

# Honeybees (*Apis mellifera*) Learn Color Discriminations via Differential Conditioning Independent of Long Wavelength (Green) Photoreceptor Modulation

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## Abstract

**Background:** Recent studies on colour discrimination suggest that experience is an important factor in how a visual system processes spectral signals. In insects it has been shown that differential conditioning is important for processing fine colour discriminations. However, the visual system of many insects, including the honeybee, has a complex set of neural pathways, in which input from the long wavelength sensitive ('green') photoreceptor may be processed either as an independent achromatic signal or as part of a trichromatic opponent-colour system. Thus, a potential confound of colour learning in insects is the possibility that modulation of the 'green' photoreceptor could underlie observations.

**Methodology/Principal Findings:** We tested honeybee vision using light emitting diodes centered on 414 and 424 nm wavelengths, which limit activation to the short-wavelength-sensitive ('UV') and medium-wavelength-sensitive ('blue') photoreceptors. The absolute irradiance spectra of stimuli was measured and modelled at both receptor and colour processing levels, and stimuli were then presented to the bees in a Y-maze at a large visual angle (26°), to ensure chromatic processing. Sixteen bees were trained over 50 trials, using either appetitive differential conditioning (N=8), or aversive-appetitive differential conditioning (N=8). In both cases the bees slowly learned to discriminate between the target and distractor with significantly better accuracy than would be expected by chance. Control experiments confirmed that changing stimulus intensity in transfers tests does not significantly affect bee performance, and it was possible to replicate previous findings that bees do not learn similar colour stimuli with absolute conditioning.

**Conclusion:** Our data indicate that honeybee colour vision can be tuned to relatively small spectral differences, independent of 'green' photoreceptor contrast and brightness cues. We thus show that colour vision is at least partly experience dependent, and behavioural plasticity plays an important role in how bees exploit colour information.

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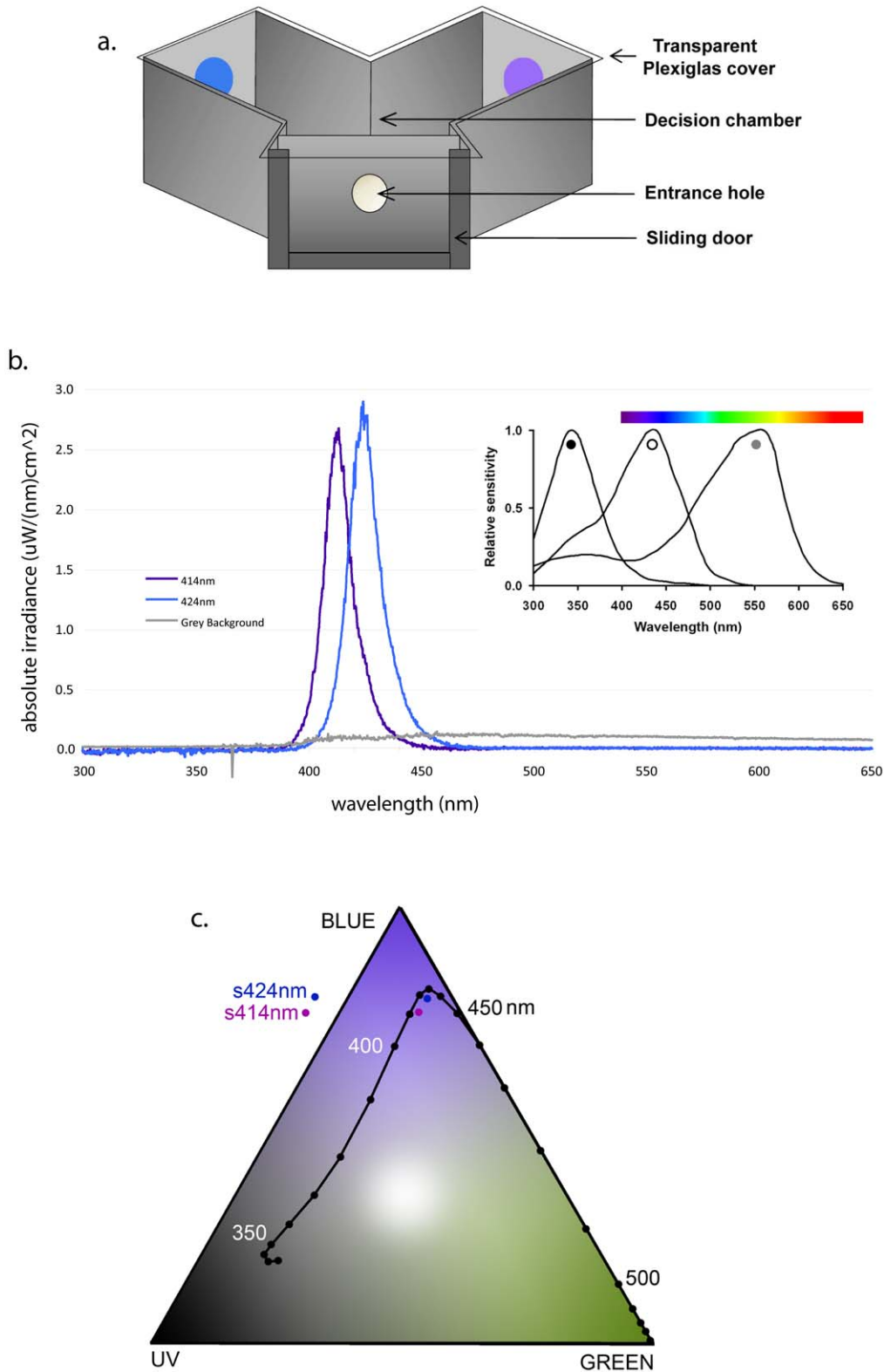
## Introduction

Colour vision allows pollinating insects to detect and discriminate flowers on the basis of reflected wavelengths, independent of potential brightness differences [1] such as those occurring in complex natural environments [2]. Indeed, there is strong evidence that flowering plants (angiosperms) have evolved spectral signals that are optimally discriminated by the visual system of hymenopterans capable of trichromatic vision [3;4]. The classic model for understanding colour vision in hymenopteran insects is the honeybee [5–14]. Individual bees tend to be flower constant, and will visit one type of flower as long as it is easily 'discriminated', facilitating efficient delivery of species-specific pollen and thus driving flower evolution [15].

Honeybee trichromatic vision is based on an 'ultraviolet' (UV) photoreceptor, which is maximally activated by 344 nm radiation, a 'blue' photoreceptor (maximally activated by 436 nm radiation),

and a 'green' photoreceptor (maximally activated by 544 nm radiation) [16]. To characterise a colour vision system in a given organism, it is necessary to empirically determine the  $\Delta\lambda/\lambda$  function, which describes the differences in wavelength that can be resolved in various regions of the electromagnetic spectrum [17]. The  $\Delta\lambda/\lambda$  function in free flying honeybees [18] yields the finest chromatic discrimination at wavelengths near 390 and 490 nm. Conversely, in the band of wavelengths between 425 and 475 nm, discrimination is relatively poor. This behavioural result dovetails with calculations based on measurements of peak absorbance and bandwidth in the three colour photoreceptor types in this species [3;16].

An important question regarding colour vision is whether it is entirely mediated by "hard-wired" physiological mechanisms [19], or is partially learned via individual experience [20]. In honeybees, two recent studies have shown that colour vision is modifiable with



**Figure 1. Testing of honeybee colour vision.** (a) Schematic diagram of Y-maze apparatus used to test free flying bees. LED array position was varied between experimental trials (see Methods). (b) Example of an absolute irradiance spectra measurement for LED stimulus arrays. Inset shows spectral sensitivity for long- (grey circle), medium- (open circle), and short- (black circle) wavelength sensitive photoreceptors in honeybees. Colour bar indicates approximate perceived colour range in humans. Adapted from Dyer et al., 2011 using the data for honeybees from Peitsch et al. 1992. (c) Plots of LED stimuli in a Maxwell triangle colour model representing the trichromatic (ultraviolet, UV; blue, B; green, G) colour vision of honeybees. Spectral loci show theoretical stimulation by spectrally pure radiation.  
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experience for perceptually similar colour stimuli. In the first study, free flying honeybees were trained on a colour discrimination task in a Y-maze apparatus using either absolute or differential conditioning [21]. Absolute conditioning involves training on a target presented in isolation, while differential conditioning presents the target stimulus in relation to a perceptually similar stimulus. Bees trained using absolute conditioning could reliably discriminate very dissimilar stimulus colours, but generalised similarly coloured stimuli [21]. In contrast, bees provided with differential conditioning could learn much finer discriminations, albeit slowly [21]. A second study tested whether the association of an aversive tasting substance with a distractor stimulus influenced learning, and concluded that honeybees can learn to resolve very similar colours if a combination of appetitive and aversive differential conditioning was used [22]. These studies suggest that colour discriminations are shaped differently depending upon individual experience [21–23]. However, as detailed below, a potential confound in both studies was the use of stimuli with the potential for incongruent modulation of the ‘green’ photoreceptor during differential conditioning.

Interpretation of behavioural experiments on colour vision with honeybees can be difficult due to the complexity of the colour visual pathway [20], with different colour channels employed in different contexts depending upon the subtended visual angle of the stimulus [24;25], or upon motion cues [26]. While the ‘UV’, ‘blue’ and ‘green’ photoreceptors all contribute to chromatic perception via opponent processing [9;20], these channels can invoke varying patterns of activation in the brain. Thus, the “UV” and “blue” photoreceptor outputs project directly to the second optic ganglia of the bee brain (the outer medulla) [27;28], while the ‘green’ photoreceptor signal is initially processed in the first optic ganglia (the lamina) [29–31]. Furthermore, behavioural experiments have shown that modulation of the ‘green’ photoreceptor is responsible for stimulus detection at small visual angles [24;32;33], broad field motion processing (as measured with the optomotor response) [34], small field motion detection at high frequencies [35;36], and some complex pattern recognition tasks, which can be learned only through differential conditioning [37].

It is currently not clear if previously described learning of fine color discriminations [21;22] can be explained solely on the basis of a simple mechanism based on the presence of contrast in the ‘green’ photoreceptor channel, or actually depends on differential conditioning and more complex colour processing that suggests neural plasticity depending upon individual experience, or tuning of attention. To evaluate these possibilities, we assessed the ability of free-flying honeybees to learn to discriminate perceptually similar chromatic stimuli which exclude modulation of the long wavelength ‘green’ photoreceptor, and were presented at a visual angle that predominantly stimulates the chromatic processing system of the honeybee. This work provides important insights into the behavioural flexibility of bees and other insects, and provides indirect information about possible neural structures involved in colour learning [20;38].

## Materials and Methods

Experiments were performed outdoors in the Jock Marshall Reserve at Monash University. Free-flying honeybees (*Apis mellifera*) were allowed to visit a gravity feeder containing 10% (vol/vol.) sucrose solution [39]. Experiments were conducted during summer on days of low wind [40] with ambient temperature between 20–30°C. The colour temperature of the foraging environment was approximately 6500°K [41;42]. Individual bees were recruited from the gravity feeder to a nearby

testing site, marked on the dorsal abdomen for identification, and trained to enter a Y-maze apparatus (Figure 1) to collect a 30% sucrose reward.

## Stimuli

Target and distractor stimuli each consisted of constantly illuminated arrays of 76 light emitting diode (LED) bulbs [individual bulb diameter 5 mm, 410 nm (405–415 nm half-bandwidth) or 420 nm (415–425 nm) manufacturer specified peak wavelength; VioLED, Inc., Taichung, Taiwan, ROC]. The LEDs were controlled on independent channels using a purpose built electronic control box. Each LED array was placed behind a 3 mm thick sandblasted UV-transparent plexiglas diffuser, yielding an evenly illuminated circular target of 7 cm diameter. During experiments each stimulus was positioned in a Y-maze (Figure 1a) arm at 15 cm from the decision chamber. This viewing distance equates to a visual angle of 26° at the decision line in the Y-maze. In addition, these stimuli encompassed an angle greater than 15° from any position in the decision chamber, thus always exceeding the visual angle required to stimulate the chromatic pathways in the honeybee visual system [10;24;25;43].

The peak wavelength separation between the LED stimuli was selected to ensure that the colour discrimination task would be perceptually difficult, being close to the known limit of discrimination ability for the honeybee [18]. The output of the LED stimuli and diffuser combination was sampled by absolute irradiance measurement using a spectrophotometer (model S2000, Ocean Optics, Dunedin, FL, USA) calibrated against known deuterium and halogen light sources (DH-2000-CAL, Ocean Optics, Dunedin, FL, USA) [44;45]. The background material surrounding the LED stimuli consisted of a grey card (HKS 92N) which was also measured with the spectrophotometer in the experimental conditions specified above.

Absolute irradiance spectra had peak wavelengths for respective stimuli of 413.9 and 424.4 nm for the ‘410’ and ‘420’ nm manufacturer-specified bulbs (Fig. 1b); LED stimuli are thus described as 414 and 424 nm for the remainder of this report. Absolute irradiance spectra were obtained at a range of outputs using the control box, and the input-output function for each LED array was then modelled for relative stimulation of the honeybee colour visual system by converting irradiance spectra to relative quantum flux, and numerically integrating at 10 nm steps with published data for honeybee photoreceptor sensitivity [16]. Power settings were calibrated so that there was less than a 3% variation in the summed brightness to the ‘UV’, ‘Blue’ and ‘Green’ photoreceptors for the chromatic processing system (equations 1–3), whilst modulation of the ‘Green’ photoreceptor was less than 5% (equation 4). This was achievable, as stimuli were specifically chosen to minimally stimulate the beta peak of the ‘green’ photoreceptor; lying in the ‘flat’ region of the trough between the alpha- and beta-peaks [46]; there was in fact little overall activation of the ‘green’ photoreceptor compared to the shorter wavelength photoreceptors (Fig. 1b-inset). Previous behavioural experiments suggest that the colour visual system of honeybees does not process brightness as a dimension in chromatic perception [5;18;47–50]; thus, the 3% variation in total stimulus intensity we empirically measured is negligible. In addition, analysis of behavioural experiments for honeybees vision suggest that brightness differences need to be greater than 14–20% for a detection task even at the correct visual angle (<15°) for the ‘green’ photoreceptor modulation [24;51], above 7.2% in a optomotor movement task [34], or ‘high’ for shape recognition tasks [52]. Indeed, studies on the potential use of Green-receptor contrast by honeybees at a large visual angle show that input is

negligible due to low sensitivity [25;53], especially when strong chromatic cues are also present [53]. In agreement with these findings, experiments on another trichromatic insect, the hawkmoth (*Macroglossum stellatarum*), shows that insect colour vision does not separately evaluate ‘green’ contrast cues in the presence of chromatic cues even when long-wavelength ‘green’ photoreceptor modulation is changed by a factor of 10 [54]. In summary, under our experimental conditions with stimuli that strongly modulate differences in the honeybee UV- and Blue- photoreceptors, but do not modulate the Green-receptor to above known threshold values, only the chromatic processing pathways could potentially enable learning of differences in stimulus properties.

$$Q_i = R \int_{310}^{650} S_i(\lambda) I(\lambda) d\lambda \quad (1)$$

Where  $Q$  is the quantum catch for ‘i’ = (1:UV, 2:Blue, 3:Green) photoreceptors respectively,  $S_i(\lambda)$  is the spectral sensitivity function of a particular photoreceptor according to data for honeybees [16], and  $I(\lambda)$  is the relative spectral photon flux of stimuli. The adaptation state of the visual system follows a von Kries normalisation (R; equation 2) [42].

$$R = 1 / \int_{310}^{650} S_i(\lambda) B(\lambda) d\lambda \quad (2)$$

where  $B(\lambda)$  is the relative spectral photon flux of the background and ‘i’ = 1–3.

$$\text{Brightness contrast} = \Sigma Q_i^{414} / \Sigma Q_i^{424} \quad (3)$$

where ‘i’ = 1–3.

$$\text{Green photoreceptor contrast} = Q_i^{414} / Q_i^{424} \quad (4)$$

where ‘i’ = 3.

$$\text{chromaticity coordinates}(uv, b, g) = Q_i / \Sigma Q_i \quad (5)$$

where ‘i’ = 1–3 and thus  $uv+b+g=1.0$  (Table 1).

The chromaticity coordinates (equation 5) were used to plot the loci of the respective test stimuli in a Maxwell Colour Triangle as this colour model is independent of brightness effects between

stimulus and the background [11;41;55–58]. This model shows that the stimuli have a negligible influence on the ‘Green’ photoreceptor (Fig. 1c) which is because the stimuli lie in the saddle of the beta-peak of the ‘Green’ photoreceptor (Fig. 1b). To enable a comparison of the current data to three previous studies that have already shown that differential conditioning is important to colour learning in honeybees (but did not specifically exclude ‘Green’ contrast), we calculated the Euclidean colour distance in the Maxwell colour triangle (Table 2).

In order to ensure that the use of LED stimuli did not introduce any new effects on learning relative to previous behavioural studies in which reflective (colour card) stimuli were employed, we replicated behavioural experiments which have previously been described for card stimuli and have shown bees are relatively insensitive to brightness differences for stimuli presented at a large visual angle [21;53]. In the first of these control experiments, bees were given appetitive absolute conditioning for 20 trials to either the 414 nm or 424 nm stimuli (4+4 bees counterbalanced), and then transfer tests that presented (i) both 414 nm and 424 nm stimuli, and (ii) the target stimulus (414 nm or 424 nm) versus a novel stimulus comprised of white LED filtered by a transparent HKS 3N yellow filter. In the second control experiment, bees were provided with appetitive differential conditioning to the 414 nm and 424 nm stimuli (counterbalanced) for 50 trials and then provided with (i) a learning test, (ii) a transfer test that presented the target stimulus at a 50% increase in stimulus brightness, and (iii) a transfer test that presented the distractor stimulus at a 50% increase in stimulus brightness.

### Behavioral testing

Reward or neutral/aversive solutions were placed on small platforms 1 cm in front of the respective LED stimuli. The platforms were made of thin wire and were identical, so did not provide a visual cue. Previous work has also shown that these solutions do not provide olfactory cues for bees [22]. The 414 and 424 nm stimuli were alternated as the target and distractor between bees in a counterbalanced experiment design. Each bee was trained for 50 trials, which is a training length that has previously been reported for fine colour and spatial discrimination tasks with differential conditioning in bees [20;44;59–62]. The first choice, defined as crossing the decision line in the Y-maze, of the bee in each trial was recorded and used for statistical analysis [63]. Correct identification of the target LED stimulus was rewarded on each learning trial with a 30% (vol./vol.) sucrose solution reward, while incorrect choices yielded either a neutral (tap water) or aversive (aqueous 60 mM quinine HCl) stimulus. After each trial, the contact surfaces and Y-maze were cleaned with 10% ethanol to preclude the use of scent marking cues on subsequent trials. Stimuli were presented in alternative Y-maze arms in a pseudo random order. At the conclusion of the 50 trial training cycle, each bee received 45 s duration unrewarded touch tests counterbalanced within subject for each arm of the Y-maze, during which the

**Table 1.** Colour distance in a Maxwell colour triangle for ‘colour’ stimuli that are only learnt by free flying honeybees that experience differential conditioning.

Study	Stimulus pair	Triangle colour distance
Giurfa 2004 [20]	HKS 37N vs 43N	0.089
Avarguès-Weber et. al 2010 [21]	HKS 43N vs 47N	0.071
Avarguès-Weber et. al 2010 [21]	HKS3N vs 68N	0.084
Dyer & Murphy 2009 [22]	Similar ‘blues’	0.043
Current	414 vs 424 nm	0.039

The stimuli used in the current study are more perceptually similar than previous studies.

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**Table 2.** Chromaticity coordinates ( $uv, b, g$ ) which are the normalised photoreceptor quantum catches [P(UV, Blue, Green)] for a given stimulus such that  $uv+b+g=1.0$ .

Stimulus	$uv$	$b$	$g$
414 nm	0.136	0.752	0.112
424 nm	0.079	0.801	0.120

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number of touches on the empty reward platform were recorded. This touch test allows for the collection of non rewarded data to exclude all possible confounds like scent marking. Between the respective touch tests, a refresher trial was presented to ensure that the bee remained motivated for the subsequent test as has been previously described [22;63].

### Data analysis

Performance during the 50 trial training cycle was evaluated by calculating the frequency of correct choices across 10 blocks of 5 trials, and the accuracy of the learned color discrimination was assessed from performance on the unrewarded touch tests within each group (neutral and aversive). Acquisition curves were plotted for each reward condition, and these data were compared to the trial block data using a 2-way repeated measures ANOVA procedure. Unrewarded touch test data were evaluated using the Wilcoxon signed rank test against an expected median correct choice frequency of 50% (chance level), and the different treatments (quinine vs. water) were compared using the Mann-Whitney U test.

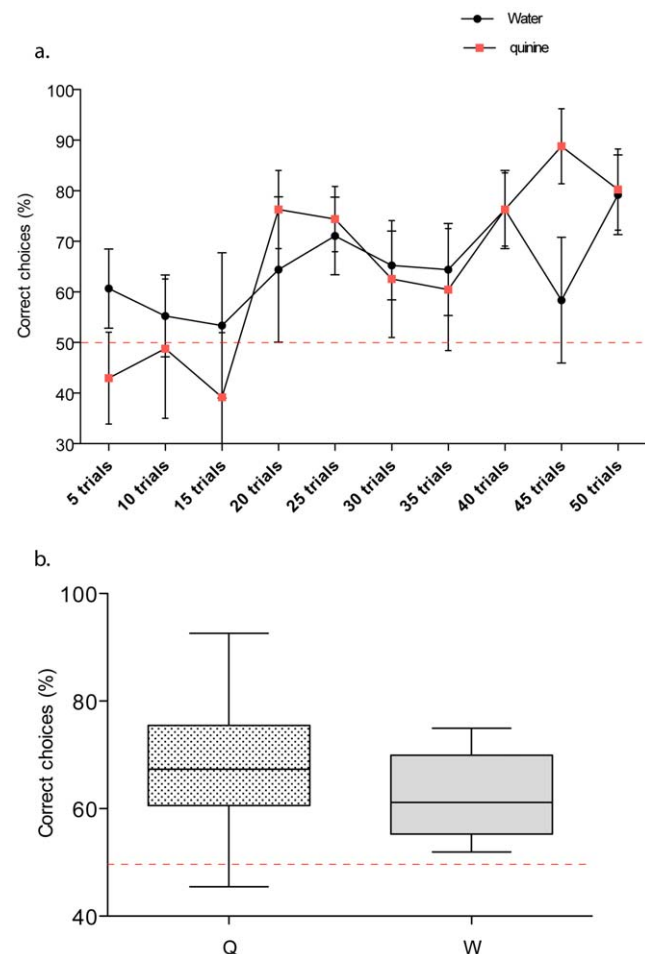
### Results

Acquisition performance for the color discrimination task over the 50 trial training cycles are presented in Figure 2a. Both the neutral (water) and aversive (quinine) distractor stimuli resulted in approximately 80% frequency of correct choices in the final trial block (trials 45–50; mean frequency of correct choice water = 79%, quinine = 80% diff = 1%, 95% CI -39–40%, ns). Improvement of correct choice frequency with repeated trials was significant for both treatment groups (2-way ANOVA, trial block X treatment,  $df = 9,1$ , trial block  $F = 2.9$ ,  $p = 0.006$ ). The learning curves clearly show that differential conditioning was important to the task as an increase in acquisition only begins after 15 trials, whilst previous empirical studies report that with absolute conditioning high levels of colour learning occur in fewer than 8 trials [21;50;64]. This behavioural finding is supported by the colorimetric modelling which shows that the colour distance between the 414 and 424 nm test stimuli was less than the colour distances between stimuli for which previous work has shown differential conditioning is important for colour learning in free flying honeybees (Table 2).

The overall rate of acquisition and correct choice frequency after the final trial block were similar for the aversive and neutral stimuli (2 way ANOVA, trial block X treatment,  $df = 9,1$ , treatment  $F = 0.001$ ,  $p = 0.977$ ), suggesting that learning of this colour discrimination task with differential conditioning was independent of aversive reinforcement. This conclusion is also supported by the relative performance of the neutral and aversive stimulus groups in the unrewarded touch test procedure. Figure 2b summarises the outcome of the post-training assessment of learning accuracy, and for both the quinine ( $z = -2.38$ ,  $d.f. = 7$ ,  $p = 0.018$ ) and water groups ( $z = -2.52$ ,  $d.f. = 7$ ,  $p = 0.012$ ) correct choice frequency was significantly different from the expected 50% level by the Wilcoxon signed rank test. No difference was observed in frequency of correct touches between the neutral and aversive distractor conditions (median and 95% confidence quinine: 68.4%, 52.8–77.1%, water: 64.4%, 58.7–70.9%; Mann-Whitney  $U = 20.5$ , 2 tailed  $p = 0.248$ , ns). Data and LED spectra are logged at Dryad; DOI: doi:10.5061/dryad.g2r32

### LED control experiments

As the current study required the use of LEDs as stimuli in order to both produce narrow-band spectra, and at the same time be



**Figure 2. Colour learning in honeybees with differential conditioning.** (a) Acquisition curves for LED colour discrimination over 50 trials. Mean  $\pm$  SEM of correct choice frequency for each 5-trial block. (b). Unrewarded touch test performance following 50 trial training interval for neutral (W: water) and aversive (Q: quinine) distractor stimuli. Mean  $\pm$  SD correct touch frequency. Note that the Y-axis in each panel is abbreviated to enhance visibility. Red dotted lines indicate expected chance performance level (50%) bee choices following differential conditioning were significantly different from chance (see text for stats). In the touch tests the mean number of choices made by bees was  $29.4 \pm 1.4$  SEM. doi:10.1371/journal.pone.0048577.g002

displayed at a large visual angle, the following tests were conducted to test that this methodology elicited colour learning consistent with previous experiments employing coloured card stimuli.

In the first LED control test,  $N = 8$  honeybees were given appetitive absolute conditioning for 20 trials to either the 414 nm or 424 nm stimuli (4+4 bees counterbalanced), followed by transfer tests comparing the target to (i) the similar distractor, and (ii) to a previously unseen yellow LED, as described in the Methods section. This experiment established that in transfer test (i) bees could not discriminate between the similar target and distractor stimuli (414 nm and 424 nm) at a level significant from chance expectation ( $56.3\% \pm 3.6$  SEM; 1-sample t-test,  $t = 1.739$ ,  $d.f. = 7$ ,  $p = 0.126$ ), but (ii) these bees had learnt to reliably discriminate the target from a novel yellow stimulus ( $95.8\% \pm 1.2$  SEM,  $t = 14.547$ ,  $d.f. = 7$ ,  $p < 0.001$ ). Two of these bees were also given a further 40 trials learning opportunity with



the target stimulus; neither bee scored greater than 55% accuracy on a final transfer test presenting the 414 nm or 424 nm stimuli.

In the second LED control test, a separate group of  $N=8$  bees were provided with appetitive differential conditioning to the 414 nm and 424 nm stimuli (counterbalanced) for 50 trials, followed by a learning test and two transfer tests which respectively varied the target/distractor stimulus brightness by 50%, as described in the Methods. In all three tests the target stimulus was preferred, and there was no significant difference between the three respective tests [test (i; learning) = 69.7% $\pm$ 3.3 SEM; test (ii; target increased brightness) = 71.8% $\pm$ 3.1 SEM; test (iii; distractor increased brightness) = 73.4% $\pm$ 3.4 SEM; one way repeated measures ANOVA Wilks' lambda = 0.879,  $F(2,6) = 0.414$ ,  $p = 0.679$ , partial eta squared = 0.121] showing that honeybees used chromatic information rather than brightness as a cue to make the discrimination between these stimuli when presented at a large visual angle.

These two control experiments are consistent with previous studies that have used coloured cards to evaluate colour processing in free flying honeybees [21;53], indicating that the use of LED based stimuli in this experiment did not introduce any additional learning artefacts for colour experiments with free flying honeybees.

## Discussion

A number of recent studies suggest that the way in which insects, including: honeybees [21;22;65], bumblebees [44;59;60], hawkmoths [66], and ants [67] learn colour discriminations is dependent upon the type of visual conditioning procedure employed. Colour vision is defined as the capacity of a visual system to discriminate between colours based on spectral differences, independent of intensity [1;68]. The current study shows that in honeybees, the capacity to learn spectral differences following extended differential conditioning is independent of modulation the 'green' photoreceptor and overall stimulus brightness. This demonstrates for the first time that differential conditioning directly affects learning for colour vision. This finding is relevant for understanding how honeybees, and probably other insect pollinators, find 'colourful' flowers in complex natural environments, where there are likely to be large and frequent variations in brightness cues available to achromatic visual systems. Indeed, the current result is consistent with recent findings in bumblebees that differential conditioning can yield very fine spectral differences for stimuli that do not specifically modulate the 'green' photoreceptor channel, i.e. flower petal iridescence [62]. The plastic colour visual system of honeybees is likely to confer an advantage for making fast decisions in foraging situations where there are salient differences between rewarding and non rewarding flowers, as well as potentially reducing energy loss in scenarios where mimic flowers might closely replicate the colour of a rewarding model flower [4;20;69].

An interesting observation in this study was the absence of a significant difference in performance between groups trained with either appetitive differential conditioning, or aversive-appetitive differential conditioning (Fig. 2). This finding indicates that even though the colour task provided to the bees was perceptually difficult and required differential condition to learn, the task difficulty was unlikely to be at the limit of honeybee colour discrimination. This explanation is consistent with a previous report that honeybees trained with extensive aversive and appetitive differential conditioning can learn to discriminate a colour distance of 0.008 hexagon units [12]. We thus do not conclude that the association of an aversive stimulus with the

distractor stimulus in differential conditioning paradigms is ineffective, but rather that the discrimination task in this current experiment was sufficiently easy as to not warrant aversive stimulation [22].

The current study shows that colour vision in bees is not exclusively mediated by mechanistic or hard-wired elements, rather it is a plastic behavioural response, modifiable with specific visual experience. Interestingly, colour vision in vertebrate visual systems has also recently been shown to have a large degree of plasticity. Dichromatic mice genetically altered to express a human red photopigment instead of the mouse green pigment on the X-chromosome exhibit perception of an extended spectral range, when provided with extensive differential conditioning [70]. Further evidence of vertebrate behavioural plasticity for colour processing was provided by gene therapy experiments which added a third cone photopigment to dichromatic squirrel monkeys, which resulted in acquired trichromatic visual behaviour even in mature monkeys [71]. This level of behavioural plasticity for colour learning in both vertebrates and invertebrates is surprising, as it requires considerable neural plasticity within a very short time frame for the individual to learn to use the spectrally different signals. However, the evidence that mice and monkeys can tune colour vision following differential conditioning in a couple of days [70;71], and honeybees can learn new colour discrimination abilities in a matter of hours, suggests that colour vision plasticity is associated with a strong evolutionary advantage.

A critical issue which remains to be addressed is where in the brain learning of colour information may occur. The current result suggests that the locus or loci is not at an early stage of visual processing, since it is known that the UV and Blue photoreceptors first project to the 2<sup>nd</sup> optic ganglia (medulla) in the honeybee [20;27]. Electrophysiological and neuroanatomical studies on colour processing in bumblebees [28;72;73] suggest that there may be two separate colour pathways in the bee brain: a relatively hard wired neural projection from the outer layers of the medulla and lobula to the posterior protocerebrum which allows for coarse colour discriminations, and a modifiable second pathway via the anterior lateral protocerebrum which could potentially allow for colour learning [20]. Intracellular recordings from neurons along the posterior pathway display colour sensitive and colour opponent response, but show little adaptation to temporal variations from LED stimuli [72;73]. In contrast, neurons along the lateral protocerebrum pathway exhibit colour sensitivity, colour opponency and temporally complex patterns including adaptation and entrainment [28;72–74]. This leads to the testable hypothesis that elements of the lateral protocerebrum pathway could include neural correlates of the behavioural plasticity observed following either absolute or differential conditioning to colour stimuli [20]. Recent studies of neuroanatomical and neurophysiological correlates of colour information processing in the anterior optic tubercle, a region within the lateral protocerebrum of the honeybee brain, suggest that this region contains different subunits that reflect segregation of complex colour information [38]. Understanding how these neural circuits respond and adapt to different types of chromatic information depending upon individual experience could yield insights into the neural bases that of behavioural plasticity observed in free flying bees, and this represents a fertile target for future investigations.

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## Author Contributions

Conceived and designed the experiments: DHR RWW MGPR AGD. Performed the experiments: RWW AGD. Analyzed the data: DHR RWW AGD. Contributed reagents/materials/analysis tools: AGD. Wrote the paper: DHR MGPR AGD.

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