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RESEARCH ARTICLE

The answer is blowing in the wind: free-flying honeybees can integrate visual and mechano-sensory inputs for making complex foraging decisions

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ABSTRACT

Bees navigate in complex environments using visual, olfactory and mechano-sensorial cues. In the lowest region of the atmosphere, the wind environment can be highly unsteady and bees employ fine motor-skills to enhance flight control. Recent work reveals sophisticated multi-modal processing of visual and olfactory channels by the bee brain to enhance foraging efficiency, but it currently remains unclear whether wind-induced mechano-sensory inputs are also integrated with visual information to facilitate decision making. Individual honeybees were trained in a linear flight arena with appetitive–aversive differential conditioning to use a context-setting cue of 3 m s^{-1} cross-wind direction to enable decisions about either a 'blue' or 'yellow' star stimulus being the correct alternative. Colour stimuli properties were mapped in bee-specific opponent-colour spaces to validate saliency, and to thus enable rapid reverse learning. Bees were able to integrate mechano-sensory and visual information to facilitate decisions that were significantly different to chance expectation after 35 learning trials. An independent group of bees were trained to find a single rewarding colour that was unrelated to the wind direction. In these trials, wind was not used as a context-setting cue and served only as a potential distracter in identifying the relevant rewarding visual stimuli. Comparison between respective groups shows that bees can learn to integrate visual and mechano-sensory information in a non-elemental fashion, revealing an unsuspected level of sensory processing in honeybees, and adding to the growing body of knowledge on the capacity of insect brains to use multi-modal sensory inputs in mediating foraging behaviour.

KEY WORDS: *Apis mellifera*, Reverse learning, Multi-modal processing, Sensory processing, Vision

INTRODUCTION

In natural environments there is an enormous amount of sensory information that needs to be either processed or ignored in order for an animal to make accurate decisions (Paulk et al., 2014; Posner et al., 1980; Sareen et al., 2011). Honeybees have emerged as an important biological model for understanding signal–receiver relationships because their goal(s) can be well mapped (Dyer and Arikawa, 2014; Morawetz and Spaethe, 2012; Von Frisch, 1967). The constrained brain size of about one million neurons in bees

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means that efficient solutions have evolved to seemingly complex problems (Srinivasan, 2010). The large existing body of knowledge on the neurobiology of bees, combined with accessible neural structures, has provided significant insight into the means of information processing in insect brains (Paulk et al., 2014; Scholl et al., 2014).

To efficiently collect nectar from flowers, bees must often fly in challenging conditions with frequent changes in climate, local weather conditions (Ravi et al., 2013) and foraging patch complexity (Steffan-Dewenter and Kuhn, 2003). The atmospheric boundary layer is the lowest region of the atmosphere and it contains the natural habitat of most flying insects. Within this region, the wind environment is highly variable, consisting of rapid and unpredictable changes in air speed and direction (Stull, 1988). While these conditions are unfavourable for flight, bees do fly stably and make accurate decisions to orientate, avoid obstacles or land on surfaces like flowers (Mirwan and Kevan, 2015; Srinivasan, 2010). Free-flying insects are endowed with a variety of sensory systems to achieve aerial locomotion, and also use fine motor-skills to maintain flight stability in adverse winds (Ortega-Jimenez et al., 2013; Ravi et al., 2013; Vance et al., 2013).

The role of optic flow in guiding insects to distant targets and flight stabilization has been well documented (Srinivasan, 2014; Srinivasan et al., 2000). Insects also receive information from sensory systems other than vision, including olfactory, inertial and mechano-sensors that are likely to aid flight control and navigation. The antennae have been identified as a key wind-encoding organ in insects such as fruit flies, locusts and honeybees (Budick et al., 2007; Gewecke, 1970; Taylor et al., 2013). The Johnston's organ present in the antenna's pedicle contains strain-sensitive sensilli (mechanosensors) that are responsible for converting the deflection of flagella due to airborne oscillations (wind or acoustic) into neural signals (Yorozu et al., 2009). Other organs that are believed to aid in wind perception include the Bhom's bristles (Sane, 2009) and hairs on the head (Alexander, 2004). While airborne, insects potentially use information from multiple sensory modalities synergistically to aid flight control, and recent research in fruit flies (*Drosophila*) has revealed that wind information from the antennae is combined with inertial and visual signals to provide a stable sensory platform (Fuller et al., 2014). A relationship between vision and wind perception has also recently been observed in honeybees, enabling them to perform abdomen-righting manoeuvres (Taylor et al., 2013). However, relatively limited information currently exists on the operational mechanics of wind-sensitive organs and the neural pathways that may enable integration of mechano-sensory information with other sensory modalities.

Apart from providing information used for flight control, visual sensory systems also play a vital role in modulating decision-making behaviours in honeybees. Bees have a trichromatic

(UV-, blue-, green-sensitive) visual system, allowing fine colour (Avarguès-Weber et al., 2010; Dyer et al., 2011; Peitsch et al., 1992) and spatial (Avarguès-Weber et al., 2015; Giurfa et al., 1999) discrimination. Bees also possess a capacity to accurately sense olfactory cues (Giurfa and Sandoz, 2012), and a capacity to perceive tactile cues on flowers via mechano-receptive sensilla trichodea (Erber et al., 1998; Kevan and Lane, 1985).

Interestingly, there is evidence that the honeybee brain allows multi-modal perception between visual and olfactory sensory inputs (Reinhard et al., 2004b). Multi-modal processing involving both visual and olfactory stimuli has become an important research topic over the past decade, with several studies exploring this new field through both behavioural observations and neural measurements (Goyret, 2010; Hebets, 2011; Leonard and Masek, 2014; Milet-Pinheiro et al., 2015; Partan, 2013). Honeybees trained in a Y-maze to solve complex delayed matching to sample problems in the visual domain can slowly learn this task, and then apply the acquired rule to solve a novel olfactory presentation of stimuli (Giurfa et al., 2001). Furthermore, bees have been shown to use visual and olfactory information in ecologically relevant foraging scenarios as scent can trigger memory for specific colours previously associated with rewarding stimuli (Leonard et al., 2011; Reinhard et al., 2004a; Srinivasan et al., 1998). This suggests that different modalities of sensation may potentially operate synergistically to enhance decision making.

Classically it has been thought that mechano-sensory processing might not require integration with other sensory modalities because in *Drosophila*, simple visual, tactile and motor memories are processed independent of the mushroom bodies (Wolf et al., 1998). However, a few studies suggest that insects may integrate such sensory channels (Budick et al., 2007), and work on honeybee psychophysics shows that integration of complex information is often experience dependent (Giurfa et al., 2003). Indeed, free-flying bumblebees show some evidence of integrating colour and tactile cues (Whitney et al., 2009), or colour and temperature cues (Dyer et al., 2006) when extensive training is provided.

Given the major influence of wind on flight, and the capacity of honeybees to perform multi-modal sensory integration depending on the foraging context, we hypothesized that bees are also capable of integrating wind-induced mechano-sensory information with vision to modulate foraging choices (Dyer et al., 2014b). We used an appetitive–aversive differential conditioning paradigm to promote attentional motivation, and we mapped colour salience to allow for some initial understanding of what sensory input into the bee brain may facilitate multi-sensory processing. Wind direction was used as a context-setting cue to predict the colour of the rewarding stimulus and to evaluate whether bees can use colour in a complex environment in a non-elemental fashion (Giurfa et al., 2003; Mota et al., 2011). We additionally explored whether bees exhibit a hierarchical preference for either spatial location or chromatic properties, while making a decision in the context of multi-modal sensory processing of wind and visual stimuli. Finally, to evaluate whether multi-modal processing increases sensory load, the learning rates of bees presented with a multi-modal sensory integration task were compared with an independent group that were presented with a simple elemental colour-learning task in the presence of a wind distracter.

MATERIALS AND METHODS
Experimental set-up

Honeybee (*Apis mellifera*, Linnaeus 1758) colonies were maintained 30 m from a feeding site where a gravity feeder provided 10% sucrose solution *ad libitum*. Individual foragers were subsequently recruited and trained using 25% sucrose solution to return to a testing site located 10 m away from the gravity feeder. Each honeybee was trained to enter a flight apparatus through a 1.5 cm wide and 10 cm long Plexiglas tube that contained gates to control bee movements. Test bees attained flight upon entering the flight apparatus (0.4×0.3×0.25 m, l×h×w), which contained a mesh roof to permit natural lighting and minimize turbulence when the side-wind stimulus was applied. The floor and side walls were lined with 'green' paper, which constituted the visual background for the coloured targets (Fig. 1).

We used a custom-made cross-wind generator consisting of 6 cm fans embedded into opposite side walls of the arena near the inlet (Fig. 1), which were operated in a synchronous direction, thus
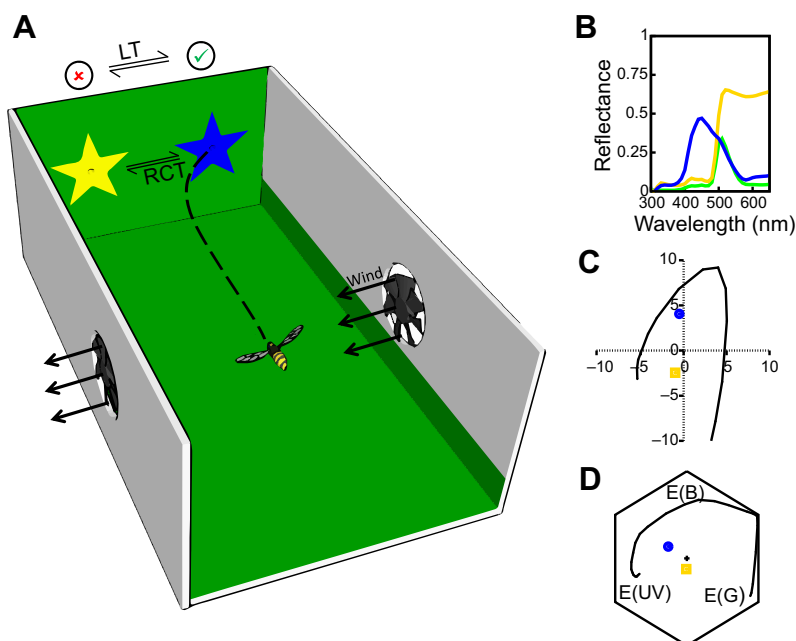

Fig. 1. Details of the experimental set-up. (A) Schematic diagram of the flight apparatus used for the experiments. As the bees entered the flight apparatus, they experienced a steady cross-wind and were subsequently presented with a 'blue' and 'yellow' stimulus containing either a reward (sucrose) or a punishment (quinine). During learning trials (LT), the stimulus containing the reward and punishment changed with the cross-wind direction; for example, in the illustration, wind from the right implied reward and punishment were placed in the blue and yellow stimulus, respectively, and in trials where the wind was from the left, the reward and punishment locations were reversed. Subsequent to learning trials, a reverse-side contingency test (RCT) was conducted where the location of the stimuli was reversed but the relationship with respect to wind direction was maintained. (B) Spectral reflectance curves from the yellow and blue colour targets and the 'green' colour making up the visual background of the flight arena. (C) Yellow and blue targets modelled in the colour-opponent coding colour space (Backhaus, 1991) and (D) the hexagon colour space (E, excitation; Chittka, 1992). The 'green' colour included in the flight apparatus was as assumed as the 'adapting' hue for colour modelling.

allowing air to flow across the flight arena with minimal overall turbulence (Fig. 1). The wind generator produced a steady cross-wind of 3 m s^{-1}, measured with a hand-held anemometer, representing an intermediate cruising velocity for bees (Riley et al., 1999).

The experimental stimuli consisted of a target and a distracter that could be either a 'blue' (B) or a 'yellow' (Y) five-point star measuring 7 cm wide, placed vertically at the end of the flight arena. Stars were used as these shapes promote fast initial learning in honeybees (Lehrer et al., 1995). A 30 mm-long tube of 15 mm diameter was placed at the centre of each visual stimulus for the bees to land on and receive a reward or punishment (Fig. 1). The size of each stimulus subtended a visual angle >15 deg from the region of cross-wind within the flight tunnel, thus promoting chromatic (colour) processing for decision making in free-flying honeybees (Giurfa et al., 1996). The target stimulus contained a reward of a 10 µl drop of 25% sucrose solution and the distracter stimulus contained a punishment of a 10 µl drop of 60 mmol l^{-1} quinine hemisulphate solution to promote learning in free-flying honeybees (Avarguès-Weber et al., 2010).

The selected colours ensured that the stimuli were easily perceived as being chromatically different considering the colour-opponent processing of honeybees (Avarguès-Weber et al., 2010; Dyer et al., 2011; Peitsch et al., 1992). The perceptual chromatic difference between the colour stimuli was measured in terms of the chromatic difference in the hexagon (Chittka, 1992) and the colour-opponent coding (Backhaus, 1991) colour spaces (Fig. 1B,C; Table S1). Modelling assumed the 'green' colour as background, a

daylight colour temperature of 6500 K (Judd et al., 1964) and spectral sensitivity functions specific for *A. mellifera* (Peitsch et al., 1992).

Behavioural training
Prior to experiments, honeybee foragers (*N*=20) were trained to fly in the flight apparatus by placing a 10 µl drop of 25% sucrose solution on a landing platform in the centre of two vertically presented and identical 5 cm squares with achromatic stripes placed at the end of the flight tunnel. Once the bees had become used to flying within the apparatus, the achromatic pre-training stimuli were replaced with the experiment stimuli (coloured stars).

For half of the bees (*N*=10), the relationship between the context-setting cross-wind direction and the rewarding colour was linked. For example, wind from the left implied that the reward solution was placed in the yellow stimulus and the blue stimulus contained quinine, whilst if the wind was from the right then the blue stimulus was rewarding and the yellow contained quinine (Fig. 2). The relationship was maintained throughout the entire flight trials for each bee, but was pseudo-randomized across individuals in the group (Fig. 2). During an experiment, after a bee entered the inlet tube leading to the entrance of the flight apparatus where its movement was controlled by gates, the cross-wind generator was engaged, and the reward/punishment solutions were placed in the corresponding stimuli following the pre-established relationship between wind direction and rewarding colour for the individual. The main arena gate was then opened to allow the bee to start foraging.
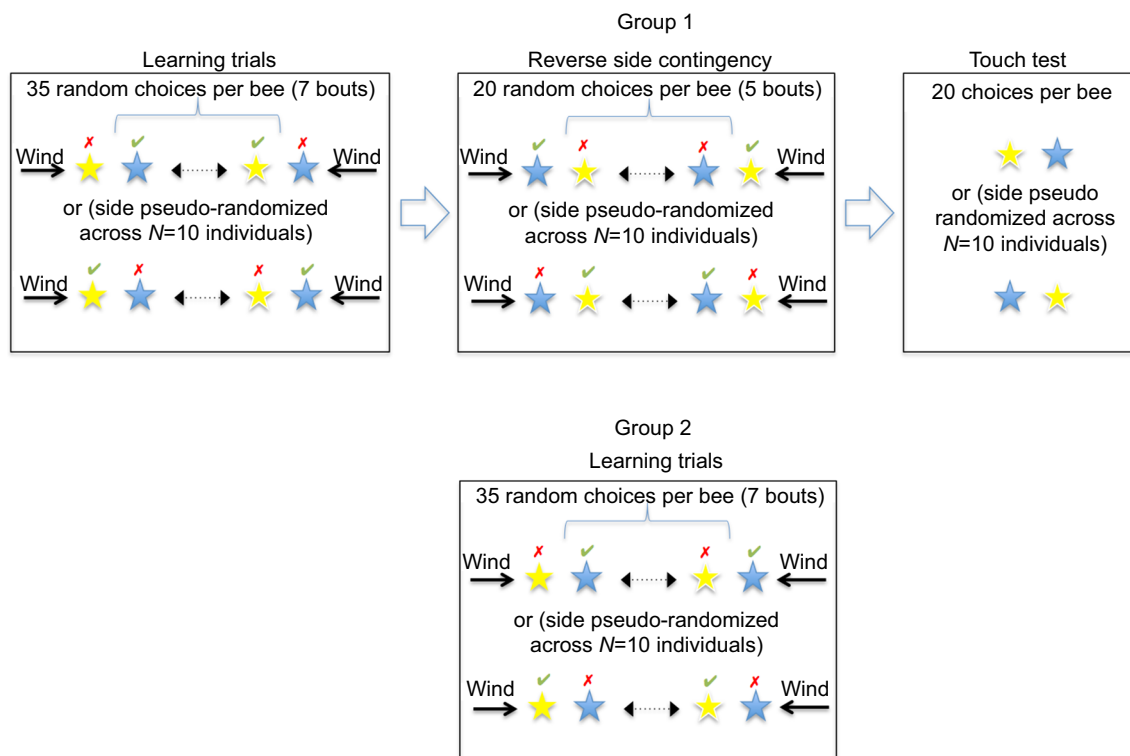

Fig. 2. Schematic representation of experiments. Group 1: in the learning trial, the relationship between wind direction and the rewarding or punishing colour stimuli was linked. Each bee (*N*=10) experienced 35 learning trials: reward contingency was counterbalanced over the group. After learning, a reverse-side contingency test was presented where the left/right position of the stimuli was reversed but the reward contingency for wind direction and colour was retained over the 20 trials. Finally, a touch test was conducted with only the two colour stimuli. Group 2: in the learning trial, wind was present but did not act as a predictor of reward colour, and each bee (*N*=10) had to learn the colour only using a simple associative mechanism. Colour position was pseudo-randomized over the 20 learning events, and colour reward contingency for yellow/blue was counterbalanced across the group.

Upon becoming airborne, bees flew through the region of cross-wind and had to subsequently make the correct choice between the two stimuli. To enable repeat testing to promote learning in a bee, we used the method developed by Morawetz and Spaethe (2012). While the bee was collecting the reward, a second drop of sucrose was placed within a vial at the end of the landing tube to entice the bee to reach the end of the tube. As the bee imbibed the second drop of sucrose solution within the vial, it was possible to carefully move the vial to the entrance of the arena and reintroduce the bee to the inlet tube to enable multiple choices per foraging bout (Morawetz and Spaethe, 2012). While the bee was in the inlet tube, the gates were closed to momentarily trap it, and the stimulus and landing surfaces were cleaned using 30% ethanol solution. This experimental procedure was repeated but the wind direction was pseudo-randomized between flight trials while maintaining the relationship between wind direction and the colour of the stimulus containing the reward. After five flight trials, a bee was satiated, at which point it returned to the hive; this set of actions was deemed as a foraging bout. A total of seven foraging bouts were performed for each bee (i.e. 35 decisions) in the initial learning phase. In this phase, the location of the stimuli remained constant but the reward or punishment changed depending on perceived wind direction to enable us to dissect what hierarchical cue (colour cue or spatial location) bees used to solve this complex problem. A reverse-side contingency test was then performed to establish whether spatial or chromatic cues were used by the bees in choosing the rewarding stimulus. In the reverse-side contingency test, the location of the chromatic stimulus was reversed within the arena while still maintaining the relationship between wind direction and rewarding colour, and this test was performed over four feeding bouts (i.e. 20 decisions) for each bee.

After the completion of both the learning and reverse-side contingency tests, a single non-rewarded touch test was performed for 20 choices where each bee did not experience cross-winds within the flight tunnel but they were presented with the blue and yellow stimuli. A non-rewarded touch test (Avarguès-Weber et al., 2010) enables the quantification of bee choices for preferring particular visual stimuli, thus providing insights into perception independent of the context-setting cue. Statistical analysis was subsequently performed to investigate the possibility of potential bias in the bees towards a particular stimulus.

For the remaining half of the bees (N=10), the colour of the rewarding stimulus remained constant irrespective of the direction of the cross-wind (Fig. 2). Half of these bees were trained to blue as the rewarding stimulus while yellow contained quinine; for the other half, yellow was the rewarding stimulus and blue contained quinine. During each trial, as the individual bee entered the arena, the reward and punishment were placed in the respective stimuli and the cross-wind was initiated. As the bees attained flight upon entering the apparatus, the cross-wind served as a potential low-level distracter stimulus on the capacity to learn colour information (Dyer, 2006). Thus, the bees' capacity to learn the rewarding colour irrespective of cross-wind direction was tested to allow for an assessment of whether elemental or multi-modal learning (as experienced by the first group of bees) occurred at a different rate of performance.

Flight path recording

The flights of a sample of the bees were acquired using a webcam (Logitech V-UBB39, Switzerland), placed vertically over the flight apparatus. In post-processing, the flight paths of the bees were obtained by digitizing the videos using custom-written scripts in MATLAB (release 2014c, The Mathworks, USA). The raw displacement data of the bees were passed through a 10 Hz fourth-order Butterworth low-pass filter to smooth the flight paths. Upon take off, the mean time taken by the bees to fly through the apparatus was generally <1.5 s, which is well within the time frame for honeybee retention of working memory for matching to sample-type problems (Zhang et al., 2005).

Statistical analysis

The statistical analysis tested the null hypothesis of no effect of training on the bees' performance in the multi-sensory associative tasks by implementing a generalized linear model (GLM). The model included as a dependent variable the percentage of correct choices made by the N=10 bees over every five trials for each of the seven bouts that consisted of the training phase. The GLM assumed binomially distributed errors and a logit link function. All analyses were performed using the routine *glm* available in the R statistical software v.3.1.5 (R Core Team 2015, cran.r-project.org). Similar methods have been used in other studies to test learning in bees (Dyer and Garcia, 2014) while further information on the statistical technique can be found in Faraway (2006).

The effect of the number of flight trials on the decision performance between the group of individuals (N=10) that were presented with a multi-modal sensory association problem and the group of individuals (N=10) presented only with the visual problem was analysed by means of an ANCOVA fitted with a GLM. The model included the percentage of correct choices (p) as dependent variable, the number of trials as continuous predictor and perceptual task, i.e. multi-modal versus visual, as categorical predictor.
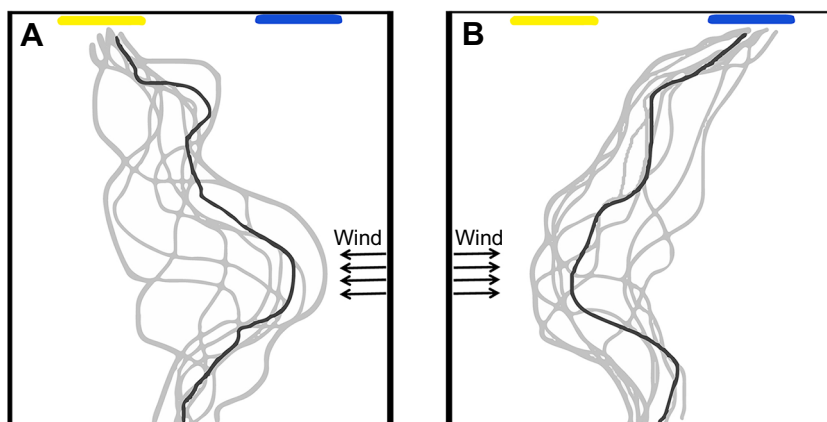

Fig. 3. Digitized flight paths of different individual bees through the apparatus during learning trials recorded with an overhead camera. The reward was placed in the yellow (A) and blue (B) stimulus, respectively. The bees typically flew into the wind by partially aligning their body with the wind direction as they traversed through the region of cross-wind. Subsequently, the bees tended to realign their flight path along the centreline of the tunnel before flying towards the chosen stimulus. To highlight the variation in flight path of the bees due to the cross-wind, the darker line in A and B is the flight path of the same individual bee during two consecutive learning trials when the wind was from the right and left, respectively.

RESULTS

Each individual bee became airborne once it entered the flight apparatus (Fig. 1A) and flew through the region of cross-wind before making a choice between the two chromatic stimuli at the end of the flight arena. Upon encountering the region of cross-wind, the bees typically deviated from the centreline of the arena and flew upstream to traverse the cross-wind, before they realigned with the centreline prior to flying towards the visual stimuli (Fig. 3).

Effect of learning on bee performance for multi-modal learning group

We found a significant effect of training on the percentage of 'correct' choices (p) made by free-flying bees after integrating both visual and mechano-sensory information ($z=4.20$, $P<0.0001$; Fig. 4A).

Reverse-side contingency test for multi-modal learning group

A reverse-side contingency test was conducted to dissect the possibility that bees use either chromatic (visual) or location

(spatial) information to solve the problem of linking the context-setting wind cue with the visual stimuli. At the end of the learning trials, bees selected the correct target with a frequency significantly different from chance [mean probability of 'correct' choices ($p_{learning}=0.800$, $\chi^2_{learning}=54.0$, $P<0.0001$; Fig. 4A,C]. When the visual stimulus locations were switched in the reverse-side contingency tests, the bees' choice for the correct colour stimulus was significantly higher than chance expectation (rst) ($p_{rst}=0.753$, $\chi^2_{rst}=38.5$, $P<0.0001$). No significant difference was found between the percentage of correct choices at the end of the training phase and start of the reverse-side contingency test ($z=0.969$, $P=0.333$; Fig. 4B,C), showing that bees use colour as the predicting cue rather than spatial position in the arena. However, these results could have potentially been explained by bees rapidly reverse learning the spatial locations of the cues as they were switched in the reverse-contingency tests; therefore, we additionally conducted analyses considering the last choice prior to the reverse-side contingency test ($p_{learning}=0.800$) to the first choice of each bee after the change ($p_{rst}=0.800$). In this case, there was also no significant change in performance ($\chi^2=0.00$, $P=1.00$), confirming that the bees used
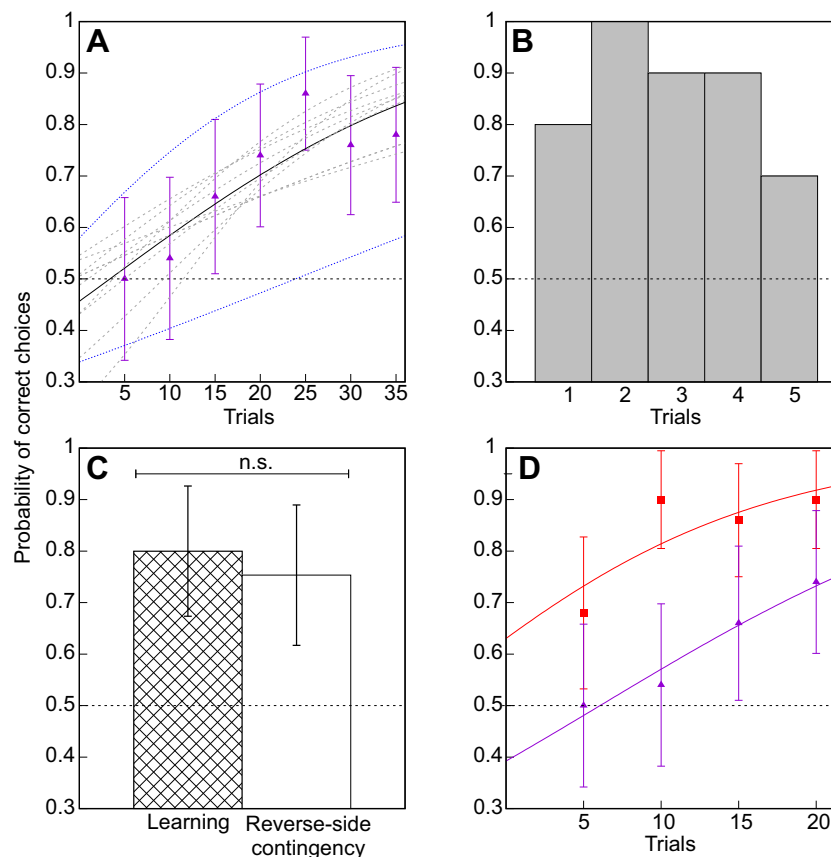

Fig. 4. Learning performance. (A) The solid black line represents the logistic function describing the performance of $N=10$ bees during the discrimination task as a function of training bouts (purple triangles and error bars represent means and 95% percentiles among all individuals for each bout). The results indicate that training had a significant effect on bee performance after the 35 trials constituting the training phase ($P<0.0001$). Dotted lines represent the 95% confidence intervals of the model and dashed grey lines correspond to logistic functions fitted to data for each individual bee. The horizontal dashed line shows the 50% chance expectation. (B) The first five choices for each of $N=10$ bees for the reverse-side contingency test, performed immediately after bees had completed the training phase. In all instances, bee performance was higher than the 50% chance expectation (dashed line), showing that bees transferred information rather than re-learning the problem. (C) Performance in the learning and reverse-side contingency test for the $N=10$ bees employed for the experiment. In both tests, the bees chose the target colour for which they had been trained more frequently than expected from chance ($P<0.001$ in both cases); however, we found no significant difference in performance between the learning and reverse-side contingency tests ($z=0.969$, $P=0.333$). (D) Performance of bees ($N=10$) selecting the 'correct' target colour in the presence of wind but without integrating information regarding colour, i.e. decoupled visual and mechano-sensory input, for 20 trials (red line) and encapsulation of the first 20 trials in A where visual and mechano-sensory information were coupled (purple line). Training had a significant effect on performance in both cases ($P=0.0002$), and there is a significant difference in performance for the two different experimental conditions ($P<0.0001$), suggesting different levels of perceptual difficulty for each visual task. Error bars indicate s.e.m. for proportion data in all cases.

colour as a cue immediately after the stimuli locations were switched in the test phase.

Non-rewarded test for multi-modal learning group

In the non-rewarded touch test for the multi-modal learning group, in the absence of the context-setting cross-wind, the bees chose the yellow stimulus 51.5% of the time. We found no evidence for preference of this colour over the blue hue option that was significantly different from chance expectation (χ^2=0.180, P=0.671).

Evaluation of learning rates between experimental groups

To evaluate the perceptual difficulty of a multi-modal task where both mechano-sensory (wind) and visual information (colour) are processed together for enabling a decision, we compared such a task with independent bees solely learning the colour of a stimulus in the presence of a cross-wind acting as a distracter.

Analyses of the effect of trials across the two groups indicated a significant effect of training on performance for the two groups [deviance (G)=14.4, P=0.0002], and a significant difference in performance resulting from using only visual or multi-modal information (G=26.8, P<0.0001). Differences in performance were also observed by the lower number of trials required by the bees to learn the target colour when only visual information was available to solve the visual task (Fig. 4D, solid red line) and the higher number of correct choices observed when only visual information was associated with the rewarding target during 20 trials (p_{visual}=0.835), compared with the performance of the bees trained with the multi-modal condition in the same number of trials ($p_{multi-modal}$=0.610).

DISCUSSION

The understanding of multi-modal sensory processing is a contemporary topic with important insights contributed by recent studies on humans (Morrot et al., 2001), and invertebrates such as honeybees (Leonard and Masek, 2014). In this study, we hypothesized that bees are capable of integrating mechano-sensory information with visual stimuli for modulating foraging choice. We sought to assess the capacity of bees to associate mechano-sensory and visual stimuli in a free-flying environment using appetitive–aversive conditioning, which promotes learning during visual association tasks (Avarguès-Weber et al., 2010). The bees had to not only encode the direction of the wind but also use it as a context-setting cue for making a choice between two saliently different colours. To be rewarded, the testing paradigm required bees to reverse learn the rewarding chromatic stimuli in the context of the wind cue over multiple flight trials. Previous work shows that honeybees are indeed capable of reverse learning saliently different colours (Von Helversen, 1974) faster than similar colours (Dyer et al., 2014a). Interestingly, an effect of stimulus salience on problem solving is also apparent for olfactory stimuli in honeybees (Mota and Giurfa, 2010).

In our experiment, the bees were clearly influenced by the wind as their flight path deviated from the centreline of the test apparatus and individuals tended to orient upstream while they traversed through the region of cross-wind (Fig. 3). Upon exiting the cross-wind region, they realigned with the centreline of the flight tunnel prior to making a choice between the two visual stimuli (Fig. 3). Wind profile is critical for not only flight-control but also higher-level decision making as it can influence foraging efficiency and colony-level energetics (Heinrich, 2004). In other studies, bees have been shown to regulate their flight path over long distances to

account for cross-winds when flying to and from foraging sites (Riley et al., 1999). Such flight path corrections would require long sampling times of the wind and one strategy to enable this could be the use of a combination of mechano-sensors that respond to different time scales of wind (Alexander, 2004). The wind profile in the outdoor environment can vary over a wide range, from intermittent gusts to sustained durations of strong winds (Stull, 1988). In our experiment, the bees learnt to associate the direction of the respective 'wind gusts' that would have induced perceivable side forces (Fig. 3) with the visual stimuli. While the sensory systems that are responsible for encoding winds varying over short duration is still unknown, the mechano-sensors present in the base of the antennae, the Johnston's organ, are likely candidates (Yorozu et al., 2009). Alternatively, the mechano-sensitive Bhom's bristles, present in the base of the antennae, provide feedback on the antennae position and could also be responsible for encoding wind-induced deflections of the antennae into relevant sensory inputs (Sane, 2009).

In the non-rewarded touch test, in which there was no context-setting cue as a predictor of the correct colour, bees from the multi-sensory learning group chose between the two differently coloured visual stimuli at a level that was not significantly different to chance expectation. This suggests that neither colour preferences (Morawetz et al., 2013) nor acquired associations during the experiments caused a colour bias that influenced the findings. This result also confirmed that the bees required the presence of wind as a type of occasion-setting cue in order to make accurate colour choices.

The reverse side contingency tests permitted us to test the preference between spatial location and chromatic properties. The colour cue appears to be more important for honeybees than spatial location in the context of an association with mechano-sensory information, as the success rate of the bees choosing the correct colour would have significantly dropped if spatial location was the cue used during the experiment; instead, bees preferred to choose stimuli on the basis of colour information (Fig. 4B,C). This is consistent with recent findings that colour is a more hierarchically represented cue in some spatial shape-processing tasks (Morawetz et al., 2013).

A potential limitation of testing multi-sensory integration in free-flying bees is the possibility that alternative cues may be used to solve the problem. For example, the bees' flight paths may be deviated by the wind such that they only use a spatial visual cue to solve the problem of which stimulus side to choose. To reduce this possibility, we used a homogeneous visual environment arena where the only salient visual information was the coloured target and distractor stimuli. These stimuli were also presented at a large visual angle to promote colour vision, whilst reducing reliance on spatial cues (Giurfa et al., 1996). Additionally, the effect of wind shows that the bees actively manoeuvred upstream and then appeared to realign with the centreline of the tunnel prior to decision making about the respective colour stimuli (Fig. 3); if bees were solely relying on spatial cues, then their performance in the reverse contingency test would have shown a significant drop, but this did not occur (Fig. 4B,C).

Colour vision is well studied in bees and by ensuring that our visual stimuli promoted colour processing (Table S1), it is possible to make some initial inferences about potential neural pathways where such signals might project to facilitate integration with mechano-sensory information that would enable the decision making we observed. Whilst the honeybees' long wavelength sensitive green photoreceptor signals are first processed in the

lamina and probably promote fast achromatic responses to stimuli (Ribi, 1975), useful for flight control, the trichromatic (UV, blue, green) receptor signals are initially processed in the outer layers of the medulla, before processing in the inner medulla where there is evidence of colour-opponent processing (Dyer et al., 2011; Kien and Menzel, 1977). These processed opponent signals subsequently project both directly to the mushroom body and also to the lobula (Dyer et al., 2011; Yang et al., 2004). Whilst little is currently known about mechano-sensory processing within the brain of bees, there is evidence from *Drosophila* brains that antenna input, via the wind-sensitive neurons in Johnston's organ, can enable an insect to have complex sensory representations of wind within the brain (Yorozu et al., 2009). As signals from the Johnston's organ do project to the mushroom bodies (Mamiya et al., 2008), this seems a plausible brain structure where both colour visual and mechano-sensory processing could be integrated.

A contemporary question in interpreting multi-sensory integration which promotes decision making is whether the different channels of information are integrated in either an elemental or non-elemental form. The experiment that tested individual bees learning the saliently different colours in the presence of a 'noise distracter' stimulus of non-predicting wind showed that bees rapidly learn a colour cue to a high level of performance, in a process that is consistent with an associative mechanism (Fig. 4D). In comparison, for the group that had to use the wind cue to predict the rewarding colour stimulus, the problem took these bees significantly more trials to reach similar levels of performance (Fig. 4D). This suggests that learning was not a simple process of interpreting the wind cue in a binary sense that might predict fast and accurate reverse learning of the colour problem; instead, the brain had to slowly acquire and process the relationships (Fig. 4A). This is consistent with a framework where occasion-setting cues are a form of non-elemental processing in bees (Mota et al., 2011), *Drosophila* (Brembs and Wiener, 2006) and some vertebrates (Bouton and King, 1983; Palmatier and Bevins, 2008; Rescorla et al., 1985). Our results suggest that similar to earlier studies, free-flying bees may also be capable of using wind as an occasion-setting cue to facilitate decision making in foraging contexts.

One potential context where the ability to integrate mechano-sensory and visual stimuli may be important is flying in complex environments where wind may affect different species of flowers like tall-stemmed daisies or tree-based flowers in different ways (Mirwan and Kevan, 2015). Additionally, sensing the wind environment could enhance decision accuracy and speed about which types of flower contain rewards (Burns and Dyer, 2008; Mirwan and Kevan, 2015). This is analogous to the capacity of the bee brain to integrate both colour and olfactory information for reducing uncertainty in decision making (Leonard et al., 2011). Additionally, the association of wind with certain visual features could also aid in improving flight efficiency, for example by avoiding edges of objects in windy conditions where strong destabilizing jets and gusts can be detrimental to flight. The wind profile can also have significant influence on the energetics of flight as it is likely that gusty conditions can render flight energetically costly because constant manoeuvres and course corrections are necessary. Thus, the ability to consider the prevailing wind profile in making foraging decisions can be beneficial in optimizing foraging strategies and improving the energetics of resource acquisition. However, our understanding of the influence of mean and transient wind on the flight energetics of insects is still limited at present and warrants future work to better understand this complex interaction.

While the evolutionary provenance of the mechanisms supporting the integration of visual and mechano-sensory perceptual channels in aiding foraging decision making are still unclear, our results indicate that the bee brain does possess such a capacity (Fig. 4B). Evolutionary principles suggest that this perceptual capacity would probably eventuate in a miniature brain if such outcomes were of high value to survival. Given the significant influence of wind on the energetics of resource acquisition for flying animals, further behavioural and neuro-physiological studies that assess the significance of such sensory integration in improving foraging efficiency in realistic environments will be very useful.

Supplementary information
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Table S1. Colorimetric specification of target stimuli

Honeybee photoreceptor contrasts, relative intensity and colour distances of the 'yellow' and 'blue' stimuli considering two models of opponent processing showing stimuli were saliently different in colour for bee perception (Chittka, 1992; Backhaus, 1991; Avargu`es-Weber et al., 2014); see Fig. 1 in main mauscript. Reflectance spectra from the coloured targets and background were measured between 300 and 800 nm at 0.7 nm intervals with a USB 2000 spectrophotometer (Ocean Optics, USA) connected to a deuterium-halogen DH-200 light source (Ocean Optics, USA) and a probe mounted at 45° by means of a bifurcated 200 μm optical fibre (Ocean Optics, USA). Reflectance profiles were measured relative to an lambertian, PTF WS-1 reflectance standard (Ocean Optics, USA). Ten readings, each one consisting of the average of 5 scans, were recorded for the targets and backgrounds and subsequently processed for obtaining mean reflectance profiles between 300 and 650 nm at 10 nm intervals (Fig. 1B, main manuscript) using custom-written code for Matlab release 2014b (The Mathworks, USA).

	'yellow'	'blue'
UV-Receptor	0.793	0.877
Blue-receptor	0.666	0.885
Green-receptor	0.786	0.618
Colour distance (COC units)	bckg: 3.45 blue: 7.00	bckg: 4.56
Colour distance (Hexagon units)	bckg: 0.123 blue: 0.263	bckg: 0.263

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