learning in Drosophila (Dill et al. 1993; Dill & Heisenberg 1995), such studies have been mainly concerned with the mechanisms involved in visual performance but not with the learning phenomena themselves. Extraction and learning of features are inculcated by consecutive learning procedures whilst learning of a template is consistent with more classical training procedures. Thus, the way an animal learns a pattern may define which neural strategy is used for pattern recognition.

Another traditional discrepancy in pattern learning and discrimination by insects refers to the extent of the visual field that is required for pattern recognition. Again, two alternatives emerge from different studies, mostly on honeybees: in recognizing a pattern presented vertically, bees may either focus on its lower part, corresponding to their frontal ventral visual field (Wehner 1972; Menzel & Lieke 1983; Giurfa et al. 1995; Lehrer 1997), or on the whole frontal image (Gould 1985, 1986; Zhang & Horridge 1992; Zhang et al. 1992; Ronacher & Duft 1996). A third alternative, recognition based on the upper part of a pattern, that is, on the upper visual field, has never been documented. Note that, in this case, the distinction between the recognition strategies does not refer to those that bees may use to extract the relevant information from the respective sampling areas (feature or template based), but to a selective increase or reduction of the visual field necessary for pattern recognition.

It is not clear why bees may recognize a pattern in one way or the other. Because bees do not possess any particular anatomical or optical specialization in the corresponding ventral frontal region of their compound eyes (Wehner 1981), an explanation may be found in the critical role that training procedures may play in determining which strategy is used. In the absence of non-rewarding stimuli, training may lead to the bees learning only part of the training pattern. Indeed, such an effect has been shown in vertebrates (Lashley 1938) and may also occur in pattern learning by honeybees (Gould 1986). Rats, Rattus norvegicus, trained to perform shape discrimination on a jumping stand apparatus learned only part of the training shape when no unrewarded alternative was present (Lashley 1938). Therefore, Lashley (1938) pointed out that ‘with very complex, irregular figures, the basis of discrimination is a part figure; the response is to some limited cue and the remainder is ignored’. In contrast, training procedures in which a discrimination between rewarded and nonrewarded patterns is already involved in the training itself might require more visual information and would, therefore, be associated with an increased visual field for pattern recognition. Training in the presence of a nonrewarding stimulus leads to inhibitory conditioning (also called conditioned inhibition; see Pavlov 1960; Mackintosh 1974) of such a stimulus. In an appetitive context, inhibitory conditioning results in learning that nonrewarding training stimuli predict the absence of reward. Inhibitory conditioning is an important learning process and its role in natural learning has been underestimated. In the honeybee, it occurs in olfactory (Hammer & Menzel 1995) and visual learning (more specifically, pattern learning; see Gould 1986). Thus bees learning simultaneously both rewarding and nonrewarding patterns may incorporate more visual information on pattern quality, and focus, therefore, on the whole pattern and not only on its lower part.

In the present study, we asked whether the conditioning procedure defines the size of the visual field required for pattern recognition. We confronted bees with similar discrimination tasks after having trained them with two conditioning procedures: absolute conditioning, in which bees should simply learn to choose a rewarded pattern when there is no alternative, and differential conditioning, in which bees should learn to choose a rewarded pattern that is paired with a nonrewarded one (Yaremko et al. 1982). The difference introduced by the procedures is thus the kind of learning that is implicit: excitatory in absolute conditioning, and excitatory and inhibitory in differential conditioning. If conditioning procedures are indeed relevant for defining visual recognition, the results of the same tests should vary with the training procedure used.

**METHODS**

**Apparatus**

We did the experiments in 1997–1998. Honeybees, Apis mellifera carnica, were individually marked with a coloured spot on their thorax and trained to enter a wooden Y maze (Fig. 1) to collect 50% (weight/weight) sucrose solution on a training stimulus (see Giurfa et al. 1996b for a more complete description of the apparatus). The Y maze was placed near a large open window in the laboratory and covered with ultraviolet-transparent Plexiglas ensuring natural daylight illumination within the maze. Only one bee at a time was present in the apparatus. The bee entered the apparatus through an open sliding door and had to pass through a hole 5 cm in diameter to gain access to the decision chamber of the maze. Only when the insect was in this small chamber, could it see both back walls of the maze simultaneously (Fig. 1). The back walls measured 20 × 20 cm and had a central orifice, 0.5 mm in diameter, through which sucrose solution was delivered with a flow rate of 25 μl/min by means of an injecting pump.

In one of the arms, termed positive, a rewarded pattern (henceforth ‘standard’) was presented vertically on a white background. A bee landing on it was rewarded
with sucrose solution. In the other arm, termed negative, either the white background alone (absolute conditioning) or an alternative pattern (differential conditioning) was visible. Both were nonrewarded. Positive and negative arms were interchanged after one or two visits in a pseudorandom way to ensure that the bees did not associate the reward with any particular arm. Patterns were changed regularly to avoid orientation by means of olfactory cues.

**Stimuli**

The patterns (Fig. 2) were circular disks, 8 cm in diameter, mounted flat and vertically on the back walls of the maze, placed 15 cm from the entrance hole. Each pattern consisted of four quadrants, each filled with a black-and-white grating having a particular orientation (0°, 45°, 90° and 135°, with respect to the vertical). Patterns were printed in black with a good quality laser printer on a white copying paper of constant quality. The period of the gratings was 1.5 cm and yielded a spatial frequency of 0.18 cycles per degree at the entrance to the decision chamber. Such a frequency corresponds to a level of 90% correct choices in experiments performed with similar gratings (Srinivasan & Lehrer 1988), in which 0.23 cycles per degree was a corner frequency yielding a level of 65% correct choices, and 0.34 cycles per degree a random choice (50%). Thus, the striped patterns were perfectly resolvable for the bees. Similar four-quadrant patterns have proven to work successfully when used for establishing the size of regions in the spatial layout in honeybees (Zhang & Horridge 1992).

In our first experiment, we used two stimuli, standard A1 and standard B1, to train different groups of bees (Fig. 2). They differed markedly from each other because the stripe orientation of each of the four quadrants was perpendicular from one pattern to the other, thus reducing to a minimum the amount of overlap between them. Also, neither the mirror image nor the rotation of one standard resulted in the other standard. The stimuli presented with the standards during extinction tests (Fig. 2) were the alternative visible in the negative arm during training (that is, the white background alone in the case of absolute conditioning, or the nonreinforced standard in the case of differential conditioning) and two novel, half-patterns composed of a homogeneous grey half and either the complementary lower (standard low) or upper part (standard high) of the training standard. The grey half was cut from a neutral, bee-grey cardboard, HKS 92N (HKS-N pigment papers: K+E Stuttgart, Stuttgart-Feuerbach, Germany) which provided a dull, even reflection throughout the bee’s visual spectrum (Giurfa et al. 1996b). Standards low and high were chosen to test the selective variation of the size of the visual field necessary for pattern recognition. If, for instance, bees recognize a pattern using only its lower part, they should confuse the trained standard with its corresponding standard low. If, however, recognition is based on the whole pattern, they should easily discriminate the alternatives.

In a second experiment, bees trained with differential conditioning with either standard A1 or B1 were afterwards tested with the previously reinforced or nonreinforced pattern paired with a checkerboard disk, 8 cm in diameter (Fig. 2). The checkerboard was made from squares measuring 2.2 × 2.2 cm. These had a period of 8.4 × 8.4° as seen from the entrance to the decision chamber and were well resolvable for the bee’s eye. In contrast to the trained standards and standards low and high, the checkerboard disk did not present information on the dominant orientation and, thus, constituted a neutral, novel pattern. We used the checkerboard to test whether bees trained with differential conditioning learned not only the features of the reinforced stimulus in an excitatory way, and thus preferred it to the checkerboard, as bees trained with absolute conditioning do, but also those of the nonreinforced pattern in an inhibitory way, and thus preferred the checkerboard to it. In other words, bees were forced to learn the pattern qualities. This is a critical test for our assumption that differential conditioning increases the demands imposed on the perceptual system to choose the reinforced stimulus.

In a third experiment, we trained bees with absolute conditioning with a new kind of standard (A2 or B2; see Fig. 2). They were afterwards tested with the standard low or the standard high paired with the checkerboard disk. The advantage of these standards is that, if bees focus only on the lower half of the trained pattern, they cannot match its memorized image with the novel standard high because of the minimal overlap resulting from the stripes being perpendicular from one half to the other. These tests allow us to determine whether bees focusing on the lower half of a pattern do so because they have a perceptual limitation (i.e. they do not ‘see’ the upper half of the standard), a learning constraint (i.e. they do see it but do not learn it) or an asymmetry in the use of visual information during the learning and/or recognition task (i.e. they learn it but give more weight to the lower half). In
that sense, the test in which the standard high is paired with the checkerboard is critical to decide between these options: if bees choose the novel patterns equally, they did not see or learn the characteristics of the upper part of the trained standard; if, however, they prefer standard high to the checkerboard, and standard low to standard high, they see and learn patterns as a whole, but assign a different weight to the lower and the upper visual field.

Procedure

Bees were either absolutely or differentially conditioned. In the former procedure, they were trained to learn a rewarded standard (A or B) against a white, stimulus-free background. In other words, they should learn to choose the arm with the pattern. In the latter procedure, they should learn to discriminate and choose correctly between a rewarded standard (A or B) and its alternative (B or A, respectively). In both cases we rewarded bees with sucrose solution at each correct choice, and we punished them at each incorrect one by tossing them away from the maze, without allowing them to get a reward. They would then enter the maze again, and we repeated the procedure until they chose the trained standard correctly (Giurfa et al. 1997).

Once the bee learned the training task (i.e., after it reached a succession of at least 10 correct choices, which was usually achieved after 20–40 rewards), we conducted extinction tests approximately twice an hour with five or six training sessions (i.e., five or six rewards) in between. Before each test, the training patterns were replaced by fresh ones on exchangeable walls that were inserted in front of the training patterns. The test patterns were never rewarded. In each test we recorded the choice of

Figure 2. (a) Training patterns used in the first and second experiments (standards A1 and B1) and in the third experiment (standards A2 and B2). (b) Conditioning procedures, illustrated with standard A1 as an example. Bees were trained either in an absolute conditioning procedure or in a differential conditioning procedure (see text). (c) Extinction tests of the first experiment. Alternatives shown here as an example correspond to a training with standard A1. (d) Extinction tests of the second experiment. Alternatives shown here as an example correspond to a training with standard A1. (e) Extinction tests of the third experiment. Alternatives shown here as an example correspond to a training with standard A2.
patterns by the bee over a 2-min period. We counted the number of approaches (flights towards a pattern that ended within ca. 2 cm of it), and the number of touches (flights towards a pattern that ended with the antennae touching the pattern surface). Choice frequencies were obtained in this way and these scores were then transformed into a percentage of correct choices. Each test with a particular pair of patterns was done twice: once with the trained pattern in one of the arms of the maze, and a second time with it in the opposite arm. This was randomized from bee to bee. In this way, we were able to rule out position tendencies in the choice of patterns by the bees. Any such tendencies were already visible during the training procedure. A bee showing such a bias was excluded because it was not able to learn the training task but simply turned to one side or other of the maze.

RESULTS

The first task was to find the most sensitive behavioural parameter that would allow us to detect differences in the choice of patterns by bees depending on the test situation. To that aim, we compared the results obtained in all test conditions for the two behaviours recorded: approaches and touches. A two-way ANOVA for repeated measures (factor 1: behaviour, with two levels; factor 2: test type, with three levels; see Zar 1985) was performed on the percentage of correct choices for each of the four experimental conditions: (1) absolute conditioning with standard A; (2) absolute conditioning with standard B; (3) differential conditioning with standard A; and (4) differential conditioning with standard B. We made specific comparisons by means of post hoc Newman–Keuls tests modified for repeated measures analysis. In all four cases, a significant difference for the factor ‘behaviour’ was found (absolute conditioning with standard A: $F_{1,36}=87.19, P<0.000001$; absolute conditioning with standard B: $F_{1,36}=151.73, P<0.000001$; differential conditioning with standard A: $F_{1,36}=435.01, P<0.000001$; differential conditioning with standard B: $F_{1,36}=149.32, P<0.000001$). The number of touches, that is, when the bee’s antennae touched the surface of the pattern, revealed the choice preferences resulting from the training procedures. Figure 3 shows as an example the performance of bees in the first experiment, after absolute conditioning with standards A1 and B1. In all cases, bees touched the trained standard significantly more than any alternative pattern tested. They were not, however, as selective in their approaches towards the patterns. Although they usually approached the trained standard significantly more than the nonrewarded alternative visible during training (i.e. white background in absolute conditioning, and the alternative standard in differential conditioning), they approached the trained standard and the novel alternatives (i.e. standard high or standard low) randomly. We concluded that touches were the parameter that should be taken into account and, therefore, based our analysis on it.

To test whether the performance of bees was the same with standards A and B, we performed a two-way ANOVA for repeated measure (factor 1: standard, with two levels; factor 2: test type, with three levels) on the percentage of touches within each conditioning procedure. The results obtained for both groups of bees trained with either standard A or B were homogeneous within each conditioning procedure (absolute conditioning: $F_{1,36}=3.39, NS$; differential conditioning: $F_{1,36}=2.84, NS$) and were therefore pooled in order to compare the performance of bees after absolute and differential conditioning.

Figure 4 shows the performance of bees after both kinds of conditioning. A two-way ANOVA for repeated measures (factor 1: conditioning procedure, with two levels; factor 2: test type, with three levels) yielded a significant variation for the conditioning procedure ($F_{1,90}=19.36, P<0.00002$), for the test performed ($F_{2,90}=15.50, P<0.00005$) and for the interaction between factors ($F_{2,90}=7.69, P<0.002$). Bees learned the training situation equally well under the two conditioning procedures: the proportions of choices for the trained standard and the alternative visible during training (background and nonrewarded standard in absolute and differential conditioning, respectively) did not differ significantly between absolute and differential conditioning (Newman–Keuls test: $P=0.80$). In the same way, the
Conditioning procedure did not influence the choice of standard versus standard high \((P=0.88)\). However, bees of both conditioning groups differed significantly in their choice of standard versus standard low. After absolute conditioning, the percentage of correct choices for the trained standard was significantly lower than after differential conditioning \((P<0.0002)\). In other words, bees used different visual strategies for the recognition of the same pattern, depending on the conditioning procedure.

Bees differentially conditioned maintained the same high level of discrimination of the trained standard in all tests \((ca. 80\% correct choices)\): comparing the results from all three tests yielded no significant difference in these bees \(test\ with\ standard\ low\ versus\ test\ with\ standard\ high\): \(P=0.80\); \(test\ with\ standard\ low\ versus\ test\ with\ nonrewarded\ standard\): \(P=0.81\); \(test\ with\ standard\ high\ versus\ test\ with\ nonrewarded\ standard\): \(P=0.82\). After absolute conditioning, discrimination of the standard decreased only when it was presented together with standard low \(test\ with\ background\ versus\ test\ with\ standard\ high\): \(P=0.71\); \(test\ with\ background\ versus\ test\ with\ standard\ low\): \(P<0.0002\); \(test\ with\ standard\ high\ versus\ test\ with\ standard\ low\): \(P<0.0002\). This shows that absolute conditioning impaired only the discrimination between the standard and standard low. The fact that bees discriminated perfectly the standard from standards high and low after differential conditioning is consistent with the use of the whole trained pattern: when either half of it was absent, discrimination was possible and very accurate. The results of absolute conditioning, on the other hand, are consistent with the use of the lower part of the trained pattern. This would explain why discrimination of the standard was very good when paired with its upper part alone, but declined significantly when it was paired with its lower part alone.

In the second experiment, bees were trained with differential conditioning and afterwards tested with the checkerboard. The results of bees trained with standards A1 and B1 did not differ significantly \(two-way\ ANOVA\) for repeated measures: factor 1: standard, with two levels; factor 2: test type: with three levels; \(F_{1,18}=3.49, NS\) and were, therefore, pooled. Figure 5 shows the performance of bees in the three tests. Since in this experiment, the relevant comparisons are performed within each test, a two-tailed binomial test was used in each case to test the null hypothesis of a random choice of stimuli. Bees learned the differential task \(N=8, P=0.004\) and significantly preferred the previously rewarded pattern to the novel checkerboard pattern \(N=8, P=0.004\). However, when they were offered a choice between the checkerboard disk and the alternative nonreinforced during differential conditioning, they significantly preferred the checkerboard disk \(N=8, P=0.004\). Thus, bees learned both the features of the reinforced pattern and those of the nonreinforced one. The former were learned in an excitatory way whilst the latter were learned in an inhibitory way. Differential conditioning results in inhibitory conditioning of the nonrewarding pattern and, thus, increases the demands imposed on the perceptual system.
to choose the rewarded stimulus. It constitutes, therefore, a learning task in which it is necessary to learn the pattern qualities and, thus, to cope with more visual information than that pertaining to absolute conditioning in which only the positive pattern per se is taken into account.

In the third experiment, bees were trained with absolute conditioning with new standards and afterwards tested with the checkerboard and the new standards low and high. If they only see and/or learn the lower part of a trained pattern, they should confuse both the checkerboard and the standard high because both were novel, and no match between them and the memorized image was possible (standard high was designed in such a way that it provided minimal overlap with the lower part of the trained standard).

The results of bees trained with the new standards A2 and B2 did not differ significantly (two-way ANOVA for repeated measures: factor 1: standard, with two levels; factor 2: test type; with four levels; $F_{1,16}=1.20$, NS) and were, therefore, pooled. Figure 6 shows the performance of bees in the four tests. Comparisons within each test were performed by means of two-tailed binomial tests. Bees learned the task as well as with the former standards ($N=8$, $P=0.004$; compare with Fig. 3, first bar of each graph) and preferred both the standard low ($N=8$, $P=0.004$) and high ($N=8$, $P=0.004$) to the checkerboard. This result clearly shows that, under an absolute conditioning procedure, bees also saw and learned the features of the upper part of a trained pattern, but assigned more weight to those of the lower part. This conclusion is confirmed by the test in which standards low and high were paired: in this case, bees significantly preferred standard low ($N=8$, $P=0.004$).

DISCUSSION

Our study shows that pattern discrimination in honeybees is affected by the conditioning procedure used. We showed that differential conditioning inculcates a visual recognition that operates on the whole pattern whilst absolute conditioning restricts it to the lower half of the pattern (Fig. 4), although, in the latter case, bees also learn the features present in the upper part (Fig. 6). In other words, bees recognize a pattern differently, depending on the kind of learning implicit to the conditioning task. Differential conditioning, which involves not only excitatory acquisition of the conditioned stimulus per se, but also simultaneous discrimination of nonreinforced stimuli (Fig. 5), results in inhibitory conditioning of the latter (Gould 1986). Such inhibitory learning is possible only if bees learn not only the features of the reinforced stimulus, but also those of the nonreinforced one; in other words, if they cope with pattern qualities and thus with more visual information than that pertaining to an absolute conditioning procedure. Differential conditioning thus increases the demands imposed on the perceptual system to choose between the conditioned stimuli and results in an extended visual field for pattern recognition. Learning tasks that do not imply simultaneous discrimination between patterns, on the other hand, may not require such an extended visual field and thus confine recognition to the lower part. In that sense, the performance of bees is consistent with the part-figure learning shown by Lashley (1938) in the case of pattern discrimination by rats trained and tested under comparable conditions. The main difference is that in Lashley’s experiments, different rats focused on different parts of the figures, whilst in our work, bees consistently focused on the lower part of the pattern trained with absolute conditioning. In absolute conditioning, bees are reinforced not to notice pattern qualities, but to learn the location and the ‘being there’ of a rewarded pattern. Since they, nevertheless, learn and remember pattern qualities as shown by the third experiment (Fig. 6), pattern learning in this case may reflect ‘incidental learning’. Pattern qualities would be stored as a by-product, just because bees are exposed to them. The difference in the recognition strategies resulting from absolute and differential conditioning could be that between an ‘incidental’ and a conditioned behavioural modification, respectively, that is, a matter of the reinforcer.

How reduced is the visual field employed in pattern recognition after an absolute conditioning procedure? Our results indicate that the rough definition established experimentally between ‘lower’ and ‘upper’ visual fields by dividing patterns arbitrarily in half may not correspond with the actual extent of each of these fields. Indeed, there is no reason to suppose that a lower visual field in insect vision is limited automatically to the lower half of a pattern, that is, regionalization of the compound eye and of the pattern should not be confused. In fact, our results indicate that such a field is more extended.
than experimentally defined. After absolute conditioning, discrimination between standard and standard low was significantly impaired, but was, nevertheless, still possible (Fig. 4). This means that bees used a pattern extent greater than the lower half only, although this was the predominant part. The lack of this additional extent in standard low is what allowed discrimination between it and the standard. If bees had used only the lower half of the patterns, choice should have been random between the standard and standard low. It will be worth characterizing more precisely the limits of the visual field employed in this case, but a deeper understanding of the optic as well as the physiological properties of the insect visual system (Vorobyev et al. 1997) and more controlled experimental procedures are necessary. In particular, a paradigm in which free flight is not allowed and, therefore, the degrees of freedom of the insect’s movement are reduced (Dill et al. 1993; Dill & Heisenberg 1995) might provide a valuable tool to define the limits of the visual field applied under an absolute conditioning procedure.

If bees trained with absolute conditioning do see and learn the features of the upper visual field (Fig. 6) why do they assign a more important weight to the lower visual field? Bees do not possess any particular anatomical or optical specialization in the corresponding ventral frontal region of their compound eyes (Wehner 1981). Although such an asymmetry in the topographic organization of the recognition task might result from the way in which bees usually approach a flower (i.e. from above, such that the target impinges on the lower ventral part of the compound eyes), many floral structures imply exactly the opposite, that is, that bees approach them from below in order to enter an inverted corolla (e.g. some Campanulaceae, and zygomorphic Lamiaeae and Scrophulariaceae). Thus, the previous argument is not straightforward and the reasons why bees prioritize the lower half after absolute conditioning are still unknown.

Our results allow us to integrate contradictory results in the field of insect vision with respect to the use of the whole image of a vertical pattern or only its lower half in pattern recognition tasks. Evidence that bees pay particular attention to the sector of vertically presented patterns projected on to their ventral frontal eye region comes from three studies that used the absolute conditioning procedure to train the insects to different kinds of patterns. After training bees to a half-white and half-black disc, Wehner (1972) performed dual-choice tests in which the trained stimulus was paired with a series of discs in which the position of the sector within the pattern was maintained but its spatial frequency was either decreased or increased with respect to the training stimulus. In this case too, best discrimination was obtained from bees trained and tested with the sectors presented in the lower visual field. In all these examples, absolute conditioning seemed to have determined that pattern recognition was restricted to the lower half of patterns.

In the same way, previous studies indicating that such tasks are accomplished over the whole angular subtense of a pattern have all used differential conditioning or equivalent procedures in the training of patterns. We review here only those that were performed under well-balanced, controlled training procedures. Gould (1985, 1986) conditioned differentially a series of patterns and afterwards performed dual-choice, extinction tests on them. He concluded that bees use the whole frontal image of patterns to memorize them as templates. He showed that discrimination declines as the number of elements in the test patterns increases, thus showing that pattern elements are analysed locally and integrated over the whole image. He also showed that bees differentially conditioned tended to avoid the nonreinforced pattern and suggested that pattern discrimination could vary with the conditioning procedure (see Introduction). Zhang & Horridge (1992) also trained bees differentially to discriminate patterns with two, four or eight sectors filled with stripes, in an attempt to find how large the angular size of a local region in the spatial layout needed to be in order to be discriminated. Their results replicated those of Gould (1985, 1986) showing that increasing the complexity of a pattern within a constant local area increases the difficulty of discrimination, and also indicated that analysis of local regions is performed and integrated over the whole image unless they are too small to be evaluated. Zhang et al. (1992) have also shown that if bees are trained with differential conditioning, pattern discrimination depends on the visual angle that local and global cues subtend at the decision point of the bees: obviously, when local cues cannot be resolved because the bees are too far from the target, global cues predominate. However, their more interesting result is that, when local and global cues are set in competition at a visual angle in which both are resolvable, global cues prevail. In other words, bees trained with differential conditioning perform a global analysis of the pattern.
instead of focusing on local features, even if these are perceived. Finally, Ronacher & Duft (1996) have shown that choice of patterns by bees trained with differential conditioning may be explained by a template-matching mechanism similar to that occurring in Drosophila (Dill et al. 1993; Dill & Heisenberg 1995). A similarity function that takes into account the overlap between the template stored and the perceived retinal image and the total area of the actual retinal image was able to describe their results. Thus, the presence of both a reinforced and a non-reinforced pattern leads, in this case too, to recognition over the whole image of a pattern. This assertion is also valid for the operant learning shown in Drosophila melanogaster flying stationary inside a flight simulator (a cylinder displaying a given panorama around the fly; Dill et al. 1993). In this case flies were trained with two discriminative stimuli (e.g. an upright and an inverted T), each one presented in one of the four quadrants of the cylinder. The flies learned to avoid particular directions of flight relative to the panorama because these were visually associated with one of the patterns and paired with a noxious stimulus such as heat from an infrared light source. The directions allowed were, therefore, associated with the alternative pattern. Patterns were learned as a whole and vertical displacement of the same associated with the alternative pattern. Patterns were learned by only 9° suppressed recognition (i.e. avoidance of the hot areas of the cylinder) because of the lack of area overlap between the memorized and actually perceived patterns.

Note that in concluding that the conditioning procedure determines whether bees use one strategy of pattern recognition or the other, one cannot distinguish whether the template or the feature strategy was used (see Introduction). In fact, behavioral experiments may conclusively show that feature extraction occurs but not that pixel-by-pixel template matching must exist because the latter possibility is also consistent with the storage of the X,Y coordinates of a pattern in the retinal field as ‘features’. Extraction and recognition of a particular pattern feature may occur in the whole image or only in the lower ventral field. In some of the experiments cited above, bees were allowed to fixate the target, and, therefore, the building of a retinotopic template could be preferred (Wehner 1972; Gould 1985, 1986; Ronacher & Duft 1996). In others, fixation was impeded by forcing bees to make a choice from a distance (Zhang & Horridge 1992; Zhang et al. 1992). In all cases, the difference leading to a recognition task based either on the lower part or on the whole pattern is determined not by the possibility of fixating the patterns, but by the way the animals learned them and thus by the nature of the reinforcer.

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