

Honeybees can recognise images of complex natural scenes for use as potential landmarks

Adrian G. Dyer^{1,2,*}, Marcello G. P. Rosa¹ and David H. Reser¹

¹Centre for Brain and Behaviour, Department of Physiology, Monash University, Clayton 3800, VI, Australia and ²Institut für Zoologie III (Neurobiologie) Johannes Gutenberg Universität, Mainz 55099, Germany

*Author for correspondence (e-mail: adrian.dyer@med.monash.edu.au)

Accepted 6 February 2008

SUMMARY

The ability to navigate long distances to find rewarding flowers and return home is a key factor in the survival of honeybees (*Apis mellifera*). To reliably perform this task, bees combine both odometric and landmark cues, which potentially creates a dilemma since environments rich in odometric cues might be poor in salient landmark cues, and *vice versa*. In the present study, honeybees were provided with differential conditioning to images of complex natural scenes, in order to determine if they could reliably learn to discriminate between very similar scenes, and to recognise a learnt scene from a novel distractor scene. Choices made by individual bees were modelled with signal detection theory, and bees demonstrated an ability to discriminate between perceptually similar target and distractor views despite similar spatiotemporal content of the images. In a non-rewarded transfer test bees were also able to recognise target stimuli from novel distractors. These findings indicate that visual processing in bees is sufficiently accurate for recognising views of complex scenery as potential landmarks, which would enable bees flying in a forest to use trees both as landmark and/or odometric cues.

Key words: spatial vision, landmark, differential conditioning, foraging, navigation.

INTRODUCTION

The honeybee *Apis mellifera* constitutes an excellent model for understanding how a miniature visual system can operate in complex environments (Capaldi and Dyer, 1995; Giurfa et al., 2001; Si et al., 2003; Vladusich et al., 2005; Zhang and Srinivasan, 2004). On a foraging flight in nature, a bee may fly several kilometres to visit a rewarding patch of flowers (Frisch, 1967), and honeybees typically forage from flowers in both simple (open fields) and complex (forests) environments (Steffan-Dewenter and Kuhn, 2003). On each foraging trip bees may visit several different locations (Menzel et al., 1996; Reinhard et al., 2004), and return several times after contributing the collected food to the colony (Collett, 1992; Frisch, 1967). To efficiently conduct these flights, bees employ a variety of sensory cues that include visual odometry, where the insect estimates the distance travelled by the flow of spatial information sensed by the visual system (Srinivasan et al., 2000; Srinivasan et al., 1997; Vladusich et al., 2005), and/or the use of landmarks (Chittka et al., 1995a; Chittka et al., 1995b; Collett and Zeil, 1997; Vladusich et al., 2005). Indeed, vision is a major modality in bee decision making for locating reliable food sources (Chittka and Tautz, 2003; Srinivasan et al., 2000; Vladusich et al., 2005), but currently the capability of bees to use their spatial vision to identify landmark cues is not well understood.

Previous work has demonstrated that bees use interactions between different visual cues to navigate. For example, honeybees searching for a feeder in a tunnel are more accurate when both odometric and landmark cues could be used in combination (Vladusich et al., 2005). If these cues are set in conflict then bees mainly rely on landmark cues for searching (Vladusich et al., 2005). However, if no odometric cues are present, the reliability with which landmarks might be used is decreased (Vladusich et al., 2005). These

findings fit well with the suggestion that the use of odometry by bees might serve as a context setting cue to help distinguish landmarks that appear similar (Collett and Collett, 2002). In complex foraging environments like forests, potential landmarks including trees might be so numerous that the discrimination between perceptually similar shapes is problematic for the limited visual acuity of bees; potentially creating a dilemma that bees might not be able to 'see the trees for the wood' when searching for salient landmarks in a visually rich environment. Currently, it remains unclear to what extent bees can use spatial vision to discriminate between similar scenes, or recognise a 'landmark' scene from a perceptually similar scene whilst flying to rewarding flowers (Collett and Collett, 2002; Vladusich et al., 2005).

Although it has previously been suspected that honeybees have relatively simple spatial visual capabilities for identifying landmarks (Horridge, 2005), recent studies of the spatial discrimination capabilities of honeybees suggest that, when provided with differential conditioning, fine spatial discriminations can be made (Giurfa et al., 1999; Stach et al., 2004; Stach and Giurfa, 2005). For example, bees can learn to discriminate between complex novel stimuli consisting of human faces, taken from a standard psychophysics test (Dyer et al., 2005). Furthermore, differential conditioning has revealed that bees can categorise perceptually similar stimuli (for example, landscapes *versus* non-landscape images) (Bernard et al., 2007; Zhang et al., 2004). Thus, there exists the possibility that despite having relatively poor spatial acuity compared with vertebrate vision (Land, 1997a; Land, 1997b), bees may be able to use their spatial vision to reliably identify and remember viewpoints of complex scenes experienced during normal navigation to and from the hive. If bees can learn to discriminate between such complex natural scenes this would permit individuals

to 'self select' landmarks that are useful for navigation, rather than have to rely only on infrequent salient landmarks.

Investigations where bees have received extended amounts of training to perceptually similar visual stimuli suggest that they make complex decisions about which stimulus to choose at a particular moment in time (Chittka et al., 2003; Dyer and Chittka, 2004a). For example, bumblebees trained to perceptually similar colours allocate more time to making decisions to improve accuracy, often choosing to abort an approach to a stimulus and reject it (Chittka et al., 2003). This is consistent with observations that during fine spatial discrimination tasks bees may learn to examine and reject a non-rewarded stimulus, in addition to learning the correct target stimulus (Giurfa et al., 1999). Importantly, if bees receive a punishment for visits to a distractor stimulus (bitter tasting quinine hemisulphate) they elect to improve accuracy at the cost of longer response times (Chittka et al., 2003), although the physiological mechanisms underlying this behavioural are not yet fully understood (de Brito Sanchez et al., 2005). When this type of complex decision making is observed in human behaviour it is useful to mathematically model the data using signal detection theory (Collishaw and Hole, 2000; Green and Swets, 1966). There is good evidence that modelling sophisticated decision making with signal detection theory is relevant to explaining behaviour in other animals including monkeys (Thompson and Schall, 2000), pigeons (Blough, 1967; Sole et al., 2003) and mice (Steckler, 2001). One recent study has shown signal detection theory to be applicable for modelling bumblebee behaviour (Lynn et al., 2005).

In this study, we used a differential conditioning procedure (Dyer and Chittka, 2004a; Dyer and Chittka, 2004b; Dyer et al., 2005; Giurfa, 2004; Giurfa et al., 1999; Stach et al., 2004; Stach and Giurfa, 2005) to test if honeybees can visually discriminate and subsequently recognise the types of visual scenes that might be encountered as potential landmarks if flying through a complex forest-like environment. To analyse the behavioural data, we used signal detection theory to model information on decisions made by individual bees (Green and Swets, 1966; Lynn et al., 2005; Marston, 1996).

MATERIALS AND METHODS

Experiments were conducted with honeybees (*Apis mellifera* L.) where individual bees were recruited from a 10% *ad libitum* feeding dish (Frisch, 1967), and then trained to visit an experimental site 5 m away where 25% sucrose solution was available for correct landings on a target stimulus. The bees were trained to visit stimuli on a 50 cm rotating screen display where two target and two distractor stimuli could be presented vertically on freely rotating hangers with a landing platform (Dyer et al., 2005; Horridge, 2005). This display presents stimuli at a number of spatially random positions, and it is possible to change stimuli and re-randomise arrangements during training. The advantage of the rotating display is that it permits a bee to view stimuli at an unconstrained visual angle, which may be important for bees potentially choosing to use either configural and/or feature extraction strategies for learning stimuli (Dyer et al., 2005; Efler and Ronacher, 2000).

Stimuli were 6 × 8 cm achromatic photographs of trees with a variety of branches, forming a complex visual scene like those a bee might encounter en route in a foraging trip (Fig. 1). The scenes were chosen to be perceptually very similar by including the only available low spatial frequency information in the lower left hand side of each image so as to avoid the possibility of bees using symmetry perception (Giurfa et al., 1996) or topological differences (Chen et al., 2003) in stimuli to solve the task. The brightness for

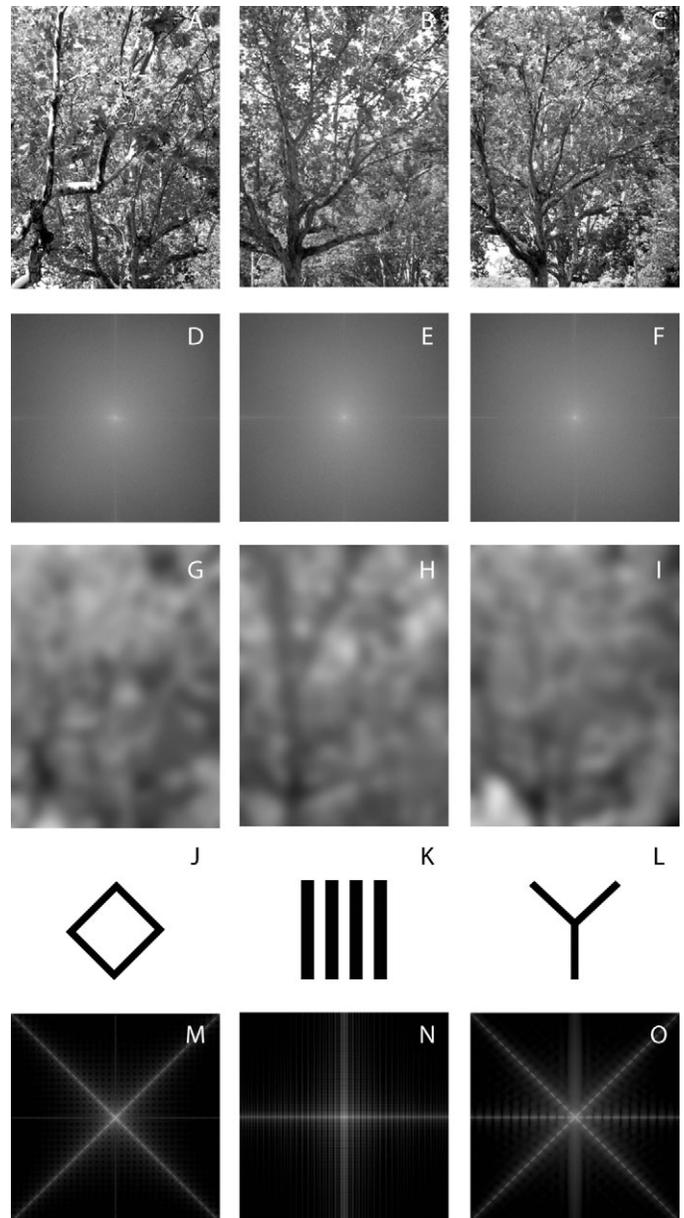


Fig. 1. Bees provided with differential conditioning can learn to discriminate between and recognise perceptually very similar complex natural stimuli. (A) A target stimulus with which bees received differential conditioning. (B) A distractor stimulus with which bees received differential conditioning. (C) A novel distractor stimulus. (D–F) Fast Fourier transforms (FFT) of images in A–C where vertical and horizontal axes show relative distribution of low (towards the centre) and high spatial (towards the edges) information in the respective images. The FFTs are almost identical (compared with images M–O below) showing that there is an approximately equivalent distribution of spatial information in the stimuli. (G–I) A representation of the images in A–C, respectively, considering the visual acuity of bee spatial vision which is approximately limited to viewing frequencies less than about $0.3 \text{ cycles deg}^{-1}$. (J–L) Angular high contrast geometric images including a diamond, square wave grating and a figure 'Y' that bees generalise to if only provided with absolute conditioning (see text for references). (M–O) FFT of angular high contrast geometric images (J–L) show relatively large differences in the distribution of low and high spatial frequency information (compare with D–F), but bees do not make use of this information to make discriminations if provided with absolute conditioning.

each image was determined in 8-bit achromatic space using ImageJ software [NIH, Bethesda, MD, USA; 1A 117 ± 63 (mean \pm s.d.); 1B 116 ± 5 ; 1C 113 ± 58], and mean luminance for respective images was measured in the experimental conditions with a Gossen Lunasix F exposure meter (Postfach, Germany) 15 cm from the stimuli (1A 316 cd m^{-2} ; 1B 316 cd m^{-2} ; 1C 316 cd m^{-2}); thus the overall intensity of the signals provided by each of the stimuli was practically identical. Fast Fourier transform (FFT) (Zhang et al., 2004) (ImageJ software) confirmed that the stimulus images (Fig. 1A–F) were highly similar with respect to spatial frequency, compared to angular high contrast geometric shapes like a diamond, square wave grating or a figure ‘Y’ (Fig. 1J–O). Previous studies have demonstrated that bees provided with absolute conditioning generalise between these high contrast shapes (Gould and Gould, 1988; Horridge, 2005).

Finally, we found that if honeybees were only provided with absolute conditioning to a target stimulus (e.g. Fig. 1A) they failed to discriminate this stimulus from the distractors, showing these were indeed perceptually similar scenes for bee vision. To visually represent how these stimuli might appear to the visual system of a bee (Chittka and Geiger, 1995b) Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA) was used to apply a 30 pixel Gaussian blur filter to each $236 \text{ pixel cm}^{-1}$ image. This application of a Gaussian filter has the effect of making a $0.3 \text{ cycles deg}^{-1}$ square wave grating (0.67 contrast) indistinguishable for a human viewer at a viewing distance of 57 cm, which approximately equates to reducing the visual resolution of each scene to available data for the limit of visual acuity of $0.3 \text{ cycles deg}^{-1}$ for honeybees (Srinivasan and Lehrer, 1988). Although the optical systems of bees and humans operate on different principles of resolving spatial information (Dyer and Williams, 2005; Land, 1997a; Land, 1997b), this technique allows a reasonable representation of how the stimuli might appear to a visual system limited to a visual acuity of approximately $0.3 \text{ cycles deg}^{-1}$ (Fig. 1G–I).

Experiment 1

During training, each individual bee ($N=10$) was first provided with absolute conditioning to target stimuli (Fig. 1A) for at least 15 visits. Each time a bee landed it was able to collect a $10 \mu\text{l}$ drop of 25% sucrose, and a second drop was also made available on a Plexiglas™ spoon presented next to the landing stage. When the bee climbed onto the spoon it was moved 1 m away from the screen so that stimuli could be exchanged and rotated (Dyer et al., 2005). Once a bee had learnt to fly to the apparatus correctly it was given differential conditioning to the target stimulus, and a similar distractor stimulus (Fig. 1B), which contained 0.012% quinine (Chittka et al., 2003). The punishment leads to motivation to perform a task to a high level of accuracy (Chittka et al., 2003). It was important that the bee first received some absolute conditioning before training with quinine on the distractor stimulus, otherwise a bee could encounter quinine before becoming highly motivated and leave the test site. When a bee became satiated it returned to the hive and all the test equipment was cleaned with 20% ethanol.

After each bee had made 120 responses to stimuli it was given a non-rewarded test with fresh stimuli where the first 20 landings on stimuli were counted to exclude any possible use of olfactory cues. After this non-rewarded test, bees were provided with reinforcement training for 10 visits to ensure motivation, and then given a non-rewarded transfer test that included the target stimulus and a novel stimulus (Fig. 1C). The two phases were thus used to separately evaluate both bee discrimination of learnt stimuli (which is a precondition to being able to recognise the target stimulus) and

then bee ability to recognise a learnt stimulus from a novel distractor.

Experiment 2

Experiment 2 tested an additional group of bees ($N=10$) to evaluate if bees can discriminate between the stimuli shown in Fig. 1B and C. Following differential conditioning to Fig. 1B as a target and Fig. 1C as a distractor, the bees were then given an additional non-rewarded transfer test to evaluate if these bees could recognise the stimulus in Fig. 1B from a novel stimulus in Fig. 1A. Experiment 2 thus controlled for the possibility of any discrimination in experiment 1 being solely on the basis of an innate preference for stimulus in Fig. 1A.

Experiment 3

Experiment 3 tested if honeybees could potentially learn complex natural stimuli in a shorter period of training than was provided in experiment 1 or 2, and to evaluate if bees might be able to learn the task in a context where the distractor stimulus did not contain any bitter solution. Experiment 3 tested an additional group of bees ($N=10$) to evaluate if bees can discriminate between the stimuli in Fig. 1B and 1C even if differential conditioning is for only 40 responses to stimuli, and the distractor does not contain any form of punishment. This experiment thus attempts to understand if bees might be able to learn fine discrimination tasks in an ecologically relevant scenario.

Analysis of bee choices

In the current study, a bee approaching a stimulus had several possible responses depending upon whether the stimulus was perceived as the target or the distractor. The bee could make a correct decision and land on the platform of the target stimulus, or an incorrect decision and land on the distractor platform. Alternatively, the bee could incorrectly reject the target stimulus, or correctly reject the distractor stimulus. A rejection was defined as a clear approach to the stimulus to a distance of less than 10 cm, slowing to look at the stimulus, and then making a saccadic turn and flying away without making any contact with the landing platform (Fig. 2).

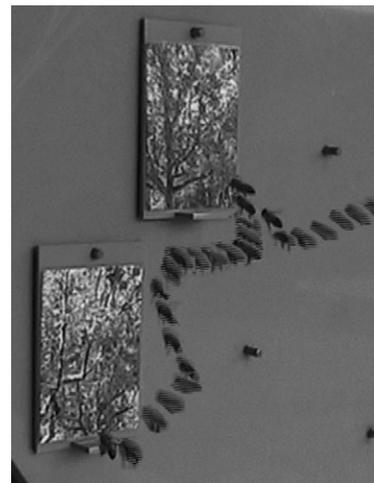


Fig. 2. Video composite at 33 ms intervals of a honeybee flying from right to left to approach a distractor stimulus (upper image), correctly rejecting it, and then viewing and correctly choosing a target stimulus (lower image). The sequence illustrates how bees make decisions to reject or select stimuli following a visual inspection.

Table 1. Possible decisions a bee can make after having made an approach to a stimulus, and the determination of proportion (probability) of either correct hits or incorrect errors

	Land	Reject	Proportion
Target	Hit	Miss	Hit/(hit + miss)
Distractor	False alarm	Correct rejection	Error/(error + rejection)

The range of possible responses a bee could make are shown in Table 1. To model this type of complex decision making, it is possible to use signal detection theory. Briefly, a matrix is determined to calculate the probability of correct choices (P_c) to the target stimulus (equation 1) and the probability of incorrect choices (P_i) to the distractor stimulus (equation 2). For each of these two probabilities it is then possible to calculate a Z score, and the difference between the Z scores defines a variable d' , where a d' score of 0 or less indicates discrimination at chance level, whilst a d' score of 3.29 corresponds with perfect performance (Collishaw and Hole, 2000; Green and Swets, 1966; Miller, 1996). To statistically evaluate if bee choices for the target stimulus could be reliably differentiated from chance performance, data was tested for normal distribution and then a one sample t -test was used to compare the d' scores for the bees against a score of 0 that might be expected for random choices. For convenience percentage discrimination (correct landings/total landings) is also provided, but all statistics were computed from d' scores (Collishaw and Hole, 2000).

$$P_c = \frac{[\text{total correct landings} / (\text{total correct landings} + \text{total incorrect aborts})]}{1}, \quad (1)$$

$$P_i = \frac{[\text{total incorrect landings} / (\text{total incorrect landings} + \text{total correct aborts})]}{1}. \quad (2)$$

RESULTS
Experiment 1

Fig. 1D–F shows FFT for the three stimuli (Fig. 1A–C) used, indicating that the overall spatial composition of each image was approximately equivalent. Following absolute conditioning to the target stimulus for 15 choices, bee choices for this stimulus were close to random (50.7% in first 10 choices; $t=0.188$, $d.f.=9$, $P=0.855$) at the beginning of the differential conditioning. This shows that the visual scenes used as stimuli were perceptually very similar for honeybees, as they generalised stimuli prior to receiving differential conditioning. However, bees gradually learnt to discriminate between very similar target and distractor stimuli (Fig. 3).

Fig. 2 shows an example of a bee visually inspecting a distractor stimulus and making a decision to reject that stimulus, before flying on to inspect a target stimulus and make a decision to land. The mean distance at which the bees hovered to visually inspect the stimuli was 5.3 ± 0.5 cm (mean \pm s.d.). The evidence that individual bees that have been provided with differential conditioning make complex decisions about whether to land or reject either the target or distractor stimulus, indicates that signal detection theory is appropriate for modelling the behavioural data. Fig. 3B shows the frequency with which bees aborted inspection of either the target or the distractor stimuli as they gained experience with the stimuli. There was a significant negative correlation of bees aborting inspection of the target stimulus with increasing experience (Spearman's $\rho=-0.633$, $N=12$, $P=0.027$). For the distractor stimulus there was not a significant correlation of aborts with increasing experience (Spearman's $\rho=0.344$, $N=12$, $P=0.273$), although there was a slight trend of more aborts which may indicate that bees extract

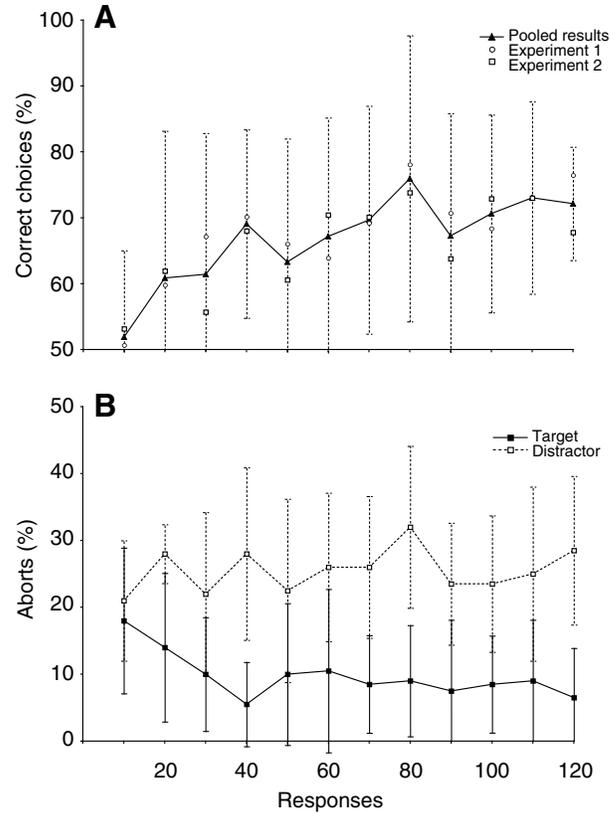


Fig. 3. Frequency of stimulus selection plotted against the total number of response decisions (landings and rejections). (A) Honeybee acquisition (± 1 s.d.) whilst being trained with differential conditioning to target and distractor stimuli representing similar complex scenes that might be encountered in a forest. Acquisition in experiment 1 is indicated by circles and in experiment 2 by squares. Experiment 2 used a different target and distractor combination; acquisition was very similar, and triangles and bold line show pooled data. (B) Pooled data for the frequency with which bees made abort flights (bees approached a stimulus and then turned away and left without making any contact); the solid line shows a significant negative correlation of aborts to target stimuli with increasing experience (see text for statistics), and the broken line shows no significant correlation of aborts to the distractor stimuli.

information about both target and distractor stimuli (Stach and Giurfa, 2005).

The mean frequency of correct choices for landings on the target stimulus (Fig. 1A vs B) in the non-rewarded test was $69.0 \pm 8.4\%$ (\pm s.d.), and the mean d' value for bee responses was 1.19 ± 0.63 . The ability of bees to correctly respond to the target stimulus was significantly different from chance (one sample t -test, $t=5.987$, $d.f.=9$, $P<0.001$), demonstrating that the bees could discriminate between the learnt target and distractor stimuli. This finding is also consistent with the last 50 bee responses (Fig. 3A) during conditioning, where the mean correct choice was $71.8 \pm 4.9\%$.

To potentially utilise these discrimination abilities in real-world scenarios, it is necessary that the bees correctly recognise the target stimulus in the presence of novel distractors. In the non-rewarded transfer test, bees were able to recognise the target stimulus from a novel but similar scene (Fig. 1A vs C) with $61.5 \pm 5.3\%$ accuracy. The mean d' value for bee responses in the recognition condition was 0.58 ± 0.34 , and the ability of bees to correctly respond to the target stimulus was significantly different from chance (one sample

t -test, $t=5.332$, $d.f.=9$, $P<0.001$). Thus, bees can resolve between photos of familiar forest scenes and novel, but perceptually similar, sylvan panoramas.

Experiment 2

The mean frequency of correct choices for landings on the target stimulus (Fig. 1B vs C) in the non-rewarded test was $70.5\pm 11.9\%$, and the mean d' value for bee responses was 1.07 ± 0.55 . The ability of bees to correctly respond to the target stimulus was significantly different from chance (one sample t -test, $t=6.108$, $d.f.=9$, $P<0.001$), demonstrating that the bees could discriminate between the stimuli used as distractors in experiment 1.

In the non-rewarded transfer test, bees were able to recognise the target stimulus (Fig. 1B) from a novel but similar scene (i.e. Fig. 1B vs A) with $70.6\pm 15.6\%$ accuracy. The mean d' value for bee responses in the recognition condition was 1.01 ± 0.52 , and the ability of bees to correctly respond to the target stimulus was significantly different from chance (one sample t -test, $t=6.214$, $d.f.=9$, $P<0.001$). This confirms that bees can recognise a familiar stimulus from a novel, but perceptually similar distractor as in experiment 1. The result also indicates that the recognition of the stimulus (as in Fig. 1A) in experiment 1 was not due to some preference by bees for this stimulus, as in experiment 2 bees learn to avoid this stimulus.

Experiment 3

Experiment 3 tested if honeybees could potentially learn complex natural stimuli in a shorter period of training than was provided in experiment 1 or 2, and to evaluate if bees might be able to learn the task in a context where the distractor stimulus did not contain any bitter solution. Following differential conditioning for only 40 responses (Fig. 4) to the same stimuli as used in experiment 2 (Fig. 1B vs C), in the subsequent non-rewarded test bees were able

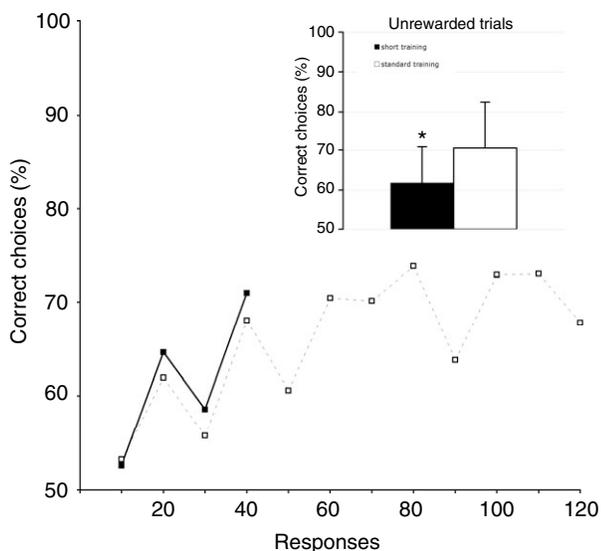


Fig. 4. Bee acquisition of target from distractor. The bold line shows bee ($N=10$) acquisition with only 40 responses to stimuli, and the broken line bee ($N=10$) acquisition for 120 responses with quinine hemisulphate used as a punishment for landings on distractor stimuli (same data as in Fig. 3). Insert shows frequency of correct choices in subsequent non-rewarded tests where bees trained for only 40 responses were significantly poorer at recognising the target, but were still able to perform significantly better than chance (see text for statistics).

to choose the target stimulus with $61.9\pm 8.9\%$ accuracy and a mean d' of 0.69 ± 0.30 , which was significantly different from chance (one sample t -test, $t=6.484$, $d.f.=9$, $P<0.001$). Thus, building on experiments 1 and 2, experiment 3 demonstrates that bees may learn a fine discrimination task of natural scenes with a relatively short amount of exposure to the stimuli, and even when the distractor does not contain any form of punishment. To compare if the results from experiment 3 differed significantly from experiment 2 (which used longer training and quinine punishment) d' data was compared with an independent samples t -test ($t=2.189$, $d.f.=18$, $P=0.042$). Thus, whilst bees can learn to discriminate complex scenes in a reasonably short time frame, continuing experience (with punishment) as in experiment 2 does convey some benefit. The current experiments do not allow, however, a dissection of the relative contribution of either the quinine and/or training length.

DISCUSSION

Consistent with the finding that bees learn to make very fine discriminations involved in recognising human faces (Dyer et al., 2005), this study shows that recognition of scenery encountered during flight may be relevant to how bees learn to visually navigate. This could enable foraging bees to use complex combinations of visual objects that exist in natural environments as navigational landmarks. Previous work has shown that bees are able to navigate maze-like structures if provided with salient visual cues (Zhang et al., 1996; Zhang et al., 2000), which could potentially provide bees with the ability to judge distance based on number of landmarks *en route* to flower resources (Chittka and Geiger, 1995a; Zhang and Srinivasan, 2004). This current study shows that experienced bees can potentially use complex natural scenes as landmarks, even when these are perceptually very similar to other novel scenes encountered between the hive and feeding sites. Although we only evaluated one type of complex natural scene (trees in a forest), it is reasonable to conclude from our findings that bees can potentially use their vision for a wide variety of natural landscapes that might be encountered during foraging (Chittka et al., 1992; Zhang et al., 2004), or when turning back to look at a feeding site and/or nest (Lehrer, 1991; Lehrer, 1993; Zeil and Wittmann, 1993).

It is clear from a number of studies that differential conditioning leads to significantly better performance in discriminating between perceptually similar stimuli than absolute conditioning (Dyer and Chittka, 2004a; Dyer and Chittka, 2004b; Dyer et al., 2005; Giurfa, 2004; Giurfa et al., 1999; Stach et al., 2004; Stach and Giurfa, 2005). The reasons underlying this could include the ability of the bee brain to learn relevant dimensions from both target and distractor stimuli (Giurfa et al., 1999) and/or the development of attention-like mechanisms (Dyer, 2007; Giurfa, 2004). In another insect model, *Drosophila*, it has recently been shown that experience with visual stimuli improves feature extraction from complex visual stimuli, and that the mushroom body region of the brain is critical in shape feature extraction (Peng et al., 2007). Two possible mechanisms by which insects might recognise visual stimuli include a retinotopic-template strategy and/or the use of specific features extracted from a scene (Efler and Ronacher, 2000; Giger and Srinivasan, 1995; Horridge, 2005; Stach et al., 2004; Stach and Giurfa, 2005). In *Drosophila* (Dill et al., 1993; Peng et al., 2007) and ants (Cartwright and Collett, 1983; Graham et al., 2007) there is evidence that these insects use a retinotopic-template strategy, and in bees there is evidence that individuals use feature extraction, which may develop into configural type processing with experience (Stach et al., 2004; Stach and Giurfa, 2005). There also exists the possibility that

individual insects use different visual strategies depending upon the context of the task to be solved (Efler and Ronacher, 2000; Giurfa et al., 1999; Stach and Giurfa, 2005), which is interesting in relation to the changes that occur in *Drosophila* visual processing depending upon level of experience (Peng et al., 2007). In this study the purpose was to evaluate if bees might be capable of solving a complex visual discrimination that is relevant to their foraging lifestyle. With complex scene stimuli, it is difficult to determine what visual strategy the bee visual system is using to solve the task, but the data is indicative that bees can solve a task of recognising complex scenes reliably.

For spatial vision discrimination tasks, differential conditioning promotes bees to learn the entire visual pattern, whereas absolute conditioning restricts learning to the visual content in the lower regions of stimuli (Giurfa et al., 1999). This is consistent with observations that for differential conditioning to complex stimuli, such as faces, honeybee visual processing is completely disrupted by stimulus inversion (Dyer et al., 2005), suggesting the possibility that animals with relatively simple nervous systems have the potential to use configural type processing to solve tasks (Stach et al., 2004; von der Emde and Fetz, 2007). In this study, analysis of the frequency of aborted flights to either target or distractor stimuli (Fig. 3B) indicated that extracting information about the target stimulus were the major visual strategy used. This is also evidenced by the observation that bees could recognise a learnt target from perceptually similar novel distractors. This finding is consistent with previous studies indicating that target stimuli contain significant visual information that the bees visual system can extract (Dyer et al., 2005; Stach et al., 2004; Stach and Giurfa, 2005).

An important consideration is whether a bee flying in a natural environment receives sufficient experience with complex visual scenes to enable learning for navigation purposes. Figs 3 and 4 indicate that the spatial task of discriminating between the target and distractor stimuli is learnt well after about 20–40 responses are made, and this is consistent with the rates with which honeybees learn to discriminate between human faces (Dyer et al., 2005) or spatial gratings (Srinivasan and Lehrer, 1988). As a forager, an individual bee typically makes 10–15 foraging bouts per day (Winston, 1987), suggesting that learning a complex visual scene as a landmark is realistic within the time frame of a bee visiting a particular flower patch within a forest. In addition, bees may learn a scene faster than estimated above if there is a voluntary effort to inspect a scene, as has been previously observed from bees at feeding sites (Horridge, 2005). Optic flow estimates (Srinivasan et al., 2000) and landmark discriminations (Chittka et al., 1995a; Chittka et al., 1995b; Collett and Zeil, 1997) are maximally useful in different types of visual environment (e.g. densely vegetated vs relatively open fields), and one can hypothesise that honeybees will take full advantage of these different types of visual information in achieving a computationally robust representation of the intended route (Vladusich et al., 2005). This current study thus demonstrates that bees potentially have the ability to visually learn to discriminate similar complex natural scenes that could be used as ‘landmarks’ even in a dense forest type scenario where there are no salient references.

In addition to showing that bees are capable of recognising complex visual scenes, the data in this study also indicate that signal detection theory is useful in quantifying the decision making of bees. In particular, Fig. 2 and Fig. 3B show that rejection of stimuli, in addition to landing choices, is an important component of the decision making process by honeybees and it is thus useful to record

these events for an optimal analysis. This is potentially a powerful analysis technique for understanding the factors determining invertebrate decision making (Lynn et al., 2005). Studies on bumblebee colour learning and evidence of peak shift discrimination suggest a dynamic behaviour where the bee brain learns to make decisions based upon level of experience with perceptually similar targets and distractors (Lynn et al., 2005). Consistent with their application of signal detection theory for cognitive learning, the present study reinforces the idea that the learning process is dynamic and involves multiple possible outcomes which the bee brain can manage to sort through to solve complex tasks. Understanding this process can, in turn, provide a more direct route for understanding the mechanisms used by insects to solve complex visual tasks, potentially improving the design of semiautonomous machines capable of operating in demanding visual environments (Franceschini et al., 2007).

We are grateful to Friederike Schweizerhof and Verena Reinhardt for assistance in conducting the bee experiments, and Michael Grant for assistance in maintaining honeybee colonies. A.G.D. is grateful to the Alexander von Humboldt Foundation, USAF AOARD (064038&074080) for support. The US Government is authorised to reproduce and distribute reprints for Government purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Research Laboratory or the US Government.

REFERENCES

- Bernard, J., Stach, S. and Giurfa, M. (2007). Categorization of visual stimuli in the honeybee *Apis mellifera*. *Anim. Cogn.* **9**, 257–270.
- Bloough, D. S. (1967). Stimulus generalisation as signal detection in pigeons. *Science* **158**, 940–941.
- Capaldi, E. A. and Dyer, F. C. (1995). Landmarks and dance orientation in the honeybee *Apis mellifera*. *Naturwissenschaften* **82**, 245–247.
- Cartwright, B. A. and Collett, T. S. (1983). Landmark learning in bees-experiments and models. *J. Comp. Physiol.* **151**, 521–543.
- Chen, L., Zhang, S. and Srinivasan, M. V. (2003). Global perception in small brains: topological pattern recognition in honey bees. *Proc. Natl. Acad. Sci. USA* **100**, 6884–6889.
- Chittka, L. and Geiger, K. (1995a). Can honeybees count landmarks? *Anim. Behav.* **49**, 159–164.
- Chittka, L. and Geiger, K. (1995b). Honeybee long-distance orientation in a controlled environment. *Ethology* **99**, 117–126.
- Chittka, L. and Tautz, J. (2003). The spectral input to honeybee visual odometry. *J. Exp. Biol.* **206**, 2393–2397.
- Chittka, L., Bonn, A., Geiger, K., Hellstern, F., Klein, J., Knoll, P., Koch, G., Meuser, S. and Menzel, R. (1992). Do bees navigate by means of snapshot memory pictures? In *Rhythmogenesis in Neurons and Networks: Proceedings of the 20th Göttingen Neurobiology Conference* (ed. N. Elsner and D. W. Richter), p. 694. Stuttgart: Georg Thieme Verlag.
- Chittka, L., Kunze, J. and Geiger, K. (1995a). The influences of landmarks on distance estimation of honeybees. *Anim. Behav.* **50**, 23–31.
- Chittka, L., Kunze, J., Shipman, C. and Buchmann, S. L. (1995b). The significance of landmarks for path integration of homing honey bee foragers. *Naturwissenschaften* **82**, 341–343.
- Chittka, L., Dyer, A. G., Bock, F. and Dornhaus, A. (2003). Bees trade off foraging speed for accuracy. *Nature* **424**, 388–388.
- Collett, T. S. (1992). Landmark learning and guidance in insects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **337**, 295–303.
- Collett, T. S. and Collett, M. (2002). Memory use in insect visual navigation. *Nat. Rev. Neurosci.* **3**, 542–552.
- Collett, T. S. and Zeil, J. (1997). The selection and use of landmarks by insects. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 41–65. Basel: Birkhäuser Verlag.
- Collishaw, S. M. and Hole, G. J. (2000). Featural and configural processes in the recognition of faces of different familiarity. *Perception* **29**, 893–909.
- de Brito Sanchez, M. G., Giurfa, M., de Paula Mota, T. R. and Gauthier, M. (2005). Electrophysiological and behavioural characterization of gustatory responses to antennal ‘bitter’ taste in honeybees. *Eur. J. Neurosci.* **22**, 3161–3170.
- Dill, M., Wolf, R. and Heisenberg, M. (1993). Visual pattern recognition in *Drosophila* involves retinotopic matching. *Nature* **365**, 751–753.
- Dyer, A. G. (2007). Windy condition affected colour discrimination in bumblebees (Hymenoptera: Apidae: Bombus). *Entomol. Gen.* **30**, 165–166.
- Dyer, A. G. and Chittka, L. (2004a). Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *J. Comp. Physiol. A* **190**, 759–763.
- Dyer, A. G. and Chittka, L. (2004b). Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* **91**, 224–227.
- Dyer, A. G. and Williams, S. K. (2005). Mechano-optical lens array to simulate insect vision photographically. *Imag. Sci. J.* **53**, 209–213.

- Dyer, A. G., Neumeyer, C. and Chittka, L.** (2005). Honeybee (*Apis mellifera*) vision can discriminate between and recognise images of human faces. *J. Exp. Biol.* **208**, 4709-4714.
- Efler, D. and Ronacher, B.** (2000). Evidence against retinoptic-template matching in honeybees pattern recognition. *Vis. Res.* **40**, 3391-3403.
- Franceschini, N., Ruffier, F. and Serres, J.** (2007). A bio-inspired flying robot sheds light on insect piloting abilities. *Curr. Biol.* **17**, 329-335.
- Frisch, K. v.** (1967). *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard University Press.
- Giger, A. D. and Srinivasan, M. V.** (1995). Pattern recognition in honeybees: eidetic imagery and orientation discrimination. *J. Comp. Physiol. A* **176**, 791-795.
- Giurfa, M.** (2004). Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften* **91**, 228-231.
- Giurfa, M., Eichmann, B. and Menzel, R.** (1996). Symmetry perception in an insect. *Nature* **382**, 458-461.
- Giurfa, M., Hammer, M., Stach, S., Stollhoff, N., Müller-Deisig, N. and Mizrycki, C.** (1999). Pattern learning by honeybees: conditioning procedure and recognition strategy. *Anim. Behav.* **57**, 315-324.
- Giurfa, M., Zhang, S., Jenett, A., Menzel, R. and Srinivasan, M. V.** (2001). The concepts of 'sameness' and 'difference' in an insect. *Nature* **410**, 930-933.
- Gould, J. L. and Gould, C. G.** (1988). *The Honey Bee*. New York: Scientific American Library.
- Graham, P., Durier, V. and Collett, T.** (2007). The co-activation of snapshot memories in wood ants. *J. Exp. Biol.* **210**, 2128-2136.
- Green, D. H. and Swets, J. A.** (1966). *Signal Detection Theory and Psychophysics*. New York: Wiley.
- Horridge, A.** (2005a). Recognition of a familiar place by the honeybee (*Apis mellifera*). *J. Comp. Physiol. A* **191**, 301-316.
- Land, M. F.** (1997a). The resolution of insect compound eyes. *Isr. J. Plant Sci.* **45**, 79-91.
- Land, M. F.** (1997b). Visual acuity in insects. *Annu. Rev. Entomol.* **42**, 147-177.
- Lehrer, M.** (1991). Bees which turn back and look. *Naturwissenschaften* **78**, 274-276.
- Lehrer, M.** (1993). Why do bees turn back and look? *J. Comp. Physiol. A* **172**, 549-563.
- Lynn, S. K., Cnaani, J. and Papaj, D. R.** (2005). Peak shift discrimination learning as a mechanism of signal evolution. *Evolution* **59**, 1300-1305.
- Marston, H. M.** (1996). Analysis of cognitive function in animals, the value of SDT. *Brain Res. Cogn. Brain Res.* **3**, 269-277.
- Menzel, R., Geiger, K., Chittka, L., Joerges, J., Kunze, J. and Müller, U.** (1996). The knowledge base of bee navigation. *J. Exp. Biol.* **199**, 141-146.
- Miller, J.** (1996). The sampling distribution of *d*. *Percept. Psychophys.* **58**, 65-72.
- Peng, Y., Xi, W., Zhang, W., Zhang, K. and Guo, A.** (2007). Experience improves feature extraction in *Drosophila*. *J. Neurosci.* **27**, 5139-5145.
- Reinhard, J., Srinivasan, M. V., Guez, D. and Zhang, S. W.** (2004). Floral scents induce recall of navigational and visual memories in honeybees. *J. Exp. Biol.* **207**, 4371-4381.
- Si, A., Srinivasan, M. V. and Zhang, S.** (2003). Honeybee navigation: properties of the visually driven 'odometer'. *J. Exp. Biol.* **206**, 1265-1273.
- Sole, L. M., Shettleworth, S. J. and Bennett, P. J.** (2003). Uncertainty in pigeons. *Psychon. Bull. Rev.* **10**, 738-745.
- Srinivasan, M. V. and Lehrer, M.** (1988). Spatial acuity of honeybee vision and its spectral properties. *J. Comp. Physiol. A* **162**, 159-172.
- Srinivasan, M. V., Zhang, S. W. and Bidwell, N. J.** (1997). Visually mediated odometry in honeybees. *J. Exp. Biol.* **200**, 2513-2522.
- Srinivasan, M. V., Zhang, S., Altwein, M. and Tautz, J.** (2000). Honeybee navigation: nature and calibration of the 'odometer'. *Science* **287**, 851-853.
- Stach, S. and Giurfa, M.** (2005). The influence of training length on generalization of visual feature assemblies in honeybees. *Behav. Brain Res.* **161**, 8-17.
- Stach, S., Bernard, J. and Giurfa, M.** (2004). Local-feature assembling in the visual pattern recognition and generalization in honeybees. *Nature* **429**, 758-761.
- Steckler, T.** (2001). Using signal detection theory for analysis of operant performance in mice. *Behav. Brain Res.* **125**, 237-248.
- Steffan-Dewenter, I. and Kuhn, A.** (2003). Honeybee foraging in differentially structured landscapes. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, 569-575.
- Thompson, K. G. and Schall, J. D.** (2000). Antecedents and correlates of visual attention and awareness in macaque prefrontal cortex. *Vis. Res.* **40**, 1523-1538.
- Vladusich, T., Hemmi, J. M., Srinivasan, M. V. and Zeil, J.** (2005). Interactions of visual odometry and landmark guidance during food search in honeybees. *J. Exp. Biol.* **208**, 4123-4135.
- von der Emde, G. and Fetz, S.** (2007). Distance, shape and more: recognition of object features during active electrolocation in a weakly electric fish. *J. Exp. Biol.* **210**, 3082-3095.
- Winston, M.** (1987). *The Biology of the Honeybee*. Cambridge: Harvard University Press.
- Zeil, J. and Wittmann, D.** (1993). Landmark orientation during the approach to the nest in the stingless bee *Trigona (Tetragonisca) angustula* (Apidae, Meliponinae). *Insectes Soc.* **40**, 381-389.
- Zhang, S. W. and Srinivasan, M. V.** (2004). Exploration of cognitive capacity in honeybees: higher functions emerge from a small brain. In *Complex Worlds from Simpler Nervous Systems* (ed. F. R. Prete), pp. 41-74. Cambridge: The MIT Press.
- Zhang, S. W., Bartsch, K. and Srinivasan, M. V.** (1996). Maze learning by honeybees. *Neurobiol. Learn. Mem.* **66**, 267-282.
- Zhang, S. W., Mizutani, A. and Srinivasan, M. V.** (2000). Maze navigation by honeybees: learning path regularity. *Learn. Mem.* **7**, 364-374.
- Zhang, S. W., Srinivasan, M. V., Zhu, H. and Wong, J.** (2004). Grouping of visual objects by honeybees. *J. Exp. Biol.* **207**, 3289-3298.