



RESEARCH ARTICLE

REVISED Honeybees (*Apis mellifera*) exhibit flexible visual search strategies for vertical targets presented at various heights [v2; ref status: indexed, <http://f1000r.es/51p>]

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Abstract

When honeybees are presented with a colour discrimination task, they tend to choose swiftly and accurately when objects are presented in the ventral part of their frontal visual field. In contrast, poor performance is observed when objects appear in the dorsal part. Here we investigate if this asymmetry is caused by fixed search patterns or if bees can increase their detection ability of objects in search scenarios when targets appear frequently or exclusively in the dorsal area of the visual field.

We trained individual honeybees to choose an orange rewarded target among blue distractors. Target and distractors were presented in the ventral visual field, the dorsal field or both. Bees presented with targets in the ventral visual field consistently had the highest search efficiency, with rapid decisions, high accuracy and direct flight paths. In contrast, search performance for dorsally located targets was inaccurate and slow at the beginning of the experimental phase, but bees increased their search performance significantly after a few foraging bouts: they found the target faster, made fewer errors and flew in a straight line towards the target. However, bees needed thrice as long to improve the search for a dorsally located target when the target's position changed randomly between the ventral and the dorsal visual field. We propose that honeybees form expectations of the location of the target's appearance and adapt their search strategy accordingly. A variety of possible mechanisms underlying this behavioural adaptation, for example spatial attention, are discussed.

Open Peer Review

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REVISED Amendments from Version 1

In accordance with the three referees' comments, we have made the following changes:

- * The title was changed as suggested by two reviewers; the term 'attention' has been removed from the title.
- * The topic 'visual attention' is now discussed more completely in the introduction.
- * More details about experimental procedure and the rationale behind the experiment were added to Material and Methods.
- * An additional analysis about error probabilities at different distances to the search screen was added. Thereby, the reference to a * 'decision line' was removed from the manuscript.
- * The location of bees during flights that terminated in an erroneous decision has been analysed.
- * A statement about the possible effects of head movements during flight was added to the discussion.

See referee reports

Introduction

When honeybees search for targets presented on a vertical plane, they show a distinct spatial asymmetry in colour and pattern learning between the ventral and the dorsal half of their frontal visual field. They easily learn a target when its defining features are perceived by the ventro-frontal area of the eye, but are less accurate when the crucial features of the target appear in the dorso-frontal area (Baumgärtner, 1928; Chittka *et al.*, 1988; Giurfa *et al.*, 1999; Lehrer, 1999; Morawetz & Spaethe, 2012; Wehner, 1972). Interestingly, this behavioural asymmetry is also found in *Drosophila melanogaster* (Sareen *et al.*, 2011) – a hint that the ventral part of the frontal eye region may play a central role in visual decision making of flying insects.

This behavioural asymmetry might in theory be explained by specialization in eye morphology. This is found in the eye of the honeybee drone, where the dorsal area is adapted to queen detection by increasing visual acuity and sensitivity, which is partly achieved by enlarged facet diameters and a reduction of the interommatidial angles (Menzel *et al.*, 1991; Seidl, 1982; Streinzer *et al.*, 2013; van Praagh *et al.*, 1980). However, such regional specialisation in eye optics is not found in the worker honeybee, where the interommatidial angles and facet diameters are similar in the frontal visual field 30° below and above the horizontal plane (Seidl, 1982).

It is also possible that regional specialisation of the visual system occurs at the neuronal level. Indeed, ascending neurons of the medulla show differences in arborisation patterns between the ventral and dorsal area (Ehmer & Gronenberg, 2002), indicating that both areas of the visual field are to some extent processed separately. Furthermore, the output of these two areas becomes segregated in the anterior optic tubercle and in the collar of the calyxes of the mushroom bodies (Ehmer & Gronenberg, 2002; Mota *et al.*, 2011). The data hint at differences in neuronal processing between these two eye regions and correspond with the behavioural evidence of a dorso-ventral differentiation (Baumgärtner, 1928; Chittka *et al.*, 1988; Giurfa *et al.*, 1999; Lehrer, 1999; Morawetz & Spaethe, 2012; Wehner, 1972).

Nevertheless, there is evidence suggesting that bees may have the ability to improve discrimination performance in the dorso-frontal area of the visual field, for example when differential conditioning is applied (Giurfa *et al.*, 1999). It is therefore possible that the dorso-ventral asymmetry might be explained by attentional mechanisms, which focus the visual processing capacity of the brain flexibly to the currently most important area in the visual field (spatial attention; Carrasco & McElree, 2001; Druker & Anderson, 2010; Geng & Behrmann, 2002; Posner, 1980; Yantis & Jonides, 1984). Attention can be thought of as a kind of 'inner' eye, focusing on a spatial subset of the information that is available from the visual sensory periphery. Attention-like processes in the context of feature learning have been considered in honeybees, bumblebees and fruit flies (Averages-Weber *et al.*, 2010; Averages-Weber *et al.*, 2015; Dyer & Chittka, 2004; Giurfa, 2004; Giurfa *et al.*, 1999; Morawetz & Spaethe, 2012; Morawetz *et al.*, 2013; Spaethe *et al.*, 2006; Van Swinderen & Greenspan, 2003; Zhang & Srinivasan, 1994). Spatial attention is well described in vertebrates, but has only recently been explored in two insect species, the fruit fly *Drosophila melanogaster* (Sareen *et al.*, 2011) and the honeybee *Apis mellifera* (Paulk *et al.*, 2014).

Spatial attention optimizes search processes in detection tasks, where the subject has an expectation of the appearance of the object, using external cueing or own experience (Posner, 1980). Search efforts can then be directed to this region which leads to faster and more accurate decisions (Carrasco & McElree, 2001). Hence, spatial attention would be a useful tool for foraging bees helping to adapt to various spatial settings of flower distribution. The dorso-ventral asymmetry observed in visual discrimination tasks could be explained by attention being focused on the ventral part of the visual field by default, but the attentional focus could be moved to other parts of the visual field, if necessary. To test if bees can overcome their dorso-ventral asymmetry in visual discrimination tasks, we confronted honeybees with three search scenarios differing in the positioning of the target in the visual field. This approach allows to analyse the changes in search performance and flight behaviour over time and therefore to identify possible adaptations of the search behaviour to the particular target presentation.

Material and methods

The experiments were conducted between July and September in 2011 on the terraces of the Biozentrum, University of Vienna, where several hives of *Apis mellifera* were located. Bees were trained to an experimental box and marked individually by applying differently coloured paint markings on the thorax. Each honeybee was trained and tested individually in the course of a single day.

Experimental setup

A wooden box (30 × 54 × 40 cm) served as experimental arena (Figure 1A; see also Morawetz & Spaethe, 2012). The bees could enter the box through a Plexiglas tube on the front and shutters in the tube allowed to control access to the box. Two video cameras with a frame rate of 25 frames/s (DCR-SR55, Sony, Minato, Tokyo, Japan) were placed above and at one side of the box to record the flights through a small-meshed net and a Plexiglas wall, respectively.

The search screen was divided into nine fields (3 rows by 3 columns) of which only the top and the bottom row were used in the

experiments. In the center of each field a platform provided reward for a correct choice (1 M sucrose solution ad libitum) and punishment for an incorrect choice (0.1% quinine solution w/w; Chittka *et al.*, 2003), respectively. Stimulus discs (K + E, Stuttgart-Feuerbach, Germany) of 9 cm diameter were cut from coloured paper; the target discs were orange (HKS 7N) and distractor discs were blue (HKS 49N, for colour details see Figure 1B, C). The stimulus discs subtended a visual angle of $>15^\circ$ on the eye of the bee when sitting at the box entrance, which enabled the bee to perceive chromatic information from the beginning of the search (Dyer *et al.*, 2008; Giurfa *et al.*, 1996). The stimuli were presented on the back wall of the arena subtending a visual field of 82° in the horizontal and 60° in the vertical on the eye of a bee (Figure 1A). Stimuli located in the top row were thus perceived by the dorsal part of the eye, while objects in the bottom row were perceived by the ventral part of the eye, when the bee entered the arena (Figure 1A).

Pre-Training

Bees were trained to enter the experimental arena and to search for the orange target on the search screen. During this phase, special care was taken in presenting the target at different heights and positions to avoid any position learning. After the first successful visit of the target, a pre-training phase of 20 foraging bouts followed. During each foraging bout, the bee was allowed to choose between the different positions until it found the target with the sucrose solution. After drinking to satiation, the bee left the arena and flew back to the colony. Only the first choice was counted (for details see ‘Data analysis’).

The position of the target was changed in a pseudo-random pattern: on average, in 50% of the foraging bouts the target was presented in

the top row, and in the other 50% in the bottom row. The target discs and feeders were exchanged with clean discs/feeders after every third foraging bout to avoid odour contamination. At the end of the pre-training, bees had learned to search for the orange target and had experienced that the target can be located either in the top or the bottom row.

Experimental phase

The pre-training phase was followed by an experimental phase, which consisted of 30 foraging bouts (five blocks, six bouts per block). Bees were divided into three experimental groups. Bees from all groups had to search for one orange target among two blue distractors, but the groups differed in the placement of the objects (see insets of Figure 3). In the ‘dorsal group’, all objects were always placed in the top row; in the ‘ventral group’, they were only presented in the bottom row. In the ‘mixed group’, target and distractors could appear in the top row as well as in the bottom row. The target position was changed in a pseudo-random order assuring that the target was presented in the top row in half of the foraging bouts and in the bottom row in the other half. The distractor positions were changed randomly between the remaining five positions of the two rows. Therefore, bees of the ‘dorsal’ and ‘ventral group’ needed to search only in a subarea of the search field (three possible positions), while bees of the ‘mixed group’ had to search the target within the entire search area (six possible positions).

These three groups allowed testing if and how bees change their search strategy over time when being confronted with targets located in ‘unfavourable’ search locations. Based on the bees’ known dorso-ventral asymmetry in visual discrimination tasks, we expected the bees to find targets in the bottom row (‘ventral group’)

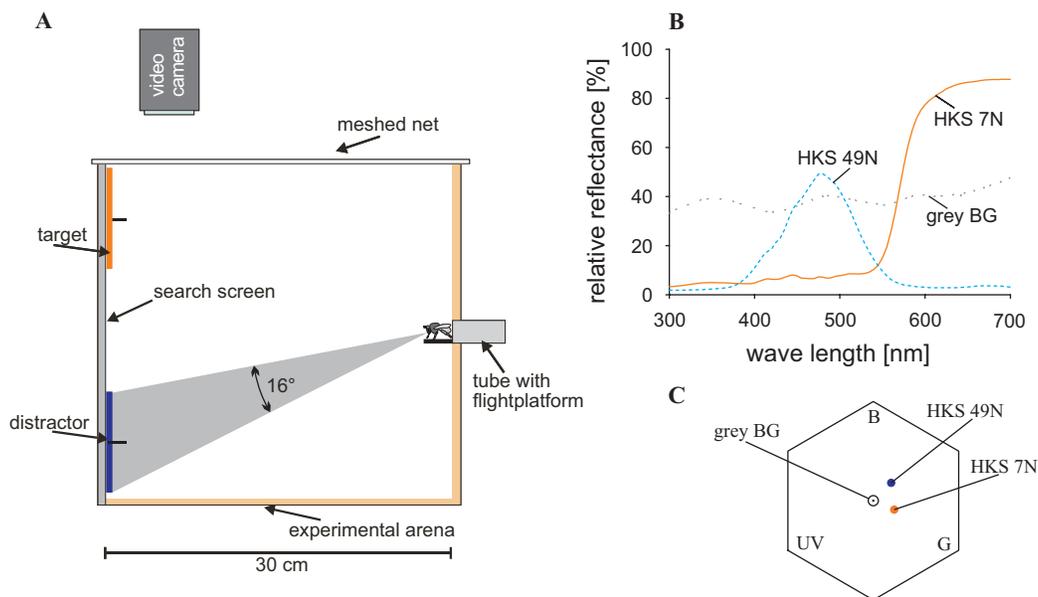


Figure 1. Experimental setup and colorimetry. **A.** Side view of the experimental arena. Bees enter the arena through a Plexiglas tube, view the objects on the search screen (back wall) and fly towards one of them. The bee’s flight could be observed and filmed through a small-meshed net from above and through a Plexiglas wall from the side. The number in the grey shaded area gives the angle of the target subtended on the bee’s eye when viewed from the entrance of the box. **B.** Reflectance curves of the search screen (grey BG - background), the orange target (HKS 7N) and the blue distractor (HKS 49N). **C.** Colour hexagon showing the colour loci of the target and the distractor (colour distance of HKS 7N to background: 0.23 hexagon units; HKS 49N to background: 0.25 hexagon units; distance between the two colours: 0.27 hexagon units); calculations after Chittka (1992).

fast and accurately, while bees might be more error-prone and take longer decision times in detecting targets in the top row ('dorsal group'; Morawetz & Spaethe, 2012; Skorupski *et al.*, 2006). The 'dorsal group' was thus confronted with objects at 'unfavourable locations' during the entire training period. To achieve a fast and targeted search comparable to that for ventrally presented targets, they would have to adapt their search strategy accordingly. Like the 'ventral group', bees of this group had the advantage that target location was predictable – here, in the dorsal part of the frontal visual field. The third group, the 'mixed group', had to search an area twice as large and were not able to predict in which area (dorsal or ventral) the target may appear.

Data analysis and statistics

Search efficiency was measured using two parameters – accuracy and decision time. This allowed to check for possible speed-accuracy trade-offs (Burns & Dyer, 2008; Chittka *et al.*, 2003). A bee's choice was counted as correct decision when she was positioned in front of the target when approaching the search screen (Morawetz & Spaethe, 2012; Spaethe *et al.*, 2006). Naturally, bees needed time to fly towards the detected target and a correct position in space is sometimes only found in the final phase of the approach. To identify the most informative distance for analysing the bees' decisions in more detail, we evaluated the error probabilities at four

different distances between bee and search screen for the first and last learning block. The accuracy of the bees' position relative to the intended target was determined at 10 cm, 7.5 cm, 5 cm and 0 cm (=landing at the target) distance from the search screen by means of video analysis. Best differentiation between the experimental groups was achieved at 5 cm distance from the search screen (Figure 2). Therefore, further detailed analyses of the search behaviour were based on the bees' position at the distance of 5 cm from the target.

For each bee and each training phase (blocks of six and three foraging bouts, respectively) the proportion of correct to incorrect choices and the median of the decision time was calculated. The decision time was defined as the time the bees needed from entering the box until they reached a distance of 5 cm to the search screen. We calculated the median of the decision time to attenuate potential effects of rare outliers.

We analysed the video recordings using The Observer XT Version 7 (Noldus, Wageningen, The Netherlands) and reconstructed the flight paths of the bees using SkillSpector 1.3.0. (Video4coach, Svendborg, Denmark). To analyse the flight directions (vertical flight angles) of the bees while crossing the arena, we measured the vertical position of the bee at different distances to the search

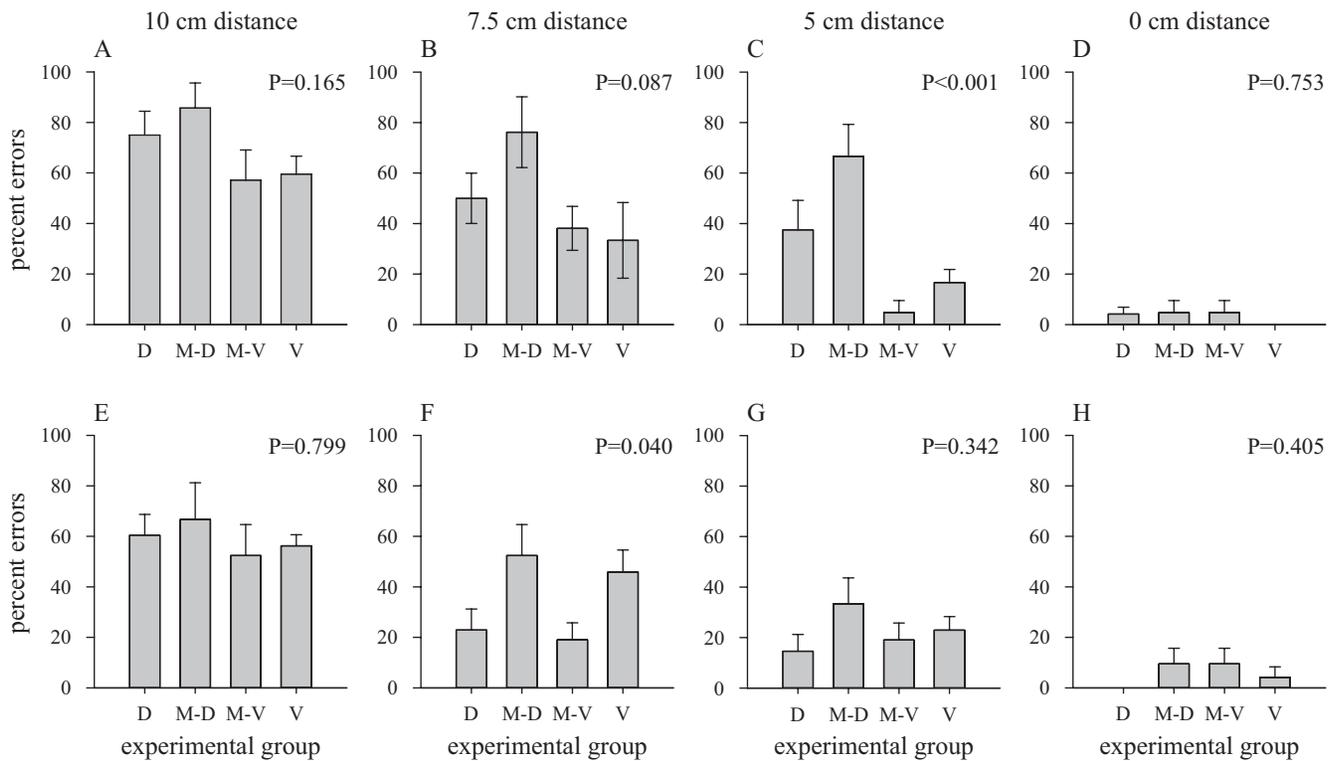


Figure 2. Decision accuracy at different distances to the search screen. The distance of the bee to the search screen has an effect on the error probability in all groups (D: 'dorsal group', M-D: flights of the 'mixed group' with targets located in the dorsal row, M-V: flights of the 'mixed group' with targets located in the ventral row, V: 'ventral group') during the first six foraging bouts (A–D) and during the last six bouts (E–H). The data show error percentages for four different distance to the search screen: 10 cm (A, E), 7.5 cm (B, F), 5 cm (C, G) and 0 cm (D, H). P values give the results of ANOVA comparisons between all four depicted groups. Bars: mean \pm s.e.m., N=8 bees for the 'dorsal group', N=7 for the other groups (videos for the lateral-view camera were incomplete for some of the 9 tested individuals).

screen and calculated the angle between the flight direction and the horizontal.

Statistics were calculated using the statistical package R version 2.14.1 (R Development Core Team, 2011). We used general linear models to test the effect of treatment group, target location and learning block on accuracy and decision time, respectively. As the behaviour of each individual bee was measured several times during experimental course (repeated measurement design), the identity of the individual bees was embedded into the model as random effect. For analysing the error data, a binomial distribution was applied using the function *lmer* of the package 'lme4' (Bates *et al.*, 2011), while for the decision time, a normal distribution was applied using the same function. Significance levels were determined by calculating a type-III ANOVA using the function *Anova* (package 'car', Fox & Weisberg, 2011). To check if the bees showed a general tendency to fly upwards or downwards during the different flight phases, the flight angles were tested against 0° (horizontal flight) with a Wilcoxon test. Figures were created using Sigma Plot 11.0 (Systat Software Inc., San Jose, USA) and Corel Draw X3 (Corel Corporation, Ottawa, Canada).

Results

Interactions of choice accuracy and distance from screen

As one might predict, bees had higher accuracy when their positions were analysed at a distance closer to the search screen ($\chi^2_{(3)}=29.00$, $P<0.001$; Figure 2A–D), likely due to the fact that they had more time to detect the target and align their flight accordingly. Bees of all groups made about 80% errors during their first six foraging bouts at 10 cm distance from the search screen, but they made nearly no mistakes when landings were counted as decisions (Figure 2A–D). During the last six foraging bouts, a similar pattern was observed ($\chi^2_{(3)}=32.13$, $P<0.001$; Figure 2E–H), although the percentage of errors dropped for most analyzed distances compared to the first bouts. Furthermore, the error probability close to 0% for landings

(0 cm distance) suggests that the difficulty of the task was not the discrimination between target and distractor but the detection of the target before reaching the back wall of the experimental box (Figure 2D, H).

Interestingly, a significant difference between the different search conditions (dorsal, mixed-dorsal, mixed-ventral, ventral) was only evident at 5 cm distance during the first six foraging bouts ($F_{(3,25)}=7.96$, $P<0.001$; Figure 2C) and at 7.5 cm distance during the last six bouts ($F_{(3,26)}=3.19$, $P=0.040$; Figure 2F). We thus performed the detailed analysis for the bees' position at 5 cm distance because the strongest effect between an easy task and a difficult task at the beginning of the experiment is most evident at this distance. However, the critical distance for analysis may shift due to the bees' experience, since after extensive training bees may detect the target earlier during flight and problems with target detection will appear earlier during the approach (compare Figure 2C, G).

Decision accuracy at 5 cm distance

During the pre-training phase, when only the target was present, bees of all treatment groups showed similar accuracy ($\chi^2_{(2)}=1.37$, $P=0.502$). At the end of pre-training they had learned to approach the target in the bottom row with high accuracy (error percentage: $15\% \pm 25\%$ s.d., t-test against change level of 83%: $t_{(25)}=-22.02$, $P<0.001$). However, they still failed to choose the target above chance level when it was located in the top row (error percentage: $65\% \pm 33\%$; $t_{(25)}=-1.46$, $P=0.157$). Hence, as predicted, bees displayed a dorso-ventral search asymmetry during pre-training, and found targets located in the bottom row four times more accurately than targets located in the top row.

At the end of the experimental period, bees of all groups were able to detect the target at all presented locations above chance level (Figure 3; 'ventral group': $t_{(8)}=-12.02$, $P<0.001$; 'dorsal group': $t_{(8)}=-11.64$, $P<0.001$; 'mixed group' – ventral target: $t_{(8)}=-9.39$,

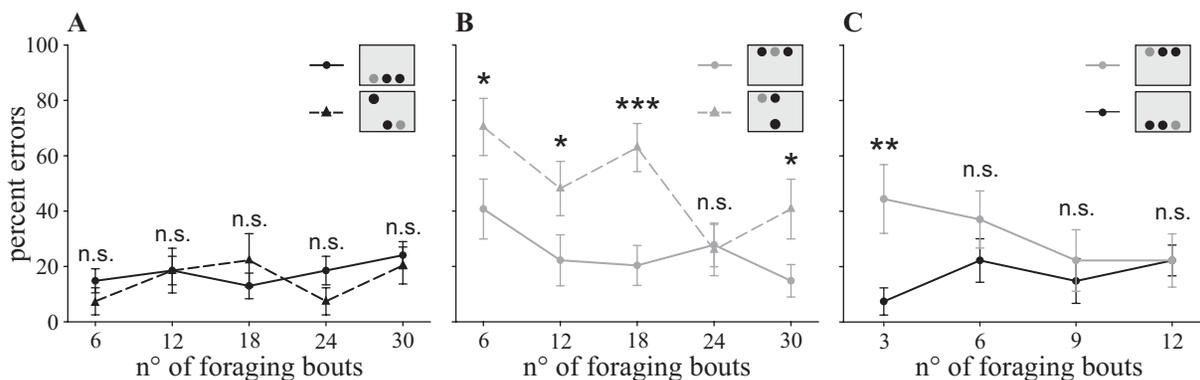


Figure 3. Search accuracy of honeybees depending on treatments. Error percentage at 5 cm distance to the search screen is shown for all three treatment groups. Treatments differed in the positioning of the rewarding target and distractors which were associated with a punishment (insets: grey circle = target, black circle = distractor). In the 'dorsal group' (straight grey line, grey circle) the target was always presented in the top row, in the 'ventral group' (straight black line, black circle) it was always located in the bottom row, and in the 'mixed group' the position of the target alternated randomly between both rows (top row: dashed grey line, grey triangle; bottom row: dashed black line, black triangle). **A, B.** Comparison between the one row condition and the two row condition separated in foraging bouts where the target appears in the bottom row (**A**) and bouts with the target in the top row (**B**). **C.** Comparison of the first 12 foraging bouts of the 'dorsal group' and the 'ventral group'. Statistics: general linear model with binomial distribution: n.s. $P>0.10$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$; data points: mean \pm s.e.m., $N=9$ individuals per group.

$P < 0.001$; 'mixed group' – dorsal target: $t_{(8)} = -3.91$, $P = 0.004$; see also [dataset 2](#)). However, the speed of improvement differed significantly between the three groups (interaction treatment group \times learning block: $\chi^2_{(8)} = 19.58$, $P = 0.012$).

Bees of the group presented with targets in the ventral visual field showed a constant performance during the experimental phase with a mean error percentage of $17\% \pm 14$ s.d. ($\chi^2_{(4)} = 2.72$, $P = 0.606$; [Figure 3A](#), straight line). In contrast, when bees were presented with targets in the dorsal visual field, they made about twice as many mistakes at the beginning of the experimental phase than at the end ($\chi^2_{(4)} = 12.84$, $P = 0.012$; [Figure 3B](#), straight line). The main change in learning performance in the 'dorsal group' took place between the first and second learning block; thus we analysed the first 12 foraging bouts in more detail, subdividing them into blocks of three ([Figure 3C](#)). During the first three bouts, bees of the 'dorsal group' made four times more mistakes than bees of the 'ventral group' ($z = 2.77$, $P = 0.006$; [Figure 3C](#)). After this initial phase, both groups achieved similar levels of accuracy (all further learning blocks: $P > 0.05$; [Figure 3C](#)).

The 'mixed group' was the only group in which the target and distractors were presented in both the ventral and the dorsal part of the visual field. Similarly to the 'dorsal group', bees improved their accuracy during the experimental phase ($\chi^2_{(4)} = 9.54$, $P = 0.049$). Bees of the 'mixed group' made fewer mistakes when the target was presented in the ventral row compared to the situation when it was presented in the dorsal row ($\chi^2_{(1)} = 5.27$, $P = 0.022$; [Figure 3A, B](#) dashed lines). Next, we analysed both data sets separately and compared them to the correspondent single row treatment group ([Figure 3A](#): flights of the 'mixed group' when the target was ventrally presented compared to the 'ventral group'; [Figure 3B](#): flights of the 'mixed

group' when target was dorsally presented compared to the 'dorsal group'). The performance of bees from the 'mixed group', when searching for a ventral target, was similar to the performance of the 'ventral group' where the target was always presented ventrally ($\chi^2_{(1)} = 0.35$, $P = 0.556$; [Figure 3A](#)). Furthermore, bees of the 'mixed group', when searching for ventral targets, showed a constant performance during the experimental phase ($\chi^2_{(4)} = 3.90$, $P = 0.420$). However, they made significantly more mistakes than bees of the 'dorsal group' in finding a dorsally positioned target ($\chi^2_{(1)} = 7.69$, $P = 0.006$; [Figure 3B](#)). Nonetheless, the 'mixed group' improved their performance for dorsal targets ($\chi^2_{(4)} = 14.05$, $P = 0.007$), and in the fourth learning block, bees managed to achieve a similarly low error percentage as the bees of the 'dorsal group' ([Figure 3B](#)).

Decision time (time between start of search and reaching 5 cm distance to target)

All three experimental groups became faster during the experiment ($\chi^2_{(4)} = 16.83$, $P = 0.002$; [Figure 4](#)), but the decision time did not differ among treatment groups ($\chi^2_{(2)} = 1.04$, $P = 0.595$) or in the interaction of both factors ($\chi^2_{(8)} = 10.00$, $P = 0.265$). However, bees of the 'mixed group' were slower when the target was presented in the top row than when it was presented in the bottom row ($\chi^2_{(1)} = 28.6$, $P < 0.001$). Bees of the 'dorsal' and 'ventral group', on the other hand, showed similar decision times during most of the time ($\chi^2_{(1)} = 0.24$, $P = 0.624$). However, during the first foraging bouts flights, bees of the 'dorsal group' were significantly slower than bees of the 'ventral group' ($t_{(13,1)} = 2.21$, $P = 0.045$; [Figure 4C](#)).

Flight path

When analysing the flight structure of the first foraging bouts in detail, it became evident that bees of the 'dorsal group' increased the steepness of their departure angle (flight angle between 0 and

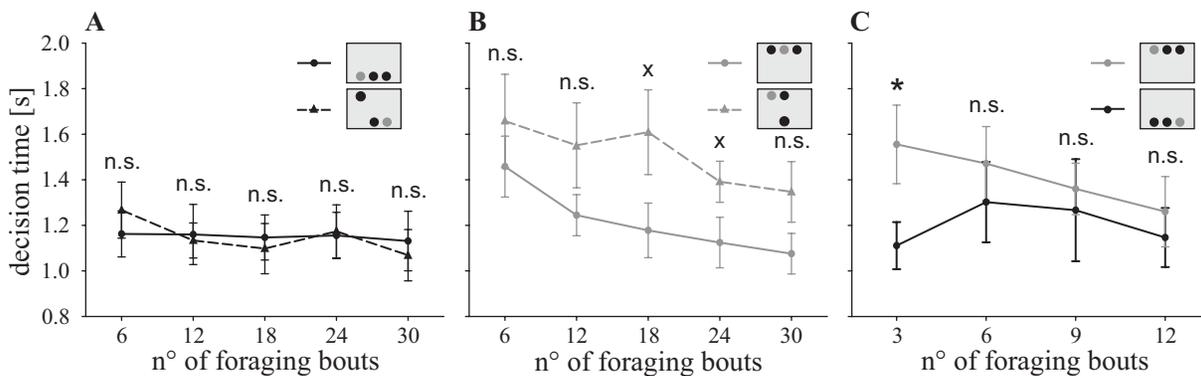


Figure 4. Decision time of honeybees depending on treatments. Decision time of the three experimental groups: 'ventral group' (black straight line, black circle), 'dorsal group' (grey straight line, grey circle) and 'mixed group' (ventral half: black dashed line, black triangle; dorsal half: grey dashed line, grey triangle). **A.** When searching for the target in the ventral half of the visual field, the bees of the 'mixed group' made their decision at a similar speed as the 'ventral group' ($\chi^2_{(1)} = 0.00$, $P = 0.979$). They did not improve their performance ($\chi^2_{(4)} = 2.25$, $P = 0.689$). **B.** Comparing the 'dorsal group' with the dorsal bouts of the 'mixed group', the bees showed no significant difference in their decision time ($\chi^2_{(1)} = 3.02$, $P = 0.082$). All bees became faster in the course of the experiment ($\chi^2_{(4)} = 22.84$, $P = 0.001$). **C.** Comparison of the 'dorsal' and the 'ventral group'. Only during the first three foraging bouts the 'dorsal group' was significantly slower than the 'ventral group' ($t_{(13,10)} = 2.211$, $P = 0.045$), afterwards bees of both groups had similar decision times (all other data points $P > 0.05$). Statistics: t test: n.s. $P > 0.10$, x $P < 0.10$, * $P < 0.05$; data points: mean \pm s.e.m., $N = 9$ per group.

5 cm distance from the entrance) significantly from the first to the third foraging bout (Figure 5; Wilcoxon test between first and third bout: $P < 0.05$). During their first bout, they started horizontally into the box (median flight angle 5.9° , Figure 5), but from the third bout on they flew upwards when taking off (median flight angle third flight: 12.1° upwards; fifth flight: 10.0° upwards). This change in flight structure indicates that the bees decided earlier to fly upwards in their third foraging bout compared to their first bout. All other experimental groups showed median departure angles between 7° downwards and 5° upwards – no significant trend in flying up- or downwards during the departure was found.

When the target was located in the bottom tier, the bees' flight was composed of downward angles during most of their flight path, suggesting a continuous downward flight (Figure 5, Figure 6). Bees searching for a dorsally located target, however, differed in

their flight structure between treatment groups: bees of the 'mixed group' flew in a steep angle downwards during the first flight period (maximum flight angle 40° downwards) and showed a significant upwards direction between 20 and 25 cm distance from the entrance (median flight angle between 33° and 58° upwards, Figure 5). During the fifth foraging bout of the experimental phase with the target in the top row, these bees lost their tendency to fly downwards at the beginning of their flight and flew straight forward for the first 20 cm and ascended steeply afterwards. Bees of the 'dorsal group' flew horizontally between the first 5 to 20 cm, with some animals already beginning to ascend. Between 20 and 25 cm they ascended in a steep angle of around 25° similar to the animals of the 'mixed group'. Therefore, all bees searching for a top row target approached the target from below. Flight paths of individual bees demonstrate the described differences of the three different groups (Figure 6).

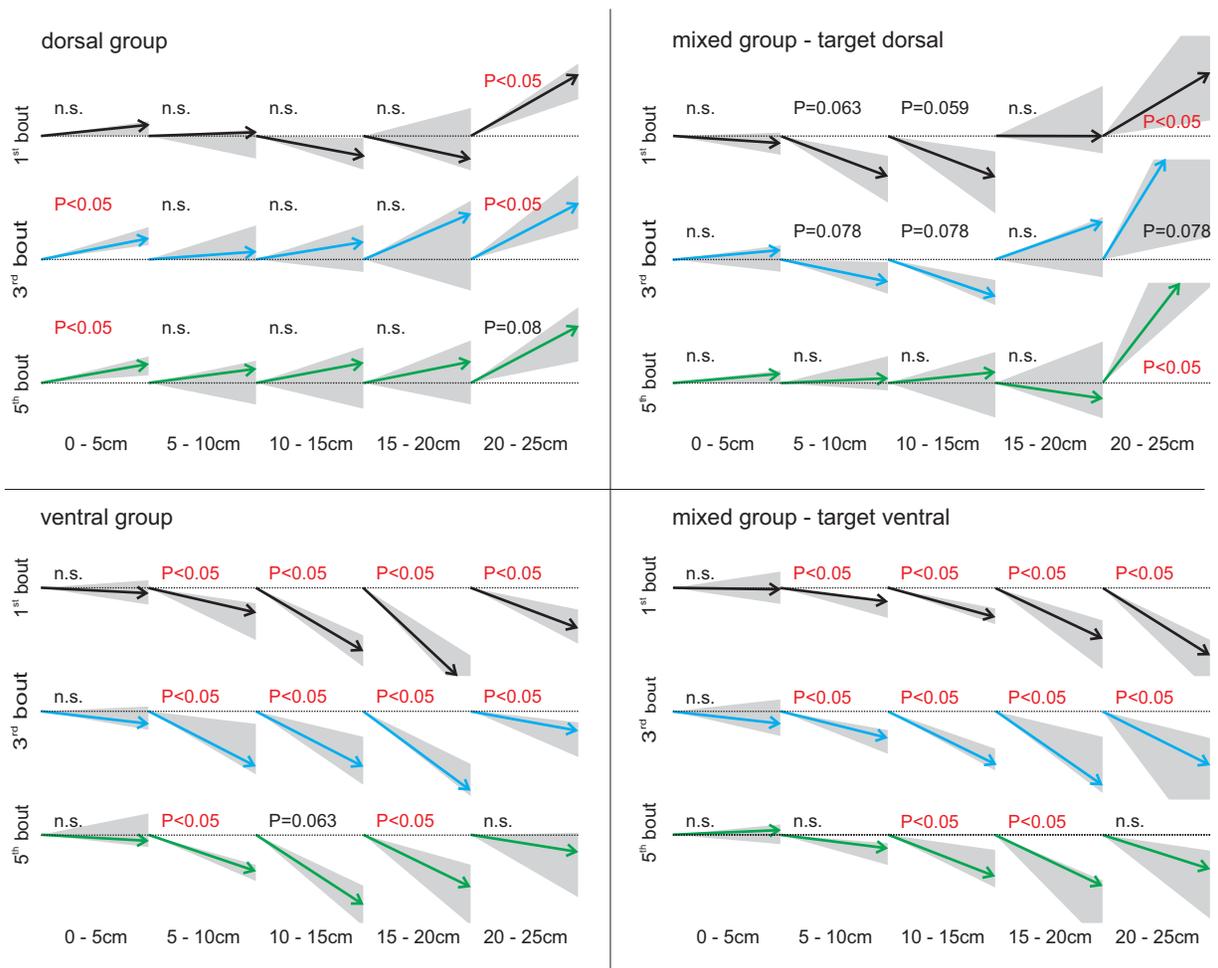


Figure 5. Flight structure of the three treatment groups. Median flight angles of different phases in the bee's flights crossing the experimental arena from the entrance (0 cm) to 25cm from the entrance (i.e. 5cm from the targets). For all four search situations the first (black arrows), the third (blue arrows) and the fifth foraging bout (green arrows) are shown (N=7 – videos for the lateral-view camera were incomplete for some of the 9 tested individuals). In the mixed group the flight number refers to number of foraging bouts absolved with the particular target location (top row or bottom row) and not to the total number of bouts the bee has absolved (all foraging bouts regardless of the target location). Arrows: median flight angles; grey area: first to third quartile; statistics: one-sample Wilcoxon test against 0° (flight without significant trend of changing flight height).

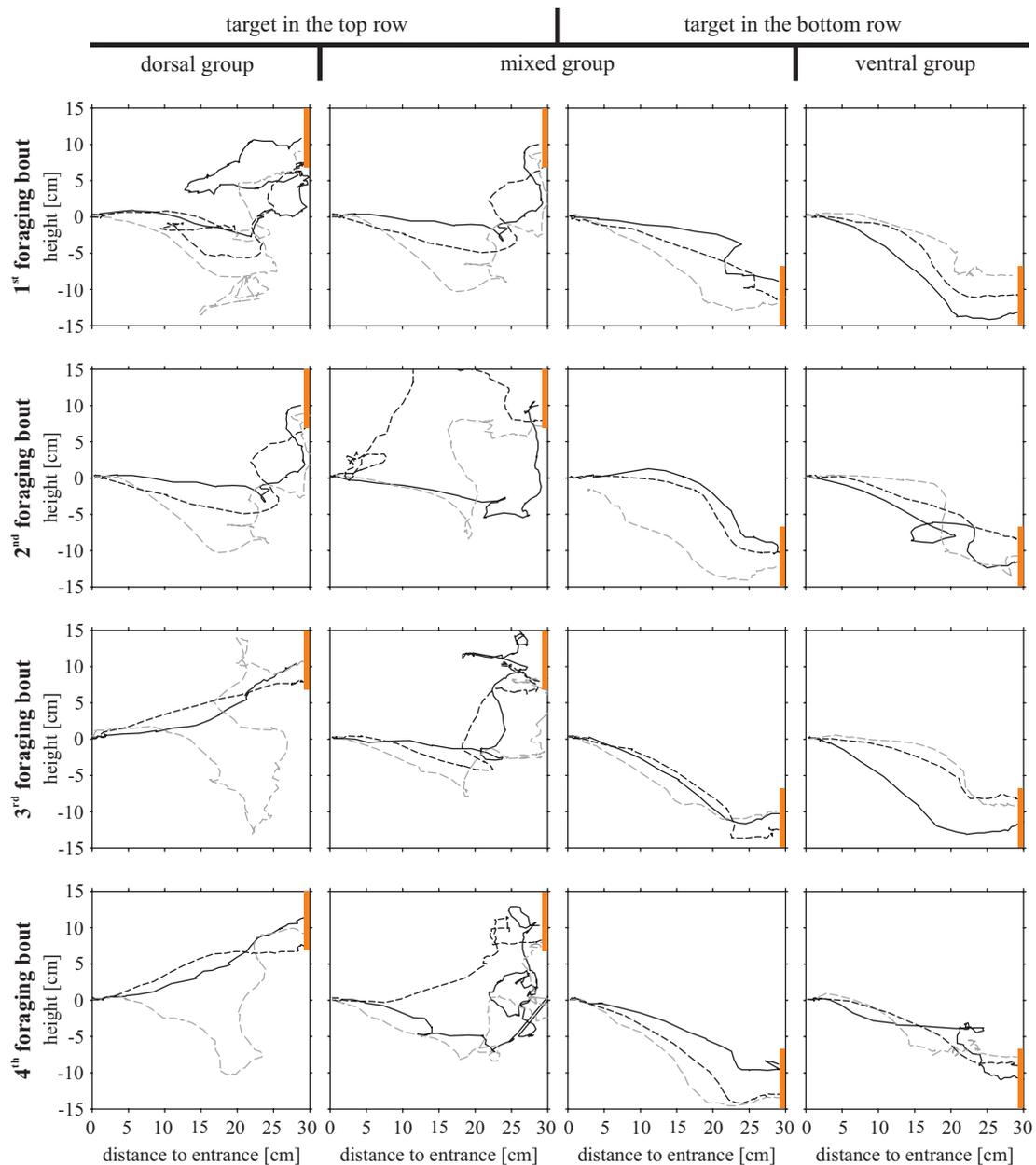


Figure 6. Flight paths of selected bees. The flight paths describe the differences in flight behaviour among the three experimental groups. The 16 squares represent cross sections of the flight box with the bee entering the box from the left side searching for the target (presented on the right side; orange bar). For each experimental group three individual bees are shown (straight black line, dashed black line, dashed grey line).

Errors in the vertical and horizontal dimension

The exploration of the flight path of the bees when making a mistake further facilitates understanding the possible reasons for erroneous choices. During most erroneous choices, bees of the 'dorsal' and 'ventral' group were at the same height as the target at 5 cm distance ('dorsal group': $\chi^2_{(2)}=18.84$, $P<0.001$; 'ventral group': $\chi^2_{(2)}=18.43$, $P<0.001$; [Figure 7](#)) – i.e. bees of the 'dorsal group' were at the height of the top row and bees of the 'ventral' group were at the height of the

bottom row. However, they were positioned at the incorrect horizontal location. In both groups, bees made most mistakes by approaching the target row in the central position ('dorsal group': 51% of all mistakes occurred in the centre of the top row; 'ventral group' 42% of all errors occurred in the centre of the top row; see also [Dataset 5](#)). This suggests that a high percentage of errors originated from situations where bees were heading for the correct tier of stimuli, but had not yet decided which of the objects to approach.

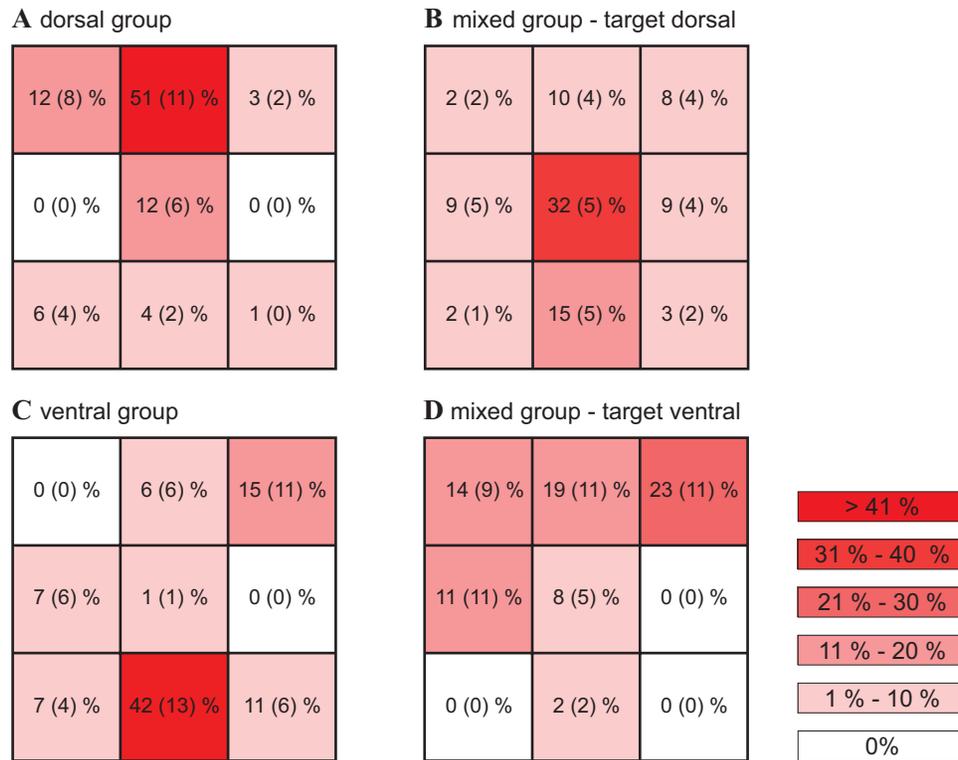


Figure 7. Positions of bees when making errors. A decision error occurs when the bee steers towards a distractor or an empty position (here shown for a distance of 5 cm from the search screen). (A–D) Error frequency for the different locations (3 rows x 3 columns) for all experimental groups (colour code see explanation on right). The numbers give the mean percentages (+/- s.e.m.) of false decisions at the respective position. N=9 bees per group.

In the mixed group, the positions of bees when making errors were more widely distributed. Bees presented with a dorsal target, when making errors, were positioned mostly in the middle row, where no object was located ($\chi^2_{(2)}=13.72$, $P=0.001$; Figure 7). Again, bees made most errors by approaching a tier of stimuli at a central position (32% of all errors at the central position of the middle row; 15% of the errors at the central position of the bottom row, where chance expectation would be 11%). When presented with a ventrally located target, bees of the mixed groups made somewhat more errors in the top row, though this was not quite significant ($\chi^2_{(2)}=5.84$, $P=0.054$; Figure 7).

In conclusion, the accuracy, decision time and flight path of bees searching for a vertically presented target was partly dependent on the target's location and partly on the bees' possibility to predict the target's location. Searching for ventrally located targets was an easy task – all bees showed high accuracy, short decision times and a direct flight path. The predictability became important in situations in which bees had to detect the target with the dorsal part of their eyes. When the target was always in the top row ('dorsal group'), bees adapted their search strategy rapidly to the situation – after three foraging bouts they detected the target swiftly, decided accurately and approached the target directly. When the target was positioned randomly in the top or the bottom row ('mixed group'), the bees

showed a more downward directed flight pattern when being confronted with dorsally located targets. As a consequence, these bees took longer in making their decisions and made more mistakes.

Datasets for strategies of *Apis mellifera* during visual search for vertical targets

1 File (5 datasets)

<http://dx.doi.org/10.6084/m9.figshare.1297290>

Discussion

We simulated three foraging situations that a bee might encounter in its natural environment: approaching flowers from above (flowers perceived by the ventral part of the eye), foraging within a 3D floral environment, either in a tree or a meadow with flowers at various heights (flowers may appear in the dorsal and ventral part of the visual field) and approaching a flower from below, for example, flying upwards on vertical inflorescences (flowers perceived by the dorsal area of the eye). We demonstrated that bees were able to adapt their detection strategy to all three foraging situations, although the course of performance improvement differs among the experimental conditions. In the following paragraphs, we discuss the possible proximate and ultimate mechanisms underlying this behavioural flexibility.

‘Ventral group’

Bees of this group were presented with one target and two distractors in the bottom row. They decided accurately and swiftly and had a downwards directed flight vector. It is therefore possible that bees, by default, focus on the ventral part of the frontal visual field when being confronted with vertically presented objects, a behaviour which might explain the dorso-ventral asymmetries in visual discrimination observed by several independent studies (Baumgärtner, 1928; Chittka *et al.*, 1988; Giurfa *et al.*, 1999; Lehrer, 1999; Morawetz & Spaethe, 2012; Wehner, 1972).

‘Dorsal group’

In the ‘dorsal group’, both target and distractors were presented in the top row. During the initial foraging bouts, bees’ search was inaccurate and slow compared to the ‘ventral group’. The bees flew straight ahead for the first 20 cm in the experimental arena and then turned upwards in a steep angle during the final approach. After the first three bouts, bees showed two major changes in their behaviour: (1) the error percentage dropped from 40 to 20% (Figure 3C) and (2) they changed their departure angle from horizontal to, on average, 11° upwards (Figure 5), a behaviour which was exclusively shown by the ‘dorsal group’.

The ultimate reason for the bees’ improvement may be explained by the ability to build up expectations about the future location of the target in space and to adapt their search strategy accordingly. This would mean that bees had learned that it is most likely that the target appears in an ‘unfavourable’ location. Two types of proximate mechanisms are possible which might enable the improvement of target detection with experience. Firstly, a top-down attentional mechanism could have accelerated processing of information from the dorsal part of the frontal visual field by shifting the attentional focus to this area. In this case, bees might have detected the target stimulus just after entering the experimental arena and started an upward flight towards the top row. Alternatively, bees could have learned to adjust their flight path to the area where they expected the target: they learned to initiate an upward flight immediately after entering the arena before detecting the target. By flying upwards they were able to analyse the top row with the ventral part of their eye and therefore increase their accuracy. Evidence for both mechanisms comes from earlier studies: bees can learn to perform directional changes and changing flight routes to optimize foraging success (Cheng & Wignall, 2006; Lihoreau *et al.*, 2010; Perry & Barron, 2013), as well as use attentional processes to selectively react to visual stimuli (Giurfa *et al.*, 1999; Paulk *et al.*, 2014; Spaethe *et al.*, 2006). However, our results do not allow discriminating between both mechanisms.

In addition, it is also theoretically possible that bees may learn to lift their head towards the target by changing their head angle and therefore perceive the top row with the ventral parts of their eyes. Unfortunately, our video resolution is not sufficient to allow measurements of head angle in relation to body axis. Further studies using high resolution cameras are necessary to investigate if bees can move their heads upwards while approaching dorsally positioned targets (Boeddeker *et al.*, 2010, Riabinina *et al.*, 2014).

‘Mixed group’

Bees of the mixed group were confronted with one target and two distractors, which were randomly distributed between the bottom and top row. During most foraging bouts, objects were located in both the bottom and the top row. Hence, the search strategy of the ‘mixed group’ was fundamentally different from the two other groups. Bees were not simply able to fly towards a group of objects, but had to decide which group of objects was relevant for them, i.e. contained the target. At the beginning of the experiment, bees flew downwards regardless of the target position – probably executing their ‘default’ behavioural pattern and flying towards the lowermost objects. This behaviour resulted in a high accuracy when the target showed up in the bottom row, but in a low accuracy and long decision time when the target appeared in the top row. Furthermore, our data indicate that bees detected ventrally located stimuli first, but analysed the chromatic features of the stimuli (which are necessary for discriminating between target and distractors) only later during flight.

Bees of the mixed group had to change their flight pattern to improve the search for targets located in the top row. A downwards orientated flight route can hamper the detection of a dorsally located target due to an unfavourable angle of view for objects in the top row. Additionally, it makes complex course corrections necessary (demonstrated by the flight curves in Figure 6). Indeed, bees of the ‘mixed group’ stopped flying downwards in the first part of their flight after their tenth foraging bout (Figure 5) and instead flew horizontally before deciding for an upwards or downwards movement.

Additionally, bees of the mixed group may have used visual attention to increase the detection performance of targets located in the top row. For example, bees could have increased the area of their attentional focus to a size large enough to cover both rows. This would have allowed to process all presented objects simultaneously (Eriksen & St. James, 1986; Pashler, 1987). In humans, an increase in the size of processing focus is normally accompanied by a decrease of processing quality (Castiello & Umiltà, 1990; Eriksen & St. James, 1986). We did not find a decrease of performance level in the search for ventrally located target in the ‘mixed group’, which makes this explanation unlikely. An alternative search mechanism might be that bees learn to move their attentional focus from the ventral part of the visual field to the dorsal part after they had not detected a target in the ventral part. This explanation fits with the constant high search performance for targets in the bottom row and with longer decision times and late upward directed flight vectors, when searching for a target in the top row. The low accuracy in the first part of the experiment probably owes to the learning process, in which the bees (1) learned to avoid flying downwards immediately after entering the arena and (2) learned to continue searching the top row when the target could not be detected in the bottom row.

Ecological relevance

When searching for a flower patch, bees often approach the meadow or tree from above (Kevan, 1990; Lehrer, 1999) – a situation represented by the conditions of the ‘ventral group’. Bees seem especially capable to deal with this type of search, as they detect ventrally

positioned targets more easily (Figure 3A; Lehrer, 1999; Morawetz & Spaethe, 2012; Skorupski *et al.*, 2006). However, when bees are foraging in a meadow with flowers at various heights, flowers can also appear in the upper part of the visual field and information received from the dorsal part of the eyes become important. For example, bees which visit raceme inflorescences tend to begin collecting nectar at the bottom of the inflorescence and ascend vertically step by step (Fisogni *et al.*, 2011; Ishii *et al.*, 2008; Pyke, 1978; Valtueña *et al.*, 2013; Waddington & Heinrich, 1979). In this situation the flowers below the just probed ones, which are perceived by the ventral visual field, are typically empty, because the bee had just visited them (Heinrich, 1975; Ishii *et al.*, 2008). To optimize foraging efficiency, bees must focus visual processing on the dorsal part of the visual field or adopt an upward directed motor pattern. We demonstrated that bees can adapt to this situation, improving their detection ability of dorsally located targets within three visits only (Figure 3C).

When foraging in a blooming tree, a bee can expect a rewarding flower appearing in the ventral and the dorsal visual field with a similar probability. Flowers are densely distributed in a tree and bees normally see several flowers simultaneously. Interestingly, large bees like *Bombus* and *Xylocopa* reveal a complex pattern of upwards and downwards movements inside a tree: when moving between neighbouring flowers, they move upwards, similar to the movement pattern on a vertical inflorescence (Kevan, 1990). However, when moving to an inflorescence at distance larger than 20 cm they tended to fly downwards (Kevan, 1990). This behaviour matches our observation that bees tend to fly to the lowermost object when approaching from a distance. However, we also demonstrated that bees can overcome this motor pattern within 10 learning trials, when it hinders the bees' foraging success. Likewise, Kevan (1990) described that movement patterns of bees differed between tree species with different flower distributions and suggested that bees optimize their foraging strategy to the particular resource distribution.

In conclusion, we showed that bees flexibly adapt to a given foraging situation by focusing their detection and discrimination effort to the

appearance of the object within their visual field. They probably use both attentional mechanisms and behavioural strategies to optimize their foraging success, although more data are needed to clearly separate between these mechanisms. This flexibility provides the ability to choose among different search strategies and to quickly adapt to various foraging environments.

Data availability

figshare: New datasets for strategies of *Apis mellifera* during visual search for vertical targets, doi: <http://dx.doi.org/10.6084/m9.figshare.1297290> (Morawetz *et al.*, 2015).

Author contributions

LM and JS conceived the study and designed the experiments. LM carried out the research and performed the data analysis. LM, LC and JS were involved in data interpretation and writing the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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Version 2

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Martin Egelhaaf

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The revised article answers most of my remarks and questions in a satisfactory way. It presents highly interesting results and deserves a broad readership.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 13 February 2015

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Martin Giurfa

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The new version answers satisfactorily my previous comments and remarks.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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This paper analyzes visual target recognition in bees and focuses on previously reported asymmetries between the dorsal and the ventral visual fields in terms of color/pattern recognition and discrimination. The authors studied whether a pattern detection/discrimination task varies when patterns are displayed either in the ventral or in the dorsal visual fields and discuss different possible mechanisms accounting for this variation. Specifically, bees were trained to choose a rewarded orange target which was presented among blue distracters either in the ventral visual field ('ventral' group' of bees), the dorsal field ('dorsal' group') or in both fields ('mixed group'), and choice errors, decision time, and flight pathways upon entering into the experimental arena were recorded.

The authors show in a **convincing way** that bees presented with targets in the ventral visual field were most efficient in detecting the rewarded target as they made rapid decisions with high accuracy and direct flight paths. In contrast, bees perceiving the patterns in the dorsal visual field made more errors and took more time to detect the pattern. Yet these 'dorsal bees' improved significantly their performance after a few learning trials as they decreased both the number of errors and decision time and flew in a straight line towards the target. Bees of the 'mixed group' needed considerably more time to improve the search of the target whose position changed randomly between the ventral and the dorsal visual field.

The conclusions drawn by the authors focus on the notion of expectation as it is said that "bees form expectations of the location of the target's appearance and adapt their search strategy accordingly". Furthermore, the authors insist on the notion of attention (more specifically, spatial attention), which is certainly attractive in the light of the results obtained, but as the authors themselves acknowledge, ***is probably not the unique explanation for their data***.

As a consequence, a more suitable title for the paper, given the results presented, would be something like: "*Honey bees exhibit flexible (plastic) visual search strategies for targets presented vertically at various heights*". It seems to me that the main finding of the paper is precisely the flexibility and adaptability of the bees' visual search strategies to the various experimental situations rather than the proof of attentional processes guiding these strategies.

A **main concern** raised by this paper relates to the criteria (unclear to me) used to define the position of the so-called "**decision line**" in the setup. Clearly such a line was very close (5 cm) to the targets so that decision counting may have under/over estimated real decisions performed (and perhaps) modified at further distances from the targets. It would be therefore interesting to contrast the decisions counted and established following the authors' method with a different one, which, for instance, may be based on quantifying angular deviations of trajectories along the flight path; one can establish a criterion for a decision based on an angular deviation from the entrance vector and quantify decision which may occur before reaching the decision line defined by the authors.

A **minor concern** relates to incomplete citations in the paper. In particular, given that the focus is on ventral/dorsal asymmetry of visual performances on bees, it would be worth considering and mentioning the work on ventral vs. dorsal target detection in bees (Giurfa *et al.*, 1999). Additionally, the statement on the relative recency of studies on attention in insects is questionable. Although it is not explicitly said - probably because of constraints imposed by reviewers - the work by van Swinderen and Greenspan (2003) constitutes a study on visual attention. Also, if one focuses on bees, the use of this concept of attention dates to 2004 (Giurfa, 2004) when it was argued that discrimination between perceptually closer

stimuli was possible after differential conditioning but not after absolute conditioning due to differences in attention inculcated by these two training procedures. The idea was further developed in later papers, so that referring the use of this concept in insects to 2011 seems a bit unfair to prior contributions which also focused on the same idea.

All in all, the manuscript is sound and well-written. The results are quite convincing (but see remark on the appropriateness of the decision line criterion) and constitute an important contribution to field of insect vision.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response (Member of the F1000 Faculty) 13 Feb 2015

Lars Chittka, School of Biological and Chemical Sciences, Queen Mary, University of London, UK

- *The conclusions drawn by the authors focus on the notion of expectation as it is said that "bees form expectations of the location of the target's appearance and adapt their search strategy accordingly". Furthermore, the authors insist on the notion of attention (more specifically, spatial attention), which is certainly attractive in the light of the results obtained, but as the authors themselves acknowledge, **is probably not the unique explanation for their data.***

As a consequence, a more suitable title for the paper, given the results presented, would be something like: "Honey bees exhibit flexible (plastic) visual search strategies for targets presented vertically at various heights". It seems to me that the main finding of the paper is precisely the flexibility and adaptability of the bees' visual search strategies to the various experimental situations rather than the proof of attentional processes guiding these strategies.

We thank the referee for the title suggestion; the title now reads ' Honeybees (*Apis mellifera*) exhibit flexible visual search strategies for vertical targets presented at various heights'

- *A **main concern** raised by this paper relates to the criteria (unclear to me) used to define the position of the so-called "**decision line**" in the setup. Clearly such a line was very close (5 cm) to the targets so that decision counting may have under/over estimated real decisions performed (and perhaps) modified at further distances from the targets. It would be therefore interesting to contrast the decisions counted and established following the authors' method with a different one, which, for instance, may be based on quantifying angular deviations of trajectories along the flight path; one can establish a criterion for a decision based on an angular deviation from the entrance vector and quantify decision which may occur before reaching the decision line defined by the authors.*

This mirrors the other referees' concern and we have now added a much more detailed analysis at various distances from the target screen.

- *A **minor concern** relates to incomplete citations in the paper. In particular, given that the focus is on ventral/dorsal asymmetry of visual performances on bees, it would be worth*

considering and mentioning the work on ventral vs. dorsal target detection in bees (Giurfa et al., 1999) . Additionally, the statement on the relative recency of studies on attention in insects is questionable. Although it is not explicitly said - probably because of constraints imposed by reviewers - the work by van Swinderen and Greenspan (2003) constitutes a study on visual attention. Also, if one focuses on bees, the use of this concept of attention dates to 2004 (Giurfa, 2004) when it was argued that discrimination between perceptually closer stimuli was possible after differential conditioning but not after absolute conditioning due to differences in attention inculcated by these two training procedures. The idea was further developed in later papers, so that referring the use of this concept in insects to 2011 seems a bit unfair to prior contributions which also focused on the same idea.

We have now added these references as suggested.

Competing Interests: No competing interests were disclosed.

Referee Report 27 August 2014

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Martin Egelhaaf

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General remarks

The amount of information that is perceived by the peripheral visual system is thought to exceed the amount of information that can be processed by subsequent stages of the brain. Based on a host of experimental data mainly obtained with humans, both overt and covert strategies were concluded to facilitate the extraction of the behaviourally relevant information and to cope with the problem that the entire visual information cannot be processed at once. In previous studies by the same authors (or a subgroup of them) it was convincingly shown that honeybees (in contrast to bumblebees) employ mainly an overt serial strategy to search for previously learnt coloured target objects, if these are embedded between distracting cues. In analogy to similar results obtained with human observers, these conclusions were interpreted as being based on a kind of serial shift of the animals' focus of attention. The analyses were based on the search performance (i.e. measuring both error rate and search time) while detecting a target object embedded in a variable number of distracting differently coloured objects.

The present study is based on a similar experimental design as employed in these previous studies and asks the highly interesting question of whether the visual system of honeybees is functionally regionalised with regard to a pattern detection/discrimination task. Honeybees were trained to detect a coloured target either in the upper or bottom row of a 3x3 matrix of possible object positions. In the test phase distracting objects were added in three different constellations to the stimulus matrix. Both target and distractor objects were placed either in the top row ('dorsal group') or only in the bottom row ('ventral group'); in a 'mixed group', target and distractors could appear in the top row as well as in the bottom row. From the way the search performance improved under the different stimulus constellations, the authors concluded that honeybees flexibly adapt to a given foraging situation by focusing their search strategy to the location of the object within their visual field. The accuracy, decision time and flight paths of honeybees were concluded to depend on both the target's location in the visual field and on the bees' possibility to predict

the target location. It is discussed whether honeybees use attentional mechanisms to increase their success rate in the detection task.

Although I am sympathetic with the overall goal of this project and agree with the authors' overall conclusion, I have to admit that I am somewhat confused by parts of the experimental design of the study. Some further explanation of the rationale behind the analysis is likely to largely raise the potential of the study.

Major issues

After the training phase the bees could not have any *a priori* expectation with regard to target position, since target position was pseudo-randomized during the training procedure. Interestingly, a clear dorso-ventral asymmetry of the error rate of target detection was found even at the end of the training period. For the test phase the bees were subdivided into the three experimental groups that were tested with just one of the stimulus constellations. The rationale behind this design is not well explained and not immediately obvious. At least at first sight, one might have expected that the different constellations were tested again with all bees in a pseudo-random fashion.

One reason that this has not been done might have been the extreme dorso-ventral performance gradient after the training phase. Even without any distractor the coloured target could be detected with only a very high median error rate of 65% when presented in the top row. Surprisingly, no strong point is made that the detection performance is not much above chance level (this issue should have been tested statistically!), when the target was presented in the top row. Theoretically, this poor performance should not increase, if in the test phase – i.e. after adding two distractor objects - the target were also presented randomly in the top and bottom row of the stimulus arrangement. Hence, it might have been scientifically meaningful to ask whether the detection performance in the dorsal part of the visual field might increase, if the bee had the chance to build up an expectation that the target is not located ventrally. This might be a rationale for subdividing the bees into the three groups. Irrespective of whether or not this supposition is correct, an explanation for the experimental design should be given.

In any case, it can be concluded from the development of error rates, decision times, but also the trajectory data that the performance of the 'dorsal group' improves over time and that this finding is consistent with the interpretation that also dorsally located targets can be readily detected, if the bee has *a priori* knowledge that the target is likely to be found in an otherwise 'suboptimal' part of the visual field. Again, it might be helpful if this kind of conclusion were drawn explicitly in the paper. Whether this finding is just a consequence of a learning process or might be also linked to attentional mechanisms can then be discussed. However, it might be hard to conceptualise how attentional mechanisms can be pinpointed experimentally in the type of experiments that were performed in this study.

The hardest issue that remains to be explained are the data obtained with the 'mixed group' of bees. Although here no expectation can develop with regard of the location of the target, the performance improves over stimulus presentations to values that are much better than those obtained at the end of the training phase – even without any distractors (!). This highly surprising issue definitely needs to be discussed. This somehow apparent contradiction raises also the question whether – given a tremendous variability between bees but also between different flights of individual bees – the data base is sufficiently large to allow for drawing strong conclusions.

Minor issues

Title of the paper:

Since the paper does not focus on the topic of attention, I would omit in the title of the paper the addendum “: a role for spatial attention?” Attention is just one possibility, in addition to several others, to explain the experimental results. Putting too much emphasis on attention already in the title may be somewhat misleading.

Abstract:

“Here we investigate if this asymmetry is caused by fixed search patterns or if bees can use alternative search mechanisms such as spatial attention, which allows flexible focusing on different areas of the visual field.” I do not see that the investigation of this alternative has been the focus of the experimental analysis of the study. At least the results do not allow answering this question in an unambiguous way.

Introduction:

1st paragraph: It appears to be surprising that the paper starts by considering whether the distinct spatial asymmetry in colour and pattern learning of honeybees as observed in previous studies might be a consequence of specialisations in eye morphology, as has been proposed for detecting the queen by the drone bee eye. It should be noted that this sort of detection task is conceptually very different: in the drone bee case we are dealing with detection thresholds where - for obvious reasons - spatial acuity and sensitivity, i.e. anatomical and physiological retinal parameters, are the relevant factors, because they determine at which distance a small target (i.e. in this case the queen) can be seen. In the learning experiments the targets were usually sufficiently large, and no spatial resolution or sensitivity problem existed for them to be detected.

Methods:

- What was the frame rate and resolution of the camera system?
- “The stimuli subtended a visual angle of ...” (and following sentence): The authors probably mean the individual discs. “stimuli” not specific enough.
- It should be explained why a virtual decision line was chosen. It might have been more intuitive to monitor the landing on the different targets. Would the error rate change, if the landings were recorded rather than the crossing of a virtual decision line?
- What happened to the bees after the individual training and test flights? Do they return to the entrance hole of the flight arena or were they caught and ‘manually’ brought back to their hive?
- Were the individual bees labelled and could be identified? This might have been a precondition to be able to monitor changes in detection performance.
- What about the orientation of body and head in 3D space? How well can these parameters be resolved? This is important information, especially if the retinal position of the objects needs to be assessed. The term ‘flight angle’ should be explained.
- “We used mixed linear models to test the effect of treatment group, target location and learning on the error rate and on the decision time, respectively. Furthermore, the identity of the individual bees was implemented into the model as a random factor.” It is not entirely clear what these sentences really mean. ‘Mixed linear models’ and ‘implementing individual bees into a model as a random factor’ should be explained.

Results:

- What does ‘overall error rate’ mean?
- Figure 3: If I understand the explanation of the figure correctly, the data point ‘6’ ‘N° of flights’ shown in A and B should be the mean of the corresponding data points ‘3’ and ‘6’ ‘N° of flights’ in C. Since in C both ‘dorsal group’ data points have a larger value than the corresponding data point in B, this interpretation cannot be correct – or something went wrong with the data. Please check.

- Legend of figure 4: “Please note that in the mixed group the flight number refers to number of trials absolved with the particular target situation and not to the total number of trials the bee has absolved”. This statement is somewhat cryptic. Please explain.

Discussion:

Most issues concerning the Discussion have already been mentioned above. Here just some minor, though relevant details:

- During the flights towards the target bees change their height. How is this done? Do they change the pitch angle of their body and, in particular, of the head when changing height as compared to the pitch angle during horizontal flights? This is important, since it pertains to the retinal area with which the target is seen. *Drosophila*, for instance, changes the pitch angle of its body when changing height, whereas it keeps the head orientation in space relatively constant (by changing the head angle relative to the body).
- “... bees could have increased the area of their attentional focus to a size large enough to cover both rows”. Obviously, by assuming such an attentional process almost every change in detection performance can be explained. However, from an epistemological point of view such a hypothesis makes only sense, if an experiment could be designed by which this hypothesis can be tested.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response (*Member of the F1000 Faculty*) 13 Feb 2015

Lars Chittka, School of Biological and Chemical Sciences, Queen Mary, University of London, UK

We thank the reviewer for the careful reading, the extensive suggestions and the help in closing gaps in the logic of the manuscript.

- *After the training phase the bees could not have any a priori expectation with regard to target position, since target position was pseudo-randomized during the training procedure. Interestingly, a clear dorso-ventral asymmetry of the error rate of target detection was found even at the end of the training period. For the test phase the bees were subdivided into the three experimental groups that were tested with just one of the stimulus constellations. The rationale behind this design is not well explained and not immediately obvious. At least at first sight, one might have expected that the different constellations were tested again with all bees in a pseudo-random fashion.*

One reason that this has not been done might have been the extreme dorso-ventral performance gradient after the training phase. Even without any distractor the coloured target could be detected with only a very high median error rate of 65% when presented in the top row. Surprisingly, no strong point is made that the detection performance is not much above chance level (this issue should have been tested statistically!), when the target was presented in the top row.

Thank you for this useful suggestion. We included this point in the analysis.

- *Theoretically, this poor performance should not increase, if in the test phase – i.e. after adding two distractor objects - the target were also presented randomly in the top and bottom row of the stimulus arrangement. Hence, it might have been scientifically meaningful to ask whether the detection performance in the dorsal part of the visual field might increase, if the bee had the chance to build up an expectation that the target is not located ventrally. This might be a rationale for subdividing the bees into the three groups. Irrespective of whether or not this supposition is correct, an explanation for the experimental design should be given.*

Yes, your explanation is correct. We apologize for the poorly crafted description of our rationale and now include a more careful explanation.

- *In any case, it can be concluded from the development of error rates, decision times, but also the trajectory data that the performance of the ‘dorsal group’ improves over time and that this finding is consistent with the interpretation that also dorsally located targets can be readily detected, if the bee has a priori knowledge that the target is likely to be found in an otherwise ‘suboptimal’ part of the visual field. Again, it might be helpful if this kind of conclusion were drawn explicitly in the paper. Whether this finding is just a consequence of a learning process or might be also linked to attentional mechanisms can then be discussed. However, it might be hard to conceptualise how attentional mechanisms can be pinpointed experimentally in the type of experiments that were performed in this study.*

Your conclusion is in accordance with our explanation, although it seems that we did not explain it sufficiently in the earlier version. In the new version, we made our point more clear by describing the rationale in the Material & Methods. Furthermore, we addressed this point in the Discussion section more extensively.

- *The hardest issue that remains to be explained are the data obtained with the ‘mixed group’ of bees. Although here no expectation can develop with regard of the location of the target, the performance improves over stimulus presentations to values that are much better than those obtained at the end of the training phase – even without any distractors (!). This highly surprising issue definitely needs to be discussed. This somehow apparent contradiction raises also the question whether – given a tremendous variability between bees but also between different flights of individual bees – the data base is sufficiently large to allow for drawing strong conclusions.*

We now discuss this issue carefully in the Discussion, suggesting a possible mechanism to cope with the search situation: we demonstrated that even in this group bees adapted their flight vectors over time, which may help them to detect the target faster and avoid difficult flight manoeuvres. It is difficult to draw conclusions from the comparison of the pre-training phase with the end of the training phase (=experimental phase) as bees had more than twice as much experience with the task at the end of the training phase than at the end of the pre-training phase and may cope more easily with the search task for this reason alone.

- *Title of the paper: Since the paper does not focus on the topic of attention, I would omit in the title of the paper the addendum “: a role for spatial attention?” Attention is just one possibility, in addition to several others, to explain the experimental results. Putting too much emphasis on attention already in the title may be somewhat misleading.*

The title has been changed to address this.

- *Abstract: "Here we investigate if this asymmetry is caused by fixed search patterns or if bees can use alternative search mechanisms such as spatial attention, which allows flexible focusing on different areas of the visual field." I do not see that the investigation of this alternative has been the focus of the experimental analysis of the study. At least the results do not allow answering this question in an unambiguous way.*

We changed that sentence into "Here we investigate if this asymmetry is caused by fixed search patterns or if bees can increase their detection ability of objects in the dorsal part of the visual field in search scenarios, where the target appears often or always in this area of the visual field."

- *Introduction, 1st paragraph: It appears to be surprising that the paper starts by considering whether the distinct spatial asymmetry in colour and pattern learning of honeybees as observed in previous studies might be a consequence of specialisations in eye morphology, as has been proposed for detecting the queen by the drone bee eye. It should be noted that this sort of detection task is conceptually very different: in the drone bee case we are dealing with detection thresholds where - for obvious reasons - spatial acuity and sensitivity, i.e. anatomical and physiological retinal parameters, are the relevant factors, because they determine at which distance a small target (i.e. in this case the queen) can be seen. In the learning experiments the targets were usually sufficiently large, and no spatial resolution or sensitivity problem existed for them to be detected.*

We understand your point of view. However, we wanted to point to the fact that a dorso-ventral asymmetry in visual pattern recognition can at least in theory be explained by eye optics. The honeybee drone is a relevant example of where such an asymmetry occurs. Even if such optical asymmetries do not explain the behavioural asymmetry in our study, this is at least a theoretical explanation for unequal performance in various parts of the visual field, so we can't disregard this possibility.

- *Methods: What was the frame rate and resolution of the camera system?*

We now include the frame rate.

- *"The stimuli subtended a visual angle of ..." (and following sentence): The authors probably mean the individual discs. "stimuli" not specific enough.*

We have changed the term into 'stimulus discs'

- *It should be explained why a virtual decision line was chosen. It might have been more intuitive to monitor the landing on the different targets. Would the error rate change, if the landings were recorded rather than the crossing of a virtual decision line?*

We now evaluate the flight behaviour in much more detail, including locations at various distances from the screen as well as errors upon landing. We find that the distance of 5 cm from the search screen allows for best differentiation between the different experimental group, and therefore focus some aspects of the evaluation on this distance.

- *What happened to the bees after the individual training and test flights? Do they return to the entrance hole of the flight arena or were they caught and 'manually' brought back to their hive?*

We have now included the following sentence into material & methods: 'After drinking to satiation, the bee left the arena to fly back to the colony.'

- *Were the individual bees labelled and could be identified? This might have been a precondition to be able to monitor changes in detection performance.*

We had stated that bees were marked individually. However, for clearer understanding we extended the sentence which now says: ' Bees were trained to an experimental box and marked individually by applying differently coloured paint markings on the thorax.'

- *What about the orientation of body and head in 3D space? How well can these parameters be resolved? This is important information, especially if the retinal position of the objects needs to be assessed. The term 'flight angle' should be explained.*

Unfortunately the video quality does not allow for analysis of orientation of body and head. We included the following sentence into material & method: "To analyse the flight directions (vertical flight angles) of the bees while crossing the arena, we measured the vertical position of the bee at different distances to the search screen and calculated the angle between the flight direction and the horizontal."

- *"We used mixed linear models to test the effect of treatment group, target location and learning on the error rate and on the decision time, respectively. Furthermore, the identity of the individual bees was implemented into the model as a random factor." It is not entirely clear what these sentences really mean. 'Mixed linear models' and 'implementing individual bees into a model as a random factor' should be explained.*

We now state: 'We used general linear models to test the effect of treatment group, target location and learning on the error rate and on the decision time, respectively. As the behaviour of each individual bee was measured several times during experimental course (repeated measurement design), the identity of the individual bees was embedded into the model as random effect.'

- *What does 'overall error rate' mean?*

This term has now been removed.

- *Figure 3: If I understand the explanation of the figure correctly, the data point '6' 'N° of flights' shown in A and B should be the mean of the corresponding data points '3' and '6' 'N° of flights' in C. Since in C both 'dorsal group' data points have a larger value than the corresponding data point in B, this interpretation cannot be correct – or something went wrong with the data. Please check.*

We used not the mean but the median of the decision time to attenuate effects of rare outlier trials. This is now clarified in Material & Methods.

- *Legend of figure 4: "Please note that in the mixed group the flight number refers to number of trials absolved with the particular target situation and not to the total number of trials the bee has absolved". This statement is somewhat cryptic. Please explain.*

The sentence was changed into 'In the mixed group the flight number refers to number of trials absolved with the particular target location (top row or bottom row) and not to the total number of trials the bee has absolved in total (all trials regardless of the target location).'

- *Discussion: Most issues concerning the Discussion have already been mentioned above. Here just some minor, though relevant details:*

During the flights towards the target bees change their height. How is this done? Do they change the pitch angle of their body and, in particular, of the head when changing height as compared to the pitch angle during horizontal flights? This is important, since it pertains to the retinal area with which the target is seen. Drosophila, for instance, changes the pitch angle of its body when changing height, whereas it keeps the head orientation in space relatively constant (by changing the head angle relative to the body).

As mentioned, we have no information about the head angle during the approach flight, nor about the pitch of the body axis. The limitations of this lack of information are now discussed.

- *"... bees could have increased the area of their attentional focus to a size large enough to cover both rows". Obviously, by assuming such an attentional process almost every change in detection performance can be explained. However, from an epistemological point of view such a hypothesis makes only sense, if an experiment could be designed by which this hypothesis can be tested.*

It is possible to test for such a hypothesis – behavioural experiments in human psychology have been testing various parameters of attentional focus (see citations in the manuscript). Theoretically these could be adapted to fit a behavioural experiment for insects.

Competing Interests: No competing interests were disclosed.

Referee Report 26 August 2014

doi:10.5256/f1000research.5123.r5787



Bruno van Swinderen

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In this study Morawetz, Chittka, and Spaethe present results from a behavioral paradigm for honeybees where they examine spatial attention. Bees were trained to select a color, using classical conditioning with sugar versus quinine. Trained bees were then presented with various scenarios whereby the targets were presented together with distracters, either in the upper or lower visual field. The authors find that targets in the ventral field led to a more efficient search strategy. Importantly, it appears that honeybees form expectations about where their target will be, in repeated flights.

These are interesting results, which help consolidate the view that insects display a selective attention. In particular, the data supporting the view that there is an expectation already formed early in the flight angle is very intriguing. It is nice to see a paper where the behavior itself is examined closely, rather than only the proportion of choice outcomes. This helps to better show how attention might be operating in these simple animals.

The paper is close to indexation quality. There are a couple questions that might need to be attended to, to further improve the quality of the work.

First, it is quite unclear how the position of the “decision line” was arrived at. While it is evidently depending on previously published work, one concern is that varying the position of this decision line might substantially change the results and conclusions. The decision line is currently 5cm away from the end wall, and both metrics (error rate and decision time) depend on this distance. Clearly, a bee flying downwards is exploiting gravity to get to the decision line, while a bee flying upwards is fighting gravity. This simple factor could account for the different flight dynamics seen for either situation (as is evident in the informative single examples shown in Figure 5). Can the authors exclude a non-trivial explanation here for the effects of gravity? With regard to the error rate for example, it is not clear whether a “mistake” is meaningful here. Maybe bees fly more erratically upwards (they do). More generally, it would be good to have a bit more of a rationale for why this 5cm point was chosen. How different would the results have been at 7cm or 10cm?

Second, the error rate metric does not seem to account for proximity. Thus, a bee crossing the decision line close to the target is as equally wrong as a bee crossing a whole square away? This seems strange. Should the error rate be weighted in some way? How might that change the results?

Minor:

Sareen et al (2011) showed that fruit fly attention is mostly ventral. This might be discussed a bit more, in connection to the current findings in the bee.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response (*Member of the F1000 Faculty*) 13 Feb 2015

Lars Chittka, School of Biological and Chemical Sciences, Queen Mary, University of London, UK

First of all, we thank the referee for the kind consideration of our manuscript and the suggestions, which helped to improve our manuscript.

- *First, it is quite unclear how the position of the “decision line” was arrived at. While it is evidently depending on previously published work, one concern is that varying the position of this decision line might substantially change the results and conclusions. The decision line is currently 5cm away from the end wall, and both metrics (error rate and decision time) depend on this distance.*

In response to this comment and those by the other referees, we have now first explored the

bees' flight structure and error rates at different distances in more detail, providing a justification why it is useful to focus on an analysis of error rates at 5cm distance from the target.

- *Clearly, a bee flying downwards is exploiting gravity to get to the decision line, while a bee flying upwards is fighting gravity. This simple factor could account for the different flight dynamics seen for either situation (as is evident in the informative single examples shown in Figure 5). Can the authors exclude a non-trivial explanation here for the effects of gravity?*

This is an interesting point. We would expect gravity to affect all bees who fly towards a dorsally located target in the same way. However, bees of the 'dorsal group' and bees of the 'mixed group' searching for a dorsally located target differed significantly in their error rate (Fig. 2), error locations (Fig. 7), flight structure (Fig. 5, 6) and decision time (Fig.4). We therefore suspect that gravity cannot provide a good explanation for the dorso-ventral asymmetry observed in this experiment.

- *With regard to the error rate for example, it is not clear whether a "mistake" is meaningful here. Maybe bees fly more erratically upwards (they do). More generally, it would be good to have a bit more of a rationale for why this 5cm point was chosen. How different would the results have been at 7cm or 10cm?*

To answer this question, we included a new video analysis into the manuscript describing the error rate in distances of 10 cm, 7.5 cm and 0 cm (fig. 3). For the first learning block it shows that 5 cm distance allows a good differentiation between direct flights (as in the ventral group) and less directed flights (as in the dorsal group). This provides a justification for our choice to analyse error rate at this distance. However, it is necessary to state, that there is no 'ideal' distance for error analysis as flight structure may change over time. This is demonstrated by the analysis of the last learning block, where differences between groups are most pronounced at 7.5 cm distance.

- *Second, the error rate metric does not seem to account for proximity. Thus, a bee crossing the decision line close to the target is as equally wrong as a bee crossing a whole square away? This seems strange. Should the error rate be weighted in some way? How might that change the results?*

This is a good point. In response, we decided to depict the different error locations in a graph (fig. 7). The graph shows that groups searching for targets at different heights differed in their way in making errors. The results corresponded well with data from flight structure analysis. For example, bees of the dorsal groups gradually developed a trend to fly upwards. At the same time, they made most errors while being located in the top row - demonstrating the distinct upwards movement of these bees during their flights.

- *Sareen et al. (2011) showed that fruit fly attention is mostly ventral. This might be discussed a bit more, in connection to the current findings in the bee.*

We thank the referee for the suggestion and included the findings of Sareen *et al.* into the introduction.

Competing Interests: No competing interests were disclosed.

