

Bees use three-dimensional information to improve target detection

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Abstract Bumblebee detection of a flat circular disc (two-dimensional (2D) presentation) and a disc which was presented 10 cm in front of a structured background (and thus provided three-dimensional (3D) cues) was compared. A dual choice test using a Y-maze apparatus was conducted to estimate the minimum visual angle at which the bees were able to detect the disc. At large visual angles of 15, 10 and 5° bees' performance between the 2D and the 3D presentation did not differ. However, when the disc subtended 3° at the bee's eye, the bees performed significantly better when 3D information was available. Overall, bees were able to detect a target subtending a 40% smaller visual angle when it was presented in front of the structured background compared to a 2D presentation. This suggests that previous reports on the limits of target detection in bees using flat stimuli might have underestimated the bees' ability to locate small flowers under natural conditions. Bees use motion

parallax, i.e. the apparent relative motion of a stationary object against a background, for perceiving the third dimension. Our data suggest that bumblebees can integrate information from at least two types of feature detectors, motion and area, to improve single target detection.

Keywords *Bombus terrestris* · Flower detection · Motion parallax · Visual perception · 3D vision

Introduction

A major role of the visual system of bees is the detection of flowers during foraging flights. Flower detection is determined by the size and shape of a flower, as well as its chromatic and achromatic contrast against the foliage. When searching for flowers, bees use two largely independent visual mechanisms: an achromatic mechanism which is mediated by the green-sensitive photoreceptors, and a chromatic mechanism, which receives input from all three photoreceptor types (ultraviolet (UV)-, blue- and green-sensitive receptors) (Dyer et al. 2008, Giurfa and Vorobyev 1998). The achromatic visual channel can resolve small targets subtending visual angles of at least 3–5° in honeybees and bumblebees and is used when sufficient contrast for the green-sensitive photoreceptors is available between target and background (Dyer et al. 2008; Giurfa et al. 1996; Lehrer and Bischof 1995). The chromatic channel has a poorer spatial resolution and is only used for the detection of targets larger than 15° in honeybees (Dyer et al. 2008; Giurfa et al. 1996). In terms of visuospatial resolution, bees are therefore relatively poorly equipped in comparison to humans when they search for small targets such as flowers. For instance, a honeybee can detect a flower measuring 3 cm in diameter only when she is less than 35 cm away

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from it, and see the colour of the flower only at a distance of no more than 11 cm. Meanwhile, many entomophilous flowers are small, often with corollas of less than 1 cm (Hempel de Ibarra and Vorobyev 2009; Menzel and Shmida 1993). However, foraging bees flying from flower to flower in their natural habitat seem to have no apparent difficulties finding even the smallest flowers.

The limits of target detection in bees are typically tested under controlled conditions using a Y-maze arena, in which individual bees are trained to associate a reward (e.g. sucrose solution) with a stimulus (Dyer et al. 2008; Giurfa et al. 1996; Macuda et al. 2001; Spaethe and Chittka 2003). The target, such as a coloured paper disc, is usually presented vertically on the back wall of one arm of the Y-maze and the bee has to enter the maze and choose the correct arm with the target. In contrast, under natural conditions, bees search for flowers which usually sit on a peduncle or a twig protruding from the foliage and thus provide three-dimensional (3D) information which might be used by the bees for detection. In humans and other vertebrates, target detection is improved when 3D cues are available (McKee et al. 1997; Pettigrew 1986, and citations therein). Bees are known to use 3D information for distance estimation, edge detection and figure-ground discrimination (Lehrer 1998; Srinivasan et al. 1990). However, whether bees can also deploy 3D information to improve object spatial resolution, such as the detection of a flower against its foliage background, has not yet been tested. Here we compare the performance of bumblebees searching for yellow targets of different sizes presented either directly on a background (two dimensional (2D)) or positioned 10 cm in front of a textured background, introducing a third spatial dimension.

Material and methods

Experiments were conducted with commercially available *Bombus terrestris* colonies (Koppert Biological Systems, The Netherlands). The colonies were housed in wooden nest boxes in an environmental chamber with controlled lighting and temperature conditions (constant temperature of 25°C, 12 h light/dark cycle provided by Osram Biolux daylight fluorescent tubes, frequency was increased to about 1 kHz with special ballasts). Bumblebees were kept on a diet of saturated sucrose solution and pollen obtained from honeybee colonies and kept fresh in a freezer.

During the experiments a nest box was connected to a wooden Y-maze (made up of an entry chamber with 45 × 32 × 30 cm and two 32 × 32 × 30 cm arms with movable back walls; Fig. 1) covered with UV transmitting Plexiglas. The entry chamber was separated from both arms by a screen with a hole (10 cm diameter) in the centre in order to prevent stimulus detection from inside the entry chamber.

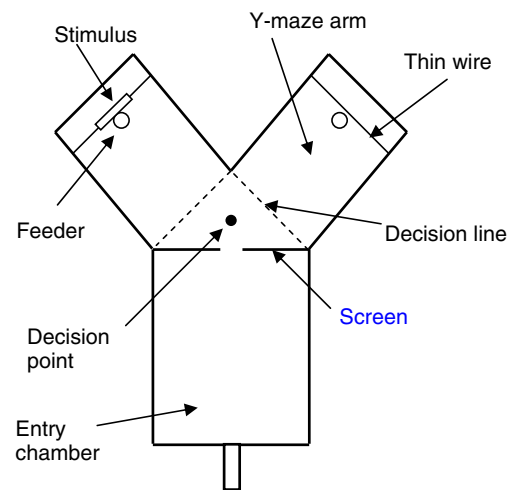


Fig. 1 Overhead view of the Y-maze apparatus. A decision for one of the two arms by the bee was made when she first crossed the (virtual) decision line

The walls of both arms and the back wall were covered by a randomly generated Julesz pattern (Julesz 1960) consisting of light and dark grey (80% and 20% overall reflection) 1 × 1 cm squares printed on cardboard.

Individual bees were trained to visit a translucent feeder with sucrose solution which was positioned at the back wall of one of the two arms on a translucent Plexiglas stand directly below a yellow target (Fig. 1). An identical feeder with a 0.02% quinine water solution was placed in the other arm (Chittka et al. 2003). During training, a large yellow stimulus (11.9 cm in diameter, subtending an angular size of 15° when viewed from 45 cm distance, see below) was presented in one of the two arms. A detailed description of the training procedure is given elsewhere (Dyer et al. 2008; Spaethe and Chittka 2003). In brief, individual bumblebees were trained by moving a feeder with sucrose solution stepwise from the entry chamber to the decision point of the Y-maze (Fig. 1). Then, the target was presented at the back of one of the two arms and the bee had to learn to fly into this arm to obtain the reward. All bees which were able to correctly associate the target with the reward also finished the complete experiment which took 2–3 days per bee. The bees were allowed to feed ad lib at each visit. After the bee visited the feeder and returned to her colony, the feeder was cleaned and exchanged together with the stimulus between the two arms in a random order. The experiment started when the bee made at least four correct choices within the previous five decisions. We started with the largest disc and then gradually reduced the size of the target (from 15 to 10, 5, 3 and 2°). The visual angle of a target was calculated as:

$$\alpha = 2 \arctan (d/2D) \quad (1)$$

where α is the visual angle subtended by a yellow disc of diameter d at a distance D from the decision point (Fig. 1;

see also Giurfa et al. 1996). In all experiments the distance D was held constant at 45 cm. In the 2D experiment the target was directly attached to the back wall. In the 3D experiment the back wall was moved 10 cm backwards and the disc was vertically attached to two thin wires (<0.1 mm diameter) which were stretched between the side walls (Fig. 1). Identical wires without target were placed in the non-rewarding arm as a control.

In total, 16 bees were randomly assigned to one of two experimental groups. The first group was tested first with 2D objects. After they had reached the smallest target size where they chose both arms randomly, they were re-tested with 3D objects, starting again with the largest angular size (15°). The second group was tested in the reverse order, i.e. first with the 3D and subsequently with the 2D stimuli to account for possible training effects. Each bee was tested at each stimulus size for five foraging bouts if no incorrect choices were made, for ten bouts in case of one error and for 20 bouts in case of two or more incorrect choices. The experiment was stopped after the performance at a certain stimulus size fell below 60% (Giurfa et al. 1996). The visual angle α_{\min} at which the bee detects the target with a probability of 0.6 was calculated as:

$$\alpha_{\min} = (\alpha_{\det} + \alpha_{\text{indet}})/2 \tag{2}$$

where α_{\det} is the smallest visual angle at which the bee made at least 60% correct choices, and α_{indet} the largest visual angle at which she failed the 60% criterion (Giurfa et al. 1996; Dyer et al. 2008).

All statistical tests were performed using Statistica 6.0. To assess the impact of training sequence, the performance for the 2D and 3D stimuli, respectively, were compared between both experimental groups using a Mann–Whitney U test. Differences in detection performance between 3D and 2D stimulus presentation at each visual angle were also tested by means of a Mann–Whitney U test. A Wilcoxon test for paired samples was used to compare α_{\min} between both groups. We adjusted α value using the sequential Bonferroni correction (Rice 1989) where multiple significance tests were performed.

Results

The comparison of the 2D and 3D stimulus presentation, respectively, between the two experimental groups (group 1: first trained to 2D object and then to 3D; group 2: vice versa) revealed no significant differences, which indicates that training sequence did not affect detection performance (data not shown). For further analysis we therefore pooled the individuals from both groups. The overall performance for the largest target (15°) was 97.5% and 96.6% of correct choices for 2D and 3D presentation, respectively (Fig. 2). Performance decreased for both 2D and 3D objects with

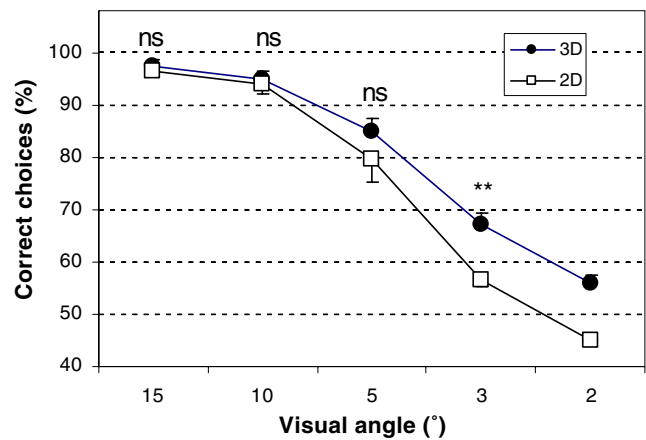


Fig. 2 Mean (±SEM) of percentages of correct choices for different stimulus sizes, showing the difference in responses to 2D and 3D stimuli. Number of tested bees per group and visual angle was: 16/16 for the 2D/3D target at 15°, 10° and 5°, 16/13 at 3° and 12/1 at 2°; ns, $p > 0.05$; **, $p < 0.01$

decreasing stimulus size but did not differ between both groups for target sizes of 15°, 10° and 5° (Fig. 2). However, at 3° performance was significantly better with 3D presentation (67.2%) compared to 2D (56.5%; $p < 0.01$, $N = 16$ and 13, respectively). Twelve individuals of the 3D group fulfilled the criterion at 3° angular size and were therefore tested at 2°. However, only one animal was able to detect the 2D target at 3° and could be tested at 2° (Fig. 2); we therefore did not perform statistics on the 2° data. The visual angle α_{\min} at which the bees detect the target with a probability of 0.6 was significantly larger with 3D compared to 2D target presentation (3D, $2.6^\circ \pm 0.2$ SE; 2D, $4.3^\circ \pm 0.4$ SE; $p < 0.002$, $Z = -3.09$; Fig. 3).

Discussion

The data show that, at large visual angles, additional 3D information does not affect target detection. However, close

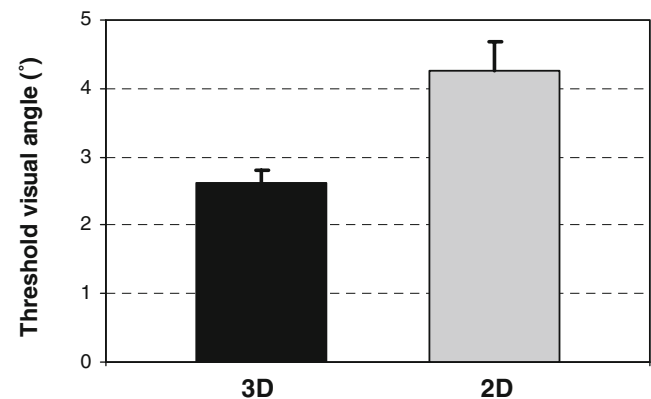


Fig. 3 Mean (±SEM) minimum visual angle α_{\min} at which a bee can detect the 2D and 3D stimulus (for calculation see Eq. 2)

to their detection threshold, bees can use 3D cues to improve object detection. Bees were able to detect a target subtending a 40% smaller visual angle when it was presented in front of the structured background compared to a 2D presentation (Fig. 3).

Many entomophilous flowers compete for access to pollinators and provide various signals and cues to attract them from a distance (Gumbert et al. 1999; Kevan and Baker 1983). Several studies have shown that visual parameters such as flower size, shape, colour and brightness significantly affect distance detection by bees (Giurfa and Vorobyev 1998; Ne'eman and Kevan 2001; Spaethe et al. 2001; Streinzer et al. 2009). Our results suggest that object perception is also dependent on 3D cues of the flower. In particular, small flowers may increase their detectability by protruding from the foliage or via wind-induced movement against a textured background, which increases the amplitude of image motion for an approaching insect. Early field experiments by Wolf (1933) showed that foraging honeybees prefer flowers swaying in the wind compared to stationary flowers. Bees can also land on these moving targets accurately by correcting for angular deviations upon approach (Zhang et al. 1990). In natural environments, flowers are almost constantly in motion, usually due to the wind, and some species even show particular morphological adaptations that facilitate flower movement even when wind is minimal (Kevan and Baker 1983; Vogel 1954).

Like most other insects, bees cannot use stereoscopic vision or lens accommodation for perceiving the third dimension due to their small size and functional restrictions by the compound eye design. Instead, they use motion parallax, i.e. the apparent relative motion of a stationary object against a background, for depth perception and object-ground discrimination (Lehrer 1998). For instance, when a bee flies over a meadow, a flower that is nearer to the bee than the background will seem to move faster than the background, thus creating relative motion between it and the background. The use of self-generated motion parallax in honeybees was shown in experiments with randomly patterned black-and-white discs (Srinivasan et al. 1990). Although we did not systematically record the flight trajectories of the bees, it became obvious that they do not fly in a straight line from the maze entrance to the target but perform slight left and right swings while approaching the target which might facilitate an increase in motion parallax.

The perception of motion parallax is mediated by the green-sensitive receptors and shown to be colour blind (Zhang et al. 1995), which also applies for the visual channel used in target detection at small subtended angles (Giurfa et al. 1997). The yellow stimulus provided both chromatic and achromatic (green-sensitive receptor) contrast with respect to the background and thus sufficient

information for the motion sensitive channel. Giurfa and Vorobyev (1998) suggested for honeybees that the green-sensitive receptors of the achromatic channel might be organised as centre-surround detectors. When parallax cues are available, compared to the 2D situation, such centre-surround detectors would be probably more stimulated in the 3D situation, especially because the bee actively moving during flight in the Y-maze and thus sampling information on motion differences between target and background which would be more reduced in the 2D situation. However, electrophysiological evidence for such centre-surround detectors in bumblebees is still wanting (Paulk et al. 2008, 2009), and further studies are necessary to unravel the spatial organisation of the achromatic detectors underlying target detection as well as the possible role of chromatic contrast in 3D perception.

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