Visual resolution of the orientation cue by the honeybee (*Apis mellifera*)

Adrian Horridge *

Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, ACT 2601, Australia

Abstract

Bees were trained to discriminate between a pattern with two or more black bars and a similar pattern with the bars at right angles. Earlier measures of the resolution of oblique black and white regular gratings of different periods were confirmed. The positions of the training bars were shifted every 5 min to prevent the bees from using their locations as cues. To measure the length of the detectors of edge orientation, the trained bees were tested with targets filled with parallel short black/white edges of various lengths. The minimum individual length of edge required to discriminate the orientation cue was found to be near 3°, and similar for vertical, horizontal and oblique edges. This is the first time that this kind of resolution has been measured in an invertebrate. The bees learn and recognize the edge orientation, not the lay-out of the pattern.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Honey bee; Resolution; Orientation; Pattern perception

1. Introduction

Bees are easily trained to discriminate between two targets, one of which presents a fixed black bar or group of parallel black bars on a white background and the other an identical target rotated by 90°, presented on a vertical surface. When there are two or more parallel bars on each target, the locations of the edges are shuffled on the target during the training so that the bees cannot use the position of an area of black on one or the other target as the critical cue (van Hateren et al., 1990). Under these conditions, the bees actually learn to discriminate the cues from the edge orientations only in the places on the targets where they occurred during the training (Horridge, 2003a). A cue is a feature, parameter or part of the pattern that is much less than the whole pattern. The summation of parallel orientation cues over large areas aids in the detection of the orientation of gradients at blurred edges (Horridge, 2000a). A current model is that the fields of vision of the orientation detectors are large, and appear to spread over the whole eye on each side, as inferred from the large angles over which there is mutual cancellation of the orientations of equal lengths of edges at right angles (Srinivasan et al., 1994).

The summation of the orientation cue over several parallel edges provides the opportunity to determine the resolution of edge orientation by presenting trained bees with patterns composed of many short edges of equal lengths in parallel. The individual lengths are then progressively reduced in tests of resolution. Measurements of this kind have not been made previously on any invertebrate. To do this, we must select training patterns from which the bees learn only the orientation cue and which will also dispose them to respond to the tests. Measuring the resolution is a convincing demonstration that the bees see the cues that they resolve, although it cannot provide evidence that the bee remembers the spatial lay-out of the training pattern.

The training pattern is designed to avoid giving the bees an opportunity to learn cues other than the orientation of edges, such as size, disruption of the pattern, and edges that are symmetrical, radial or tangential relative to a fixation point. These other cues are discriminated by separate processing channels in parallel (Horridge, 2000b), and we can anticipate that each cue has its own measure of resolution.

* Tel.: +61-2-6125-4532; fax: +61-2-6125-3808.
 E-mail address: horridge@rsbs.anu.edu.au (A. Horridge).

2. Materials and methods

2.1. The apparatus

The methods have been published many times (Horridge, 2000a, 2003a-c). The experiments are done under a roof with open front 3 m wide and 3 m high in indirect sunlight. The top of the Y-choice apparatus (Srinivasan and Lehrer, 1988) is of clear Perspex, the walls are of white card (Fig. 1). The baffles, of transparent artists' drawing film, 0.13 mm thick, are set in a cardboard frame 1 cm wide. They control the angle of about 55° subtended by the pattern at the bees' decision point and allow the observer to make a sharp decision at each choice.

The targets carry the patterns on white cards which can be rotated. During training the target that displays the positive pattern and the reward with it is interchanged with the non-rewarded (negative) target every 5 min to prevent the bees from learning which arm of the apparatus to choose. The reward is a solution of sugar solution of a strength just sufficient to keep the bees coming without attracting recruits. In the illustrations of training patterns, the rewarded pattern is always shown on the left (labelled + at the top).

A new group of bees is used for each experiment. A small group of 10–15 bees are marked individually with spots of fabric paint and other bees excluded. In our conditions, this number ensures a reasonable spacing of the

arrivals. The bees require 20 or so visits to build up a discrimination between the two training patterns. The criterion for the score is when the bee passes through the hole in one baffle or the other. After an initial training period of 2–3 h, while training continued, the first choice of each individual bee in each 5 min period was recorded. This prevents two choices being recorded if the bee flies back out and enters again within a 5 min period. On the recording sheet, each individual bee has a column, and each horizontal line across the page represents a 5 min period, so that each choice of each identified bee is separately recorded. These results are labelled "train" and, with the training patterns, the totalled results appear first in the illustrations.

Next, a different pair of patterns is substituted for those in the training and the bees' first choices are recorded in a period of 5 min as before, first on one side of the apparatus and then on the other. These results when totalled are labelled "test". The tests must be carefully controlled. It is essential to give a reward otherwise the bees continue to search in the Y-maze, and will not go away, but there must be precautions so that the bees do not influence each other. Our apparatus has a narrow entrance which allows only one bee at a time to fly through. If another bee follows, it is easily waved off and made to wait outside the apparatus.

To prevent the bees from learning from the tests, several different tests with different patterns were always interleaved between continued periods of training, so the trained bees do not become familiar with any one test. In the tests, the bees get a reward after they have made their only choice in that 5 min period, and when they return the patterns have changed and the side may have changed. Any one test is not repeated until at least an hour has passed. We give a reward to first one test pattern and then, at another time, to the other. Tests for 5 min were alternated with 20 min periods of continued training, so filling up each bee's individual score column. The days records are examined, and from the score sheets, it is easy to observe the performance of each bee individually, for example, to see when they learn the task, that they do not change their performance in successive tests, or that they fail to appear. Many checks have been made in previous years to show that the bees do not learn from the test patterns if this routine is followed. It is a matter of observation that the results for a given test pattern do not change in a consistent way over time. When the bees fail in a test, it is not because they have been rewarded at different times on both targets. If the bees fail, they fail from the start of the test. The totalled score on the test pattern is then compared with the score on the training pattern within a short time, with the same sample of bees.

An advantage of the study of resolution is that different tests can be scrambled so that the bees cannot learn from the mixture of failures and successes, and the per-
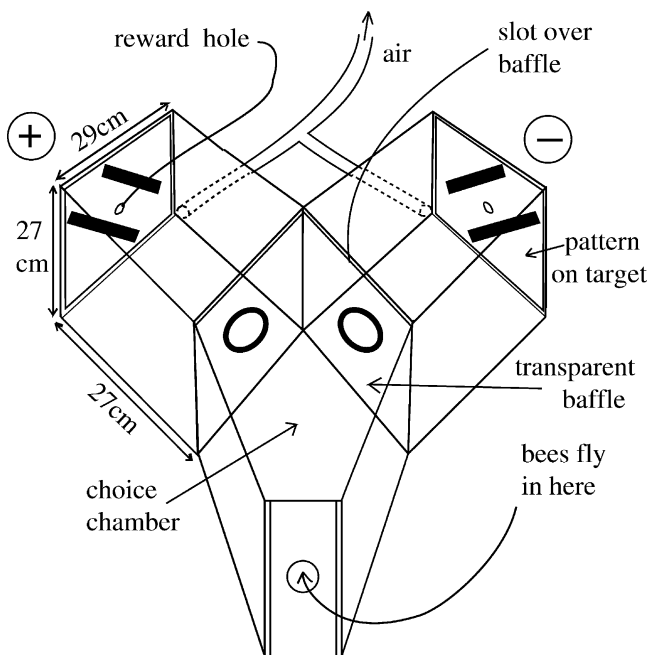

Fig. 1. The Y-choice apparatus. The bees enter at the front through a hole 5 cm diameter into a choice chamber from which they can see both targets. They decide to enter through one of the baffle orifices 5 cm wide. They can exit via the slots over the baffles. To prevent the bees from learning which side to go, the targets and the reward change sides every 5 min. Odours are extracted by the air pipe.

formance is limited by the resolution, not by the details of the training and tests.

2.2. Scoring and statistics

Tests are repeated until 200–300 choices are recorded, which usually takes several days, while training continues. Two calculations are made. In the first, the correct choices are counted in each successive block of 20 choices. The standard deviation (S.D.) between 10 and 15 of these blocks is calculated, together with the total number of choices. The percentage of correct choices and the value of the S.D. is placed after each score.

In the second method (van Hateren et al., 1990), an estimate of the S.D. is the value of $\sqrt{[p(1-p)/n]}$ where p is the fraction of correct choices and n is the total number of choices. This method assumes that there are no trends, that the individual choices are independent and they have a binomial distribution about the mean. The S.D. estimated from this formula is given in brackets after each score. By this method, a score of 57% based on 200 choices is more than twice the estimated S.D. away from the null (random) hypothesis of 50%.

3. Results

3.1. The resolution of regular gratings

As a preliminary check of the resolution towards regular gratings, a group of bees was trained to discriminate between two orthogonal black and white gratings of period 18°, one at +45° to the vertical, the other at −45° (Fig. 2a). The targets were rotated by 180° every 5 or
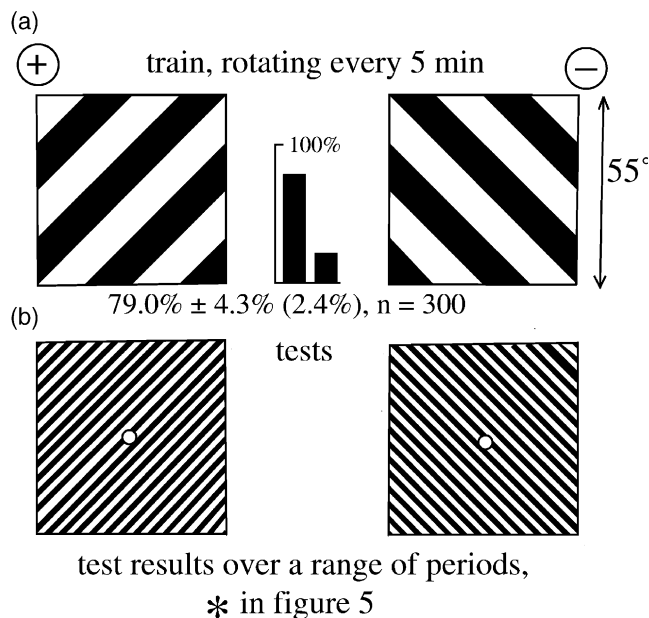
Fig. 2.   The resolution of oblique gratings. (a) The bees were trained on coarse gratings with shuffled bar positions and baffles in place. (b) A representative test. For test results with oblique gratings of various periods, see Fig. 5.
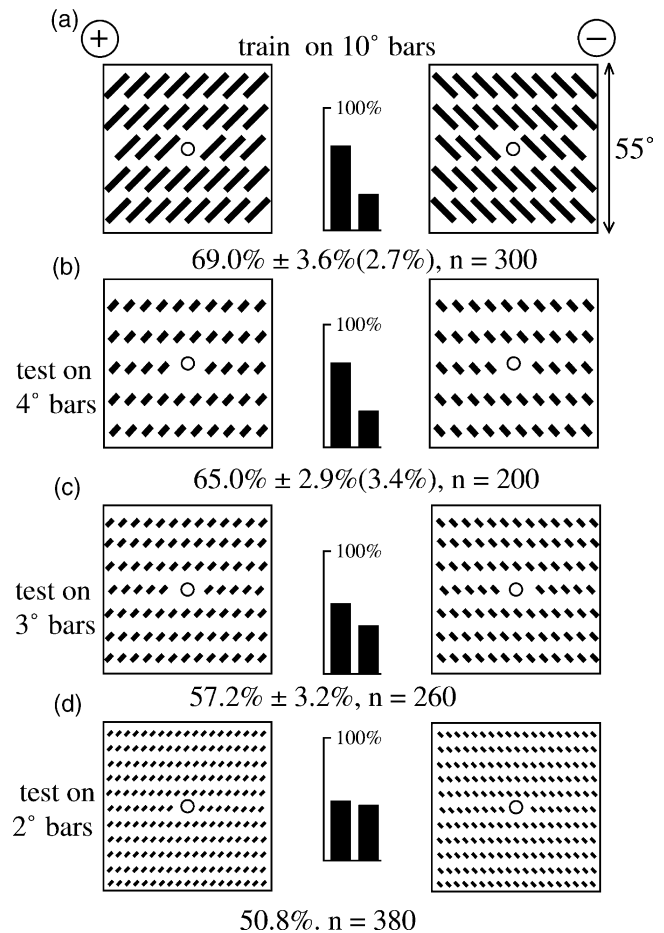
Fig. 3.   The resolution of the orientation of oblique bars. (a) The bees were trained on numerous oblique bars, each subtending 10° by 2°, and tested with pairs of patterns of oblique bars each subtending (c) 4° by 2°, (d) 3° by 1.5°, (e) 2° by 1°. For complete test results, see Fig. 5.

10 min so that the locations of the bars could not be used as cues. After 3 h training, the result was 79.0 ± 4.3% (2.4%), $n = 300$. Because the training gratings are oblique, a difference in the modulation caused by horizontal scanning motion, or yaw in flight, cannot be a possible cue. The trained bees were tested with equally spaced oblique black and white gratings of various smaller periods in interleaved tests (Fig. 2b). The performance drops to 58.5% at a period of 4°, 52.5% at 3°, and 50.5% at 2.5° (Fig. 5). These are almost the same values as those obtained previously with vertical/horizontal gratings (Srinivasan and Lehrer, 1988).

3.2. The limiting length of short bars

The limit of resolution of gratings, as measured above, depends on the resolution of spatial frequencies when done with oblique gratings, and on differences in the modulation caused by relative motion of the two targets when done with horizontal versus vertical gratings

(a)

⊕ train, rotate by 180° every 5 min ⊖

55°

75.3% ± 3.3% (2.4%), n = 300

(b)

for test results, see Fig. 5

train, rotate by 180° every 5 min

(c)

○

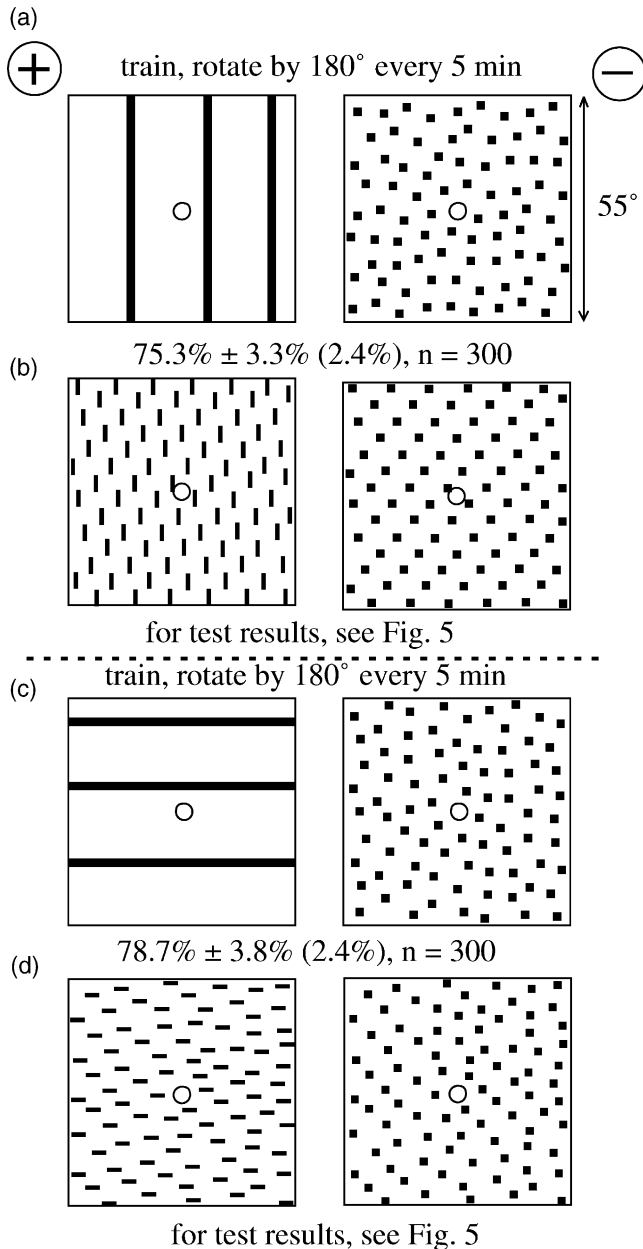78.7% ± 3.8% (2.4%), n = 300

(d)

for test results, see Fig. 5

Fig. 4. The separate resolutions of the orientation of vertical and horizontal bars. (a) The bees were trained on offset and shuffled vertical black bars, versus scattered squares of the same total area. (b) A representative test with short vertical bars versus squares of the same areas and position. This test was repeated over a range of bar lengths from 2.5° to 7.5°. (c) New bees were similarly trained on horizontal bars versus scattered squares. (d) A representative test with short horizontal bars, repeated over a range as before. For complete results, see Fig. 5.

(Horridge, 2003b), but tells us nothing about how much of the edge is needed for the detection of orientation. The next series of experiments is designed to determine the limit of resolution with respect to the length of edge.

To investigate this, a group of bees was trained to discriminate between a pattern of bars (subtending 10° by 2°) oriented at 45° and a similar pattern at −45° (Fig. 3a). These patterns show no modulation difference if the
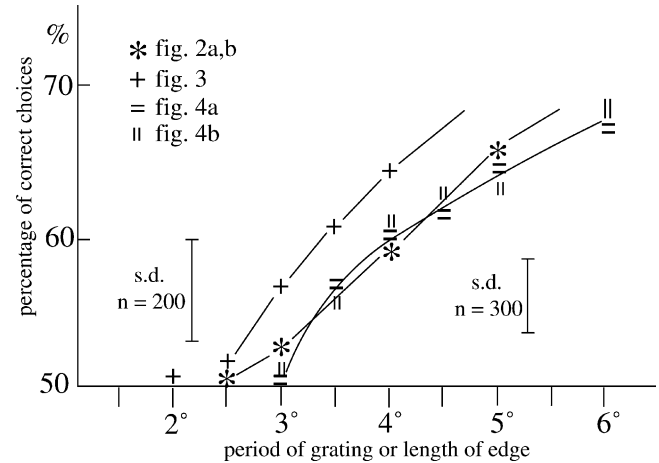

Fig. 5. Summary of results referenced to the illustrations. The standard deviations shown are the calculated ones.

bees scan them with horizontal movements in flight. After training for 3 h the result was 69.0 ± 3.6% (2.7%), n = 300. The trained bees were tested with a similar pair of patterns with progressively shorter lengths of edge. With bars subtending 4° by 2°, the result was 65.0 ± 2.9%, n = 200. With bars subtending 3.5° by 2°, the result was 61.0 ± 3.6%, n = 200. With smaller bars (3° by 1.5°), the result was 57.2 ± 3.2%, n = 260, and with still smaller bars (subtending 2° by 1°), the result was 50.8%, n = 380 (Fig. 3b–d). Results are plotted in Fig. 5. A length of at least 3° is required for discrimination of orientation.

3.3. Vertical and horizontal edges tested separately

The results above do not tell us whether the minimum length of edge depends on the direction of the edge on the eye. In the following experiment, vertical and horizontal bars were tested separately against squares of the same area and locations. The first positive training pattern consisted of three vertical bars 1 cm wide by 27 cm long (subtending 2° by 55°), offset from the centre so that their positions are shuffled when the target is rotated by 180° every 5 min. The negative training target displayed an irregular pattern of 81 squares (each subtending 2°) which was also rotated by 180° every 5 min (Fig. 4a). After training for 3 h, the performance with the training targets was consistently over 70% correct during a series of interleaved tests.

The positive test patterns consisted of short black bars all 1° wide, not lined up, with a range of lengths subtending 2.5–7.5° (Fig. 4b). The negative test patterns consisted of small black squares, each of the same area and centred at the same place as the corresponding bar.

The whole series was then repeated with the training and test bars horizontal (Fig. 4c,d). It makes no difference whether the small bars or squares are arranged regularly (Fig. 4b), or randomly (Fig. 4d).

The system of orientation detectors sums the edges over the whole target, but the scores with bars 1° wide are not as high as with bars 2° wide (Figs. 3b and 5). Discrimination falls to 50% (random choice) for bars 3° long, and the minimum lengths for vertical and horizontal edges are indistinguishable (Fig. 5).

3.4. The limiting size of circular dipoles

A similar kind of resolution limit is the minimum diameter of circular dipoles which are half black and half white on a grey background of 50% black, when the task is the discrimination of the orientation of the edges between the two halves (Fig. 6a–c).

The bees were trained with shuffled oblique bars similar to those in Fig. 7a. The performance reached 77.5 ± 3.9% (2.9%), $n = 200$, after only 3 h of training. The trained bees were tested with pairs of patterns of dipoles, one with the edges oriented at 45° and the other similar with the edge at −45°, for a range of angular diameters. In the first test, one target had 54 dipoles, subtending 4° (Fig. 6a). The result was 61.0 ± 3.0% (3.4%), $n = 200$. With a test diameter of 3.5°, the result was 56.7 ±

(a) train on figure 7a, rotating every 5 min

(a) test, 4°, 61.0% ± 3.0% (3.4%), n = 200

(b) test, 3°, 48.0%, n = 200

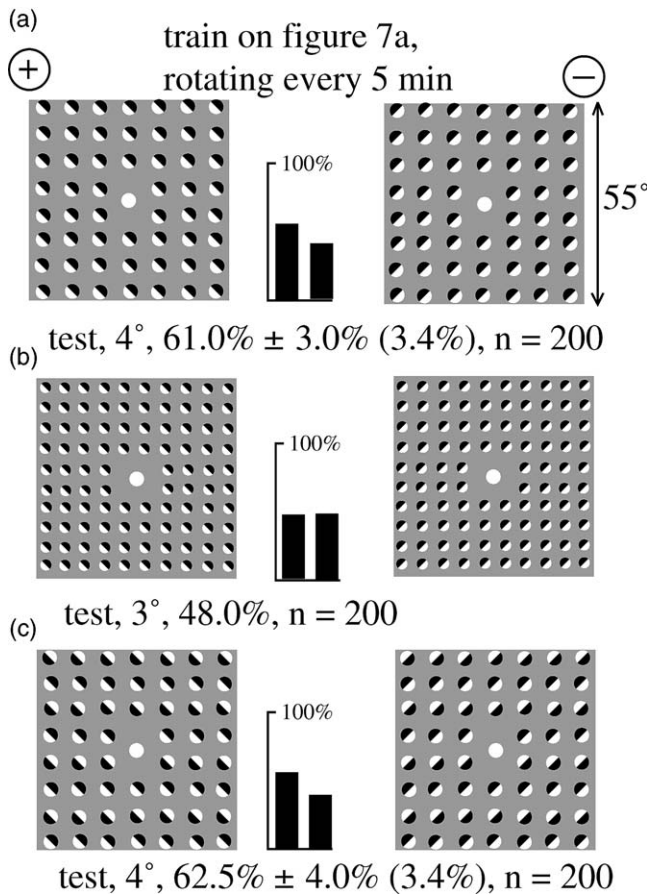(c) test, 4°, 62.5% ± 4.0% (3.4%), n = 200

Fig. 6. The resolution of oblique dipole spots. The bees were trained on shuffled oblique bars (Fig. 7a). (a) The trained bees were tested on dipoles of diameter 4°. (b) Tests on 3° dipoles. (c) Tests with alternate rows of 4° dipoles inverted.

(a) train, alternating 1,2

(b) 72.5% ± 3.9% (3.3%), n = 200

test with 8° squares

(c) 49.5%, n = 200

test

(d) 63.5% ± 3.9% (3.4%), n = 200

test

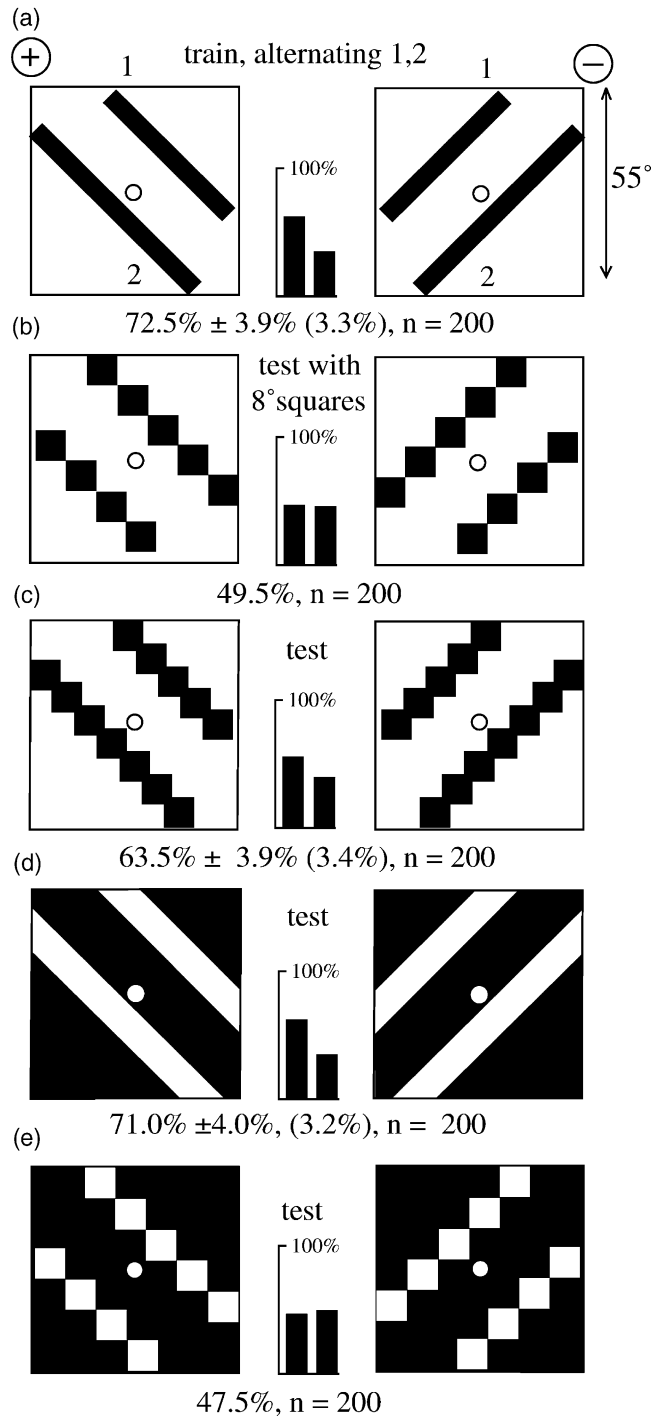(e) 71.0% ±4.0%, (3.2%), n = 200

test

47.5%, n = 200

Fig. 7. Tests with oblique rows of squares. (a) Training with rotation by 180° every 5 min, as shown by 1, 2. (b) Test with rows of 8° squares. (c) With the squares closer together. (d, e) Tests with black and white interchanged.

2.9% (2.8%), $n = 300$. With a test diameter of 3°, the result was 48.0%, $n = 200$ (Fig. 6b). The minimum length of edge for discrimination is near 3.5°. These results are indistinguishable from those based on the short bars (Fig. 4).

The trained bees were also tested with similar patterns in which alternate rows of 4° dipoles were turned through 180° (Fig. 6c), but this made no difference to the discrimination. The result was 62.5 ± 4.0% (3.4%), $n = 200$. This suggests that the mechanism of detection of the edge orientation precedes the summation of similar orientations.

3.5. Stepped bars

In the next group of experiments, the bees were first trained to discriminate the orientation of two oblique black bars on each target (Fig. 7a), with shuffled locations of black. The trained bees were tested on bars of the same area having edges with square steps of differing size.

During the tests, the performance with the training patterns exceeded 70% correct choices. When tested with diagonal rows of 8° squares (Fig. 7b), the trained bees fail to discriminate the global orientation. The stepped edges at right angles cancel the local orientation cues, and the lines of squares are not assembled into a global orientation. With lines of 2° black squares, placed corner to corner, the individual steps are not resolved and the bees discriminate the orientation very well. The result was 64.5 ± 3.3% (3.4%), $n = 200$ (not illustrated). As the size of the squares increases, the response rapidly falls to random choice. With 3.0° squares the result was 58.0 ± 3.7% (3.5%), $n = 200$. With 3.5° squares the result was 56.0%, and for squares subtending 4.0° the result was 51.5%, $n = 200$. The limit of square size for the resolution of the row is near 3.5°. This is the length of edge which is large enough to be discriminated as an orientation, and each edge cancels an equal length of edge at right angles, leaving no residual orientation cue.

Care is needed, however. When black squares subtending 8° are pushed closer together to make steps that subtend 6°, the result was 63.5% (Fig. 7c). The trained bees discriminate the global orientation very well, although they must be able to resolve the separate steps. The explanation may be that, when the image is blurred by the vision of the bee, the continuity of black along the centre is not cancelled by the square steps, and an intensity gradient persists along each side of the bars (Horridge, 2000a).

3.6. Reversed contrast

The bees trained above (Fig. 7a) were also tested with white bars on a black background (Fig. 7d), with a result of 71%, $n = 200$. They were also tested with bars composed of white 8° squares placed corner to corner on a black background (Fig. 7e), but in this case they failed to discriminate the global orientation, just as they did with black squares on white (Fig. 7b). As with dipoles

(Fig. 6c), the exchange of black and white makes little difference to the orientation cue.

3.7. The bees detect the cue, not the pattern

In a new type of resolution experiment, a group of bees was trained to discriminate between four horizontal versus four vertical bars, with both targets rotated by 180° every 5 min to shuffle the positions of the bars on the targets. The trained bees were tested with the four horizontal bars versus a scatter of small horizontal bars of equal size and the same total area, with different bar lengths in different tests (Fig. 8). The tests with small bars of one length were interleaved with tests with small bars of other lengths, and every test was made first with one target, and, at a different time, with the other target rewarded. When the small bars subtended 6° in length, they were not discriminated from the full length bars. The result was 51.5%, $n = 200$. The result at 4° was 58.3%, $n = 300$; at 3° was 68.0%, $n = 300$; at 2.5° was 72.0% and at 2° was 81.6%, $n = 300$. As the small bars
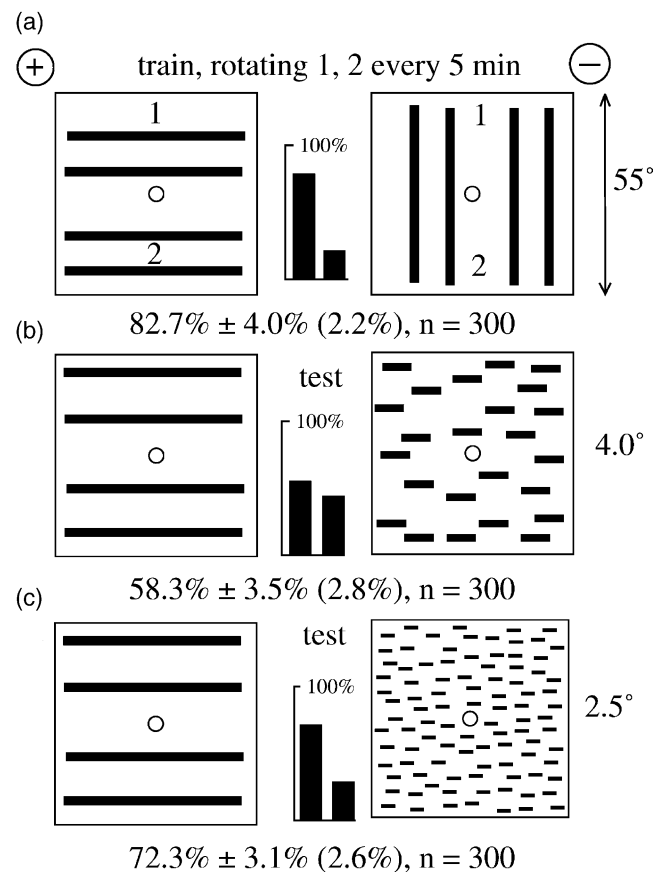

Fig. 8. The bees remember the cue, not the pattern. (a) Training on four horizontal versus four vertical long bars with shuffled positions 1, 2. (b) Test with four horizontal training bars versus a scattering of bars subtending 4° with the same orientation. (c) The same with bars subtending 2.5° and same total area. Results of tests with further lengths of bars are given in the text.

are made shorter in different tests, the discrimination from the long bars improves. Thus, with bars of 3° or less in tests, the bees strongly prefer the long bars that they were trained on. The transition is very sharp and occurs with a surprisingly small lengths of the test bars. This result implies that the bees see a difference between the patterns of long bars and patterns of bars subtending 3° or less, only because the short lengths of oriented edges are not resolved. The bees do not detect a difference between the long bars that they were trained on and bars longer than 4° because they carry an equal orientation cue, and the lengths of the training bars were not learned in the training.

4. Discussion

4.1. Receptor fields and spacing

At the front of the honeybee eye, the angle between the visual axes of the ommatidia, as calculated from measurements made by an optical technique (Seidl and Kaiser, 1981), in the horizontal direction (2°) is about twice that between horizontal rows in the vertical direction (1°). For the worker honeybee, the field width of the receptors at the frontal region of the eye, at the 50% sensitivity contour, is $2.57 \pm 0.12°$ and symmetrical (Labhart, 1980), but the receptors may not have been fully light-adapted. The resolution towards a grating agrees with the angular sensitivity of the individual receptors, and is the same for vertical and horizontal gratings when separately tested against grey (Srinivasan and Lehrer, 1988). From this result, these authors inferred that the resolution of a grating is limited by the minimum modulation detectable in the receptors, not by the angle between the optical axes of adjacent ommatidia. Bees discriminate the difference in modulation caused by vertical and horizontal edges as the bee turns in flight, so the receptor angular sensitivity sets the limit, but when the targets are orthogonal oblique gratings, there is no difference in modulation and the limit is set by the orientation detectors. The two cues are quite distinct. The bee is colour blind to the orientation cue but not to the modulation cue (Horridge, 2003b). Because this was discovered in time, most of the experiments have been made with oblique edges (Figs. 2, 3, 6 and 7).

4.2. The length of the primary orientation detectors

In the present experiments, the bees are taught to ignore the spatial locations of black bars on the targets, and there is no modulation difference because the edges are oblique. Therefore, the bees learn to look for an orientation cue. The discrimination of orientation requires edges subtending at least 3.5° in length, which is approximately twice the angle between adjacent ommatidia in the horizontal rows and three times the angle on the diagonal. We can infer that behind the retina is an array of primary edge detection units each with a field of 3.0–3.5° long, which cannot relate to more than three receptors in a straight horizontal row and four receptors in the oblique rows. This is the minimum length of the detector of orientation, here measured for the first time.

The orientation is detected when the receptors projecting to a unit orientation detector are simultaneously modulated. Therefore, each orientation detector could project from a single row of three or four receptors. The results support a model of orientation detection in which short primary detectors with the same axis of orientation in all parts of the eye sum into deeper units (Horridge, 2000b). There must be at least three arrays of primary orientation detectors with orientation axes at 120° to each other, and the shortness of the primary detectors accounts for the poor angular sensitivity of the large-field detectors, which is near 90° wide at the 50% sensitivity level (Srinivasan et al., 1994).

Like the resolution of the direction of motion, the shortest length of edge that gives an orientation cue depends on the interommatidial angle. Directional motion detection depends on the successive modulation of adjacent visual axes; orientation detection depends on the simultaneous modulation of adjacent visual axes, but the ultimate resolution of a grating may still be limited by the angular sensitivity of the receptors and not by their separation.

With a set of different training and test patterns, it has been shown that the bee has no orientation detectors that can detect the global orientation of a row of squares or spots. Individual squares or round spots carry no orientation cue, and the global orientation of a straight line of them is not discriminated if they are separately resolved (Horridge, 2003c). The maximum possible length of the orientation detector found in that earlier study is indistinguishable from the smallest length of edge that can be detected, as measured in the present work. Therefore, the short orientation detectors are not strung together to detect longer lengths of edge, even straight edges, and they cannot collaborate to span gaps. Bees that have learned only the orientation cue are unable to discriminate whether individual edges are long or short. There is summation of the orientation cue but no re-assembly of the pattern. This places a serious restriction on the bees' ability to discriminate patterns.

The final effect is a remarkable resolution of the average direction of orientation, which is summed in large fields over the whole of each eye, so that the sensitivity to average orientation is increased but different local orientations are summed and individually lost. Nevertheless, an isolated orientation cue is not recognized if it is moved from its expected place, as seen from the point of choice during the training (Horridge, 2003a). Two

features that bees can discriminate very well are the average orientation of blurred edges or gradients of intensity (Horridge, 2000a) and the average orientation of many short edges in large areas of textures, as illustrated here. Perhaps also, the summation also improves vision of the edges of large objects in dim light. It follows from this model that if the bees learn only the orientation cue, they will not be able to discriminate between long bars and a scatter of short bars with the same orientation cue, as indeed is the case (Fig. 8).

The experiments reported here make honeybee vision look very different from our own, especially in the way that the orientation of edges is processed in many independent units in the early visual pathways, with mutual cancelling of edges at right angles. In the bee, there is no evidence of mechanisms that could re-assemble the orientations in even a simple pattern. The bees detect the averaged orientation cue in its expected place, by coarsely tuned large-field detectors, not the pattern.

Acknowledgements

References

van Hateren, J.H., Srinivasan, M.V., Wait, P.B., 1990. Pattern recognition in bees: orientation discrimination. Journal of Comparative Physiology A 167, 649–654.

Horridge, G.A., 2000a. Pattern vision of the honeybee (*Apis mellifera*). What is an oriented edge? Journal of Comparative Physiology A 186, 521–534.

Horridge, G.A., 2000b. Seven experiments on pattern vision of the honeybee, with a model. Vision Research 40, 2589–2603.

Horridge, G.A., 2003a. The effect of complexity on the discrimination of oriented bars by the honeybee (*Apis mellifera*). Journal of Comparative Physiology A (in press).

Horridge, G.A., 2003b. Visual resolution of gratings by the compound eye of the bee (*Apis mellifera*). Journal of Experimental Biology 206, 2105–2110.

Horridge, G.A., 2003c. The visual system of the honeybee (*Apis mellifera*); the maximum length of the orientation detector. Journal of Insect Physiology 49, 621–628.

Labhart, T., 1980. Specialized photoreceptors at the dorsal rim of the honey bee's compound eye: polarization and angular sensitivity. Journal of Comparative Physiology A 141, 19–30.

Seidl, R., Kaiser, W., 1981. Visual field size, binocular domain and ommatidial array of the compound eyes in worker honey bees. Journal of Comparative Physiology A 143, 17–26.

Srinivasan, M.V., Lehrer, M., 1988. Spatial acuity of honeybee vision and its spectral properties. Journal of Comparative Physiology A 162, 159–172.

Srinivasan, M.V., Zhang, S.W., Witney, K., 1994. Visual discrimination of pattern orientation by honeybees. Philosophical Transactions of the Royal Society of London B 343, 199–210.