Spatial coincidence of cues in visual learning by the honeybee
(*Apis mellifera*)

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Abstract

The discrimination of patterns was studied in a Y-choice chamber fitted with a transparent baffle in each arm, through which the bees had a choice of two targets via openings 5 cm wide. The bees see the positive (rewarded) and the negative (unrewarded) targets from a fixed distance. The patterns were bars (subtending 22° × 5.4° at the point of choice) presented in one-quarter of each target. The bars were moved to a different quarter of the target every 5 min, to make the location of black useless as a cue. A coincident presentation is when the bar on the left target is on the same side of the target as the bar on the right target. The bees learn the orientation cue when the presentation is coincident but otherwise cannot learn it. This experiment shows that bees do not centre their attention on the individual bars, otherwise they would always discriminate the orientation. Centring the target as a whole precedes learning. Having learned with the bar on one side of the targets, bees do not recognize the same cue presented on the other side. A separate orientation cue can be learned on each side. A radial/tangential cue is preferred to a conflicting orientation cue. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In investigations of the discrimination of edge orientation by bees up to 1990, black and white targets were presented in a vertical plane, were fixed in one place throughout the learning process and subtended a large angle at the point of choice. In these conditions, the bees use the locations of areas of black as cues in discrimination tasks (Horridge, 1996b) and other cues are not necessarily used at all. Besides the location of an area of black, they can also learn one obvious orientation cue (Wehner, 1971). This kind of memory appears to be related to that of landmarks (Horridge, 1996d).

Under these conditions, the memory of location is thought to be restricted to the part of the eye where it is learned. The evidence is that when the upper or the lower parts of the eyes were covered separately, it was found that a simple cue learned in one place was not recognized when tests were made on a different part of the eye, or on the other eye (Wehner, 1972). These results led to the idea of an ‘eidetic’ image, that is, a memory fixed on the eye region where it is learned. However, the targets subtended a large angle at the point of choice, and were fixed in one position during the whole learning period. There was therefore no need for the bees to learn anything but one stimulus location during the training.

A similar result, with an orientation as well as a location cue, was obtained recently when the bees learned to discriminate fixed orientation cues subtending a large angle on one side of the path to the reward, and therefore with only one eye. When the orientation cues were presented to the other, naïve, eye, the bees were unable to discriminate them (Giger and Srinivasan, 1997). Thus transfer of this particular task did not occur between one eye and the other. Again, however, the technique determined the result: when a large image is fixed in one location during the training period the bees recognize the locations of its outer edges (Horridge, 1996b). These results are consistent with the finding that neurons of the lobula that are sensitive to the orientation of a bar, have fields that cover one eye but not both eyes (Yang and Maddess, 1997). On the other hand, in learning experiments, cues derived from image motion are
transferred between the two sides (Lehrer, 1994; Lehrer and Wehner, 1990). The transfer of other cues, such as object size (Horridge et al., 1992) or radial and tangential cues (Horridge, 1994, 1996a, 1997b; Horridge and Zhang, 1995), has not been investigated.

Even with fixed patterns, Wehner (1971) showed that bees can discriminate between two average orientations and then use this cue to distinguish between patterns that they have not previously encountered. By using gratings of random period in the Y-choice apparatus Van Hateren et al. (1990) also demonstrated the discrimination of one average orientation irrespective of the locations of edges and black areas or pattern disruption. In these experiments, one orientation was presented in all parts of the positive target and another in the negative target, so the two cues could be compared in corresponding regions of the two targets. Similarly, when single bars were used in other work, they lay across the centres of the two targets (Horridge, 1994, 1996a; Srinivasan et al., 1994; Zhang and Srinivasan, 1994). In these experiments the Y-choice apparatus had no baffles, so the bees flew directly to one target or the other. Again, the results correspond with the finding that lobula neurons sensitive to the orientation of a bar have fields that cover the whole of one eye, and therefore they sum all the orientation cues within their field (Yang and Maddess, 1997).

The baffles illustrated in Fig. 1 were developed in 1994 to control the target size as seen from the point of decision (Horridge, 1996b), and to make it easier to count the decisions of the bees. When baffles were introduced, however, some differences were noticed in the behaviour of the bees, which now were obliged to pause at the baffles and look through them or through the access holes at the targets from a fixed distance. At the same time, the technique of changing the pattern every 5 min was introduced to make the locations of edges or areas of black useless as cues, but to keep constant other features to be discriminated.

From the point of view of studying the learning mechanism, however, the important events occur in the comparison of the positive cue with the negative one, not after the memory is in place. Therefore the cues should be compared in corresponding locations on the targets during the learning process, or alternatively they should be in separate locations. This question of coincidence of presentation of the positive and negative cues during the learning process has not been previously studied.

2. Materials and methods

The experiments were done in the Y-choice apparatus, modified by the addition of the baffles and a circular entrance hole 5 cm in diameter to keep out newly recruited bees (Fig. 1). The baffles were made of transparent 'Articcare Drawfilm', 0.13 mm thick, set in a cardboard frame 1 cm wide. The hole at the centre of each baffle was 5 cm in diameter and was surrounded by a black annulus 0.5 cm wide. The bees flew through this central opening without landing on it. The targets had a hole 2 cm in diameter at the centre; in positive targets this allowed access to the reward of sugar solution, but in negative ones it led to a blind tube. The bees can see this hole from the orifice in the baffle, as shown by the way that they fly towards it when the targets are blank. The side of the positive target and the reward with it, are changed every 10 min during training to prevent the bees from learning which arm of the apparatus to choose, but in the illustrations the rewarded pattern (labelled +) is always shown in the left column.

The bees were trained on one pair of patterns at a time, but in most of the experiments the patterns were exchanged for a different pair every 5 min to make some potential cues useless. The bees had to ignore those features that were not constant and find a consistent feature of the positive patterns as opposed to the negative ones.

The bees usually spent some time looking through a baffle but not necessarily hovering in front of the central hole. The criterion for a score was when the bee passed through the baffle. With the baffle at a distance of 27 cm, the square targets of side 26 cm, on a slightly larger back plate, subtend an angle of about 55° at the point of choice. After an initial 2 h training period, the bees' choices were counted in each period of 10 min while training continued. These results were labelled 'Train and test'. In Figs 4 and 5 the trained bees were then tested for periods of 5 min on each side with a pair of...
patterns that they had not seen before, first with the positive pattern on one side and then with the sides reversed. These results are labelled 'Test'. The full procedure has been repeatedly described (Horridge, 1996a, b; Srinivasan and Lehrer, 1988; Van Hatere et al., 1990).

To change patterns, the targets were rotated or moved to the other arm of the apparatus, rather than being replaced by other targets. This procedure acted as a control against differences in odour, brightness or unexpected cues. Quite arbitrarily it was considered that training for 2 hours was more ecologically realistic than training for several days, because the intention was to analyse the discrimination system, not to realize the utmost capabilities of the bees.

The experiments were designed to show whether or not the bees could or could not make a discrimination, which is usually obvious from their behaviour. All the conclusions are based on decisions made by the bees after a comparable number of visits. Each bee was marked with a colour code and scored separately. The choices of the bees were independent. The group of 8 to 16 bees made a total of 10 to 25 visits in each of the 12 to 20 periods of 10 min between the changes in the position of the reward. The numbers of correct choices and the total number of choices were scored, and the fraction of correct choices was calculated for each of the 10-min periods. This distribution of 12 to 20 fractions was tested by a \( \chi^2 \) test for a difference from a similar distribution about a chance probability (0.5). The same distribution was used to calculate the standard deviation and the fractions were all converted to percentages. The statistical procedure is described in detail by Van Hateren et al. (1990).

3. Results

Some of the cues proposed in the past, such as differences in pattern disruption, symmetry or size, do not apply when the pattern is a single bar of constant size and shape. The only cues relevant to the present situation that are known from previous work are the orientation of the bar (Wehner, 1971), its location on the target, and the perception of it as a radius or a tangent (Horridge, 1994, 1996a; Horridge and Zhang, 1995).

3.1. Coincident presentation

A group of bees was trained with a single bar pointing NW-SE (North at the top) on the positive target, and a similar bar pointing SW-NE on the negative target. The positive and negative targets were interchanged every 10 min. The bar on both targets was moved every 5 min to one of four positions in succession (1-4) in such a way that at each choice the bars on the positive and negative targets occupied corresponding quarters for equal periods of time (Fig. 2). This has the effect that, as they are moved around, both bars alternated between being radial and tangential relative to the centre, so that these features and the locations of the bars were not useful as cues. The orientation cue, on the other hand, was consistent. After 2 h of preliminary training, the result was 61.7 ± 3.1%, \( n = 237, P < 0.0005 \) scored over the next 2 h of continued training and testing. The bees were taught not to pay attention to the bar location because it was similar in the positive and negative targets at any one time, and they could not have learned all four separate pairs of locations within the short learning period of 2 h. The result shows that orientation of a single bar is discriminated when tangents, radii or location of black areas have all been shuffled. Before discussing the significance of the result, let us consider the next experiment.

![Train and test with 4 different coincident locations](image)

61.7% ± 3.1%, \( N = 237, p < 0.0005 \)

Fig. 2. Discrimination of orientation with coincident presentation. The bars on the positive and negative targets are at right angles to each other and are constant in orientation. Every 5 min the bar in both targets is moved to a different location, 1, 2, 3 and 4 (coincident on the two sides), so that location is a useless cue. The bars alternate between being tangents and radii, so this feature is also a useless cue, and the bees discriminate the difference in bar orientation.
3.2. Non-coincident presentation

A new group of bees were trained with the same patterns but with a sequence of negative patterns $1^-$, $2^-$, $3^-$, $4^-$, changed so that the single bar is now at opposite sides of the target at each presentation (Fig. 3). The bees failed to learn, even though the training and testing was extended beyond the usual period. Starting tests after 2 h of preliminary training, the result was $50.7 \pm 0.4\%$, $n = 219$, which is in marked contrast to the first result (Fig. 2).

The difference between the two experiments was whether the bars that were to be discriminated from each other, lay on the same or on opposite sides of the target at each choice. If the bees centre their attention on the bars individually, then changing the positions of the bars from coincident to non-coincident should have no effect on the orientation discrimination. Conversely, if the bees centre their attention on the reward holes or on the geometry of the target as a whole, then the bars fall on the same side of each target at each presentation in the first experiment but on opposite sides in the second experiment. The comparison between the results shown in Fig. 2 and those in Fig. 3 implies that the bees do not learn to discriminate between the orientation cues unless they are presented on the same side of the target. The difference also implies that the bees centre their attention on the target as a whole, not on the individual bars. They place the bar in its location relative to the centre of the target. The result may also suggest that the left side of the target is processed by the left eye and the right side by the right eye, but the actual observations have no bearing on this question.

3.3. Orientation is not the strongest cue

In a third experiment, a new group of bees was trained with the bars in coincident locations but now fixed in place for the whole period of the training (Fig. 4a). The result, scored after 2 h of preliminary training, was $78.4 \pm 3.1\%$, $n = 193$, $P < 0.0001$. The bees discriminate very well, but cues are now available from the location of black, and radial/tangential edges, as well as from the orientation. When tested with the bars in the same orientations but in different locations, the bees disregard the orientation cue, and use the cue provided by the tangent or the radius (Fig. 4b). The result was $73.1 \pm 3.3\%$, $n = 159$, $P < 0.0001$. The reason for alternating the radial/tangential cue in Fig. 2 and Fig. 3 now becomes obvious, because the bees prefer this cue to the orientation cue, and perform better with it. The locations of the ends of the bars could not have been the cue in Fig. 4a because they are changed to new locations in the test.

3.4. Alternation between two positions

In a fourth experiment, the bars alternate between only two non-coincident positions, both on the right side of the positive target and both on the left side of the negative target, with radial and tangential features not useful as cues (Fig. 4c). The bees failed to learn, even though training and testing was continued for 4 hours. The result, with the count starting after 2 h of training, was $50.8 \pm 4.5\%$, $n = 221$. The bees clearly did not centre their attention on the individual bars, for if they did they would discriminate the orientation. This experiment shows that the bees did not learn the separate locations of the bars, even when they were alternated between only two positions, so they were unlikely to have learned the four combinations of bar positions in Fig. 2.

3.5. Failure of transfer

A new group of bees was trained with an orientation cue and coincident presentation, but they were alternated between only two positions. Both positive and negative patterns had the bar on the right side (Fig. 5a). As expected for a coincident presentation, and when the use of radial/tangential cues was excluded, the bees learned to discriminate orientation. The result was $66.5 \pm 3.0\%$, 

![Diagram showing non-coincident bar locations](image-url)
Fig. 4. (a) The bar positions are fixed and the bees learn to discriminate, but the orientation is not the only cue. (b) When tested with the bars in different positions, the bees prefer the tangent to the radius, as they did in the training situation, although the orientation cue is now contrary to that in the training. The radial/tangential cue is more effective than the orientation cue. (c) With only two alternating locations of the bars and non-coincident presentation, the bees fail to discriminate the consistent positive and negative orientations.

\[ n = 193, \quad P < 0.0001 \text{ after } 2 \text{ h of training.} \]

The trained bees were then tested with the bars on the left side, in tests that were alternated between the upper (Fig. 5b) and the lower quarters (Fig. 5c). The bees fail in the tests, showing that the learnt discrimination is not transferred from one side of the target to the other. This result also suggests that the flying bees processed the two sides of the target separately, although they were free to turn and direct either eye towards the individual bars.

3.6. Two parallel bars

A new group of bees were trained with the positive target bearing two parallel bars that sloped down towards the mid-line, and the negative target with two similar bars that sloped upwards towards the mid-line, with non-coincident presentation. The two bars were moved to the other side of the target every 5 min (Fig. 6a), so that the orientations on each target were alternated between positions 1 and 2, and there was no average orientation cue. The result, after 2 h of preliminary training, was \[ 52.1 \pm 3.4\%, \quad n = 214. \]

We discriminate the consistent component of the positive pattern as half a funnel and the negative one as half a roof-line. The bees fail to find this consistent difference in bar orientation relative to the midline or in the many possible conjunctions of cues available from the individual bars. There is no evidence that they compared the patterns or even saw the individual bars, or their lay-out in relation to the midline. This failure shows that no cue was available.

With a new group of bees, the presentation of the same patterns 1 and 2 provided a consistent difference
in the orientation between corresponding sides of the positive and negative cues. A comparison of the orientation could now be made, first on one side and then on the other (Fig. 6b). Not surprisingly, as there was now a strong orientation cue, with coincident presentation, the bees performed well, with a result of 70.1 ± 3.3%, n = 195 after only 1 h training. Opposite orientations on the two sides evidently did not interfere with each other in the recognition mechanism. The bees were capable of remembering the two different orientations in the two sides of the target. This in turn implies that the target was centred innately before the bars were accepted as being in one side of the target or the other.

A new group of bees, trained and tested on patterns that were the sums of the pairs of bars (Fig. 6c), gave the result 68.7 ± 2.7%, n = 135, exactly as expected from these particular patterns (Horridge, 1996a, c). As in all patterns that are symmetrical about a vertical midline, the presentation is coincident on both sides and there are opposite orientation cues on the two sides. Comparison of Fig. 6c with Fig. 6a shows that the bees did not take the sum of the two cues presented successively in the same location in Fig. 6a. Similarly, consideration of Fig. 3 shows that the bees did not remember the sums of targets that were presented successively, so summation of successive targets is unlikely to be an explanation in Fig. 2. The only possible interpretation of the results is that the different orientation cues presented alternately on the two sides of the targets were independently placed relative to the centre and separately built into memory if they corresponded in location at each presentation. As mentioned in the discussion, this is not a new result.

4. Discussion

As mentioned in the introduction, in previous experiments in which bees were trained at one region of one eye and tested at a different region, a large pattern was fixed for the whole period of the learning. These are the conditions that lead to the learning of the location of a contrast while offering little information about the nature of the cue. The image that was projected during learning to one eye region was not recognized by another eye region in subsequent tests (Wehner, 1972). The results led to the idea that bee pattern vision is 'eidetic', that is, fixed on the region of the eye that was occupied by the image of the cue. Such a mechanism is appropriate for the memory of landmarks, which are useless unless their direction is remembered. However, an eidetic image, like a photograph, is insufficient for vision, which involves spatial interaction between responses of separate receptors. An important feature of vision, generalization over a range of certain related images, but not others, can be explained by a processing mechanism with coarsely tuned filters, but not by an eidetic image, or universal learning mechanism.

The learning of the location of black on the target is a characteristic of fixed targets subtending 130° but not necessarily of targets subtending up to 50° at the point of choice (Horridge, 1996b, 1997b). On the other hand, experiments in which the locations of the features are
shuffled or randomized show that bees learn certain cues very well when they are obliged to do so, although there is no possibility of an eidetic image. When regions of black are shuffled, bees discriminate the disruption of the pattern irrespective of pattern, orientation or location (Hertz, 1933; Horridge, 1997a), orientation irrespective of location, pattern, reverse contrast or size (Van Hateren et al., 1990; Wehner, 1971), radial and tangential cues irrespective of pattern (Horridge, 1996a; Horridge and Zhang, 1995), size irrespective of location or range (Horridge et al., 1992), and bilateral symmetry about an axis irrespective of pattern (Horridge, 1996c). In all these experiments, the distinction between transfer of a memory and correspondence in the presentation during learning did not arise.

Learning a generalization of one of the above cues occurs when location of black is shuffled or alternated during training. On the other hand, eidetic memory is a result of a training procedure with large targets that are fixed in location during the training period. Another reason to shuffle unwanted cues is to make all except one of them useless, as illustrated in Fig. 4b. The bees prefer the radial/tangential cues to the orientation cues when both are available, but alternation of the unwanted cues between two opposite states was sufficient to make them useless (Fig. 4c).

When the bars are non-coincident at each presentation (Fig. 3), the bees failed to use the consistent orientation cue, and shows that they do not centre their attention on the individual bars. If they had centred their attention on the individual bars, they would have discriminated the consistent difference in orientation. In Fig. 3, the bees behave as if they compare the cues only after they see the bars in relation to a centre that is obtained from the geometry of the apparatus. The use of a radial or tangential cue (Fig. 4b) also requires the innate centring of the target as a whole. In each case, the bees cannot be centring their attention on the individual bars. A coloured spot also acts as a centre, a point that is fundamental for the processing of flower shape on a textured background (Horridge, 1997c). The innate centring of the target before learning the cues is fundamental for this kind of form vision.

In previous work on targets subtending up to 50°, it has been shown that the bees fail to discriminate the shapes formed by the spatial relations between bars, even in the simplest patterns of two bars, whether or not they are fixed during the training period (Horridge, 1996a, 1997b). They also fail to see the relation between two bars with different orientations on the same side of the target (Horridge, 1997b). The failure to separate the bars or discriminate the spatial relations between them is consistent with the idea that the fields of all the cue detectors are large and they may fill the whole field of view of an eye, but rules out an eidetic image.

Previous work has shown that bees can remember two different orientations of bars of different sizes that are easily distinguished on the same target (Zhang et al., 1992, Fig. 4). They can also discriminate when there is one orientation on one side of the target and the orthogonal one on the other side (Zhang and Horridge, 1992, Fig. 2; Horridge, 1997b, Fig. 6). The same result was obtained with patterns of two pairs of orthogonal bars, as in Fig. 6c (Horridge, 1996a). Despite these findings, there is no evidence that they see the spatial relations between separate bars.

Finally, bees that are trained to discriminate between two orientations on one side of the target failed to discriminate when the test bars were moved to the other sides (Fig. 5b and Fig. 5c). In this experiment an eidetic image has been ruled out and the orientation cue was separated from the cue of location on the target. In all the experiments with the Y-choice apparatus, we refer only to the two sides of the target, not to the two eyes, but the results are compatible with the recent finding that the fields of orientation-sensitive neurons are restricted to the whole field of the eye on each side (Yang and Maddess, 1997). Using targets that subtended much larger angles at the point of choice, Giger and Srinivasan (1997) found recently that established learning of orientation was not transferred from one eye to the other.

The experiments show that the geometry of the whole scene is important in the learning of a simple cue in front of the bee. Bees in free flight centre their attention on the centre of the target, with the effect that the two sides of the target are processed separately, although the bees are free to turn. The implications of this behaviour are rather broad. For example, in mirror images, oblique orientations are in opposite directions on the two targets, and the cues may or may not be on corresponding sides. Only some pairs of mirror images are discriminated, depending on whether a suitable global cue is available (Horridge, 1996a, 1997b).

The results presented here are also relevant to possible mechanisms for detecting symmetry about a vertical line, when the left and right sides of the target are mirror images of each other, and the choice of cue on one side is the reverse of that on the other (Horridge, 1996c). In the discrimination between a symmetrical and an unsymmetrical flower, the relevant cues are duplicated on the two sides. A coloured spot can also act as a centre (Horridge, 1997c). The common thread running through these ideas is that the bee first centres the target by use of the geometry of the whole scene. The ‘turning to look’ by many insects (Horridge, 1977) is also related to the centring of the target by innate mechanisms. It is also possible that the panning manoeuvres made by bees or wasps as they leave a food or nest site (Lehrer, 1996; Zeil, 1993), are a way to use landmarks to establish the position of a centre that will be useful, irrespective of range, when the insect returns to the scene. Without a
centre, information about symmetry, location, size or radial/tangential cues, is useless.

The baffles were brought into use to control the distance between the choice point and the target, but the subtle interaction between the direction of attention and the required coincidence of presentation during the learning of orientation cues would not have been revealed without them.

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References


