

The preferences of the honeybee (*Apis mellifera*) for different visual cues during the learning process

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Abstract

By working with very simple images, a number of different visual cues used by the honeybee have been described over the past decades. In most of the work, the bees had no control over the choice of the images, and it was not clear whether they learned the rewarded pattern or the difference between two images. Preferences were known to exist when untrained bees selected one pattern from a variety of them, but because the preferences of the bees were ignored, it was not possible to understand how natural images displaying several cues were detected. The preferences were also essential to make a computer model of the visual system. Therefore experiments were devised to show the order of preference for the known cues in the training situation.

Freely flying bees were trained to discriminate between a rewarded target with one pattern on the left side and a different one on the right, versus a white or neutral target. This arrangement gave the bees a choice of what to learn. Tests showed that in some cases they learned two or three cues simultaneously; in other cases the bees learned one, or they preferred to avoid the unrewarded target. By testing with different combinations of patterns, it was possible to put the cues into an order of preference.

Of the known cues, loosely or tightly attached to eye coordinates, a black or blue spot was the most preferred, followed by strong modulation caused by edges, the orientation of parallel bars, six equally spaced spokes, a clean white target, and then a square cross and a ring. A patch of blue colour was preferred to yellow.

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1. Introduction

The concept that visual cues were detected by feature detectors, which were single neurons or groups of neurons near the periphery of the visual system, was popularized in the 1960s with evidence from the retinal ganglion cells of the frog and the primary visual cortex of the cat (Hubel and Wiesel, 1962). In the periphery of these visual systems, there were arrays of neurons that acted as detectors of edges, spots, edge orientation or directional movement. About the same time, it became apparent that similar ideas were essential for the development of artificial visual systems and could be expressed mathematically.

Adopting these ideas, and summarizing a variety of responses of ants and bees, Jander (1964) proposed that

simple peripheral feature detectors could be the universal mechanism in arthropod vision. Size or area had long been recognized as a cue (Hertz, 1933; Cruse, 1972; Anderson, 1977; Horridge et al., 1992). Modulation at the receptor level, caused by contrast at edges as the eye moved, was also demonstrated as a cue that was independent of pattern (Hertz, 1933; Horridge, 1997). When parallel bars were shuffled in position and spatial frequency during the learning process, the orientation was learned irrespective of pattern (Hateren et al., 1990). In fixed targets the bees learned to look at the places where the cues were presented during the training (Horridge, 2003a, 2005a). Another high priority cue for bees, the memory of the position of the centre irrespective of pattern, was discovered in experiments on the discrimination of shape. With thick fixed bars the bees detected the position irrespective of the shape or edge orientation, but with thin fixed bars the cue was the orientation (Horridge, 2003b). In radially symmetrical

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patterns, the local cues were radial or tangential edges irrespective of the rotation of the pattern (Horridge and Zhang, 1995; Horridge, 1996). The positions of the centres of symmetry, or hubs, of radial and tangential edges were also learned (Horridge, 2006a). There has recently been abundant evidence that these local cues were detected and learned independently together with the retinotopic coordinates where they were presented during the training (Horridge, 2003a, b, 2005a, 2006b). In no case was there any evidence that the various cues were re-assembled in the brain to form a spatial representation of the pattern on the retina. The trained bees responded in a variety of tests, irrespective of the pattern, because their feature detectors detected cues, not patterns.

The relation between the feature detectors and the cues became clear when the field sizes were related together. Feature detectors for receptor modulation subtended 2° , those for edge orientation subtended 3° (Horridge, 2005b), but the field sizes for the corresponding cues were larger. Feature detectors for colours were single receptors, but the photon flux was summed over many ommatidia in the discrimination of colour (Baumgärtner, 1928). The cues were the sums of the corresponding feature detectors within a larger field, and the total label on a pattern or landmark was a coincidence of cues (Horridge, 2006b).

Naturally, during the course of all this work, many of the patterns displayed more than one cue or more than one difference between the two targets in each choice. For example, size (measured as area), modulation (often measured as length of edge), and the position of the centre (the centroid) are features of all patterns in different proportions, although when they were similar on the two targets the bees learned to ignore them.

In most of the training experiments during the past century the patterns were selected by the experimenter, giving the bees no choice. In some cases the trained bees learned only one cue, as shown by failure when it was omitted, but in most cases the number of cues was unknown, because there was no information about the number of cues available, the preferences for different cues or the different speeds of learning them.

The present work was initiated as an exploration of this theme, using one experimental procedure with pairs of simple patterns that were closely related to the cues discovered in previous studies. The bees were trained with two simple patterns on the rewarded target, versus a white or a neutral target. The trained bees were tested with each of the two training patterns in turn, and with the positions of the two patterns reversed to see whether the relative positions had been learned. Other tests followed, as required.

2. Methods

The bees came from a local hive within 100 m of the experimental apparatus, and they could return in 10 min for another reward. The experiments were done in the

Y-choice apparatus (Srinivasan and Lehrer, 1988), modified by the addition of the baffles and a circular entrance hole 5 cm in diameter that helped to keep out newly recruited bees (Fig. 1). The apparatus was placed outside under a roof with an open front 3 m wide and 3 m high, with the targets facing bright daylight. The walls of the apparatus were of white card, the top was of clear Perspex. The baffles, of transparent Perspex, 1 mm thick, were set in a frame 1 cm wide with a 5 cm hole at the centre. They controlled the angle subtended by the target at the bees' decision point, and allowed the observer to make a sharp decision about the success or failure of each choice. The bees could also exit by walking under or over the baffle. The targets had a hole 2 cm in diameter at the centre, in positive ones for access to the reward and in negative ones leading to a blind tube.

The patterns were printed in black on standard white paper. Calibrations of the luminance of the white paper and the reflectance spectra of the coloured papers, Canson No. 595 blue and No. 384 fawn, were indistinguishable from those published with previous work (Horridge, 1999, 2000).

The bees were individually marked with a two-colour code. The reward was a fresh aqueous solution of sucrose with the concentration adjusted to keep the marked bees making regular visits without attracting recruits, which

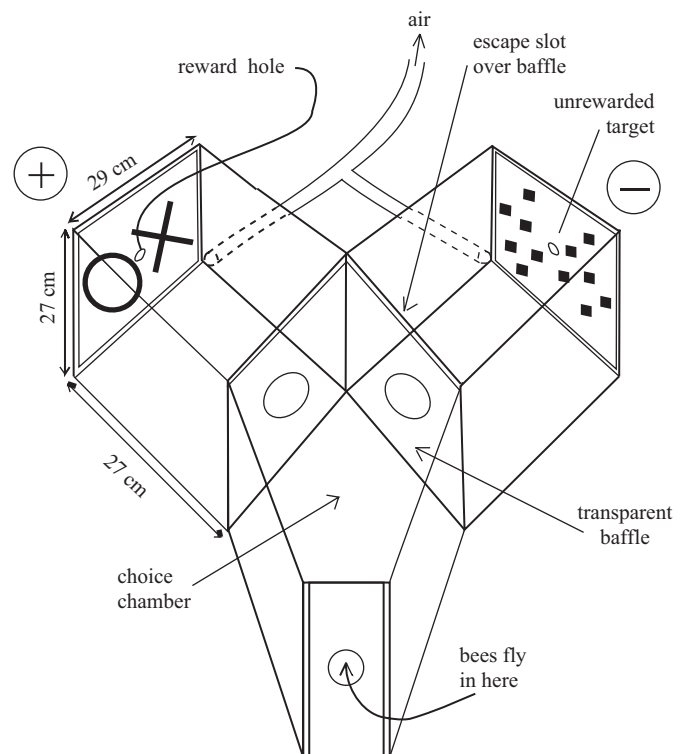


Fig. 1. The modified Y-choice apparatus. The bees entered at the front through a hole 5 cm diameter into a choice chamber from which they could see both targets. They selected one side, passed through or over one of the baffles, reached the reward hole, then when satisfied, exited by the way they came. To make the bees look at the patterns, the targets and the reward changed sides every 5 min. Odours were extracted by the air pipes.

differed from week to week depending on the weather and availability of natural nectar sources. During the training and tests the side of the positive target and of the reward with it were changed every 5 min to prevent the bees from learning which arm of the apparatus to choose, but in the illustrations the rewarded pattern (labelled +) was always shown on the left.

A small group of 10–15 bees from a local hive was trained to select one of the two targets while in flight in the central chamber (Fig. 1). They usually spent some time looking through the baffles. Each bee was identified by its colour code, and the criterion for a score was when it passed one baffle or the other. The bees required 20 or so visits to build up a memory of a single pattern. Only the first choice of each bee was recorded in each 5 min period. After an initial training period, the bees' first choices were counted in each period of 5 min while training continued. These results were labelled "train". To obtain results labelled "test", a different pair of patterns was substituted for those in the training, and the first choices were recorded for 5 min. All tests were made with one pattern rewarded, and then at a different time with the other pattern rewarded. In tests it was essential to give a reward, otherwise the bees continued to search. All tests were repeated with the patterns reversed in the two arms of the apparatus to compensate for possible side preferences. Different tests were interleaved between continued periods of training, and the trained bees did not repeat a test for some hours, so the trained bees did not become familiar with any one test. All the tests were distributed through the whole experiment. Each time the bees returned the patterns had been changed, so that their choices were independent. Testing in this way proceeds smoothly because the bees detect familiar cues in unfamiliar patterns.

Training began in the early morning and the experiment was usually completed within the week. The tests were continued between periods of training until 100–250 choices were counted for each test. By watching the bees in the choice chamber, one could see whether they decided quickly. If they were baffled they spent a long time examining first one target then the other. The main requirement was a decision whether the bees could or could not discriminate in the test, and there was no intention of comparing the abilities of bees trained on different patterns. Even the performances of the same bees in different tests are not usefully compared because they switch between different cues. In many of the tests the bees failed, so they were unlikely to learn new cues from that particular test. In the tests the bees got a reward after they had made their only choice in that 5 min period, and when they returned the patterns had always changed. There was then a long period of training and other tests before any test was repeated on the same bees, ensuring that choices were independent.

Two statistical calculations were made with samples of constant size. In the first, the correct choices were counted in each block of 20 successive choices, for the training or

the tests. The standard deviation (s.d.) between 10 and 20 of these blocks was calculated and placed after each significant score. The method was arbitrary because the size of the blocks was arbitrary, and any change in the performance during the repeated tests made the s.d. too large.

In the second method (Friedlaender, 1931), an estimate of the s.d. was the value of $\sqrt{[p(1-p)/n]}$ where p was the fraction of correct choices and n was the total number of choices. This method assumed that there were no trends during counting, that the individual choices were independent and they had a binomial distribution about the mean. The s.d. estimated from this formula was given in brackets after each score. By this method a score of 57% based on 200 choices was twice the estimated s.d. away from the null (random) hypothesis of 50%.

Almost all samples were of 200 successive choices, so the following p values give the significance of the difference from 50%. If the s.d. was used, $p < 0.05$ for a score of 57%, $p < 0.01$ for a score of 60%. If the chi-squared test was used, $p < 0.05$ for a score of 60%, $p < 0.01$ for a score of 62.5%, and $p < 0.002$ for a score of 65%.

3. Results

3.1. Training on two patterns versus a white target

Bees learn rapidly to go to a rewarded target that displays a pattern in one arm of the Y-choice apparatus in preference to a white target in the other arm. To investigate a preference for learning one simple pattern rather than another, the rewarded target displayed three vertical bars on the left side and a pattern of six spokes on the right (Fig. 2a). The bars and the spokes both displayed similar cues of modulation by edges, area of black, and position of the centre, but the bars displayed orientation strongly and the spokes displayed a radial cue. Scores over 90% were reached in 2 h of training. Between periods of continued training the trained bees were repeatedly given four different tests over the course of the day.

The trained bees could discriminate between the rewarded training pattern and the mirror image of the same pattern (Fig. 2b), but not very well, with a score of 67.0%. In explanation of this poor score, they may have learned to avoid the white negative target, or they omitted to learn which side of the target was occupied by the bars or the spokes. In next two tests, the bars alone or the spokes alone, were presented versus the white target (Figs. 2c, d). The scores were also rather poor, at 66.0% and 67.5%. These two results showed that the high score in the learning was not because the bees had learned to avoid the negative target, and also that the bars, the spokes and some avoidance of the negative were all required for good discrimination. The mirror image test (Fig. 2b) showed that the bees had learned something more than to go towards anything black, but none of these tests demonstrated a preference.

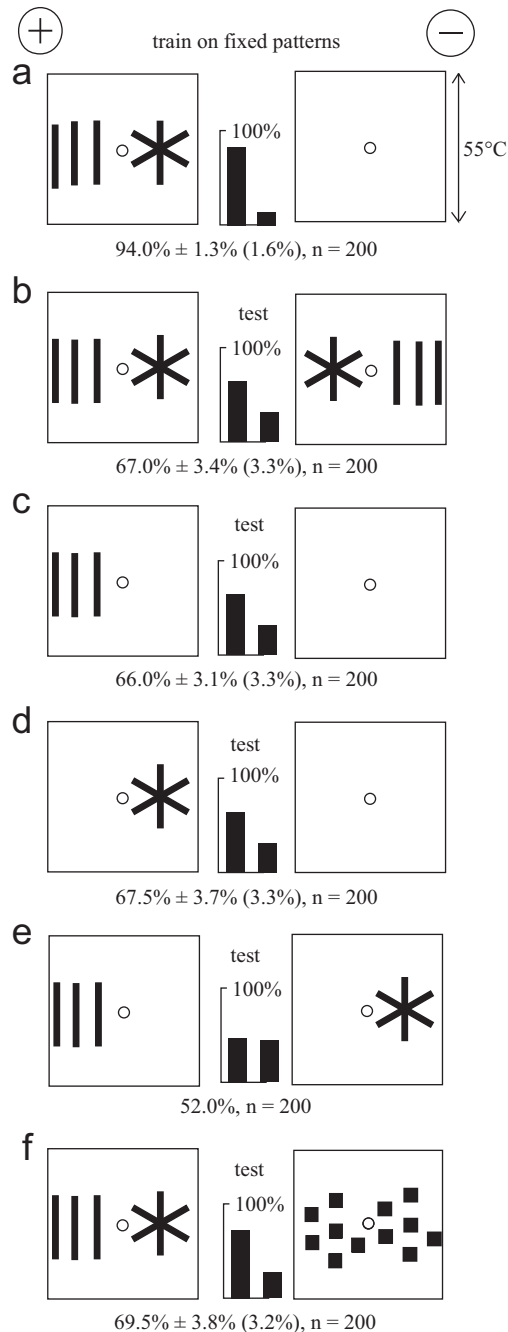


Fig. 2. Bars and spokes were equal cues of medium priority and the relative positions on the rewarded target were learned. (a) Training on three vertical bars and six spokes versus a white target. Learning was rapid and to a high score. (b) The rewarded training pattern was discriminated from the mirror image of the same pattern. (c) The score with the bars alone was much worse than in the training. (d) The score with the six spokes alone was also much worse than in the training. (e) There was equal preference for the bars and the spokes. (f) The high training score was partially due to attraction for black and avoidance of the blank.

With the bars on one target and the spokes on the other target, each on their proper side, the trained bees again showed no preference (Fig. 2e). Examination of the scores of individual bees revealed no suggestion that some bees preferred one pattern while others preferred the other.

In tests with a neutral pattern of spots versus the white target, it was clear that the bees had learned the bars and the spokes, but not well because the test score was very poor compared to the training score (Fig. 2f). This experiment therefore suggested a similar preference for the bars and the spokes.

3.2. Training on two colours versus a blank

In this experiment, the rewarded target displayed a large fawn spot (subtending 20° from the point of choice) on the left side and a blue spot of the same size on the right (Fig. 3a). The fawn spot was made of Canson 384 paper and the blue was Canson 595, as documented in previous papers (Horridge, 1999, 2000). The intention was not to determine a scale of preference for different colours during learning, but to demonstrate that bees can have a strong preference against learning two colours simultaneously or choosing between them. The spots had similar areas and equal contrast to the green receptors against the background, so they would excite the motion detector system equally, but they differed strongly in the contrast to the blue receptors, and of course, in their chromatic contrast. The unrewarded target was plain white paper. The bees clearly found this task difficult. They scanned the targets repeatedly and at first refused to choose. Scores of only 75% were reached in 3 h of training. Between periods of continued training the trained bees were repeatedly given five different tests over the course of the next few days.

With a choice between the rewarded training pattern and an irregular pattern of black squares (Fig. 3b), the score fell to 57.5%, showing that the bees did not readily recognize the coloured pattern that they were trained on. This was a surprising result. Moreover, when tested with the irregular pattern of black squares versus a white target, they avoided the white target with almost the same score as in the training (Fig. 3c). Therefore, in the training, they must have ignored the colours and learned to avoid the white negative target. This result shows that the choice of which target the bees learn depends on their preferences for the cues that are available, and not on the conditioning procedure.

Given a choice between the coloured training pattern and its mirror image (Fig. 3d), the bees could not discriminate, with a score of 55.5%. The white target was no longer available as a cue, and they had also omitted to learn which sides of the target were occupied by the two colours.

Next, the trained bees were tested with the fawn or the blue spot alone on its proper side of one target versus the white target (Figs. 3e, f). In both cases the trained bees discriminated well, as they would if they had learned to avoid the white target.

Finally, the trained bees were tested with the fawn spot on the left versus the blue spot on the right, in an analogous test to that in Fig. 2(e). The bees were totally confused by the test, as if they looked for the white target.

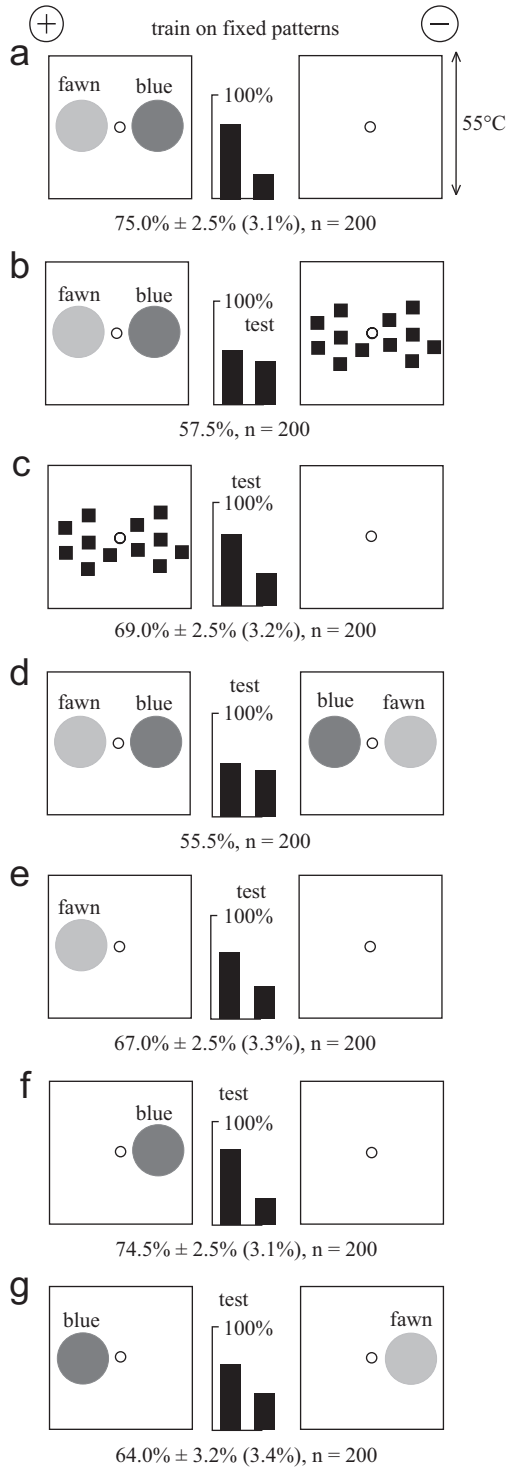


Fig. 3. With two differently coloured spots on one target the bees learned to avoid the white unrewarded target. (a) Training with a fawn and a blue spot on the rewarded target versus a white target. (b) Poor discrimination with the training target versus a pattern of small black squares. (c) The trained bees preferred an unfamiliar pattern of black squares to the white target. (d) The trained bees could not discriminate between the training target and its mirror image. (e,f) With a single spot in its expected position versus the white target the blue spot gave a larger score than the fawn spot. (g) The trained bees preferred the blue spot, as they would with no training at all.

When they finally made a choice they showed a slight preference for the blue spot (Fig. 3g).

It was concluded from this experiment that the bees simply learned to avoid the negative target which had none of the familiar cues. It is well established, however, that when trained with a forced choice, bees discriminate between the same fawn and the blue spots (see Fig. 4), and they learn to discriminate between two targets that display different layouts of these two colours (Horridge, 2000). In this example (Fig. 3a), for some reason, the bees preferred not to learn to go to the target with the blue and fawn spots. Earlier work has shown that, for successful discrimination, the eye must be stabilized in the horizontal plane, so that the two colours are presented consistently at different places on the eye (Horridge, 1999). In Fig. 3a, therefore, the bees did not first stabilize their eyes on the target.

3.3. Training on a large fawn spot versus a blue one

To show that the bees can discriminate the fawn spot from the blue one, and to investigate the preference when the bees had no choice of the patterns in the learning

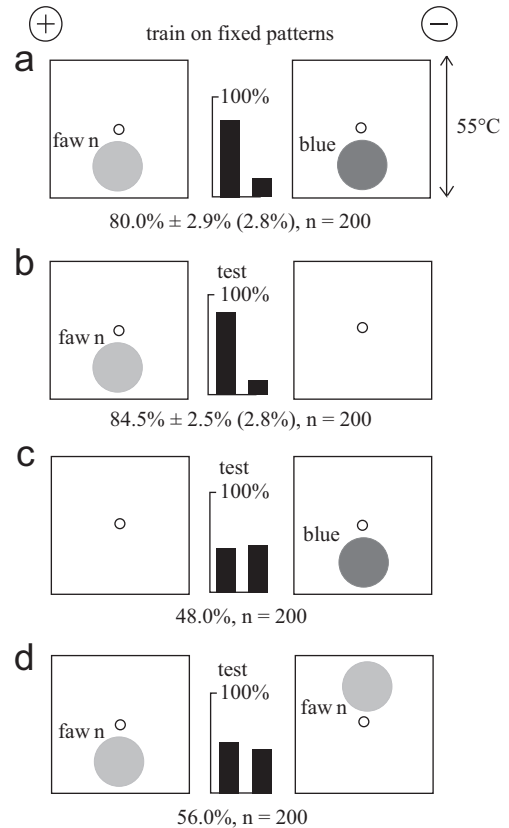


Fig. 4. Two large spots of different colours were not learned equally. (a) With a fawn spot versus a blue spot, in corresponding positions on the two targets, learning was rapid and to a high score. (b) With the fawn spot tested versus a white target, the score was better than in the training. (c) With the white target tested versus a blue spot, the bees could not discriminate. (d) Although the trained bees had learned to recognize the fawn spot, they could not remember its position during the training.

process, the bees were trained with the fawn spot rewarded versus the blue spot (Fig. 4a). When the training started, the bees preferred the blue spot, and took an hour to reach a score of 50%, and 2 h to reach 80% correct. As shown by the tests, there was still a residual innate attraction of the trained bees for the blue spot on the negative target.

When tested with the fawn spot versus a white target, the score was larger than the training score because the innate attraction of the blue spot was removed (Fig. 3b). When tested with the white target versus the blue spot (Fig. 4c), the score was 48.0%, showing that the trained bees had learned to overcome the initial preference for the blue spot. The bees arriving in the apparatus were slow to make a choice, and their behaviour suggested that they were searching for the fawn spot. If the bees had learned to avoid the blue spot completely, the score would have been much less.

When tested with a fawn spot in the expected place, versus a fawn spot at the top of the target (Fig. 4d), the score was 56%, showing that they had not learned well the position of the fawn spot on the training target. In conclusion, the bees in training learned to ignore the blue spot and to look for the fawn one, but the initial preference for blue was reduced, not removed.

3.4. Training on two patterns versus 12 squares

In the following experiments, the unrewarded target displayed a neutral pattern of 12 black squares. Similar cues of position, black, and modulation by edges were therefore presented on both targets. The bees were less likely to learn these cues displayed in common and were more likely to reveal preferences for other more useful cues.

3.4.1. Bars and spokes

In this experiment, the rewarded target displayed three vertical bars with an obvious orientation cue on the left side and a pattern of six spokes with an obvious radial cue on the right. The unrewarded target displayed an irregular pattern of six small squares on each side (Fig. 5a). This was a difficult learning task and the score reached 64% after 3 h of training. Between periods of continued training the trained bees were given three different tests.

With a choice between the rewarded training pattern and the mirror image of the same pattern (Fig. 5b), the bees could discriminate as well as in the training, with a score of 66.0%. Therefore (as in Fig. 2) they had learned the relative positions of the bars and the spokes, and they did not rely on avoidance of the negative target. In next two tests, the spokes or the bars, together with some squares, were presented versus the pattern of squares (Figs. 5c, d). The scores were also similar to those in the training, at 63.5% and 68% correct.

These results demonstrated that the bars and the spokes were equally involved in the learning process, as in Fig. 2. Therefore the unrewarded target had no influence on the preferences. Either of the cues was sufficient for the

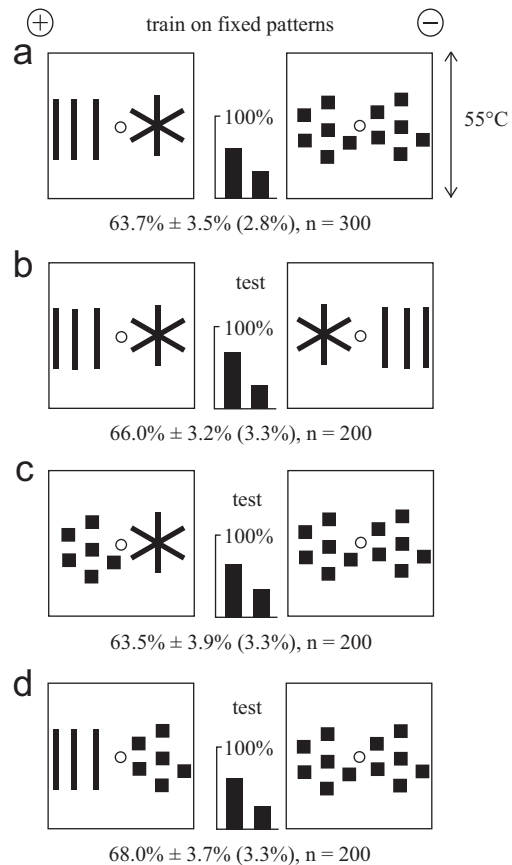


Fig. 5. Bars and spokes were both medium priority cues and the relative positions on the rewarded target were learned. (a) Training on three vertical bars and six spokes versus 12 squares. Learning was slow and moderate. (b) The rewarded training pattern was discriminated from the mirror image of the same pattern, showing that they had learned the relative positions of the cues and did not rely on the unrewarded pattern. (c) They had learned to recognize the six spokes. (d) They had learned the three vertical bars as well as the spokes.

discrimination, and the bees did not rely on avoidance of the unrewarded target (Fig. 5b).

3.4.2. Spokes and strong modulation

The spokes were the same as in the last experiment. They displayed cues of area of black, the position of the centre of black, modulation at edges, and a radial cue. The second pattern consisted of 26 small black squares which provided only three known cues for the bees, the total area of black, the position of the centre of the combined black area, and the strong modulation caused by movement of the eye across the large total length of edge (Fig. 6a). The unrewarded pattern was the same pattern of 12 squares as before. With this combination, the bees were slow to learn and achieved a score of 69.0% after 3 h of training.

When tested with the rewarded training pattern and the mirror image of the same pattern (Fig. 6b), the trained bees could not discriminate at all, with a score of 51%, showing that they had not learned the relative positions of the two patterns. When tested with the six spokes versus the

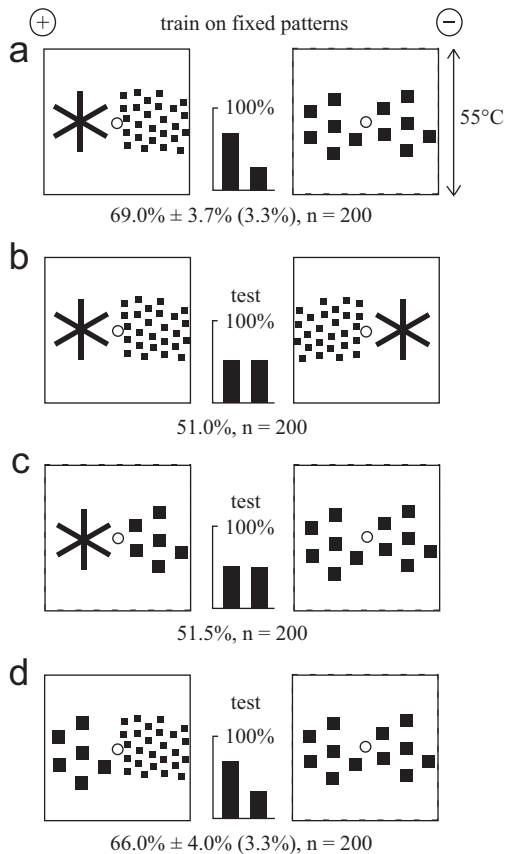


Fig. 6. Strong modulation was a higher priority cue than spokes, and their relative positions were not learned. (a) Training on six spokes and 26 small spots versus 12 squares. Learning was slow and moderate. (b) The relative positions were not learned. (c) The six spokes in their expected position were not detected when tested versus the unrewarded pattern. (d) The 26 small spots alone were discriminated as well as the training pattern, suggesting that they comprised the whole cue.

unrewarded pattern, the result was the same, failure to discriminate (Fig. 6c). This showed that they had not learned to avoid the unrewarded pattern. However, when tested with the numerous small squares versus the unrewarded target (Fig. 6d), the score was similar to that in the training, showing that the whole of the learning was due to the strong modulation cue.

3.4.3. Strong modulation and a large black spot

In this experiment the patch of small squares was combined with a large black spot (subtending 15°) versus the same negative target as before (Fig. 7a). The patch of small squares displayed strong modulation while the spot displayed an obvious area of black with little modulation. Compared to the previous experiments, learning was rapid and the score reached 85% after 3 h training.

When tested with the rewarded training pattern and the mirror image of the same pattern (Fig. 7b), discrimination was very poor, with a score of 58%, showing that the bees had learned little about the relative positions of the two patterns. When tested with the spot versus the unrewarded pattern (Fig. 7c), the result was 74%, and when tested with

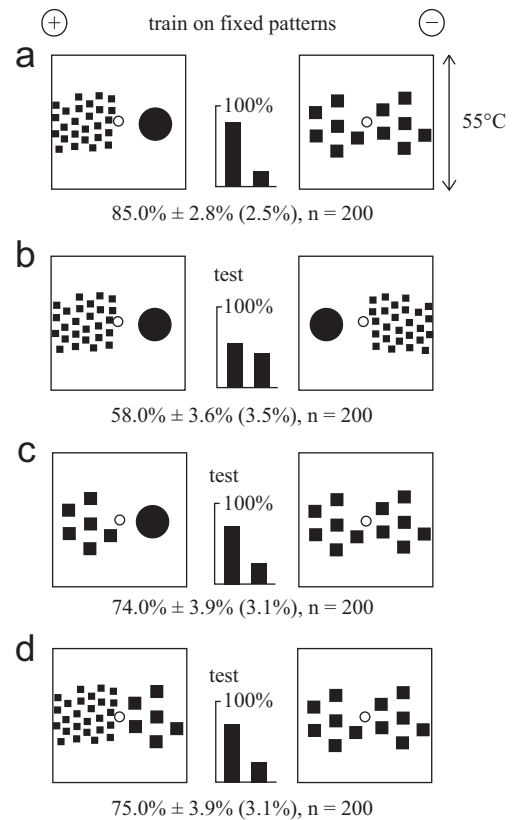


Fig. 7. Strong modulation and the black spot were both high priority cues but their relative positions were not learned. (a) Training on 26 small spots and a large black spot versus 12 squares. Learning was rapid and to a high score. (b) The rewarded training pattern was scarcely discriminated from the mirror image of the same pattern. (c) The large spot alone was discriminated very well. (d) The 26 small spots alone were also discriminated very well.

the numerous small squares versus the unrewarded pattern (Fig. 7d), the result was 75%.

These results showed no distinction between the contribution of the small squares and the black spot, and the bees did not rely on avoidance of the unrewarded pattern. It was concluded that the modulation and the black spot were both strong signals that provided equal cues, but they were poorly localized.

3.4.4. Three vertical bars and a large black spot

In this experiment the three vertical bars (as in Fig. 5) were combined with a black spot (subtense 15°), versus the same negative target as before (Fig. 8a). The vertical bars displayed a strong orientation cue while the spot displayed an obvious area of black. As in the previous experiment, learning was rapid and the score reached 83% after 3 h training.

When tested with the rewarded training pattern versus the mirror image of the same pattern (Fig. 8b), discrimination was excellent, with a score of 75.5%, showing that the trained bees had learned something about the relative positions of the two patterns. When tested with the spot on

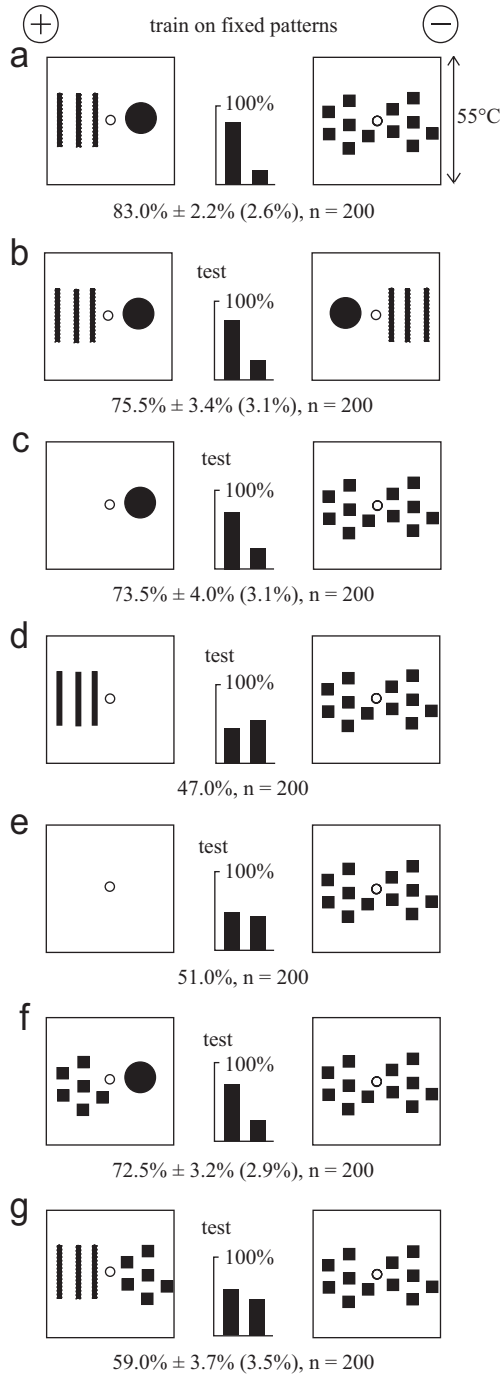


Fig. 8. When trained on parallel bars and a spot on the rewarded target, the bars contributed nothing. (a) Training on three vertical bars and a large black spot versus the pattern of 12 small squares. (b) The rewarded training pattern was well discriminated from the mirror image of the same pattern. (c) The spot alone was well discriminated. (d) The bars alone were not recognized. (e) A white target was not discriminated from the pattern of squares, showing that the bees had not learned to avoid the latter. (f, g) The spot was recognized but not the bars when there was an equal amount of black on both test targets.

a white background versus the unrewarded pattern (Fig. 8c), the result was 73.5%, but when similarly tested with the three bars versus the unrewarded pattern (Fig. 8d), the result was only 47.0%.

These results showed that the bees did not rely on avoidance of the unrewarded pattern (Fig. 8b), and their success in learning depended on the black spot (Fig. 8c). The same conclusion came from the results of tests with a white target versus the negative pattern (Fig. 8e), and from tests with the spot or the bars with equal amounts of black on the two targets (Figs. 8f, g). It was concluded that the black spot provided a strong signal but the bars contributed little to the learning process. It was also observed in the above experiments that the bees had not learned to avoid the negative pattern of 12 black squares when no other cue was available (Fig. 8e).

3.4.5. Symmetry and orientation cues in symmetrical oblique bars

In a search for the preferences between the cues of symmetry and edge orientation, bees were trained to go to a symmetrical pattern of six thin oblique black bars versus the same negative target as before (Fig. 9a). The bars offered a strong symmetry cue that was readily open to analysis. A score of 72% was achieved after 2 h of training.

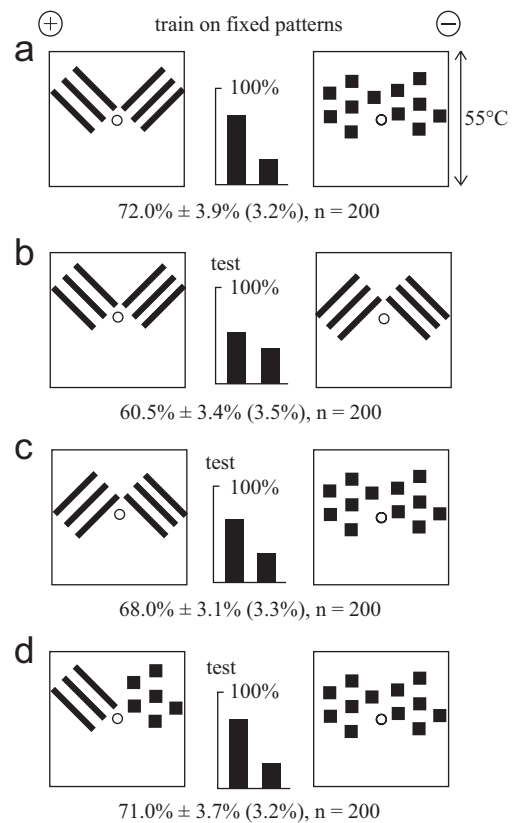


Fig. 9. Strong bilateral symmetry is a stronger cue than edge orientation. (a) Training on three oblique bars and three orthogonal bars versus 12 squares. Learning was moderate but definite. (b) The rewarded training pattern was discriminated weakly from the same pattern rotated by 180°, showing that they had learned some orientation cues in the rewarded pattern. (c) The rewarded training pattern rotated by 180° was well discriminated from the 12 squares, suggesting that the length of edge or the symmetry, or both, was a high priority cue. (d) The three parallel bars were discriminated, suggesting that the bees had learned the orientation of the bars in the expected place.

When tested with the rewarded training pattern versus the same pattern rotated by 180° (Fig. 9b), the score was 60.5%, showing that the trained bees had learned something about the relative positions of the two orientations. When tested with the rotated pattern versus the negative training pattern (Fig. 9c), discrimination was similar to that in the training, suggesting that the preferred cue was the symmetry and not the edge orientations. As before, the discrimination did not rely on avoidance of the negative target.

When one orientation alone was presented in its expected location versus the negative target (Fig. 9d), the score was similar to that in the training, showing that the orientation was also a powerful cue (or else the symmetry would not be a strong cue). The lower score in Fig. 9b was because the symmetry was detected on both targets, and the orientation cues were partially cancelled by the edges at right angles to each other (Horridge, 1996).

3.4.6. A large spot and a small spot versus the negative target

In this experiment, the rewarded target displayed a large black spot (subtending 15° from the point of choice) on the left side and a small black spot (subtending 5°) on the right. The questions were whether the bees preferred the small or the large spot, or both, and whether learning of spot position depended on spot size. The unrewarded target was 12 black squares as before (Fig. 10a). It was clear from their behaviour that the bees found this task very easy, and scores of 75% were reached in 2 h of training and 89% in 3 h. Between periods of continued training the trained bees were repeatedly given seven different tests over the course of the next few days.

When tested with the rewarded training pattern versus the mirror image of the same pattern (Fig. 10b), the score was 69.0%, showing that the trained bees had learned the position of the centre of the black area or the expected position of the large spot. It did not demonstrate that the two spots were separately detected (Horridge, 2003b).

When tested with the large spot on a white background versus the unrewarded pattern (Fig. 10c), the result was 83.0%, but when similarly tested with the small spot versus the unrewarded pattern (Fig. 10d), the result was only 54.5%. These results suggested that the small spot counted

for little, and the bees did not rely on avoidance of the unrewarded pattern.

When tested with a white target versus the negative pattern (Fig. 10e), the score was 54.0%, and when tested with the rewarded training pattern versus a white target

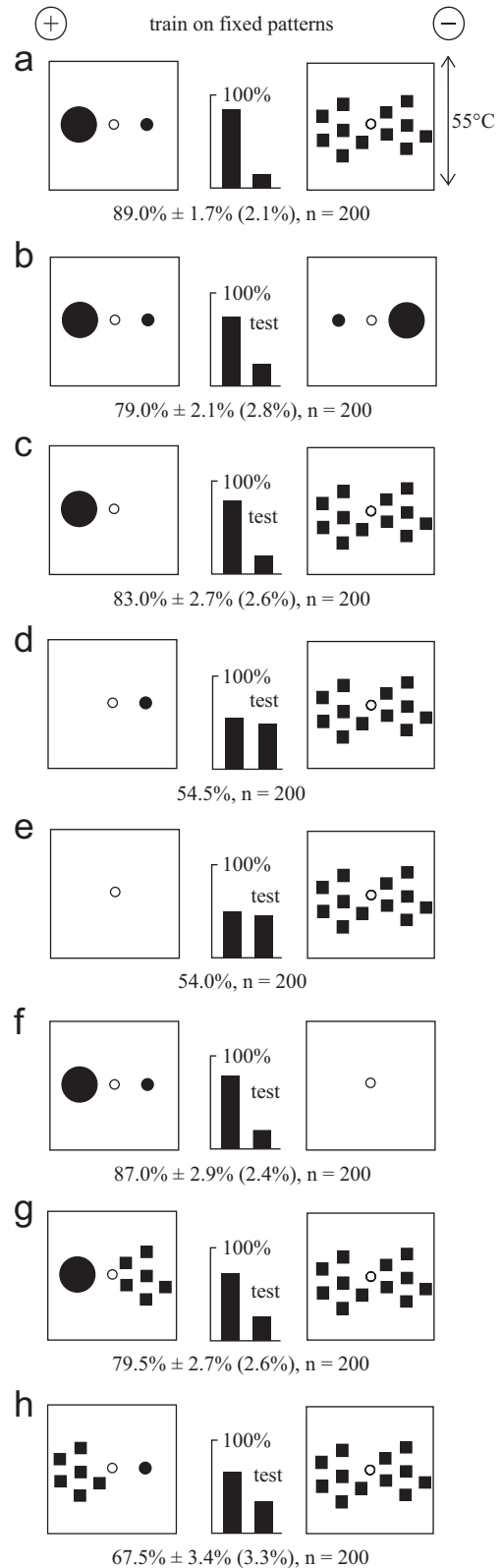


Fig. 10. A large and a small black spot on the same target were not separated, so this method did not measure a preference. (a) Training on a large and a small spot versus the pattern of 12 squares. (b) The rewarded training pattern was discriminated from its mirror image. (c) The large spot alone was well discriminated from the 12 squares. (d) The small spot alone was not recognized and the trained bees did not avoid the 12 squares. (e) A white target was discriminated from the 12 squares, suggesting that the bees had learned to avoid the latter. (f) The rewarded training pattern was well discriminated from a white target. (g) The large spot with six added squares was well discriminated from the 12 squares. (h) The small spot with six added squares was also discriminated from the 12 squares, suggesting, along with the other results, that the cue was the position of black on the left of the target.

(Fig. 10f), the score was 87.0%, which was similar to the training score. These two results again showed that the trained bees had learned the position of the large spot but not the negative pattern.

The trained bees discriminated when tested with the large spot in its expected position and additional black squares on the test pattern to help balance the modulation and amount of black on the two sides (Fig. 10g). The same arrangement with the small spot was weakly discriminated from the negative training target (Fig. 10h). The results from all these tests put together showed that the preferred cue in the rewarded training pattern was the position of the large spot (as in Fig. 8) with a smaller contribution from the greater modulation in the unrewarded pattern.

3.4.7. A ring and a cross versus 12 squares

With a large ring and a large cross on the rewarded training target versus the negative target as before (Fig. 11a), there was no learning. The score was 49.0% after 4 h training and did not rise above 54% despite training for the rest of the day. Although the ring, the square cross and the pattern of squares were familiar patterns that looked totally different to human eyes, they provided only the cues of black area, length of edge or modulation, and position of the centre, all of which in this case were similar on the two targets. Therefore the bees did not detect the ring or the cross because they could detect no cues that were sufficiently different on the two targets. This result illustrated dramatically the anti-intuitive nature of honeybee vision, which detects cues and their positions, not patterns. It also illustrated the usefulness of the 12 squares as a neutral pattern with no specific cue.

3.4.8. Training on a ring and a cross versus a blank

To investigate the ring and the cross further, a group of bees were trained with the ring on the left and the cross on the right of the rewarded target versus a white unrewarded target (Fig. 11b). Learning was rapid and to a high score of 85.0% after 2 h training. When the trained bees were tested with the 12 squares versus a white target (Fig. 11c), the score was 78.5%, which suggested that the bees had learned to go to black or to avoid the negative target.

When the rewarded target was tested versus the 12 squares (Fig. 11d) the result was 51.0%, which suggested that the trained bees had learned nothing about the shapes of the ring or cross. When tested with the rewarded training pattern versus the mirror image of the same pattern (Fig. 11e), the score was 60.5%, possibly showing that the trained bees had learned a little about the relative positions of the centres of the ring and the cross (Horridge, 2006b).

When the ring alone was tested versus the same ring moved up on the target (Fig. 11f), or the cross in its expected position was tested against the same cross moved upwards (Fig. 11g), the bees detected the difference in the position of black in each case, but they would have done this whatever the pattern (Horridge, 2003b).

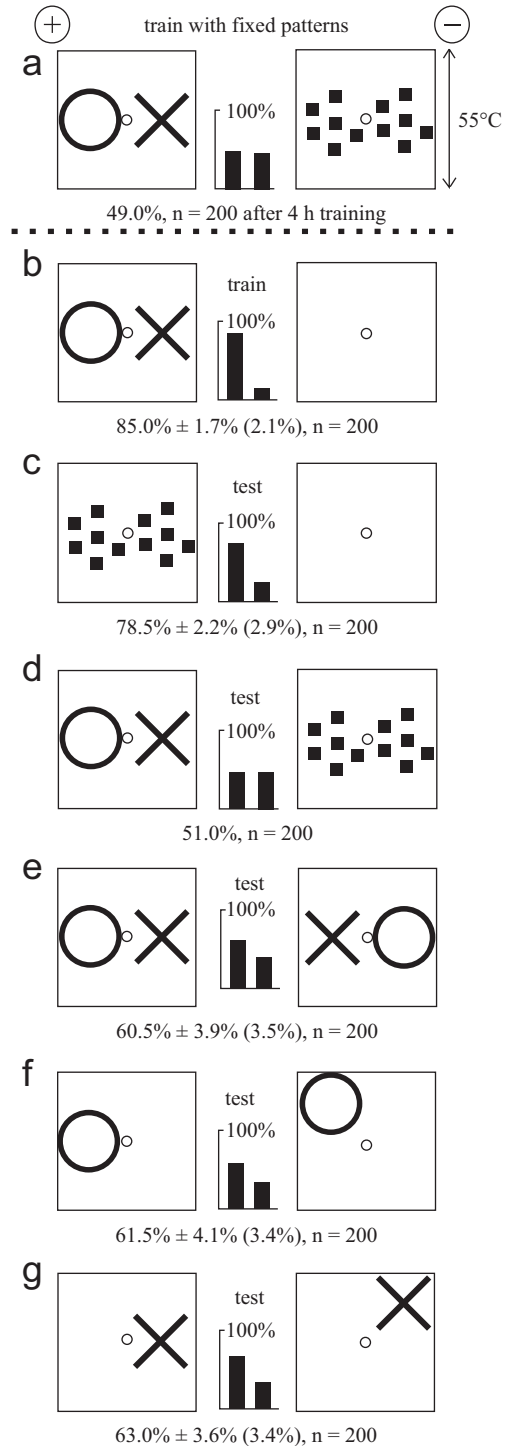


Fig. 11. Two obviously different patterns displaying similar non-specific cues. (a) The bees failed to learn to discriminate the ring and the cross on one target from the 12 squares on the other. (b) The bees readily learned to discriminate the ring and the cross on one target from a white target. (c) The trained bees discriminated the 12 squares equally well from the white target. (d) The trained bees failed to discriminate the ring and the cross from the 12 squares. (e) The ring and the cross were poorly discriminated from the mirror image, suggesting that radial and tangential edges were distinguished a little. (f, g) The ring and the cross were separately discriminated from the same moved upwards on the target, so the position of the black areas had been learned.

It was concluded from this experiment that the bees had learned to go to black or to avoid the unrewarded target, and the results confirmed earlier conclusions that, when offset from the centre, the square cross and the ring offered no cue other than black, position, and modulation at the edges.

4. Discussion

4.1. *The bees learned coincidences of cues, not patterns*

As shown in many earlier training experiments, the bees learned coincidences of a few simple cues and their positions, not patterns. When given an appropriate test, the trained bees could not recognize the pattern that they were trained on (Figs. 3b, c, 4c, 6b, 7b, 8e, 11a, d). If they were able to discriminate, one or more of the known simple cues was always available, and if they were unable to discriminate, none of these known cues were different on the two sides.

Some of the cues, such as the large black or coloured areas and the strong modulation, were detected in quite large fields, as shown in tests when the bees were unable to remember their positions. The feature detector for colour, however, is a single receptor type, that for modulation subtends only 2° , and the feature detectors for edge orientation subtend about 3° (Horridge, 2005b). The cues are therefore composed of numerous identical feature detectors that are excited in each array and summed in fields of various sizes. Some of the cues, such as modulation and area of black, were clearly quantified as well as having an order of preference.

4.2. *Preferences*

In previous experimental work the patterns were selected by the experimenter, but the preferences of the bees during the learning situation had an effect because the images always displayed more than one cue, and the bees learned first the most preferred of them. Even so, often it was not clear whether the bees learned the rewarded pattern, the difference between the rewarded and the unrewarded patterns, to avoid familiar cues on the unrewarded target, or to avoid unfamiliar cues that were not in the training patterns at all. More to the point, it was not clear how many cues were learned in parallel and in what order. This situation was due to the lack of sufficient tests of trained bees, which in turn was a fault of the design of the experiments.

The preferences were evident in earlier work where bees were trained to come to randomized checkerboard patterns and then tested with a variety of unfamiliar patterns. When test patterns were of the same kind, the bees preferred low over high spatial frequencies, i.e., large over small areas of black or colour (not strong modulation). When patterns were of different types, the bees preferred patterns radiating from a centre and avoided circular patterns or

random patterns. They also preferred symmetry to asymmetry (Lehrer et al., 1995).

Very early on, it was accepted that bees would readily learn to come to the patterns that they spontaneously preferred (Hertz, 1933). Preferences also revealed themselves as differences in the rate of learning and the maximum score achieved. Easy patterns were linked with preferred cues, but comprehensive experiments on preferences were not done because most of the cues had not been described.

Preferences were easily demonstrated by the new strategy. Single black spots and strongly modulated patterns were powerful cues, irrespective of the pattern. Large black spots were stronger cues than small spots. Radial spokes and parallel bars were weaker cues, similar in strength. The bees could learn a difference between radial and tangential edges, and the positions of their centres of symmetry, but could not remember the patterns. A ring, a square cross, or a group of small squares provided cues of area of black, modulation and position of the centre of black, none of which were unique to these patterns. With circular patterns the bees learned to overcome an avoidance tendency. Symmetry in a pattern of bars was preferred as a cue over the edge orientations that generated the symmetry.

When a weak and a strong cue were presented together, the weak one was scarcely noticed. The same principle applied to colours. When two colours were presented side by side on the rewarded target or on separate targets, the bees had difficulty in learning both at the same time. They learned the blue in preference to the fawn or yellow, even if this implied learning to avoid the blue.

Results were often anti-intuitive, e.g., parallel bars and radial spokes were ignored in preference to modulation or a spot. The bees had difficulty with two colours centred at the same horizontal level. When presented with a pattern on each target they ignored the cues that were displayed on both targets. When no preferred cue was associated with the reward, they learned to avoid a white unrewarded target. There was nothing in the results to suggest that the bees learned the layout of a pattern or anything more than the detection and memory of the same independent local cues that have been previously described (Horridge, 2005a, b, 2006a, b).

4.3. *Salience versus retinotopic cues*

In previous work, when a broad black bar or spot was moved more than 10° after training, the bees did not recognize it in its new place, showing that the memory was a map of the identified locations (Horridge, 2003a). In the examples described above, with three parallel bars or a large black spot (Figs. 2, 5, 8, 10) the training pattern was distinguished from its mirror image, so the fields of these cues must be restricted to one side of the target. In the case of the modulation cue (Figs. 6 and 7), even though the training was successful, the field was clearly much larger.

With the symmetry cue (Fig. 9) quite different images were confused because the symmetry was displayed on both sides.

The sensitivity to displacement was related to the field size of the cue. Cues of orientation were more retinotopic and therefore detected in smaller fields; modulation was detected over larger displacements and therefore in larger fields, so that it was more salient. Small fields implied some failures to detect; large fields implied some failures to localize and loss of resolution, but improved salience. Each cue had its own field size that was a compromise between salience and localization.

4.4. *The ring and the cross*

In earlier experiments with the large black centred ring versus a white target, the bees learned only to avoid the white target. Discrimination failed in a test with the ring versus a pattern of spots, or any other pattern of similar area and edge length. When the bees were trained with the centred ring versus the centred cross, again discrimination failed in tests with the ring or the cross versus a pattern of spots (Horridge, 2006a). Unpublished tests showed that in the training the bees had learned the presence or absence of black close to the reward hole at the centre. The bees would not learn to recognize the centred ring as a ring.

Similarly, bees could not be trained to discriminate a centred square cross from the same cross that was rotated by 45° (Srinivasan et al., 1994), and when bees were trained with a centred square cross versus a white target, they could not discriminate the cross from different patterns of similar size (Horridge, 2006b).

The ring, the cross, and the pattern of squares or spots are examples of patterns that display similar cues of area of black, position of black and position of the centre (and nothing else found so far), so are discriminated with difficulty although they look very different to the human eye (Fig. 11a). Bees learn to discriminate between the centred ring and cross only by the black around the reward hole, as shown by failure in tests when they are both offset equally from the centre. It was not surprising, therefore, that the bees showed no preference for these patterns, but preferred to learn to avoid a white target.

4.5. *Training on one or two patterns*

When the bees were trained with a pattern on each side of one target versus a white target or versus a neutral target, they usually learned several cues in order of preference. They had a low preference for learning to avoid the pattern of 12 squares but would do so when the rewarded pattern displayed fewer cues (Fig. 10e). They would learn to avoid the white target when the rewarded pattern was of two different colours, a square cross, or a ring (Figs. 3 and 11).

The preferences for parallel bars and six spokes were not influenced by the training regime, as shown by the close

similarity between the scores in Figs. 2 and 5. Similarly, the difficulty of learning two colours at the same time, and the preference for blue, was not influenced by whether they were on two targets or one (Figs. 3 and 4). Training on a rewarded pattern versus a blank, however, in some cases gave a false impression that the bees learned the rewarded pattern (Fig. 11b). This was sufficient reason to use a neutral pattern for the unrewarded target.

An aspiring critic will already have noted that the bees may have looked at the two targets with the same part of one eye, or at the centre of each target so that each eye looked at its own half of each target; or any part of the eye may have been used to look at either side of either target. Therein lies a good deal of future observation that would relate to the question of differences between eye regions and other retinotopic processing of the inputs, but this cannot be done before the cues and their preferences have been described because it is essential to understand what the bees actually detect.

When like was compared with like, and there were sufficient tests to reveal exactly what the bees detected, the same preferences for the same few cues were found. There were no coded descriptions of patterns, and at each training the bees learned afresh only the cues for one task. There was no need for a large memory or any kind of higher processing.

5. Conclusion

These simple experiments point the way towards more exhaustive measurements of the preferences for the known cues in a wider variety of images in the natural environment. Also, the preferences were required to be incorporated into a computer model of the visual system of the honeybee. A successful model, if taken into the bees' environment, might give us some idea of what the bees normally detect there, or at least, what they remember about what they detect.

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