

RESEARCH ARTICLE

Can bees see at a glance?

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ABSTRACT

Primates can analyse visual scenes extremely rapidly, making accurate decisions for presentation times of only 20 ms. We asked whether bumblebees, despite having potentially more limited processing power, could similarly detect and discriminate visual patterns presented for durations of 100 ms or less. Bumblebees detected stimuli and discriminated between differently oriented and coloured stimuli when presented as briefly as 25 ms but failed to identify ecologically relevant shapes (predatory spiders on flowers) even when presented for 100 ms. This suggests an important difference between primate and insect visual processing, so that while primates can capture entire visual scenes ‘at a glance’, insects might have to rely on continuous online sampling of the world around them, using a process of active vision, which requires longer integration times.

KEY WORDS: Active vision, *Bombus terrestris*, Insect vision, Rapid feature extraction, Visual processing

INTRODUCTION

Do bigger brains confer advanced sensory or behavioural capabilities? Larger brains could provide increased representational capacity of objects and greater capabilities for parallel processing (Chittka and Skorupski, 2011). Conversely, animals with smaller brains might rely on continuous, active sampling of their environment. Insect brains are orders of magnitude smaller than human brains. A typical bee brain contains fewer than a million neurons (Menzel and Giurfa, 2001; Witthöft, 1967) compared with an estimated 85 billion neurons for the human brain (Azevedo et al., 2009; Roth and Dicke, 2005; Williams and Herrup, 1988). Bees are nonetheless capable of remarkable feats of visuo-cognitive behaviour. They can not only learn to associate coloured or black and white patterns with rewards (Menzel and Lieke, 1983; Wehner, 1967; Zhang et al., 1992) but also use visual features to categorise natural scenes, as well as novel visual objects based on shared properties (Zhang et al., 2004). They can learn simple relational ‘rules’ (e.g. above/below-ness) between stimuli regardless of basic stimulus properties (Avarguès-Weber et al., 2011). They have also been shown to learn to choose different targets in different contexts (Dale et al., 2005; Lotto and Chittka, 2005) and to use colour cues in different spatial contexts to navigate (Zhang et al., 1996). They can learn sequences of movements in response to visual stimuli (Collett et al., 1993) and their behaviour when navigating along series of identical landmarks conforms to the basic criteria of

numerosity (Chittka and Geiger, 1995; Dacke and Srinivasan, 2008). Bees thus display the capability to perform several visual behaviours that are traditionally thought to be the domain of much larger brained animals, including humans.

Much of the research on comparative animal cognition has focused, perhaps too narrowly, on identifying shared capacities between humans and other animals (Shettleworth, 2001; Penn et al., 2008). Undoubtedly, such research has led to remarkable successes in placing human cognition into an evolutionary framework, and also dismissed the myth that large brains automatically convey superior cognition (Emery and Clayton, 2004; Giurfa, 2013). However, an obsession with animal ‘cleverness’ and a focus on human-like capacities of animals might obscure significant differences in cognition between species, and indeed unique cognitive adaptations and constraints (Bolhuis and Wynne, 2009; Chittka et al., 2012; de Waal and Ferrari, 2010).

One important difference between primate and insect visual cognition might relate to visual search. Humans are capable of discriminating between scenes at a glance (Kirchner and Thorpe, 2006) and using parallel search (Wolfe, 2000). Honeybees, by contrast, may be restricted to serial visual search (Spaethe et al., 2006). Target identification is substantially slowed by increasing the number of distractors in a scene, even when target and distractors differ only in one stimulus dimension such as colour (Spaethe et al., 2006). In humans, visual search would be parallel in such a situation, with the target ‘popping out’ rapidly, independent of the number of distractors (Wolfe, 2000). It has therefore been suggested that insects might use ‘active vision’ to compensate for their lack of parallel processing power, actively scanning the scene for key targets rather than using a rapid snapshot (Chittka and Skorupski, 2011; Collett et al., 2013). Indeed, flying bees make systematic side to side scanning movements (‘peering’) during target approach (Boeddeker and Hemmi, 2010; Ings et al., 2012), which stabilise translational image flow by cancelling out rotational motion of the retinal image (Dittmar et al., 2010). Such scanning in the translational plane could theoretically increase spatial resolution, if bees correlated self-generated scanning movements with the resulting image translation. This would, however, lead to increased image acquisition time as well as greatly impaired visual discrimination in situations where eye movements (meaning, for a bee, head and whole-body movements) are not possible. In fact, it appears that immobilised bees are visually impaired: although associative olfactory learning has been extensively studied in tethered bees, attempts to condition tethered bees to visual stimuli have only been successful for large-field, coarse colour discrimination (Niggebrügge et al., 2009).

Honeybees can extract at least simple visual features (grating orientation) at presentation times of as little as 2 ms (Srinivasan et al., 1993). In addition, a recent study indicates that bumblebee visual search is not affected by the number of distractors and that they are capable of parallel search (Morawetz and Spaethe, 2012). Simulations of different search mechanisms suggest that these different search strategies might reflect different ecological environments of

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honeybees and bumblebees (Bukovac et al., 2013). Thus bumblebees, at least, might be able to recognise some targets rapidly when distractors are also present in a scene. No study has, however, tested whether bees can recognise complex targets 'at a glance' as well as simple visual features. We explored whether bumblebees, *Bombus terrestris* (Linnaeus 1758), can distinguish both simple visual features and complex, ecologically relevant shapes at a glance, when presented for increasingly brief durations (<100 ms) and therefore without the possibility to scan the visual scene.

RESULTS

Bees foraged in an arena with one wall containing an LCD computer screen (Fig. 1A). Bees were pre-trained to collect droplets of sucrose solution from landing platforms positioned in front of the screen, three on each side. Stimuli were presented on the screen directly behind the platforms.

Bees were trained individually on one of five tasks. Correct choices led to a reward of sucrose solution while incorrect choices were penalised with aversive saturated quinine hemisulphate solution in all tasks. In every task, stimuli were presented continuously in the first condition. In three subsequent conditions, stimuli were presented for durations of 100, 50 and 25 ms. After training on each condition of each task, we tested bees on the same task and condition with distilled water presented across all stimuli rather than sucrose or quinine and noted the proportion of correct choices made by the bees.

Stimulus presence/absence detection

This task required bees to detect the presence of a yellow rectangular bar at an angle of 45 deg (Fig. 1B). Bars were presented behind three randomly chosen landing platforms with no stimulus behind the other platforms. All 10 tested bees learnt to detect yellow bars, regardless of the brevity of presentation (Fig. 2A). The mean number of choices taken to learn the task to criterion was close to 20 for all durations of presentation, with no significant differences for stimulus duration (Fig. 3; Friedman test, $\chi^2=3$, d.f.=3, $P=0.39$).

Bees also chose correct stimuli significantly above chance in tests (all feeding posts with 50 μ l of water, i.e. no reward or punishment) that followed the training conditions (t -tests, all $t>7.1$, $P<0.001$; the mean \pm s.d. proportion of correct choices for static presentation and durations of 100, 50 and 25 ms was 0.76 ± 0.09 , 0.72 ± 0.09 , 0.71 ± 0.07 and 0.71 ± 0.07 , respectively; Fig. 2B). The proportion of correct choices also did not differ significantly across presentation durations (repeated measures ANOVA, $F_{3,27}=1.1$, $P=0.37$). Thus, for this simple stimulus absence/presence task, the brevity of stimulus presentation did not constrain either learning speed or levels of accuracy.

Discrimination of stimulus edge orientation

Here, bees had to discriminate between rectangular bars oriented at an angle of 45 deg from otherwise identical bars at an angle of -45 deg (Fig. 1C). All 10 tested bees also learnt to discriminate between differently oriented bars when presented either statically or for 100 ms (Fig. 2A). Nine of the 10 bees successfully distinguished the stimuli when presented for 50 ms and seven of these remaining nine bees (78%) also discriminated the stimuli when presented for 25 ms (Fig. 2A). The mean number of choices taken by the successful bees to learn the task to criterion was near 20 for static and 100 ms presentation but increased slightly to near 22 for very short presentation times (Fig. 3). The number of choices taken to learn the discrimination did not, however, differ across durations of presentation (Friedman test, $\chi^2=3$, d.f.=3, $P=0.39$). Bees chose the correct stimuli significantly above chance in the test bouts for all stimulus durations (t -test, all $t>3.0$, $P<0.05$; the mean \pm s.d. proportion of correct choices for static presentation and durations of 100, 50 and 25 ms was 0.73 ± 0.13 , 0.68 ± 0.05 , 0.61 ± 0.07 and 0.64 ± 0.12 , respectively; Fig. 2B). The differences between the proportions of correct choices across durations of presentation were not significant (repeated-measures ANOVA, $F_{3,18}=1.42$, $P=0.27$). Presentation duration thus had no significant effect on either the time taken to learn the task or the accuracy of performance on this task.

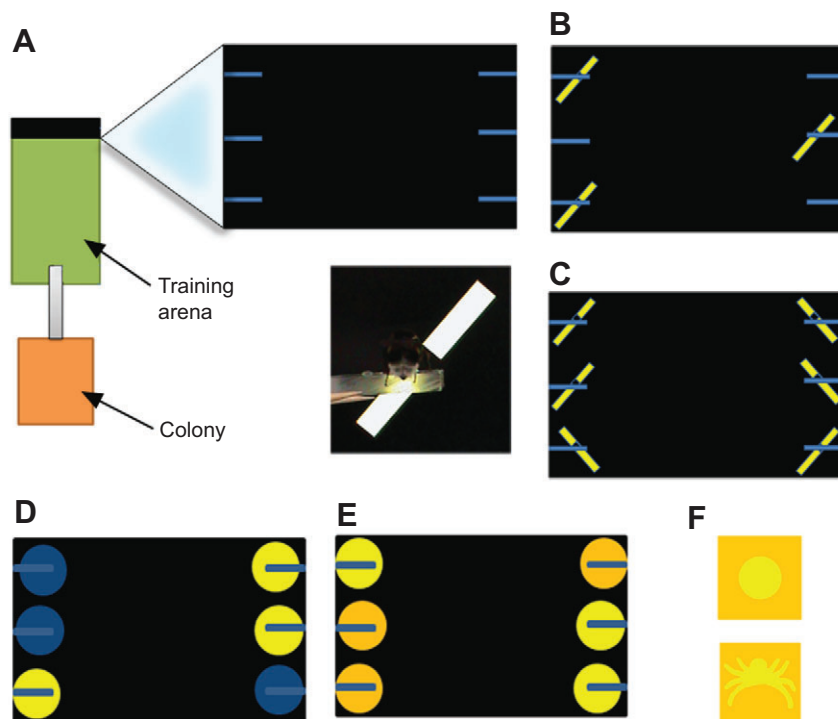


Fig. 1. Experimental set-up and stimuli. (A) Bumblebee colony box linked by a Perspex tunnel to the training arena, containing a computer monitor inserted into the wall opposite the arena entrance, shown in black. Expanded view of the monitor depicts the screen and six Perspex feeding platforms. Inset shows a bee feeding on a platform with the rewarding stimulus behind it. (B–F) Representation of the stimuli displayed on the screen during the tasks: (B) task 1: stimulus detection; (C) task 2: discrimination of edge orientation; (D) task 3: colour discrimination: yellow and blue; (E) task 4: colour discrimination: yellow and orange; (F) task 5: complex shape discrimination: spiders and circles.

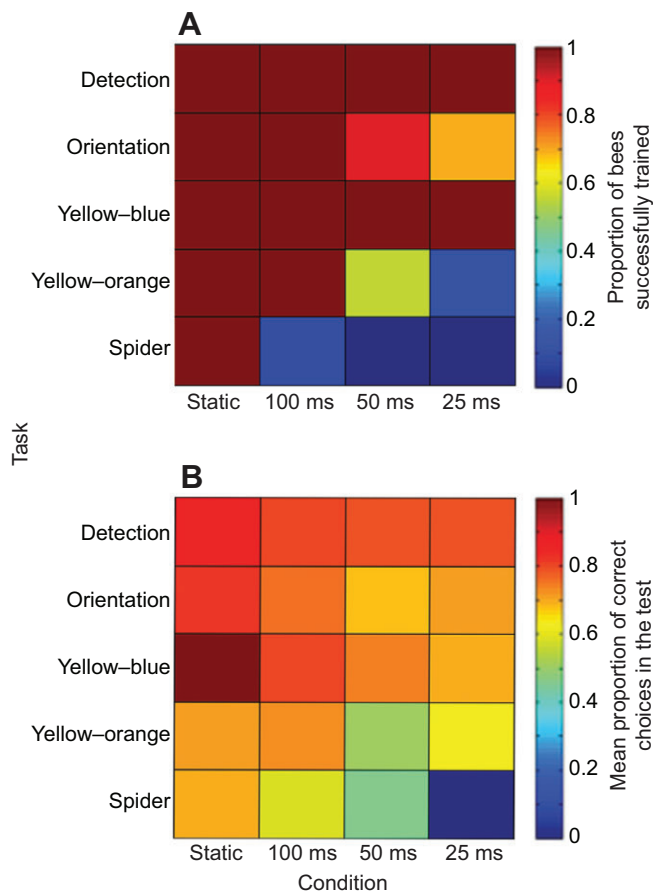


Fig. 2. Performance of bees during training and test. Fifty bees were tested in total, with 10 bees tested in each of the five tasks across all conditions. Rows correspond to tasks and columns correspond to durations of stimulus presentation as indicated. The square colour codes the value for each combination of task and condition as indicated by the colour bar on the right. (A) Proportion of bees performing significantly above chance (*t*-tests, $P < 0.05$) for each of the training conditions of each task. (B) Mean proportion of correct choices made by successfully trained bees during tests. Proportions above 0.6 are significantly different from chance (*t*-tests, $P < 0.05$).

In theory, bees might have acquired visual information even in a 25 ms window by rapidly scanning the visual stimulus. Visual inspection of the bees' flight movements revealed that bees typically inspected the screen from a distance of between 2 and 5 cm, and initiated their landing manoeuvre from this range of distance. Given that the length of the stimulus in the first two tasks was 5 cm, this suggests that the bees were not recognising it by actively scanning the entire length but perceived it rapidly while remaining relatively stationary: at the distance at which they made their decisions (typically within 5 cm of the screen), they would need to scan an angle of 38.2 deg or more subtended by the stimulus within the 25 ms presentation time. Using rotational movements, this would require an angular velocity of around 1528 deg s⁻¹, far higher than has previously been observed in bees: 410–480 deg s⁻¹ (Srinivasan et al., 1996). The bees could not therefore have scanned the stimulus with rotational movements. Thus, while they were indeed extracting visual features, they were apparently doing so independently of movement.

Colour discrimination

We tested bees on their ability to discriminate between circles of differing colours: blue versus yellow and yellow versus orange

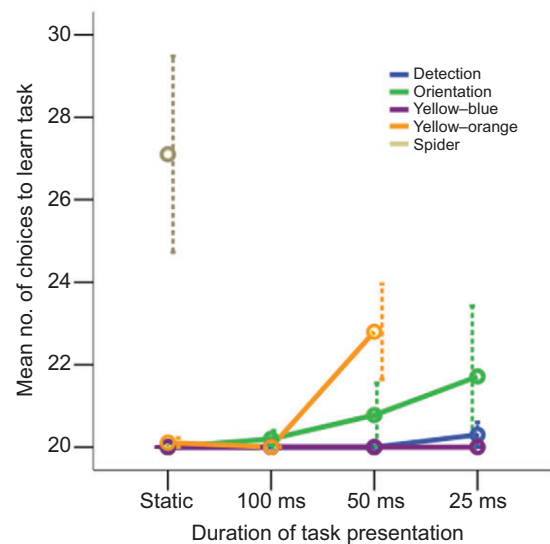


Fig. 3. Number of choices taken by bees to learn tasks presented for different durations. The mean number of choices taken by bees to learn tasks varied with both task and presentation duration. Different colours indicate different tasks, dashed bars indicate the standard error. Means were only calculated for those combinations of tasks and durations where more than one bee successfully learnt the task.

(Fig. 1D,E). All 10 bees tested learnt to discriminate between blue and yellow circles across all durations of presentation (Fig. 2A). The mean number of choices taken to learn this task was 20 across all durations of presentation, again indicating that this was a relatively easy task for the bees to learn. Bees also chose correct stimuli significantly above chance in tests (all feeding platforms with 50 μ l of water, i.e. no reward or punishment; *t*-tests, all $t > 3.3$, $P < 0.01$; the mean \pm s.d. proportion of correct choices for static presentation and durations of 100, 50 and 25 ms was 0.90 \pm 0.11, 0.72 \pm 0.20, 0.67 \pm 0.04 and 0.62 \pm 0.11, respectively; Fig. 2B). The proportion of correct choices differed significantly across different durations of presentation (repeated-measures ANOVA, $F_{3,27}=9.47$, $P < 0.01$). Thus, while the experience needed to reach criterion did not vary between stimulus durations, accuracy levels deteriorated with shorter presentation times for this simple colour discrimination task.

When tested for discrimination of similar colours, all bees distinguished between yellow and orange circles presented either statically or for 100 ms (Fig. 2A). When the same stimuli were presented for 50 and 25 ms, the proportion of the bees that learnt to discriminate them successfully was only 0.6 and 0.1, respectively (Fig. 2A). The mean number of choices taken by successful bees to learn this task was 20 for static and 100 ms presentation times, but increased to 23 for the 50 ms presentation time (Fig. 3). This difference was significant across presentation durations (Friedman test, $\chi^2=6$, d.f.=2, $P=0.05$). In this difficult colour discrimination task, bees also chose correct stimuli significantly above chance only in the tests with stimuli presented statically or for 100 ms (*t*-test, all $t > 5.5$, $N=9$, $P \leq 0.001$) but not for stimuli presented for 50 ms (*t*-test, $t=-1.2$, $N=9$, $P=0.27$). The mean \pm s.d. proportion of correct choices for static presentation and presentation durations of 100, 50 and 25 ms was 0.63 \pm 0.06, 0.65 \pm 0.08, 0.45 \pm 0.13 and 0.57 \pm 0.10, respectively (Fig. 2B), and these proportions differed significantly across presentation durations (repeated-measures ANOVA, $F_{3,24}=10.07$, $P < 0.01$). Thus, presentation duration affected both the time taken to learn this task and the accuracy of performance on the task.

Complex pattern detection and discrimination

We tested bees on their ability to integrate information about edges and colour differences by requiring them to discriminate between a biologically relevant stimulus, the silhouette of a yellow crab spider (an ambush predator hunting for pollinators on flowers), and yellow circles, both presented against an orange background. To ensure that the bees learnt this task when stimuli were presented statically, we first pre-trained the bees in a 'robotic spider' arena (Ings and Chittka, 2008) where we could simulate predatory attacks if bees approached the spider shapes.

All bees learnt to discriminate yellow spider shapes from yellow circles when presented statically. The mean (\pm s.d.) number of choices taken to learn the task (27 ± 7.5) was higher than for all the tasks previously described (Fig. 3). All but one bee, however, failed to learn the task when stimuli were presented for 100 ms and none of the bees completed the task with a stimulus presentation duration of 50 ms (Fig. 2A). Bees also chose correct stimuli significantly above chance only in the static presentation condition (*t*-test, $t=4.172$, $N=10$, $P<0.003$; mean \pm s.d. proportion of correct choices: 0.63 ± 0.10 ; Fig. 2B). However, for the presentation duration of 100 ms, performance (0.53 ± 0.26 correct choices; Fig. 2B) did not exceed chance levels (*t*-test, $t=0.339$, $N=10$, $P=0.74$). The proportion of correct choices for presentation durations of 50 ms was even lower (0.42 ; Fig. 2B). Thus, bees could only successfully learn the biologically relevant pattern identification task in the static condition and failed when these complex stimuli were presented for short durations. Control experiments ascertained that bees trained on static stimuli were able to generalise to stimuli presented for 50 ms or above in tests: bees trained on static stimuli chose the correct stimulus significantly above chance in bouts with a presentation duration of 50 ms or above (*t*-tests, all $t>2.5$, all $P<0.05$) but not for presentation durations of 25 ms (*t*-test, $t=0.3$, $P=0.75$). The failure of bees at non-static tasks is thus not explained by their training to static stimuli.

Comparison of performance between tasks

The number of choices taken to learn the tasks in the static condition differed significantly across tasks (Kruskal–Wallis test, $H_4=32.8$, $P<0.001$). *Post hoc* tests revealed that this difference was entirely due to significant differences between the number of choices taken to learn the pattern discrimination task and all the other tasks (Mann–Whitney *U*-tests, $U_4=10$, all $P\leq 0.001$) indicating that this task was more difficult. The number of choices taken to learn the tasks, however, is not an indication of how long bees take to perform the tasks in a given trial and their accuracy should depend on presentation duration of a particular task regardless of task difficulty. Indeed, our results show that bees varied significantly in the proportion of correct choices during the test across tasks and presentation durations (general linear model; task, duration, task \times duration; $F=4.45$, 5.64 , 2.51 ; $P=0.002$, 0.001 , 0.009). There was a significant effect of both task ($P=0.002$) and stimulus presentation duration ($P=0.001$) as well as a significant interaction effect between the two ($P=0.009$), which is not explained solely by differing performance on the final task. Interestingly, except for the simple colour discrimination task, bees also took more choices to learn the tasks as the duration of presentation decreased (Fig. 3). Although not all the differences were significant, there was a clear trend for the rate at which learning performance decreased (as measured by the number of choices) being different for each of the tasks (Fig. 3). Thus, the bees had longer learning periods as well as performing worse because of specific combinations of tasks and presentation durations.

DISCUSSION

Our results demonstrate that bumblebees can detect and discriminate between elementary visual features, even when presented as briefly as 25 ms, but not between similar colours and complex patterns. Experiments with humans and monkeys have shown that they are capable of ultra-rapid categorisation of natural versus manmade scenes even when scenes are presented for durations of only 6–20 ms (Bacon-Macé et al., 2007; Girard et al., 2008; Kirchner and Thorpe, 2006; Thorpe et al., 1996).

The fact that bees are capable of extracting simple features at this time scale, but seem to fail to analyse complex scenes, suggests an important difference between primate and bee visual processing: the higher computational power of bigger brains could enable the analysis of whole visual scenes at a glance, whereas insects might require longer integration times for any but the most simple visual identification tasks. Extended integration times might involve continuous 'online' sampling of the world around them. Such active vision, where animals sometimes follow repeated paths to memorise contingencies between own movements and object views for target recognition has been observed in a number of insect species (Collett et al., 2013; Collett and Zeil, 1996), and also in birds (Dawkins and Woodington, 2000; Gall and Fernández-Juricic, 2010). Active vision has also been suggested to play a role in colour perception (Skorupski and Chittka, 2011). As ommatidia contain a variety of different sets of colour receptor types (Spaethe and Briscoe, 2005; Wakakuwa et al., 2005), a single-coloured object might be perceived as consisting of multiple pixels each with different colours – unless insects move their eyes over the object to generate a temporal integration (Skorupski and Chittka, 2011). This could explain discrimination of similar colours being limited to presentation durations above 50 ms. Complex pattern discrimination is further limited to durations above 100 ms.

The rapid categorisation of the kind seen in humans and monkeys might rely on pre-attentive feed-forward processing of low level features (Joubert et al., 2009; Walker et al., 2008). Extraction of image components at a glance in primates implies a time course that seems compatible with only a single sweep of sensory information along the known delays of the visual system (VanRullen and Thorpe, 2002). A single sensory snapshot might be less useful for a miniature brain, such as that of an insect, simply because there is less capacity for off-line processing (processing involving significant neural delays). In active perception, the time taken to build up a picture of the world will depend on how much can be sampled at once, and in a miniature brain there may be less capacity to process a single 'sensory snapshot' and consequently a greater dependence on continuous online sampling of the scene. It is therefore conceivable that reduced representational capacity may lead to fundamentally different perceptual sampling strategies in large and small brains (Chittka and Skorupski, 2011).

Our hypothesis is that more than a static retinal image (a glance) is required for visual pattern recognition in bees (unlike in humans). We suspect that active scanning is required, which depends upon systematic translations of the retinal image generated by the bee's own exploratory body movements. The fast photoreceptors of the bee's visual system would allow for efficient analysis of such image translations (Skorupski and Chittka, 2010), but the time for perceptual analysis would be significantly longer, incorporating motor loops and the actual durations of exploratory movements. This might explain why significantly longer exposure times are required for pattern recognition in bees.

Further evidence for this suggestion comes from the observation that visual learning is severely impeded in tethered bees

(Niggebrügge et al., 2009). There might be a simple explanation: if visual perception depends on active visual probing of the environment by means of self-generated movement, then tethered bees (which cannot move their heads to scan visual patterns) are constrained to fail in any but the most simple discrimination tasks (Chittka and Skorupski, 2011). Insects might simply rely on active vision to perform more complex discriminations (Justice et al., 2012) and therefore fail when scanning is not possible, for example as a result of the short presentation durations in our experiment.

In our experiments, the flight movements of bees were not evaluated in detail; it would be highly informative to explore how flight paths near the targets depend on the nature of the discrimination or detection task (Philippides et al., 2013), and to explore whether different individuals have acquired individually different strategies for tracing visual patterns (Collett et al., 2013; Dawkins and Woodington, 2000). An alternative strategy might be to hinder bees from following their accustomed flight paths near the target, to measure the extent to which this impedes target recognition (Dawkins and Woodington, 2000). An ideal experimental scenario would be to work with tethered bees in a virtual flight arena, and to record the bee's self-generated motor output and use it to control the visual simulation in predictable ways (Liu et al., 2006; Luu et al., 2011). The sensorimotor loop will thus remain closed, which we hypothesise to be essential for any complex form of visual learning.

In conclusion, our results indicate that while comparative analyses of cognition and visual processing rarely integrate information from taxa as diverse as insects and primates (but see Chittka and Jensen, 2011; Chittka and Niven, 2009), there is nonetheless much to be gained by doing so. As discussed in the Introduction, previous studies have found that bees can learn several cognitive tasks similar to primates, calling into question what the minimum neural requirement may be for several of these tasks. Our results and those of others (Spaethe et al., 2006) conversely shed light on the limitations of miniature nervous systems and point towards capabilities that might be unique to primates and their bigger brains.

MATERIALS AND METHODS

Animals

Bumblebees were obtained from Syngenta Bioline (Weert, The Netherlands). They were individually tagged with Opalith number tags (Christian Graze KG, Weinstadt-Endersbach, Germany) and transferred to one compartment of a bipartite wooden nest box (28×16×11 cm length×width×height) along with the queen and the colony. The floor of the other compartment was covered with cat litter to allow bees to discard refuse. On non-experimental days, bees were given 50% (v/v) sucrose solution supplied by a gravity feeder in the arena. Bees were fed pollen on alternate days. On experimental days, sucrose solution was only provided as part of the pre-training and training conditions, to ensure that the bees remained motivated throughout the experiment.

Experimental set-up

Bees entered a training arena (60×70×40 cm length×width×height) via a 45 cm long Perspex tunnel (Fig. 1A). The entrance to the arena was blocked by a square of cardboard with only a 10 mm gap, approximately equal to the width of a bumblebee's body. This ensured that the bees were facing the back wall of the arena when they exited from the entrance tunnel into the arena. The stimuli were not visible from within the tunnel but became visible immediately after the bees emerged from the tunnel into the flight arena. At the entry point, stimuli (at their widest dimension; shapes described below) subtended >5.8 deg of the bee visual field, indicating that they were just detectable from that distance (Kapustjansky et al., 2010). After entering the arena, bees flew to the screen and typically made their decisions from less than 5 cm away from the screen.

The arena was covered with a UV-transparent Plexiglas lid and illuminated with high frequency fluorescent lighting (TMS 24F lamps with HF-B 236 TLD ballasts, Philips, The Netherlands; fitted with Activa daylight fluorescent tubes, Osram, Germany) which flicker at around 42 kHz – well above flicker fusion frequency for bees (Skorupski and Chittka, 2010; Srinivasan and Lehrer, 1984).

In order to have precise control of the timing of stimuli presentation, we used a novel paradigm of presenting visual stimuli to the bees on a computer monitor. The flat screen LCD Samsung SyncMaster 2233RZ monitor was set vertically in grey acoustic foam (Simply Foam Products Ltd, Bilston, UK) to form the back wall of the flight arena. The foam had a 30 cm wide by 26 cm tall gap cut into it through which the screen of the monitor was visible. The monitor had a refresh rate of 8.33 ms, which set the theoretical lower limit to how briefly a complete stimulus could be presented. In addition, use of an LCD monitor ensured that there would be no flicker in the presentation of stimuli. The distance between the monitor and the entry point of the bee into the arena was 34 cm and the screen subtended an angle of 47.6 deg when viewed from the entry point. Six Perspex chips were fixed in the foam in front of the screen to serve as feeding platforms. Each chip was square with a side of 25 mm and 4 mm thickness. The platforms on each side were 20 cm apart. On each side, the vertical distance between platforms was 8 cm.

We used MATLAB (MathWorks) and the Psychophysics Toolbox (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997) to create and display visual stimuli on the screen of the monitor and specify the time interval for which they were to be presented.

Pre-training

Bees were pre-trained in a group to feed off the feeding platforms with no stimuli behind them for two to three bouts. Individual bees were identified by previously attached numbered tags on their thoraxes or paint marks that had been applied to the thorax. Other bees were prevented from entering in the arena by the insertion of card barriers through slots in the entry tunnel.

For the final task (complex pattern discrimination/spider detection), bees were initially pre-trained to discriminate between the stimuli in a flight arena containing robotic spiders (Ings and Chittka, 2008) in order to make the task ecologically relevant and to ensure that bees learnt the task as the discrimination involved was potentially difficult. Bees were presented with printouts of the stimuli described above on removable squares with a landing balcony directly in front of the stimuli. Bees could land on this balcony and extend their proboscis through a hole in the wall. If this was a correct choice (circle), bees were rewarded with 20 µl of 50% (v/v) sucrose solution. If this was an incorrect choice (i.e. one for a flower with a spider), bees were lightly pinched with sponge-covered clamps that were fitted on either side of the landing balcony, thus experiencing a simulated spider attack (Ings and Chittka, 2008). Four rewarding flowers and four flowers with spiders were presented. Bees were free to revisit flowers but rewards were only replenished if the bee had visited all other correct flowers or after 2 min, whichever was sooner. The positions of the rewarding and unrewarding flowers were changed between bouts according to a previously generated random order. Bees were trained until they had chosen the correct stimulus significantly above chance in the last 20 choices in at least two different bouts. In order to ensure that bees remembered the stimuli across different experimental conditions, the bees were pre-trained on this set-up before every training condition.

Training

During training, each bout contained three possible correct choices and three incorrect choices. Correct stimuli had a 50 µl drop of 50% sucrose solution on the feeding posts in front of the stimuli, while posts in front of incorrect stimuli had a 50 µl drop of 0.12% (saturated) quinine hemisulphate solution on them, which bees find aversive (Chittka et al., 2003). The positions of rewarding or unrewarding stimuli were independently randomised between left and right for each pair of landing platforms in the bottom, middle and top rows for every training bout. A training bout lasted 4 min or until the bee returned to the colony, whichever occurred sooner. Each training condition consisted of around five to 12 training bouts (with two to six choices per bout) and lasted until

the bee made significantly more correct choices (14 choices out of 20; binomial probability test, $N=20$, $k=14$; exact binomial probability $P=0.04$) out of the last 20. As our criterion was measured in a window of 20 choices, bees could make several choices before reaching the criterion in this window and the total number of choices made thus served as a measure of how long bees took to learn the task. If the criterion was not achieved after 50 choices had been made, the condition was terminated.

During the non-static training conditions, stimuli were presented multiple times for the specific duration with a blank interval of 500–800 ms in between presentations. The exact duration of the blank interval was chosen by randomly picking a number between 500 and 800 ms. Presenting the stimuli multiple times but with a long enough blank duration in between presentations ensured that the bee would not miss seeing the stimuli but at the same time would be unable to temporally integrate over multiple presentations of the stimuli. The irregularity of the duration of the interval also ensured that the bee would be unable to learn a rhythm of presentation.

Stimuli

Stimulus presence/absence detection

The stimuli in the detection task consisted of three yellow (RGB scale: 255 255 0) rectangular bars (5 mm wide by 50 mm long) at an angle of 45 deg against a black background (Fig. 1B). Table 1 gives the coordinates of the yellow in bee colour space as well as those of the other colours used in the following tasks.

Discrimination of edge orientation

Three bars as described above were presented along with three similar bars oriented at an angle of -45 deg. The bars were interrupted in the centre by a black square (5×5 mm) centred on the feeding platform so that bees would not learn features of only that region during training (Fig. 1C).

Easy colour discrimination task

Bees had to discriminate between three blue (RGB scale: 0 0 255) circles (3.5 cm diameter) and three yellow (RGB scale: 255 255 0) circles of the same dimensions (Fig. 1D). The distance between the colour loci corresponding to these two lights in the bee colour hexagon is 0.67 (where 1 is the distance between the centre of the hexagon and any of its vertices). This is a very large colour distance indicating easy discriminability for bees (Dyer and Chittka, 2004).

Difficult colour discrimination task

Bees had to discriminate between three yellow (RGB scale: 255 255 0) circles (3.5 cm diameter) and three orange (RGB scale: 255 200 0) circles of the same dimensions as in the easy colour discrimination task (Fig. 1E). The colour hexagon distance between the colour loci of the yellow and orange computer-generated lights is 0.07, i.e. one-tenth of the distance between yellow and blue. The colour distance indicates that these colours are distinguishable for bees, but only with some difficulty (Dyer and Chittka, 2004).

Table 1. Colour parameters of computer-generated stimuli

	Yellow	Orange	Blue
E_u	0.16	0.15	0.2
E_b	0.36	0.34	0.72
E_g	0.77	0.69	0.36
X	0.53	0.47	0.14
Y	-0.11	-0.08	0.44

Receptor excitations of a bumblebee's UV (E_u), blue (E_b) and green (E_g) receptors for the three colours used in the experiments, as well as X/Y-coordinates in the bee's colour space, the colour hexagon. The quantum output of the LCD Samsung SyncMaster 2233RZ monitor for the yellow, orange and blue stimuli was measured from 300 to 700 nm, and converted into receptor excitations, E_i , using spectral sensitivity functions of UV, blue and green receptors of the bumblebee *Bombus terrestris* (Skorupski et al., 2007). These were converted into colour loci in the colour hexagon following procedures described elsewhere (Chittka, 1992).

Complex pattern recognition task

Bees were presented with three yellow (RGB scale: 255 255 0) silhouettes of a crab spider set in an orange (RGB scale: 255 200 0) square (side: 3.5 cm) and three yellow (RGB scale: 255 255 0) circles of the same area (diameter: 1.7 cm) also set in an orange (RGB scale: 255 200 0) square (side: 3.5 cm) (Fig. 1F).

Testing

At the end of each training condition, the bees were subjected to a test bout. During this bout, all feeding platforms had a 50 μ l drop of distilled water on them regardless of the stimulus presented behind them on the screen. The bouts lasted 4 min or until the bee returned to the colony, whichever occurred first. The choices of the bees, including repeated choices, were noted but only the proportion of choices in the first 2 min of the bout was calculated as a measure of the bee performance post-learning to guard against bees potentially choosing randomly in the later part of the bout once they realised that the feeding platforms only had water droplets on them. The proportion of correct choices made during tests for different tasks and presentation durations was tested for normality using a Kolmogorov–Smirnov test and compared with chance values (0.5) using a one-sample *t*-test.

Control experiment

To ascertain that bees trained on static stimuli could generalise to briefly presented stimuli in a test, we pre-trained 10 bees as above and then trained them to recognise a yellow (RGB scale: 255 255 0) circle of diameter 5.5 cm as a rewarding stimulus. Training procedures were similar to the stimulus detection task above. We then tested the bees with only distilled water presented on the platforms and the stimuli presented behind three feeding platforms for 500, 100, 50 and 25 ms in separate test bouts. Each of the test bouts was followed by three static training bouts so that the bees were re-trained to stimuli in between different tests. The sequence of test bouts with different presentation durations was randomised and the choices of the bees in each test were noted.

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Competing interests

The authors declare no competing financial interests.

Author contributions

V.N., P.S. and L.C. designed the experiments, V.N. conducted the experiments and analysed the data, V.N., L.C. and P.S. wrote the manuscript.

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