Discrimination of closed coloured shapes by honeybees requires only contrast to the long wavelength receptor type

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Floral shape is a visual cue used by pollinators to discriminate between competing flower species. We investigated whether discrimination is possible between closed shapes presenting the same colour and lacking a centrally presented fixation point. Free-flying honeybees, Apis mellifera L., had to discriminate between a solid square and a solid triangle of the same colour presented on the back walls of a Y-maze. Different colours were used to vary chromatic contrast and receptor-specific contrasts. Discrimination was possible whenever shapes presented contrast to the long wavelength receptor but was independent of chromatic contrast, overall intensity contrast or short and middle wavelength receptor contrast. We suggest that the bees used the edges of the closed shapes to solve the task. Bees failed when shapes were rotated, showing that a single shape edge was not sufficient for recognition.

Flowers are not only colourful, but also variable in their morphology. The variation in shape and pattern provided by flower corollas represents an additional source of information that pollinators can use to discriminate between competing flower species. Although the flower image for an insect becomes coarser with increasing distance (Land 1993; Vorobyev et al. 1997), the fact that flowers have evolved a large variety of shapes and patterns suggests that such visual information is important for close-up flower recognition (Wehner 1981; Dafni et al. 1997; Giurfa & Lehrer 2001).

The honeybee, Apis mellifera, has been a powerful model for investigating the mechanisms by which insects learn and recognize patterns (reviewed by Wehner 1981; Srinivasan 1994; Dafni et al. 1997; Ronacher 1998; Giurfa & Lehrer 2001). Studies on honeybee pattern recognition have identified two essential strategies used to process and recognize visual patterns: (1) retinotopic template matching and (2) feature extraction. The former assumes that the template of a learned pattern is stored in a way that preserves the retinotopic coordinates (Wehner 1967, 1972; Cruse 1972; Gould 1985, 1986; Dill et al. 1993; reviewed by Heisenberg 1995). Recognition depends on the amount of overlap between an actual retinal image and the stored template. Consistent with this hypothesis, it has been shown in the context of scene recognition during navigation, that bees can store landmark shapes as retinotopic snapshots in which the retinal positions of the edges and not the area overlap are important (Cartwright & Collett 1983; reviewed by Collett 1996). The feature extraction hypothesis assumes that in pattern discrimination, selective attention and processing are focused on a single feature (orientation, symmetry, contour density, size, etc.) such that recognition depends on the presence or absence of such a feature and its specific values (van Hateren et al. 1990; Srinivasan et al. 1994; Horridge & Zhang 1995; Giurfa et al. 1996a; Ronacher 1998).

Early studies suggested that bees do not discriminate between closed shapes, such as a square, a disc or a triangle, either in the horizontal (von Frisch 1914, 1965) or in the vertical (Baumgärtner 1928) plane of presentation. In addition, dissected shapes, that is, shapes with many contours, could not be discriminated from each other but could be easily distinguished from closed shapes. Such experiments led to the conclusion that the spatial scale of a figure (i.e. its degree of dissectedness or ‘figural intensity’) was the primary cue used for pattern discrimination by bees (Hertz 1929, 1933; Zerrahn 1933; Wolf & Zerrahn-Wolf 1935). However, researchers later doubted that contour density alone was sufficient to describe the bees’ discrimination abilities for patterns.
Hertz (1933) could not explain all of her results using only this parameter, and Schnetter (1968) found that bees could discriminate between broken, star-like patterns that had similar spatial scale.

One possible reason for the disagreement between studies was the mass training and testing of bees, which made the study of individual discrimination strategies impossible. In addition, different response characteristics that did not always yield consistent results were analysed (Giurfa et al. 1999; reviewed by Wehner 1981). Once researchers understood that patterns presented on a horizontal plane might change their appearance from one visit to the next, depending on the direction of the bees’ approach, vertical presentation of the patterns became the standard method used to study pattern perception (reviewed by Wehner 1981). It was then shown that rotational invariance is not a necessary condition for shape discrimination and recognition (Wehner 1967, 1972), because the bee visually fixates the landing point and adjusts its position in space before landing (Wehner & Flatt 1977). This would allow the bee always to map a given shape or pattern onto the same parts of its ommatidial lattice, and to carry out a retinotopic comparison of the image actually seen and an image stored in the memory. Thus the fixation of the landing point was identified as crucial for retinotopic template building and matching (Wehner 1972, 1981) because it precludes any translational or rotational displacements of shapes. In all later experiments, a clearly visible central fixation point, a large black entrance tube leading to a reward chamber behind the stimuli, was presented in the middle of a target, easily resolvable for the approaching bee.

Recently, the topic of closed shape discrimination by bees has been revisited (Ronacher & Duft 1996; Hempel & Giurfa 2001; Lehrer & Campan 2001). Hempel & Giurfa (2001) and Lehrer & Campan (2001) asked explicitly whether closed shapes are discriminable for bees. Campan & Lehrer (2002) answered the question affirmatively for achromatic (black-and-white) patterns presented vertically and displaying a prominent central fixation point. We investigated discrimination between coloured closed shapes presented vertically and lacking a central fixation point. Bees were free to choose any possible fixation point if required. By varying chromatic and achromatic properties we could address specific receptor channels and determine the visual pathway involved in this task.

METHODS

Set-up

We trained honeybees, A. m. carnica, to collect 50% sucrose solution (weight/weight) on vertically presented stimuli on the back walls of a wooden Y-maze. The maze was covered with a UV-transparent Plexiglas ceiling and illuminated by natural daylight (Fig. 1; also see Giurfa et al. 1996b; Hempel et al. 2001). A coloured dot was painted with gouache paint on the bee’s thorax or abdomen for individual recognition. Only one bee was present in the maze at a time. The bee entered the maze through a hole (5 cm in diameter) in the front panel of the maze. Once through the entrance hole, the bee was in the decision chamber from which it could for the first time simultaneously see both back walls (20 × 20 cm each) of the maze and the stimuli on them.

Stimuli

The bee had to discriminate two closed shapes, an equilateral triangle (sides 7.0 cm) and a square (sides 5.7 cm). The shapes were 20 cm from the entrance hole such that both subtended a visual angle of 30° (in their maximal extension from their respective centres) for the bee when it was at the central point of the maze decision chamber (Fig. 1). The shapes did not have a central fixation point and thus differed from stimuli commonly used, in which a central hole in the middle allows access to a reward chamber. Both shapes had a central hole, 3 mm in diameter, through which sucrose solution was dispensed. This hole could not act as a fixation point for the bees in the decision chamber because its small size and the poor spatial resolution of the bees’ compound eyes rendered it undetectable (Giurfa et al. 1996b; Giurfa & Vorobyev 1998). During the test phase, it was invisible because the newly inserted back walls displaying the test shapes covered it.

The two shapes had the same colour and were presented on a grey background. Different colours were used with different groups of bees to determine whether addressing specific chromatic or achromatic pathways affects the discrimination performance. Yellow, violet, blue, cyan and brown shapes were cut from high-quality coloured HKS-N cardboards (K+E Stuttgart, Stuttgart-Feuerbach, Germany; yellow: 3N; violet: 33N; blue: 41N; cyan: 50N; brown: 82N); UV-white shapes were cut from cardboard painted with a mixture of transparent varnish.
Receptor-specific contrasts were calculated from the spectral reflection curves of each stimulus as receptor quantum catches normalized to the grey background. $S$, $M$, $L$ = short, middle and long wavelength receptor types.

Chromatic contrast to background was calculated according to the COC model of colour vision for honeybees (Backhaus 1991). If chromatic contrast exceeds the threshold value of 1 model unit (bold numbers), bees can distinguish the stimulus from its background by chromatic mechanisms. In the case of receptor-specific contrasts, values close to 1 (e.g. 0.9) indicate an absence of contrast with respect to the background while higher or lower values (bold) indicate a significant deviation of receptor excitation from the adaptation state given by the background. Overall intensity contrast was calculated as the sum of the three receptor signals.

Table 1. Spectral properties of the colours used

<table>
<thead>
<tr>
<th>Colour of stimuli pair</th>
<th>Chromatic contrast $Q_i$</th>
<th>Receptor-specific contrasts $S$, $M$, $L$</th>
<th>Overall intensity contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey background</td>
<td>0</td>
<td>1,1,1</td>
<td>3</td>
</tr>
<tr>
<td>UV-white</td>
<td>0.4</td>
<td>10.6,7.7,7.6</td>
<td>25.9</td>
</tr>
<tr>
<td>Yellow</td>
<td>8.8</td>
<td>0.9,0.4,3.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Cyan</td>
<td>1.7</td>
<td>3.1,5.0,3.9</td>
<td>12.0</td>
</tr>
<tr>
<td>Violet</td>
<td>4.0</td>
<td>2.4,2.4,0.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Brown</td>
<td>4.3</td>
<td>0.7,0.4,0.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Blue</td>
<td>3.0</td>
<td>0.6,0.9,0.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Receptor-specific contrasts were calculated from the spectral reflection curves of each stimulus as receptor quantum catches normalized to the grey background. $S$, $M$, $L$ = short, middle and long wavelength receptor types. Overall intensity contrast of a stimulus against the background was calculated as the sum of the relative absorbed quanta in the three receptor types. Blue and brown were dim and cyan and UV-white were bright stimuli.

To determine the chromatic properties of the stimuli, we used a model of honeybee colour vision, the Colour Opponent Coding (COC) model (Backhaus 1991), which has been derived from experimental data and correctly predicts the spectral threshold sensitivity function in the honeybee (Vorobyev & Brandt 1997). The two chromatic mechanisms describe the bee’s perceptual colour space, which is represented in a two-dimensional plane, the so-called ‘COC-diagram’. Coordinates $A$ and $B$ of colour loci in the COC diagram are calculated as:

$$A = \sum_i Q_i / (1 + Q_i)$$  \hspace{1cm} $B = \sum_i b_i Q_i / (1 + Q_i)$ \hspace{1cm} (2)

with $a_i = \{-9.86, 7.70, 2.16\}$ and $b_i = \{-5.17, +20.25, -15.08\}$. Chromatic contrast ($\Delta S$) refers to the colour difference of a given stimulus against its background ($A=0; B=0$) and is given in standard units in the COC-diagram:

$$\Delta S = |\Delta A| + |\Delta B|.$$  \hspace{1cm} (3)

Procedure

The conditioning procedure was always differential. Each bee was trained either with a rewarded closed square against a nonrewarded closed triangle or vice versa. In experiment 1, after a pretraining of three to five rewards, bees were trained during 30 visits to discriminate between the two shapes. If the bee chose the correct shape, it was rewarded with sucrose solution ad libitum. If the bee chose the wrong shape, we gently tossed it away from the maze by hand and it had to re-enter through the entrance.

and BaSO$_4$ powder. The grey background was provided by HKS-92N cardboard. Spectral reflectance of the stimuli and background were measured with a flash photometer (SR01, Gröbel UV-Elektronik, resolution 1 nm).

All stimuli presented a relatively high chromatic contrast (>1; Table 1) against the grey background except UV-white, which was achromatic to the bees against that background but could nevertheless be detected well (Hemple et al. 2000). Colours also differed in their receptor-specific contrasts, that is, in the relative number of quanta absorbed by each receptor type (short ($S$-), middle ($M$-) and long ($L$-) wavelength type) with respect to the background (Table 1). In the honeybee the $S$-receptor has its maximal sensitivity in the UV ($344$ nm), the $M$-receptor in the blue ($436$ nm) and the $L$-receptor in the green part of the spectrum ($556$ nm, Menzel & Backhaus 1991). The relative excitation of each photoreceptor type $i$, that is, the receptor-specific contrast $Q_i$, was calculated by integrating the illumination function $I$, the reflection spectrum of the stimulus $R_{st}$ and of the background $R_{bg}$, respectively, and the receptor’s sensitivity curve $S_i$ over the range of visible wavelengths ($300$ nm $<\lambda<$ $700$ nm, equation 1).

$$Q_i = \frac{300}{700} \int I(\lambda) R_{st}(\lambda) S_i(\lambda) \, d\lambda,$$  \hspace{1cm} $i = S, M, L$ - receptor \hspace{1cm} (1)

We chose colours to abolish one of the three receptor-specific contrasts at a time (Table 1). $S$-receptor contrast was absent in the yellow stimulus and $M$-receptor contrast was absent in the blue stimulus. $L$-receptor contrast was not present in the brown and the violet stimuli (Giurfa et al. 1996b) and was highest in the UV-white achromatic stimulus. Yellow, blue and cyan presented both chromatic and $L$-receptor contrast to the background and thus fulfilled the two conditions necessary for efficient colour detection by bees (Giurfa et al. 1996b). Overall intensity contrast of a stimulus against the background was calculated as the sum of the relative absorbed quanta in the three receptor types. Blue and brown were dim and cyan and UV-white were bright stimuli.

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The conditioning procedure was always differential. Each bee was trained either with a rewarded closed square against a nonrewarded closed triangle or vice versa. In experiment 1, after a pretraining of three to five rewards, bees were trained during 30 visits to discriminate between the two shapes. If the bee chose the correct shape, it was rewarded with sucrose solution ad libitum. If the bee chose the wrong shape, we gently tossed it away from the maze by hand and it had to re-enter through the entrance.
hole and make a new decision. We repeated this until the bee chose the correct shape, but only the first choice was counted. The rewarded stimulus was assigned to the left or the right arm of the maze according to a pseudorandom sequence. We gave bees two test phases of 2 min each at the end of the training phase. For the test phases, we presented fresh and nonrewarded test stimuli, identical to the training ones, by introducing new back walls without the small central hole. Between the two test phases a refreshment training of 10 visits was conducted. In the second test phase we repeated the procedure but reversed the test stimuli between sides. During tests, we recorded approaches towards the stimuli if the bee crossed the imaginary line separating the decision chamber from the arms of the maze. If the bee touched or landed on the test stimulus, we scored contact independently of the number of consecutive touches or landings following that contact in the chosen arm. The next contact was scored only after the bee returned to the decision chamber from which it could again see both test stimuli. Approaches were distributed randomly between the stimuli, which is consistent with previous studies (Giurfa et al. 1999; Stach & Giurfa 2001), showing that this parameter usually does not allow one to determine whether the stimuli are discriminated. We used the binomial test (Zar 1999) to test whether stimuli were discriminated from each other ($P_o=0.5, \alpha=0.05$).

To assess the possible role of the area of the shapes and the orientation of single edges, we performed a second experiment in which an additional group of bees was trained during 20 visits to the cyan square versus an unrewarded cyan triangle as described previously. After completing the first test (same procedure as above), the bees had 10 additional refreshment trials and were then tested with transformed or rotated shapes in two consecutive tests separated by 10 refreshment trials (for details see also Stach & Giurfa 2001): (1) an enlarged triangle with the same area as the rewarded training square and a reduced square with the same area as the nonrewarded training triangle; (2) the rotated presentation of the trained shapes resulting in a rhombus and a triangle lying on its side (triangle rotated 90°).

If bees learned to choose between shapes on the basis of the area difference, they should prefer the enlarged triangle to the smaller square. If the orientation of a single edge was sufficient for shape recognition, discrimination would be reduced to recognition of a vertical versus a diagonal edge. In this case, bees should choose the triangle lying on its side with a vertical edge and not the rhombus, which presented diagonal edges.

**RESULTS**

Three colours, UV-white, cyan and blue, allowed reciprocal discrimination of the square and the triangle (binomial test: UV-white square: number of choices, $n=256$, number of bees, $N=8$; UV-white triangle: $n=383, N=8$; cyan square: $n=138, N=8$; cyan triangle: $n=78, N=8$; blue square: $n=172, N=8$; blue triangle: $n=146, N=7$; all $P<0.04$; Fig. 2). Bees could discriminate the yellow square from the yellow triangle when the former was rewarded (binomial test: $n=196, N=9$, $P<0.001$) but were unsuccessful in the same discrimination task when the latter was rewarded (binomial test: $n=427, N=14$, $P=0.42$). Since bees discriminated the shapes when rewarded on the yellow square, we assume that they chose randomly after being rewarded on the yellow triangle not because they could not discriminate but for stimulus-specific reasons. Finally, violet and brown did not allow discrimination: in both cases shapes were chosen randomly in all tests (binomial test: violet square: $n=168, N=8$; violet triangle: $n=361, N=12$; brown square: $n=208, N=8$; brown triangle: $n=104, N=5$; all $P>0.86$).

Figure 3 shows the performance of the bees in the three kinds of test performed in the second experiment. The first test showed that the bees learned to discriminate the shapes (binomial test: $n=91, N=7$, $P<0.001$; Fig. 3a), whereas the test with the shapes modified in their area yielded no discrimination (binomial test: $n=68, N=6$, $P=0.45$; Fig. 3b). The bees also showed no preference for one of the rotated shapes (binomial test: $n=60, N=6$, $P=0.65$; Fig. 3c). In all three tests the total number of contacts matched the choice rate measured as number of approaches with contacts (paired $t$ tests: square versus triangle: $t_s=5.25, n=201, P<0.01$; smaller square versus larger triangle: $t_s=0.88, n=90, P=0.42$; rotated shapes: $t_s=1.41, n=83, P=0.22$), indicating that the bees made their decision before or during the approach flight, but not when directly in front of the stimuli.

**DISCUSSION**

Contrary to early experiments on shape discrimination in honeybees (von Frisch 1914; Baumgärtner 1928; reviewed...
by von Frisch 1965) which suggested that bees do not discriminate between closed shapes with geometrical outlines, our results show that such discrimination is possible between coloured shapes even in the absence of a central fixation point. The different colours we used allowed us to determine which processing pathways were involved in closed-shape discrimination. L-receptor contrast was of fundamental importance for discrimination of closed shapes. Shapes lacking such a contrast (brown and violet) could not be distinguished. Since bees use L-receptor contrast for edge detection (Lehrer et al. 1990), this result suggests that bees might have used the edges of the closed shapes for discrimination. Discrimination could not be related to any other spectral cue such as chromatic contrast, overall intensity or the presence of S- or M-receptor contrast (Table 1).

Ronacher & Duft (1996) showed that honeybees discriminate achromatic (black-and-white) closed shapes with a central, visible hole, which allowed for central stimulus fixation. It was therefore not surprising that these authors could interpret their findings in terms of retinotopic template acquisition and matching. Later, Efler & Ronacher (2000) also used stimuli with a central rewarding tube. They found that although bees relied strongly on the area adjacent to the central fixation hole (rewarding tube) of their stimuli, they were not using retinotopic matching for pattern recognition. In our study the shapes did not have a central fixation point, but the free-flying bees approaching the stimulus may have used an edge or a vertex of a shape as a fixation point.

Since the reward was given in the centre during the training phases, bees might also have used the centre of gravity of the shapes as a fixation point (Ernst & Heisenberg 1999). Thus, one could argue that in our experiments bees were also matching the shapes pixel by pixel and using the area overlap between the rewarded shape and the alternative shape as the main cue for discrimination (Heisenberg 1995; Ronacher & Duft 1996). This possibility, however, seems unlikely for several reasons. First, to estimate the overlap between the rewarded and the alternative shape, bees could align both shapes at their lower or upper edges or at their centres of gravity using a given fixation point (Fig. 4). The largest overlap between our shapes was obtained when they were aligned at their lower edge (96.8% coincidence when the rewarded shape was the triangle and 62.6% when it was the square): if bees used a template of the rewarded shape they would always confuse the shapes after being trained to the triangle, which we did not observe. Second, although the shapes differed in area (triangle: 21.2 cm²; square: 32.5 cm²), in the case of the violet and brown shapes discrimination was not possible. Third, bees are able to discriminate shapes with a central rewarding tube displaying the same area (Campan & Lehrer 2001). Fourth, our bees did not use just the difference in area for shape discrimination: in the test with the transformed shapes (Fig. 3b), they did not prefer the cyan test triangle with a larger area than the cyan test square. We cannot rule out that area was used as an additional cue for discrimination but it was not the main discrimination cue.

We thus suggest that shape discrimination as observed in our experiments was based on edge- and not on area-related information. Such a conclusion is in line with the results of Cartwright & Collett (1983), Judd & Collett (1998) and Campan & Lehrer (2002). It is unlikely that the bees relied on the difference in the vertical position of the centres of gravity of the shapes, which tethered flies use for pattern discrimination.
Aligning both shapes at their lower or their upper edge (Fig. 4) yields a difference in the vertical position of their centre of gravity of 4’ and 3’, respectively. Such a small difference can be barely resolved by the bees and was also inconsequential for Drosophila flies (Ernst & Heisenberg 1999).

If edge-based information provided the main discrimination cue, the question arises of how many edges are needed for shape recognition and whether recognition is edge invariant, for example if it is possible when shapes are rotated or changed in size. Free-flying bees can discriminate between two orientations of a single edge (Wehner 1967; Menzel & Lieke 1983) and extract the orientation of stripes as a feature when trained to do so (van Hateren et al. 1990; Srinivasan et al. 1994; Giger & Srinivasan 1995, 1996). These studies used stimuli with a unique dominant orientation, for example horizontal versus vertical stripe patterns. Discrimination therefore involved recognizing a single orientation. This condition was not met in our closed shapes in which various orientations coexisted (e.g. horizontal and vertical edges in the square, and two different diagonal edges and a horizontal edge in the triangle). It is incorrect to assume that the discrimination of these shapes can be reduced to the discrimination of a single orientation (a vertical edge in the square versus a diagonal edge in the triangle), as shown by the test in which the square and the triangle were rotated (Fig. 3c) such that the vertical edge of the square was now available in the rotated triangle and the diagonal edge of the triangle in the rotated square. Under these experimental conditions bees chose randomly, thus showing that a single edge is not enough to discriminate between closed shapes.

We suggest that bees might have solved the discrimination task by learning the different orientations defining a shape and by integrating them into a configural representation in which the spatial relationships between the elementary orientations are preserved (Stach & Giurfa 2001). The resulting representation is different from a retinotopic template in the classical sense, as the bees would learn only the outline of a shape without paying attention to its pixel-based information. This possibility explains the results of experiments in which bees were trained with a randomized succession of changing stimuli, but are especially sensitive to edges. This assumption is consistent with our results and with those of Lehrer et al. (1990) and Lehrer & Srinivasan (1993) on edge detection by honeybees.

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References


