RESEARCH ARTICLE

Seeing near and seeing far; behavioural evidence for dual mechanisms of pattern vision in the honeybee (*Apis mellifera*)

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SUMMARY

Visual perception is a primary modality for interacting with complex environments. Recent work has shown that the brain and visual system of the honeybee is able, in some cases, to learn complex spatial relationships, while in other cases, bee vision is relatively rudimentary and based upon simple elemental-type visual processing. In the present study, we test the ability of honeybees to learn 4-bar asymmetric patterns in a Y-maze with aversive–appetitive differential conditioning. In Experiment 1, a group of bees were trained at a small visual angle of 50 deg by constraining individuals to the decision chamber within the Y-maze. Bees learned this task, and were able to solve the task even in the presence of background noise. However, these bees failed to solve the task when the stimuli were presented at a novel visual angle of 100 deg. In Experiment 2, a separate group of bees were trained to sets of 4-bar asymmetric patterns that excluded retinotopic matching and, in this case, bees learned the configural rule describing stimuli at a visual angle of approximately 50 deg, and this allowed the bees to solve the task when the stimuli were presented at a novel vision angle of 100 deg. This shows that the bee brain contains multiple mechanisms for pattern recognition, and what a bee sees is very dependent upon the specific experience that it receives. These multiple mechanisms would allow bees to interact with complex environments to solve tasks like recognising landmarks at variable distances or quickly discriminating between rewarding/non-rewarding flowers at reasonable constant visual angles.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/3/379/DC1

Key words: bee, landmark, Y-maze, visual angle.

INTRODUCTION

The ability of a visual system to construct meaningful representations of a scene is one of the primary ways that animals interact with their environment. Whilst honeybees have the capacity to sense their environment using a variety of sensory modalities, including olfaction (Reinhard et al., 2004), taste (Avarguès-Weber et al., 2010a; de Brito Sanchez et al., 2005), magnetoreception (Hsu and Li, 1994; Srinivasan, 2011) and tactile perception (Kevan and Lane, 1985; Scheiner et al., 2005), vision is the primary sensory channel for the remote detection and discrimination of the spatial characteristics of stimuli (Avarguès-Weber et al., 2010c; Avarguès-Weber et al., 2011; Horridge, 2009a; Horridge, 2009b; Srinivasan, 2011). Indeed, honeybees have a complex visual system that allows for reliable colour (Dyer et al., 2011) or spatial (Avarguès-Weber et al., 2010b; Avarguès-Weber et al., 2011; Horridge, 2009a; Horridge, 2009b; Srinivasan, 2011) vision.

The types of visual tasks that honeybees perform are quite varied. For example, a foraging honeybee may leave a hive and (i) fly several hundred metres through a treed landscape using odometric and landscape cues to judge the distance to a patch containing rewarding flowers (Dyer et al., 2008a; Srinivasan et al., 2000; Vladusich et al., 2005), (ii) at the flower patch detect potential target flowers at a distance and recognise rewarding flowers at 'close' range against background noise using spatial (Dafni et al., 1997), achromatic (Hempel de Ibarra and Vorobyev, 2009) and colour cues (Chittka and Wells, 2004; Dyer et al., 2008b; Giurfa et al., 1996;

Hempel de Ibarra and Vorobyev, 2009), and (iii) finally, fly back to the hive and recognise the precise location using a variety of orientation and landmark cues (Dyer et al., 2008a; Srinivasan, 2011; Vladusich et al., 2005). These different tasks are likely to place very different demands upon a visual system.

An enduring question about sensory systems is the nature in which they can be trained based upon individual experience. At least in primate models there is overwhelming evidence of behavioural plasticity; what a subject perceives from a particular stimuli set is heavily dependent upon the level of experience with the stimuli. For example, the capacity for humans to read (Ahissar et al., 2009), recognise faces (Collishaw and Hole, 2000; Maurer et al., 2002) or even identify fingerprints (Busey and Vanderkolk, 2005) and abstract 'Greeble' figures (Gauthier and Tarr, 1997) is dependent upon the level of experience with a particular class of stimuli. Whilst it has been assumed that comparatively simple insect brains only allow for vision that is mediated by 'hard-wired' physiological mechanisms (Backhaus et al., 1987; Horridge, 2000; Horridge, 2009a; Horridge, 2009b), several recent studies have shown that what a honeybee perceives is very dependent upon the type of visual experience that an individual animal receives (Avarguès-Weber et al., 2011; Dyer et al., 2008a; Giurfa et al., 1999; Stach et al., 2004; Stach and Giurfa, 2005), and comparative studies of cognitive capacity are of high value (Zhang and Srinivasan, 2004; Dyer, 2012). For example, considering pattern vision, the level of discrimination by an individual animal is very dependent upon whether a target

stimulus is learned in isolation, which is termed absolute conditioning, or whether a target stimulus is learned in relation to a perceptually similar distractor, which is termed differential conditioning (Giurfa et al., 1999). A plausible explanation for this very different learning of the same 'target' stimulus is that there is a development of selective attention in a honeybee brain depending upon the level of experience (Giurfa, 2004; Giurfa et al., 1999). The work on pattern vision has been subsequently extended to show that the length of training with differential conditioning significantly influences the amount of generalisation or specificity that an individual honeybee demonstrates (Stach and Giurfa, 2005). Following extensive differential conditioning, honeybee brains can learn to assemble complex patterns (Stach et al., 2004), which can even lead to the reliable recognition of face stimuli using configural-type visual mechanisms (Avarguès-Weber et al., 2010b; Dyer et al., 2005; Dyer and Vuong, 2008). These findings largely confirm the conceptual framework of configural or holistic processing proposed for honeybee vision by previous researchers (Hertz, 1929; Wehner, 1967; Wehner, 1981).

Fine levels of discrimination capability for honeybee vision following differential conditioning (Avarguès-Weber et al., 2010b; Dyer et al., 2005; Dyer et al., 2008a; Dyer and Vuong, 2008; Zhang et al., 2004) are not consistent with other studies, which reveal that, in many cases, honeybee spatial vision is simple and based on low-level feature detectors that combine to create elemental cues (Horridge, 1996; Horridge, 2000; Horridge, 2003a; Horridge, 2003b; Horridge, 2009a; Horridge, 2009b), and the coincidence of these elemental cues are remembered as a retinotopic label for a target image such that bees generalise between stimuli containing similar cues (Horridge, 2009a). For example, when training honeybees in a Y-maze apparatus to a 4-bar asymmetric target and distractor pair, it has been claimed that honeybees can learn to discriminate these stimuli if presented at a large visual angle of 100 deg but bees fail to learn this discrimination task if the stimuli are presented at a relatively small visual angle of 50 deg (Horridge, 2009a; Horridge, 2009b; Horridge, 1996). This basic methodology of training honeybees in a Y-maze has been a fundamental basis for many of the claims about insect vision, which have been used to construct elaborate models of mechanistic visual processing in insects (Horridge, 1996; Horridge, 2000; Horridge, 2003a; Horridge, 2003b; Horridge, 2009a; Horridge, 2009b). However, whilst a Y-maze apparatus may be used to control for the visual angle at which a stimulus is presented and has been very useful for detection experiments (Dyer et al., 2008b; Giurfa et al., 1996), experiments conducted in a Y-maze may potentially lead to false negative findings for discrimination tasks. In particular, at small visual angles a bee may not comply with the experimental rules and just fly over the decision line to improve image resolution, because honeybee resolution is relatively poor compared with vertebrate lens eyes (Srinivasan and Lehrer, 1988). Hence, an experimental finding of no discrimination of stimuli in a Y-maze at a small visual angle may not actually mean that the visual system is not able to perform the task; it may simply reflect that it is a perceptually difficult task that requires an extended training time to learn or that the bee is simply employing a different strategy like trading off accuracy for speed in order to collect nutrition (Burns and Dyer, 2008). For example, recent work has shown that honeybee discrimination of colour stimuli is not only constrained by mechanistic aspects of visual processing in an insect. If a differential conditioning procedure is used where a target stimulus is associated with a reward and distractor stimulus with an aversive substance then bees learn to discriminate between very similar colours that are otherwise generalised (Avarguès-Weber et al., 2010a). Currently, it is unknown how honeybees may learn difficult spatial patterns if aversive–appetitive differential conditioning is used.

Another potential discrepancy that currently exists in understanding honeybee spatial vision is that several studies have reported behavioural results that support the idea that honeybees have a retinoptic mechanism for recognising patterns (Giger and Srinivasan, 1995; Gould, 1985; Wehner, 1967; Wehner, 1981), whilst several other studies reveal impressive pattern recognition in experiments that specifically exclude retinoptic cues by using a variety of training stimuli that differ in terms of the exact retinotopic layout to the final test stimuli (Avarguès-Weber et al., 2010c; Avarguès-Weber et al., 2011; Stach et al., 2004). A number of studies have discussed the possibility of multiple systems potentially underlying some of the discrepancies observed in different experiments (Dyer et al., 2005; Efler and Ronacher, 2000; Giger and Srinivasan, 1995). Although clear evidence of the use of multiple mechanisms remains outstanding, with many papers favouring one strategy over another, here we investigate if there is evidence of multiple strategies that can be used based on the experimental methodology.

In this present study, we address two important and unresolved questions about spatial vision in honeybees. First, we apply an aversive–appetitive differential conditioning procedure to determine if honeybees can learn a configured 4-bar stimulus pair at a small visual angle if constrained to having to solve this task from within the decision chamber of a Y-maze. Second, we test if different stimulus training conditions including either a fixed target–distractor pair or, alternatively, sets of configured target–distractor pairs that exclude retinotopic matching evoke different types of visual pattern matching in individual bees. In this context, we specifically test whether bees trained in alternate ways can extrapolate from a learned visual task when test stimuli are presented at a novel visual angle.

MATERIALS AND METHODS

Experiments were performed at the Jock Marshall Reserve, Monash University, Australia. A hive of honeybees (*Apis mellifera* Linnaeus) was maintained 10 m from a gravity feeder that provided 5% (vol.) sucrose solution. Individual bees were collected from the feeder site on a small Plexiglas® spoon, and were transferred to a test site that was 15 m from the feeder and 25 m from the hive. All experiments were performed on fine days of low wind (Dyer, 2007), with ambient temperature in the range of 20–30°C.

At the test site individual bees were marked with a colour spot on their thorax and trained and tested in a Y-maze apparatus that was covered by an ultraviolet (UV)-transparent Plexiglas® ceiling to ensure the presence of natural daylight. The entrance of the Y-maze led to a decision chamber, where the honeybee could choose between the two arms of the maze. Each arm was 40×20×20 cm (length×height×width). Visual target stimuli (20×20 cm) were achromatic parameterised line figures (Fig. 1) printed on UV-reflecting white paper (Reflex Australia, Melbourne, VIC, Australia) (Dyer, 2001) presented vertically on the back walls of the Y-maze (Fig. 2), at a distance of either 9 cm or 27 cm (27±3 cm in Experiment 2, see below) from the decision chamber. The line thickness of the bars was 2 cm and was above the discrimination threshold for bee vision (Srinivasan and Lehrer, 1988). A marked bee was trained to enter the Y-maze and collect sucrose from a vertically mounted, transparent Plexiglas® screen positioned on the decision line (Fig. 2, insert) and contained a small 4 mm diameter transparent tube to hold fluid. The tube was present on both target and distractor stimuli and so did not provide a cue. The transparent screen allowed the bees
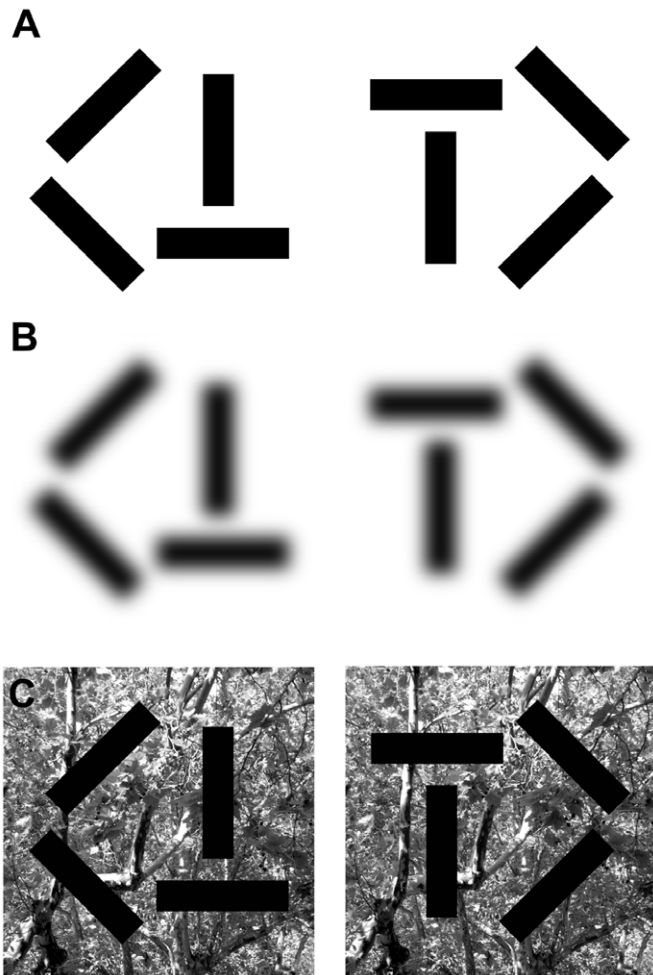
Fig. 1. Target and distractor stimuli. (A) Stimuli that were counterbalanced during the experiments. (B) Representation of stimuli considering bee acuity. (C) Stimuli presented on background noise.

to view and learn the stimuli at different visual angles but avoided the confound that a bee might fly over a decision line for stimuli set further back in order to have a higher acuity view.

Pre-training used absolute conditioning for an initial five bouts so that a bee became highly motivated to participate in the experiment prior to the introduction of an aversive stimulus. The main training of bees used aversive–appetitive differential conditioning as this promotes the ability of individual honeybees to solve difficult visual tasks, possibly through the development of selective attention mechanisms (Avarguès-Weber et al., 2010a). Thus, the sucrose (30% vol.) was associated with the target stimulus, whilst a bitter tasting quinine ($60\,\mathrm{mmol\,l^{-1}}$) solution was associated with the 'incorrect' distractor stimulus. Bees cannot detect these solutions *via* olfaction (Avarguès-Weber et al., 2010a). This conditioning procedure was important because the relatively short distance between the decision lines in the Y-maze could have led to speed–accuracy trade-offs for perceptually difficult visual tasks (Burns and Dyer, 2008; Chittka et al., 2003), but the presence of an aversive substance with the distractor strongly encourages bees to perform close to the perceptual limit allowed by sensory apparatus (Avarguès-Weber et al., 2010a; Chittka et al., 2003; Dyer and Neumeyer, 2005).

During training the side of the rewarded stimulus (left or right) in the Y-maze was interchanged using a pseudorandom sequence

in order to avoid positional cues affecting the results. If the bee chose the rewarded stimulus, it could imbibe a full crop of solution, whilst if it chose the non-rewarded stimulus it was observed to just taste the quinine (Avarguès-Weber et al., 2010a) and then fly to the rewarded target; in this case only the first choice was recorded but the bee was allowed to drink the sucrose to encourage motivation to continue the experiment after tasting the quinine. Once a bee was satiated it was allowed to return to the hive. At this stage the equipment was washed with 10% ethanol.

When the training was completed, non-rewarded transfer tests with novel stimuli and learning tests with fresh training stimuli were conducted. In these tests the dependent variable was the frequency of contacts (Avarguès-Weber et al., 2010c; Stach et al., 2004) with the Plexiglas® surface positioned in front of the respective stimuli, thus maintaining control over the visual angle of the respective stimuli during tests. Each test lasted for 45s and was conducted twice to counterbalance sides, and these data were used to determine the frequency of correct choices for the stimulus representing the training stimulus. Between tests bees were provided with two refresher bouts to promote motivation for participation in subsequent tests. Only a single frequency value was calculated for each bee in a particular test to avoid pseudoreplication effects. Data were arc-sine transformed and analysed with SPSS v.18 software (SPSS Inc., Chicago, IL, USA).

Experiment 1

Experiment 1 investigated the possibility that the previously reported evidence revealing that honeybees do not recognise a 4-bar stimulus at a small visual angle might be due to a false negative artefact produced by the experimental apparatus. Furthermore, the experiment sought to understand if these bees were able to recognise the pattern of the training stimuli in the presence of background noise, and if the bees could recognise the stimuli when displayed at a large novel angle.

In this experiment, only a single set of training stimuli was used [Fig. 1A; Fig. 1B shows a representation considering honeybee visual acuity (Dyer et al., 2008a)]. Eight bees were trained and tested, and the target–distractor stimuli were counterbalanced for half of the bees. The stimuli were placed at a distance of 27cm from the decision chamber in the Y-maze, which represented a relatively small visual angle of 50deg. Importantly, at this visual angle previous work suggests that honeybees cannot discriminate between the target and distractor pair (Horridge, 2009a; Horridge, 2009b). Training used aversive–appetitive differential conditioning (Avarguès-Weber et al., 2010a) for 60 trials on the basis that this is the length of training previous work suggests that bees require for learning difficult spatial vision problems (Dyer et al., 2005; Dyer et al., 2008a; Dyer and Vuong, 2008; Stach and Giurfa, 2005).

The first transfer test was presented to the bees where the stimuli were presented on a noisy background of tree branches (Dyer et al., 2008a) (see Fig. 1C). This noise was common to both stimuli and thus did not provide a cue for bees to solve the visual task but potentially could disrupt visual processing.

The second transfer test presented the original training stimuli at a large visual angle of 100deg (stimuli distance set at 9cm from the decision point) to test if bees were able to demonstrate size invariance if a specific training pair of stimuli is employed.

Finally, the bees received a non-rewarded learning test to assess if the task had indeed been learned at a small visual angle.

Experiment 2

Experiment 2 tested whether bees taught with four different training stimuli, which varied in exact retinotopic match but shared a similar
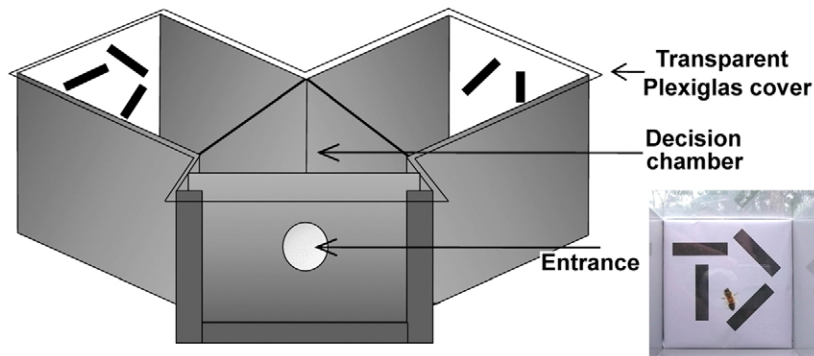
Fig. 2. Y-maze apparatus, which allows stimuli to be set at a particular distance from the decision chamber so that there is control over the visual angle. An individual bee can enter the Y-maze and then in the decision chamber can view stimuli on the back walls. Ultraviolet (UV)-transparent vertical barriers at the two edges of the decision chamber constrains the bee to making a decision at a given visual angle; appetitive or aversive solution is presented in a small tube in the vertical barriers for the target and distractor stimuli, respectively. Insert shows a photograph where a bee has landed on the feeder tube and is imbibing sucrose solution after correctly choosing the target stimulus at a distance of 27 cm (relatively small visual angle of 50 deg).

spatial configuration (Fig. S1 in supplementary material), could learn the configured rule of the pattern and use this information to recognise novel target stimuli. There were five different target and distractor combinations that varied in layout but shared the same basic configuration; within a particular pair of target and distractor stimuli these were matched and thus shared exactly the same centre of gravity and spatial frequency cues (Avarguès-Weber et al., 2010c). For half of the bees the target and distractors were reversed so that the experiment was counterbalanced. During training four target–distractor pairs were used (Fig. S1 in supplementary material), and these were presented at 24 cm, 27 cm or 30 cm in a pseudorandomised order to exclude honeybees using a retinotopic mechanism of recognising the target stimulus. Training used aversive–appetitive differential conditioning for 60 trials. In non-rewarded transfer tests these bees were presented with the fifth, novel, target–distractor stimuli pair (Fig. S1 in supplementary material) on a noisy background. Bees were next tested with the fifth, novel, target–distractor stimuli pair stimuli at a novel distance of 9 cm from the decision point of the Y-maze (equivalent visual angle of 100 deg). Finally, the bees were presented with a learning test with the fifth, novel, target–distractor stimuli pair at the visual angle (50 deg) used during training. Pilot control experiments showed that bees could discriminate the novel stimulus from the other stimuli of similar configuration (Fig. S1 in supplementary material). To solve these tasks, the bees had to use relationship processing in Experiment 2 (Avarguès-Weber et al., 2011; Avarguès-Weber et al., 2010c), as retinotopic matching had been specifically excluded as a mechanism to solve the visual task.

RESULTS
Experiment 1

In Experiment 1, honeybees were trained with aversive–appetitive differential conditioning using a fixed target and distractor pair and there was a gradual acquisition of information. The bees started at a level that was not significantly different from chance in the first 10 choices [mean=48.8±9.7% (±s.d.), one sample t-test, t=0.355, d.f.=7, P=0.733], indicating that there was no ability to choose the target following the brief absolute conditioning at the start of the experiment; however, with continued differential conditioning, there was a significant increase in the ability of bees to recognise the target stimulus (repeated measures ANOVA; Wilks' lambda=0.03, $F_{(3,5)}$=19.278, P=0.017, multivariate η^2=0.970). This shows that by the end of 60 decisions bees had learned the visual task at a small visual angle (Fig. 3A).

In the first transfer test the bees chose the target stimulus *versus* the distractor stimulus with a frequency of 70.3±7.3% (±s.d.) (Fig. 3B, column 1), and these choices were significant from chance expectation (one sample t-test, t=7.658, d.f.=7, P<0.001), even

though the stimuli were presented on a noisy background. Thus, when the stimuli were at the same visual angle as the training condition, bees in Experiment 1 could recognise a learned pattern on a noisy background.

In the second transfer test the bees were presented with the test patterns at a novel visual angle of 100 deg. In this transfer test the frequency of correct choice was 49.5±4.8% (±s.d.) (Fig. 3B, column 2), and these choices were not significant from chance expectation (one sample t-test, t=0.276, d.f.=7, P=0.791). Thus, these bees trained with fixed stimuli failed to recognise the target stimuli when it was presented at a novel visual angle.

Finally, a non-rewarded learning test was conducted at the initial visual angle of 50 deg, and in this test the bees chose the target with an accuracy of 71.2±8.7% (±s.d.) (Fig. 3B, column 3), which was significantly different from chance expectation (one sample t-test, t=6.511, d.f.=7, P<0.001). This test showed that bees could learn the target at a small visual angle, which is consistent with the first transfer test, and that the failure to recognise the target in the second transfer test described above could not have been due to a motivation effect, as the learning test was conducted last.

Experiment 2

In Experiment 2, honeybees were trained with aversive–appetitive differential conditioning to variable target and distractor stimuli. Whilst bees started at a level that was not significantly different from chance in the first 10 choices [mean=52.9±8.3% (±s.d.), one sample t-test, t=0.981, d.f.=7, P=0.359], there was a gradual acquisition of information with increasing experience (repeated measures ANOVA; Wilks' lambda=0.06, $F_{(3,5)}$=9.612, P=0.046, multivariate η^2=0.941). Thus, by the end of 60 decisions the bees had learned the visual task at a small visual angle (Fig. 4A). We also observed the distance from the Plexiglas® screen that the bees hovered to inspect the stimuli prior to making a decision to abort or land on the feeding tube; this distance was 2±1 cm and was thus independent of the changes in visual angle to the stimuli due to the 6 cm range of presentation variations.

In the first transfer test the bees chose the target stimulus *versus* the distractor stimulus with a frequency of 69.2±7.6% (±s.d.), and these choices were significantly different from chance expectation (one sample t-test, t=6.909, d.f.=7, P<0.001) (Fig. 4B, column 1), even though the stimuli were presented on a noisy background. Thus, when the stimuli were at the same visual angle as the training condition, bees in Experiment 2 could recognise a learned pattern on a noisy background.

In the second transfer test the bees were presented with the test patterns at a novel visual angle of 100 deg. In this transfer test the frequency of correct choice was 67.9±6.9% (±s.d.) (Fig. 4B, column 2), and these choices were significantly different from chance
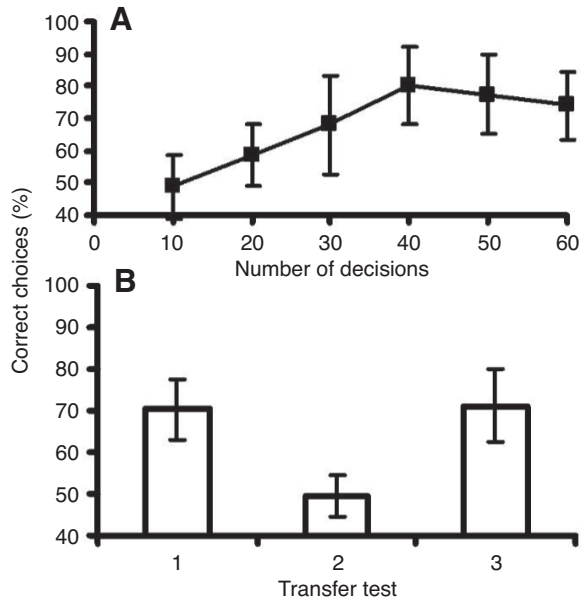
Fig. 3. Experiment 1. Behavioural data for honeybees discriminating patterns at different distances following differential conditioning to target and distractor stimuli at a set visual angle [mean *N*=8 bees (±s.d.)]. (A) Acquisition curves show that bees can slowly learn a 4-bar stimulus at a small visual angle of 50 deg. (B) In transfer test 1, trained bees could reliably recognise the target stimulus when presented on a noisy background. In transfer test 2, bees failed to recognise the target stimulus at a novel visual angle of 100 deg. A learning test shown in column 3 acts as a control and confirmed that bees could recognise stimuli that had been learned at a small visual angle. See text for statistics.
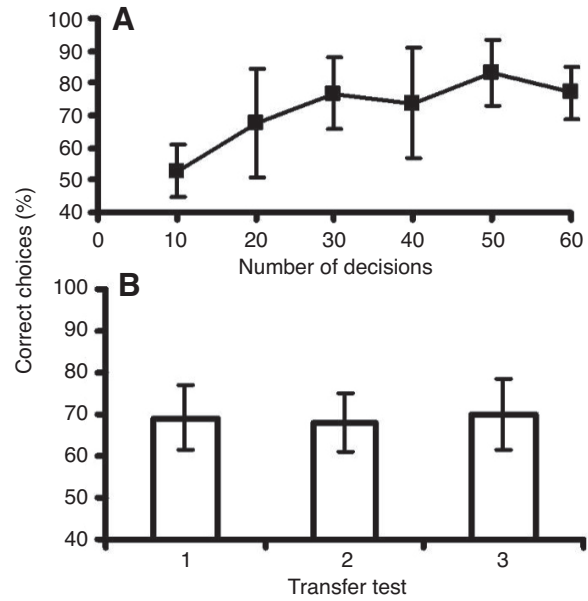

Fig. 4. Experiment 2. Behavioural data for honeybees discriminating patterns at different distances following differential conditioning to variable target and distractor pairs at a range of different visual angle [mean *N*=8 bees (±s.d.)]. (A) Acquisition curves show that bees can slowly learn a 4-bar stimulus at a small visual angle of 50 deg. (B) In transfer test 1, trained bees could reliably recognise the target stimulus when presented on a noisy background. In transfer test 2, bees could reliably recognise the target stimulus at a novel visual angle of 100 deg. A control learning test shown in column 3 confirmed that bees could recognise stimuli that had been learned at the small visual angle used during the training phase. See text for statistics.

expectation (one sample *t*-test, *t*=6.990, d.f.=7, *P*<0.001). Thus, the bees in Experiment 2 trained with variable target and distractor pairs were able to recognise the target stimuli at a novel visual angle.

Finally, a non-rewarded learning test was conducted at the initial visual angle of 50 deg, and in this test the bees chose the target with an accuracy of 70.1±8.5% (±s.d.) (Fig. 4B, column 3), which was significantly different from chance expectation (one sample *t*-test, *t*=6.286, d.f.=7, *P*<0.001).

DISCUSSION

Honeybees have to forage in very complex natural environments and thus encounter a number of different visual tasks. In some cases stimuli may appear at a reasonably constant visual angle, such as when a forager bee is collecting nectar rewards from a patch of flowers, whilst in other cases bees may have to recognise patterns like a landmark tree at variable distances (Dyer et al., 2008a; Zhang et al., 2004). However, even in the case of flowers that might be viewed at a reasonably constant visual angle by a foraging bee, it is likely that natural variability in flower size and shape would, in some cases, make reliable recognition a challenging visual task. Experiment 1 shows that bees can learn to discriminate between 4-bar asymmetric stimuli at a small visual angle of 50 deg. This is a new result that was unsuspected based upon previous experiments and models of bee vision (Horridge, 2009a; Horridge, 2009b). The methodology that constrains bees to the decision-making chamber of a Y-maze and promotes high levels of learning with aversive–appetitive conditioning shows that discrimination-type experiments conducted in a Y-maze apparatus are prone to false negative results if the decision line is the criteria for determining

choice frequency. This is because a bee may cross this line to get a clearer view of stimuli (and random choices are thus recorded), even though the visual system of the bee can actually solve the task at a small visual angle. Experiment 1 also showed that bees were able to recognise the learned pattern in the presence of noise, which is consistent with one previous report (Avarguès-Weber et al., 2010c), but the bees failed to recognise the patterns when presented at a novel visual angle of 100 deg. Experiment 2 showed that bees could learn to discriminate between 4-bar asymmetric stimuli at a small visual angle of 50 deg when the stimuli excluded the possibility of bees using retinotopic template matching, which is consistent with other studies that specifically exclude retinoptic cues (Avarguès-Weber et al., 2010b; Avarguès-Weber et al., 2010c; Avarguès-Weber et al., 2011; Stach et al., 2004). Interestingly, in Experiment 2 the bees were also able to use the learned rule to then make correct decisions for stimuli that were presented at a novel visual angle of 100 deg. This behavioural evidence shows that the visual system of honeybees is very likely to have multiple mechanisms of recognising spatial patterns, and it will be of high value for future work to examine the neurophysiological basis that underlie these different behavioural results.

Several previous studies have considered the possibility that honeybee spatial vision is mediated either by retinotopic (Giger and Srinivasan, 1995; Gould, 1985; Wehner, 1967; Wehner, 1981), configural mechanisms (Avarguès-Weber et al., 2011; Avarguès-Weber et al., 2010c) and/or a combination of multiple mechanisms (Dyer et al., 2005; Efler and Ronacher, 2000). Experiment 1 is consistent with a retinotopic mechanism as once the bees had learned the task they were not able to transfer the learned rule to novel

stimuli; specifically there appears to have been a requirement that the stimulus elements should have a fixed relationship to what was learned for correct decisions to be made. Future work should consider the limits of this retinotopic-type mechanism for fine discrimination tasks like 'face' (Dyer et al., 2005) or 'natural scene' recognition (Dyer et al., 2008a; Zhang et al., 2004). However, Experiment 2 does not fit this model, as bees that were trained to a stimuli set that excluded retinotopic matching were able to learn the configured rule and then apply this to a novel situation. In this case the bees had learned a relationship rule that enabled extrapolation to a visual task that was well beyond the range of variation encountered in the training phase of the experiment. This builds on recent research that the bees' visual system can learn relationship rules to solve complex spatial tasks that incorporate multiple elemental features (Avarguès-Weber et al., 2010c; Avarguès-Weber et al., 2011). Our work shows that how a bee sees is a complex process and it cannot be fully explained by simple elemental-type models (Horridge, 2009a; Horridge, 2009b).

The result for Experiment 2, where bees were able to use a learned rule to solve a visual task at a novel visual angle contrasts with a previous experiment that trained bees to different viewpoints of faces and reported that interpolation was possible but not extrapolation (Dyer and Vuong, 2008). This difference in experimental results is likely to be due to differences in how rule-based configural processing or averaging of learned representations is applied differently by the visual system. It would appear that the rule-based mechanism of visual learning provides for a more robust mechanism for solving difficult visual tasks in complex environments, e.g. a bee may be required to identify a particular tree at a range of distances that would exclude reliable retinotopic matching. It is plausible that an experienced bee that had viewed a tree from a range of different distances and viewpoints would learn the configured rule to enable more efficient and reliable recognition from a novel distance when returning from a distant foraging venture.

In other cases, it may be beneficial for bees to use a retinotopic mechanism as this mechanism could allow faster recognition (or require a shorter learning time) if there is low variability in stimuli viewing distance. For example, bees are able to solve delayed-matching-to-sample (DMTS) tasks where an individual bee must fly through an apparatus and store a particular 'target' image in working memory to then make a choice between two stimuli, with the correct choice being the stimulus that matches the target (Zhang et al., 2005). In this type of DMTS experiment, the 'target' stimulus is often changed to a different image, e.g. a pattern, a colour, a tree, a landscape or a flower (Giurfa et al., 2001; Zhang et al., 2004). In this type of situation, rapidly storing and retrieving different images through a retinotopic mechanism may be more efficient than using a rule-based configural mechanism, because rules could require a longer period of time to learn (Avarguès-Weber et al., 2010c; Avarguès-Weber et al., 2011). In this regard, the time course for working memory retaining a particular stimulus in a DMTS task decays as an exponential function, and is only reliable for up to approximately 5 s (Zhang et al., 2005). A biologically plausible example of this type of situation is when bees collect nectar from rewarding flowers in the field, and at a reasonably fixed distance in front of a flower must decide if it appears to be the same shape as the previously rewarding flower. Thus, there are examples of why bees would benefit from a visual system that is flexible and can both recognise stimuli at a fixed distance as shown in Experiment 1 or at a range of distances over long time frames as shown in Experiment 2. However, the suggestion that a retinoptic mechanism may be faster in some circumstances is not completely consistent

with the learning rate of the bees in Experiments 1 and 2 (learning rate was similar in the respective experiments; Fig. 3A and Fig. 4A, respectively), and thus the potential value of a retinotopic mechanism is a question that would be of high value to explore the ecological relevance of in future work. One plausible reason for why we did not observe faster learning for Experiment 1 may have been that the complexity of using the apparatus impaired bee learning rate for the comparative experiments.

The behavioural evidence of multiple mechanisms of spatial vision in honeybees leads to the question of how spatial information is managed in a bee brain? For visual tasks, this is largely unknown, because the recently reported rich visual behaviour of learning very detailed or complex spatial tasks (Avarguès-Weber et al., 2010b; Avarguès-Weber et al., 2011; Dyer et al., 2005; Dyer et al., 2008a; Stach and Giurfa, 2005; Stach et al., 2004; Zhang et al., 2004) is currently only possible in free-flying bees. Interestingly, for difficult visual tasks involving colour processing, free-flying bees can learn very fine colour discriminations with differential conditioning (Avarguès-Weber et al., 2010a; Dyer and Neumeyer, 2005) but harnessed bees can only learn relatively coarse colour discriminations (Niggebrugge et al., 2009). Thus, it appears there is currently poor access to directly understanding the underlying neural mechanisms of fine discrimination vision in bees. Whilst some studies have inferred the processing architecture of the bee brain from behavioural experiments and the existing knowledge of bee brain anatomy (Horridge, 2009a; Horridge, 2009b), the present study shows these models are not a complete explanation for the range of rich visual learning in free-flying honeybees. Interestingly, discrepancies also exist in our current understanding of brain anatomy and how complex patterns are processed for mammalian vision. For example, in the primary visual cortex there are single neurons with selectivity for orientation and retinal position (Ahissar and Hochstein, 2004; Hubel and Wiesel, 1962), and psychophysics experiments of Vernier discrimination tasks (involving the fine discrimination of two lines) are solved at early stages of visual processing in either the striate cortex (V1) or the extrastriate cortex level (V2), where neurons are very selective (Herzog and Fahle, 1998). However, more complex tasks such as direction discrimination of moving dots are most likely solved in the middle temporal (MT) area (Seitz et al., 2006), and it has been demonstrated that the MT in monkeys can be modified as a result of perceptual training with random dot motion discrimination tasks (Zohary et al., 1994). Whilst this suggests the complexity of an image is constructed at successively higher levels of processing *via* feed-forward connections, recent work suggests that there are complex interactions between different processing levels where higher level processing may recruit resources from lower levels of processing *via* feedback connections to allow specificity for fine discrimination tasks, termed the reverse hierarchy theory of visual processing (Ahissar and Hochstein, 2004; Ahissar et al., 2009). Specifically, this theory asserts that with extended perceptual learning, there is a top-down cascade of information processing that tunes neural responses at earlier levels of visual processing (Ahissar and Hochstein, 2004; Ahissar et al., 2009). For example, training results in a feedback process that enhances the capacity of individuals to make Vernier discriminations (Fahle, 2009; Herzog and Fahle, 1997). Whilst these ideas on visual processing and expertise with perceptual learning in the primate brain have not yet been applied to our understanding of how invertebrates may process their visual environment, it is plausible that the honeybee brain can use top-down information to assist in making complex spatial discriminations. There is evidence that the bee brain has both innate

templates for biologically relevant patterns (Lehrer et al., 1995), and that experienced bees can use top-down information in solving complex visual tasks (Zhang and Srinivasan, 1994; Zhang and Srinivasan, 2004). Bees can clearly process complex spatial information like patterned sectors (Stach et al., 2004; Stach and Giurfa, 2005), faces (Avarguès-Weber et al., 2010c; Dyer et al., 2005; Dyer and Vuong, 2008), natural scenes (Zhang et al., 2004) or above/below spatial relationships (Avarguès-Weber et al., 2011), and in the context of current models, we suggest that this relationship processing (consistent with Experiment 2) is likely to occur at levels beyond the lamina, medula or lobula in the honeybee brain, which have only been implicated in elemental processing (Horridge, 2009a; Horridge, 2009b) (consistent with our Experiment 1).

The experiments also showed that bees in both Experiments 1 and 2 could correctly identify stimuli following differential conditioning, even when the stimuli were presented on a noisy background. This finding is consistent with reports that honeybees could recognise configured features when viewed on a novel 'face' background (Avarguès-Weber et al., 2010c), and suggests that both retinotopic and configural mechanisms of bee vision efficiently extract important salient features from irrelevant noisy background information. These results suggest that foraging bees have reliable mechanisms for the visual tasks of landmark recognition in noisy environments like forests (Dyer et al., 2008a) or identifying flower shape from a noisy background. To date only a few studies have considered how the visual system of honeybees is able to extract meaningful spatial information from background noise (Avarguès-Weber et al., 2010c; Zhang and Srinivasan, 1994; Zhang and Srinivasan, 2004). In mammalian systems it is proposed that learning to perceive stimuli in noise is a result of selective inhibition of noise stimuli and excitation of the target stimuli (Dosher and Lu, 1998). Given that there is now evidence that bees can exclude background spatial noise from learned salient stimuli, it would be of high value to systematically address this question further with a range of target salience variations.

We conclude, based on behavioural results, that honeybees have a more complex visual system than has been proposed in some recent models (Horridge, 2009a; Horridge, 2009b). The visual system is likely to have multiple mechanisms that either (i) only permit a reasonably fixed representation of learned stimuli and thus may be explained by retinotopic-type mechanism, or (ii) are based on a rule-learning or configural-type mechanism that permits robust visual discriminations of multiple elements at novel visual angles. These two models, respectively, would allow for reliable vision in ecologically valid scenarios of collecting nutrition from flowers at close range where the visual angle for decision making is reasonably constant or recognising landmark features which has to be done at a range of visual distances. It is likely that the visual system may use a combination of these mechanisms for different visual problems depending upon experience level, which partially explains discrepancies between different results in different studies. The possibility that the bee brain uses information from different levels of visual processing in a way that is analogous to mammalian systems and the reverse hierarchical hypothesis of visual learning would be an interesting conceptual framework for future work to explore.

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