Seeing the light: Illumination as a contextual cue to color choice behavior in bumblebees

R. Beau Lotto*† and Lars Chittka‡

*Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V 9EL, United Kingdom; and ‡School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, United Kingdom

The principal challenge faced by any color vision system is to contend with the inherent ambiguity of stimulus information, which represents the interaction between multiple attributes of the world (e.g., object reflectance and illumination). How natural systems deal with this problem is not known, although traditional hypotheses are predicated on the idea that vision represents object reflectance accurately by discounting early in processing the conflating effects of illumination. Here, we test the merits of this general supposition by confronting bumblebees (*Bombus terrestris*) with a color discrimination task that can be solved only if information about the illuminant is not discounted but maintained in processing and thus available to higher-order learned behavior. We show that bees correctly use the intensity and chromaticity of illumination as a contextual cue to guide them to different target colors. In fact, we trained bees to choose opposite, rather than most similar, target colors after an illumination change. This performance cannot be explained with a simple color-constancy mechanism that discounts illumination. Further tests show that bees do not use a simple assessment of the overhead illumination, but that they assess the spectral relationships between a floral target and its background. These results demonstrate that bees can be color-constant without discounting the illuminant; that, in fact, they can use information about the illuminant itself as a salient source of information.

color vision | ecology | vision

We see a world composed of differently colored objects of various sizes, orientations, and locations in 3D space, which is extraordinary given the fact that the 2D patterns of light that fall on the eye completely lack these attributes. However, this lack of isomorphism would be trivial if there were a direct relationship between a stimulus and its source. The problem, however, is that each spectral stimulus is, in fact, determined by multiple real-world factors, e.g., reflectance and illumination. Indeed, the fundamental challenge for generating useful visually guided behavior for all animals is that the light that meets the eye is inherently ambiguous. Vision must somehow overcome this ambiguity, which is like trying to solve the equation $xy = z$ for x without ever knowing y. Although a seemingly impossible task, visual animals from bees to humans can recognize objects under different illuminants, a phenomenon called color constancy (1–8).

Most explanations of color constancy, although differing in detail and/or focus, assume that it is achieved by "discounting," as H. L. F. von Helmholtz so famously put it (9), the effects of the illuminant early in visual processing, leading to a veridical perception of object reflectance. Thus, in the words of E. H. Land, ". . . in determining color . . . [the visual system] has evolved to see the world in unchanging colors, regardless of always unpredictable, shifting, and uneven illumination." (10). This general view, which in this case was expressed in 1977, remains a basic supposition of contemporary research (e.g., ref. 11), which attempts to determine how illumination could, at least in principle, be explicitly deduced and then discounted by visual processing. Indeed, several strategies have been proposed, e.g., indirectly from spectral highlights, by looking directly at the light source (5), or by differentially adapting the receptors to the average spectral power in the stimulus (12–16). Although each strategy has its advantages and disadvantages, a necessary corollary of this supposition is that higher-order processing and perception should be blind to changes in illumination, because the relevant information is ultimately lost. It could be argued, however, that this would be an ecologically poor strategy for many animals, because information about the illuminant itself could be of biological relevance (17–21). Indeed, experiments on humans, for instance, have shown that adapting the average spectral quality of the stimulus is not a robust explanation for color constancy (e.g., refs. 22–24). We therefore tested whether bumblebees can take advantage of changes in information about the illuminant as a contextual cue for guiding foraging behavior.

Materials and Methods

Illumination for the experiments was generated by using six Duro-Test 40-W True-Lite tubes (Duro-Test, Philadelphia) and one 36-W blacklight tube (Osram, Berlin) mounted 65 cm above the arena floor. The frequency of tube flicker was converted to 1,200 Hz by using special ballasts (Osram Quicktronic QT-Eco 1 × 58/230-240) (8). No natural daylight was admitted into the laboratory. The light from the tubes was diffused by a single sheet of UV-transmitting white diffusion screen (no. 216, Rosco, Munich) to provide an even, homogenous illumination. Blue illumination was generated by two Rosco 061 blue filter sheets placed on top of the arena cover (simulating a blue skylight foraging environment). Green illumination (providing an illumination similar to that found under forest canopy) was provided by placing a Rosco 121 filter sheet on top of the flight arena. Dim light was generated by covering the arena with three sheets of neutral density filters (400.15 ND, Arri, Munich), each of which transmits 70% of the illumination, so that total transmittance was 34.3% of the illumination provided under bright light conditions. Spectral irradiance of the illumination was measured with an S2000 spectrometer (Ocean Optics, Dunedin, FL) relative to a calibrated deuterium/halogen radiation source DH 2000-CAL (Ocean Optics). Measurements are in $\mu W \cdot cm^{-2} \cdot nm^{-1}$ and had to be converted into quantum-based spectra.

Spectral reflectance functions of the artificial flowers and the background was measured by using an S2000 spectrometer with deuterium/halogen light source DH 2000. The relative amount of light absorbed by each photoreceptor color type was determined as described in ref. 25, and the calculation of hexagon color loci was from the description in ref. 26. Note that the distance between the color loci in the color hexagon is correlated with the degree to which two stimuli are perceived as differently colored, with the background color lying at the center of the hexagon. The distance from the center to any of the hexagon's corners is unity. Therefore, the maximum distance between two opposite corners of the hexagon is a value of 2. Only the relevant subregion of this space is shown in the figures described below.

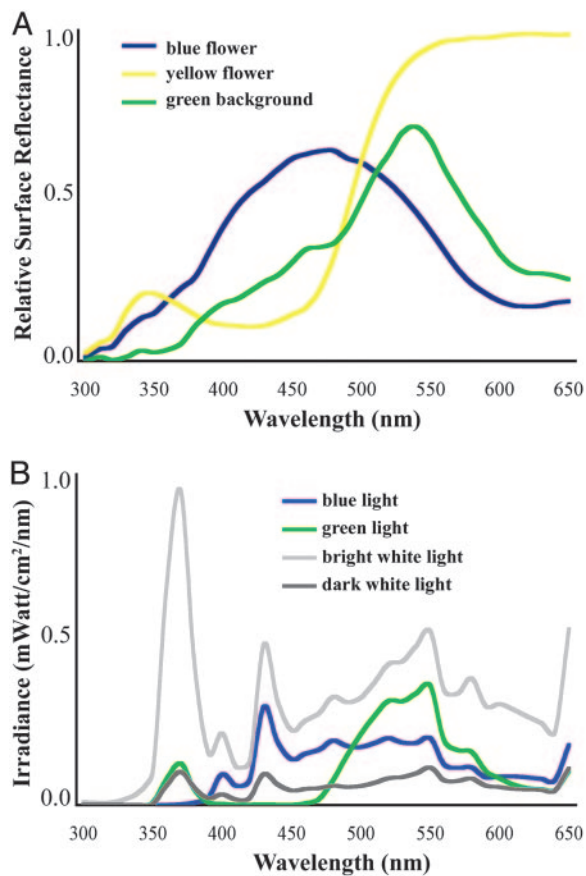†To whom correspondence should be addressed. E-mail: lotto@ucl.ac.uk.

Fig. 1. Relative spectral reflectances of artificial flowers and background (*A*) and relative spectral distribution of illumination (*B*).

A bumblebee (*Bombus terrestris*) nest box was connected to a flight arena (120 cm × 100 cm × 35 cm). Worker bees were first acquainted with the arena for 24 h, during which time they foraged from artificial flowers (clear, UV-transmittent Plexiglas squares (25 mm × 25 mm) placed on clear glass cylinders (diameter = 10 mm; 40 mm height). During this phase, illumination mimicked natural daylight. After 24 h, the bees were trained to forage from 20 clear Plexiglas flowers, 10 on yellow and 10 on blue glass cylinders, placed at random locations across the floor of the flight arena. Training involved 8–12 consecutive sessions of alternating illumination, each session lasting 30–90 min (actual duration was randomly determined so that bees could not use the temporal schedule itself as a guide for behavior). Illumination conditions were simulated with gel filters placed over the arena (see Fig. 1 for the spectral quality of the illuminants and surfaces used). In the first experiment, the chromatic illuminants were blue and green. When the illuminant was green, yellow flowers were rewarding (with 15 μl of 2 M sucrose solution), and blue flowers were filled with 15 μl of 0.12% (mass) quinine hemisulfate solution (Q-1250, Sigma), which is an aversive stimulus (27). Under blue light, the opposite was the case (both conditions are shown schematically in Fig. 2*A*). Immediately after training, individual bees were tested with one of the illuminants (flowers were unrewarding during testing). Visits to each flower color were recorded for 1 min. Only the first 10 visits were used for analysis, because foraging becomes increasingly random after a protracted series of unrewarded visits. The results for this experiment are shown in Fig. 2*C*.
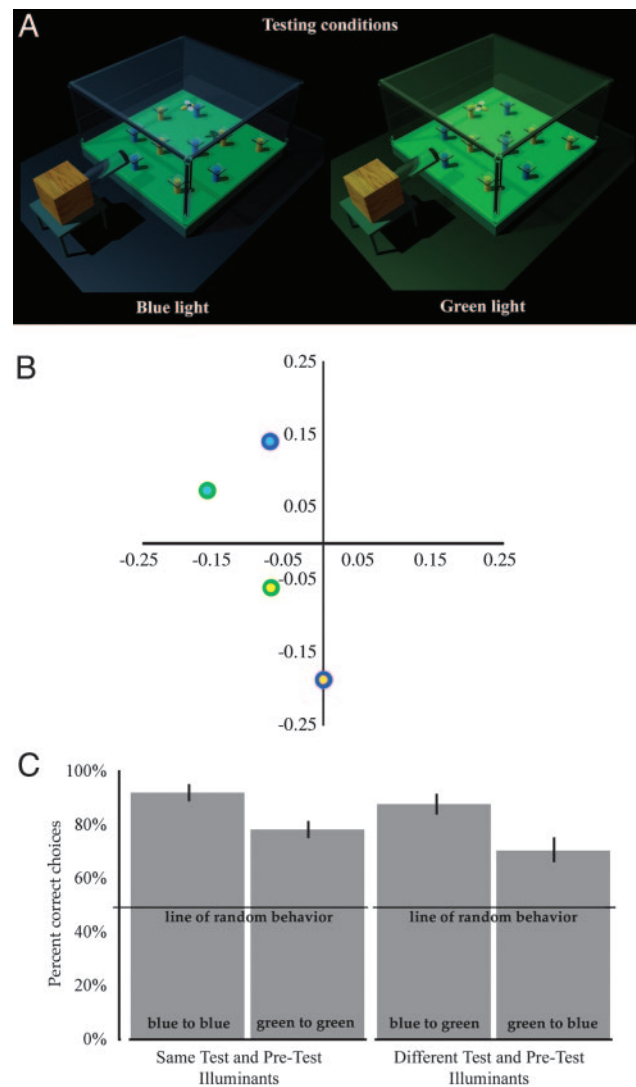

Fig. 2. Experimental setup, color loci, and behavioral results with two chromatic illuminants. (*A*) Schematic illustration of the two testing conditions: blue illumination on the left and green illumination on the right. (*B*) Location of spectral stimuli arising from the flowers within bee color space (blue and yellow, inner dots) under blue and green illumination (outer rings). Note that the space presented is the central portion of the color hexagon. The *x* axis represents UV vs. green antagonism, and the *y* axis represents blue vs. UV–green antagonism. (*C*) Results of testing the ability of bees to use changes in chromatic illumination to guide visual behavior. "Correct" indicates choosing a target color consistent with the test illuminant. Error bars indicate the standard deviation of each population, which combines bees from each of the two paradigms, because their χ^2 distributions did not vary significantly from each other. Significance of difference from random behavior (noted in the plot) was determined by using the χ^2 test.

Results

When the pretest illuminant (i.e., the last illuminant used in the training schedule) and test illuminant were the same (e.g., either both green or both blue), an average of 8.6 of the first 10 flowers visited were correct for the test illuminant (Fig. 2*C*). Choice values were significantly different from random both under blue light [one-sided χ^2 goodness-of-fit test; Yates corrected $\chi^2 = 39$, df = 1 (here and everywhere else); $P < 0.0001$] and under green light ($\chi^2 = 11.9$; $P < 0.0003$). When bees were tested under conditions in which the pretest and test illuminants were different, which, in contrast to the previous experiment, requires changing foraging preference (from one flower color to an-

other), bees still chose highly accurately: on average, 7.9 of the first 10 visits were correct for the test illuminant (Fig. 2C). Under green light, yellow flowers were chosen significantly more often ($\chi^2 = 7.2$; $P < 0.0036$), whereas under blue light, blue flowers were preferred ($\chi^2 = 31.5$; $P < 0.0001$). Together, then, the results show that bees chose colors correctly according to current illumination, irrespective of the color of the pretest illuminant, i.e., independently of the color on which they had most recently been rewarded.

These data are consistent with the bee visual system using information in the stimulus about the nature of the illuminant to guide behavior. It also is possible, however, that the bee visual system encoded the actual spectral quality of the rewarded targets (one arising from blue flowers under blue light and the other from yellow flowers under green light; Fig. 2B) or the spectral quality of the overhead acetate itself, which, although directly correlated with the nature of the illuminant, also provides an explicit contextual cue. An additional test was therefore performed to tease these possibilities apart.

The training of the bees was identical to that above. In the test situation (shown schematically in Fig. 3 A and B), the bees were confronted with white light, where no overhead acetate sheets covered the arena. A single layer of blue filter sheet was placed on the arena's floor under the flowers (note that the effects of the single filter on the ground plane are doubled, because the light reflected by the floor must pass through the filter twice: once on the way down and once on the way up). This manipulation had two effects. By eliminating the explicit contextual cue to trained behavior (the overhead acetate), the bees were directly exposed to a large, extended, novel white light source, thereby making the information arising from the bees' upper visual field inconsistent with the scene being under blue light. And, when the blue acetate was replaced to the floor of the arena, the information arising from the bees' lower visual field remained consistent with their recent experience of the scene being blue light. In addition to this more global manipulation, half of the blue flowers were wrapped with a single layer of the blue filter used to simulate blue illumination during training. Consequently, the blue-filtered blue flowers were empirically consistent with the information provided by the spectral return from the background, whereas the unfiltered blue flowers were consistent with the information provided directly by visible overhead light source. (Note, however, that if considered independently of the bees' training experience, the combined information is most consistent with a novel scene composed of a new set of flowers on a bluish floor under white light.)

The question is whether, under these conditions, bees would (*i*) select the blue-filtered blue flowers that would have been correct had the scene actually been under blue light (which it was not); (*ii*) select the unfiltered blue flowers, thereby demonstrating a degree of color-constant behavior; or (*iii*) disregard their recent experience altogether, and forage from neither flower type. As shown in the left column of Fig. 3D, the answer is (*i*); the bees were indeed fooled into choosing the non-color-constant ("incorrect") blue-filtered blue flowers 82% of the time ($\chi^2 = 32.4$; $P < 0.0001$), and the color-constant blue flowers only 6% of the time. When, however, the same bees were presented with the scene without the blue acetate on the arena's floor, which shifts the color locus of the nontarget blue-filtered blue flowers closer to the trained target (compare the color maps in Fig. 3 C and D), the bees were now 8-fold more likely to select the unfiltered blue flowers than in the previous condition ($\chi^2 = 36.7$; $P < 0.0001$).

The experiments, thus far, focus on chromatic changes in illumination. In the final experiment, we tested whether bees can use illumination intensity as a contextual cue to identify the correct flower color. Because current models of bee color vision assume that receptor adaptation will fully compensate for
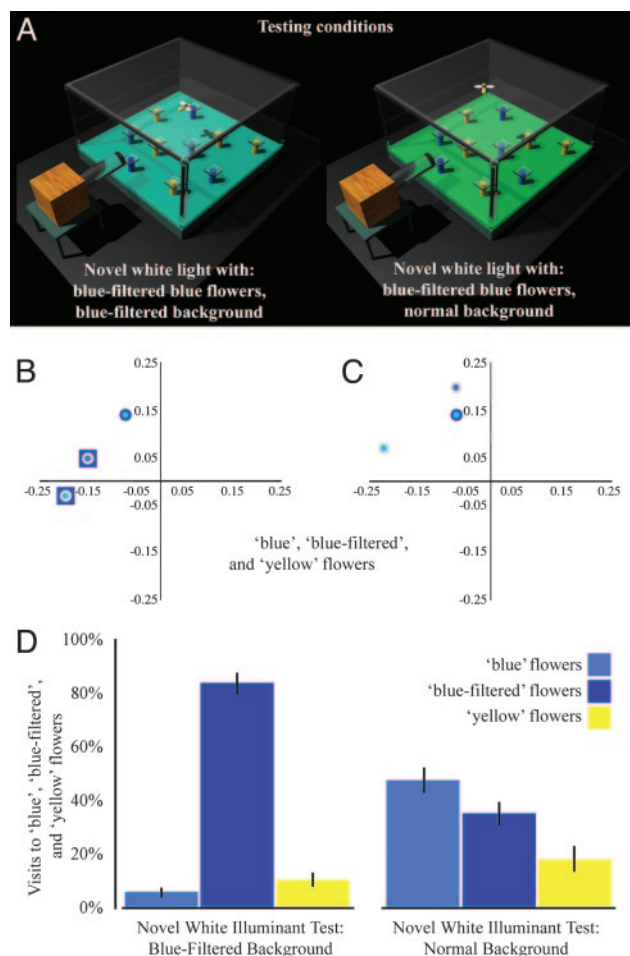

Fig. 3. Experimental setup, color loci, and behavioral results with white light but two differently colored arena floors. (*A*) Schematic illustration of the two testing conditions, which are described in the text. (*B* and *C*) Location of spectral stimuli arising from the flowers within bee color space under blue or white light. Blue-filtered blue flowers are represented as darker blue circles. Outer rings represent the color of the illumination. Surrounding squares in *C* represent the blue-filtered green background. The *x* axis represents UV vs. green antagonism, and the *y* axis represents blue vs. UV–green antagonism of the color hexagon. (*D*) Results of testing the ability of bees to use changes in chromatic illumination to guide visual behavior.

changes in illumination intensity (see *Materials and Methods*), bees should not be able to use illumination intensity as a meaningful source of information. The training conditions in these experiments were identical to those above (and are shown schematically in Fig. 4A), with the exception that the yellow flowers were rewarding in dim illumination (provided by neutral density filters), whereas blue flowers were rewarding in bright white light (unfiltered light). Although the shifts in the color locus of the blue and yellow targets under bright and dim light were negligible (Fig. 4B), the intensity of the dark light was 3-fold dimmer than the bright light. Nonetheless, if one assumes perfect von Kries adaptation, then this intensity shift on either luminance or green-receptor contrast is negligible and should therefore be irrelevant to behavior. And yet, the results clearly demonstrate that bee visual behavior can indeed be guided by differences in illumination intensity (Fig. 4C). Independently of the intensity of the pretest illuminant, bees visited the flowers that were correct for the test illuminant ≈75% of the time. Bees chose yellow flowers in dim light significantly more often, independently of whether they had most recently been rewarded
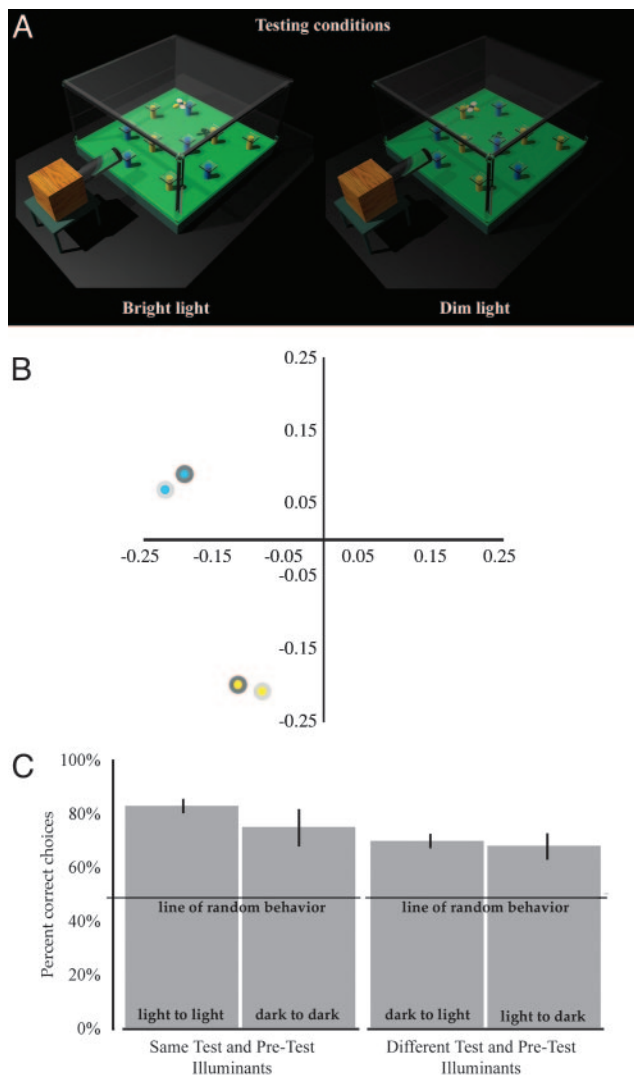
Fig. 4. Experimental setup, color loci, and behavioral results with two different illumination light intensities. (*A*) Schematic illustration of the two testing conditions, which are described in the text. (*B*) Location of spectral stimuli arising from blue and yellow flowers (dots) under bright and dim light (rings) within bee color space. Color loci of the *x* axis represent UV vs. green antagonism, and color loci of the *y* axis represent blue vs. UV–green antagonism. (*C*) Results of testing the ability of bees to use changes in illumination intensity. ''Correct'' indicates choosing a target color consistent with the test illuminant.

interpret them as errors: if a change in illumination results in a minor change of object appearance, then animals would choose targets that most closely resemble those that had previously been entrained (typically referred to as imperfect color constancy). But in ecological terms, it makes good sense not to throw away information about illumination, because it may be useful when assessing, among other things, object structure (28), weather (18), time of day (29), and direction (20). And our results show that bees can indeed be trained to use the illuminant as a contextual cue to choose opposite (yellow vs. blue) rather than the most similar colors after an illumination change, demonstrating that insects can use illumination as a valuable source of information.

That bees can use contextual cues to help solve ambiguous foraging problems has been established previously (30–34). In one classic experiment, Collett and Kelber (34) trained freely flying bees to choose between feeders positioned close to either yellow or blue cylinders but in two different outdoor huts. In one hut, yellow was correct, and in the other, blue was correct. The interiors of both huts were exactly the same; there were no cues as to which was the correct color, once the bee was inside. Nevertheless, bees were able to use the contextual cue (the spatial position of the hut) to choose either the yellow or the blue cylinders (34). In other studies, bees were able to use time of day (33, 35), signposts (36, 37), and sequences of landmarks encountered en route (31) as contextual cues.

In these previous studies, the contextual cue (e.g., position in space) is distinct from the conditioned stimuli (e.g., target color). Both are perceived at different times, and sometimes even with different sensory and memory modalities, (e.g., where color is a contextual cue to retrieve a motor pattern; refs. 36 and 37). Illumination, as a contextual cue, is fundamentally different, however, because it is inherently entangled with the stimulus. Nevertheless, the bees solved the task at hand, demonstrating that they neither discount the illuminant nor interpret illumination-induced changes in object appearance as errors. But because illumination and reflectance are inseparable at the level of the visual periphery and because the phasic (although not tonic) component of photoreceptor adaptation is an inevitable consequence of changes in illumination (16), how did bees resolve this ambiguity?

The most straightforward strategy would have been to use the overhead stimulus information, measured by receptors in the dorsal eye region and/or, in the case of light intensity, the ocelli (38), because it most directly represents, in physical and probabilistic terms, the scene's illuminant (5). Processing (and storing in memory) this information separately from the spectral information generated by the scene below would therefore be an effective means by which to guide illumination-dependent behavior in these experiments. When, however, the overhead, blue acetate was removed, enabling the bees to directly view the large, extended white light source, and the floral background was covered with the same blue acetate that was used to color the illuminant during training, the foraging behavior of the bees was almost completely determined by the color of the background: bees selected the blue-filtered blue flowers, which would have been correct if the scene had actually been under blue light (as implied by the spectral similarity between the testing background and the bees' recent experience of the scene under blue light), and avoided the unfiltered blue flowers, which were in fact the correct flowers when one assumes that color constancy should aid simply in recovery of reflectance spectrum.

Although these results are unexpected if bees discount the illuminant through von Kries adaptation and/or by directly viewing the illuminant, the results are well explained by the argument that color-constant behavior is generated by encoding in behavior (and therefore processing) the past significance of contrast relationships in scenes, in this case, between the high spatial frequency infor-

on yellow flowers under dim light ($\chi^2 = 9.6$; $P = 0.001$) or blue flowers under bright light ($\chi^2 = 5.3$; $P < 0.011$). Likewise, in bright light conditions, bees correctly chose blue targets both when they had most recently been rewarded on yellow flowers in dim light ($\chi^2 = 11.9$; $P < 0.0003$) and when they had been rewarded on blue flowers in bright light ($\chi^2 = 26.9$; $P < 0.0001$). Thus, bees successfully identified the correct target color according to the intensity of illumination as a contextual cue.

Discussion

A basic task of natural vision is to guide behavior according to the spectral similarities and differences of objects in the natural world. A widespread notion in vision research is that animals do this by recalibrating their visual system in such a way that the information about object illumination is itself discounted (12–16). In this view, deviations from perfect color constancy are the result of mechanistic limitations, and the visual system should

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mation arising from the flowers and the low spatial frequency information arising from the background (2, 6, 22–24, 39). A corollary of these findings and subsequent argument is that understanding how neural systems resolve the fundamental challenge of vision, namely the inherent ambiguity of stimuli, must move beyond traditional notions of constancy, in which sensations and the neural machinery that generate them are assumed to be aimed at veridical representations of one real-world attribute (in this case, reflectance), at the expense of another.

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