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Visual Discrimination of Pattern Orientation by Honeybees: Performance and Implications for `Cortical' Processing

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Visual discrimination of pattern orientation by honeybees: performance and implications for 'cortical' processing

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SUMMARY

The ability of honeybees (*Apis mellifera*) to discriminate pattern orientation was evaluated by examining their choice behaviour in a twin-choice Y-maze apparatus which offered two differently oriented patterns, one of which was associated with a reward. The distinctive feature of this experimental arrangement was that it forced the freely flying bees to choose between the two patterns at a distance, thus preventing the bees from fixating the patterns and discriminating between them on the basis of an eidetic ('photographic') image. Training and testing experiments using a number of different pairs of patterns, such as gratings, stripes and plaids, led to the following results: (i) bees perform well at discriminating the orientation of unidirectional patterns, such as single stripes or gratings; (ii) trained bees learn to prefer the orientation that is associated with the reward, as well as to avoid the unrewarded orientation; (iii) bees perform poorly at discriminating the orientation of right-angled crosses or plaids, but well at discriminating the orientation of 45° crosses or plaids; and (iv) orientation discrimination appears to be mediated primarily by signals from the green-receptor channel of the bee's visual system. These findings, together with recent work (Srinivasan *et al. Nature*, *Lond.* **362**, 539–540 (1993)), suggest that the honeybee's visual system analyses orientation in much the same manner as the mammalian cortex. Our data suggest the participation of at least three orientation-sensitive channels with different preferred orientations, and broad tuning curves with half-widths of *ca.* 90°.

1. INTRODUCTION

Despite intensive investigation, the principles by which animals recognize visual patterns remain an enigma. The 'vocabulary' of the visual system continues to be a mystery, and we are still largely in the dark as to what 'tokens' the visual system uses to represent, analyse and recognize patterns. This is true for a wide variety of organisms, including man.

Recently, we have been investigating the principles by which honeybees memorize and recognize visual patterns (van Hateren *et al.* 1990; Zhang & Horridge 1992; Zhang *et al.* 1992). We have shown that bees can learn the orientation of a pattern, and use this as a parameter to distinguish between other patterns which they have never previously encountered (van Hateren *et al.* 1990). We have also shown that, under the conditions of our experiments, the orientation of a pattern can be analysed even if the rewarded pattern is not memorized 'photographically'. It thus appears that bees are able to abstract certain general properties of patterns, without memorizing the patterns literally.

How does the visual system of the bee abstract the orientation of a pattern? Recently, we examined the possibility that pattern orientation is analysed in terms of the directional movement signals that the pattern generates in the eye as the bee approaches or flies past it. A pattern consisting of vertically oriented stripes, for example, would generate predominantly leftward or rightward movement signals in the flying bee, whereas a pattern comprising horizontal stripes would generate primarily upward or downward signals (Srinivasan & Lehrer 1988; Horridge 1991). This is a simple and attractive hypothesis, given that the visual systems of many flying insects possess four classes of directionally selective movement-detecting neurons tuned to movement in the leftward, rightward, upward and downward directions, respectively (Hausen & Egelhaaf 1989; Goodman *et al.* 1991). However, a recent test of this hypothesis, using moving patterns and flashed patterns has shown that bees do not use directional movement cues to analyse orientation (Srinivasan *et al.* 1993). Rather, it appears that patterns are analysed in terms of their geometry per se, and independently of their motion.

How can the orientation of a pattern be represented in a purely geometrical fashion by the nervous system? One way would be to encode orientation in terms of the responses that the pattern evokes in a number of orientation-sensitive channels, each tuned to a different range of orientations, as in the mammalian

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visual cortex (Hubel & Wiesel 1968). Is it possible that orientation is analysed similarly in the visual system of the bee? Recent physiological and behavioural evidence from our laboratory strongly favours this notion (Srinivasan *et al.* 1993; O'Carroll 1993).

Here we describe a series of experiments to measure the performance of bees at discriminating orientation, and to further explore the possibility that bees possess a functional visual 'cortex'.

2. MATERIALS AND METHODS

(*a*) *Apparatus*

Worker honeybees (*Apis mellifera*) were marked and trained to enter a Y-shaped, dual-tunnel apparatus, similar to that described in Srinivasan & Lehrer (1988). Bees entered the apparatus through an aperture in the window of the laboratory, and could simultaneously view two patterns, each presented in the vertical plane on the end wall of a tunnel. One of the patterns (termed positive) offered a reward of sugar water, dispensed by a feeder located in a box behind the pattern, accessible to the bee via a small tube. The other pattern (termed negative) offered no reward. The significance of the design of the apparatus is that the bees must make a choice at the entrance to either tunnel, which is at a considerable distance (27 cm) from the pattern being viewed. As demonstrated by van Hateren *et al.* (1990), this arrangement prevents the bees from discriminating between the patterns on the basis of a 'photographically' memorized image, because they are unable to fixate the patterns steadily at such a large viewing distance. Details of the apparatus are given in Srinivasan & Lehrer (1988).

(*b*) *Training and testing procedure*

Each experiment was commenced by training a fresh, naive group of four to seven bees to enter the apparatus and collect the reward. On a warm day, all of these bees would visit the apparatus roughly twice every 10 min (the hive was about 50 m from the laboratory in which the experiments were conducted). The positions of the positive and negative patterns were interchanged every 10 min. The reward box was also moved, so that it stayed with the positive pattern. This interchanging was performed continually throughout the experiment, in order to ensure that the bees did not associate the reward with a particular tunnel.

In the testing procedure we presented the bees with two patterns, the rewarded one called positive and the other called negative. The reward was offered behind the positive pattern. Tests were of three kinds: (i) in tests to assess whether learning had occurred (learning tests), the positive and negative patterns were identical to those used in the training; (ii) in control tests, the two patterns were identical, to check for the possibility that the bees were using olfactory cues from the reward box. Such tests, and similar tests conducted in an earlier study (van Hateren *et al.* 1990) assured us that olfactory cues did not play a significant role in the bees' choice behaviour in our experimental setup; (iii) in critical tests, the positive, negative or both patterns were different from those used in the training.

The tests were conducted for short periods of about 10 min, during which each bee was rewarded only four times, on average: twice in one tunnel, and twice in the other. The brevity of the tests, together with the fact that tests were interspersed by lengthy periods of training, ensured that the bees did not learn to discriminate the test patterns on the basis of being rewarded at one of them. (Several controls against this possibility, showing that the behaviour is not influenced by the brief tests, are described in van Hateren *et al.* (1990).) In the tests, a bee's choice was scored as correct if it entered the tunnel leading to the rewarded pattern, or as incorrect if it entered the other tunnel. Only the first choice of each bee on each visit was taken into account, to eliminate the possibility that the second choice might be influenced by the outcome of the first. This was particularly important if the first choice happened to be incorrect, so that the bee inspected one pattern at close range and then went to the other.

The bees' responses were analysed in terms of the choice frequency, α, in favour of the positive pattern. Thus, $\alpha = 0.5$ implies that the bees do not discriminate between the two patterns, whereas $\alpha = 1$ indicates perfect discrimination. A $\chi 2$ test was used to determine whether a measured α was significantly different from random choice behaviour ($\alpha = 0.5$). Further details are given in van Hateren *et al.* (1990).

(*c*) *Stimuli*

Stimuli were prepared on cardboard disks, 24 cm in diameter. Except for the chromatic patterns (see below), all patterns consisted of black or grey stripes on a white background. The white background was cut out of photocopying paper. The black stripes were cut out of thin, matt cardboard and the grey stripes out of photocopied 50% Letratone which was carefully screened to avoid flaws. These stripes were glued on to the background. Their dimensions and spacings were such that individual stripes should have been clearly resolvable by the bee's visual system, based on its known visual acuity (see Srinivasan & Lehrer 1988). A variety of patterns were used: random gratings, periodic gratings, single-stripe patterns, cross patterns, and plaid patterns. The details of each pattern are specified in the description of the corresponding experiment in § 3.

3. RESULTS

(*a*) *Orientation discrimination of gratings*

The ability of bees to discriminate pattern orientation was first examined by using patterns consisting of random, one-dimensional, black-and-white gratings. Each grating consisted of 12 bars, each 2 cm wide, with each bar having an equal probability of being

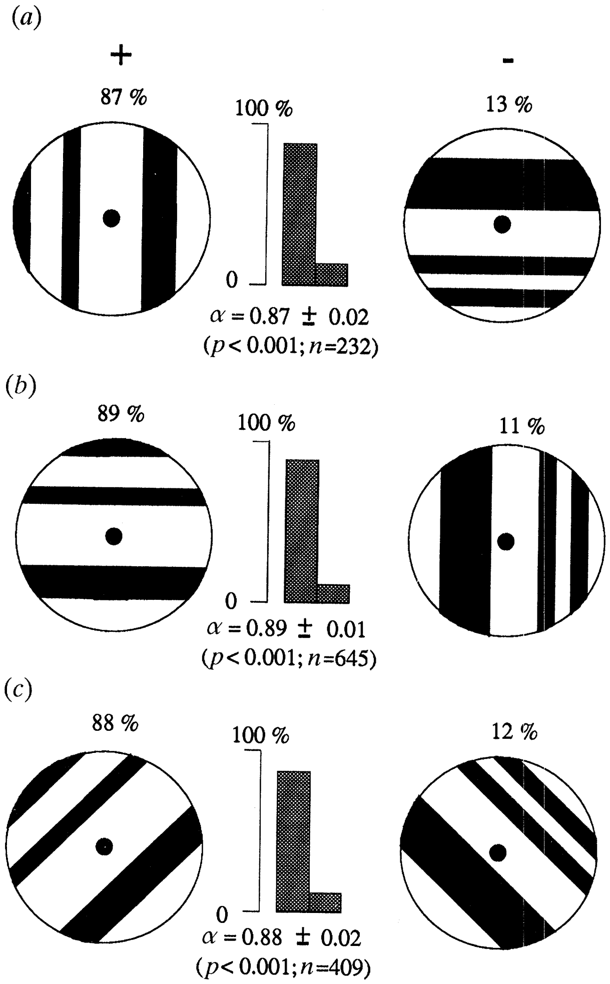

Figure 1. Bees perform well at distinguishing between random gratings oriented in two mutually perpendicular directions. The rewarded (positive) orientation is vertical in (*a*), horizontal in (*b*) and tilted 45° clockwise with respect to the vertical in (*c*). In this and following figures, the histograms show the choice frequencies with respect to the positive (left) and negative (right) stimuli. The figures below each histogram give the choice frequency in favour of the positive pattern (*a*), the number of choices evaluated (n) and the probability, p, of α being significantly different from random choice (see § 2).

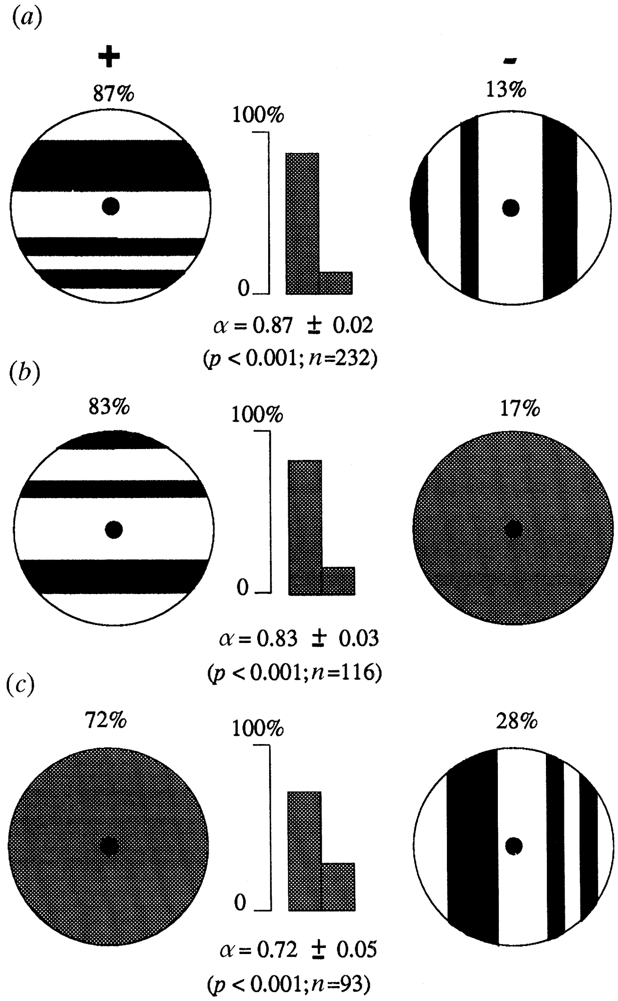

Figure 2. Evidence that bees trained to distinguish between the horizontal and vertical orientation (*a*) learn not only to prefer the rewarded orientation (*b*), but also to avoid the unrewarded orientation (*c*).

black or white. Some examples are shown in figure 1 (see van Hateren *et al.* (1990) for details). During training the gratings were chosen randomly, in pairs, from a pool of ten such randomly constructed gratings. With each pair of gratings, each bee was rewarded four times, on average: twice with the positive pattern in the left-hand tunnel and twice with the positive pattern in the right-hand tunnel. The training was then continued with another randomly chosen pair of gratings. This procedure ensured that the bees learned to discriminate the patterns on the basis of orientation, and not on the basis of a 'photographically' memorized image of the positive pattern (see van Hatern *et al.* 1990). It also eliminated the effects of any preferences that bees might display for a particular tunnel.

Bees, trained as described above to discriminate random gratings oriented in two mutually perpendicular directions, display a strong preference for the rewarded orientation ($\alpha = 87\%$, see figure 1). This is true regardless of whether the rewarded orientation is vertical, horizontal or oblique (figure 1*a–c*). In an earlier study, we have shown (in agreement with the findings of Wehner (1971)) that bees trained in this way can learn the rewarded orientation and apply it to distinguish between patterns which they have never previously encountered (van Hateren *et al.* 1990).

Bees trained to favour a horizontal grating over a vertical one (figure 2*a*) prefer the horizontal grating to a grey disc in subsequent tests (figure 2*b*). However, the same bees prefer a grey disc over a vertical grating (figure 2*c*), indicating that they have also learned to avoid the vertical orientation. Thus, bees trained on a specific orientation learn not only to favour the positive (rewarded) orientation, but also to avoid the negative (unrewarded) one.

Bees can also be trained to distinguish the orientation of patterns that are composed of a single stripe (as in figure 3), or a periodic grating (as in figure 4). The single-stripe patterns were composed of a black stripe, 3.0 cm wide, against a white background. The periodic grating consisted of alternate grey-and-white stripes, each 2.0 cm wide.

The ability to distinguish between two orientations

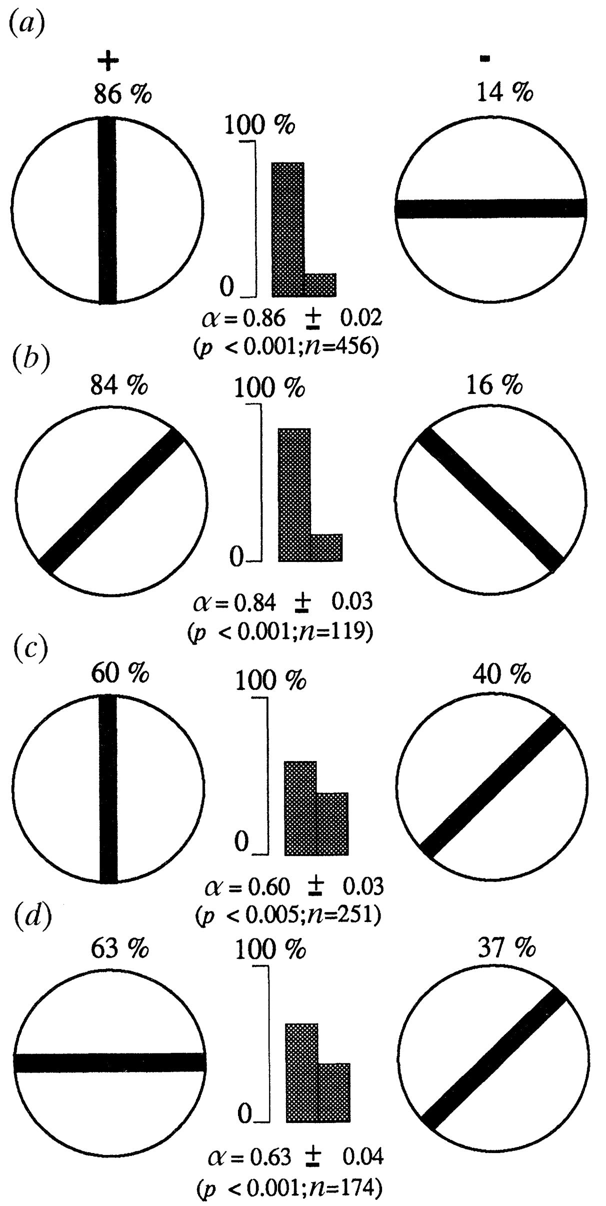

Figure 3. Bees perform well at distinguishing between single-stripe patterns that are oriented mutually perpendicularly (*a*,*b*). They are also able to distinguish between stripes oriented at 45° to each other (*c*,*d*) although discrimination is not as good as in the former case.

is poorer, but nevertheless significant when the angle separating them is reduced from 90° to 45°. This is true regardless of whether the orientation is defined by a single stripe (figure 3), a periodic grating (figure 4) or a random grating (data not shown). The discrimination of a 45° difference in orientation is slightly better with gratings (*ca.* 70%, figure 4*b*,*c*) than with single stripes (*ca.* 62%, figure 3*c*,*d*). Thus, orientation discrimination is enhanced by increasing the number of stripes. The discrimination of orthogonal orientations, however, is largely unaffected by the introduction of extra stripes: the choice frequency in favour of the rewarded orientation is at least 80% in all cases (compare figures 1*a*–*c*, 3*a*,*b* and figure 4*a*). Presumably, in this case the difference in orientation is large enough to cause the orientation-discrimination signals to saturate.

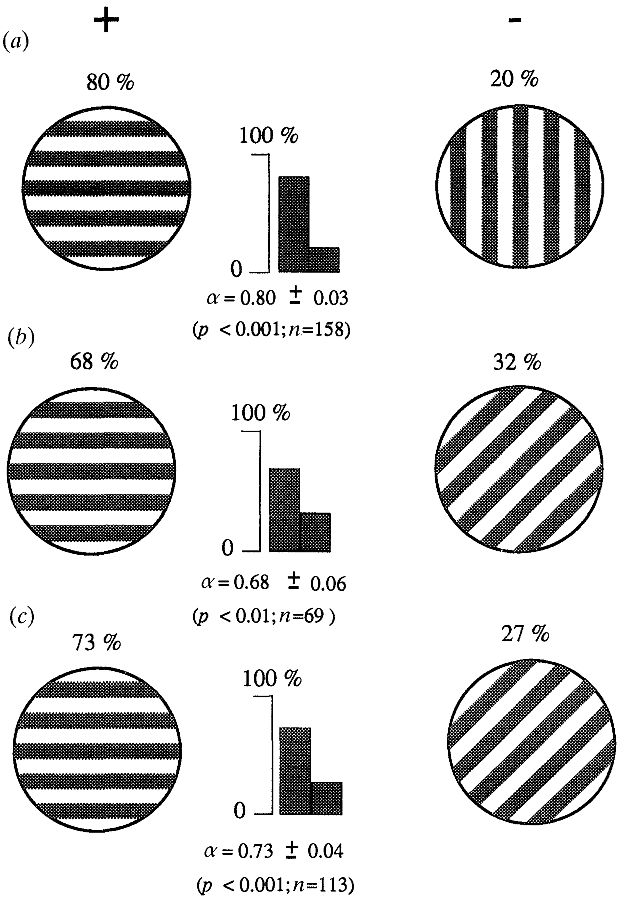

Figure 4. Bees perform well at distinguishing between periodic gratings that are oriented mutually perpendicularly (*a*). Bees trained on this task can also distinguish between gratings that are oriented at 45° to each other (*b*). Bees can also be trained to discriminate gratings whose orientations differ by 45° (*c*). In these experiments the gratings consisted of 50% grey bars on a white background.

(*b*) *Orientation discrimination of crosses and plaid patterns*

Next, we examined the ability of bees to distinguish the orientation of more complex patterns, such as crosses or plaids, which presented more than one orientation (figures 5 and 6). A cross was composed of two black stripes, each 3.0 cm wide, arranged either at right angles (figure 5*a*) or at 45° to each other (figure 6*a*). A plaid consisted of a periodic grating superimposed on another one at a different orientation. The two component gratings were either mutually perpendicular (figure 5*b*) or oriented at 45° to each other (figure 6*b*,*c*). Each component grating was constructed from 2.0 cm wide stripes that were alternately grey (50% density) and white. The intersections of the grey stripes were black, as required by linear superposition.

We found, curiously, that bees are unable to discriminate a right-angled cross from a 45° rotated version of the same cross (figure 5*a*). This is paradoxical, given that each individual stripe of the cross can be discriminated from a 45° rotated version of it (see figure 3*c*,*d*). A similar result is obtained with a plaid pattern, composed of a superposition of two mutually perpendicular gratings. Such a plaid is discriminated very poorly from a 45° rotated version of itself (figure

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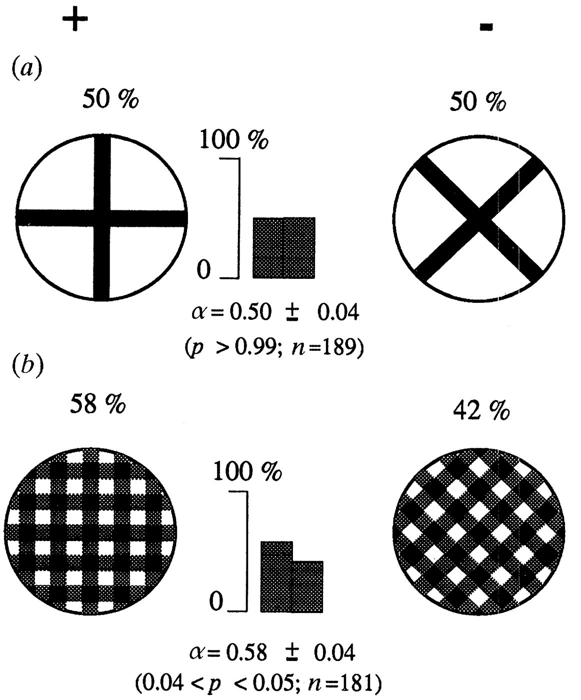

Figure 5. Bees are very poor at distinguishing between a right-angled cross and a 45° rotated version of the same cross (*a*). They are also poor at distinguishing between a 90° plaid pattern and a 45° rotated version of the same plaid (*b*).

5*b*), although each component grating of the plaid is discriminated well from a 45° rotated version of itself (see figure 4*b*,*c*).

It is conceivable that the difficulty in discriminating the orientation of crosses and plaid patterns is simply due to general confusion caused by the increased complexity of these patterns, or by the fact that each pattern presents more than one orientation. However, the experiments of figure 6 indicate that neither of these factors is responsible. Bees have no difficulty in discriminating 90°-rotated crosses (figure 6*a*) or plaids (figure 6*b*) when the angle separating the two components of each pattern is reduced from 90° to 45°. Plaid patterns of this kind can be discriminated even when one of them is rotated only by 45° relative to the other (figure 6*c*).

We then examined discrimination of patterns which simultaneously present three different orientations. We found that bees cannot be trained to distinguish between a right-angled cross and a cross which is augmented by adding another stripe (of similar width) along the bisectrix, as shown in figure 7*a*,*b*. They also do not distinguish between such an augmented pattern and a 90° rotated version of it (figure 7*c*). However, when the cross is augmented by three parallel stripes, bees are able to distinguish this pattern from a 90° rotated version (figure 7*d*).

(*c*) *Chromatic properties of orientation discrimination*

We investigated the role of colour in orientation discrimination by creating single-stripe patterns which presented contrast exclusively to the green receptor channel or to the blue receptor channel. In these patterns the background was a specific shade of blue, and the colour of the stripe was chosen to be a shade of yellow such that the boundary between the stripe and the background provided a strong contrast (44%) to the green receptors of the bee's visual system and a weak contrast (2%) to the blue receptors (figure 8*a*) or, conversely, a strong contrast (66%) to the blue receptors and a weak contrast (2%) to the green receptors (figure 8*b*). Details of the pigment papers used to construct these patterns, and of the contrast calculations are given in Srinivasan & Lehrer (1988). The stripe was 3 cm wide.

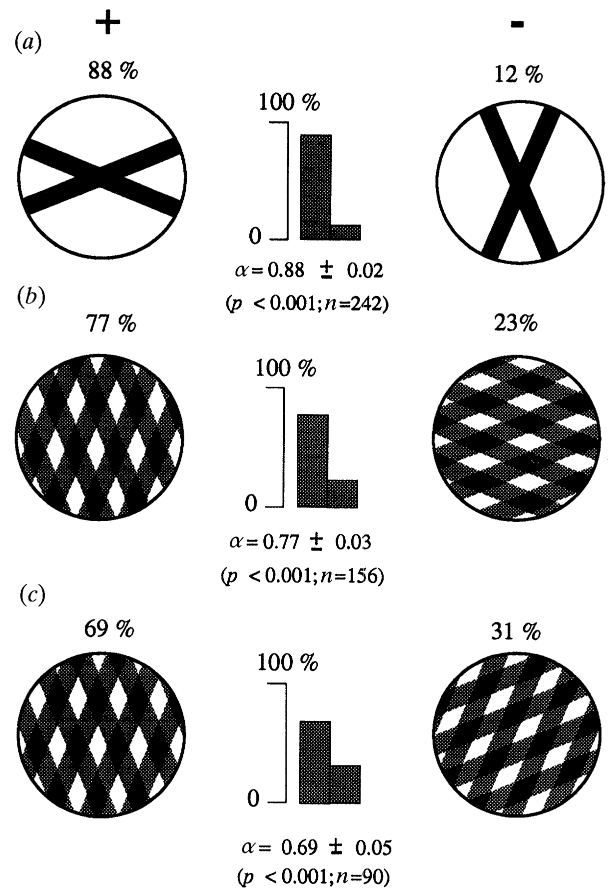

Figure 6. Bees perform well at distinguishing between a 45° cross and a 90° rotated version of the same cross (*a*). They can also discriminate a 45° plaid and versions of the same plaid that are rotated by 90° (*b*) or even by 45° (*c*).

It is evident from figure 8 that orientation discrimination is much better with green-contrast than with blue-contrast. This is in spite of the fact that the green-contrast offered by the patterns in figure 8*a* is smaller in magnitude compared to the blue-contrast offered by the patterns in figure 8*b* (see § 2). It appears, therefore, that orientation discrimination is dominated by signals from the green receptor channel. The blue channel does, however, make a small, but significant contribution (see also Lehrer *et al.* 1985; Srinivasan & Lehrer 1988).

4. DISCUSSION

Our results show that bees can discriminate unambiguously the orientation of a unidirectional pattern,

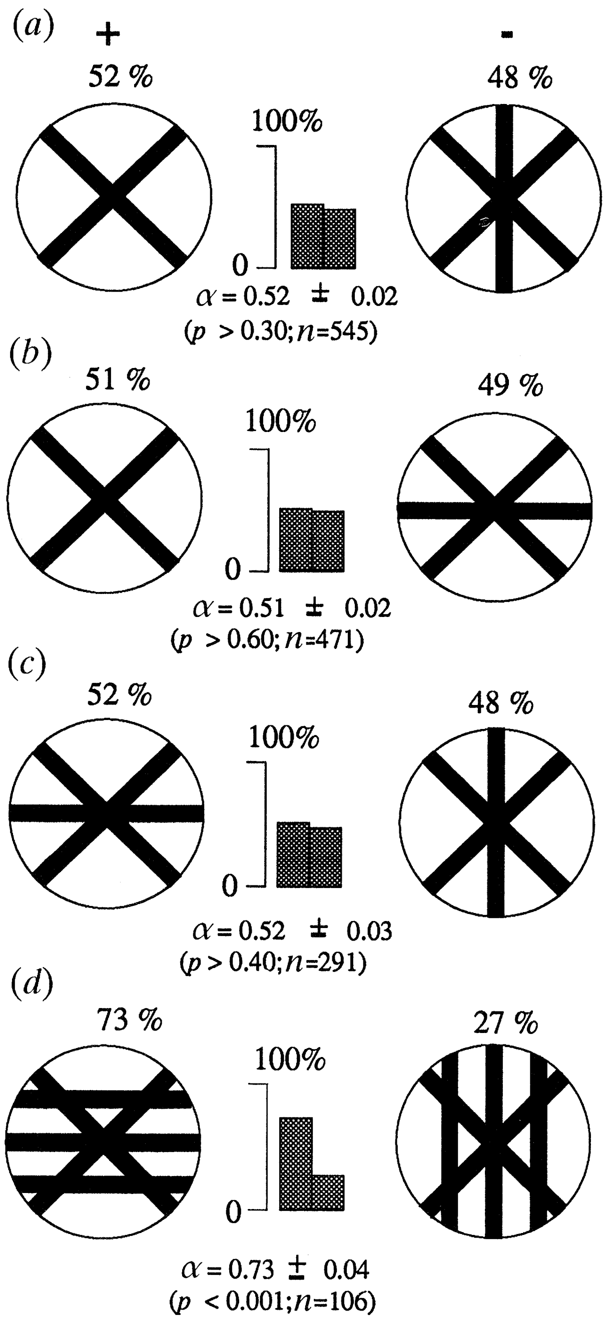

Figure 7. Bees perform poorly at distinguishing a right-angled cross from the same cross augmented by a stripe (*a*,*b*) or at distinguishing the augmented cross from a 90° rotated version (*c*). However, when the cross is augmented by three stripes, they are able to distinguish this pattern from a 90° rotated version (*d*).

such as a single stripe or a grating (figures 1, 3 and 4). We also find that bees that are trained to distinguish between two different pattern orientations learn not only to favour the positive pattern, but also to avoid the negative one (figure 2). On the other hand, bees cannot discriminate the orientation of bidirectional patterns (such as crosses or plaids) when the two component orientations of each pattern are separated by 90° (figure 5). The orientation of bidirectional patterns can be discriminated, however, if the angular separation of the components is considerably different from 90° (figure 6).

How are the bees distinguishing these patterns? Are they using visual mechanisms based on an eidetic ('photographic') memory of the rewarded pattern, as was suggested, for example, by Wehner (1981) and Gould (1985)? If the bees were indeed using eidetic imagery, one would not expect them to have any difficulty in distinguishing, for example, a right-angled cross from a 45° rotated version of itself. But it is clear from the experiment of figure 5*a* that the bees find this discrimination very difficult (figure 5*a*), at least under the experimental conditions imposed by our setup. This finding is in agreement with earlier work using the same apparatus, where we have shown that bees do not use eidetic imagery when they are forced to discriminate between patterns at a distance (Van Hateren *et al.* 1990).

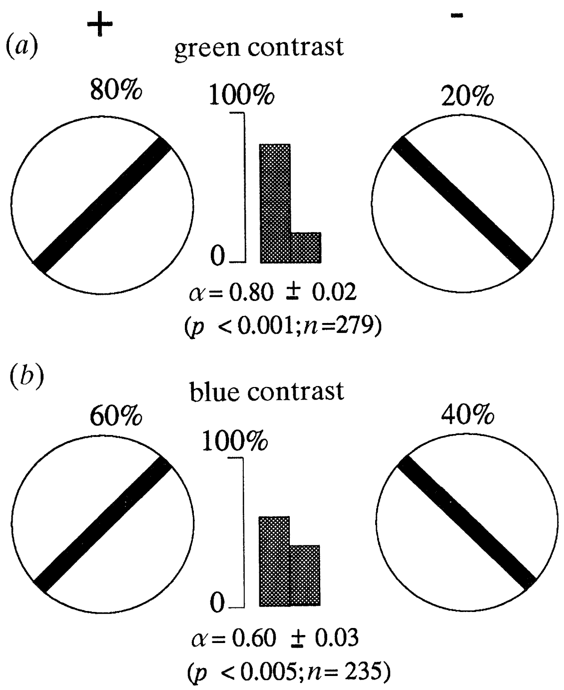

Figure 8. Chromatic properties of orientation discrimination. Bees perform better at discriminating the orientation of a stripe when its boundaries present contrast exclusively to the green receptors of the bee's eye (*a*), than when these boundaries present contrast exclusively to the blue receptors (*b*).

It is possible, however, that bees do use eidetic cues when they are given the opportunity to fixate the patterns at a close distance, as Wehner (1981) has suggested. We have examined this question by repeating the training experiment of figure 5*a* (right-angled cross versus 45° rotated version), and carrying out two types of tests to measure the bees' discrimination performance. In one kind of test, the reward was retained and we noted the relative frequencies with which the trained bees' first entries corresponded to the correct and incorrect tunnels, as already described in § 2. This yielded a choice frequency of 51.3% in favour of the positive pattern (three tests, $n = 230$), indicating, in agreement with the results shown in figure 5*a*, that the bees were unable to distinguish the two patterns when they viewed them from the tunnel entrances ($p > 0.95$, χ^2 test). In another kind of test, the discrimination performance of the same group of trained bees was measured by removing the reward, replacing the patterns by identical but fresh, unscented ones and measuring the relative frequencies with which the bees touched and landed on the entrance tubes corresponding to the positive and negative patterns (for details of this method of measuring discrimination performance, see Lehrer *et al.* 1985). This yielded a choice frequency of 96.3% in favour of the positive pattern (five tests, $n = 333$), indicating, in agreement with Wehner (1967), that the trained bees are indeed capable of distinguishing between the two patterns when they are able to view them close up and fixate them ($p < 0.001$, χ^2 test). These findings suggest that bees indeed use an eidetic mechanism for discriminating patterns when the patterns can be fixated or viewed close up. However, when patterns have to be discriminated at a distance, other mechanisms, which seem to be capable of extracting more 'general' attributes (such as orientation, colour, etc.) seem to play a dominant role.

An alternative interpretation of the above experiment would be that orientation-sensitive mechanisms mediate pattern discrimination at all distances – near and far – and that the dramatic improvement in the bees' ability to detect the rotation of the right-angled crosses when they are viewed close up is simply a consequence of the angular size of the visual patches over which orientation is computed by the nervous system. Thus, the crosses of figure 5*a* are not distinguished from afar because they fall entirely within one patch, but are distinguished when they are viewed close up because the individual arms are then viewed by different patches. While this interpretation needs further investigation, it is unlikely in the light of the observation that fixating bees can distinguish well between identical black-and-white sectored radial gratings when one grating is rotated by half a period relative to the other (Wehner 1981; Srinivasan & Lehrer 1988). Here the two patterns cannot be distinguished purely on the basis of orientational cues, because they possess identically oriented edges. But they can be distinguished on the basis of an eidetic image, because the black sectors of one pattern correspond to the white sectors of the other, and vice versa. Thus, it seems very likely that fixating bees do indeed use eidetic imagery in discriminating patterns.

How does the visual system of the bee compute the orientation of a remote pattern? Our recent study (Srinivasan *et al.* 1993) rules out the possibility that orientation is analysed through directional motion cues: bees continue to discriminate the orientation of a pattern even when the pattern is in motion, or when it is presented for very brief periods. Thus, it appears that orientation is analysed in terms of the geometry of the pattern, rather than on the basis of its apparent motion. How is orientation analysed in a geometrical sense? Although one can think of several ways to do this mathematically, a realistic possibility – based on what we know about biological vision – is that orientation is analysed by a number of orientation-sensitive channels, each with a different preferred orientation, as in the mammalian cortex (Hubel & Wiesel 1968). Here we shall pursue this hypothesis, and interpret the present findings in terms of a multichannel model of orientation computation.

(*a*) *Number of orientation-sensitive channels*

True discrimination of orientation requires that orientation be determined independently of the structure or contrast of a pattern.

Can orientation be determined unambiguously by a single orientation-sensitive channel? If the bee were to roll about its long axis as it approaches the pattern, the output of a single orientation-sensitive channel would provide the necessary information: the orientation would correspond to that at which the channel produced the strongest response. However, we have never observed bees performing such manoeuvres. Moreover, the observation that bees can determine the orientation of even briefly presented gratings (Srinivasan *et al.* 1993) argues strongly against such a sequential process, and favours a scheme in which orientation is analysed simultaneously by a multiplicity of channels with different preferred orientations.

A single, stationary orientation-tuned channel would not provide unambiguous orientation information, because the output of the channel would confound orientation with other attributes of the pattern, such as structure or contrast. Furthermore, the response of a single channel would not distinguish between orientations that are equally inclined to the channel's preferred orientation, but lie on opposite sides of it. Our observation that bees are able to learn the orientation of a unidirectional pattern oriented along any direction, without confusing it with other orientations makes it very unlikely that this analysis is carried out by a single channel.

Further evidence to support this contention is provided by the results of figure 2, which show that bees learn not only to favour the rewarded orientation, but also to avoid the unrewarded one. A single orientation-sensitive channel (tuned to, say, the vertical orientation) would enable discrimination of a horizontal grating from a vertical one, by signalling a strong response to the vertical grating and a weak response to the horizontal grating. However, such a channel would not distinguish between a horizontal grating and a grey disc, because it would respond weakly to either pattern. Our results show that bees trained to favour a vertical grating over a horizontal one distinguish between a horizontal grating and a grey disc, by showing a clear preference for the latter (figure 2*c*). Thus, at least two orientation-sensitive channels are required – one having a near-vertical, and the other a near-horizontal preferred orientation – to account for the findings shown in figure 2.

However, a system comprising a vertical channel and a horizontal channel is not adequate either: for example, such a system would not be capable of distinguishing between orientations that are symmetrically inclined about the horizontal or vertical (including +45° and −45°, which the bees are clearly able to distinguish, see figures 1*c* and 3*b*). A third channel, with a different preferred orientation, would eliminate this ambiguity. It can be shown theoreti-

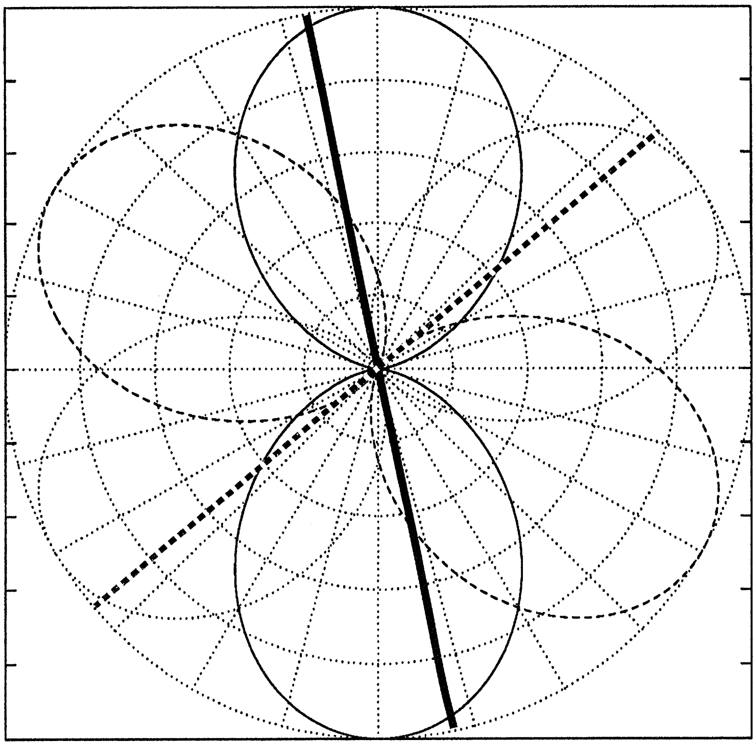

Figure 9. Illustration of how the outputs of three orientation-sensitive channels can determine the orientation of a unidirectional pattern unambiguously, independently of pattern structure or contrast. The solid curve shows the polar orientation-sensitivity plot of a channel with a vertical preferred orientation. It is clear that this channel on its own would (i) confound the orientation of a pattern with its contrast and structure, and (ii) not distinguish between patterns that are identical, but inclined equally and oppositely to the channel's preferred orientation. The addition of a second channel with a different preferred orientation (dashed curve) would remove these ambiguities partially: the ratio of the outputs of the two channels would depend upon the orientation of the pattern and be independent of its structure and contrast, and thus provide an indication of pattern orientation. However, the ratio would not specify orientation uniquely: each value of the ratio would correspond to two orientations. The two orientations corresponding to one particular value of the ratio (2.0) are illustrated by the continuous and dashed lines. The addition of a third channel with a distinct preferred orientation (dotted curve) would eliminate the remaining ambiguity: the pairwise ratios of the outputs of the three channels would then specify pattern orientation uniquely, and independently of structure or contrast.

cally that three channels – each with a different preferred orientation, but with overlapping orientation-tuning curves – are sufficient to determine orientation unambiguously, i.e. independently of the contrast or structure of the pattern (see figure 9). Three channels with preferred orientations separated by 60° would be optimal, as they would enable nearly uniform discrimination of orientation in all directions. It is worth noting that the hexagonal arrangement of the ommatidia of the compound eye lends itself readily to the construction of receptive fields that would endow channels with preferred orientations separated by 60°. The mathematical basis for requiring three channels is identical to that concerning the determination of the orientation of the plane of polarization of a beam of light. There it has been shown that three polarization-sensitive receptors, with different preferred orientations, are sufficient to determine the plane of polarization unambiguously and independently of intensity or degree of polarization (Kirschfeld 1973; Bernard & Wehner 1977).

Our present findings, taken together with those of van Hateren *et al.* (1990) indicate that bees are capable of determining the orientation of unidirectional patterns unambiguously, and independently of pattern structure. In the light of the above discussion, this finding suggests that the bee's visual system must comprise at least three orientation-sensitive channels with different preferred orientations.

(*b*) *Tuning of orientation-sensitive channels*

How sharply are the channels tuned to their preferred orientations? One would expect this to be difficult, if not impossible to ascertain from behavioural experiments. Fortunately, a curious finding may provide a useful insight. We have seen that bees can distinguish between two bars oriented at 45° to each other (figure 3*c*,*d*), but not between two right-angled crosses or two right-angled plaids oriented at 45° to each other (figure 5). This apparent paradox can be explained in terms of the tuning of the orientation-sensitive channels. Let us assume that each channel possesses an orientation tuning curve in the form of a raised cosine with a half-width of 90°, as shown in figure 10. This tuning curve is described by the function $0.5(1+\cos 2\theta)$ where θ is the orientation of the pattern relative to the channel's preferred

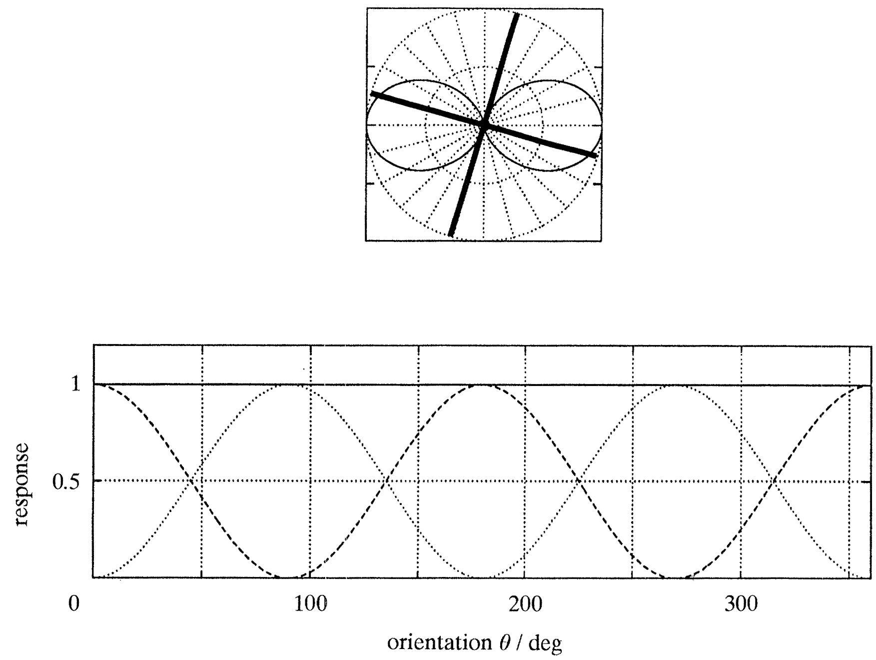

Figure 10. Modulation of the response of a hypothetical orientation-sensitive channel with an angular half-width of 90°, as it views a rotating right-angled cross. One arm of the cross induces the response shown by the dashed curve, and the other arm the response shown by the dotted curve. The response to the cross, which is the sum of the two outputs, shows no modulation (solid curve).

orientation. As the orientation of a bar is varied, the response of this channel would vary as shown by the dotted line in figure 10.

It is easy to see that the orientation of the bar can be determined from the outputs of three such channels, each with a different preferred orientation, as illustrated in figure 9. Consider now the response of one of these channels to a right-angled cross. This response would be the sum of the responses elicited by each stripe of the cross (sum of the dashed and dotted curves in figure 10). It turns out that this summed response is constant and independent of the orientation of the cross, as shown by the continuous line in figure 10. The reason is that the angular separation between the two stripes of the cross (90°) is equal to the half-width of the channel's orientation tuning curve (90°). As a result, the sum of the contributions to the response from the two orthogonally-oriented stripes of the cross is

$$0.5[1+\cos 2\theta]+0.5[1+\cos 2(\theta+90°)]=1.0,$$

which is a constant that is independent of θ. Thus, if the channels have tuning curves with an angular half-width of 90°, each channel would produce a constant, unmodulated output as the orientation of the cross is varied. The outputs of the channels would then carry no information on the orientation of the cross. Increasing the number of channels would be of no avail, as long as the orientation tuning curve of each of the channels is 90°. This property is not crucially dependent upon the shape we have assumed for the channels' orientation tuning curves. For example, the tuning curves can be triangular, rather than cosinusoidal, without affecting the result; and if the half-width of the tuning curve deviates from 90°, the output of each channel would show some modulation as the orientation of the cross is varied, but this modulation will be less than 8%, for example, if the angular half-width is varied between 80° and 110° (M. V. Srinivasan, unpublished calculations). In the light of the experimental observation that bees are unable to discriminate the orientation of right-angled crosses or right angled plaids (figure 5), we suggest that each of the orientation-sensitive channels in the bee's visual system possesses an orientation tuning curve with a half-width of approximately 90°.

One can predict the consequences of this hypothesis for crosses that are not right-angled. The response elicited by a 45° cross in a channel with an orientation tuning curve of 90° half-width is shown in figure 11. The response shows a strong modulation as the orientation of the cross is varied, predicting that bees should be able to discriminate the orientation of a 45° cross, or of a plaid formed by the superposition of two gratings at an angle of 45° to each other. The results of the experiments shown in figure 6 demonstrate that this is indeed the case. These findings are therefore consistent with our hypothesis that the orientation-sensitive channels have tuning curves with half-widths of 90°.

What does such a model predict about the bees' ability to discriminate the orientation of a right-angled cross augmented by the addition of a single stripe as in figure 5*a*–*c*)? The response that would be elicited by such a pattern in a channel with an orientation tuning curve of 90° half-width is shown in figure 12. The response does show a modulation as the orientation of the pattern is varied, but, expressed as a percentage of the mean response, this modulation is much weaker than that elicited by a single stripe (see

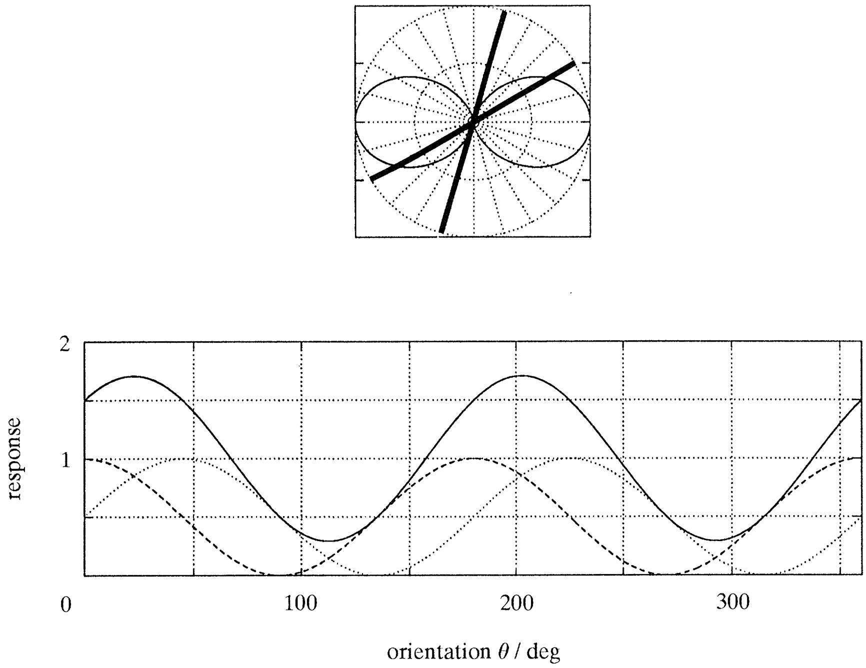

Figure 11. Modulation of the response of a hypothetical orientation-sensitive channel with an angular half-width of 90°, as it views a rotating 45° cross. The two arms of the cross induce the responses shown by the dashed and dotted curves, respectively. The response to the cross, which is the sum of the responses induced by the two arms, shows a strong modulation of 71% of the mean value (solid curve).

figure 10) or by a 45° cross (see figure 11). This is because the right-angled cross in the augmented pattern adds a constant value to the response at all orientations, thereby decreasing the percentage of modulation that the additional stripe would have produced on its own. Our model therefore predicts that bees should discriminate the orientation of such an augmented pattern poorly, if at all, and that they should also experience difficulty in distinguishing such a pattern from a right-angled cross. This prediction is in agreement with the experimental results of figure 7*a–c*.

Consider now a pattern consisting of a right-angled cross augmented by not one, but three stripes, as shown in figure 7*d*. The response elicited by such a pattern in a channel with an orientation tuning curve of 90° half-width is shown in figure 13, where we have assumed that the three augmenting stripes are three times as effective as one augmenting stripe in stimulating the channel. Clearly, the response of the channel shows a strong modulation as the orientation of this pattern is varied. The model therefore predicts that bees should discriminate the orientation of such a pattern well: if the three augmenting stripes produce a stronger response in the channel than does a single stripe. The results described in figure 7*d* show that bees are capable of discriminating the orientation of this pattern quite well. This finding indicates that the 'strength' of an orientation signal can be increased by increasing the number of stripes (or edges) along that orientation.

Details of the model can be modified to improve its fit with the data. Consider, for example, the slight discrepancy between the prediction made by the model, of weak orientation discrimination of the cross patterns that are augmented by a single stripe (figure 12), and the experimental observation of virtually no discrimination (figure 7*a–c*). This discrepancy can be reduced by assuming that the output of each channel is not a linear function of its stimulation, but rather, a mildly saturating or adapting function. Alternatively, one can assume that the orientation tuning curve of each channel (the response versus orientation for a pattern consisting of a single bar, see figure 10) is associated with a modulation of less than 100%; in other words, the response to the worst orientation is not zero, but a substantial fraction of the response to the optimal orientation. Either of these refinements would reduce the percentage modulations of the channel responses, and therefore make the orientation-computing system less sensitive to small changes in the structure or orientation of the pattern.

In summary, our findings are consistent with the hypothesis that orientation is measured by a system of at least three orientation-sensitive channels, each having a rather broad angular tuning curve of *ca.* 90° and a different preferred direction.

(*c*) *Neurophysiological correlates of orientation-sensitive channels in insect vision*

The present findings, considered together with those of van Hateren *et al.* (1990) and Srinivasan *et al.* (1993), predict the existence of orientation-sensitive neurons that display orientation tuning curves with angular half-widths of about 90°, and whose responses are not directionally selective to motion. So far, such units have not been reported in the bee. However, D. O'Carroll (1993) has recently discovered neurons with precisely such properties in the lobula of the dragon-

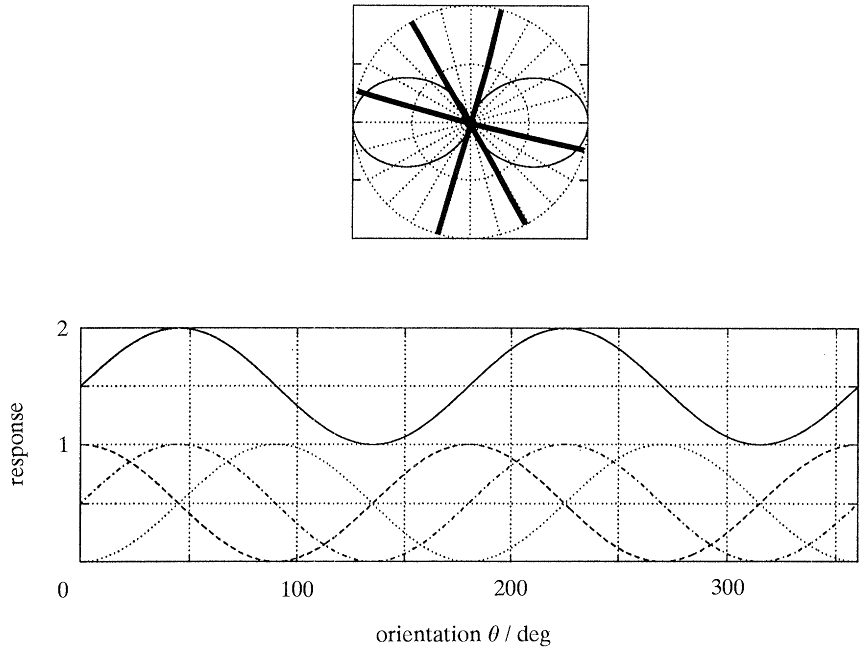

Figure 12. Modulation of the response of a hypothetical orientation-sensitive channel with an angular half-width of 90°, as it views a rotating right-angled cross augmented by a stripe. The three arms of the pattern induce the responses shown by the dashed, dotted and dash-dotted curves, respectively. The response to the pattern, which is the sum of the responses to the three arms, shows a relatively weak modulation of 33% of the mean value (solid curve).

fly. These neurons exhibit responses to a moving bar which depend upon the orientation of the bar, but not upon the direction in which it is moved along the perpendicular axis. The profiles of the orientation tuning curves of these units are surprisingly close to sinusoidal, and they possess angular half-widths that are close to 90°, as in our model. Further work is needed to determine whether such cells also exist in the lobula of the bee, and if so, to ascertain the distribution of their preferred orientations, and to establish, above all, whether cells of this kind are indeed involved in the analysis of pattern orientation.

The case for a rudimentary form of 'cortical' analysis in the insect visual pathway has been gaining

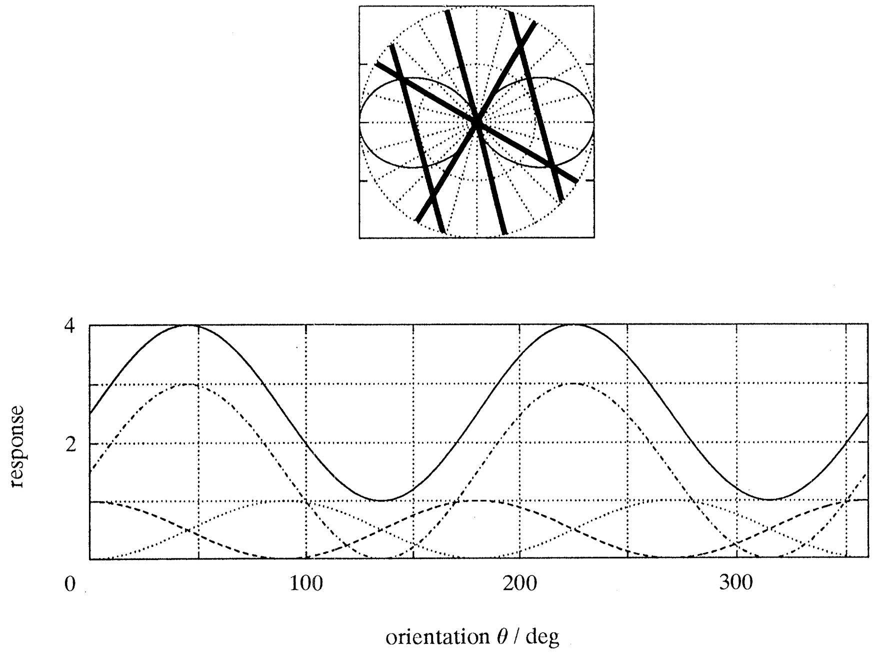

Figure 13. Modulation of the response of a hypothetical orientation-sensitive channel with an angular half-width of 90°, as it views a rotating right-angled cross augmented by three parallel stripes. The two crossed arms of the pattern induce the responses shown by the dashed and dotted curves, respectively, while the three parallel stripes induce the response shown by the dot-dashed curve. The response to the pattern, which is the sum of the responses evoked by the individual components, shows a relatively strong modulation of 60% of the mean value (solid curve).

ground steadily in recent years. For example, it has been found that bees perceive illusory contours (van Hateren *et al.* 1990; Horridge *et al.* 1992). Orientation-sensitive cells which respond to elongated bars, 'end-stopped' cells, and even cells that respond to illusory contours have recently been discovered in the dragonfly lobula (Horridge *et al.* 1992; O'Carroll 1993). Furthermore, it has recently been shown that bees analyse orientation simultaneously on more than one spatial scale (Zhang *et al.* 1992), a property reminiscent of cortical processing and human vision (e.g. De Valois *et al.* 1982; Bihrle *et al.* 1989). The second and third ganglia of the insect visual pathway (the medulla and the lobula) are each organized in retinotopic columns, with most of the neurons in each column viewing a small patch of visual space, not unlike the situation in a cortical hypercolumn (Strausfeld 1976). These observations reinforce the possibility that insect vision may well embody some of the computational principles that are manifest in primate visual cortex (von der Heydt & Peterhans 1989). Unfortunately, the response properties of neurons in the medulla and lobula of the bee remain largely unexplored.

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