1. INTRODUCTION

Almost a century has elapsed since Karl von Frisch presented empirical evidence that honeybees had the capacity to learn to distinguish between stimuli based upon spectral differences [1]. Electro生理ological recordings from a number of hymenopteran species [2,3], and subsequent phylogenetic analyses suggest that the ultraviolet-sensitive (short-wavelength-sensitive; SWS), blue-sensitive (medium-wavelength-sensitive; MWS) and green-sensitive (long-wavelength-sensitive; LWS) trichromatic vision is remarkably conserved among flowering-plant visiting hymenopteran insects [4]. For example, figure 1 shows the similar spectral sensitivities of photoreceptor cell types in honeybees (Apis mellifera) and bumblebees (Bombus terrestris), over the range of ‘light’ wavelengths to which bees respond visually. These photoreceptor sensitivities are representative of hymenopteran trichromatic vision that predates the evolution of flowering plants (angiosperms) [5]. In addition to the three dorsal ocelli [6] (figure 2a), the visual system of worker honeybees has two compound eyes each containing approximately 5000 ommatidia [7]. With the exception of ommatidia [8], most of the ommatidia in honeybee vision contribute to colour perception and can be divided into three distinct types [9]. Specifically, while all three honeybee ommatidial types contain six LWS receptors, type I ommatidia (44% of ommatidia) contain one SWS and one MWS receptor, type II ommatidia (46%) contain two SWS receptors, and type III ommatidia (10%) have two MWS receptors. There is also a basal ninth receptor in each ommatidium, but its exact sensitivity and function remain unclear [9].

The distribution of the three ommatidial types appears to be random [9], which is consistent with similar studies on bumblebees [10] and butterflies [11,12]. However, the spectral sensitivities and distribution of photoreceptors in an animal are only one component of colour information processing. An important and somewhat understudied question is: how do individual insects learn to recall and apply previously acquired spectral information for decision-making in complex visual environments?

2. INITIAL PROCESSING IN THE LAMINA

The three photoreceptor (figure 1) outputs are segregated with respect to wavelength sensitivity prior to processing in the bee brain (figure 2). Colour vision requires the discrimination of stimuli independent of brightness, and we thus describe cells that respond to chromatic differences independent of brightness as having colour sensitivity. The lamina receives input principally from the LWS photoreceptor, which provides input to the achromatic motion-processing pathway in flies [13,14] and bees [15–17] (figure 2b). In bees, the lamina contains many neurons that exhibit relatively little response variation across a wide range of wavelengths [15]. The achromatic pathway LWS photoreceptors generally exhibit faster response times than photoreceptors involved in colour processing [18], and it has been suggested that processing colour information may come at the cost of reduced processing speed [18]. However, even at the ganglion of the lamina, experience-dependent modification of coarse colour responses has been reported, with bees reared in UV light exhibiting reduced synaptic connections from the green-sensitive lamina neurons [19], while bees...
ject into the outer medulla (layers 1–3) [25,27]. As neurons, as well as SWS and MWS photoreceptors, project orthogonally to the long axis of the columns. Laminae exhibit a distal-proximal laminated architecture consisting of eight histologically identified layers, oriented equally; (i) broad-band neurons that respond to multiple wavelengths of light broadly grouped into three classes: (i) broad-band neurones in the bee visual system [26]. Medulla neurons are organized into a retinotopic columnar pattern, with horizontal connections evident between adjacent columns [16,17]. This is in contrast to the lamina, which has few horizontal connections [16,17]. In addition, the medulla exhibits a distal-proximal laminated architecture consisting of eight histologically identified layers, oriented orthogonally to the long axis of the columns. Lamina neurons, as well as SWS and MWS photoreceptors, project into the outer medulla (layers 1–3) [25,27]. As discussed below, this anatomical segregation of lamina and photoreceptor input is accompanied by a divergence of neuronal responses to colour stimuli among medulla neurons and their target structures.

In the medulla and other visual areas of both honeybees and bumblebees, neural responses to colour can be broadly grouped into three classes: (i) broad-band neurons that respond to multiple wavelengths of light equally; (ii) narrow-band neurons that respond to input from a single photoreceptor type; and (iii) colour-opponent neurons (electronic supplementary material, table S1) [33]. Recent experiments using intracellular recordings and dye-filling of individual medulla neurons have demonstrated that the chromatic sensitivity of medulla neurons is layer-dependent, with narrow-band and broad-band responses observed in the outer layers, while colour-opponent responses were observed more frequently in the inner medulla layers (figure 2b) [27]. A schematic of the intracellular recording technique and representative types of visual stimuli used to test colour-sensitive neurons is shown in electronic supplementary material, figure S1.

The apparent divergence of colour responses observed in the medulla is preserved and amplified in its downstream targets, particularly in the lobula, which also contains many narrow-band and colour-opponent neurons (figure 2b) [22,24,27,32–35]. As in the medulla, the lobula is a layered structure [36], with two major regions: the motion-sensitive, largely achromatic outer layers (layers 1–4) and colour-sensitive inner layers (layers 5–6) [25,32]. The projection patterns of colour-sensitive neurons in the lobula also suggest a segregation of colour processing in downstream regions of the central brain, particularly a region called the lateral protocerebrum (figure 2b). Cells in the outer lobula layers, which are more likely to exhibit narrow-band or broad-band responses, project preferentially to the posterior lateral protocerebrum (figure 2b), and are likely to be involved in the achromatic visual motion system [32]. In contrast, neurons with branches predominantly located in the inner lobula layers, which are more likely to exhibit colour opponency and complex responses to coloured stimuli, project preferentially to the anterior lateral protocerebrum, including central brain structures called the mushroom bodies (MBs; figure 2b) [31,32].

Segregation of medulla and lobula neurons applies to the observed temporal response properties as well as chromatic responses. In intracellular recordings, 47 per cent of inner medulla and 69 per cent of inner lobula neurons adapted to repeated 1 Hz flashes from coloured light emitting diodes (LEDs; see electronic supplementary material, figure S1), compared with 20 and 14 per cent of outer medulla and outer lobula neurons, respectively [27,32]. Moreover, colour-opponent neurons found in the inner layers of both the lobula and medulla exhibit more complex temporal response patterns than cells in the outer layers, including habituation, stimulus entrainment (continuation of responses to a 1 Hz stimulus beyond the end of the stimulus train) and temporal summation [24,32] (figure 2b).

3. SEGREGATION OF INFORMATION IN EARLY COLOUR PATHWAY COMPONENTS

Beyond the level of the lamina, visual processing occurs in specialized regions of the honeybee brain, which has been imagined at high resolution [21]. This allows clarification of the relationships between different brain structures, as schematized in figure 2a. Despite volumetric differences, the architecture of the honeybee and bumblebee brains has been shown to be similar, even at the level of individual neurons [22–25].

The next level in the visual pathway after the lamina is the medulla (figure 2b), a structure that contains the most neurons in the bee visual system [26]. Medulla neurons are organized into a retinotopic columnar pattern, with horizontal connections evident between adjacent columns [16,17]. This is in contrast to the lamina, which has few horizontal connections [16,17]. In addition, the medulla exhibits a distal-proximal laminated architecture consisting of eight histologically identified layers, oriented orthogonally to the long axis of the columns. Lamina neurons, as well as SWS and MWS photoreceptors, project into the outer medulla (layers 1–3) [25,27]. As discussed below, this anatomical segregation of lamina and photoreceptor input is accompanied by a divergence of neuronal responses to colour stimuli among medulla neurons and their target structures.

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4. COLOUR PROCESSING IN CENTRAL BRAIN STRUCTURES

Recent findings suggest that the segregation of temporal response properties in colour neurons is also apparent at more central areas of the bee brain, along neural pathways that are roughly divisible across anterior and posterior central brain structures. For example, inner-layer lobula and inner medulla neurons, which display complex temporal responses as described above, send projections to anterior brain areas, particularly to the MBs and the lateral protocerebrum [27,31,32,37] (figure 2b). By contrast, outer lobula and both inner and outer medulla neurons project to the posterior
In bees, the MBs exhibit multi-modal experience-dependent plasticity, and they receive major visual input, some of which has been shown to be colour-sensitive [24,37,44–49]. The anatomical and physiological segregation of colour responses in the bee visual pathway represents a potential neural correlate for the differences in behavioural responses for either coarse or fine colour discriminations, which will be discussed below. Specifically, we hypothesize that: (i) difficult colour discriminations may preferentially invoke circuits in the inner lobula/inner medulla to MB/lateral protocerebrum pathway; (ii) differential conditioning to similar stimuli may depend on multi-modal integration, with the MBs as probable sites for this process; and (iii) task learning in both experimental and naturalistic conditions may alter connections along this pathway in an experience-dependent manner.

5. THE IMPORTANCE OF BEE COLOUR VISION

The way that pollinators like honeybees and bumblebees use their colour vision is of considerable interest owing to the important role of these species as pollinators of angiosperms. Individual bees have a tendency to be flower-constant [50,51] and tend to forage from one plant type so long as it is rewarding. There is good evidence that for angiosperms from the Middle East and Europe, where bees are known to be important pollinators, the evolution of flower colour correlates with discrimination thresholds at which bees can best resolve colour differences between stimuli [52]. Furthermore, the
6. COLOUR LEARNING BY BEES

How do bees learn to use their colour vision in complex environments, which may contain a variety of similar or dissimilar colours? Early work showed that bees learn to respond appropriately to distinct colours very quickly, typically in one to seven trials, and that performance was independent of whether a colour was learned in isolation (absolute conditioning) or in the presence of colour distractors (differential conditioning) [56, 57]. However, recent studies have extended this important work and shown that for both honeybee [58] and bumblebee [53, 59, 60] colour vision, bees can also learn very fine colour discriminations when differential conditioning is provided for an extended training period. This suggests different levels of behavioural plasticity in bee colour decision-making for either dissimilar or similar colours.

Two plausible hypotheses for the learning of fine colour discriminations by bees when provided with extended differential conditioning are: (i) though it cannot initially resolve the colour differences, the peripheral visual system and the neurons beyond the level of the photoreceptors can be slowly tuned to increasingly salient chromatic stimuli (colour tuning at a sensory level hypothesis); or (ii) the visual system can initially resolve fine colour differences in chromatic stimuli, but improved learning performance is the result of higher-level cognitive processing of stimuli (cognitive hypothesis). These two hypotheses may not be mutually exclusive, and evidence suggests both possibilities may underlie fine colour discrimination processing following extended differential conditioning. Honeybees trained to a fine colour discrimination task presented in a Y-maze apparatus (electronic supplementary material, figure S2) fail to exhibit fine discrimination if only appetitive differential conditioning is used, but bees trained with a combination of appetitive and aversive differential conditioning do learn to discriminate the similar colour cues [61], suggesting that selective attention is important for fine colour discrimination by honeybees [58, 61]. However, studies on learning in individual bumblebees reveal that, even after extensive experience of 90 landings on artificial colour stimuli to discriminate the target from perceptually similar distractors, highly trained bees still generalize new similar distractor stimuli, and discrimination of these stimuli are only learned if differential conditioning is provided to a specific colour pair [59]. Figure 3 shows that bees that have sequentially received extensive amounts of differential conditioning to colours of 'low', 'medium' and 'high' similarity still initially generalize between colour stimuli of 'very high' similarity. These bees had received an extensive opportunity to learn the procedure of the experiment and target colour; thus, if it was the case that the bees had to only allocate a 'selective attention' type mechanism to make the 'very high' similarity colour discrimination, then we should expect that the bees would exhibit a rapid acquisition function for the 'very high' similarity colour task. However, the acquisition for the 'very high' similarity colour task does not resemble a rapid function (figure 3), suggesting that the bee brain contains a mechanism that slowly tunes itself to discriminate between a particular set of similar colour stimuli.

Thus, behavioural data have shown that individual bees can learn colour stimuli with either a rapid but coarse form of processing [56, 57, 62], or a form of fine discrimination colour processing that is learned slowly and is dependent upon individual experience with stimuli [53, 58–61]. Indeed, individual honeybees that have received extensive differential conditioning to colours can discriminate colour stimuli near the threshold for human vision [63–65].

These behavioural results suggest that the bee brain learns colour information in complex ways, and we thus hypothesize that the bee visual system possesses at least two systems for processing colour information. One pathway is for encoding coarse, rapid discrimination of easily resolved chromatic components of the visual scene [56, 57, 62] along a ‘hard-wired’ series of connections, which may have parallels to the use of unique hues to construct a colour representation in primate colour vision research [66–68]. As discussed above, the outer medulla/lobula to posterior protocerebrum pathway may represent this ‘hard-wired’ visual processing pathway used for coarse discrimination.

The putative second pathway would incorporate circuits modifiable via experience-dependent plasticity for improved discrimination over repeated exposures. Selective pressure, which could result in the evolution of two
colour pathways, may be found in the foraging lifestyle of individual bees. Specifically, the rapid, coarse pathway could facilitate quick decisions in situations where salient differences exist between the colours of rewarding and non-rewarding flowers, while, if required, the discrimination of rewarding flowers from perceptually similar mimics [54,55] could invoke a slow, learning-dependent pathway to help manage the risk of accidentally rejecting correct colour stimuli, or accepting incorrect colour stimuli.

7. SPEED–ACCURACY TRADEOFFS AND COLOUR DISCRIMINATION

Solving perceptually difficult tasks often results in a trade-off between speed and accuracy [69,70]. For either bumblebees [71] or honeybees [72] tested with perceptually similar target and distractor colours, there is a significant correlation between the response time individuals allocate to making a decision and their choice accuracy. If honeybees are trained to collect sucrose associated with different colour cues at different times in the day, there is a circadian modulation of memory retrieval [73]. Traditionally, circadian timing [74,75] and interval timing [75,76] were thought to involve different mechanisms in invertebrates. However, recent attempts to find universal laws that might govern perception suggest that there may be common underlying principles to these mechanisms of time perception [77]. However, currently there is a paucity of data on how neurons in the bee brain process temporal information, although complex responses to temporal variations in stimulus (such as stimulus entrainment, temporal summation and habituation in the inner lobula, and central brain structures) have been reported [27–29,31–35,37]. One plausible mechanism for processing of timing information in the brain would be an oscillating circuit to which neurons could synchronize their responses as described for olfactory neurons in the locust MBs [43,78], and oscillations have been reported in honeybee brains [79,80]. Further studies of oscillatory activity in the bee visual pathway would be useful for understanding the interplay between colour learning and time perception in bees, and thus how free-flying bees employ colour vision for efficiently collecting nectar in complex environments [72,81,82].

8. FLOWER DETECTION

Given the relationship between angiosperms and bee pollinators, an interesting question to consider is how bees detect different flower colours depending upon the visual angle that the colour stimulus subtends in the eye of a bee. For honeybees, the visual angle at which colour information can be detected is dependent upon which of the photoreceptors are stimulated. Stimulus-like coloured paper discs in a Y-maze (electronic supplementary material, figure S2) that provide contrast modulation to the LWS photoreceptor are reliably detected at visual angles greater than 5°, while stimuli that provide only chromatic contrast modulation to the SWS and/or MWS photoreceptors require stimuli to subtend visual angles greater than 15° [83]. Interestingly, stimuli subtending a visual angle greater than 15° required chromatic contrast for efficient detection [84]. This suggests that honeybees have a separate low-resolution chromatic channel for processing colour information, and a relatively high-resolution achromatic channel for processing input only from the LWS photoreceptor. A stimulus comprising a ring providing contrast to the LWS photoreceptor around a disc with low contrast to the LWS photoreceptor yields a good detection limit of 6.5°, while if this pattern is reversed (a low-contrast ring) then the stimulus is only detected if it subtends a relatively large visual angle of more than 10°, possibly owing to neurons with a centre-surround type of organization [85]. Recent work has related this centre-surround organization of visual processing in honeybees to the possible evolution of flower patterns in radial symmetric bee-pollinated flowers, and found a significant positive relationship with flower detection [86]. There is some evidence of neurons with complex receptive fields, including spatially antagonistic receptive fields, where light presented in one area of the receptive field can inhibit the response to light presented in another area of the receptive field [22,32]; however, true centre-surround neurons as found in the vertebrate colour visual system [87] have yet to be found in the bee brain.

Two recent studies have tested the colour detection thresholds of bumblebees when compared with honeybees and reported significant differences between species [64,88]. When the colour discrimination capabilities of honeybees and bumblebees were tested following extensive differential conditioning, bumblebee colour discrimination was significantly poorer, but the trade-off was that bumblebees could detect colour stimuli at a much smaller visual angle than honeybees [64]. This suggests that while many hymenopteran species may have very similar sets of colour photoreceptors [2,4] (figure 1) and neural processing (figure 2), there are significant differences between species in the way the receptor signals are pooled and then processed, and how these results can be related to the visual ecology of the foraging environment [86].

While psychophysics experiments described above are highly valuable for inferring possible physiological mechanisms of colour vision in bees, it is also important to remember that bees must operate in very complex natural environments incorporating many factors. The sensory perception of animals is likely to have evolved to make use of multiple cues to aid reliable flower recognition [53,89–91]. For example, a recent study investigated the ability of male long-horned bees (Tetralonia berlandi) to detect orchid flowers in natural settings. When these bees approach flowers from a distance of greater than about 30 cm, the bees first show evidence of detecting the presence of flower by odour, but when closer to the flower colour vision becomes the dominant cue. If the ‘pink’ penianth of the flower is removed in an experiment, then detection by a bee is significantly slower than for intact flowers that combine both olfactory and visual cues [92]. This study suggests that bees can integrate multiple cues to find flowers, and also fits well with the data on the visual acuity of bees that colour information is only an important parameter at relatively close range [83,92]. Future behavioural work could explore how multiple cues are used independently of each other, depending rather upon the visual range of stimuli.
[89,92], or how the different cues may sometimes be used in a more compound fashion [90,93–95].

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