HAL
open science

New vistas on honey bee vision

Aurore Avarguès-Weber, Theo Mota, Martin Giurfa

To cite this version:

Aurore Avarguès-Weber, Theo Mota, Martin Giurfa. New vistas on honey bee vision. Apidologie, 2012, 43 (3), pp.244-268. 10.1007/s13592-012-0124-2 . hal-01003646

HAL Id: hal-01003646

https://hal.science/hal-01003646v1

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Apidologie (2012) 43:244–268
© INRA, DIB and Springer-Verlag, France, 2012
DOI: 10.1007/s13592-012-0124-2

Original article

New vistas on honey bee vision

Aurore Avarguès-Weber[1,2,3], Theo Mota[1,2], Martin Giurfa[1,2]

[1]Centre de Recherches sur la Cognition Animale, Université de Toulouse (UPS), 118 route de Narbonne, 31062 Toulouse Cedex 9, France
[2]Centre National de la Recherche Scientifique (CNRS); Centre de Recherches sur la Cognition Animale, 118 route de Narbonne, 31062 Toulouse Cedex 9, France
[3]School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS UK

Received 5 August 2011 – Revised 14 December 2011 – Accepted 18 January 2012

Abstract – The honey bee is a traditional animal model for the study of visual perception, learning, and memory. Extensive behavioral studies have shown that honey bees perceive, learn, and memorize colors, shapes, and patterns when these visual cues are paired with sucrose reward. Bee color vision is trichromatic, based on three photoreceptor types (S, M, L), which peak in the UV, blue, and green region of the spectrum. Perceptual color spaces have been proposed to account for bee color vision, and the anatomy of the visual neuropils in the bee brain was described to a large extent. In the last decade, conceptual and technical advances improved significantly our comprehension of visual processing in bees. At the behavioral level, unexpected cognitive visual capacities were discovered such as categorical and conceptual categorization. At the neurobiological level, molecular analyses of the compound eye revealed an intricate heterogeneity in the distribution of photoreceptors in the retina. Spatial segregation and integration of visual information in the bee brain has been analyzed at functional levels so far unexploited. These recent discoveries associated with the perspective of accessing the bee brain of harnessed bees while they perceive and learn visual cues open new avenues toward a comprehension of the neural substrates of visual perception and learning in bees. Understanding how the miniature brain of bees achieves sophisticated visual performances is a fundamental goal for the comparative study of vision and cognition.

vision / visual processing / visual cognition / honey bee / *Apis mellifera* / insect / invertebrate

1. INTRODUCTION

Honey bees exhibit impressive learning and memory capabilities (Giurfa 2007), which underlie their flower recognition ability. Bees are in fact flower-constant, which means that in their foraging bouts, they visit and exploit a single flower species as long as it offers valuable nectar or pollen resources (Grant 1950; Chittka et al. 1999). Recognition of the flower species exploited is based on rapid and reliable learning and memorization of the flower's characteristics (von Frisch 1914; Menzel 1999). Moreover, bees are central-place foragers which need to navigate long distances in a complex environment and return always to the same nest. Landmarks and celestial cues (azimuthal position of the sun, polarized light pattern of the sky) ensure efficient navigation in a complex environment (Rossel and Wehner 1986; Giurfa and Menzel 1997; Collett 1996; Collett and Zeil 1998; Collett and Collett 2002; Collett et al. 2003). Flower color and shape recognition are based on visual perception as shown by the pioneering work of Karl von Frisch and co-workers (von Frisch 1914; Kühn and Pohl 1921; Kühn 1927; Hertz 1929, 1933, 1935).

Corresponding author: M. Giurfa, giurfa@cict.fr
Manuscript editor: Bernd Grünewald
Aurore Avarguès-Weber and Theo Mota contributed equally to this review.

Although honey bee vision has been intensively studied during several decades (see reviews in Menzel and Backhaus 1991; Srinivasan 1994; Giurfa and Menzel 1997; Srinivasan 2011), novel findings have yielded a fresh view on the visual capacities of this insect. This review aims at presenting recent major advances in the study of honey bee vision both at the behavioral and neurobiological levels and at discussing how they open exciting and novel research perspectives for understanding vision in a behavioral, neurobiological, and ecological dimension.

2. BEHAVIORAL STUDIES ON HONEY BEE VISION

2.1. A historical survey: from Von Frisch to nowadays

Every description of studies on honey bee visual perception should start with the pioneer work of Karl von Frisch (von Frisch 1914, 1967) who established the experimental method for training and testing individually marked, free-flying bees to choose and discriminate visual targets that offer a drop of sucrose solution. Bees trained in this way search for sugar on the rewarded target for hours, traveling regularly back and forth between the hive and the experimental site. Testing the trained bees with various alternatives allows concluding which visual characteristics of the targets are perceived and memorized. In this way, von Frisch was the first to demonstrate the existence of color vision in bees, whose visual spectrum spans from 300 to 650 nm (von Frisch 1914; Kühn and Pohl 1921; Kühn 1927). Honey bee color vision is trichromatic as shown by color matching experiments performed by Daumer (1956). Later electrophysiological studies confirmed that honey bees possess three kinds of photoreceptors in their retina (Autrum and Zwehl 1964; Peitsch et al. 1992): S (short wavelength type) with an absorption peak at 344 nm (UV), M (medium wavelength type) with an absorption peak at 436 nm (blue), and L (large wavelength type) with an absorption peak at 544 nm (green).

Further experiments aimed at understanding the neural bases of color processing in the bee brain. Physiological studies starting in the early seventies by Randolf Menzel and coworkers provided fundamental insights into the mechanisms of color vision in bees. Single-neuron recordings performed at different stages of visual circuits in the bee brain revealed the presence of color opponent neurons (Kien and Menzel 1977b), which constitute the hallmark of color processing in nervous systems. These neurons antagonize the input from photoreceptor types (e.g., UV+ vs. B− G− and B+ vs. UV− G−, where "+" indicates excitation and "–" inhibition) and served to model bee color perception in terms of a two-dimensional space defined by two classes of color opponent neurons (UV vs. BG and B vs. UG) (Backhaus 1991). This model—the color opponent coding model (COC; Backhaus 1991)—was based on behavioral data obtained in color discrimination experiments with free-flying honey bees and on neurobiological data obtained in recordings of honey bee color opponent neurons; it was consequently designed for honey bees. Later, a different model, the color hexagon (Chittka 1992), was proposed to account for color perception in honey bees and other hymenopterans. This model did not use biologically relevant opponencies, as was the case of the COC model, and attempted to provide a more general account of bee color vision. This generality may, therefore, be a main defect as it is not grounded on consistent neurobiological data. A third general color opponency model based on the noise properties of photoreceptors has been lately proposed (Vorobyev and Osorio 1998; Vorobyev et al. 2001).

Studies on bee vision did not only focus on color vision but also on shape and pattern perception and discrimination (Hertz 1929, 1933, 1935; Wehner 1967, 1971; Lehrer 1994; Srinivasan 1994; Dafni et al. 1997). Training free-flying bees to find sugar reward on a black shape lying flat on a white, horizontal background indicated that bees discriminate certain shapes and patterns based on parameters such as spatial frequency, pattern disruption, or percentage of black surface (von Frisch 1914; Hertz 1933, 1935). Later, when it was realized that horizontal stimulus presentation induced bees to

use local cues corresponding to their approach direction instead of focusing on the trained global shape, vertical presentation of the stimuli was adopted in order to facilitate a global view upon frontal approach (Wehner 1967). This training method showed that bees are able to discriminate shapes and patterns by means of diverse parameters such as global orientation (van Hateren et al. 1990; Campan and Lehrer 2002; Giurfa et al. 1995), radial (Horridge 1996), or bilateral symmetry (Giurfa et al. 1996a) and concentric or ring-like structure of the patterns (Horridge and Zhang 1995).

A further experimental innovation in studies on pattern and shape perception and discrimination was the introduction toward the end of the eighties of a Y-maze in which free-flying bees had to choose between two visual targets (Srinivasan and Lehrer 1988; see a schematic representation of a Y-maze in Figure 2a). The maze added to the vertical presentation of stimuli the possibility of controlling the distance at which the decision is made by the bee and thus the possibility of evaluating which cues were used at which distance from the targets. Using colored stimuli, it was shown, for instance, that L-photoreceptors mediate fine spatial vision, i.e., visual detection of targets subtending small visual angles (from 5° to 15°) (Giurfa et al. 1996b, 1997), while chromatic channels intervene when targets subtend larger visual angles (from 15° on) (Giurfa and Vorobyev 1997). Further studies also showed the fundamental role of these receptors in pattern or shape vision (Zhang et al. 1995; Hempel de Ibarra and Giurfa 2003) as well as in motion detection (Srinivasan and Lehrer 1984, 1985; Zhang et al. 1995). These and other behavioral studies exploited the robust learning and memorization capacities of bees for visual cues and besides providing a rich picture of visual perception in this insect, consolidated the bee as a major invertebrate model for understanding visual object recognition.

2.2. The cognitive revolution

Studies on bee visual capacities focused during decades on the sensory processing of distinct visual cues. Researchers wanted to know essentially which cues were used by bees to discriminate particular sets of visual stimuli, but they rarely focused on how they learned them and which cognitive implications had their learning performances. At the end of the nineties, however, a significant change in perspective occurred as the honey bee started to be used as a model for studying higher-level, visual-problem solving. Such interest took place in the context of the cognitive revolution that occurred in the field of animal behavior after Donald Griffin's (1992) studies. Although invertebrates were traditionally considered as simple reflex machines, incapable of any sophisticated form of learning, experiments performed with free-flying honey bees in the last decade showed that this prejudice had no justification.

Several studies had already documented the bee's capability to generalize among visual stimuli (Wehner 1971; Anderson 1972; van Hateren et al. 1990; Srinivasan 2006; Horridge 2009). Generalization is a basic faculty of any recognition system allowing the transfer of a response learned to a given stimulus to stimuli which differ from the learned one but which are nevertheless similar to it along a specific dimension (Spence 1937; Pearce 1987; Shepard 1987; Ghirlanda and Enquist 2003). Generalization thus progressively decays along this dimension so that it is maximal for similar stimuli and minimal for different stimuli.

A more sophisticated way to treat similar and dissimilar stimuli is the capacity to categorize them in broader classes. This faculty is at the core of *categorization* abilities which have been intensively studied by cognitive psychologists and neuroscientists in the last decades (Herrnstein 1990; Lamberts and Shanks 1997; Zayan and Vauclair 1998; Zentall et al. 2002; Murphy 2010). Categorization consists in grouping together stimuli that are recognized as explicitly different but which are classified as similar based on shared attributes. Any unknown exotic bird will be recognized as a "bird" based on the presence of attributes defining this category such as wings, feathers, a beak, etc. Categorization therefore differs from strict generalization as categories present abrupt borders (either the stimulus is or is not in the category) instead of a progressive decay in responses with decreasing similarity.

Categorization has attracted the interest of cognitive scientists as it promotes cognitive economy by reducing the cost of learning by trial-and-error every new object encountered in our environment (Zayan and Vauclair 1998). At the end of the 1990s, a first study asked whether free-flying honey bees are able to categorize visual targets based on their bilateral symmetry (Giurfa et al 1996a). When bees were trained to associate various bilaterally symmetric visual stimuli with sucrose reward, they were able to extract the common feature predicting the reward, i.e., the symmetry, and transferred their choice to novel, unknown symmetric stimuli (Giurfa et al. 1996a). A similar result was obtained when bees were trained to choose asymmetric stimuli, i.e., bees transferred their choice to novel asymmetric stimuli. Interestingly, similar classification abilities were found previously in the case of pattern orientation (i.e., classifying patterns as exhibiting, irrespective of their spatial details, a global orientation of 45° vs. 135°; van Hateren et al. 1990), radial vs. concentric organization (Horridge and Zhang 1995), or spatial frequency (Horridge 1997). Yet none of these works, contrary to the one on bilateral symmetry, was framed into a categorization perspective as researchers interpreted their findings in terms of generalization abilities.

Since then, several studies have shown the bees' capacity to categorize visual stimuli in the strict sense of term, using a specific feature defined by the experimenter (see Benard et al. 2006 for review). More recently, bees had been shown to learn more complex categories based on multiple, combined features (reviewed in Benard et al. 2006 and Avarguès-Weber et al. 2011a). Such categories are essential in natural encounters with varying yet similar objects of the environment such as flowers, trees, or predatory hornets, which can be recognized based on several perceptual criteria. Zhang et al. (2004) trained bees with pictures of realistic objects that were divided into four categories: star-shaped flowers, circular flowers, landscapes, and plant stems. When rewarded on a particular category, bees were able to transfer their choice to novel pictures of the trained category that differed in color, size, outline, etc. (Zhang et al. 2004). It thus seems bees construct a generic representation of the trained category based on the salient features of the category (e.g., five radiating edges for the star-shaped flowers). This interesting work presented, however, the deficit of using complex, non-controlled pictures, thus leaving open the possibility (certainly remote given the a posteriori controls presented by the authors) of bees using low-level cues (such as contrast or percentage of the stimulus covered by the picture) to categorize pictures.

To avoid this problem, Stach et al. (2004; Figure 1) and Avarguès-Weber et al. (2010; Figure 2) used simple stimuli to determine whether bees combine visual features in a configural representation used to classify novel stimuli. In the first case, stimuli were composed of four edges oriented differently (Stach et al. 2004; Figure 1). Bees had to remember these four orientations simultaneously in their appropriate relative positions in order to recognize the appropriate rewarding stimulus. Bees had to extract the orientation layout of different patterns, irrespective of their spatial details, and determine whether or not, it corresponded to trained layout. Bees succeeded in this task thus showing that they extracted the four orientations in their specific topographic arrangement and classified novel patterns as presenting or not this arrangement. In the second case (Avarguès-Weber et al. 2010), bees were trained to discriminate face-like stimuli (two dots in the upper part, a vertical bar below, and a horizontal bar in the lower part) from non-face stimuli made of the same simple elements placed at random positions (Avarguès-Weber et al. 2010; Figure 2). The category "face" was thus defined by a particular spatial configuration of the elements. Bees succeeded in learning the face category, thus preferring novel faces never seen before to novel non-faces. They responded on the basis of a specific layout of features and recognized this configuration in the novel stimuli.

The honey bee is thus capable of extracting regularities from its visual environment and of combining them in complex layouts by estab-

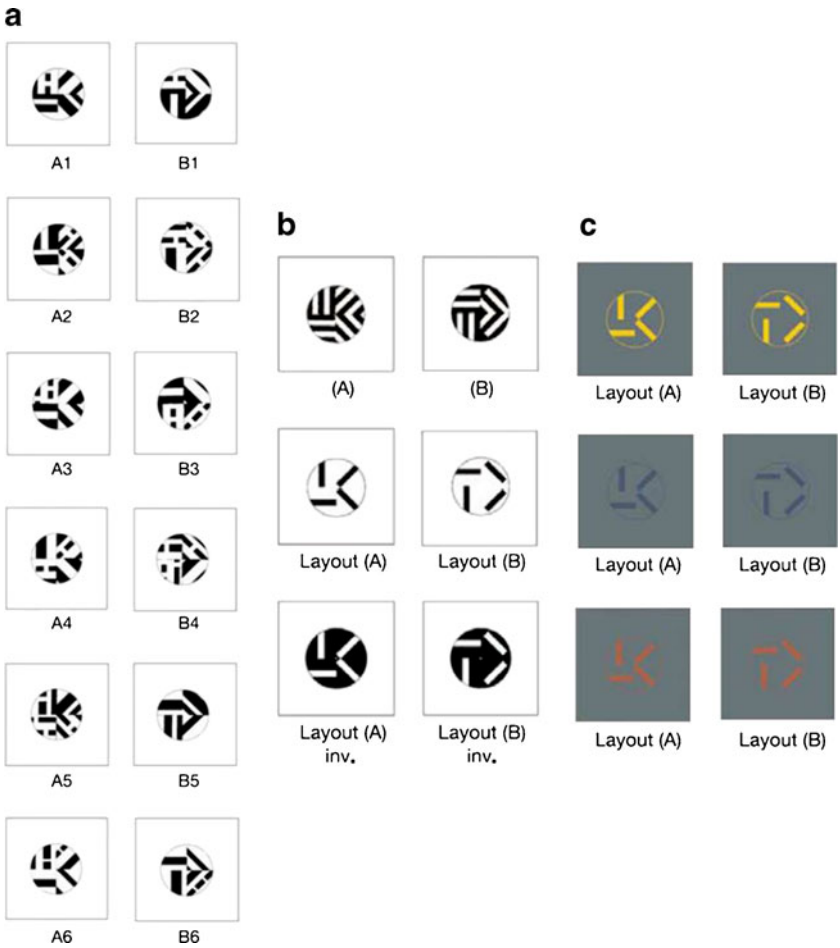

Figure 1. Stimuli used in the study of Stach et al. (2004) showing the honey bees' ability to build categories based on multiple-features. **a** Stimuli used for training in a Y-maze. The stimuli are divided into four quadrants, each presenting one given orientation. At each trial, the bee faced one stimulus from the category A (rewarded) vs. one from the category B (non-rewarded). Each category is defined by a particular layout composed of four orientations. **b** Stimuli used in non-rewarded tests to assess the bee's capability to transfer the learned layout to novel stimuli. **c** Colored stimuli used in non-rewarded tests to investigate which type of photoreceptor mediates the layout information. The results showed that the L photoreceptors are essential for such visual processing.

lishing correspondences between correlated features. Such ability, considered before as a prerogative of vertebrates, may allow the bees to group objects of their environment into functional categories.

In the categorization experiments reviewed above, a critical aspect for deciding if a novel stimulus belongs or not to a learned category is its perceptual similarity to the known items of the category, i.e., whether or not it presents the same physical attributes defining the category. A more sophisticated level of stimulus classification is based on *concept learning*, in which animals solve problems on the basis of abstract rules instead irrespective of any physical similarity (Thompson 1995; Lamberts and Shanks 1997; Murphy 2002; Zentall et al. 2002, 2008; Doumas et al. 2008; Halford et al. 2010; Mareschal et al. 2010; Murphy 2010). Indeed, humans and some animals can be trained to

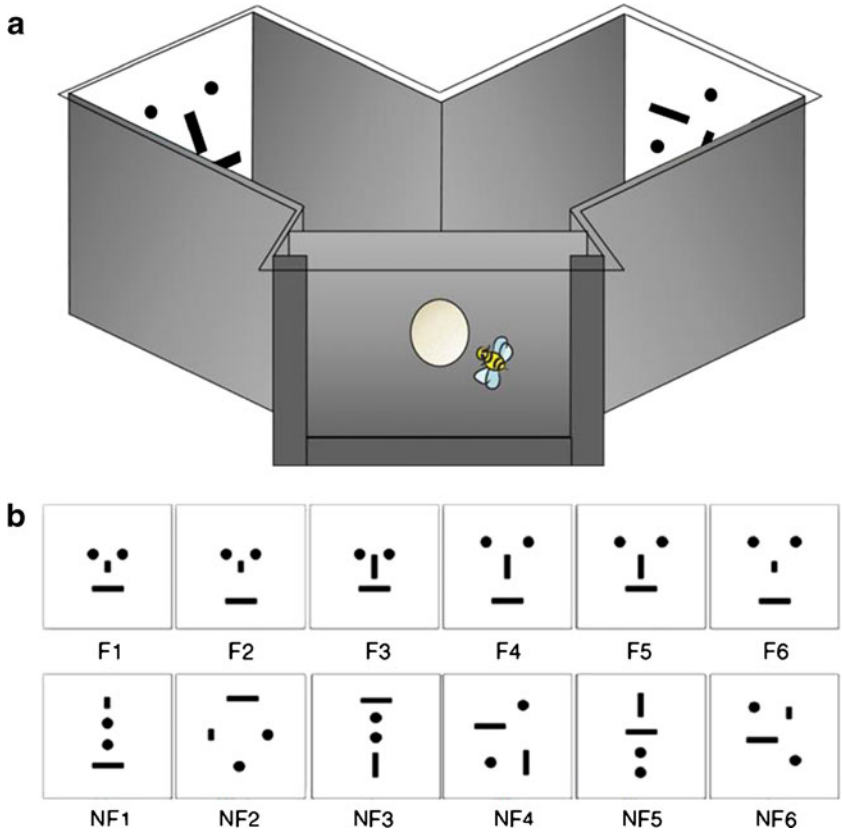

Figure 2. Apparatus and stimuli used in the study of Avarguès-Weber et al. (2010) showing the honey bees' ability to build categories based on a spatial configuration. **a** Schematic representation of a Y-maze used in several studies on visual discrimination by free-flying honey bees. At each trial, the bee faced one stimulus from the face-like category (rewarded) vs. a stimulus from the non-face category (non-rewarded). From trial to trial, the bee extracted the shared configuration to create a category and was then able to choose appropriately between novel items of both categories in non-rewarded tests. **b** Stimuli used. The non-face (*NF*) category presented the same elements as the face-like (*F*) category but in scrambled spatial positions. All stimuli are resolvable for the honey bee.

solve problems in which they have to extract that a specific relationship is the cue to be positively rewarded. Relationships such as "same," "different," "above," or "bigger" can be used to this end. Individuals have to recognize whether a given relationship is fulfilled or not independently of the objects linked by this relation and thus of their physical nature.

Such a powerful level of abstraction has been also found in free-flying honey bees (Giurfa et al. 2001). Bees were trained to master a delayed-matching-to-sample task in a Y-maze, in which they had to choose the arm of the maze presenting the same stimulus as a sample encountered at the entrance of the maze (Giurfa et al. 2001; Zhang et al. 2005). After having been trained to choose the same color displayed at the entrance (i.e., "choose blue vs. yellow when blue displayed at the entrance" and vice versa), bees succeeded in transferring the rule to black and white gratings displaying a vertical or horizontal orientation. Transfer was even successful between colors and odors (Giurfa et al. 2001). More recently, bees were shown to

master spatial relational concepts such as "above" vs. "below" (Avarguès-Weber et al. 2011b). Free-flying bees were trained in a Y-maze to choose a variable target located above or below a black bar that acted as constant referent throughout the experiment. In another experiment, two visual stimuli were aligned vertically, one being the referent, which was kept constant throughout the experiment, and the other the target, which was variable. In both experiments, the distance between target and referent, and their location within the visual field was systematically varied. In both cases, bees succeeded in transferring the learned concept to novel stimuli preserving the trained spatial relation thus showing an ability to manipulate this relational concept independently of the physical nature of the stimuli (Avarguès-Weber et al. 2011b; Figure 3).

Finally, bees have been shown to possess *basic forms of numerosity* as they can find a food source positioned after a given number of landmarks (Chittka and Geiger 1995; Dacke and Srinivasan 2008) or resolve a delayed-matching-to-sample task in which they have to choose the stimulus containing the same numbers of items as the sample (Gross et al. 2009). However, none of these studies fulfills standard criteria necessary to demonstrate real counting abilities in bees (Gallistel 1993): Indeed, a demonstration that numerosity labels can be applied to any collection of objects, or test conditions is still missing.

The experiments reviewed in this section show that the last decade induced an important shift in behavioral experiments with free-flying bees trained to solve visual tasks: from elemental problem solving, researchers focused more and more on higher cognitive processing, and uncovered, in this way, unsuspected cognitive abilities in these insects. Thanks to these recent studies, honey bees are no longer viewed as reflex machines but rather as cognitive animals capable of impressive capacities of abstraction. However, the mechanisms underlying such sophistication are still unknown. Indeed, even if spatial or numerical concepts can be useful in navigation tasks to create a map-like representation of the environment (Menzel et al. 2005), we ignore so far the neural mechanisms, which in the miniature brain of bees mediate these capacities. Clearly, a limiting factor to dissect the neural bases of these performances is the fact that they were all uncovered by experiments in which bees freely flew between the hive and the laboratory thus precluding the use of any invasive method to study brain activity.

2.3. Behavioral protocols to access the neural mechanisms of visual cognition

A transfer of behavioral experiments with free-flying bees to laboratory conditions in which bees exhibit the same cognitive richness but in harnessing conditions seems crucial for any study aiming at accessing the neural mechanisms underlying such richness. Invasive techniques such as electrophysiology (Hammer 1993; Mauelshagen 1993; Abel and Menzel 2001; Denker et al. 2010), neuropharmacology (Müller 1996; Hammer and Menzel 1998; Lozano et al. 2001; Farooqui et al. 2003; Vergoz et al. 2007), RNA interference (Farooqui et al. 2004), or calcium imaging of central neural activity (Faber et al. 1999; Sandoz et al. 2003; Fernandez et al. 2009; Roussel et al. 2010) require immobilizing the bees during training and testing. Furthermore, performing experiments of harnessed bees allow a better control of stimulus timing and perception. Such a combination of behavioral and neural analyses has been fully successful in the case of olfactory learning performances (see Giurfa 2007 for review); yet a similar strategy has been so far impossible in the case of studies on honey bee visual learning. In the case of olfactory learning, the behavioral protocol allowing the use of invasive techniques is the olfactory conditioning of the proboscis extension reflex (PER) (Takeda 1961; Bitterman 1983; reviewed in Giurfa 2007). In this Pavlovian protocol, harnessed bees learn to associate a neutral odor (the conditioned stimulus or CS) with a reward of sucrose solution (the unconditioned stimulus, US). The sucrose solution delivered to the antennae elicits a reflexive extension of the proboscis, which allows the bee to imbibe the solution. When the associ-

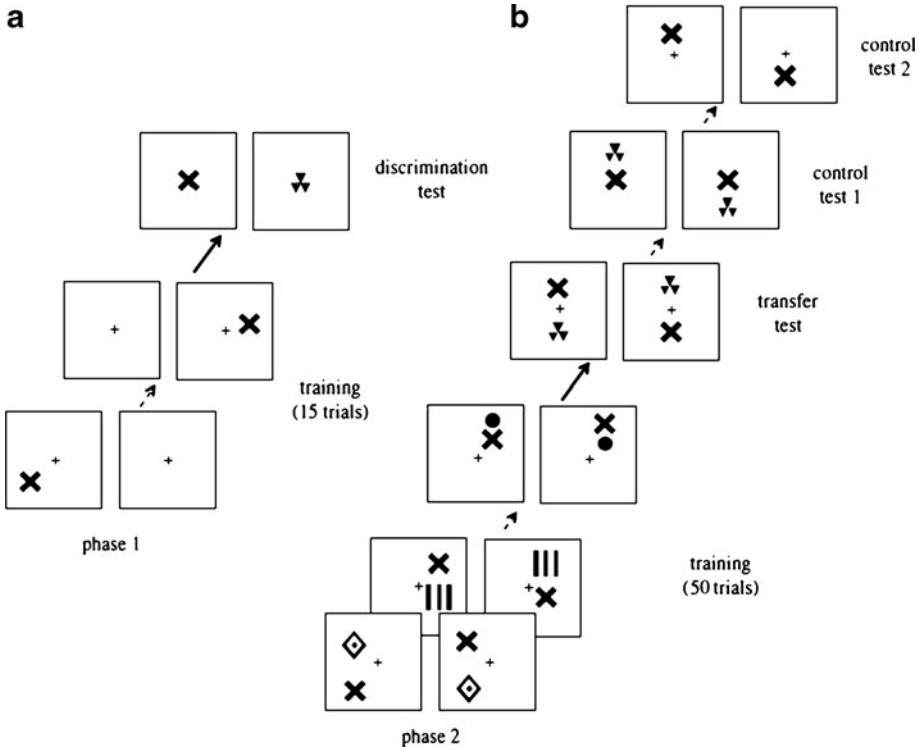

Figure 3. Procedure allowing to show the honey bees' ability to use a spatial relational concept as a category definition (Avarguès-Weber et al. 2011b). **a** In the first training phase, the bees had to choose in a Y-maze the stimulus presenting the black cross, independently of its position and were then tested for their ability to discriminate the learned symbol form an alternative one. This phase allows the bee to acquire the referent symbol (the *cross*) used in the next phase. **b** In this main phase, bees were trained in a category formation paradigm with various versions of a stimulus composed of the referent symbol presented *above* another symbol vs. the same referent symbol presented *below* the other symbol. One given spatial relationship is always rewarded along the training. In the non-rewarded transfer test, bees were tested for their capability to transfer the spatial concept to novel stimuli. Finally, control tests were performed to verify that the absolute position of the referent symbol in the background was not used as a simpler cue to solve the task.

ation is made, the bee extends its proboscis to the odor presented alone, despite the harnessing conditions. In this framework, the proboscis extension is thus a marker of olfactory learning.

Similar attempts to develop a visual conditioning of PER have not been so successful. Kuwabara (1957) found that bees learn to associate colored lights with sucrose reward but learning performances were poor. These results were not replicated until recently, when Hori et al. (2006) noticed that a critical step in Kuwabara's procedure was the cutting of the bees' antennae, which was done to avoid any potential learning of an association between the water vapor coming from the spoon containing the sucrose solution and the sucrose itself. Thus, by cutting the antennae and letting harnessed bees resting for two days, Hori et al. (2006) were able to condition bees with colored lights paired with sucrose solution. Yet, conditioning lasted 2 days and learning performances were poor and slow. Similar results were obtained with motion stimuli paired with sucrose solution (Hori et al. 2007). Furthermore, when conditioned responses were reported to be faster and to

reach higher levels, discrimination performances were coarse and retention performances poor (Niggebrugge et al. 2009).

Until now, no explanation is available to account for the fact that cutting the antennae is necessary to obtain visual learning in harnessed bees. Sensory inputs from the antennae may be more salient for harnessed bees, thus competing with visual cues. Alternatively, PER may be more easily triggered by conditioned olfactory cues in intact bees than by visual cues, or PER is not the appropriate behavioral readout for visual stimulus learning, which might nevertheless occur despite not being observable through this behavior. In any case, antennae deprivation has deleterious consequences on bees' fitness, thus reducing their general responsiveness to external stimuli. It was shown that antennae-ablated bees respond significantly less to tarsal sucrose stimulation than intact bees (de Brito Sanchez et al. 2008). Thus, antennae deprivation can disrupt appetitive-US (sucrose) responsiveness, thereby affecting appetitive learning. In other words, the poor visual learning performance in appetitive training of antennae-ablated bees could result from the effect of impaired sucrose (US) perception or from the inhibitory physiological processes resulting from antennae amputation or from a combined effect of both.

Interestingly, even though harnessed intact bees are unable to directly associate visual stimuli with sucrose reward, they can perceive and discriminate colors. Gerber and Smith (1998) studied potential blocking of odor learning by yellow-light pre-conditioning and showed that a pre-trained color did not block odor when delivered in a compound but facilitated olfactory learning. Despite the facilitatory effect exerted by the color, it did not elicit responses per se after compound training similarly to what has been reported for intact bees in which color conditioning of PER was unsuccessfully attempted. Recently, the capacity to perceive and discriminate colors by intact harnessed bees was shown by means of an occasion setting protocol in which colors acted as occasion setters for appropriate responding to an odor that could be either rewarded or non-rewarded (Mota et al. 2011a). In this framework, the odor was associated with sucrose when coupled with one colored light but not when a different colored light was presented (Mota et al. 2011a; Figure 4a). This protocol offers interesting perspectives for studying multimodal, non-elemental learning in an appetitive framework.

An alternative option to study visual learning in harnessed bees in the laboratory is the use of an aversive framework. Harnessed honey bees learn an association between an odor as CS and an electric shock as US (Vergoz et al. 2007). The conditioned response is, in this case, the sting extension reflex (SER), which is normally produced when confronting aversive, noxious stimuli. As for the appetitive olfactory PER conditioning, aversive olfactory SER conditioning could be successfully combined with invasive techniques to measure or manipulate neural activity (Vergoz et al. 2007; Giurfa et al. 2009; Roussel et al. 2010). Recently, attempts to condition SER using visual stimuli (colored lights) paired with electric shock proved to be successful, without necessity of cutting the antennae (Mota et al 2011b; Figure 4b). Antennae ablation was not only unnecessary for learning to occur but it even impaired visual SER conditioning due to a concomitant reduction of responsiveness to the electric shock. In this way, the first visual conditioning protocol of harnessed honey bees which does not require injuring the experimental subjects was established, thus opening new doors for accessing the neural correlates of visual learning and memory in honey bees.

Finally, a further possibility that needs to be explored in order to access the neural mechanisms of visual performances in bees is the development of experiments using tethered bees placed in controlled visual environments such as the flight simulator designed for studies on visual learning in fruit flies *Drosophila melanogaster* (Goetz et al. 1979; Wolf and Heisenberg 1991; Ernst and Heisenberg 1999). In this apparatus, a single fly, glued to a small hook of copper wire and attached to a torque meter, is flying stationary in the center of a cylindrical panorama (arena). In the flight simulator mode (closed loop), the

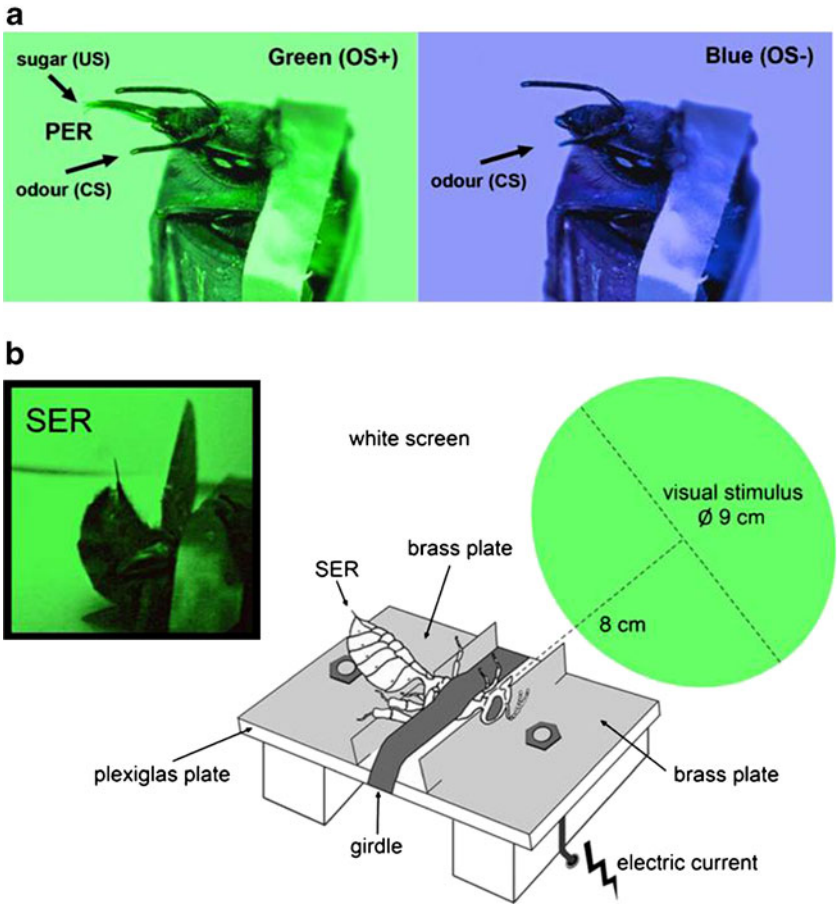

Figure 4. New experimental paradigms available for the study of visual learning and memory in harnessed or tethered bees under controlled laboratory conditions. **a** In an occasion-setting (bimodal) appetitive paradigm (Mota et al. 2011a), the honey bee learns to respond with proboscis extension to a green light (*left*) but not to a blue light (*right*) when the green light is a positive occasion setter (OS+) and the blue light is an negative occasion setter (OS−) indicating the contingency of a same odor (conditioned stimulus, *CS*). **b** Experimental setup for visual conditioning of the sting extension reflex (*SER*; Mota et al. 2011b). Visual stimulation is produced by a monochromator and projected from behind onto a white screen, thus producing a 9-cm-diameter colored disk. The white screen is placed at a distance of 8 cm from the bee eye so that the colored disc subtends a visual angle of 59° to the bee eye. In this visual SER-conditioning paradigm, the bee learns to extend its sting for the color that is punished with electric shock (e.g., green, *left inset*). **c** Experimental setup used by Luu et al. (2011) in which tethered bees exhibit a flight behavior in response to artificial optic flow in a simulated flight tunnel.

rotational speed of the arena is made proportional to the fly's recorded yaw torque around its vertical body axis. This enables the fly to stabilize the rotational movements of the panorama (i.e., to fly straight) and to adjust certain flight directions with respect to particular visual landmarks (e.g., visual patterns presented on the cylinder wall). A step toward a comparable experimental situation adapted for honey bees was made in a recent study by Luu et al. (2011), who analyzed the flight behavior of tethered bees surrounded by screens mimicking optic flow that

occurs in the field due to the relative motion of landmarks. The following years thus promise to overcome current experimental limitations to access the neural bases of visual learning in honey bees. In this way, the cognitive richness exhibited by free-flying bees in experiments reviewed above could be finally understood at the mechanistic level.

3. PHYSIOLOGICAL STUDIES OF HONEY BEE VISION

The physiology of photoreceptors in the bee retina as well as the visual circuits in the bee brain have been studied essentially by means of in vivo electrophysiological techniques applied, respectively, at the photoreceptor (e.g., Menzel 1979; Menzel et al. 1986; Peitsch et al. 1992) or single neuron level (e.g., Menzel, 1974; Kien and Menzel 1977a, b; Riehle 1981; Hertel 1980; Homberg 1985; Hertel et al. 1987; Hertel and Maronde 1987; Milde 1988). These studies, mostly performed during the 1970s and 1980s, characterized the spectral sensitivity of bee photoreceptors and identified different individual neurons in central visual circuits and their visual-processing properties. Together with neuroanatomical descriptions of the central organization of the visual system (e.g., Ribi 1975a, b; Ribi and Scheel 1981; Mobbs 1984), these studies also allowed understanding to a large extent how different visual neuropils in the bee brain are interconnected.

3.1. The periphery: the compound eye and the photoreceptors in the bee retina

Honey bee workers detect visual cues by means of their compound eyes, which are made of approximately 5,500 ommatidia, each of them hosting nine photoreceptor cells (R1-9, Gribakin 1975). The visual pigments present in each photoreceptor cell are localized in microvilli which are oriented toward the central axis of the ommatidium and form a photoreceptive rhabdomere. The R1-9 rhabdomeres build together a fused rhabdom along the central axis of the ommatidium (Gribakin 1975). The main photoreceptors R1-8 contribute to the microvilli along the entire length of the rhabdom, but the basal cell R9 contributes microvilli only at the base of the ommatidium (Gribakin 1975; Menzel and Blakers 1976). Three types of photoreceptors, S, M, and L (for short-, mid-, and long-range wavelength), peaking in the UV (344 nm), blue (436 nm), and green (544 nm) regions of the spectrum, respectively, have been identified in the honey bee retina (Menzel, 1979; Menzel et al. 1986; Peitsch et al. 1992; Figure 5a). Behavioral, psychophysical experiments revealed that achromatic information is processed by the L receptor channel (Giurfa et al. 1996b, 1997), while chromatic vision uses the three receptor channels in an antagonistic way (Menzel and Backhaus 1991; Backhaus 1991).

From the 1970s to the 1990s, the localization of the photoreceptors in an ommatidium was essentially studied by means of electrophysiological recordings coupled with dye injection (Menzel and Blakers 1976) and by analyzing light-induced structural changes in specific photoreceptor types (Gribakin 1975). These studies established a long-held view that the ommatidia in the main part of the eye contain an identical set of spectral receptors, with three UV (R1, 5, and 9), two B (R4 and 8), and four G (R2, 3, 6, and 7) receptors (Menzel and Blakers 1976; Waterman 1981; Menzel and Backhaus 1991; Peitsch et al. 1992). These studies stated that M and L receptors project to the first visual neuropil (the *lamina*; termination of the short visual fibers), while S receptors project to the second visual neuropil (the *medulla*; termination of the long visual fibers; Menzel and Blakers 1976; Meyer 1984). In the last 10 years, however, the advent of the honey bee genome (Honey Bee Genome Sequencing Consortium 2006) and the new molecular tools available have changed this view as they allowed studying photoreceptor distribution in the bee retina and their projections into the brain in a more precise way (Spaethe and Briscoe 2004, 2005; Velarde et al. 2005; Wakakuwa et al. 2005).

In situ hybridization studies localized specific opsins in the bee retina and demonstrated that the traditional view positing that ommatidia in

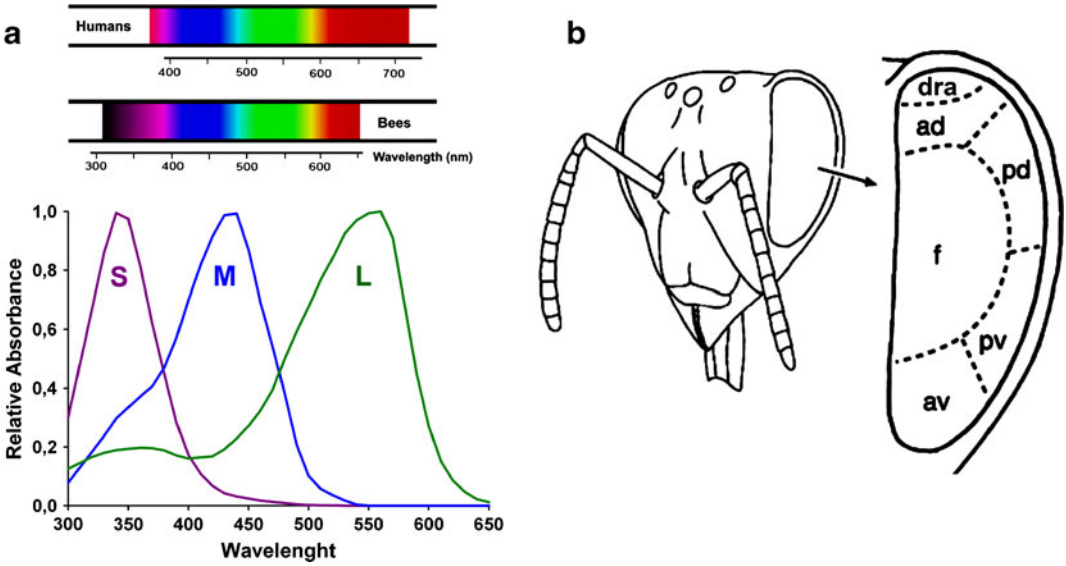

Figure 5. The compound eye and the photoreceptors in the bee retina. **a** The spectral range of honey bee vision is shifted toward the ultraviolet when compared to that of humans. Three types of photoreceptors, S, M, and L (for short-, mid-, and long-range wavelength) peaking in the UV, blue and green regions of the spectrum, respectively, have been identified in the honey bee retina (Peitsch et al. 1992). **b** The compound eye of *Apis mellifera* and its different eye regions (Wakakuwa et al. 2005): dorsal rim area (*dra*), anterior dorsal (*ad*), posterior dorsal (*pd*), frontal (*f*), anterior ventral (*av*), posterior ventral (*pv*).

the main part of the eye contain an identical set of spectral receptors is incorrect (Menzel and Blakers 1976; Waterman 1981; Menzel and Backhaus 1991; Peitsch et al. 1992). Ommatidia are, on the contrary, heterogeneous with respect to their photoreceptor-type composition (Spaethe and Briscoe 2005; Wakakuwa et al. 2005). Wakakuwa et al. (2005) provided the most accurate description of ommatidial types in the honey bee eye and identified three types of photoreceptors: All of them contain six L (green) receptors; type I ommatidia (44% of ommatidia) contain additionally one S (UV) and one M (blue) receptor, type II ommatidia (46%) contain in addition to the 6 L receptors two S receptors, and type III ommatidia (10%) present two M receptors together with the 6-L receptors. Each ommatidium also presents a basal ninth receptor (formerly described as an UV receptor) whose exact sensitivity remains unclear (Wakakuwa et al. 2005). In situ hybridization studies show that these cells are rather stained by green-probe, and not by UV-probe (Spaethe and Briscoe 2005; Wakakuwa et al. 2005). Combined molecular and physiological (intracellular recording and staining) experiments should help uncovering the physiological properties and the role of this enigmatic photoreceptor cell in visual processing. Furthermore, further studies should confirm that the molecular probes used are indeed selective for the different bee rhodopsins.

The distribution of these three different ommatidial types appears to be mostly random in the bee retina (Wakakuwa et al. 2005; Figure 5b). However, two exceptions to this random distribution are (1) the dorsal rim area (*dra*), which is a polarization-sensitive specialized eye region (Rossel and Wehner 1984) and where the more numerous S (UV) receptors are oriented strictly orthogonal to each other thus leading to polarized light sensitivity in the UV spectrum (Wehner and Strasser 1985; Labhart

and Meyer 2002; Spaethe and Briscoe 2005), and (2) the anterior ventral region (*av*), whose function remains unclear and in which type III ommatidia are more frequent, thus constituting a region with a density of M (blue) receptors that is higher than that of the other regions of the retina (Wakakuwa et al. 2005; Figure 5b). Concentration of M receptors in the ventral region of the eye (also found in *Manduca sexta* by White et al. 2003) may be related to a better ventral detection of targets providing contrast to M receptors (Giurfa et al. 1999) and/or to enhanced colored target detection by this eye region (Menzel and Lieke 1983; Lehrer 1998, 1999).

The six L (green) receptors present in all ommatidia were identified as R2, 3, 4, 6, 7, and 8 ommatidial cells (Wakakuwa et al. 2005). The higher amount of green receptors in the bee eye is coherent with their involvement both in chromatic and achromatic pathways, while UV and blue receptors essentially participate in chromatic pathways (Giurfa et al. 1996b, 1997). In the same way, a higher concentration of UV receptors in the dorsal rim area of the eye makes sense given the implication of this region of the eye in skylight analysis and navigation. Interestingly, a recent study showed that the light-adapted impulse responses recorded in green receptors are significantly faster than those recorded in UV and blue receptors (Skorupski and Chittka 2010). These faster responses of green photoreceptors seem to agree with their role in fast achromatic vision, while processing of chromatic information may come at the cost of reduced processing speed (Skorupski and Chittka 2010).

From all these studies, it can be concluded that the compound eye of bees has a more complex organization than previously thought. This organization is probably related to the remarkable asymmetries in visual performances involving the different honey bee eye regions, as revealed by extensive behavioral experiments (see Lehrer 1998 for review).

Studies on molecular biology of photoreceptor distribution in the bee eye also changed our knowledge about how photoreceptors project into the bee brain. In situ hybridization studies showed that UV and blue-sensitive opsins are exclusively expressed in R1 and R5 cells (Spaethe and Briscoe 2005; Wakakuwa et al. 2005) and terminate in the *medull*a. This result corrects the interpretation that all receptors with axons penetrating the lamina and reaching the medulla are only UV receptors (Menzel and Blakers 1976). Therefore, it seems that while L (green) photoreceptors project to the lamina, axons of both S (UV) and M (blue) receptors project to the medulla (Dyer et al. 2011).

3.2. Central processing: organization of the visual neuropils in the bee brain

Photoreceptors are peripheral components of visual processing conveying visual information to the bee brain, which uses this information to create visual percepts by means of various forms of neuronal processing. A fundamental question is, therefore, how visual neuronal circuits are organized at successive stages within the bee brain to generate such percepts.

The lamina is the first visual neuropil in which the axons of the photoreceptors connect to first order processing interneurons, the lamina monopolar cells (LMCs; Menzel 1974). In honey bees, the lamina was shown to contain mainly neurons exhibiting relatively little response variation across a wide range of wavelengths (Menzel 1974; Kien and Menzel 1977a; Ribi 1975a). This neuropil is made of thousands of optical cartridges, each receiving an axon bundle (containing the nine photoreceptor cell axons) from the overlying ommatidium, as well as the axons of four different types of monopolar cells. Additionally, tangential, centrifugal, and horizontal fibers can be found within each cartridge. The spatial arrangement of photoreceptor axons and LMCs within a cartridge remains constant throughout the lamina, thus retaining the retinotopic organization.

The outer chiasm forms the connection between the lamina and the second visual neuropil, the medulla, a structure that contains most of the bee visual system neurons (Ribi and Scheel 1981). Fibers coming from the anterior part of the lamina project to the posterior medulla while posterior fibers from the lamina project to the

anterior medulla. Thus, the retinotopic organization is retained but reversed in the medulla, which is also organized into a columnar pattern. Medulla columns are highly connected by horizontal fibers (serotoninergic or GABAergic) in contrast with the lamina that has few horizontal connections (mostly serotoninergic; Ribi 1975b; Bicker et al. 1987). In addition, the medulla exhibits a distal proximal laminated architecture consisting of eight identified layers, oriented orthogonally to the long axis of the columns (Ribi and Scheel 1981). Neurons in the medulla already respond with spectral opponency, i.e., with opponent excitation or inhibition depending on photoreceptor-type input (Kien and Menzel 1977b; Hertel 1980; Hertel et al. 1987). These color-opponent neurons, which exhibit combination-sensitive excitatory and/or inhibitory interactions between two or three photoreceptor classes, represent the principal basis of color-vision in honey bees. But since data stem from few individual color-opponent neurons, it is so far difficult to understand whether and how color sensations may arise in the bee brain at the neuronal network level.

The third visual neuropil is the lobula, where columnar stratification and retinotopic organization are preserved mainly in the outer part (Hertel et al. 1987). The inner chiasm forms the connection between the medulla and the lobula, in which the retinotopic organization is again reversed anteroposteriorly. Chromatic properties of neurons in the medulla are preserved and amplified in the lobula, which was also shown to contain distinct color-opponent neurons (Kien and Menzel 1977b; Hertel 1980; Hertel and Maronde 1987). Moreover, different types of spatial opponent neurons (i.e., with opponent excitation or inhibition depending on the visual field region or on direction in which the stimulus is presented) were also described in the lobula (Hertel et al. 1987; Hertel and Maronde 1987).

Different tracts connect the optic lobes with the mushroom bodies, higher-order brain structures associated with multimodal sensory integration and cognitive phenomena (Mobbs 1984; Menzel 1999; Giurfa 2007). In bees, some of the major visual afferences received by the mushroom bodies are color-sensitive (Gronenberg 1986; Mauelshagen 1993; Ehmer and Gronenberg 2002). In addition, extrinsic medulla and lobula neurons project into different tracts connecting these neuropils to the mushroom bodies of both brain hemispheres (Mobbs 1984). Diverse interhemispheric commissures connect the medulla and lobula of both brain hemispheres (Mobbs 1984; Ehmer and Gronenberg 2002), and two of these commissures seem to be involved on chromatic processing: the *posterior optic commissure* (approximately 200 neurons; Mobbs 1984) and the *anterior optic commissure* (approximately 3,200 neurons; Mobbs 1984). Furthermore, the medulla and lobula are highly connected to the lateral protocerebum of the bee central brain (Hertel 1980; DeVoe et al. 1982; Hertel and Maronde 1987). Until recently, little was known about how visual information is processed in this central brain region. Yet, in the last years, considerable advances were reached in our understanding of visual processing in different optic neuropils in the bee brain, including those located in the lateral protocerebrum (Yang et al. 2004; Paulk et al. 2008, 2009a, b; Paulk and Gronenberg 2008; Mota et al. 2011c).

Extensive intracellular recordings in different regions of the bee brain allowed identifying distinct visual pathways for the processing of chromatic and achromatic cues (Yang et al. 2004; Paulk et al. 2008, 2009a, b; Paulk and Gronenberg 2008). Yet, most of this recent work was done on bumblebees and not on honey bees (Paulk et al. 2008, 2009a, b; Paulk and Gronenberg 2008). In bumblebees, intracellular recordings from 105 morphologically identified neurons in the lobula revealed that these cells have anatomically segregated dendritic arborizations confined to one or two of six lobula layers. Lobula neurons exhibit physiological characteristics common to their respective input layer. Cells with arborizations in layers 1–4 are generally indifferent to color but sensitive to motion, whereas layer 5–6 neurons often respond to both color and motion cues (Paulk et al. 2008). Furthermore, further recordings from neurons in the central bumblebee brain (the lateral protocerebrum) and the medulla and the lobula showed that visual neurons projecting

to the anterior central brain are generally color sensitive, while neurons projecting to the posterior central brain are predominantly motion sensitive (Paulk et al. 2009a, b). The temporal response properties differed significantly between these areas, with an increase in spike time precision across trials and a decrease in average reliable spiking as visual information processing progressed from the periphery to the central brain. These data suggest that neurons along the visual pathway to the central brain not only are segregated with regard to the physical features of the stimuli (e.g., color and motion) but also differ in the way they encode stimuli, possibly to allow for efficient parallel processing to occur (Paulk et al. 2009a, b). In a further study on bumblebees, recordings of optic lobe neurons conveying visual information to the mushroom bodies revealed that some input neurons were color sensitive while a subset was motion sensitive (Paulk and Gronenberg 2008). Additionally, most of the mushroom body input neurons would respond to the first, but not to subsequent presentations of repeated stimuli (Paulk and Gronenberg 2008).

Anatomical and physiological studies in the lateral protocerebrum of bees have allowed the description of unknown visual pathways in this central brain region (Paulk et al. 2009a; Mota et al. 2011c). Moreover, a novel technique of optophysiological calcium imaging allowed recording for the first time the activity of whole visual circuits in the bee brain upon visual stimulation of the compound eye (Mota et al. 2011c). All these studies considerably improved our knowledge of visual-information processing, segregation, and integration within distinct bee brain regions.

3.3. Central processing: segregation of achromatic and chromatic visual pathways in the bee brain

Recent works using intracellular recordings coupled with single neuron staining in diverse regions of the bumblebee brain allowed the identification of segregated visual pathways for the processing of achromatic and chromatic cues (Paulk et al. 2008, 2009a, b; Paulk and Gronenberg 2008; Dyer et al. 2011). This remarkable segregation found in different levels of visual processing within the bee brain may constitute the neural basis of the independency between achromatic and chromatic vision revealed in behavioral studies (Werner et al. 1988; Giurfa et al. 1996b, 1997; Hempel de Ibarra et al. 2000, 2002).

The lamina, the first visual neuropil (see above), receives input essentially from the L-receptors which provide information to the achromatic motion-processing pathways (Menzel 1974; Ribi 1975a; Meyer 1984). Axons of lamina monopolar cells (see above) and M- and S-receptors proceed to the second visual neuropil, the medulla (Meyer 1984; Wakakuwa et al. 2005; see Section 3.2). In this structure information from the three photoreceptor types (S, M, and L) are combined in an opponent way and chromatic processing occurs as revealed by a remarkable amount of color-opponent neurons (Kien and Menzel 1977b; Hertel et al. 1987; Paulk et al. 2009a). Recent intracellular recordings of diverse medulla neurons in the bee brain demonstrated that chromatic-sensitive responses are observed more frequently in the inner medulla layers, while the outer medulla layers are largely achromatic and mostly sensitive to motion (Paulk et al. 2009a).

The same type of segregation between achromatic and chromatic pathways was also recently observed in the third visual neuropil, the lobula. At least eight new types of color-opponent neurons were lately described in the bee lobula (Yang et al. 2004; Dyer et al. 2011). As the medulla, the lobula is a layered structure in which color sensitivity was found to be more pronounced in inner layers, whereas the outer layers are mostly motion-sensitive and involved in achromatic processing (Paulk et al. 2008, 2009b). Moreover, chromatic and achromatic pathways in the central bee brain also segregate into the anterior and the posterior protocerebrum, respectively (Paulk et al. 2009b). Inner-layer lobula and medulla neurons, which are more likely to exhibit color-sensitive responses, send projections to anterior brain areas, particularly to the mushroom bodies and the anterior lateral protocerebrum (Paulk and Gronenberg 2008; Paulk et al. 2008, 2009a, b;

Dyer et al. 2011). By contrast, outer lobula and both inner and outer medulla neurons project to the posterior protocerebrum (Paulk et al. 2008, 2009a, b; Dyer et al. 2011). It seems therefore that achromatic and chromatic pathways are largely segregated in different steps of visual processing in the bee brain.

3.4. Central processing: visual processing in the lateral protocerebrum of the bee brain

Physiological studies of the bee lateral protocerebrum were scarce until recently. Few isolated visual neurons were identified and recorded in this brain region (Hertel 1980; Hertel and Maronde 1987) and little was known about how visual information from the medulla and lobula is processed in the lateral protocerebrum of bees. In the last years, however, the anatomical organization and new visual pathways in the lateral protorecebrum of bees have been described (Paulk et al. 2009b; Mota et al. 2011c).

In flies, anatomical mapping of the lateral protocerebrum recently identified various glomerular centers (termed "optic glomeruli") involved in the processing of visual information (Strausfeld et al. 2007; Strausfeld and Okamura 2007). In bees, the lateral protocerebrum can be divided in at least five main regions: the superior lateral protocerebrum, the inferior lateral protocerebrum, the posterior protocerebrum, the lateral horn, and the anterior optic tubercle (Paulk et al. 2009b). With the exception of the lateral horn, which is involved in olfactory processing (Mobbs 1984; Abel and Menzel 2001; Kirschner et al. 2006), all the other protocerebral regions receive visual input from the medulla and/or lobula and participate in visual processing (Paulk et al. 2009b). As mentioned before, anteroposterior segregation of achromatic and chromatic processing was found in the input from the medulla and lobula to the lateral protocerebrum of bees (Paulk et al. 2008, 2009a, b; Dyer et al. 2011). Moreover, this same gradient of achromatic/chromatic segregation in the anteroposterior brain axis seems to be retained at the level of lateral protocerebrum neurons (Paulk et al. 2009b).

The most prominent optic neuropil in the anterior region of the lateral protocerebrum is the anterior optic tubercle (AOTu). The neural organization and connectivity of the AOTu of the honey bee was recently described, revealing novel pathways of visual processing in this central brain region and a clear segregation of visual information along these pathways (Mota et al. 2011c). The AOTu of bees was found to be compartmentalized in four distinct units (Mota et al. 2011c; Figure 6). The AOTu receives substantial input from the medulla and lobula via the anterior optic tract and send output to the median protocerebrum (lateral accessory lobe) via the tubercle accessory lobe tract (Figure 6). Furthermore, two distinct tracts interconnect the AOTus of both brain hemispheres: the ventral inter-tubercle tract and the medial inter-tubercle tract. In addition to these four tracts, a specific neuron provides input from the vertical lobe of the mushroom bodies to the AOTu (Mota et al. 2011c; Figure 6).

Interestingly, visual information from the dorsal and ventral parts of the bee eye segregate within different AOTu compartments, both at the level of the visual input via the anterior optic tract and of the visual output to the contralateral AOTu via intertubercle tracts (Mota et al. 2011c; Figure 7). Therefore, visual processing in the AOTu of bees includes a notable spatial component, which is chiefly characterized by the segregation of dorsoventral visual information within distinct compartments (Figure 7). This segregation of dorsoventral visual information in the AOTu of bees may be related to the specializations uncovered in the dorsal and ventral parts of the bee retina (see Section 3.2). Moreover, it may be part of the neural mechanisms behind the remarkable asymmetries in behavioral performances involving the dorsal and ventral eye regions (Menzel and Snyder 1974; Anderson 1977; Menzel and Lieke 1983; Rossel and Wehner 1986; Giger and Srinivasan 1997; Lehrer 1998, 1999; Giurfa et al. 1999).

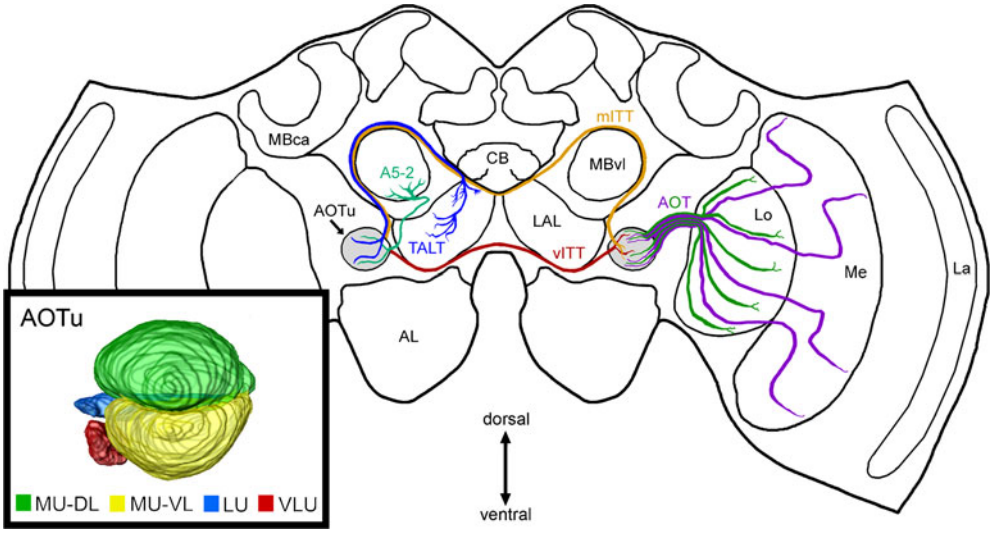

Figure 6. Three-dimensional structure and neural connectivity of the anterior optic tubercle (*AOTu*). Three-dimensional reconstruction showing the different AOTu compartments (*left inbox*): major unit dorsal lobe (*MU-DL*; *green*), major unit ventral lobe (*MU-VL*; *yellow*), ventrolateral unit (*VLU*; *red*), and lateral unit (*LU*; *blue*). The schematic diagram summarizes neural pathways connecting the AOTu with other brain neuropils. *La* lamina, *Me* medulla, *Lo* lobula, *AL* antennal lobe, *MBvl* mushroom body vertical lobe, *MBca* mushroom body calyx, *CB* central body, *LAL* lateral accessory lobe, *AOT* anterior optic tract, *vITT* ventral inter-tubercle tract, *mITT* medial inter-tubercle tract, *TALT* tubercle-accessory lobe tract; A5-2 neuron previously described by Rybak and Menzel (1993).

Functional segregation of visual information in the AOTu of the honey bee was studied using a novel optophysiological calcium-imaging technique that allowed recording for the first time the activity of whole visual circuits in the bee brain upon visual stimulation of the compound eye (Mota et al. 2011c; Figures 7b and 8). Although intracellular electrophysiology allows recording activity of single neurons and relating this activity to the potential role of a certain brain region, the large amount of neurons within visual circuits and the intricate interactions among them represent considerable obstacles to understand visual processing in a more functional, network level.

Optophysiological recording has been successfully applied to study olfactory circuits in the bee brain (Joerges et al 1997). The possibility of achieving optophysiological recordings of neural activity in visual circuits of the bee brain, which has been recently established (Mota et al. 2011c), will hopefully open new doors to understand the coding of visual information and the function of distinct visual neuropils in the bee brain.

Another useful technique recently available for studying neuronal circuits in the insect brain is the use of extracellular multi-electrode probes (e.g., Christensen et al. 2000; Daly et al. 2004; Denker et al. 2010). This method was recently used for studying olfactory processing and learning-dependent changes in the moth (Christensen et al. 2000; Daly et al. 2004) and the bee brain (Denker et al. 2010), but it has not been used so far in studies of visual processing. Multi-electrode electrophysiology allows the simultaneous recording of several individual units (neurons) participating in a certain neuronal circuit and studying interactions between these distinct units. This level of analysis is inaccessible in optophysiological recording of whole circuit activity which cannot identify single neurons. Conversely,

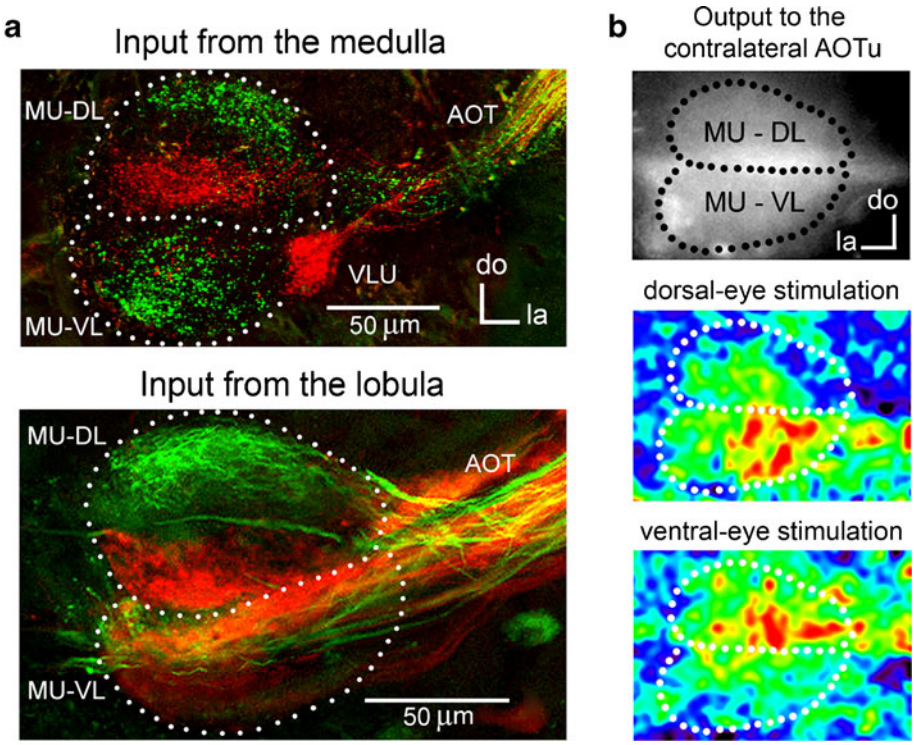

Figure 7. Segregation of visual information from the dorsal and ventral parts of the bee eye within different AOTu compartments. **a** Simultaneous injections of two fluorescent tracers with *different colors* into the dorsal (*red*) and ventral (*green*) parts of the medulla and lobula show that input from these neuropils is segregated into different AOTu regions. **b** Calcium-imaging recordings of AOTu output neurons showing dorsoventral segregation also at the output level. The false color-coded activation maps reveal that stimulation of the dorsal-eye region induces activity mostly in the MU-VL, while stimulation of the ventral-eye region induces activity mostly in the MU-DL. Both in **a** and **b**, the contours of MU-DL and MU-VL are indicated by *dashed lines.*

multi-electrode electrophysiology does not allow accessing the whole circuit dimension and the activation pattern resulting from all the interactions within a neuronal network. Thus, using both techniques, optophysiological recordings of whole circuit activity and multi-unit electrophysiology, constitutes a promising strategy to improve our knowledge of visual circuit physiology in different regions of the bee brain.

4. CONCLUSION

Novel techniques, either molecular or optophysiological, have opened new doors for research on honey bee vision. New discoveries such as the higher complexity and spatial heterogeneity of the bee retina, or the functional segregation of information along visual pathways in the bee brain, shed new light on the mechanism of visual processing in the bee nervous system. They provide for instance neural correlates to interpret behavioral asymmetries in recognition performance depending on the position of visual targets in the visual field or on their chromatic or achromatic cues.

In parallel, a conceptual shift has been incorporated in studies of honey bee visual learning and recognition so that bees have emerged as a powerful model to study cognitive visual behaviors. Bees, for instance, do not

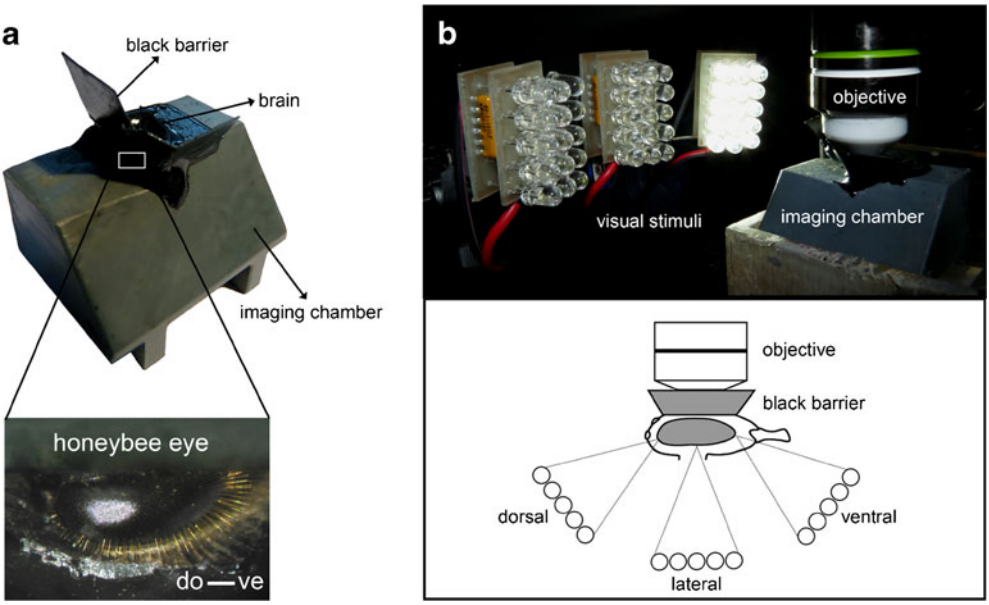

Figure 8. Optical recordings at the neuronal circuit level in the visual system of honey bees. **a** Bees in which visual neuronal circuits were stained with calcium indicator dye (fura-2 dextran) are placed individually in opaque recording chambers. The brain area is optically isolated from the compound eye area where visual stimuli are given, using opaque barriers glued to the bee head with custom black wax. **b** Visual stimulation produced by three rectangular LED arrays disposed in a half-circle allows the stimulation of different parts of the visual field (dorsal, lateral, or ventral) of the bee eye.

necessarily rely on static template recognition (a pixel-by-pixel memory of a visual object) but rather extract and combine the specific features of an image to create simplified sketches, which are the basis of functional categories. They can also classify items in terms of conceptual relationships, thus revealing considerable plasticity and abstraction abilities. Further studies are nevertheless still necessary to combine this level of analysis with the physiological one, in order to understand how the bee brain achieves such sophisticated performances.

To this end, it is necessary to establish on a firm ground new visual conditioning protocols allowing the reproduction of the cognitive richness of the free-flying bee in controlled laboratory conditions in harnessed bees. In this way, it will be possible to combine visual learning protocols with in vivo physiological techniques such as calcium-imaging or multi-units electrophysiology of visual circuits in the bee brain. Using these integrative approaches should allow studying for the first time experience-dependent changes in the bee brain related to visual learning, a goal that has remained elusive until now.

The honey bee is a fascinating and promising insect model for studies on visual perception, processing, learning, and memory. The novel perspectives described here can have wide implications not only for the insect scientific community but also for scholars in fields as diverse as comparative cognition, philosophy, or robotics and artificial intelligence. Understanding the mechanisms underlying the cognitive visual abilities of the honey bee could allow for instance a description of the minimal neuronal architecture required for complex visual processing (Chittka and Niven 2009) and to implement similar architectures in the conception of intelligent robots or problem-solving algorithms (Rind 2004; Srinivasan 2011).

ACKNOWLEDGMENTS

We thank two anonymous reviewers and A. Dyer (Melbourne, Australia) for productive collaboration in the field of this review. We also thank the French Research Council (CNRS), the University Paul Sabatier (Project APIGENE), and the National Research Agency (ANR: Project Apicolor) for generous support. A. Avarguès-Weber was supported by the French ministry of Research and T. Mota by a doctoral scholarship from the CAPES Foundation and the Brazilian government. M. Giurfa thanks the Program Raíces of the Argentinean Research Ministry for a Milstein Grant.

Nouvelles perspectives sur la vision de l'abeille

Vision / sens de la vue / cognition visuelle / abeille / *Apis mellifera*/ Insecta

Neue Blicke auf den Gesichtssinn der Bienen

Sehen / Gesichtssinn / visuelle Wahrnehmung / visuelle Kognition / Honigbiene / *Apis mellifera* / Insekt / wirbelloses Tier

REFERENCES

Abel, J.R., Menzel, R. (2001) Structure and response patterns of olfactory interneurons in the honey bee, *Apis mellifera*. J. Comp. Neurol. **437**, 363–383

Anderson, A.M. (1972) The ability of honey bees to generalize visual stimuli. In: Wehner, R. (ed.) Information Processing in the Visual Systems of Arthropods. Springer, Berlin.

Anderson, A.M. (1977) Parameters determining the attractiveness of stripe patterns in the honey bee. Anim. Behav. **25**, 80–87

Autrum, H.J., Zwehl, V. (1964) Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol. **48**, 357–384

Avarguès-Weber, A., Deisig, N., Giurfa, M. (2011a) Visual cognition in social insects. Annu. Rev. Entomol. **56**, 423–443

Avarguès-Weber, A., Dyer, A.G., Giurfa, M. (2011b) Conceptualization of above and below relationships by an insect. Proc. R. Soc. B **278**, 898–905

Avarguès-Weber, A., Portelli, G., Benard, J., Dyer, A. G., Giurfa, M. (2010) Configural processing enables discrimination and categorization of face-like stimuli in honey bees. J. Exp. Biol. **213**, 593–601

Backhaus, W. (1991) Color opponent coding in the visual system of the honey bee. Vision Res. **31**, 1381–1397

Benard, J., Stach, S., Giurfa, M. (2006) Categorization of visual stimuli in the honey bee *Apis mellifera*. Anim. Cogn. **9**, 257–70

Bicker, G., Schäfer, S., Rehder, V. (1987) Chemical neuroanatomy of the honey bee brain. In: Menzel, R., Mercer, A. (eds.) Neurobiology and Behavior of Honey Bees, pp. 202–224. Springer, Berlin

Bitterman, M.E. (1983) Classical conditioning of proboscis extension in honey bees (*Apis mellifera*). J. Comp. Psychol. **97**, 107–119

Campan, R., Lehrer, M. (2002) Discrimination of closed shapes by two species of bee, *Apis mellifera* and *Megachile rotundata*. J. Exp. Biol. **205**, 559–572

Chittka, L. (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J. Comp. Physiol. A **170**, 533–543

Chittka, L., Geiger, K. (1995) Can honey bees count landmarks? Anim. Behav. **49**, 159–164

Chittka, L., Niven, J. (2009) Are bigger brains better? Curr. Biol. **19**, R995–R1008

Chittka, L., Thomson, J.D., Waser, N.M. (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften **86**, 361–377

Christensen, T.A., Pawlowski, V.M., Lei, H., Hildebrand, J.G. (2000) Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles. Nat. Neurosci. **3**, 927–931

Collett, T.S. (1996) Insect navigation *en route* to the goal: multiple strategies for the use of landmarks. J. Exp. Biol. **199**, 227–235

Collett, T.S., Zeil, J. (1998) Places and landmarks: an arthropod perspective. In: Healy, S. (ed.) Spatial Representation in Animals. Oxford University Press, Oxford.

Collett, T.S., Collett, M. (2002) Memory use in insect visual navigation. Nat. Rev. Neurosci. **3**, 542–552

Collett, T.S., Graham, P., Durier, V. (2003) Route learning by insects. Curr. Opin. Neurobiol. **13**, 718–725

Dacke, M., Srinivasan, M.V. (2008) Evidence for counting in insects. Anim. Cogn. **11**, 683–689

Dafni, A., Lehrer, M., Keyan, P.G. (1997) Spatial flower parameters and insect spatial vision. Biol. Rev. **72**, 239–282

Daly, K.C., Wright, G.A., Smith, B.H. (2004) Molecular features of odorants systematically influence slow temporal responses across clusters of coordinated antennal lobe units in the moth *Manduca sexta*. J. Neurophysiol. **92**, 236–254

Daumer, K. (1956) Reizmetrische Untersuchungen des Farbensehens der Bienen. J. Comp. Physiol. **38**, 413–478

de Brito Sanchez, M.G., Chen, C., Li, J., Liu, F., Gauthier, M. (2008) Behavioral studies on tarsal gustation in honey bees: sucrose responsiveness and sucrose-mediated olfactory conditioning. J. Comp. Physiol. A **194**, 861–869

Denker, M., Finke, R., Schaupp, F., Grün, S., Menzel, R. (2010) Neural correlates of odor learning in the honey bee antennal lobe. Eur. J. Neurosci. **31**, 119–133

DeVoe, R.D., Kaiser, W., Ohm, J., Stone, L.S. (1982) Horizontal movement detectors of honey bees: directionally-selective visual neurons in the lobula and brain. J. Comp. Physiol. **147**, 155–170

Doumas, L.A.A., Hummel, J.E., Sandhofer, C.M. (2008) A theory of the discovery and predication of relational concepts. Psychol. Rev. **115**, 1–43

Dyer, A.G., Paulk, A.C., Reser, D.H. (2011) Colour processing in complex environments: insights from the visual system of bees. Proc. Biol. Sci. **278**, 952–959

Ehmer, B., Gronenberg, W. (2002) Segregation of visual input to the mushroom bodies in the honey bee (*Apis mellifera*). J. Comp. Neurol. **451**, 362–373

Ernst, R., Heisenberg, M. (1999) The memory template in Drosophila pattern vision at the flight simulator. Vision Res. **39**, 3920–3933

Faber, T., Joerges, J., Menzel, R. (1999) Associative learning modifies neural representations of odours in the insect brain. Nat. Neurosci. **2**, 74–78

Farooqui, T., Robinson, K., Vaessin, H., Smith, B.H. (2003) Modulation of early olfactory processing by an octopaminergic reinforcement pathway in the honey bee. J. Neurosci. **23**, 5370–5380

Farooqui, T., Vaessin, H., Smith, B.H. (2004) Octopamine receptors in the honey bee (*Apis mellifera*) brain and their disruption by RNA-mediated interference. J. Insect Physiol. **50**, 701–713

Fernandez, P.C., Locatelli, F.F., Person-Rennell, N., Deleo, G., Smith, B.H. (2009) Associative conditioning tunes transient dynamics of early olfactory processing. J. Neurosci. **29**, 10191–10202

Gallistel, C.R. (1993) A conceptual framework for the study of numerical estimation and arithmetic reasoning in animals. In: Boysen, S.T., Capaldi, E.J. (eds.) The Development of Numerical Competence: Animal and Human Models, pp. 211–223. Lawrence Erlbaum Associates, Hillsdale

Gerber, B., Smith, B. (1998) Visual modulation of olfactory learning in honeybee. J. Exp. Biol. **201**, 2213–2217

Ghirlanda, S., Enquist, M. (2003) A century of generalization. Anim. Behav. **66**, 15–36

Giger, A., Srinivasan, M.V. (1997) Honey bee vision: analysis of orientation and colour in the lateral, dorsal and ventral fields of view. J. Exp. Biol. **200**, 1271–1280

Giurfa, M. (2007) Behavioral and neural analysis of associative learning in the honey bee: a taste from the magic well. J. Comp. Physiol. A **193**, 801–24

Giurfa, M., Menzel, R. (1997) Insect visual perception: complex ability of simple nervous systems. Curr. Opin. Neurobiol. **7**, 505–513

Giurfa, M., Vorobyev, M. (1997) The detection and recognition of color stimuli by honey bees: performance and mechanisms. Israel J. Plant Sci. **45**, 129–140

Giurfa, M., Backhaus, W., Menzel, R. (1995) Color and angular orientation in the discrimination of bilateral symmetric patterns in the honey bee. Naturwissenschaften **82**, 198–201

Giurfa, M., Eichmann, B., Menzel, R. (1996a) Symmetry perception in an insect. Nature **382**, 458–461

Giurfa, M., Zaccardi, G., Vorobyev, M. (1999) How do bees detect coloured targets using different regions of their compound eyes. J. Comp. Physiol. A **185**, 591–600

Giurfa, M., Vorobyev, M., Kevan, P., Menzel, R. (1996b) Detection of coloured stimuli by honey bees: minimum visual angles and receptor specific contrasts. J. Comp. Physiol. A **178**, 699–709

Giurfa, M., Vorobyev, M., Brandt, R., Posner, B., Menzel, R. (1997) Discrimination of coloured stimuli by honey bees: alternative use of achromatic and chromatic signals. J. Comp. Physiol. A **180**, 235–243

Giurfa, M., Zhang, S., Jenett, A., Menzel, R., Srinivasan, M.V. (2001) The concepts of 'sameness' and 'difference' in an insect. Nature **410**, 930–933

Giurfa, M., Fabre, E., Flaven-Pouchon, J., Groll, H., Oberwallner, B., et al. (2009) Olfactory conditioning of the sting extension reflex in honey bees: memory dependence on trial number interstimulus interval intertrial interval and protein synthesis. Learn. Mem. **16**, 761–765

Goetz, K.G., Hengstenberg, B., Biesinger, R. (1979) Optomotor control of wing beat and body posture in *Drosophila*. Biol. Cybern. **35**, 101–112

Grant, V. (1950) The flower constancy of bees. Botanic Rev. **16**, 379–398

Gribakin, F.G. (1975) Functional morphology of the compound eye of the bee. In: Horridge, G.A. (ed.) The Compound Eye and Vision of Insects, pp. 154–176. Clarendon, Oxford

Griffin, D.R. (1992) Animal Minds: Beyond Cognition to Consciousness. University Chicago Press, Chicago

Gronenberg, W. (1986) Physiological and anatomical properties of optical input-fibres to the mushroom body in the bee brain. J. Insect Physiol. **32**, 695–704

Gross, H.J., Pahl, M., Si, A., Zhu, H., Tautz, J., et al. (2009) Number-based visual generalisation in the honey bee. PLoS ONE **4**, e4263

Halford, G.S., Wilson, W.H., Phillips, S. (2010) Relational knowledge: the foundation of higher cognition. Trends. Cogn. Sci. **14**, 497–505

Hammer, M. (1993) An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honey bees. Nature **366**, 59–63

Hammer, M., Menzel, R. (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honey bees. Learn. Mem. **5**, 146–156

Hempel de Ibarra, N., Giurfa, M. (2003) Discrimination of closed shapes by honey bees requires only contrast to the long wavelength receptor type. Anim. Behav. **66**, 903–910

Hempel de Ibarra, N., Vorobyev, M., Brandt, R., Giurfa, M. (2000) Detection of bright and dim colours by honey bees. J. Exp. Biol. **203**, 3289–3298

Hempel de Ibarra, N., Giurfa, M., Vorobyev, M.V. (2002) Discrimination of coloured patterns by honey bees through chromatic and achromatic cues. J. Comp. Physiol. A **188**, 503–512

Herrnstein, R.J. (1990) Levels of stimulus control: a functional approach. Cognition **37**, 133–166

Hertel, H. (1980) Chromatic properties of identified interneurons in the optic lobes of the bee. J. Comp. Physiol. **137**, 215–231

Hertel, H., Maronde, U. (1987) The physiology and morphology of centrally projecting visual interneurons in the honey bee brain. J. Exp. Biol. **133**, 301–315

Hertel, H., Schäfer, S., Maronde, U. (1987) The physiology and morphology of visual commissures in the honey bee brain. J. Exp. Biol. **133**, 283–300

Hertz, M. (1929) Die Organisation des optischen Feldes bei der Biene. I. Z. vergl. Physiol. **8**, 693–748

Hertz, M. (1933) Über figurale Intensität und Qualität in der optische Wahrnehmung der Biene. Biol. Zbl. **53**, 10–40

Hertz, M. (1935) Die Untersuchungen über den Formensinn der Honigbiene. Naturwissenschaften **23**, 618–624

Homberg, U. (1985) Interneurons of the central complex of the bee brain (*Apis mellifica L.*). J. Insect Physiol **31**, 251–264

Honey Bee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honey bee *Apis mellifera*. Nature **443**, 931–949

Hori, S., Takeuchi, H., Arikawa, K., Kinoshita, M., Ichikawa, N., et al. (2006) Associative visual learning, color discrimination, and chromatic adaptation in the harnessed honey bee *Apis mellifera* L. J. Comp. Physiol. A **192**, 691–700

Hori, S., Takeuchi, H., Kubo, T. (2007) Associative learning and discrimination of motion cues in the harnessed honey bee *Apis mellifera* L. J. Comp. Physiol. A **193**, 825–833

Horridge, A. (1997) Pattern discrimination by the honey bee: disruption as a cue. J. Comp. Physiol. A **181**, 267–277

Horridge, A. (2009) Generalization in visual recognition by the honey bee (*Apis mellifera*): a review and explanation. J. Insect Physiol. **55**, 499–511

Horridge, G.A., Zhang, S.W. (1995) Pattern vision in honey bees (*Apis mellifera*): flower-like patterns with no predominant orientation. J. Insect Physiol. **41**, 681–688

Horridge, G.A. (1996) The honey bee (*Apis mellifera*) detects bilateral symmetry and discriminates its axis. J. Insect Physiol. **42**, 755–764

Joerges, J., Küttner, A., Galizia, C.G., Menzel, R. (1997) Representation of odours and odour mixtures visualized in the honey bee brain. Nature **387**, 285–288

Kien, J., Menzel, R. (1977a) Chromatic properties of interneurons in the optic lobes of the bee. I. Broad band neurons. J. Comp. Physiol. A **113**, 17–34

Kien, J., Menzel, R. (1977b) Chromatic properties of interneurons in the optic lobes of the bee. II. Narrow band and colour opponent neurons. J. Comp. Physiol. A **113**, 35–53

Kirschner, S., Kleineidam, C.J., Zube, C., Rybak, J., Grünewald, B., et al. (2006) Dual olfactory pathway in the honey bee, *Apis mellifera*. J. Comp. Neurol. **499**, 933–952

Kühn, A. (1927) Über den Farbensinn der Bienen. Z. vergl. Physiol. **5**, 762–800

Kühn, A., Pohl, R. (1921) Dressurfähigkeit der Bienen auf Spektrallinien. Naturwissenschaften **9**, 738–740

Kuwabara, M. (1957) Bildung des bedingten Reflexes von Pavlovs Typus bei der Honigbiene, *Apis mellifica*. J. Faculty Sci., Hokkaido Univ., Series VI. Zoology **13**, 458–464

Labhart, T., Meyer, E.P. (2002) Neural mechanisms in insect navigation: polarization compass and odometer. Curr. Opin. Neurobiol. **12**, 707–714

Lamberts, K., Shanks, D. (1997) Knowledge, Concepts, and Categories. Psychology, Cambridge

Lehrer, M. (1994) Spatial vision in the honey bee: the use of different cues in different tasks. Vision Res. **34**, 2363–2385

Lehrer, M. (1998) Looking all around: honey bees use different cues in different eye regions. J. Exp. Biol. **201**, 3275–3292

Lehrer, M. (1999) Dorsoventral asymmetry of colour discrimination in bees. J. Comp. Physiol. A **184**, 195–206

Lozano, V.C., Armengaud, C., Gauthier, M. (2001) Memory impairment induced by cholinergic antagonists injected into mushroom bodies of the honey bee. J. Comp. Physiol. A **187**, 249–254

Luu, T., Cheung, A., Ball, D., Srinivasan, M.V. (2011) Honey bee flight: a novel 'streamlining' response. J. Exp. Biol. **214**, 2215–2225

Mareschal, D., Quinn, P.C., Lea, S.E.G. (2010) The Making of Human Concepts. Oxford University Press, Oxford

Mauelshagen, J. (1993) Neural correlates of olfactory learning paradigms in an identified neuron in the honey bee brain. J. Neurophysiol. **69**, 609–625

Menzel, R. (1974) Spectral sensitivity of monopolar cells in the bee lamina. J. Comp. Physiol. A **93**, 337–346

Menzel, R. (1979) Spectral sensitivity and colour vision in invertebrates. In: Autrum, H. (ed.) Invertebrate Photoreceptors—Handbook of Sensory Physiology, pp. 503–580. Springer, Berlin

Menzel, R. (1999) Memory dynamics in the honey bee. J. Comp. Physiol. A **185**, 323–340

Menzel, R., Snyder, A.W. (1974) Polarized light detection in the bee, *Apis mellifera*. J. Comp. Physiol. **88**, 247–270

Menzel, R., Blakers, M. (1976) Colour receptors in the bee eye—morphology and spectral sensitivity. J. Comp. Physiol. **108**, 11–33

Menzel, R., Lieke, E. (1983) Antagonistic color effects in spatial vision of honey bees. J. Comp. Physiol. **151**, 441–448

Menzel, R., Backhaus, W. (1991) Color vision in insects. In: Gouras, P. (ed.) Vision and Visual Dysfunction the Perception of Color, pp. 262–288. Macmillan, London

Menzel, R., Ventura, D.F., Hertel, H., de Souza, J.M., Greggers, U. (1986) Spectral sensitivity of photoreceptors in insect compound eyes: comparison of species and methods. J. Comp. Physiol. A **158**, 165–177

Menzel, R., Greggers, U., Smith, A., Berger, S., Brandt, R., et al. (2005) Honey bees navigate according to a map-like spatial memory. Proc. Natl. Acad. Sci. USA **102**, 3040–3045

Meyer, E. (1984) Retrograde labelling of photoreceptors in different regions of the compound eyes of bees and ants. J. Neurocyt. **13**, 825–36

Milde, J.J. (1988) Visual responses of interneurones in the posterior median protocerebrum and the central complex of the honey bee *Apis mellifera*. J. Insect Physiol. **34**, 427–436

Mobbs, P.G. (1984) Neural networks in the mushroom bodies of the honey bee. J. Insect Physiol. **30**, 43–58

Mota, T., Giurfa, M., Sandoz, J.C. (2011a) Color modulates olfactory learning in honey bees by an occasion-setting mechanism. Learn. Mem. **18**, 144–155

Mota, T., Roussel, E., Sandoz, J.C., Giurfa, M. (2011b) Visual conditioning of the sting extension reflex in harnessed honey bees. J. Exp. Biol. **214**, 3577–3587

Mota, T., Yamagata, N., Giurfa, M., Gronenberg, W., Sandoz, J.-C. (2011c) Neural organization and visual processing in the anterior optic tubercle of the honey bee brain. J. Neurosci. **31**, 11443–11456

Müller, U. (1996) Inhibition of nitric oxide synthase impairs a distinct form of long-term memory in the honey bee, *Apis mellifera*. Neuron **27**, 159–168

Murphy, G.L. (2002) The Big Book of Concepts. MIT, Cambridge

Murphy, G.L. (2010) What are categories and concepts? In: Mareschal, D., Quinn, P.C., Lea, S.E.G. (eds.) The Making of Human Concepts. Oxford University Press, Oxford

Niggebrugge, C., Leboulle, G., Menzel, R., Komischke, B., de Ibarra, N.H. (2009) Fast learning but coarse discrimination of colours in restrained honey bees. J. Exp. Biol. **212**, 1344–1350

Paulk, A.C., Gronenberg, W. (2008) Higher order visual input to the mushroom bodies in the bee, *Bombus impatiens*. Arthropod Struct. Dev. **37**, 443–458

Paulk, A.C., Phillips-Portillo, J., Dacks, A., Fellous, J.-M., Gronenberg, W. (2008) The processing of color, motion, and stimulus timing are anatomically segregated in the bumblebee brain. J. Neurosci. **28**, 6319–6332

Paulk, A.C., Dacks, A.M., Gronenberg, W. (2009a) Color processing in the medulla of the bumblebee (Apidae: *Bombus impatiens*). J. Comp. Neurol. **513**, 441–456

Paulk, A.C., Dacks, A.M., Phillips-Portillo, J., Fellous, J.-M., Gronenberg, W. (2009b) Visual processing in the central bee brain. J. Neurosci. **29**, 9987–9999

Pearce, J.M. (1987) A model for stimulus generalization in Pavlovian conditioning. Psychol. Rev. **94**, 61–73

Peitsch, D., Fietz, A., Hertel, H., Souza, J., Ventura, D.F., et al. (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. J. Comp. Physiol. A **170**, 23–40

Ribi, W.A. (1975a) The first optic ganglion of the bee. I. Correlation between visual cell types and their terminals in the lamina and medulla. Cell Tissue Res. **165**, 103–111

Ribi, W.A. (1975b) The neurons of the first optic ganglion of the bee (*Apis mellifera*). Adv. Anat. Embryol. Cell Biol. **50**, 1–43

Ribi, W.A., Scheel, M. (1981) The second and third optic ganglia of the worker bee: Golgi studies of the neuronal elements in the medulla and lobula. Cell Tissue Res. **221**, 17–43

Riehle, A. (1981) Color opponent neurons of the honey bee in a heterochromatic flicker test. J. Comp. Physiol. A **142**, 81–88

Rind, F.C. (2004) Bioinspired sensors: From insect eyes to robot vision. In: Christensen, T. A. (ed.) Methods in Insect Sensory Neurosciences. CRC, Boca Raton.

Rossel, S., Wehner, R. (1984) How bees analyse the polarization patterns in the sky. Experiments and model. J. Comp. Physiol. A **154**, 607–615

Rossel, S., Wehner, R. (1986) Polarization vision in bees. Nature **323**, 128–131

Roussel, E., Sandoz, J.C., Giurfa, M. (2010) Searching for learning-dependent changes in the antennal lobe: simultaneous recording of neural activity and aversive olfactory learning in honey bees. Front. Behav. Neurosci. **4**, 1–12

Rybak, J., Menzel, R. (1993) Anatomy of the mushroom bodies in the honey bee brain: the neuronal connections of the alpha-lobe. J. Comp. Neurol. **334**, 444–465

Sandoz, J.C., Galizia, C.G., Menzel, R. (2003) Side-specific olfactory conditioning leads to more specific odor representation between sides but not within sides in the honey bee antennal lobes. Neurosci. **120**, 1137–1148

Shepard, R.N. (1987) Toward a universal law of generalization for psychological science. Science **237**, 1317–1323

Skorupski, P., Chittka, L. (2010) Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee, *Bombus terrestris*. J. Neurosci. **30**, 3896–3903

Spaethe, J., Briscoe, A.D. (2004) Early duplication and functional diversification of the opsin gene family in insects. Mol. Biol. Evol. **21**, 1583–1594

Spaethe, J., Briscoe, A.D. (2005) Molecular characterization and expression of the UV opsin in bumblebees: three ommatidial subtypes in the retina and a new photoreceptor organ in the lamina. J. Exp. Biol. **208**, 2347–2361

Spence, K.W. (1937) The differential response in animals to stimuli varying within a single dimension. Psychol. Rev. **44**, 430–444

Srinivasan, M.V. (1994) Pattern recognition in the honey bee: recent progress. J. Insect Physiol. **40**, 183–194

Srinivasan, M.V. (2006) Honey bee vision: in good shape for shape recognition. Curr. Biol. **16**, R58–R60

Srinivasan, M.V. (2011) Honey bees as a model for the study of visually guided flight, navigation, and biologically inspired robotics. Physiol. Rev. **91**, 413–460

Srinivasan, M.V., Lehrer, M. (1984) Temporal acuity of honey bee vision: behavioural studies using moving stimuli. J. Comp. Physiol. A **155**, 297–312

Srinivasan, M.V., Lehrer, M. (1985) Temporal resolution of colour vision in the honey bee. J. Comp. Physiol. A **157**, 579–586

Srinivasan, M.V., Lehrer, M. (1988) Spatial acuity of honeybee vision, and its spectral properties. J. Comp. Physiol. A **162**, 159–72

Stach, S., Benard, J., Giurfa, M. (2004) Local-feature assembling in visual pattern recognition and generalization in honey bees. Nature **429**, 758–61

Strausfeld, N.J., Okamura, J.Y. (2007) Visual system of calliphorid flies: organization of optic glomeruli and their lobula complex efferents. J. Comp. Neurol. **500**, 166–188

Strausfeld, N.J., Sinakevitch, I., Okamura, J.Y. (2007) Organization of local interneurons in optic glomeruli of the dipterous visual system and comparisons with the antennal lobes. Dev. Neurobiol. **67**, 1267–1288

Takeda, K. (1961) Classical conditioned response in the honey bee. J. Insect Physiol. **6**, 168–179

Thompson, R. K. R. (1995) Natural and relational concepts in animals. In: Roitblat, H., Meyer, J. A. (eds.) Comparative Approaches to Cognitive Science. MIT, Cambridge.

van Hateren, J.H., Srinivasan, M.V., Wait, P.B. (1990) Pattern recognition in bees: orientation discrimination. J. Comp. Physiol. A **167**, 649–654

Velarde, R.A., Sauer, C.D., Walden, K.O., Fahrbach, S. E., Robertson, H.M. (2005) Pteropsin: a vertebrate-like non-visual opsin expressed in the honey bee brain. Insect Biochem. Mol. Biol. **35**, 1367–1377

Vergoz, V., Roussel, E., Sandoz, J.C., Giurfa, M. (2007) Aversive learning in honey bees revealed by the olfactory conditioning of the sting extension reflex. PLoS ONE **2**, e288

von Frisch, K. (1914) Der Farbensinn und Formensinn der Bienen. Z. Jhb. Physiol. **37**, 1–128

von Frisch, K. (1967) The Dance Language and Orientation of Bees. Belknap Press of Harvard University Press, Cambridge

Vorobyev, M., Osorio, D. (1998) Receptor noise as a determinant of colour thresholds. Proc. Biol. Sci. **265**, 351–358

Vorobyev, M., Gumbert, A., Kunze, J., Giurfa, M., Menzel, R. (2001) Colour threshold and receptor noise: behaviour and physiology compared. Vision Res. **41**, 639–653

Wakakuwa, M., Kurasawa, M., Giurfa, M., Arikawa, K. (2005) Spectral heterogeneity of honey bee ommatidia. Naturwissenschaften **92**, 464–467

Waterman, T.H. (1981) Polarization sensitivity. In: Autrum, H. (ed.) Handbook of Sensory Physiology VII/6B, pp. 281–469. Springer, Berlin

Wehner, R. (1967) Pattern recognition in bees. Nature **215**, 1244–1249

Wehner, R. (1971) The generalization of directional visual stimuli in the honey bee. Apis mellifera. J. Insect Physiol. **17**, 1579–1591

Wehner, R., Strasser, S. (1985) The POL area of the honey bee's eye: behavioural evidence. Physiol. Entomol. **10**, 337–349

Werner, A., Menzel, R., Wehrhahn, C. (1988) Color constancy in the honey bee. J. Neurosci. **8**, 156–159

White, R.H., Xu, H., Münch, T.A., Bennett, R.R., Grable, E.A. (2003) The retina of *Manduca sexta*: rhodopsin-expression, the mosaic of green-, blue-, and UV-sensitive photoreceptors and regional specialization. J. Exp. Biol. **206**, 3337–3348

Wolf, R., Heisenberg, M. (1991) Basic organization of operant behavior as revealed in *Drosophila* flight orientation. J. Comp. Physiol. A **169**, 699–705

Yang, E.C., Lin, H.C., Hung, Y.S. (2004) Patterns of chromatic information processing in the lobula of the honey bee. Apis mellifera L. J. Insect Physiol. **50**, 913–925

Zayan, R., Vauclair, J. (1998) Categories as paradigms for comparative cognition. Behav. Process. **42**, 87–99

Zentall, T.R., Galizio, M., Critchfied, T.S. (2002) Categorization, concept learning, and behavior analysis: an introduction. J. Exp. Analys. Behav. **78**, 237–248

Zentall, T.R., Wasserman, E.A., Lazareva, O.F., Thompson, R.K.R., Rattermann, M.J. (2008) Concept learning in animals. Comp. Cogn. Behav. Rev. **3**, 13–45

Zhang, S.W., Srinivasan, M.V., Collett, T. (1995) Convergent processing in Honey bee vision: multiple channels for the recognition of shape. Proc. Natl. Acad. Sci. USA **92**, 3029–3031

Zhang, S., Srinivasan, M.V., Zhu, H., Wong, J. (2004) Grouping of visual objects by honey bees. J. Exp. Biol. **207**, 3289–3298

Zhang, S., Bock, F., Si, A., Tautz, J., Srinivasan, M.V. (2005) Visual working memory in decision making by honey bees. Proc. Natl. Acad. Sci. USA **102**, 5250–5255