Chapter II

Configural categorization of visual stimuli in a mini brain

Abstract

Categorization is a fundamental cognitive ability that allows treating similar stimuli as equivalents, and thus responding to them in the same manner. It promotes cognitive economy, since it allows adaptive responses to novel objects in the environment. While the importance of categorization in animal behaviour is widely acknowledged (Pearce, 1997), the question of how animals form categories remains unanswered. In insects, the demonstration of visual categorization has been limited to the classification of stimuli on the basis of a single feature. Honeybees can categorize visual stimuli on the exclusive basis of bilateral (Giurfa et al., 1996) and radial symmetry (Horridge & Zhang, 1995), concentric pattern organisation (Horridge & Zhang, 1995), edge orientation (van Hateren et al., 1990), and pattern disruption (Horridge, 1997). Here we show that bees can categorize visual patterns on the basis of configurations of visual features. Bees trained with a series of complex patterns sharing a common configuration made up of four edge orientations extracted all four orientations and integrated them into a configural representation, which allowed them to respond to novel stimuli that preserved the trained configuration. Stimulating the achromatic L- and M- photoreceptor channels was necessary for configural categorization. The mini-brain of the honeybee can thus extract regularities in its environment and establish correspondences among correlated features in order to build configural stimulus representations. It can thus generate a large set of object descriptions and categories from a finite set of elements.

The idea that insect visual recognition is based on rigid and stereotyped mechanisms has been progressively abandoned since recent research has shown that insects are capable of highly sophisticated and plastic visual recognition strategies (Giurfa & Menzel, 1997). Honeybees are particularly suited for studies on visual recognition as they can be easily trained to fly toward a given stimulus in order to obtain a sucrose solution reward on it (von Frisch, 1967). Using this approach, bees were shown to categorize visual stimuli as they appropriately transferred their choice to novel sets of stimuli, which were not experienced during training, but which shared a common feature with the trained stimuli. Up to now, examples showing visual categorization in bees have been restricted to the use of a single feature at a time. This kind of performance is a rather limited option when compared to configural categorization exhibited by humans (Maurer et al., 2002). Here we investigated whether categorization on the basis of configurations of visual features is also possible in honeybees, and which physiological pathways are involved in the categorization task.

Individually-marked honeybees, *Apis mellifera*, were trained with a series of six randomly changing pairs of patterns presented vertically on the back walls of a naturally illuminated Y-maze. Only one bee was present in the maze at a time. Each bee entered the maze by flying through an aperture in the middle of an entrance wall. The entrance led to a decision chamber, where the bee could choose between two arms. Each arm had one stimulus, presented vertically. Only the stimulus defined by the experimenter as positive was rewarded with sucrose solution. Under such a differential conditioning with two stimuli, one rewarded and the other not, bees learn the patterns as a whole, not only their local cues (Giurfa et al., 1999). Patterns were circular disks divided into four quadrants, each presenting a different orientation. Depending on the experiment, the training patterns were either black and white on a white background (Fig. 1a) or coloured on a grey

background (not shown). Bar width and grating period could be well resolved by the bees' visual system within the maze (Srinivasan & Lehrer, 1988). Patterns labelled 'A' shared a common configuration defined by the four different orientations (Fig 1a). Patterns labelled 'B' also shared a common orientation configuration, which was perpendicular to that of the A patterns (Fig. 1a).
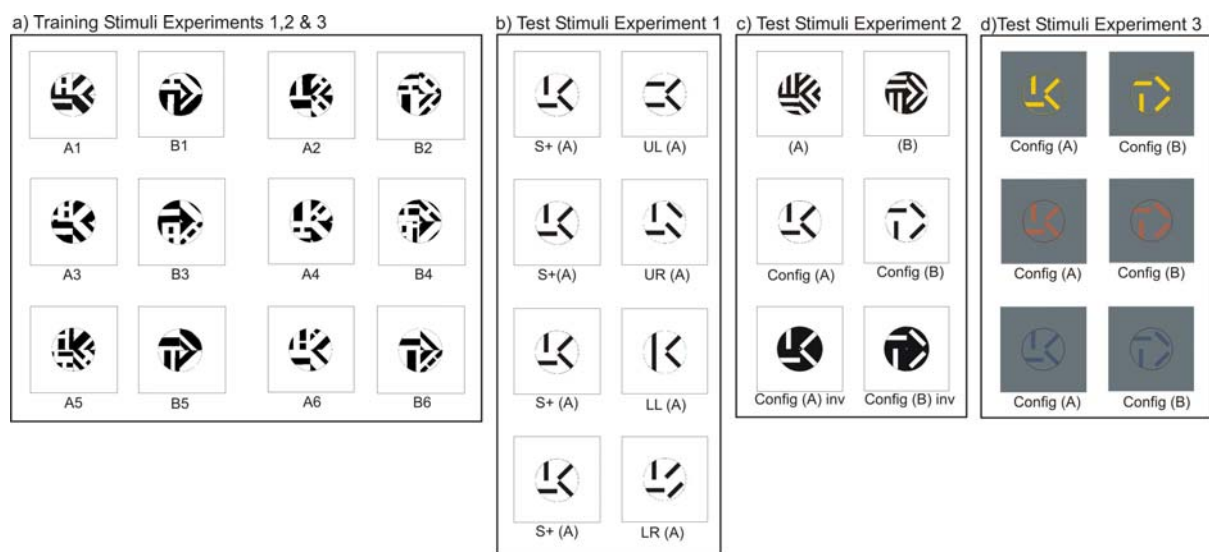

Figure 1: Training (a) and test (b, c, d) stimuli. **a** Achromatic (black and white) training stimuli used in the first and second experiments. Six pairs of A (A1 to A6) and B (B1 to B6) patterns were used. A patterns differed from each other but shared a common configuration defined by the spatial arrangement of orientations in the four quadrants; B patterns also shared a common configuration, which was perpendicular to that of A patterns. In the third experiment, the training patterns were identical but were coloured against a grey background (not shown). **b** Test stimuli used in the first experiment. The four test pairs shown correspond to the group of bees rewarded on A patterns during the training. S+: simplified configuration of the rewarded training patterns, UL: <u>u</u>pper <u>l</u>eft bar rotated, UR: <u>u</u>pper <u>r</u>ight bar rotated, LL: <u>l</u>ower <u>l</u>eft bar rotated, LR, <u>l</u>ower <u>r</u>ight bar rotated. Equivalent tests were performed with bees rewarded on B patterns during the training (not shown). **c** Test stimuli used in the second experiment ('Config': simplified configuration of training patterns; 'Config inv': same as 'Config' but with black and white areas inverted). **d** Test stimuli used in the third experiment. Coloured patterns were used to suppress S-receptor contrast (yellow patterns), M- and L-receptor contrast (blue patterns) and L-receptor contrast (brown patterns).

Our first experiment consisted in training individual bees during 42 training trials (i.e. 42 foraging bouts) with a random succession of the six pairs of black and white A and B patterns (Fig. 1a). For one group of bees the A patterns were rewarded and the B patterns were not, and vice-versa for the other group of bees. Both groups learned equally well to chose the appropriate stimulus within each pair, and the results were therefore pooled. Pooled acquisition increased significantly and reached a proportion of 0.67 correct choices at the end of the training (Fig. 2a: F = 5.2; *df*: 6,96; p < 0.001). To test whether bees were capable of extracting the configuration common to the rewarded patterns, they were presented in dual-choice situations with novel test stimuli that were all non-rewarded and had not been seen by the bees beforehand. In each quadrant, orientation cues were reduced to a single oriented bar (Fig. 1b). One of the test stimuli always preserved the previously rewarded configuration (positive configuration (S+)) whilst the other differed in a single orientation. To this end, the orientation of one of the four bars was rotated by 90° with respect to the positive configuration (Fig. 1b). Four different alternatives were thus possible and each one was contrasted to the simplified positive configuration in a separate test. These tests allow deciding whether or not bees can extract the four-stripe configuration, in which case they should always prefer the complete positive configuration to any stimulus in which one of the four bars was rotated. Bees of both groups preferred the test patterns with the complete positive configuration S+ to any of the four modified alternatives, regardless of the quadrant in which the bar was rotated (Fig. 2b, p < 0.005 in all four cases). Thus the bees were able to extract a common configuration made up of four different orientations, and used such a simplified representation to categorize novel patterns. Within this simplified representation, all four quadrants had the same importance.
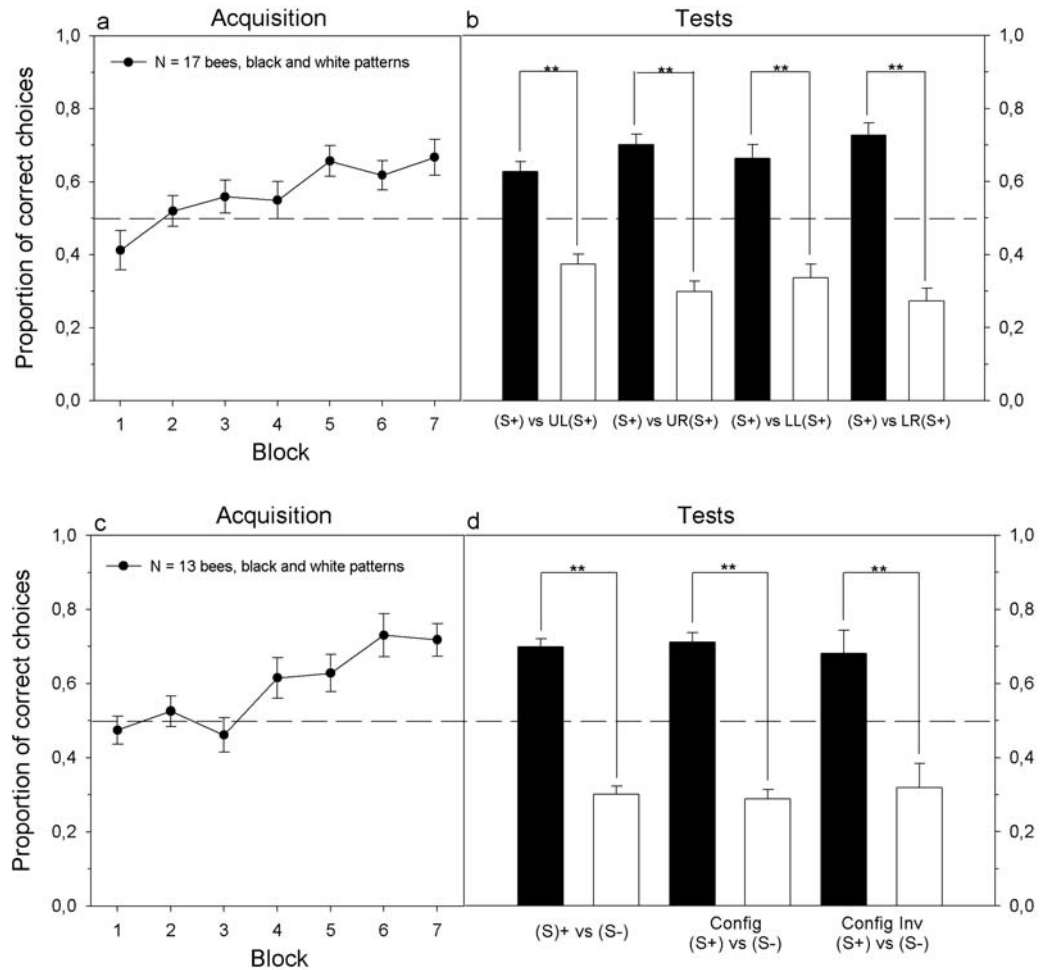
Figure 2: First (a, b) and second experiment (c, d) (S+ and S-: configurations of the training patterns previously rewarded and non-rewarded, respectively). **a** Acquisition in the first experiment during training with 6 pairs of black and white patterns. The figure shows the proportion of correct choices along 7 blocks of 6 consecutive visits. **b** Proportion of correct choices in the tests of the first experiment (see Fig. 1b). In all cases, bees preferred the novel stimulus displaying the simplified configuration of the training patterns previously rewarded (S+) to any alternative in which a single bar was rotated; **: $p < 0.005$. **c** Acquisition in the second experiment. Training was identical to that of the first experiment. **d** Proportion of correct choices in the tests of the second experiment (see Fig. 1c). In all cases, bees preferred the novel patterns displaying the simplified configuration of the training patterns previously rewarded (S+); **: $p < 0.005$.

In a second experiment, in order to promote a configural pattern representation, we trained the bees as in the first experiment. Pooled acquisition again increased significantly

and reached a proportion of 0.71 correct choices at the end of the training (Fig. 2c: F = 5.4; *df*: 6,72; p < 0.001). Such performance did not differ from that observed in the first experiment (F = 0.42; *df*: 1,28; NS; compare Figs. 2a and 2c). We then tested the flexibility of configural categorization by confronting bees with three pairs of novel test patterns differing in their appearance but not in their orientation configurations (Fig. 1c). In all cases, and independent of pattern quality, bees preferred the novel pattern corresponding to the previously rewarded configuration (Fig. 2d, p < 0.005 in all cases). They were thus able to categorize different kinds of unknown patterns on the basis of a common configuration of orientations.

We next studied the involvement of different visual processing channels in configural categorization. Three kinds of photoreceptors exist in the bee retina: S, M, and L, which have their maximum sensitivity in the ultraviolet (S- or UV receptor; λ_{max} = 344 nm), blue (M- or blue receptor; λ_{max} = 436 nm, and green regions of the spectrum (L- or green receptor; λ_{max} = 544 nm), respectively (Menzel & Backhaus, 1991). Achromatic vision in bees is mediated by single receptor channels, which provide achromatic contrasts between stimulus and background for different behavioural tasks (Giurfa & Menzel, 1997). Global orientation of a pattern is processed through the achromatic channel provided by the L-receptor ('green receptor type') (Giger & Srinivasan, 1996). Thus, in a third experiment, we trained bees as in the first and second experiments to promote configural categorization, but the training stimuli were now coloured gratings on a grey background, instead of being achromatic. Three different colours were chosen in order to suppress different receptor-specific contrasts to the grey background against which the stimuli were presented: yellow patterns in which contrast to the S-receptor type was suppressed, blue ones in which contrasts to the S- and the M-receptor type were suppressed, and brown ones in which contrast to the L-receptor type was suppressed (see Table 1).

Receptor-specific contrasts

	S	M	L
Background	**1.0**	**1.0**	**1.0**
Yellow	**1.1**	1.4	3.0
Brown	0.6	0.4	**0.9**
Blue	**1.0**	**1.0**	0.7

Table 1. Receptor-specific contrasts for the stimulus-background combinations. Bold values indicate coincidence in receptor-specific contrasts, i.e. lack of achromatic contrast.

Bees could only learn the training task efficiently (i.e. to choose the appropriate stimulus within each of the six training pairs) in the case of the yellow stimuli, which provided M- and L- contrast. Performance increased significantly during training and reached a proportion of 0.84 correct choices in the last block (Fig. 3a: F = 10.7; *df*: 6,72; p < 0.001). The task could not be learned in the case of the blue and brown stimuli (Fig. 3a: blue patterns: F = 1.6; *df*: 6,66; NS; brown patterns: F = 1.8; *df*: 6,72; NS) although a positive tendency was visible at the end of training. In the case of yellow stimuli, acquisition was significantly better than that obtained with the achromatic patterns (F = 25.23; *df*: 1,41; p < 0.001; compare with Fig. 2a), thus showing that chromatic cues improve performance in this task. Tests with simplified yellow, blue and brown patterns (Fig. 1d) showed that bees preferred the correct simplified configuration (p < 0.005, Fig. 3a) only in the case of the yellow stimuli in which M- and L-contrast were available. When the test patterns were blue or brown, bees chose randomly between them or slightly

preferred the wrong alternative, respectively. Thus, absence of S-contrast did not affect configural visual categorization, whereas absence of M- and L-contrast impaired it.
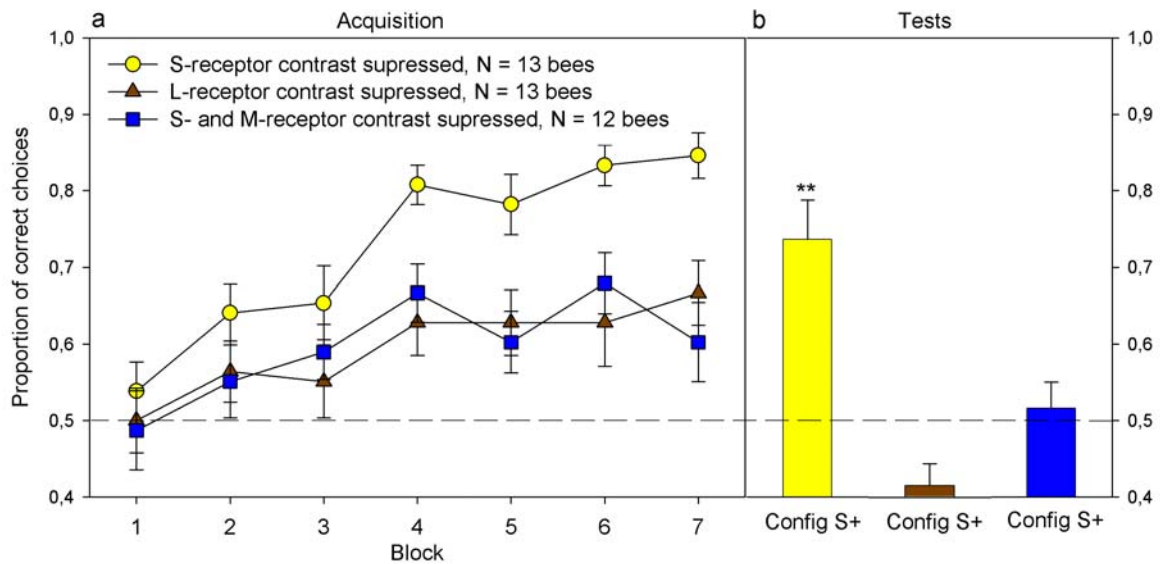

Figure 3: Third experiment. **a** Acquisition during training with 6 pairs of chromatic patterns, yellow, blue or brown, identical to those of Fig. 1a. The figure shows the proportion of correct choices among 7 blocks of 6 consecutive visits. Performance increased significantly only for yellow stimuli in which S-contrast was suppressed, but M- and L- contrast was displayed. **b** Proportion of correct choices in the tests of the third experiment (see Fig. 1d); **: $p < 0.005$.

Our results show for the first time that bees can categorize visual patterns on the basis of a global configuration made from four different orientations, common to a series of different patterns. In our experiments, bees built a generic stimulus representation in which the four orientations coexisted in a specific spatial configuration, which allowed categorization of novel stimuli. The consequence of this result is that bees could build generic categories for flowers, plants, landscapes and other objects in the environment based on a common morphology. The small brain of the honeybee, with its 950 000

neurons, is therefore capable of generating large sets of object descriptions and categories from finite sets of elements.

In responding to stimuli preserving a learned configuration bees may look for stimuli in which each of the four orientations appears in the appropriate retinal field. This possibility refers to elemental theories of compound processing in which a compound is treated as the mere sum of its elements (Rescorla & Wagner, 1972). On the other hand, bees could treat a stimulus presenting the four orientations as an entity, different from the simple sum of the individual orientations. This possibility refers to non-elemental theories of compound processing (Pearce, 1994). Non-elemental processing and learning of olfactory (Deisig et al., 2001) and visual (Fauria et al., 2000; Schubert et al., 2002) compounds has been shown in bees, thus suggesting that such a non-linear strategy may underlie visual configural categorization.

The accessibility of the bee visual system, in which pathways for processing chromatic and achromatic stimuli have been identified (Menzel & Backhaus, 1991), allows addressing the question of the chromatic channels involved in configural categorization. Suppression of S-receptor contrast (yellow stimuli), did not affect categorization, whereas the suppression of M- and L-receptor contrast (blue and brown stimuli, table 1) impaired it. This result is consistent with the importance of L-contrast for edge detection (Giger & Srinivasan, 1996), but raises the question of the role of M-contrast in this performance. A possible explanation is that our stimuli lacking M-receptor contrast might not be well detected due to their low L-receptor contrast (see Table 1). Orientation detectors with small visual fields are required for the implementation of an orientation configuration like the one demonstrated by our experiments. Such detectors have been found in the visual neuropiles of the insect brain (Osorio, 1987; James & Osorio, 1996). The response of these

cells with receptive fields from 2 to 20° suggests a role as edge detectors. It is thus conceivable not only that they respond to specific orientations but also that their simultaneous activation provides the basis for pattern representation in terms of a specific set of orientations.

The belief that insects have limited categorization capabilities allowing them to use a single categorical feature at a time is questioned by our results. Despite the apparent simplicity of their nervous systems, bees can access visual recognition strategies that yield surprising similarities to results obtained by strategies employed by vertebrates (Biedermann, 1987; Greene, 1983; Troje et al., 1999; Werner & Rehkämper, 1999; Gauthier et al., 1998).

Methods

Individual bees were trained to collect 50% (weight/weight) sucrose solution in a Y-maze. The maze was covered by an ultraviolet-transparent Plexiglas ceiling and shielded from direct sunlight. Bees entered the maze by flying through an aperture, 5 cm in diameter, in the front panel of the maze. During training and tests only one bee was present in the apparatus at one time.

Stimuli

Visual stimuli were discs of 10 cm in diameter, presented in the vertical plane at a distance of 15 cm from the entrance hole and thus subtending a visual angle of 37°. The black and white patterns were produced with a high-resolution laser printer on standard copy paper of constant quality. The coloured patterns were printed with a high-resolution ink jet printer on the same paper. The circular patterns were divided into four quadrants, each presenting a grating of a different orientation (0°, 45°, 90° and 135° with respect to the vertical). The

width of stripes in the training patterns varied but it was always at least 1 cm, which corresponds to a visual angle of 4° as seen from the entrance hole. This minimal width ensured that stripes were always well resolvable for the bees' eyes (Srinivasan & Lehrer, 1988). The reward was provided in the centre of the patterns.

Colour parameters

The reflectance spectra of the coloured stimuli were measured by means of a spectrophotometer (Ocean Optics SD2000 with a DT1000 mini light source (200 – 1100 nm) and the R400-7 UV/VIS optical fibre). The receptor quantum catches Q_i were calculated as follows:

$$Q_i = \int\limits_{300}^{700} I(\lambda) S_i(\lambda) R(\lambda) d(\lambda)$$

where i is S,M or L, λ is the wavelength, $I(\lambda)$ is the illumination spectrum (D65 standard function), $S_i(\lambda)$ is the spectral sensitivity function of receptor i (Menzel & Backhaus, 1991) and R is the measured reflectance spectrum of the coloured stimulus. For each colour/background combination the receptor specific contrasts q_i (see Table 1) were calculated as:

$$q_i = Q^t_i / Q^b_i$$

where Q^t_i and Q^b_i are the quantum catches of receptor i for the stimulus (target) and the background colours.

Procedure

During training, the side of the rewarded pattern (left or right arm of the maze) was interchanged in a pseudorandom way to avoid positional learning. Each of the six pairs of

training patterns was presented seven times in a randomised sequence, thus resulting in a total number of 42 training trials (approximately one day of training per bee). This amount of training is sufficient to ensure learning in this task (Giurfa et al., 1999). Acquisition curves were established by dividing the 42 trials into 7 blocks of 6 trials each. Choice proportions were calculated from the number of choices for the positive stimuli (correct choices) per block. After the training, transfer tests with different non-rewarded test patterns were performed. Contacts with the surface of the patterns were counted for two minutes. The choice proportion for each of the two test patterns was calculated. Each test was done twice, interchanging the sides of the patterns in order to control for possible side preferences. Refreshing trials were intermingled among the tests.

One-way ANOVA was performed in order to detect possible differences in performance between bees rewarded on A patterns and those rewarded on B patterns. In all cases, no significant differences were found, such that data were pooled for all subsequent treatments. To detect differences in test performances a Wilcoxon paired-sample test was performed on the absolute number of choices for each of the two test patterns. ANOVAs for repeated measures were performed to detect whether the bees' acquisition significantly improved during training. The alpha-level was set to 0.05 for all analyses.

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