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Visual learning in social insects: from simple associations to higher-order problem solving

Martin Giurfa

Contents

Abstract

Visual learning allows the acquisition of new environmental information, which in turn allows adaptive responses when viewing already experienced events again. This capacity is crucial in contexts such as search for food, partner recognition, navigation and defense against potential enemies. It admits different levels of complexity, from simple associative link formation between a visual stimulus (e. g. a specific color) and the consequence of it (e. g. reward or punishment), to more sophisticated performances such as categorization of objects (e. g. animal vs. non-animal) or apprehending abstract rules applicable to unknown visual objects (e. g. "larger than" or "on top of"). In principle, mastering categories and rules allows flexible responses beyond simple forms of learning. Not surprisingly, higher-order forms of visual learning have been mainly studied in vertebrates with larger brains, while the study of simple visual learning has been restricted to animals with small brains such as insects. However, this dichotomy has recently changed, as research on visual learning in social insects (mainly bees and wasps) has yielded surprising results in terms of the sophistication of the tasks that can be mastered. In parallel, the accessibility and small size of insect brains have allowed the characterization of some neural mechanisms of visual learning. Here I review a spectrum of visual learning forms in social insects, from color and pattern learning, visual attention, and top-down image recognition to

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Martin Giurfa
Université Paul Sabatier – Toulouse III
Centre de Recherches sur la Cognition Animale CNRS
118 Route de Narbonne
31062 Toulouse cedex 4, France
e-mail: giurfa@cict.fr

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inter-individual recognition, conditional discrimination, category learning and rule extraction. I discuss the necessity and sufficiency of simple associations to account for complex visual learning and profit from the extensive knowledge on brain organization in insects to discuss neural mechanisms underlying these visual performances.

1.

Introduction

Visual learning refers to an individual's capacity to acquire experience-based information pertaining to visual stimuli so that adaptive responses can be produced when viewing such stimuli again. This capacity, which is present in almost all living animals capable of seeing, intervenes in contexts as diverse as the search for food, partner recognition, navigation and orientation, and defense against potential enemies. It admits different levels of complexity as it varies from the establishment of a simple associative link connecting a visual stimulus (e. g. a specific color) and the consequences that result (e. g. a reward or a punishment), to more sophisticated performances such as learning to categorize distinct objects (e. g. animal vs. non-animal) or apprehending abstract rules applicable to unknown visual objects (e. g. "larger than," "on top of" or "inside of).

The first situation, the establishment of unequivocal, unambiguous links between a visual target and its outcome, constitutes a case of elemental learning. What is learned for a color is valid only for that color and not for different ones. Red light means not crossing the street while green light means that crossing is allowed. In this context, however, blue light, does not mean anything. In contrast, learning about categories and rules constitutes a case of non-elemental learning, which is not based on one stimulus – one consequential relationship, as an appropriate response can be then transferred to novel, unknown stimuli for which the subject has no personal experience. We are able to categorize a dodo as a bird, or as an animal, even if we have never observed one of these extinct creatures. Similarly, if trained to classify objects based on a size rule (e. g. 'larger than') we would be able to respond appropriately to unknown objects just by considering their size relative to each other. In these cases, the subject's response is flexible and relatively independent of the physical nature of the stimuli considered.

Social Hymenoptera, particularly bees (*Apis* sp. and *Bombus* sp.) and wasps (several genera), which are at the center of this article, are interesting models for the study of visual learning because in the context of their natural behavior they have to solve a diversity of visual problems of varying complexity. For instance, these insects learn and memorize the local cues characterizing the places of interest, which are essentially the hive and the food sources (Menzel 1985; Menzel et al. 1993; Zeil et al. 1996; Fauria et al. 2000, 2002). Honeybees, and to a minor extent bumblebees too, are 'flower constant'. They forage on a particular flower species as long as it offers profitable nectar and/or pollen reward (Grant 1951; Heinrich 1979; Chittka et al. 1999). This capacity is partly based on visual cues provided by flowers such as colors or patterns. Learning and memorizing the visual cues of the exploited flower through their association with nectar and/or pollen reward is what allows a bee forager tracking a particular species in the field (von Frisch 1965; Menzel 1985). Similarly, learning abilities for landmark constellations and for celestial cues used in navigation (azimuthal position of the sun, polarized light pattern of the blue sky) ensure a safe return to the nest and enhance foraging efficiency (Collett and Collett 2002; Collett et al. 2003).

Visual capacities are highly developed in social Hymenoptera, especially in those species that fly freely. Bees and wasps see the world in color (Beier and Menzel 1972; Menzel and Backhaus 1991; Chittka et al. 1992; Campan and Lehrer 2002; Dyer and Chittka 2004; Lehrer and Campan 2004, 2005; Lotto and Chittka 2005), perceive shapes and patterns (Wehner 1981; Srinivasan 1994; Giurfa and Lehrer 2001; Lehrer and Campan 2004, 2005; Dyer et al. 2005) and resolve movements with a high temporal resolution (Srinivasan et al. 1999). They implement these capacities in navigational journeys that may lead them several kilometers away from their nest to which they nevertheless return after each successful foraging trip. The complexity and richness of their natural life is therefore appealing in terms of the opportunities it offers for the study of visual learning and memory. Such an appeal would be, however, useless if these phenomena were not amenable to controlled laboratory conditions. Indeed, one of the reasons why bees and wasps constitute an attractive model for the study of visual learning resides precisely in the existence of controlled experimental methods for the study of this capacity at the individual level.

2.

Visual conditioning of bees

Visual conditioning of freely-flying honeybees (von Frisch 1914) has allowed the uncovering of the perceptual capabilities of these insects and has been used to this end for more than nine decades. The common experimental protocol exploits the fact that free-flying honeybees learn visual cues such as colors, shapes and patterns, depth and motion contrast, among others (von Frisch 1914; Wehner 1981; Giurfa and Menzel 1997; Lehrer 1997; Giurfa and Lehrer 2001) when these are presented together with a reward of sucrose solution (Fig. 1a). Each bee is individually marked by means of a color spot on its thorax or abdomen so that performances of individuals can be recorded. In general the marked bee is displaced by the experimenter towards a site (called experimental place) where it is rewarded with sucrose solution in order to promote its regular return. Such pre-training is performed without presenting the training stimuli in order to avoid uncontrolled learning. When the bee starts visiting the experimental place actively (i. e., without being displaced by the experimenter), the training stimuli are presented and the choice of the appropriate visual target rewarded with sucrose solution.

Bees have to be trained and tested *individually* to achieve a precise control of the experience made by each subject. It is also important to control the distance at which a choice is made because visual orientation and choice are mediated by different visual cues at different distances or angles subtended by the target (Giurfa et al. 1996; Giurfa and Menzel 1997; Giurfa and Lehrer 2001). The time between visits to the experimental place has also to be recorded as it reflects the appetitive motivation of the bee (Núñez 1982) and thus its motivation to learn. Bees coming irregularly to the experimental place are not highly motivated and their performances are therefore unreliable.

Several actions can be used to quantify the bees' choice in these experiments. Contacts (i. e. flights towards a target that end with a contact of the bee's antennae or legs with the stimulus surface) and landings on a given visual target are usually recorded. The associations built in this context can be either classical, operant or both, i. e. they may link a visual stimulus (conditioned stimulus

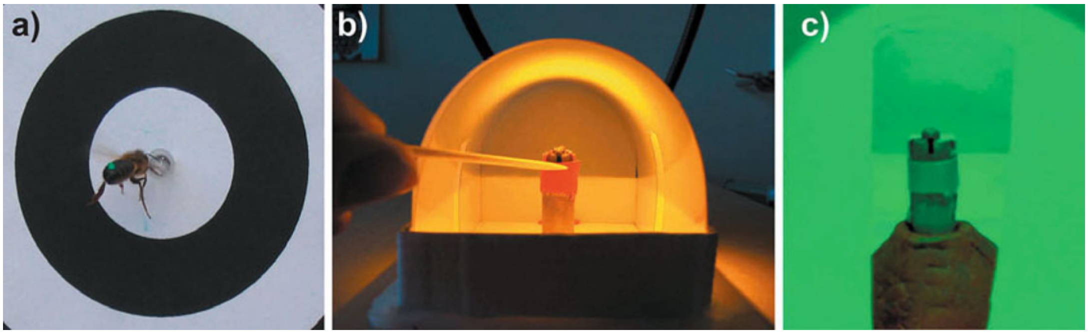

Fig. 1 **Experimental protocols** for the study of visual learning and perception in honeybees. **a** *Visual appetitive conditioning of free-flying bees*. A bee marked with a colored spot on the abdomen is trained to collect sugar solution in the middle of a ring pattern. **b, c** *Color conditioning of the proboscis extension reflex in harnessed honeybees*. **b** A hungry honeybee immobilized in a tube extends its proboscis when its antennae are touched with a drop of sucrose solution. **c** Pairing a chromatic (green) illumination with such a reward leads to the establishment of a color – reward association that makes the bee extend the proboscis when presented with the chromatic illumination alone (see Hori et al. 2006)

or CS) and sucrose reward (unconditioned stimulus or US), the response of the animal (e. g. landing) and the US, or both, respectively. The experimental framework is nevertheless mainly operant as the bee's behavior determines whether it obtains the sucrose reinforcement or not. The same experimental method has been successfully adapted to train other freely flying social insects to a variety of visual targets (bumblebees: Dyer and Chittka 2004; Lotto and Chittka 2005; Dyer et al. 2005; solitary bees: Campan and Lehrer 2002; Menzel et al. 1988; stingless bees: Menzel et al. 1988; wasps: Beier and Menzel 1972; Lehrer and Campan 2004, 2006).

Visual conditioning of freely-flying bees presents a fundamental problem for researchers interested in the mechanistic basis of visual learning as it does not allow the study of visual learning at the cellular level. Because bees fly freely during the experiment, to simultaneously study the neural activity in visual centers in the brain so far has remained impossible. Recently, however, a protocol for visual conditioning of harnessed bees has been developed (Hori et al. 2006, 2007). It is based on pioneer studies by Kuwabara (1957) and consists in training a harnessed bee to extend its proboscis to colors (Hori et al. 2006) or visual motion cues (Hori et al. 2007) paired with sucrose solution (Fig. 1b). Hungry bees reflexively extend the proboscis when their antennae are touched with sucrose solution, the equivalent of a nectar reward. In this protocol, colors or patterns are paired with a sucrose reward to create a Pavlovian association in which the visual stimuli are the conditioned stimuli (CS) and sucrose is the unconditioned stimulus (US). With some effort (learning takes two days and acquisition levels remain relatively low at 40%), bees learn the visual task only if their antennae have been previously cut. The reasons for this apparent interference of the antennae on visual learning remain unknown but it may simply be that cutting the antennae affects the general motivation of the bee so that the sucrose reward is not as attractive as expected by the experimenter (de Brito Sanchez et al. 2008). Improving this protocol is a priority for future research on visual learning as it will allow a combination of behavioral quantification with access to the nervous system. As in honeybee olfactory learning (see Giurfa 2007 for review), the fact that the bee is immobilized while it learns allows access to the nervous system and the study of it by the application of invasive methods.

3.

Attentional and experience-dependent modulation of visual learning

The first study of bee learning and memory that used controlled protocols for characterizing individual learning and memory, employed colors as rewarding stimuli (Menzel 1967). Freely flying honeybees were trained to choose a rewarded monochromatic light and then presented with the rewarded light versus an alternative color on a horizontal plane in dual choice situations. This early study reported learning curves for different wavelengths and showed that, under the given experimental conditions, bees learned all the wavelengths after only a few learning trials. Some wavelengths, particularly 413 nm, were learned faster than others, requiring only one to three acquisition trials (Menzel 1967; but see below). This finding argued in favor of innate biases in color learning, probably reflecting the particular biological relevance of the color signals that are learned faster (Menzel 1985). Indeed, in their first foraging flight color-naïve honeybees prefer those colors that experienced bees learn faster (Giurfa et al. 1995), and preliminary findings indicate that these very colors may correspond to floral colors that are strongly associated with a profitable nectar reward (Giurfa et al. 1995).

Visual learning, as studied in these color conditioning experiments, is elemental as bees are merely presented with a single color target paired with sucrose solution. It was intended to be a fast form of learning (Menzel 1967; see above), compared, for instance, to learning of visual patterns which usually takes longer (20 or more trials). Recent studies on bumblebee and honeybee color learning (Dyer and Chittka 2004; Giurfa 2004) have, nevertheless, introduced a new twist to these conclusions. It was long thought that what an animal sees and visually learns is constrained by its perceptual machinery with little or no room for experience-dependent modulations of perception. However, studies of honeybees (Giurfa 2004; Dyer and Neumeyer 2005) and of bumblebees (Dyer and Chittka 2004) have shown that this idea is incorrect: In some cases, learning one and the same color may need few trials only but in other cases it may take more than twenty trials (Fig. 2a). The critical feature is *how* the bees learn the task. For instance, *absolute conditioning*, in which a subject is trained with a single color rewarded with sugar water, in general yields fast learning. *Differential conditioning*, in which the same subject has to learn to discriminate between a rewarded from a non-rewarded color, takes more trials, even if the rewarded color is the same as in absolute conditioning. When these animals are asked to discriminate between colors in a test, their performance differs dramatically. While bees trained in differential conditioning can discriminate between colors that are very similar (Fig. 2c), bees trained in absolute conditioning cannot discriminate between the same pair of colors (Fig. 2b; Giurfa 2004). Interestingly, similar results were obtained for bumblebees (Dyer and Chittka 2004) and for ants trained to discriminate between colors in a Y-maze (Camlitepe and Aksoy 2010). Differential conditioning promoted fine color discrimination while absolute conditioning did not.

Comparable results were obtained in a study on pattern learning and discrimination by honeybees (Giurfa et al. 1999). While differential conditioning results in a visual recognition strategy that uses the cues present

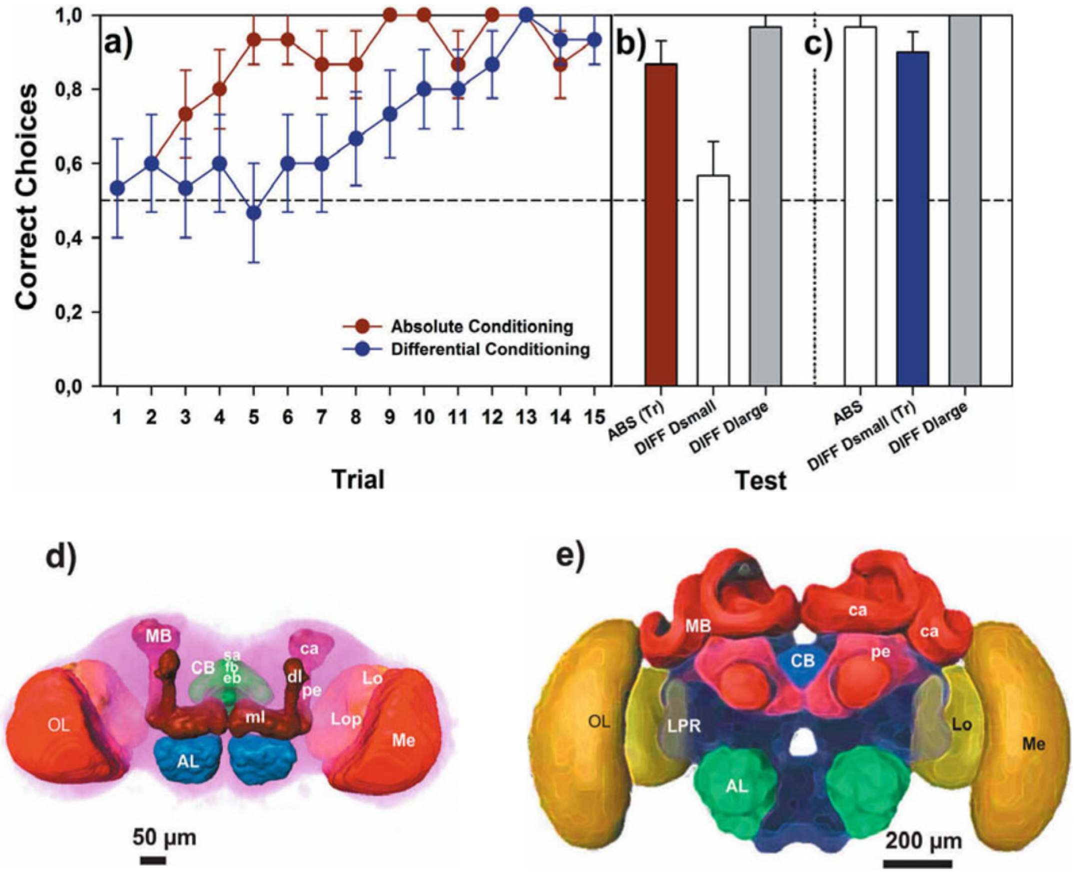

Fig. 2 a, b, c Attention-like processes in honeybees. Performance of the free-flying bees trained with colors under absolute (one color rewarded) and differential conditioning (one color rewarded vs. a color non-rewarded). Adapted from Giurfa (2004) **a** ***Acquisition*** along 15 trials (mean ±S. E.; n = 15 bees for each curve). Red circles: absolute conditioning; blue circles: differential conditioning. **b** ***Performance in the tests of the group trained in absolute conditioning.*** *Red Bar:* test presenting the trained situation (ABSTr), i. e. the single color that was previously rewarded. *White Bar*: test presenting a novel differential situation (DIFF D_{small}), i. e. the color that was previously rewarded vs. a new color that was very similar to the trained one. *Grey Bar:* test presenting a novel differential situation (DIFF D_{large}), i. e. the color that was previously rewarded vs. a new color that was very different from the trained one. **c** ***Performance in the tests of the group trained in differential conditioning***. *Blue Bar:* test presenting the trained situation (DIFF D_{small}Tr), i. e. the previously rewarded and the non-rewarded colors which were very similar. *White Bar:* test presenting onlyt the previously rewarded color (ABS). *Grey Bar:* Test presenting the previously rewarded color vs. a novel color very different from the rewarded one (DIFF D_{large}). **d** ***Three-dimensional reconstruction of a fruit fly brain***; AL: antennal lobe; OL: optic lobe; Me: medulla; Lo: lobula; Lop: lobula plate; MB: mushroom body; Ca: calyx; dl: dorsal lobe; ml: medial lobe; pe: peduncle; CB: central body; eb: ellipsoid body; fb: frontal bridge; fb: fan-shaped body; sa: superior arch. **e** ***Three-dimensional reconstruction of a honeybee brain***; LPR: lateral protocerebrum

in the whole pattern, absolute conditioning results in a recognition strategy that restricts cue sampling mainly to the lower half of the pattern. In other words, bees recognize a pattern differently, depending on the kind of learning implicit to the conditioning task. In both cases (color and pattern learning), however, differential conditioning increases the demands imposed to the perceptual system of the bees. They not only have to go where a rewarded stimulus is presented (absolute conditioning) but to discriminate it from a non-rewarding alternative (differential conditioning). The difference in performance suggests, therefore, that attentional processes are involved. In differential conditioning the bee has to focus on the difference and not on the mere presence of a visual target, thus making learning slower. In any case, the result contradicts the idea that the difference between two colors is a fixed, immutable property constrained by the visual machinery.

At the time at which Menzel first characterized color learning (1967, 1968; see above) studies on pattern perception were performed by Wehner (1967, 1971, 1972) and others (e. g. Anderson 1972), continuing the tradition started by von Frisch's students (Hertz 1929a,b, 1933; Wolf 1933, 1934; Wolf and Zerrahn-Wolf 1935). In contrast to Menzel's work, these studies did not focus on learning but on the perceptual capabilities of bees confronted with pattern discrimination tasks. Certainly, visual conditioning was also used in these and in later works on pattern perception (for review see Wehner 1981; Srinivasan 1994; Lehrer 1997; Srinivasan and Zhang 1997) but there was no quantification of acquisition curves and/or a characterization of pattern memory. This tradition was continued in the 1970s, 80s and even 90s when visual learning was used mainly as a tool to answer questions of visual perception and discrimination close to the feeding place. The questions asked by these works focused on visual spatial resolution, shape discrimination, orientation detection, movement perception and distance estimation based on image movement on the retina (the so-called parallax), among others. An accurate control of the bees' individual experience prior to the tests to which they were subjected, would have been desirable. As mentioned above, in pattern vision, like in color vision, what a bee sees depends on its previous visual experience and on possible attentional processes. Zhang and Srinivasan (1994) showed, for instance, that the previous visual experience of a bee can speed up the analysis of the retinal image when a familiar object or scene is encountered. They first attempted to train bees to distinguish between a ring and a disk when each shape was presented as a textured figure placed a few centimetres in front of a similarly textured background (Fig. 3a). In principle the figures were detectable through the relative motion of the figure outlines, whose distance differed from that of the background when bees flew towards the targets. Despite intensive training, the bees were incapable of learning the difference between a ring and a disk (Fig. 3a), a discrimination task that usually poses no problems when the bees see the stimuli as plain (non-textured) shapes. Zhang and Srinivasan (1994) then trained a group of bees to solve the 'easy' problem, presenting a plain black disk and ring positioned a few cm in front of a white background (Fig. 3b). As expected, the bees easily learned the task. They were then confronted with the 'difficult' problem of learning the textured disk versus the ring and this time they immediately solved the discrimination task (Fig. 3c). Thus, pre-training with plain stimuli primed the pattern recognition system in such a way that it was able to detect shapes that otherwise could not be distinguished. Again, it may be that such pretraining triggers attentional processes that allow a better focus on the targets that have to be discriminated between.

Thus, color and pattern vision studies in bees have introduced the idea that visual capabilities are modulated by attentional and experience-dependent processes. This idea has been explicitly studied in honeybees trained to choose a colored disc ('target') among a varying number of differently color-

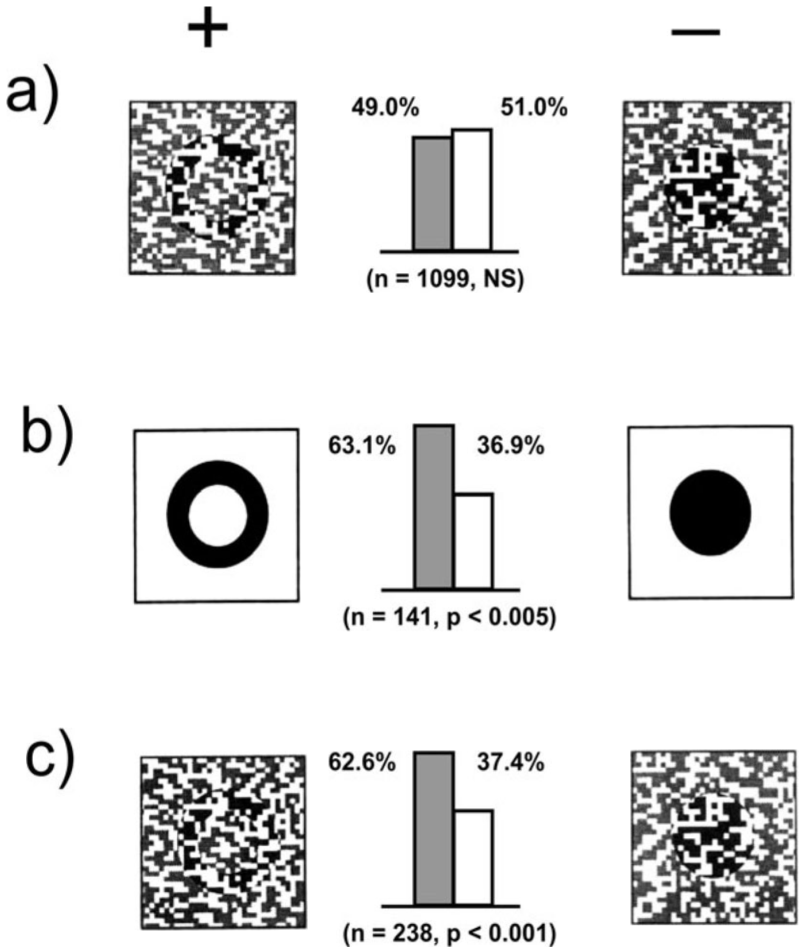

Fig. 3 **Prior experience** enhances pattern discrimination in honeybees (adapted from Zhang and Srinivasan 2004). **a** Bees were trained in a dual-choice Y-maze to distinguish between a ring (rewarded) and a disk (non-rewarded) when each shape was presented as a textured figure placed a few cm in front of a similarly textured background. Despite intensive training, the bees were incapable of learning the difference between a ring and a disk (n: number of choices; the percentages correspond to the choice of stimuli presented). **b** When these stimuli were presented as plain (non-textured) shapes, few cm in front of a white background, the bees could, as expected, easily learn the task. **c** They were then confronted with the difficult problem of learning the textured disk vs. the ring and this time they solved the discrimination challenge. Pre-training with the plain stimuli may trigger attentional processes that allow a better focus on the targets whose discrimination is difficult

ed discs ('distractors') (Spaethe et al. 2006). Accuracy and decision time were measured as a function of distractor number and color. For all color combinations, decision time increased and accuracy decreased with increasing distractor number, whereas performance increased when more targets were present. These findings are characteristic of a serial search in primates, when stimuli are examined sequentially, thus indicating that at the behavioral level, the strategies implemented by bees converge with those of animals in which attention is commonly studied (Spaethe et al. 2006).

In addition, other results have shown that the visual strategies used by bees to accomplish visual discrimination may be affected by the amount of experience accumulated at the moment of a test (Giurfa et al. 2003; Stach and Giurfa 2005). Quantifying individual experience and reporting visual acquisition curves is therefore crucial even for the simplest visual discrimination experiment that involves training to a single visual target.

Uncovering how attentional processes and learning modulate visual perception constitutes an unexplored and promising research field. The existence of attentional processes in insect brains is not far-fetched and recent research has been able to locate such processes in identified structures of the insect brain. In the fruit fly *Drosophila melanogaster*, attention can be demonstrated and characterized at the physiological level (van Swinderen and Greenspan 2003). A tethered fruit fly within a circular arena and tracking a visual object (a vertical black bar) moving at a constant frequency around it, exhibits anticipatory behavior consistent with attention for the bar it tracks. The neural correlate of such an anticipatory tracking is a transient increase in a 20–30 Hz local field-potential recorded in a region of the brain called the medial protocerebrum (Fig. 2d; van Swinderen and Greenspan 2003). The neural response is not only anticipatory, but also selective to the stimulus presented, increased by novelty and salience and reduced when the fly is in a sleep-like state (van Swinderen and Greenspan 2003). Moreover, the use of mutants showed that a subset of neurons of the mushroom bodies, which are a higher-order structure of the insect brain (Fig. 2d, e), are required for both the tracking response and the 20–30 Hz response. Mutants in which the output of these neurons was suppressed did not retain either of these characteristics, thus showing that the neural basis of attentional processes can be located in a specific set of neurons of the fruit fly brain (van Swinderen and Greenspan 2003). This result is consistent with the finding that mushroom bodies are required for choice behavior in *Drosophila* facing contradictory visual cues (Tang and Guo 2001). In this case, a tethered fly flying stationarily is trained in a circular arena in which one kind of visual stimulus (say, a T pattern) represents a permitted flight direction, while another kind of visual stimulus (say, an inverted T pattern) represents a forbidden flight direction associated with an unpleasant heat beam on the thorax. Tang and Guo (2001) conditioned flies to choose one of two directions in response to color and shape cues; after the training, the flies were tested using contradictory cues. Wild-type flies made a discrete choice that switched from one alternative to the other as the relative salience of color and shape cues gradually changed. However, this ability was greatly diminished in mutant flies with miniature mushroom bodies or in flies with chemically ablated mushroom bodies. Obviously, mushroom bodies mediate the assessment of the relative saliency of conflicting visual cues (Tang and Guo 2001, Xi et al. 2008) and are also involved in improving the extraction of visual cues after pre-training in *Drosophila* (Peng et al. 2007). The mushroom bodies of hymenopterans may play similar roles (Fig. 1e), favoring attention processes and better problem solving and discrimination in the visual domain.

Yet, visual learning and the neural circuits mediating it are still poorly understood in the fruit fly. The mushroom bodies, which are the main site for olfactory memories, are not directly involved in visual learning. In *Drosophila*, different from hymenopterans, there is no direct input from the visual areas of the brain to these structures (Wolf et al. 1998). Recent studies succeeded in precisely identifying the neuronal substrates of two forms of visual memory in the *Drosophila* brain, outside the mushroom bodies (Liu et al. 2006). Memory for pattern elevation and orientation, two parameters whose variations can be easily distinguished by flies, were retraced to different regions of the central complex, a structure in the centre of the insect brain (Fig. 2d). Two neuronal layers of the central complex are required for visual discrimination based on pattern elevation and orientation, respectively. Because in all cases only visual short-term memory was studied, the localization of visual long-term memory remains an open question.

In bees and wasps, the localization of visual memories may differ from that in *Drosophila*. In contrast to the fruit fly, the visual areas of the hymenopteran brain do provide direct input to the mushroom bodies (Ehmer and Gronenberg 2002). The mushroom bodies may well, therefore, be the seat of visual memories in addition to the central complex (Fig. 2d).

4.

Complex forms of visual learning (that may not be so complex)

Only in the 1990s, when researchers became interested in the existence of cognitive processing in insects and the honeybee, was the model chosen to address most of the questions asked at that time. The delay with respect to the general "cognitive revolution" which already flourished at the end of the 1970s and the early 1980s (Miller 2003) was due to the reluctance to view invertebrates, and therefore insects, as cognitive organisms (by cognitive we understand here as 'capable of non-elemental', higher-order forms of learning). For instance, the main idea about visual pattern learning, which is still sometimes defended, was that insects can only view isolated spots, blobs and bars without having the capacity to integrate them in a given configuration (Horridge 2000, 2003, 2006, 2009). Even a basic capacity of recognition such as generalization, the ability to respond to stimuli that despite being different are perceptually similar to a trained target (Spence 1937, Shepard 1987), was and still is considered by some researchers as being too high-level for a honeybee (Horridge 2006, 2009). Yet, dozens of works had already shown that honeybees generalize their choice of visual patterns to novel figures that have some similarity with those that have been trained (e.g. Wehner 1971, Anderson 1972). This denial of generalization capacities is consistent with the preconception that bees and insects in general have limited plasticity and should instead be viewed as reflex machines or robots reacting to specific features in the environment to which they are attuned.

In the last decade, however, researchers have shown that bees are not robots but exhibit visual learning capabilities that to date had only been attributed to various vertebrates. Some of these capacities are surprising and may be viewed as non-elemental. However, alternatively one might argue that it is possible to explain them as being based on simple, elemental associations. The experiments reviewed in the next section were not conceived to address these opposite views, so we are currently unable to determine whether the fascinating performances observed are forms of elemental or higher-order learning.

4.1 Visually-based recognition of individuals in wasps

The capacity to recognize the distinctive identity of individuals has long been dismissed in social insects because of the cognitive requirements that such performance may impose in colonies with thousands of individuals. For instance, Wilson (1971) stated that "*insect societies are, for the most part, impersonal* [...]. *The sheer size of the colonies and the short life of the members make it inefficient, if not impossible, to establish individual bonds*". Not all social insects live, however, in huge overcrowded societies. Small colonies of bumble bees, wasps and some ant species are based on dominance hierarchies where individual recognition may be crucial for responding appropriately to a conspecific. Indeed, recent studies have shown that queens of the ant *Pachycondyla villosa* recognize each other using olfactory cuticular cues (d'Ettorre and Heinze 2005). In the visual domain, studies on the paper wasp *Polistes fuscatus* have shown that individual recognition is achieved through learning the yellow-black patterns on the wasp faces and/or abdomens (Tibbetts 2002) (Fig. 4). More variable patterns with larger black components were found to be carried by individuals ranking higher in the nest hierarchy. Altering these facial and/or abdominal color patterns induced aggression against such animals, irrespective of whether their patterns were made to signal higher or lower ranking. These results, therefore, are in favor of the capacity to learn visual features allowing the recognition of individual wasps (Tibbetts and Dale 2004). This capacity should not necessarily be a surprise with regard to its cognitive implications. Each individual facial mask would have an unambiguous significance in terms of its ranking in the social structure (i. e. mask A →α individual; mask B →β individual, etc.) Thus, wasps would learn a series of elemental associations between mask patterns and social ranking. Given the small size of colonies in which five to ten individuals can coexist, storing several memories, one for each individual, seems to be a plausible possibility. If this were the case, a fundamental research goal would now be to characterize the storage capacity of the visual memory and its accuracy in relation to colony size (number of individuals).

4.2 Learning by observation in bumblebees

According to recent studies on bumblebees (Leadbeater and Chittka 2005, 2007; Worden and Papaj 2005), these insects copy the learnt foraging preferences of other bees by observing their choices of visual rewarded targets. Bumblebees, *Bombus terrestris,* are influenced by other conspecifics when sampling unfamiliar flowers. They land on unknown flowers where other bees are already present (Leadbeater and Chittka 2005). This behavior is exhibited even when naïve bees are separated from experienced foragers by a transparent screen such that they can neither sample the flowers by themselves nor interact with their foraging conspecifics (Worden and Papaj 2005). Similarly, naïve

Fig. 4 Portraits of nine *Polistes dominulus* wasp foundresses showing the diversity of facial patterns (from Tibbets and Dale 2004)

bumblebees would abandon an unrewarding flower species and switch to a more rewarding alternative more quickly when accompanied by experienced foragers (Leadbeater and Chittka 2007).

As surprising as this performance may appear, it can be accounted for by an elemental form of associative learning called second-order conditioning (Pavlov 1927), which involves two connected associations. In this scenario, an animal first learns an association between a conditioned stimulus (CS) and an unconditioned stimulus (US). It then experiences a pairing between a new conditioned stimulus CS2 and CS1 so that CS2 becomes meaningful through its association with CS1, and indirectly with the US. How would this apply to the observational learning of bumblebees? One could propose that naïve bumblebees first associate the presence of a conspecific (CS1) with reward (US) simply by foraging close to experienced foragers. Subsequently, the observation of a conspecific landing on a given color (CS2) may allow establishing an association between color (CS2) and conspecific (CS1) (Leadbeater and Chittka 2007). The hypothesis of connected elemental links is supported by the fact that honeybees can learn 2nd-order associations while searching for food. They learn to connect both two odors (Odor 1 + Sucrose Reward; Odor 2 + Odor 1; Bittermann et al. 1983) and one odor and one color (Odor + Sucrose Reward; Color + Odor; Grossman 1971).

4.3 'Symbolic matching to sample' and other forms of conditional discrimination in bees and wasps

'Symbolic matching to sample' is a term used to describe an experimental situation in which the correct response to a problem depends on a specific background or condition. In other words, animals have to learn, for instance, that in condition A response C is correct while in condition B response D is correct. This is why symbolic matching to sample is considered to be a form of *conditional discrimination* because a given stimulus, the sample (also called the 'occasion setter'), sets the condition for the next choice. Using this design, Zhang et al. (1999) trained honeybees to fly through a compound Y-maze consisting of a series of interconnected cylinders. The first cylinder carried the sample stimulus (e. g. a vertical or a horizontal black-and-white grating). The second and third cylinders each had two exits. Each exit presented a visual stimulus so that the bee had to choose between them. In the second cylinder, bees had to choose between a blue and a green square whereas in the third cylinder they had to choose between a radially sectored and a ring pattern. Correct sequences of choices were '*Vertical – Green – Ring*' and '*Horizontal – Blue – Radial*'. Only after making a succession of correct choices both in the second and in the third cylinder a bee could reach the feeder with sucrose solution. The bees learned to master these successive associations between different kinds of visual cues (Zhang et al. 1999). This finding was also extended to other sensory modalities; the same principle applied when visual cues were combined with odors in a similar symbolic matching protocol (Srinivasan et al. 1998).

Conditional learning admits other variants that, depending on the number of occasion setters and discriminations involved, have received different names. For instance, another form of conditional discrimination involving two occasion setters is the so-called *transwitching problem*. In this experiment an animal is trained differentially using two stimuli, A and B, and two different occasion setters, C and D. When C is available, stimulus A is rewarded but not stimulus B (A+ vs. B-), while the opposite is the case (A- vs. B+) when D is available. The transwitching problem is also considered a form of contextual learning because the occasion setters C1 and C2 can be viewed as contexts determining the appropriateness of each choice. Bumblebees have been trained in a transwitching problem to choose a 45° grating and to avoid a 135° grating to reach a feeder, and to do the opposite to reach their nest (Fauria et al. 2002) (Fig. 5). Here, the nest and the feeder provide the appropriate contexts defining what has to be chosen. Bumblebees can also learn that an annular or a radial disc must be chosen, depending on the disc's association with a 45° or a 135° grating either at the feeder or the nest entrance: At the nest, access was allowed by the combinations 45° + radial disc and 135° + annular disc, but not by the combinations 45° + annular disc and 135° + radial disc; at the feeder, the opposite applied (Fauria et al. 2000). In both cases, the potentially competing visuo-motor associations were insulated from each other by being set in different contexts.

Solving this kind of problem can be viewed as a form of non-elemental learning and thus as a sophisticated form of cognitive visual

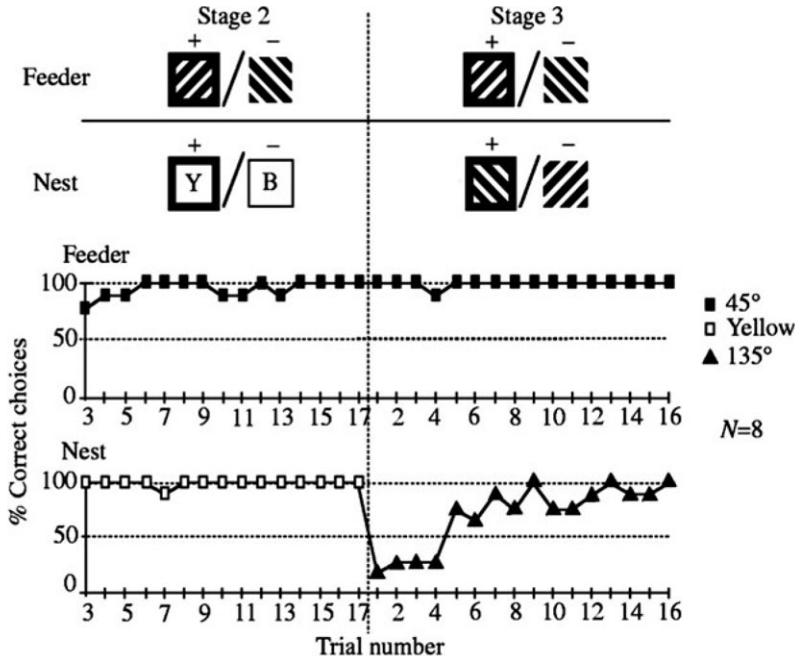

Fig. 5 Performance of bumblebees in a transwitching problem in which they had to choose between two gratings, which have different outcomes depending on the site (feeding place or nest entrance) in which they are presented (adapted from Fauria et al. 2002). **Top:** Stimuli used to train the bumblebees in phases 2 and 3 of the experiment; B: blue; Y: yellow. The stimuli framed in bold are those that allowed animals to access either the food (grating oriented at 45°) or the nest (yellow in phase 2, and grating oriented at 135° in phase 3). While phase 2 poses a simple, non-ambiguous problem to the bees, phase 3 constitutes a case of transwitching as the grating oriented at 135° (and not at 45°) becomes positive at the nest entrance (i. e. allows accessing the nest). **Bottom:** Percentage of correct choices of groups of eight bees plotted against trial number for phases 2 and 3. The curves show the performance with 45° *versus* 135° gratings at the feeder and yellow *versus* blue at the nest in phase 2, and with 45° *versus* 135° at the feeder and 135° *versus* 45° at the nest in phase 3. In phase 2, bees were assisted in trials 1 and 2, so these trials are not plotted. Changing the stimuli from phase 2 to 3 affected performance at the nest but left intact that at the feeder. At the end of phase 3, the bees mastered both problems simultaneously

processing. Indeed, as for other forms of conditional discrimination, one could describe this protocol as CA+, CB- (if C then A but not B), and DA-, DB+ (if D then B but not A). Each stimulus, A, B, C, and D, is rewarded as often as it is non-rewarded so that solutions cannot be based on the mere consequence of A, B, C or D. A higher-order solution would then be to learn the outcome of each particular configuration CA, CB, DA, DB. However, an alternative explanation could argue that what the insects do is to establish hierarchically simple associations like those underlying 2nd-order conditioning (see above). Indeed, one could imagine that bees learn to associate a radial disc with sucrose reward and that they then learn to associate a 45° grating with the radial disc. This is a relatively simple strategy probably used by bees for navigational purposes (Zhang et al. 1996) when they are confronted with successions of different landmarks en route to the goal.

A critical factor determining the application of one strategy or the other may, therefore, be the temporal order of stimulus presentation. If these are presented *serially*, learning chains of simple associations could be primed while *simultaneous* presentation of stimuli may prime learning of configurations and their specific consequences. An example of the latter is the case of honeybees trained to solve a *biconditional discrimination* AC+, BD+, AD-, BC- in which all four stimuli were presented simultaneously, and were as often rewarded as non-rewarded (Schubert et al. 2002). Four different gratings combining one color (yellow or violet = A or B) with one orientation (horizontal or vertical = C or D) were used in such a way that bees had to learn that, for instance, yellow-horizontal (AC) and violet-vertical (BD) were rewarded while yellow-vertical (AD) and violet-horizontal (BC) were non-rewarded. Bees learned to choose the rewarded stimuli despite the fact that colors and orientations were ambiguous when considered alone. They thus learned the configurations and not the specific outcome of each element (Schubert et al. 2002).

The last example shows that it is possible to distinguish between elemental and non-elemental visual learning. However, leaving this exception aside, despite their sophistication the visual performances presented in this section can be accounted for by elemental associations. Further research should determine to what extent visual performances, which appear complex to us, are indeed based on higher-order or on simple associations.

5.

Non-elemental visual learning

A higher level of complexity is reached when animals respond in an adaptive manner to novel stimuli *that they have never encountered before and that do not predict a specific outcome per se based on the animals' past experience*. Such a positive transfer of learning (Robertson 2001) is therefore different from elemental forms of learning, which link known stimuli or actions to specific rewards (or punishments). In the previous section, symbolic matching to sample, for instance, does not pose this kind of problem to a bee: Horizontal leads always to Blue which in turn leads always to Radial which in turn always leads to a reward (Zhang et al. 1999). In the cases considered in this section, the insects' response seems to reflect a *rule* guiding the animal's behavior. Examples are relational rules such as 'on top of' or 'larger than' which can be applied irrespective of the physical similarity of the stimuli presented or the previous 'knowledge' that the animal has of these stimuli.

5.1 Categorization of visual stimuli in honeybees

Positive transfer of learning is a distinctive characteristic of categorization performances. Visual categorization refers to the classification of visual stimuli into defined functional groups (Harnard 1987). It can be defined as the ability to group distinguishable objects or events on the basis of a common feature or set of features, and therefore to respond similarly to them (Troje et al. 1999; Delius et al. 2000; Zentall et al. 2002). Categorization deals, therefore, with the extraction of defining features from objects of the subject's environment. A typical categorization experiment trains an animal to extract the basic attributes of a category and then tests it with novel stimuli that were never encountered before and that may or may not present the attributes of the category learned. If the animal chooses the novel stimuli based on these attributes it classifies them as belonging to the category and therefore exhibits a positive transfer of learning. Categorization differs between generalizations because generalization implies a gradual decrease in responses along a perceptual dimension while categories have abrupt borders. In other words, if we are trained to search for a specific fruit of a given red color, we may respond less and less (we generalize less) to fruits whose hue varies progressively from the known red to different red hues. However, if the task we learned is to search for red objects in general and not for blue ones, we may respond equally to very different reds. We assign the different reds to the same category, even though we can distinguish them well. As long as we identify the presence of red, we respond positively. However, if the hue is changed to an extent which makes us decide that we are out of the category, we will stop responding abruptly.

According to several recent studies, free-flying honeybees trained to discriminate between different patterns and shapes are indeed able to visually categorize. For instance, van Hateren et al. (1990) trained bees to discriminate between two given gratings presented vertically and in other orientations (e. g. 45° vs. 135°) by rewarding the choice of only one of these gratings with sucrose solution. Each bee was trained with a changing succession of pairs of different gratings, one of which was always rewarded and the other not. Despite the difference in pattern quality, all the rewarded patterns had the same edge orientation and all the non- rewarded patterns had a common orientation as well (perpendicular to the rewarded one). Under these circumstances, the bees had to extract and learn the orientation common to all rewarded patterns to solve the task. This was the only cue predicting reward delivery. In the tests, bees were presented with novel patterns, which they had never been exposed to before. These patterns were all non-rewarded but had the same stripe orientations as the rewarding and non-rewarding patterns employed during the training. In such transfer tests, bees chose the appropriate orientation despite the novelty of the structural details of the stimuli. Thus, bees could categorize visual stimuli on the basis of their global orientation.

Bees can also categorize visual patterns based on their bilateral symmetry. When trained with a succession of changing patterns to discriminate bilateral symmetry from asymmetry, they learn to extract this information from very different figures and indeed transfer it to novel symmetrical and asymmetrical patterns (Giurfa et al. 1996). Similar conclusions apply to other visual features such as radial symmetry, concentric pattern organization and pattern disruption (see Benard et al. 2006for review) and even photographs belonging to a given class (e. g. radial flower, landscape, plant stem) (Zhang et al. 2004).

How could bees classify different photographs of radial flowers appropriately if these vary in color, size, outline, etc.? An explanation was provided by Stach et al. (2004) who expanded the demonstration that bees can categorize visual stimuli based on their global orientation. The authors showed that different coexisting orientations can be considered at a time, and can be integrated into a global stimulus representation that is the basis for the category (Stach et al. 2004). Thus, a radial flower would in fact be the conjunction of five or more radiating edges. Besides focusing on a single orientation, honeybees were shown to assemble different features to build a generic pattern representation, which could be used to respond appropriately to novel stimuli sharing such a basic layout. Honeybees trained with a series of complex patterns sharing a common layout comprising four edge orientations remembered these orientations simultaneously in their appropriate positions, and transferred their response to novel stimuli that preserved the trained layout (Fig. 6). Honeybees also transferred their response to patterns with fewer correct orientations, depending on their match with the trained layout. Obviously honeybees extract regularities in their visual environment and establish correspondences among correlated features such that they generate a large set of object descriptions from a finite set of elements.

This capacity can also explain the recent claim that honeybees can learn to recognize human faces (Dyer et al. 2005). Bees were rewarded with sugar water to choose a photograph of a person's face and to distinguish it from other persons' photographs. They were indeed able to do that. Does this mean that bees do recognize human faces? Not really. For the bees rewarded on the photographs these were just strange flowers. The more interesting question is which information contained in the photographs was used to recognize the correct stimulus. This question was recently tackled by a work that studied whether bees can bind the features of a face-like stimulus (two dots in the upper part as the eyes, a vertical line below as the nose, and a horizontal line in the lower part as the mouth) and recognize faces using this basic configuration (Avargues et al. 2010). Bees did indeed distinguish between different variants of the face-like stimuli, thus showing that they discriminate between these options, but they grouped the stimuli and therefore reacted similarly to faces if trained to do so. Stimuli made of the same elements (two dots, a vertical and a horizontal line) but not preserving the configuration of a face were not recognized as positive. This shows that bees learn that the rewarded stimulus consists of a series of elements arranged in a specific spatial configuration. Interestingly, the recognition of face-like stimuli by bees is flexible enough to transfer the choice towards real photographs which obviously preserve the basic configuration learned (Avargues et al. 2010). Furthermore, when trained with real faces, bees can learn to recognize novel views of a face by interpolating between or 'averaging' views they have experienced (Dyer and Vuong 2008).

In any case, honeybees show a positive transfer of learning from a trained to a novel set of stimuli, and their performances are consistent with the definition of categorization. Visual stimulus categorization is not, therefore, a privilege of certain vertebrates. At the same time this finding may not be surprising as it admits (again) an interpretation based on elemental learning. To understand this interpretation, the possible neural mechanisms underlying categorization have to be considered. If we admit that visual stimuli are categorized on the basis of specific features such as orientation, the neural implementation of category recognition could be relatively simple. The feature(s) allowing stimulus classification would then have to activate specific neuronal detectors

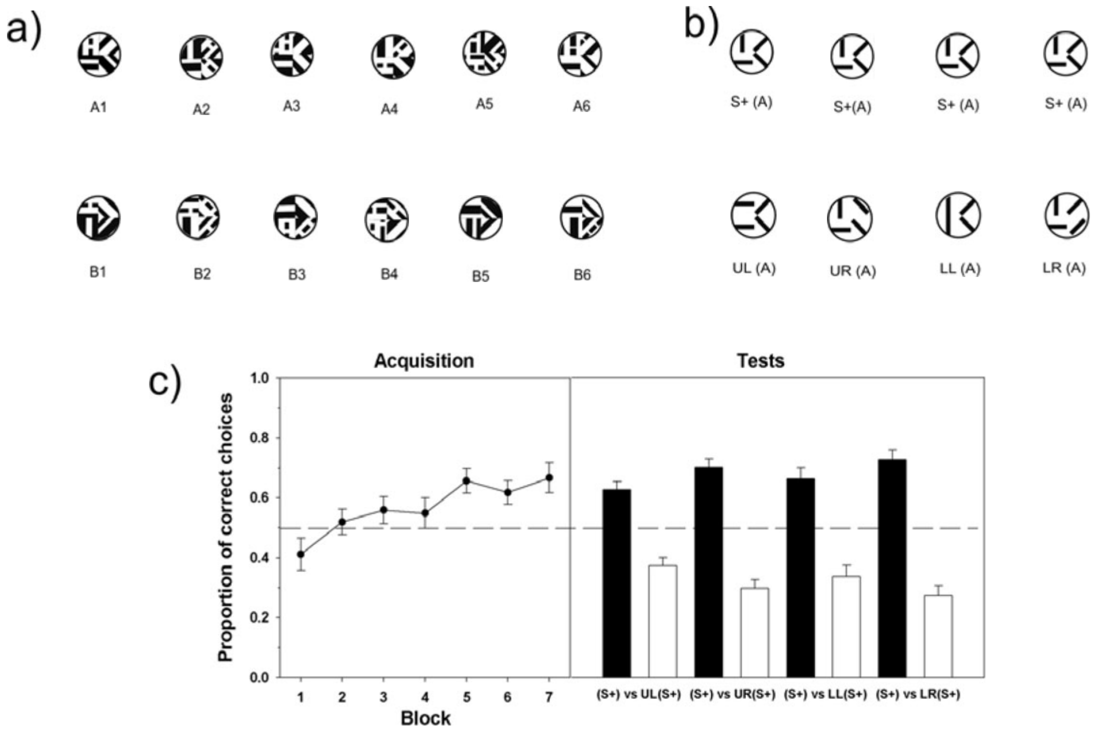

Fig. 6 Categorization of visual patterns based on sets of multiple features (adapted from Stach et al. 2004). **a** Training stimuli used in Stach et al.'s experiments (2004). Bees were trained to discriminate A from B patterns during a random succession of A vs. B patterns. A patterns (A1 to A6) differed from each other but shared a common layout of orientations in the four quadrants. B patterns (B1 to B6) shared a common layout perpendicular to that of A patterns. **b** Test stimuli used to determine whether bees extract the simplified layout of four bars from the rewarded A patterns. S+, simplified layout of the rewarded A patterns; UL, upper-left bar rotated; UR, upper-right bar rotated; LL, lower-left bar rotated; LR, lower-right bar rotated. **c** *Left panel*: acquisition curve showing the pooled performance of bees rewarded on A and B patterns. The proportion of correct choices along seven blocks of six consecutive visits is shown. Bees learned to discriminate between the rewarding patterns (A or B and improved significantly their correct choices during training. *Right panel*: proportion of correct choices in the tests with the novel patterns. Bees always preferred the simplified layout of the training patterns previously rewarded (S+) to any variant in which one bar was rotated

in the optic lobes, the visual areas of the bee brain. Examples of such feature detectors are orientation detectors whose selectivity for stimulus orientation was already characterized electrophysiologically by recording from neurons in the honeybee optic lobes (Yang and Maddess 1997). Thus, responding behaviorally to different gratings with a common orientation of, say, 60°, is simple because they all will elicit activity in the same set of neural orientation detectors despite their different structural quality. In the case of category learning, the activation of an additional neural element is needed. Such an element would be a 'reward neuron' whose activity substitutes for the sucrose reward. A neuron with these properties has been identified in the honeybee brain. It is called VUM_{mx1} (from 'ventral unpaired median' neuron located in the maxillar neuromere 1; Hammer 1993). VUM_{mx1} mediates olfactory learning in the honeybee as it contacts the olfactory circuit at its key processing stages in the brain. In other words, when an odor activates the olfactory circuit, concomitant sucrose stimulation activates VUM_{mx1} thus providing the basis for neural coincidence between odor and reward. The branching of VUM_{mx1} makes it specific for the olfactory circuit and thus for olfactory learning (Hammer 1993). Other VUM neurons whose function is still unknown are present in the bee brain (Schroter et al. 2006). One or a few of them could be the neural basis of reward in associative visual learning. Category learning could thus be reduced to the progressive reinforcement of a neural circuit relating visual-coding and reward-coding neurons to each other, similar to the behavior observed in simple associative (e. g. Pavlovian) conditioning. Even if categorization is viewed as a non-elemental learning form because it involves the positive transfer of learning, it may simply rely on elemental links between conditioned and unconditioned stimuli.

An even simpler alternative may account for the categorization task described. The mechanism just explained could be viewed as a form of supervised learning, in which a visual network is instructed by the external signal of the reinforcement neuron to respond to the right combination of features. Recent modeling work on the vertebrate visual system has shown that visual networks can learn to extract the distinctive features of a category without any kind of supervision (Masquelier et al. 2007). The model relies on the fact that neurons strongly activated fire first, a mechanism that encodes image information, and on the presence of the so-called spike timing dependent plasticity (STDP), which is a rule that concentrates high synaptic weights on afferents that systematically fire early. When a network with these properties is repeatedly presented with natural images belonging to a given category, neurons become selective to the features that are predictive of the category, while their latencies decrease. Thus, those features that are both salient and consistently present in the images are highly informative and enable robust object recognition (Masquelier et al. 2007). Testing whether similar neural mechanisms underlie object categorization in the insect visual system would be a fascinating task.

5.2 Rule learning in honeybees

In rule learning, positive transfer occurs independently of the physical nature of the stimuli considered. The animal learns relations between objects and not the objects themselves. Typical examples are the so-called rules of *sameness* and *difference*. They are demonstrated through the protocols of delayed matching to sample (DMTS) and delayed non-matching to sample (DNMTS), respectively. In DMTS, animals are presented with a sample and then with a set of stimuli, one of which is identical to the sample. Choice of this stimulus is rewarded while choice of the different stimuli is not. Since the sample is regularly changed, animals must learn the sameness rule, i. e. *'always choose what is shown to you (the sample), independent of what else is shown to you'*. In DNMTS, the animal has to learn the opposite, i. e. *'always choose the opposite of what is shown to you (the sample)'*. Honeybees foraging in a Y-maze learn both rules (Giurfa et al. 2001). They were trained in a DMTS experiment in which they were presented with a changing non-rewarded sample (i. e. one of two different color disks or one of two different black-and-white gratings, vertical or horizontal) at the entrance of a maze (Fig. 7). The bees were rewarded only if they chose the stimulus identical to the sample once within the maze. Bees trained with colors and presented in transfer tests with black-and-white gratings that they had not experienced before solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained

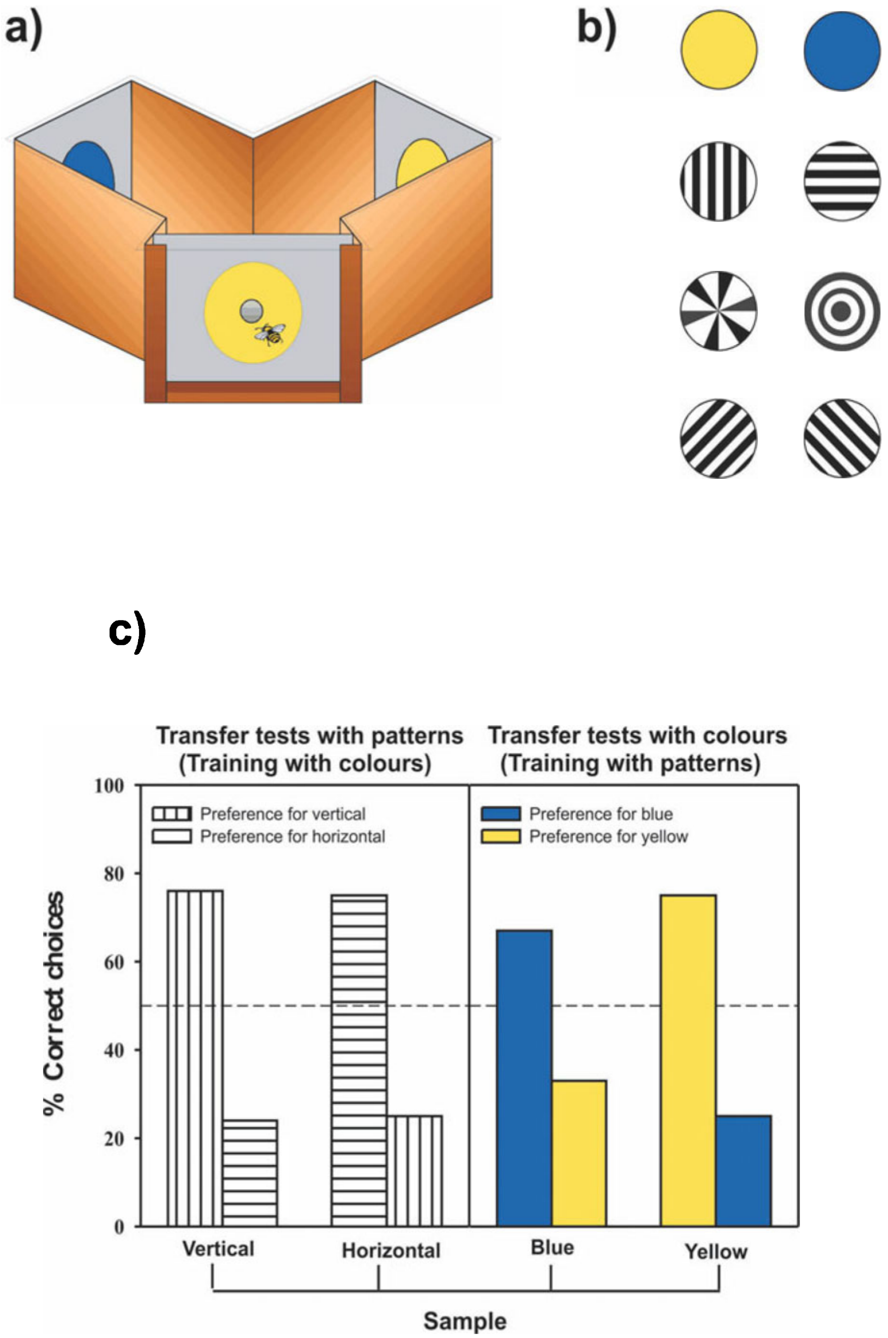

Fig. 7 Rule learning in honeybees (adapted from Giurfa et al. 2001). **a** Honeybees were trained to collect sugar solution in a Y-maze. **b** A series of different patterns was used to train a rule of sameness. A sample was shown at the maze entrance and bees had to match their choice to the sample once they entered the maze. When the bees mastered the trained discrimination, they were presented with novel simuli. **c** Transfer tests with novel stimuli. In Experiment 1, bees trained with the colors were tested with the gratings. In Experiment 2, bees trained with the gratings were tested with the colors. In both cases the bees chose the novel stimuli corresponding to the sample shown at the entrance of the maze, although they had no experience with such test stimuli

with the gratings and tested with colors in transfer tests also solved the problem and chose the novel color corresponding to that of the sample grating at the maze entrance. Transfer was not limited to different types of visual stimuli (pattern vs. color), but could also operate between drastically different sensory modalities such as olfaction and vision (Giurfa et al. 2001). Bees also mastered a DNMTS task, thus showing that they learn a rule of difference between stimuli as well (Giurfa et al. 2001). These results document that bees learn rules relating to stimuli in their environment.

The capacity of honeybees to solve a DMTS task has recently been verified and studied with respect to the working memory underlying it (Zhang et al. 2005). It was found that the working memory for the sample underlying the solving of DMTS lasts for approximately 5 s (Zhang et al. 2005). This length of time coincides with the duration of other visual and olfactory short-term memories characterized in simpler forms of associative learning in honeybees (Menzel 1999; see above). Moreover, bees trained in a DMTS task can learn to pay attention to one of two different samples presented successively in a flight tunnel (either to the first or to the second) and can transfer the learnt relevance of the sequence to novel samples (Zhang et al. 2005).

Despite the honeybees' evident capacity to solve relational problems such as the DMTS or the DNMTS tasks, such capacities are not unlimited. In some cases, biological constraints may impede the solving of a particular problem for which the extraction of a rule is necessary. It is therefore interesting to focus on a different example of rule learning which bees could not master, the *transitive inference problem* (Benard and Giurfa 2004). In this problem, animals have to learn a transitive rule, i. e. A > B, B > C, then A > C. Preference for A over C in this context can be explained as the result of two strategies: 1) deductive reasoning (Fersen et al. 1990) in which the experimental subjects construct and manipulate avlinear representation of the implicit hierarchy A > B > C; or 2) responding as a function of the effective number of reinforced and non-reinforced experiences with the stimuli (A is always reinforced while C is always non-reinforced) (Terrace and McGonigle 1994).

To determine whether bees learn a transitive rule, they were trained using five different visual stimuli A, B, C, D, and E in a multiple discrimination task: A+ vs. B–, B+ vs. C–, C+ vs. D–, D+ vs. E– (Benard and Giurfa 2004). Training involved the overlap of adjacent premise pairs (A > B, B > C, C > D, D > E), which underlie a linear hierarchy A > B > C > D > E. After training, the bees were tested by presenting B vs. D, a non-adjacent pair of stimuli that were never explicitly trained together. In theory, B and D have equivalent associative strengths because they are, in principle, equally associated with reinforcement or its absence during training. Thus, if the bees were guided by the associative strength of the stimulus, they should choose randomly between B and D. If, however, the bees used a transitive rule, they should prefer B to D. Honeybees learned the premise pairs as long as these were trained as uninterrupted, consecutive blocks of trials (Benard and Giurfa 2004). However, if shorter and interspersed blocks of trials were used, such that the bees had to master all pairs practically simultaneously, performance collapsed and the bees did not learn the premise pairs. The bees' choice was significantly influenced by their experience with the last pair of stimuli (D+ vs. E–) such that they preferred D and avoided E. In the tests, no preference for B to D was found. Although this finding agrees with the idea of an evaluation of stimuli according to their associative strength (see above), during training the bees more often visited B when it was rewarding than they visited D. Therefore a preference for B should have been expected if only the associative strength were guiding the bees' choices. From the experimental results it was concluded that bees do not establish transitive inferences between stimuli but rather guide their choices by the joint action of a recency effect (preference of the last rewarded stimulus, D) and by an evaluation of the associative strength of the stimuli (in which case preference for B should be evident). As the former supports choice of D while the latter supports choice of B, the equally frequent choice of B and of D in the tests could be explained (Benard and Giurfa 2004). In any case, memory constraints (simultaneous mastering of the different premise pairs was not possible and the predominance of the last excitatory memory over previous memories) impeded the learning of the transitive rule. Recently, Cheng and Wignall (2006) demonstrated that the failure to master several consecutive visual discrimination tasks is due to response competition occurring when animals are tested. This may explain why bees in the transitive inference protocol were unable to master the successive short blocks of training with different premise pairs.

Conclusion

Almost one hundred years of research on visual learning in bees and other social Hymenoptera, starting with Karl von Frisch's (1914) first demonstrations of color and pattern learning in bees, have yielded an impressive amount of information about how honeybees, bumblebees and wasps see the world and learn about visual cues in their environment. New discoveries in this field, inspired by the 'cognitive revolution,' which had a delayed influence on studies of insect perception and learning, have shown that besides simple forms of visual learning whose relevance can be easily conceived in the life of a bee (e. g. associating a flower color and nectar reward), social Hymenoptera also master complex forms of visual learning, ranging from conditional discriminations and observational learning to rule learning. Visual learning capabilities, therefore, allow the extraction of the logical structure of the perceptual world of insects. Such capabilities reach different levels of complexity but are not unlimited. Although the cognitive capabilities of bees and wasps may surprise due to their sophistication, limitations related to natural life seem inescapable. For instance, in the case of wasps learning facial mask patterns of conspecifics, one could imagine that memory has specific size constraints related to the small sizes of the colonies in which such wasps live. In other words, interindividual recognition is certainly possible but probably has limitations in terms of the number of individuals that can be learned and remembered. Similarly, mastering simultaneously several different associations would be facilitated if these are organized *serially or hierarchically* in chains of associations that can mediate successful navigation in a complex environment. But if these associations have to be mastered *simultaneously* at the same place, learning them would be probably difficult given the bees' biological specialization as a serial forager. In this case, learning configurations of stimuli may be more adaptive than learning each component separately.

Considering that social bees and wasps exhibit such complex forms of visual learning, the question arises, which kind of limitation do these insects present as models for the study of the mechanisms of these phenomena? So far the main limitation resides in the impossibility of addressing questions related to the cellular and molecular level. This is especially obvious when considering the achievements reached in *Drosophila*. The learning protocols used have the advantage of not restraining the animals' movements so that the behaviors recorded reflect the potential of the mini brains of social Hymenoptera. However, the protocols so far used are limiting because in a flying bee no access to the brain is so far possible. As mentioned above, new protocols in which bees learn color – reward and motion cues– reward associations under restrained conditions (Hori et al. 2006, 2007), are promising because they allow access to the neural circuits involved in these learning forms (Giurfa 2007). The critical question would then be to what extent experimental constraints limit the expression of forms of visual learning more complex than elemental associations. Why should bees and wasps continue to be attractive for research into visual cognition despite this technical limitation? The answer is simple: because the sophisticated visual performances that they exhibit, reviewed in this article, have not been found so far in the fruit fly. Future research should benefit from an analysis comparing the visual performances and mechanisms of bees and flies. The historic burden of not having a window open to the neural and molecular basis of visual learning (irrespective of the level of complexity considered), as is the case in bees, has to be overcome.

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