

Chapter IV

Detectability of grouped colour targets for bees is enhanced through edge density

Abstract Bees use a brightness cue, mediated by their long wavelength (L-) receptor, to detect and discriminate single coloured targets at small angular subtenses. At threshold, such targets vanish in the bee's perception although more than one ommatidium is still excited. If the target is patterned it requires the input from an increased number of excited ommatidia in order to be detected. These previous studies indicate that the underlying visual processing is based on the interaction between signals from several neighbouring ommatidia. Here we investigated how the detection limit of patterns formed by grouped targets is affected by the operation of such mechanisms. Individual bumblebees of similar size and honeybees were trained to single coloured discs or groups of them arranged in a triplet pattern in such a way that optical fusion of the triplet elements at small angular subtenses was prevented. To distinguish the participation of the L-receptor mediated visual system, we used colours that selectively did or did not present a brightness cue. In bumblebees and honeybees, the detection limits of single discs and triplet patterns were improved when targets presented colours with a brightness cue. More importantly, the triplet pattern was in this case also detected over a larger distance range than predicted by detectability results obtained with a single disc as compared to a spatial summation of the pattern's parts. We thus present further evidence that the L-receptor mediated visual system of bees operates through units with centre-surround receptive fields whose outputs can be integrated spatially.

Introduction

Flowers are often arranged in a variety of inflorescences, which has been of great interest to botanists but has not been assessed from a pollinator's visual perspective. It is usually assumed that flowering plants enlarge their coloured display by grouping single flowers to be more conspicuous to an insect. However, it is not known, how spatial patterning of flower and background colours in complex plant displays affect the insect's perception of targets. In a previous study, we trained free-flying bees to detect single targets that displayed a coloured pattern. The detectability of such patterned targets varied in dependence of the spatial distribution of brightness contrast, provided through the long wavelength (L-) receptor, within the target's area (Hempel de Ibarra *et al.* 2001). Here, we ask how spatial cues may interact with target detection if single-coloured targets are grouped. We used both honeybees *Apis mellifera* and bumblebees *Bombus terrestris* as a comparative model.

The detection of single-coloured targets depends on two size-tuned visual mechanisms (Giurfa *et al.* 1996, 1997, Giurfa and Vorobyev 1998). If angular subtenses are small (5-15°), i.e. the bee is relatively far away from a target, the signals of the long wavelength receptors are fed into an achromatic visual system. As angular subtenses increase (>13-15°), i.e. during approach of the bee, target detection is mediated by the chromatic system which receives input from all three photoreceptor types of the bee eye. The same is true for targets that display a bicoloured concentric pattern (Hempel de Ibarra *et al.* 2001). However, the minimum angle at which the target can be detected by the bee eye is decreased. A critical parameter that determines the varied detectability of the bicoloured target is the distribution of L-receptor contrasts within the target. If a central disc with a weak (dim) L-receptor contrast was surrounded by a ring with strong (bright) L-receptor contrast, the target yielded a detection limit of 6.5°. The opposite arrangement of the target's pattern, i.e. where the ring was dim and the central disc was bright, was detected only when its visual angle subtended more than 10°. Taking into account the low resolution of bee eyes, stimuli with a bright outer part and a dim inner part have enhanced edges, while stimuli with a dim inner part and a bright outer part have blurred edges. The impaired detectability of targets with blurred edges is a plausible consequence, if the neurons that process visual information have a centre-surround organisation (Giurfa and Vorobyev 1998). In the present study we trained bees to a pattern that consisted of a group of discs – such patterns display a greater density of borders in their area than larger single discs do. If detector units with centre-surround receptive fields

mediate target detection, we expect the target detectability to be more strongly influenced by the amount and density of borders rather than by the area of targets.

Material and Methods

Honeybees *Apis mellifera*, marked individually, were trained to find a sucrose reward on a target, a concentric disc, presented in a Y-maze, that was located at a large open window of a laboratory room (for details see Hempel de Ibarra *et al.* 2001). Bumblebees *Bombus terrestris* (purchased from STB Control, Germany) were trained individually to a similar maze and target within a flight cage, situated in a glass house.

One arm of the Y-maze presented a rewarded target on a grey vertical background while the other arm only displayed the unrewarded grey background (detection paradigm; Fig. 1). After entering the maze an animal was able to see both back walls simultaneously before it had to decide for one of the arms. It learned to decide in favour of the arm containing a visible target in the decision chamber. When the animal entered the arm with the rewarded stimulus, its choice was recorded as correct and it was allowed to feed *ad libitum*. If it entered the unrewarded arm, it was either allowed to return to the maze entrance or it was gently pushed out of the maze to repeat its choice until it found the reward. Such a choice was recorded as incorrect. The target was moved along the maze arm away from the entrance in systematic steps to vary the visual angle of the target. One step, i.e. the target at a specific visual angle, was regarded as being successfully detected, if an animal completed a series of visits with a performance above the 60% percent threshold of correct choices as determined by the Binomial distribution (Giurfa *et al.* 1996, Hempel de Ibarra *et al.* 2001). Since the flight cage of the bumblebees limited the length of the maze arms, reduction of the target's angular size was also achieved by reducing its aerial size (Giurfa *et al.* 1996). If targets were not detected, a maximum of 30 visits were had to be completed, which were followed by a repetition of performance recording at the closest experimental distance.

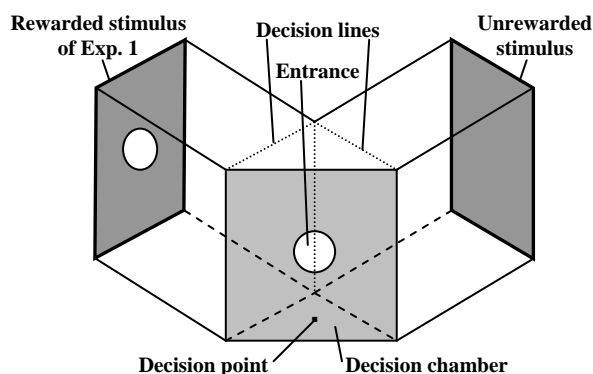


Fig. 1 Experimental set-up: After entering the Y-maze the bees had to decide for one arm within the decision chamber. The arms presented the rewarded stimulus (coloured discs or triplets) or the unrewarded background only, in pseudo-random order. Distance to the stimulus was measured from the decision point.

Single bees were trained to detect a single coloured disc with a diameter of 8 cm (bumblebees) or 4.6 cm (honeybees) or to detect a triplet pattern consisting of three discs of the same colour and size (each 4.6 cm in diameter) arranged in an triangle. A distance of 4.6 cm was kept between the borders of neighbouring discs to prevent a merging of the triplet elements at small angular subtenses. Stimulus detection was tested with angular subtenses of 29.9°, 16.9°, 13.0°, 10.2°, 7.6°, 7.0°, 5.1°, 4.3°, 2.5°, 2.25°, 1.3° for bumblebees and 17.4°, 5.9°, 4.1°, 3.3° for honeybees. We chose a yellow and a violet colour which were both chromatic to the bees, but differed in their L-receptor contrast to the background. The same colours for stimuli and background were used in our previous studies, cut from standard graphic papers (HKS 3 N, 33 N, 92 N; K + E Druckfarben, Germany; Giurfa *et al.* 1996, for calculations see Hempel de Ibarra *et al.* 2001). The flight cage illumination, measured with a calibrated spectrometer (SD 2000, Ocean Optics Inc., USA) differed slightly from illumination during honeybee series. Thus, the yellow stimuli in the bumblebee series presented a slightly smaller L-receptor contrast (3.2) to the background compared to the stimuli in the honeybee series, whereas violet stimuli had equivalent, i.e. no L-receptor contrast (0.98). Since eye size is correlated with thorax width (Spaethe *et al.* 2001), we measured the thorax width of the tested bumblebees (4.0 ± 0.06 mm \pm SD) and concluded that all animals were similarly sized.

Bees selected to be tested for target discrimination learned the rewarded target in a detection phase. The following discrimination phase proceeded at the same angular subtense (for details see Hempel de Ibarra *et al.* 2002). During the discrimination phase the rewarded target (disc triplet) was presented in one arm, while the other arm showed an unrewarded alternative target being a single disc with an area equal to the summed areas of the triplet elements. Since the bees failed to show discrimination with both yellow and violet stimuli at all angular subtenses (Niggebrügge 2003), we changed the experimental procedure. Bumblebees were trained in the discrimination paradigm during 30 visits and their behaviour was subsequently recorded in an unrewarded test with unused targets of the same colour and size. The animals were free to move within the maze during 2 minutes. The bees' approaches, touches and landings were recorded. After refreshment training of three visits, the same test was repeated with targets inversely presented. In this experiment we used two angular subtenses: 1) 30° for the single disc and 56.2° / 17.5° for the triplet (triplet circumference/triplet element); 2) 7.1° and 14.1° / 4.1°.

Results

Bumblebees showed different responses to yellow and violet stimuli. The single yellow disc subtended a visual angle of $\alpha_{\text{det}} = 2.3^\circ$ when it was last detected by the bumblebees (Binomial test, $p = 0.035$) and $\alpha_{\text{indet}} = 1.3^\circ$ when it was not detectable anymore ($p = 0.99$). This performance leads to an angular limit of $\alpha_{\text{lim}} = 1.8^\circ$ (Fig. 2). The violet disc was last detected at a smaller distance as compared to the yellow target, subtending $\alpha_{\text{det}} = 4.3^\circ$ ($p < 0.001$) and not detected anymore subtending $\alpha_{\text{indet}} = 2.5^\circ$ ($p = 0.79$). Thus, the absence of L-receptor contrast resulted in an impaired spatial range of detection of the violet disc ($\alpha_{\text{lim}} = 3.1^\circ$, Fig. 3). These results are in line with previous findings for honeybees where the presence of L-receptor contrast in a coloured target increased the distance over which a stimulus was detected and discriminated (Giurfa *et al.* 1996, 1997, Hempel *et al.* 2001, 2002). The detection limit for the yellow target in those and the present studies was about 5° for honeybees (honeybees, $n = 9$: $\alpha_{\text{lim}} = 4.5^\circ$, since $\alpha_{\text{det}} = 5.9^\circ$ ($p = 0.008$) and $\alpha_{\text{indet}} = 4.1^\circ$ ($p = 0.63$), Fig. 4). The lower resolution of the honeybee eye can be attributed to the smaller size of its eyes as compared to the larger-sized bumblebees tested in our study (Land and Nilson 2001, Macuda *et al.* 2001, Spaethe and Chittka 2003).

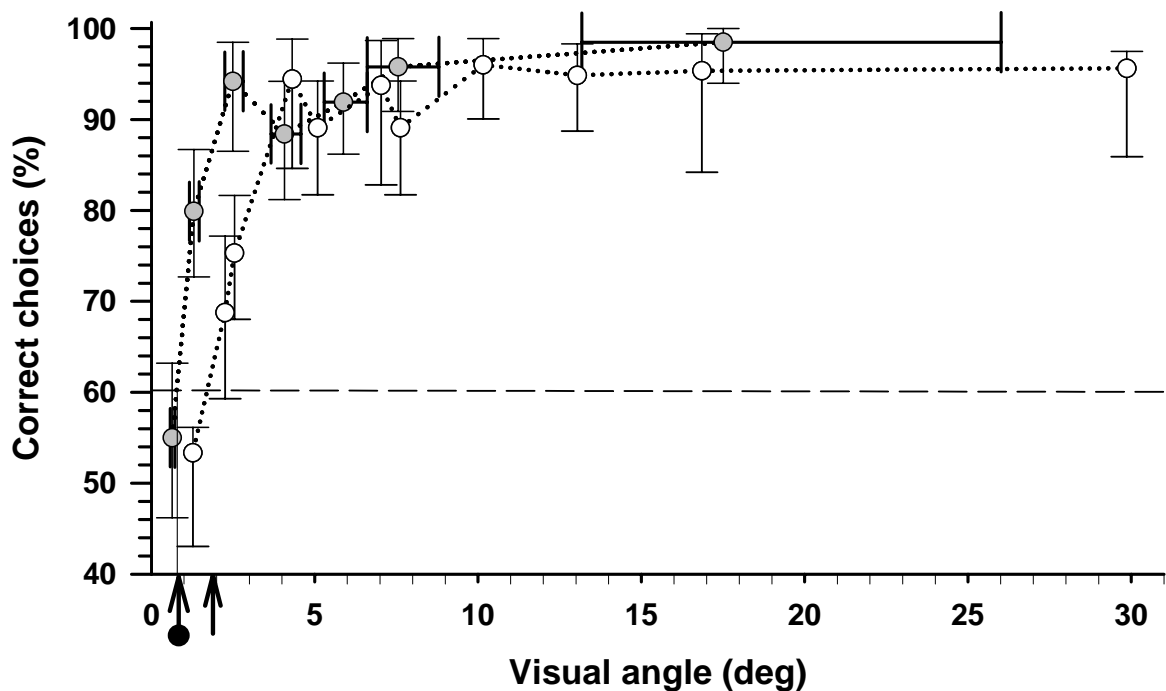


Fig. 2 Detection of yellow single and grouped discs by bumblebees. Shown is the detection performance of bees as a function of the angular subtense of a single disc presented either alone (open symbols) or as an element of a triplet (filled symbols). The colour of the discs provided chromatic contrast as well as L-receptor contrast. The arrows indicate the detection limit for a disc presented alone (arrow with dot) which improved for the discs presented in a triplet

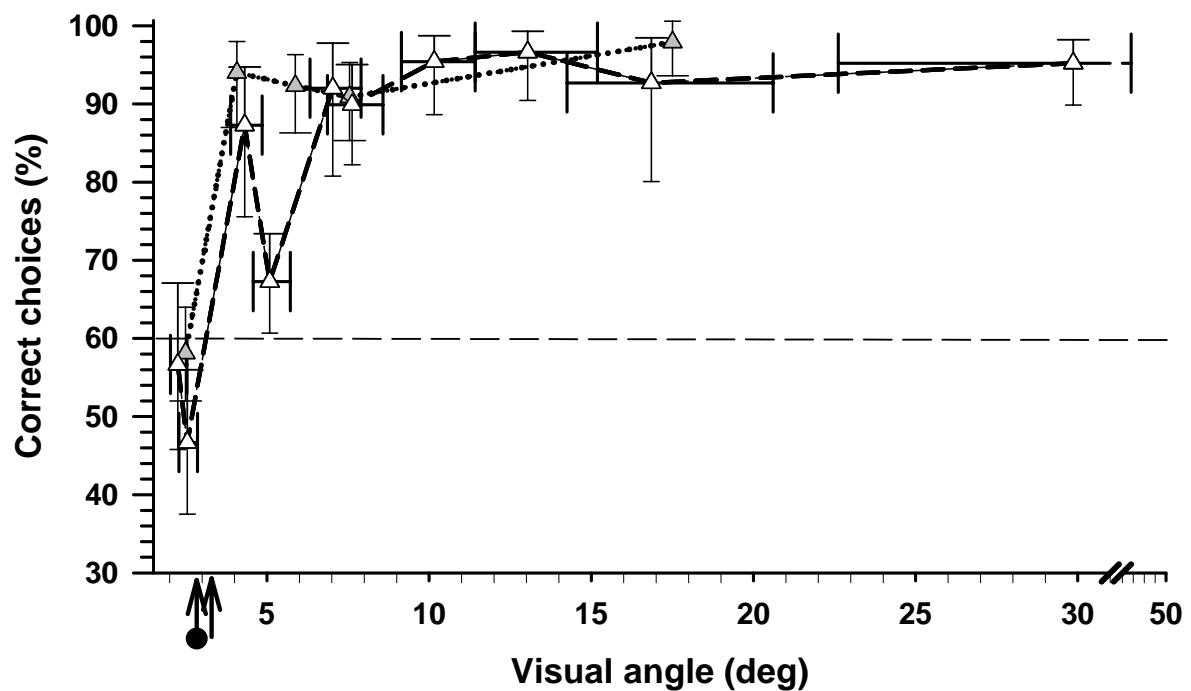


Fig. 3 Detection of violet single and grouped discs by bumblebees. Shown is the detection performance of bees as function of the angular subtense of a single disc presented either alone (open symbols) or as element of a triplet (filled symbols). The colour of the discs provided only chromatic contrast but no L-receptor contrast. The arrows indicate similar detection limit for a disc presented alone (arrow with dot) or in a triplet.

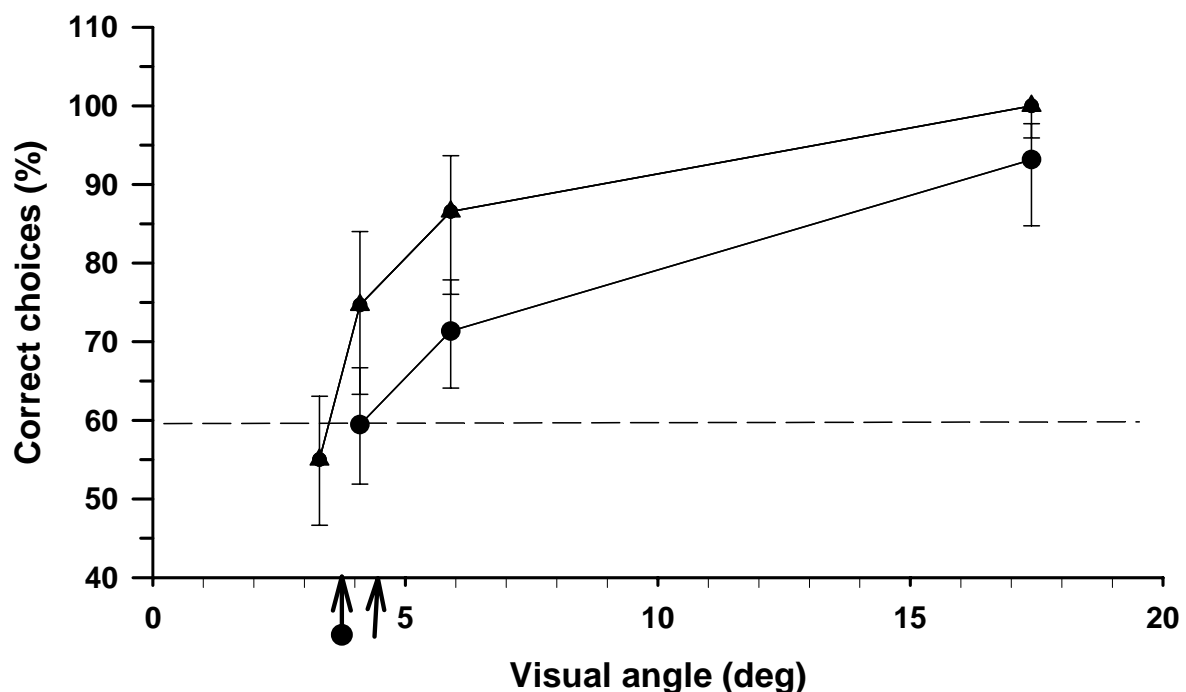


Fig. 4 Detection of yellow single and grouped discs by honeybees. Shown is the detection performance of bees as function of the angular subtense of a single disc presented either alone (disc symbols) or as element of a triplet (triangle symbols). The colour of the discs provided chromatic contrast as well as L-receptor contrast. The arrows indicate the detection limit for a disc presented alone (arrow with dot) which improved for the discs presented in a triplet.

The detection limit of the triplet pattern was predicted to depend on the detectability of the single widely spaced discs. This was indeed the case for the violet triplet but not for the yellow triplet. Bumblebees were able to detect the violet triplet, presenting only chromatic contrast to the background but no L-receptor contrast, at a distance where a triplet element subtended $\alpha_{\text{det}}^e = 4.5^\circ$ and the whole triplet in its circumference $\alpha_{\text{det}}^t = 14.0^\circ$ (Binomial test, $p < 0.001$, Fig. 2). When the single element subtended $\alpha_{\text{det}}^e = 2.2^\circ$ and the whole triplet $\alpha_{\text{det}}^t = 8.7^\circ$, the bees were unable to detect the stimulus. Thus the detection limit for the violet triplet was $\alpha_{\text{lim}}^e = 2.6^\circ$ and $\alpha_{\text{lim}}^t = 8.8^\circ$ (Fig. 3). Since the bumblebees trained with the single violet disc were reaching a detection limit between $\alpha_{\text{det}} = 4.3^\circ$ and $\alpha_{\text{indet}} = 2.5^\circ$ the result indicates that the triplet's detectability was defined by the detectability of the single element and not the triplet as a whole.

When training bees to detect the yellow triplet (Fig. 4) the bumblebees showed an increase of the distance over which the triplet was detectable compared to the violet triplet. They were performing well at a large distance where the triplet's element subtended $\alpha_{\text{det}}^e = 1.3^\circ$ and the triplet as a whole $\alpha_{\text{det}}^t = 4.5^\circ$ (Binomial test, $p < 0.001$). To compare: A single disc was not detected by the bumblebees at this angular subtense (Fig. 4). In the next step, when the angular subtense was decreased to $\alpha_{\text{indet}}^e = 0.6^\circ$ and $\alpha_{\text{indet}}^t = 2.2^\circ$, the bees showed a lower, but significant performance (Binomial test, $p = 0.03$). We concluded that this angular subtense was close to the detection limit. Even assuming that the last tested angular subtense was at the threshold, we can conclude that the detection limit for the triplet was enhanced as a result of integration over the triplet and not limited by the detectability of single elements.

We aimed to train bumblebees in a discrimination paradigm to estimate the perceptual properties of the triplet pattern at small angular sizes (Hempel *et al.* 2002). However, they failed to show that they discriminated the triplet pattern from a single disc at any angular subtense when they had to make their choice at the maze entrance (data not shown; groups of 6-7 bees for each of the four training combinations, e.g. rewarded yellow disc vs. unrewarded yellow triplet or *vice-versa*). When tested in less restricted conditions in an unrewarded discrimination test (data not shown), they performed quite inconsistently indicating that such a task was difficult for them.

Knowing the optical properties of the honeybee eye, we were able to simulate the appearance of the triplet pattern at small angular subtenses (Vorobyev *et al.* 1997) and to compare them with our behavioural results. The yellow triplet was detected by honeybees subtending 4.1° ($n = 6$, Binomial test, $p = 0.006$) when the disc presented alone was undetectable (Fig. 4) which paralleled the result obtained with the bumblebees. At 3.3° , however, bees were not

able to detect the yellow triplet anymore ($p = 0.82$). We projected the triplet pattern onto the honeybee ommatidial lattice subtending the tested visual angles (Vorobyev *et al.* 1997) (Fig. 5). The disc elements covered clearly separated ommatidia at angular subtenses, at which they would not be detected when presented alone. Thus, the improvement of detectability was not due to a merging of the pattern elements.

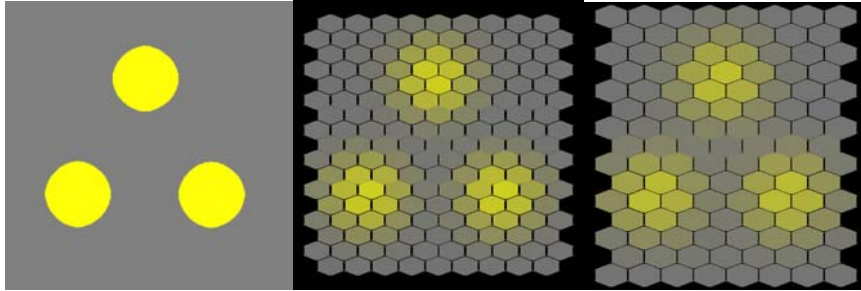


Fig. 6 Projection of the triplet grouped discs on the honeybee's ommatidial lattice. Middle panel – the triplet subtending 4.1° (last detected by honeybees), right panel – the triplet subtending 3.3° (not detected).

Discussion

What insects with a low-resolution eye may see depends crucially on the distance to an object and the object's size, i.e. the angular subtense. If a limiting lowest number of ommatidia is excited, an object will be detected or recognised. However, it is assumed that the limiting size of a detectable object should match the spatial resolution of the viewing array of receptors to optimise the performance of the eye. For a bee's eye, this is the single ommatidium. The apparent mismatch indicates that object detection is mediated by more complex detectors which may help to enhance important visual object features, primarily borders, and thus to functionally optimise detection. The present study provides further evidence for such a mechanism in the bees' L-receptor mediated visual system.

For bees, the detectability of single and grouped targets depends on their spatial and spectral properties. Thus, the distance over which stimuli are detectable is larger if the target's colour provides contrast to the long wavelength (L-) receptor compared to targets without L-receptor contrast. The present study thus generalises a result from honeybees to bumblebees (Giurfa *et al.* 1996, 1997, Hempel *et al.* 2001). In the latter case, the target's detectability varied depending on the presence but not on the magnitude of L-receptor contrast. Thus, the L-receptor contrast is the only signal needed to achieve target detection at small angular subtenses (Giurfa and Vorobyev 1998). If, however, the L-receptor contrast is not uniform

over the target's area, e.g. in a two-coloured concentric pattern, the spatial range of detectability decreases (Hempel de Ibarra *et al.* 2001). Detection of large-sized targets is not influenced by L-receptor contrast (Giurfa and Vorobyev 1998, Niggebrügge and Hempel de Ibarra 2003) but is mediated by the chromatic visual system requiring a large number of excited ommatidia.

Results obtained for target detection at small angular subtenses indicate an L-receptor mediated neural mechanism with an upper and lower limit of sensitivity which is characteristic for antagonistic receptive fields. A linear model of detector units with centre-surround receptive fields (Giurfa and Vorobyev 1998) predicts a number of experimental results mentioned above. Such detectors do not respond to large-sized targets but rather to small ones due to the subtraction of signals originating from the centre of the receptive field and from the surround (Kuffler 1953). They are also sensitive to borders (Fiorentini *et al.* 1990), which would explain our results with a more discontinuous spatial pattern – single objects were grouped such that they would not merge into a single stimulus when seen from a large distance. We found that the target's detectability was improved compared to a single disc, which suggests some form of a spatial integration over elements of the triplet pattern. Following the original model of detector units in the L-receptor mediated system of bees, we propose that the output of such detectors has been integrated leading to an improved detectability of grouped targets. Such performance would be dependent on the density of edges rather than areas of the elements.

Acknowledgements

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