

HONEYBEE VISION: ANALYSIS OF ORIENTATION AND COLOUR IN THE LATERAL, DORSAL AND VENTRAL FIELDS OF VIEW

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Summary

The ability of honeybees (*Apis mellifera*) to learn and recognise peripherally presented patterns was investigated by training bees in a Y-maze which presented patterns on the side walls, the ceiling or the floor. We found that pattern orientation is learnt and recognised in the lateral and frontal visual field, but not in the dorsal or ventral fields. Colour information, in contrast, is used in the lateral and frontal as well as the ventral visual field, but not in the dorsal field. If pattern orientation is different on opposite sides of the visual field during training, both patterns are

learned, but each on its own is sufficient for the bees to recognise the learnt stimulus. However, unilaterally learnt pattern information, be it orientation or colour, cannot be accessed when the test pattern is viewed on the other side. That is, interocular transfer of such information does not occur.

Key words: honeybee, *Apis mellifera*, vision, learning, pattern recognition, colour vision, orientation.

Introduction

The analysis of pattern orientation in the honeybee has been the subject of several recent studies. After the bee's use of orientation as an abstract pattern parameter was demonstrated by van Hateren *et al.* (1990), it was found that honeybee orientation analysis is based on geometrical cues (Srinivasan *et al.* 1993) and is possibly mediated by neurones similar to the orientation detectors in the mammalian cortex (Srinivasan *et al.* 1994). These neurones appear to rely almost exclusively on input from the green receptor channel and are therefore 'colour blind' (Giger and Srinivasan, 1996). The aim of the present study was to determine how pattern orientation is used in the lateral, ventral and dorsal parts of the visual field and whether there is interocular transfer of laterally learnt pattern orientation. For comparison, the honeybee's ability to discriminate colour under the same conditions was also investigated.

Few behavioural studies have investigated the honeybees' use of visual stimuli in the peripheral visual field, i.e. laterally, ventrally or dorsally presented patterns. Wehner (1979) described experiments in which honeybees were trained to access a food source through one of 89 holes in a vertical wall. An opaque, white cylinder, protruding from that wall and surrounding the whole arrangement of holes, obscured the visual background in the honeybees' peripheral visual field while they were searching for the correct hole in the frontal wall. The internal surface of the cylinder could be used to

introduce artificial visual marks in the lateral regions of the honeybee's visual field. In the absence of such marks, the honeybees were unable to locate the reward-bearing hole: they searched the array of holes basically at random. With two lateral black bars marking the horizon, however, the target (which was always the central hole) was pinpointed quite accurately, particularly its vertical position. Similar results were achieved with black bars in the ventral and dorsal visual field, or in any intermediate position, but the honeybees performed best with the horizontal arrangement of bars.

Very similar experiments, producing the same results, were reported by Lehrer (1990). In addition, she showed that the honeybees used image motion rather than the angular bar size to determine the target position between the two bars. Furthermore, by using a modified, rectangular arrangement and coloured patterns offering either only blue contrast or only green contrast, she found that the honeybees' localisation performance is mediated by the green-sensitive receptor channel.

In a later study, Lehrer (1994) used the same rectangular arrangement to test for interocular transfer of this positional information. She trained honeybees with a bar or edge on the left side and a plain wall on the right. In subsequent tests with the mark on the right and nothing on the left, she found that the honeybees were still able to localise the target, suggesting that interocular transfer indeed occurred (see Discussion).

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The studies mentioned above demonstrated that honeybees memorise and use the position of peripheral marks to pinpoint a frontally positioned target. The present study goes one step further in asking whether honeybees can recognise specific attributes (other than position) of patterns presented in the peripheral visual field. The experiments discussed here were undertaken with two main questions in mind: (i) is pattern orientation used in peripheral regions of the visual field and, if so, (ii) is there interocular transfer of a unilaterally learnt orientation? For comparison, the same questions were also asked of the honeybees' colour discrimination.

Materials and methods

The experimental arrangement consisted of a transparent Y-maze with inserted opaque structures (or 'baffles') that served both to restrict the honeybees' movements and strategically to obscure their view of the patterns (Fig. 1). For each experiment, a group of 10–20 individually marked honeybees

(*Apis mellifera*) was trained to enter the apparatus and collect a reward of sugar water associated with one of two visual patterns (or pattern configurations). The reward was offered inside a dark box accessible through a tube (diameter 1.9 cm) in the centre of the back wall of the tunnel carrying the rewarded pattern (the 'rewarded tunnel'). An identical tube in the other tunnel (containing the unrewarded pattern) was blocked at a depth of 3 cm. Throughout the experiment, both the reward and the training patterns were switched between the two tunnels every 10 min in order to prevent the honeybees from associating the reward with a particular tunnel.

The training and test patterns were usually either black-and-white gratings with a period of 4 cm (e.g. Fig. 1Bii) or uniform colours (blue or yellow). They were presented on cardboard discs (diameter 16 cm) and placed on the side walls, the ceiling or the floor of each tunnel. Specially shaped baffles (shown in grey in Fig. 1) ensured that bees entering the Y-maze could not view the stimuli from the tunnel entrances, but only after they had nearly passed the baffles (see also Discussion). The baffles

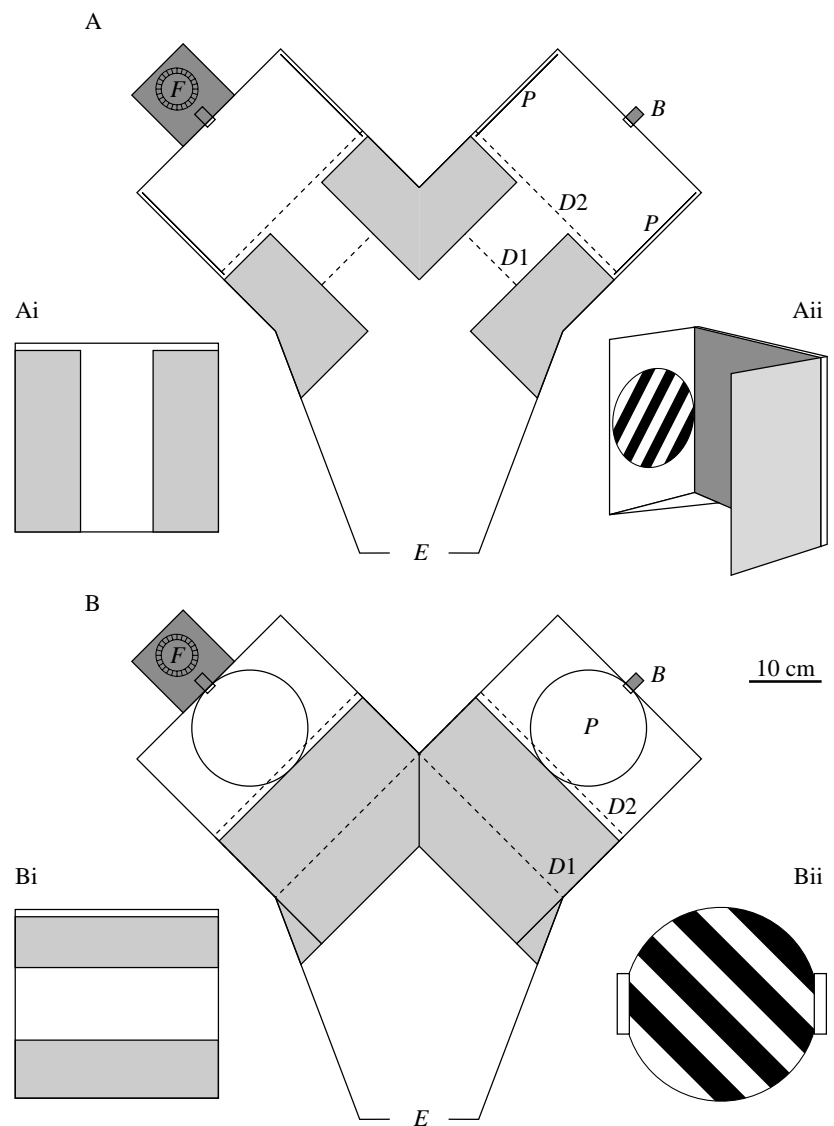


Fig. 1. Experimental arrangement. Grey areas represent space occupied by inserted paper baffles (except in Aii). *B*, blocked tube. *D1*, *D2*, first and second decision line, respectively. *E*, entrance to the apparatus. *F*, feeder inside reward box. *P*, patterns. (A) Schematic ground plan of arrangement for lateral presentation of patterns. (Ai) Cross section of either tunnel through *D1*. (Aii) Perspective view of removable back wall of either tunnel with attached cardboard panels carrying the patterns. (B) Schematic ground plan of arrangement for dorsal and ventral presentation of patterns. (Bi) Cross section of either tunnel through *D1*. (Bii) View of dorsal pattern from below with the two cardboard pieces fixing the pattern to the ceiling.

were constructed out of white photocopying paper printed with randomly arranged black dots (diameter 3 mm; approximately $0.45 \text{ dots cm}^{-2}$ on average). Care was taken to ensure that both tunnels showed exactly the same arrangement of dots. The baffles could easily be removed and re-inserted, but could not be moved by the honeybees.

The two different arrangements used are represented schematically in Fig. 1. Apparatus A was used for experiments involving laterally presented patterns. In this case, the paper baffles were placed along the side walls of the tunnels, creating a passage 10 cm wide and 26 cm high (see cross section in Fig. 1Ai). Between the baffles and the ceiling of the tunnel, a gap of 1 cm was left for honeybees leaving the apparatus to crawl through. No honeybee was ever observed to enter a tunnel *via* this gap. Apparatus B was used for experiments on patterns presented dorsally and/or ventrally. Here, the baffles were placed on the floor and under the ceiling of the Y-maze, leaving a passage 10 cm high and 28 cm wide (see cross section in Fig. 1Bi). Again, departing honeybees were provided with a 1 cm gap under the ceiling.

The laterally presented patterns were fixed to white cardboard panels (16.5 cm × 25 cm) which provided a visual background (Fig. 1Aii). The dorsally presented patterns were held in place by two small pieces of cardboard, and could be slid in and out (Fig. 1Bii). The patterns presented ventrally were simply laid on the floor of the tunnels, carefully aligned using fine pencil marks. All the patterns were round discs, 16 cm in diameter, positioned such that their circumferences touched the back wall of the tunnel. Their centres were always

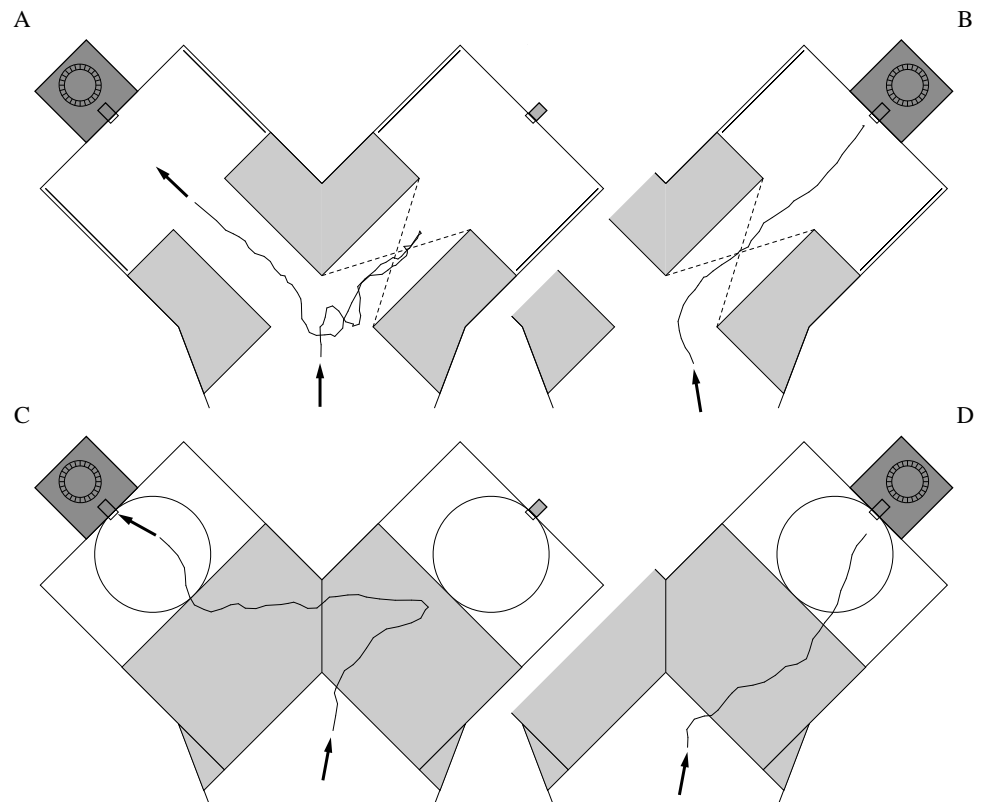
placed at the same height as the entrance to the feeder box (when presented laterally) or along the midline of the tunnel (when presented dorsally and ventrally). Unless stated otherwise, the back walls of the tunnels were covered with 50% grey paper, with a reflectance spectrum corresponding to that of 'Letratone 50%' as given in Fig. 2a of Srinivasan and Lehrer (1988).

The black-and-white gratings were created on a laser printer. The reflectance spectra of the white and black regions are given in Srinivasan and Lehrer (1984). The blue and yellow stimuli were constructed from commercially available coloured paper (Spectrum, Typofot AG, Wohlen, Switzerland). The reflectance spectra of these papers are given in Fig. 2 of Lehrer and Bischof (1995) as 'Violet 1' and 'Yellow 2' respectively.

In both apparatus A and apparatus B, the honeybees had to enter one of the two tunnels at random (or based on expectations irrelevant to the experiment), since the two tunnels looked exactly the same from what used to be the decision chamber. Only after they had entered one of the tunnels were they offered a clue as to whether they had made the right choice. At that instant, they had to decide whether to accept the tunnel as the rewarded one and fly on to collect the reward, or to turn back when they had entered the wrong tunnel. Therefore, each test with these arrangements actually consisted of two separate tests, one in each tunnel, since a honeybee exploring one tunnel had no way of knowing what the other tunnel contained.

To quantify the honeybees' behaviour, two imaginary decision lines were defined for each tunnel (Fig. 1). The first

Fig. 2. Four examples of the flight trajectories of honeybees with laterally (A,B) and ventrally (C,D) presented patterns. Fine broken lines delimit areas from which either or both patterns of the correct (rewarded) tunnel are at least partially visible. These sample trajectories were recorded using a CCD camera with a 16 mm lens and a 25 Hz video recorder. The position of the honeybee's head and abdomen (not shown), respectively, in each video frame was digitised on a PC equipped with a frame grabber card. (A) Behaviour D, recorded during the training shown in Fig. 3A. (B) Behaviour A, recorded during the same training. (C) Behaviour D, recorded during the training equivalent to that shown in Fig. 3B. (D) Behaviour A, same training as in C.



decision line ($D1$) was a line connecting the corners of the tunnels of the Y-maze. The second decision line ($D2$) was 1 cm beyond the edge of the paper baffles closest to the reward, i.e. if a honeybee stopped within 1 cm of that edge, it did not cross $D2$. On the basis of these imaginary lines, four possible behaviours can be defined. The honeybees could (A) enter the correct tunnel and fly on (i.e. cross $D1$ and $D2$), (B) enter the correct tunnel and turn back (i.e. cross $D1$, but turn back before $D2$), (C) enter the incorrect tunnel and fly on (i.e. cross $D1$ and $D2$), or (D) enter the incorrect tunnel and turn back (i.e. cross $D1$, but turn back before $D2$).

Examples of behaviours A and D in well-trained honeybees are depicted schematically in Fig. 2. Note that, after turning back in one tunnel, the honeybees either immediately turned into the other tunnel (as shown in the examples) or flew around in the decision chamber before making a new decision which could be in favour of the same tunnel again. However, only the first decision of each honeybee entering the apparatus was recorded.

On the basis of the four behaviours defined above, the honeybees' responses to a given pattern combination can be described by the acceptance frequency (af) for the tunnel bearing that pattern combination. In any test, the acceptance frequency for the positive (correct) tunnel (af_p) can be calculated as $A/(A+B)$, while the acceptance frequency for the negative (incorrect) tunnel (af_n) equals $C/(C+D)$. The choice frequency for the positive tunnel (cf_p), i.e. the frequency at which the honeybees enter the positive tunnel, is defined as $(A+B)/(A+B+C+D)$.

At the second decision line ($D2$), the honeybees faced an asymmetric task. If they had not learnt anything, they always flew on to look for the reward at the end of the tunnel they had entered, irrespective of which tunnel that was. If they had learnt the task perfectly, they would still have flown on when they encountered the positive pattern, but would always have turned back in response to the negative pattern. However, even a low proportion of honeybees turning back in the unrewarded tunnel indicates that they had already learnt the task to some extent. A further source of asymmetry is that it presumably takes more effort to stop in mid-flight and turn back than to keep going. This could have decreased the frequency with which the negative tunnel was rejected.

For the statistical analysis of the data collected in this study, we made use of the hypergeometric distribution (Sachs, 1982; 'exact treatment of 2×2 tables'; Fisher, 1970) and the binomial distribution (Sachs, 1982). The former was used to test the significance of differences in the honeybees' behaviour in the two tunnels, while the latter was used to decide whether the honeybees preferred one tunnel over the other.

Results

Pattern orientation

The first experiment of this series, designed to test whether honeybees use laterally presented patterns at all, is depicted in Fig. 3. A naive group of bees was trained to a tunnel offering

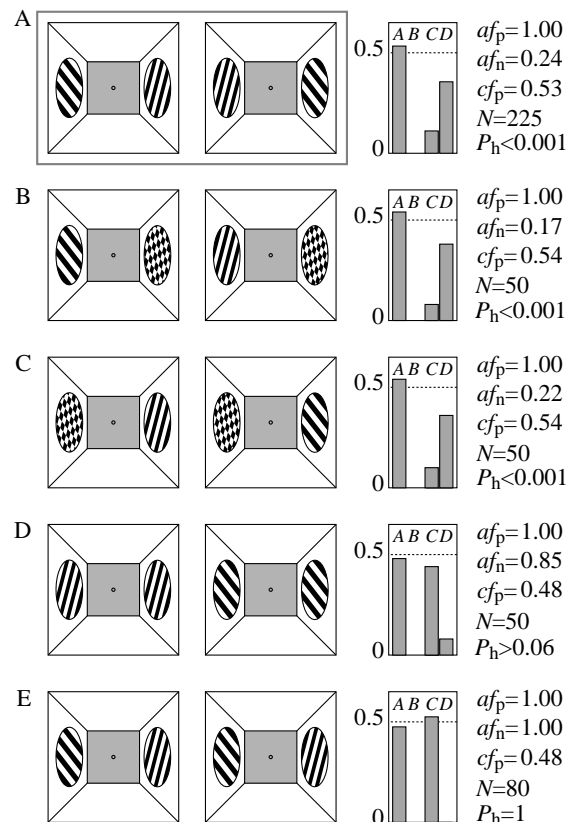


Fig. 3. Training on orientation on either side (lateral visual field). Left side: schematic representation of experimental situations. The framed situation (A in this figure) depicts the training. All the situations given below the training represent tests performed on the same group of bees, after successful training. Each panel is a (pseudo)perspective view of a tunnel with its back wall (central rectangle) and its side walls, ceiling and floor (trapezoids on the sides, top and bottom, respectively). The circular patterns are distorted accordingly. The rewarded tunnel is always shown in the left-hand panel. Right side: results. In the graphs, the height of the bars represents the recorded frequency of the four behaviours A–D. af_p , acceptance frequency for the positive tunnel, i.e. $A/(A+B)$. af_n , acceptance frequency for the negative tunnel, i.e. $C/(C+D)$. cf_p , choice frequency for the positive tunnel, i.e. $(A+B)/(A+B+C+D)$. N , number of choices. P_h , probability of the behaviour of the honeybees in the positive tunnel being the same as that in the negative tunnel, based on hypergeometric distribution. Details of the experiments are given in the text.

a grating oriented at 150° on the left wall and the same grating oriented at 60° on the right wall (Fig. 3A, left side, left panel). (Throughout this study, the angle of orientation is deemed to increase with counter-clockwise rotation, with the horizontal orientation defined as 0° .) The unrewarded tunnel contained a grating oriented at 60° on the left wall and a grating oriented at 150° on the right wall (Fig. 3A, left side, right panel), i.e. the orientation on either wall of this tunnel was perpendicular to the orientation on the corresponding wall of the rewarded tunnel.

The result of this training can be read from the graph in Fig. 3A. The four columns labelled A–D represent the

frequencies with which the honeybees displayed the respective responses described in Materials and methods. Accordingly, the left half of the graph (columns *A* and *B*) denotes honeybees first entering the positive tunnel and then either flying across the second decision line (*A*) or turning back (*B*). The right half of the graph, likewise, denotes honeybees that entered the negative tunnel and then either flew on (*C*) or turned back (*D*).

If the training was successful, i.e. the bees had learned something, we would expect the responses in the negative tunnel to be different from the responses in the positive tunnel. That is, the bees should turn back more often when confronted with the negative pattern combination. In this particular training (Fig. 3A), this was obviously the case. The acceptance frequency for the positive tunnel (af_p) is 1.00, i.e. none of the honeybees entering the positive tunnel turned back. In contrast, af_n is only 0.24, i.e. 76% of all honeybees entering the negative tunnel recognised the negative pattern combination as such and turned back. The difference between the two acceptance frequencies is highly significant (see Materials and methods for details of statistical tests).

The figures also state the ratio of visits (cf_p) in which the honeybees first entered the positive tunnel, to the total number of visits. This can be used as a first control for any biases towards the rewarded tunnel (e.g. olfactory cues). Since the honeybees cannot see the patterns from the tunnel entrance, both tunnels should appear the same to them when they make their first decision. Therefore, they should enter both the tunnels equally often, i.e. we would expect cf_p to be close to 0.5. During the test shown in Fig. 3A, 0.53 of the honeybees' first choices were in favour of the positive tunnel.

From Fig. 3A, we can conclude that honeybees can learn to recognise and avoid the unrewarded tunnel if the two tunnels differ only in the orientation of two gratings presented in their lateral visual field, one on either side. The remainder of Fig. 3 depicts four different tests performed on the bees trained to the pattern configuration shown in Fig. 3A. For one of these tests (Fig. 3B), the patterns on the right wall of either tunnel were replaced by checkerboard patterns (with one of its two orientations parallel to the training orientation). Hence, the bees had to base their decision whether to fly ahead or to turn back on the pattern on the left wall only. Similarly, in the reciprocal test presented in Fig. 3C, only the pattern on the right wall of each tunnel offered a cue. In both of these tests, the bees' performance differed very little from their performance in the learning test (Fig. 3A). Therefore, although the bees were trained with two patterns, one on either side, information presented on one side only was sufficient for the recognition and avoidance of the negative tunnel.

In the test reported in Fig. 3D, the bees were presented with conflicting information in both tunnels. The positive tunnel (left-hand panel) bore the correct orientation (i.e. the orientation encountered on the same side in the rewarded tunnel during training) on the right-hand side, but the incorrect orientation (i.e. the same as in the unrewarded tunnel during training) on the left-hand side. In the other tunnel, the left-hand side showed the same orientation as the rewarded tunnel, and

the orientation on the right-hand side was incorrect. If the bees lent the same weight to both sides of the visual field when making their decision, we would expect them to behave in the same way in both tunnels, either always flying on or always turning back. If there was a bias towards one eye, however, the bees would turn back in one tunnel and fly on in the other. Since individual bees would probably have different biases, the expected outcome of the test in this case (for the pooled data) would be that approximately 0.32 (average of *A* and *C* in learning test) of the bees fly on in both tunnels. The result of this test, presented in Fig. 3D, suggests that the bees treated both the tunnels equally, always flying on. However, with a P_h value of only 0.065 for this test, the behaviour in the tunnel labelled negative comes very close to being significantly different from the behaviour in the tunnel labelled positive. Therefore, it cannot be excluded with certainty that some of the bees displayed a bias towards one eye (all of them favouring the right eye) when both eyes saw an orientation. Yet, this possible bias appears insignificant in the light of the results in Fig. 3B,C, where the bees' performance was the same with either eye on its own.

From Fig. 3B–D we can conclude that both eyes perform equally well when tested individually and that neither of them markedly predominates when they are tested against each other.

Finally, Fig. 3E shows a test in which both tunnels contained the same orientations as the rewarded tunnel during training. One bore exactly the same patterns (left-hand panel), while the gratings in the other tunnel were shifted by half a period, resulting in a reversal of their intensity contrast. The bees accepted both tunnels as the positive one, indicating that they did not make use of the intensity distribution in the patterns. This test also acts as a control against the availability of any cues unknown to the experimenter, such as smell or visual markings on the back walls of the tunnels.

After this demonstration of the bees' ability to learn and use pattern orientation in the lateral visual field, we went on to train bees on orientation presented on one side only and to test whether interocular transfer occurred. As the neutral pattern (i.e. the pattern on the other side, offering no global orientation), we used three different patterns in three different trainings: (i) a 50% grey disc, (ii) white concentric circles on a black background (with average intensity 50%), and (iii) the checkerboard patterns used in some of the tests of Fig. 3. The training success with these different neutral patterns varied considerably, af_n ranging from 0.76 with the grey disc (poor training) and 0.54 with the concentric circles to 0.13 with the checkerboard (very good training, Fig. 4A). It seems that, in order for the bees to acquire pattern orientation information on one side, the pattern on the opposite side has to elicit a similar degree of intensity modulation when the bees are flying past. Therefore, in all subsequent experiments to test for interocular transfer, we used the checkerboard as the neutral pattern.

Fig. 4 depicts the training on pattern orientation (60° versus 150°) presented to the right eye, using checkerboards as neutral patterns (Fig. 4A). The bees trained on orientation

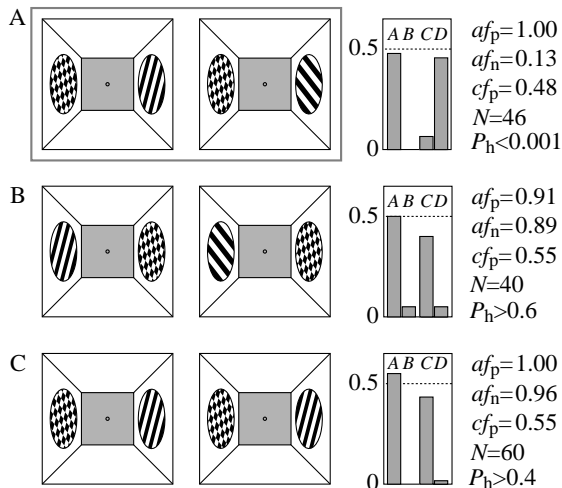


Fig. 4. Trainings on orientation on one side only (lateral visual field). Same notation as in Fig. 3. See text for details.

offered to one eye were then tested on the same orientation presented to the other eye (Fig. 4B). (To present the 'same orientation' on the opposite wall of the tunnel, the pattern was rotated around a vertical axis midway between the two walls.) In this test, the bees did not differentiate between the two tunnels, accepting both as the positive one. Therefore, interocular transfer of the unilaterally learnt pattern orientation information did not occur. Bees that have learnt an orientation on one eye prefer neither the same orientation (rotated about the vertical axis, as described above) nor the 'mirror' orientation when they are presented to the other eye. The same result was obtained in the experiments with grey discs and concentric circles, respectively, as neutral patterns.

Fig. 4C represents a control experiment in which both tunnels contained the pattern combination rewarded during training, except for a contrast reversal of the grating in the right panel. The latter demonstrates that the bees' performance is not based on the patterns' intensity distribution, while the test generally serves as a control for possible systematic errors in the apparatus or the training.

The experiments presented so far dealt with the bees' use of pattern orientation in the lateral visual field. The next step was to find out how orientation is used in the dorsal and ventral visual field. To do so, the experimental arrangement was modified so as to allow the presentation of patterns on the ceiling and on the floor of the two tunnels. In the training shown in Fig. 5A, the rewarded tunnel was marked with a grating on the ceiling, oriented at 45° to the bee's flight direction, and a 50% grey disc on the floor. The unrewarded tunnel was marked with the same patterns, but the grating was oriented at 135° , i.e. perpendicular to the rewarded orientation. Evidently, the bees were unable to learn the difference between these two situations, showing that pattern orientation is not used in the dorsal visual field.

An attempt to train bees on the same gratings, but presented on the floor, failed as well (Fig. 5B). We can conclude,

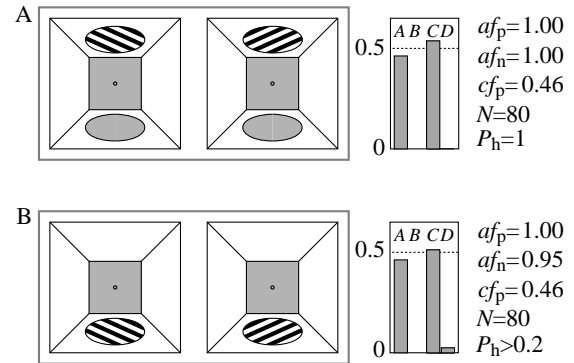


Fig. 5. Trainings on orientation on the ceiling and on the floor (dorsal and ventral visual field, respectively). Same notation as in Fig. 3. See text for details.

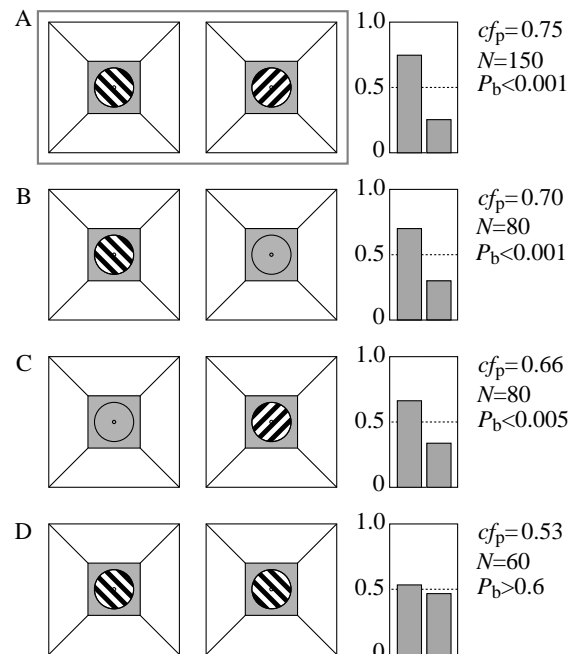


Fig. 6. Training on orientation on the back wall (frontal visual field). Same notation as in Fig. 3, except that here the bars represent the recorded choice frequencies for the two tunnels. P_b , probability of the choice behaviour of the honeybees being random, based on binomial distribution. For details of experiments, see text.

therefore, that pattern orientation is not used in the ventral visual field either.

Experiments on the use of pattern orientation in the frontal visual field are presented in Fig. 6. Here, the paper baffles used to obscure the patterns on the floor and ceiling of the tunnels in the experiments described above were removed. The patterns were now presented at the back wall of each tunnel, centred around the tube leading to the feeder box. Apart from a hole for that tube, these patterns were the same as the gratings used for the lateral, dorsal and ventral presentation. The rewarded pattern was oriented at 135° , perpendicular to the unrewarded pattern. The bees' first choices were recorded at

the entrance to the tunnels (*D1*). To compare the graphs in this figure with the four-bar graphs in the preceding figures, we can therefore think of the left-hand column in this figure (correct choices) as *A+B* and the right-hand column as *C+D*.

As expected, on the basis of the findings of earlier studies on orientation discrimination (e.g. van Hateren *et al.* 1990), the bees learn to discriminate pattern orientation in the frontal visual field quite well (Fig. 6A; $cf_p=0.75$). In the test presented in Fig. 6B, the unrewarded pattern was replaced by a 50% grey disc. This reduced the bees' choice frequency for the positive pattern only slightly, indicating that the positive training pattern on its own is sufficient for the pattern to be learnt. When the bees were tested on the negative pattern alone, replacing the positive pattern with a grey disc, a similar result was obtained (Fig. 6C): the bees recognised and avoided the negative pattern in absence of the positive pattern. Therefore, they must have learnt the negative pattern as well.

Finally, we can infer from the test shown in Fig. 6D that the bees' performance in the other tests of this experiment was indeed based on the patterns and not on any other cue, such as smell. Furthermore, the bees cannot have used the intensity distribution of the patterns, since the two patterns in Fig. 6D are contrast-inverted versions of the same grating and were not discriminated.

Colour

For comparison, the experiments on the use of the orientation of a single grating in different regions of the visual field were repeated using coloured discs. The purpose of these experiments was to examine how well colours are distinguished in the same regions and whether there is interocular transfer of learned colours.

Two different experiments with training on a coloured disc on one side wall and a grey disc as neutral pattern on the opposite wall are summarised in Fig. 7 (for the equivalent experiments with pattern orientation see Fig. 4). In both trainings, the colour information was presented to the right eye. In the training shown in Fig. 7A, the rewarded tunnel was marked with a blue disc, while the unrewarded tunnel bore a yellow disc. For the other training (Fig. 7D), these two colours were interchanged, so that yellow was rewarded and blue was not. In both cases, the training was equally successful, with an acceptance frequency for the negative tunnel of 0.35 and 0.39, respectively. This performance broke down, however, when the coloured discs and the grey neutral patterns were swapped (Fig. 7B,E). In Fig. 7B, the fact that two bees turned back in the negative tunnel ($D=0.10$) while no bee turned back in the positive tunnel ($B=0.00$) might indicate that the yellow disc on the left wall was recognised as the negative pattern. However, the number of recorded choices in this test ($N=20$) is arguably too small to draw any conclusions at all. This is reflected in the fact that the difference between the behaviour of the bees in the two tunnels is not statistically significant. Furthermore, taking the result of Fig. 7E (where only one choice involved turning back in each tunnel) into account as well, we can conclude that colour information acquired

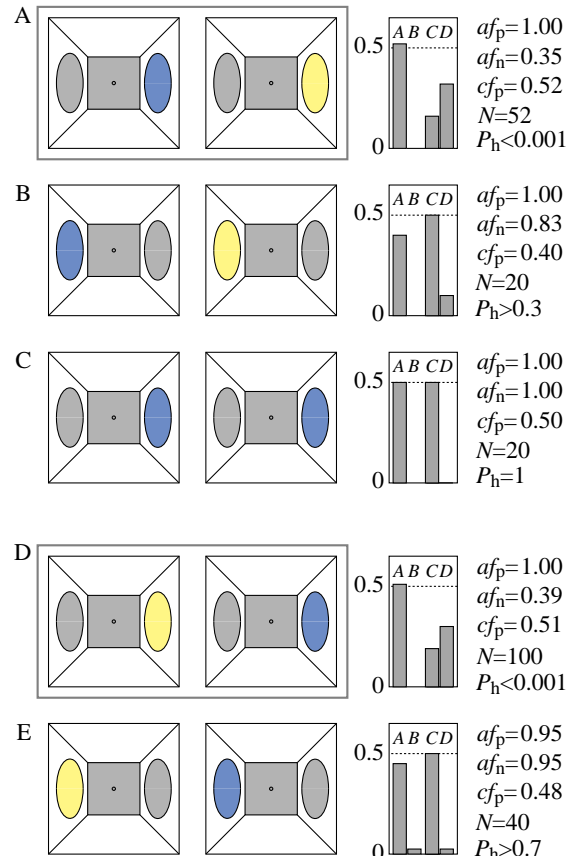


Fig. 7. Trainings on colour on one side only (lateral visual field). Same notation as in Fig. 3. See text for details.

through one eye is not transferred to the other eye. Fig. 7C depicts another control experiment to test for olfactory cues and other possible biases.

The experiments on the bees' use of colour information in the dorsal and ventral visual field, respectively, are presented in Fig. 8 (for comparison with pattern orientation see Fig. 5). As with the differently oriented gratings, we failed to train bees to discriminate between a blue disc (rewarded) and a yellow disc (unrewarded) when both discs were fixed to the ceiling of the respective tunnels (Fig. 8A). When the same discs were presented (to a different group of bees) on the floor, however, the training was very successful (Fig. 8B). In other words, the bees were very good at acquiring and using colour information presented in the ventral visual field. This result contrasts with the bees' inability to learn pattern orientation presented on the floor (compare with Fig. 5B).

The bees trained on coloured discs in the ventral visual field were then tested on the same discs presented in the dorsal visual field, i.e. on the ceiling of the respective tunnels. In this situation, the bees did not differentiate between the two tunnels (Fig. 8C). They did not recognise the negative pattern as such, i.e. the learnt colour information was not transferred from the ventral visual field to the dorsal visual field.

Fig. 8D,E shows two different control experiments demonstrating that a given pattern configuration elicited the

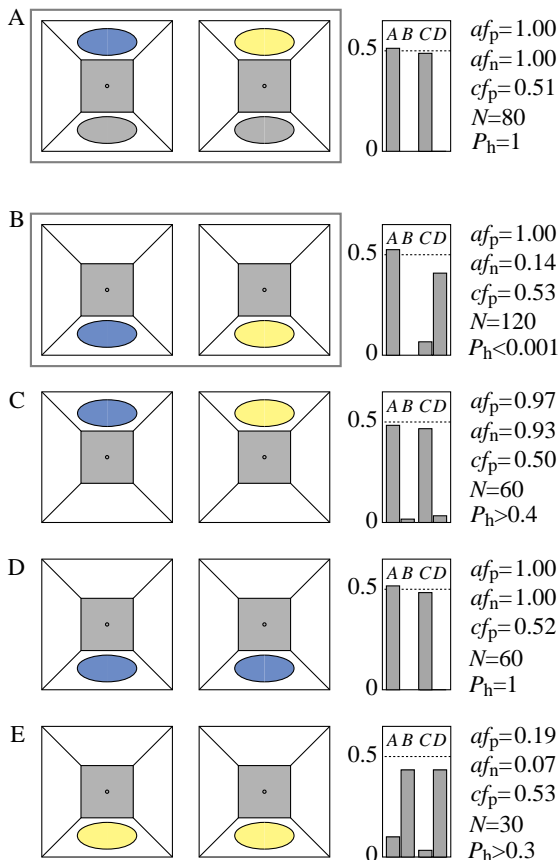


Fig. 8. Trainings on colour on the ceiling and on the floor (dorsal and ventral visual field, respectively). Same notation as in Fig. 3. See text for details.

same response whether it was located in the rewarded tunnel or in the unrewarded tunnel. Both tunnels were accepted as the positive tunnel when they both contained the rewarded pattern configuration (Fig. 8D). When both tunnels bore the negative pattern, however, they were both rejected (Fig. 8E).

To complete this series of experiments, the training shown in Fig. 6 (pattern orientation in the frontal visual field) was repeated using coloured discs (Fig. 9). Again, the obscuring walls within the Y-maze were removed, and the patterns were presented on the back wall of either tunnel, the tube leading to the reward (or a dead end, respectively) protruding through their respective centres. As can be seen from Fig. 9A, the bees learned very well to discriminate between a blue disc (rewarded) and a yellow (unrewarded) disc, favouring the correct tunnel in 95% of their choices. By analogy with the corresponding experiment on pattern orientation, these trained bees were then tested with either the positive or the negative pattern on its own, offering no pattern (i.e. only the white background) in the alternative tunnel. When only the positive pattern was offered, the bees accurately recognised and preferred this pattern (Fig. 9B), while the negative pattern, when presented on its own, was recognised and avoided just as accurately (Fig. 9C). Fig. 9D shows a control experiment in which both patterns were missing. In this test, the bees were

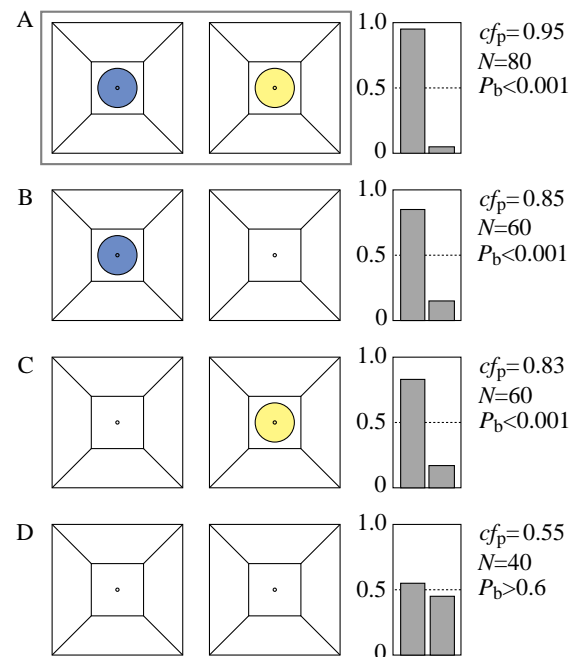


Fig. 9. Training on colour on the back wall (frontal visual field). Same notation as in Fig. 6. See text for details.

left without visual clues to the rewarded tunnel and showed no preference for either tunnel.

We can conclude that colour information – like pattern orientation – is acquired and used accurately in the frontal visual field. Furthermore, both the positive and the negative patterns are learnt.

Discussion

The results of the experiments presented here can be summarised as follows. Bees can learn to use patterns presented in the peripheral visual field to navigate their way to a food source. Pattern orientation is learnt and recognised in the lateral and frontal visual field, but not in the dorsal nor the ventral visual field. Colour information, in contrast, is used in the lateral and frontal as well as the ventral visual field, but not in the dorsal visual field. If pattern orientation is different on opposite sides during training, both patterns are learnt, but each on its own is sufficient for the bees to perform their task. Unilaterally learnt pattern information, be it orientation or colour, cannot be accessed when the test pattern is viewed on the other side, i.e. interocular transfer does not occur (this is documented further below). Bees make use of both the orientation and the colour of frontally presented patterns. In this case, both the positive and the negative patterns are learnt.

Analysing the data we present here, an interesting and somewhat surprising additional observation can be made. In the experiments involving non-frontal pattern presentation, the bees reacted to unfamiliar pattern configurations (i.e. configurations they did not identify as either the positive or the negative configuration) in the same way that they reacted to

the positive pattern configuration (Figs 4B, 7B,E, 8C), namely by mostly flying ahead. In other words, the bees turned back only when they saw the negative configuration, but not when they encountered an unfamiliar one. This phenomenon could be a result of the asymmetry of the discrimination task, i.e. the fact that bees presumably find it easier to continue along their path, while it takes an effort to stop and turn back. An alternative explanation, however, is that the bees are not looking for the positive pattern combination to lead them to the reward, but rather learn only the negative configuration, which signals them to turn back. In this case, the bees would never recognise the positive pattern configuration as such, but simply assume that they are in the correct tunnel if they do not encounter the negative configuration. Thus, in our experimental situation, the bees seem to treat the peripherally presented patterns as landmarks, learning only the *unrewarded* pattern configuration in order to recognise navigational mistakes made on the way to the food source.

Peripheral presentation of patterns

So far, we have assumed that patterns presented on the side walls, ceiling or floor are viewed laterally, dorsally or ventrally, respectively. This, however, is not necessarily the case, especially for patterns on the side walls. It is very unlikely, if not physically impossible, that the bees rotate around their transverse axis (i.e. pitch) to face the dorsally and ventrally presented patterns frontally. Cinematographic studies of bees approaching a target (Baumgärtner, 1928), hovering in front of the entrance to a feeder (Wehner, 1972; Wehner and Flatt, 1977) and searching for a target in a vertical wall (Lehrer, 1990) show that the pitch angle of both the head and body axis of the bee vary very little during free flight. Moreover, common sense alone suggests that the physics of flight would prevent a bee from pitching by up to 90° without seriously disrupting its flight pattern. However, the bees should find it very easy to turn around their dorso-ventral axis (i.e. yaw) to move laterally presented patterns into their frontal visual field.

This frontal viewing of the patterns cannot be prevented in our apparatus, but it can be detected by filming the bees and analysing their position and bearing frame by frame. During the learning phase of the experiment, i.e. the initial training, the bees' flight trajectories are typically very convoluted as they explore the apparatus and search for the reward. Later on in the training, however, the bees 'know' where to go, and their flight paths become much straighter, leading directly to the feeder's entrance (Fig. 2B,D provides two examples of such flight paths). It is this stage that we chose for our video analysis.

Magnifying the relevant portions of the bee's trajectories (i.e. those areas in which the bees turning back made their decision), we determined the patterns' position in the bee's visual field for each digitised position in four trajectories of bees making the correct decision. It was found that in at least three of these trajectories the bees had never turned to view the indicative pattern frontally. This seems to verify the assumption that laterally presented patterns are effective in the lateral visual field.

Interocular transfer

Interocular transfer is said to occur if the memory of a visual stimulus, acquired with only one eye, can be retrieved when the same stimulus is presented to the other, naive eye only. This does not necessarily require an actual transfer of information, but can be due to the convergence of the signals from both eyes onto the same memory site.

To demonstrate interocular transfer beyond any doubt, one has to ensure that, at the time of testing, the naive eye is really naive, i.e. has never seen the stimulus before. This requirement is very difficult to fulfil in experiments involving freely moving animals. In our experiments with patterns presented on only one side wall, the bee's 'naive' eye is frequently exposed to the training stimulus, both during the initial training (when the bee is exploring the apparatus) and throughout the experiment (each time the bee leaves the feeder and flies back the way it came). The only way around this problem is to assume that no learning occurs in those situations, i.e. that stimulus learning commences only when the bees start to approach the feeder entrance directly.

This assumption made, the next requirement is to ensure that the pattern does not enter the field of view of the trained eye during the test in which the stimulus is presented to the 'naive' eye. This can be done *a posteriori* by analysing video sequences of the bees' trajectories, as was done in the previous section. Close to the horizon, the binocular visual field of the worker bee covers approximately 24° (Seidl and Kaiser, 1981). In the above analysis, we found that in at least three of the four analysed trajectories the patterns never entered this binocular area. We can therefore conclude that the patterns on each side were viewed exclusively by the corresponding eye. For the results presented here, however, this finding is not important, since interocular transfer could not be demonstrated.

The work by Lehrer (1994) suggests that interocular transfer does occur in the honeybee – if one assumes that learning takes place during the bee's approach only (see above). Among other experiments, she trained bees to use a bar offered in the left peripheral visual field to localise the rewarded hole in a vertical array of identical holes. She then found that, in subsequent tests with the same bar presented only on the right, the bees were still able to localise the target. This was true regardless of whether the training bar was presented equatorially or in an elevated or depressed position in the visual field of the left eye. Video recordings of bees searching in front of the array showed that the bees kept their longitudinal body axis fairly perpendicular to the array, thus viewing the bar on the right only with the right eye. Lehrer (1994) concluded that interocular transfer of the positional information does occur. This conclusion must be qualified, however, by the fact that the training in these experiments was conducted with the bar presented only in the equatorial region of the visual field. Therefore, the apparent transfer of visual performance to the other eye may have simply reflected an innate tendency to place the bar in the equatorial region of the eye, rather than a learned behaviour.

In experiments on the desert ant *Cataglyphis fortis*, Wehner

and Müller (1985) showed that interocular transfer occurs in that animal when it navigates using the pattern of polarised light in the sky, but not when it uses landmarks for orientation. The former is to be expected since, in reading the polarisation pattern of the sky, both eyes act as part of the same filter. Covering one eye simply reduces the input to that filter by half, regardless of which eye is covered. In other words, the signals from both eyes converge onto one channel that does not discriminate between the two inputs. The positional information of landmarks, in contrast, is necessarily fixed somehow to the animal's internal representation of space. Since the two eyes cover different regions of this space, a transfer of information from one eye to the other could have devastating effects on the animal's orientation.

Comparing Lehrer's (1994) results and our findings reported in the present paper, it is possible to arrive at an analogous interpretation. In Lehrer's experiments, the bees had to localise a target along a vertical axis, i.e. in one dimension only. The vertical position of a bar can be safely transferred from one eye to the other, since both eyes share the same height. By analogy with the celestial compass, we could therefore – very hypothetically – propose a channel coding for vertical position which receives simultaneous input from both eyes. In our experiments, however, the honeybees had to recognise a particular attribute (orientation or colour) of a pattern presented in a particular position in the visual field. By analogy with the landmark orientation experiments, this information is fixed in space and cannot be transferred from one eye to the other.

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