

Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well

Martin Giurfa

Received: 17 February 2007 / Revised: 21 April 2007 / Accepted: 22 April 2007 / Published online: 17 July 2007
© Springer-Verlag 2007

Abstract Equipped with a mini brain smaller than one cubic millimeter and containing only 950,000 neurons, honeybees could be indeed considered as having rather limited cognitive abilities. However, bees display a rich and interesting behavioral repertoire, in which learning and memory play a fundamental role in the framework of foraging activities. We focus on the question of whether adaptive behavior in honeybees exceeds simple forms of learning and whether the neural mechanisms of complex learning can be unraveled by studying the honeybee brain. Besides elemental forms of learning, in which bees learn specific and univocal links between events in their environment, bees also master different forms of non-elemental learning, including categorization, contextual learning and rule abstraction, both in the visual and in the olfactory domain. Different protocols allow accessing the neural substrates of some of these learning forms and understanding how complex problem solving can be achieved by a relatively simple neural architecture. These results underline the enormous richness of experience-dependent behavior in honeybees, its high flexibility, and the fact that it is possible to formalize and characterize in controlled laboratory protocols basic and higher-order cognitive processing using an insect as a model.

Keywords Perception · Cognition · Learning · Memory · Honeybee

This paper is dedicated to the memory of Guillermo ‘Willy’ Zaccardi (1972–2007), disciple and friend beyond time and distance, who will always be remembered with a smile.

M. Giurfa (✉)
Research Centre on Animal Cognition,
CNRS – University Paul Sabatier, 118 route de Narbonne,
31062 Toulouse cedex 9, France
e-mail: giurfa@cict.fr

Abbreviations

AL	Antennal lobe
CS	Conditioned stimulus
DMTS	Delayed matching-to-sample
DNMTS	Delayed non matching-to-sample
MB	Mushroom body
mRNA	Messenger ribonucleic acid
PER	Proboscis extension reflex
RNAi	Ribonucleic acid interference
SER	Sting extension reflex
US	Unconditioned stimulus
VUM _{mx1}	Ventral unpaired median neuron of the maxillary neuromere 1

Introduction

French naturalist Buffon (1707–1788) became famous for the 36 volumes of his Natural History, an entire life’s work where he covered subjects as diverse as the origin of the solar system, the fossilization processes, the classification of flora and fauna and the origin of Mankind. Following a peculiar vision of animal intelligence, he expressed admiration for some creatures while he fervently rejected others. Among the despised animals, an insect gathered his anger and devastating criticisms. It was neither an irritating mosquito nor a creeping cockroach. It was the honeybee. Buffon was impressed by the reproductive capabilities of a honeybee queen which “produces thirty or forty thousands flies” (bees were indistinctly called bees and flies in his works) thus constituting “the largest known multiplication in the animal kingdom”. This led him to conclude that “the most abject, vilest and smallest species are the most abundant ones” (Buffon 1749a, pp. 13–14). He went farther and argued that “it is forceful to conclude that bees, taken individually, have less genius than

a dog, a monkey and the vast majority of living animals; we shall also agree that they have less docility, less attachment and less feelings, in a word, less qualities relative to our own ones" (Buffon 1749b, pp. 93–94).

This animadversion contrasts with the admiration expressed by another famous scientist, who devoted his life to the study of honeybees. Karl von Frisch (1886–1982) became famous for the discovery of the honeybee dance, a ritualized behavior that allows a successful bee forager to inform other bees within the hive about the distance and direction of a profitable food source (Frisch 1967). This was not the only contribution made by von Frisch. He left us an amazingly rich and accurate body of evidence on honeybee behavior that spans studies on honeybee navigation, vision, olfaction, taste and magnetic sensing among others (Frisch 1967). Von Frisch liked to describe honeybees as a "magic well" for discoveries in biology because the more is drawn from it, the more is to draw. Surprisingly, this fascination ended at a particular point, in which, ironically, von Frisch could eventually join some of Buffon's ideas. He expressed his view on the plasticity underlying honeybee behavior in the following way: "*The brain of a bee is the size of a grass seed and is not made for thinking. The actions of bees are mainly governed by instinct*" (Frisch 1962, p. 78). Certainly, von Frisch expressed this view in relation to communication behavior but it is nevertheless striking that a tendency to dismiss the cognitive capacities of bees—and of insects in general—has been perpetuated throughout different centuries.

Despite this prolonged skepticism, in the last three decades honeybees have become a useful model for the study of learning and memory (Menzel and Erber 1978; Menzel et al. 1993; Menzel 1985). More recently they have also acquired a new reputation in the framework of studies addressing higher-order cognitive capacities that for long time seemed to be the exclusive patrimony of some vertebrates such as monkeys, pigeons or dolphins, which are reputed for their good learning abilities. In this review, I will analyze the contributions made by research on honeybee learning and memory that facilitated such an evolution. I will present findings and open questions that show the extent to which honeybees have increased our current understanding of cognitive processing both at the behavioral and the cellular level. I hope, in this way, to underline the power and potential of the honeybee in the framework of cognitive neurosciences.

Elemental and non-elemental forms of associative learning

Because this review intends to present the different levels of complexity that honeybees can reach in mastering different learning tasks, it is worth starting with operational defini-

tions that allow discerning the 'simple' from the 'complex'. I will focus on associative learning and introduce the distinction between elemental and non-elemental learning, which may be useful as a boundary between simple and complex forms of learning.

Associative learning is a capacity that is widespread among living animals and that allows extracting the logical structure of the world. It consists in establishing predictive relationships between contingent events in the environment so that uncertainty is reduced and adaptive behavior results from individual experience with such events. Two major forms of associative learning are usually recognized: in *classical conditioning* (Pavlov 1927), animals learn to associate an originally neutral stimulus (conditioned stimulus, CS) with a biologically relevant stimulus (unconditioned stimulus, US); in *operant conditioning* (Skinner 1938), they learn to associate their own behavior with a reinforcer. Both forms of learning allow, therefore, predicting reliably reinforcement, either appetitive or aversive, and admit different levels of complexity. In their most simple version, both rely on the establishment of elemental links connecting two specific and unambiguous events in the animal's world. What has been learned for a given tone in terms of its outcome is valid for that tone but not necessarily for another stimulus like a light. The outcome of a given behavior, like pressing a lever, is valid for that behavior but not for a different one like pulling a chain. These forms of learning, which have been intensively studied by experimental psychologists, are also particularly interesting for neuroscientists interested in the neural bases of learning because they allow tracing to the level of neural circuits and single neurons the basis of associations underlying learning. Because these forms of learning rely on specific stimuli (e.g. a given CS and a given US), it is possible to study where and how in the central nervous system such stimuli are represented, where and how their neural pathways interact in order to facilitate association and how experience modifies their respective neural representations. Both at the behavioral and neural level, these forms of learning have in common the univocal and unambiguous relationships established between events in the world. Due to the fact that they can be characterized through specific links between unique events, simple forms of associative learning are termed '*elemental learning*' forms. Typical examples of elemental learning are *absolute conditioning* (A+), in which a single stimulus A is reinforced (+), and *differential conditioning* (A+ vs. B-), in which one stimulus, A, is reinforced (+), while another stimulus, B, is non-reinforced (-) (see Table 1). In the former, an animal has to learn to respond to A, which is unambiguously associated with reinforcement; in the latter, it has to learn to respond to A and not to B because both are unambiguously associated with reinforcement and with the absence of it, respectively.

Table 1 Examples of elemental and non-elemental conditioning protocols

Conditioning task	Training	Processing
Absolute conditioning	A+	Elemental
Differential conditioning	A+ vs. B–	Elemental
Feature positive discrimination	AB+ vs. B–	Elemental
Negative patterning	A+, B+ vs. AB–	Non-elemental
Biconditional discrimination	AB+, CD+ vs. AC–, BD–	Non-elemental

Simple, unambiguous links between a stimulus and reinforcement allow solving *absolute conditioning* (A → Reward) *differential conditioning* (A → Reward; B → No Reward) and *feature-positive discrimination* (B ambiguous but A → Reward). Elemental solutions can neither account for *negative patterning* nor for *biconditional discrimination* in which each element is as often rewarded as non-rewarded

However, other forms of associative learning are possible, in which unique links connecting specific events are useless because ambiguity characterizes the events under consideration (see Table 1). For instance, in the so-called patterning problems, animals have to learn to discriminate a stimulus compound from its components, a task that is not necessarily trivial. Consider, for example, *negative patterning*, a problem in which an animal has to learn to discriminate two single components reinforced from their non-reinforced binary compound (A+, B+ vs. AB–). This situation is challenging because each element A and B appears as often reinforced as non-reinforced. Relying on elemental links between A (or B) and reinforcement (or absence of reinforcement) does not allow solving this problem. Different strategies, like treating the binary compound in a non-linear form (i.e. as being different from the simple sum of A and B) have to be implemented to solve this kind of problem. A profuse literature has shown that some vertebrates can solve this kind of non-linear processes and has put the accent on the nervous circuits and brain structures required for this kind of cognitive processing (Rudy and Sutherland 1995; O'Reilly and Rudy 2001; Bucci et al. 2002; Alvarado and Bachevalier 2005; Moses et al. 2005; Borlikova et al. 2006; Jacobs 2006).

Having introduced these two forms of learning, which define in a formalized and operational way different levels of complexity in cognitive processing, I will present findings showing that it is possible to dissect and understand basic mechanisms underlying these two levels of processing using honeybees as a model system. I will demonstrate that this insect exhibits elemental and non-elemental forms of learning which are relevant in its natural life and which are amenable to the laboratory, thus allowing controlled study and access to the underlying nervous system.

The honeybee as a model for studies on learning and memory

Several reasons justify the use of the honeybee as a model for the study of learning abilities. In a natural context and despite their small size, honeybees exhibit an extremely rich behavioral repertoire (Frisch 1967). A social lifestyle is obligatory, and a single bee cannot survive very long separated from its mates. Outside of the hive a bee travels over distances of several kilometers and visits hundreds of flowers in a quick and efficient succession for gathering food (nectar and/or pollen). It also collects resin or water, or roams for information-gathering purposes. Sensory capacities and motor performances are highly developed. Bees see the world in color (Menzel and Backhaus 1991), perceive shapes and patterns (Srinivasan 1994; Giurfa and Lehrer 2001) and resolve movements with a high temporal resolution (Srinivasan et al. 1999). Their olfactory sense is able to distinguish a large range of odors (Guerrieri et al. 2005a) and mechanosensory perception is also extremely rich due to thousands of hair cells all around the body and proprioceptors inside the body.

In a natural context, bees 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). In the case of food sources, learning and memory are the very basis of floral constancy, a behavior exhibited by foragers which consists in foraging on a unique floral species as long as it offers profitable nectar and/or pollen reward (Grant 1951; Chittka et al. 1999). Only when such an offer becomes unprofitable, bees will switch to a different species. Learning and memorizing the sensory 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. 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).

Honeybees communicate information about important locations around the hive through ritualized body movements, called the “waggle dance”, a communication system that transmits information about the vector flown toward an attractive food source or nest site (Frisch 1967). Hive bees attending such a dance decode from the speed of dance movement the distance to the food source and from the angle of the wagging phase relative to gravity the flight direction relative to the sun. In this context, specific associations are built as dance followers learn to associate the odor of nectar brought by a dancer with the nectar that it regurgitates and passes them through trophallactic contacts (Farina et al. 2005, 2006; Gil and de Marco 2005, 2006).

Usually, many such dances occur in parallel within a colony. Individual and collective decision-making result from multiple and independent decisions without reference to full knowledge of all potential options available (Seeley 1995).

The complexity and richness of the honeybee's life is therefore appealing in terms of the opportunities it offers for the study of learning and memory. Such an appeal would be, however, useless if these phenomena would not be amenable to controlled laboratory conditions. However, several protocols have been developed to allow experimental access in terms of controlled training and testing conditions, thus underlining the remarkable plasticity of this insect which can learn even under restrictive (in terms of movement, for instance) or stressful (in terms of the aversive reinforcement experienced) conditions.

Experimental access to learning and memory in honeybees

Honeybees can be easily trained individually to solve different kinds of discrimination problems (Frisch 1967). Differently to the en-masse training commonly used in other insects (e.g. *Drosophila*; Tully and Quinn 1985), which does not always allow to control the exact experience of the experimental subjects, different experimental protocols have been implemented to study learning and memory in honeybees at the individual level. Such an individual approach is important because learning and memory result from individual experience and because a neurobiological approach can be then undertaken and correlated with individual learning and memory scores only if such scores have been recorded in a precise way. Four main protocols developed to study honeybee learning and memory can be mentioned here: (1) conditioning of the approach flight towards a visual target in free-flying bees, (2) olfactory conditioning of the proboscis extension reflex in harnessed bees, (3) mechanosensory conditioning of the proboscis extension reflex in harnessed bees, and (4) olfactory conditioning of the sting extension reflex in harnessed bees. The first three protocols exploit the appetitive context of food search as in both cases bees are rewarded with sucrose solution as an equivalent of nectar. The fourth protocol represents a case of aversive learning as bees learn to associate odorants paired with the noxious reinforcement of an electric shock. In all four cases, and with different possible modifications derived from particular experimental needs, the basic experimental design comprises an acquisition or training phase in which the bees experience a particular stimulus or perform a given task that is reinforced, and a test or retrieval phase without reinforcement in which the bees are presented with the

trained situation in order to assess the memory created by training. Eventually, novel stimuli can be presented in the test together with the trained stimulus in order to study generalization and discrimination capabilities. Transfer to novel stimuli (i.e. in absence of the trained stimulus) can also be tested to characterize the flexibility of the bee's choice (see below).

Conditioning of the approach flight towards a visual target in free-flying bees

Free-flying honeybees can be conditioned to visual stimuli such as colors, shapes and patterns, depth and motion contrast, among others (Frisch 1914; Wehner 1981; Giurfa and Menzel 1997; Lehrer 1997; Giurfa and Lehrer 2001). In such a protocol, each bee is individually marked by means of a color spot on the thorax or the abdomen so that individual performances can be recorded. The marked bee is generally displaced by the experimenter towards the training/test place where it is rewarded with sucrose solution to promote its regular return (Fig. 1a). 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 reinforced with sucrose solution. As pointed out above, bees have to be trained and tested *individually* to achieve a precise control of the experience of each subject when it enters into a particular test. It is also important to control the distance at which a choice is made because orientation and choice are mediated by different visual cues at different distances or angles subtended by the target (Giurfa and Menzel 1997; Giurfa and Lehrer 2001). The time between visits to the experimental place is also an important variable to be recorded as it reflects the appetitive motivation of the bee (Núñez 1982) and thus its motivation to learn. For a food source distance of approximately 100 m from the hive, motivated bees take between 2 and 10 min between foraging bouts. Longer intervals may reflect a lower appetitive motivation and thus unreliable data.

Several behaviors can be used to quantify the bees' choice in these experiments. Touches (i.e. the 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 stimulus are usually recorded to this end. The associations built in these context can be either operant, classical or both, i.e. they may link visual stimuli (CS) and reward (US), the response of the animal (e.g. landing) and the US, or both. The experimental framework is nevertheless mainly operant as the bee's behavior is determinant for obtaining or not the sucrose reinforcement.

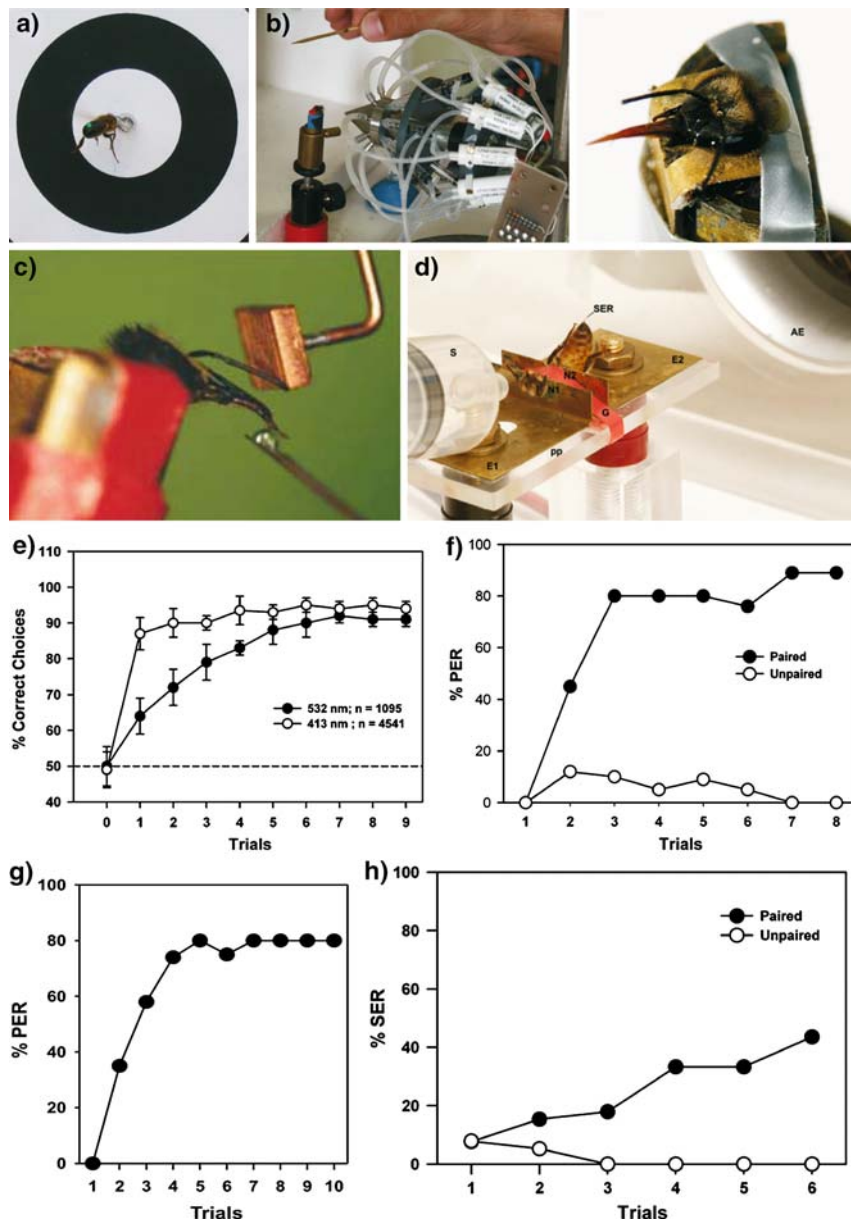


Fig. 1 Experimental protocols for the study of learning and memory in honeybees. **a** Visual appetitive conditioning of free-flying bees. A bee marked with a green spot on the abdomen is trained to collect sugar solution in the middle of a ring pattern. **b** Olfactory appetitive conditioning of harnessed bees. Left panel A bee immobilized in a metal tube faces an olfactory stimulation device controlled by a computer. The toothpick soaked in sucrose solution allows delivering reward to the antennae and mouthparts. Right panel Proboscis extension reflex (PER). **c** Tactile learning of harnessed bees (courtesy of R Scheiner). An immobilized bee contacts with its antennae a metal surface presenting a given texture and receives a reward of sucrose solution delivered to its proboscis. **d** Olfactory aversive conditioning of harnessed bees. A bee is immobilized by an elastic girdle (G) between two metal plates (E1, E2) on a Plexiglas plate (pp) through which a mild electric shock is delivered. Odorant presentation is achieved by a syringe (S) and olfactory contamination is avoided by an air extractor (AE). **e** Acquisition curves for bees trained with colors in dual-choice experiments (adapted from Fig. 4 in Menzel 1967). The curves depict the percentage of correct choices along conditioning for two wavelengths, 413 nm (human violet) and 532 nm (human green). Trial 0 constitutes a “spontaneous-choice” test in which bees freely choose between the color that will be trained and an alternative. Bees learned faster 413 nm than 532 nm. *n*: number of choices recorded. **f** Acquisition curves for bees trained to associate and odorant with sucrose solution (adapted from Fig. 2 in Bitterman et al. 1983). The curves depict the percentage of PER along conditioning trials for a group of bees trained with explicitly paired presentations of odorant and reward (‘paired’, black dots) and for another group trained with explicitly unpaired presentations of odorant and reward (‘unpaired’, white dots). Only the paired group learned the odorant-reward association thus showing that learning has an associative basis. **g** Acquisition curve in tactile learning of bees trained to associate a vertical pattern texture with sucrose solution (adapted from Fig. 3a in Erber et al. 1998). The curve depicts the percentage of PER along ten conditioning trials. **h** Acquisition curves for bees trained to associate and odorant with an electric shock (adapted from Fig. 2 in Vergoz et al. 2007). The curves depict the percentage of SER along conditioning trials for a paired group (black dots) and for an unpaired group (white dots). Only the paired group learned the odorant-reward association thus showing that learning has an associative basis

taneous-choice” test in which bees freely choose between the color that will be trained and an alternative. Bees learned faster 413 nm than 532 nm. *n*: number of choices recorded. **f** Acquisition curves for bees trained to associate and odorant with sucrose solution (adapted from Fig. 2 in Bitterman et al. 1983). The curves depict the percentage of PER along conditioning trials for a group of bees trained with explicitly paired presentations of odorant and reward (‘paired’, black dots) and for another group trained with explicitly unpaired presentations of odorant and reward (‘unpaired’, white dots). Only the paired group learned the odorant-reward association thus showing that learning has an associative basis. **g** Acquisition curve in tactile learning of bees trained to associate a vertical pattern texture with sucrose solution (adapted from Fig. 3a in Erber et al. 1998). The curve depicts the percentage of PER along ten conditioning trials. **h** Acquisition curves for bees trained to associate and odorant with an electric shock (adapted from Fig. 2 in Vergoz et al. 2007). The curves depict the percentage of SER along conditioning trials for a paired group (black dots) and for an unpaired group (white dots). Only the paired group learned the odorant-reward association thus showing that learning has an associative basis

Olfactory conditioning of the proboscis extension reflex in harnessed bees

Apart from visual stimuli, honeybees can be conditioned to olfactory stimuli (Takeda 1961; Bitterman et al. 1983). In such a protocol, each bee is restrained in an individual harness such that it can only freely move its antennae and mouth-parts (mandibles and proboscis) (Fig. 1b). The antennae are the bees' main chemosensory organs. When the antennae of a hungry bee are touched with sucrose solution, the animal reflexively extends its proboscis to reach out to and suck the sucrose (proboscis extension reflex or PER). Neutral odorants blown to the antennae do not release such a reflex in naive animals. If, however, an odorant is presented immediately before sucrose solution (forward pairing), an association is formed which enables the odorant to release the PER in a following test (Fig. 1b) This effect is clearly associative and constitutes a case of classical conditioning (Bitterman et al. 1983), i.e. the odorant can be viewed as the conditioned stimulus (CS) and the sucrose solution as the rewarding, unconditioned stimulus (US). Within this framework, bees learn to associate the odorant with the sucrose reward.

As in any learning protocol, it is important to ensure the appropriate appetitive motivation of the experimental subjects. Immobilized bees in the laboratory have therefore to be starved prior to conditioning. Two to three hours or a whole night are usually used as starvation periods in which bees have to be kept in a calm, darkened, humid environment. In olfactory PER conditioning, the response recorded is the extension of the proboscis which is a dichotomous response (1 or 0). The duration of PER can also be recorded in order to provide a continuous, instead of a discrete, measure of acquisition (Hoster and Smith 2000). To quantify learning, responses to the CS (the odorant) have to be measured *before* US delivery in each acquisition trial. Quantifying responses to the US is also important to control for the presence of the unconditioned reaction and thus for the maintenance of the appetitive motivation of the bee throughout the experiment. A useful practice is to check the integrity of PER before and after the experiment by touching the antennae with sucrose solution. Animals not exhibiting PER in these control assays should not be included in the experimental analyses as negative responses during acquisition and/or retrieval can be due to sensory-motor deficits instead of learning and/or memory deficits.

Mechanosensory conditioning of the proboscis extension reflex in harnessed bees

Different protocols of mechanosensory conditioning have been developed, which exploit the principle that harnessed honeybees can associate a mechanosensory stimulation of

their antennae or specific antennal movements with a reward of sucrose solution delivered to the proboscis. In one of these protocols, the bee is rewarded when its frequency of antennal contacts with an object (a plate close to the bee's head) exceeds a certain threshold (Kisch and Erber 1999); as a result of this operant conditioning, bees increase their frequency of antennal contacts with the reinforced object. In another protocol, bees are rewarded after scanning with their antennae the surface of a given object in order to learn its texture properties (Fig. 1c; Erber et al. 1998; Scheiner et al. 1999; Scheiner et al. 2001a); the associations established in this form of conditioning are probably both operant and classical. In a third variant, bees are rewarded whenever their antennae, left, right or both, are mechanically stimulated by the experimenter (Giurfa and Malun 2004). In this case, and contrarily to the two previous protocols, the antennal response of the bees is not crucial for obtaining the reward so that bees learn a Pavlovian association between mechanosensory stimulation and sucrose reward. The same experimental remarks apply as for olfactory conditioning of PER as the same response is measured here: PER integrity has to be measured before and after the experiment and responses to the CS (the mechanosensory stimulation) have to be measured *before* US delivery. As the mechanosensory stimulation of the antennae may be confounded with visual cues (the approach of the mechanosensory stimulus to bee head), a common procedure is to paint the eyes of the harnessed bees with black paint before conditioning. In this way undesirable visual cues are excluded from the association.

Olfactory conditioning of the sting extension reflex in harnessed bees

Contrarily to the previous protocols, this new form of conditioning (Vergoz et al 2007) offers the opportunity to study aversive instead of appetitive learning in honeybees. In this case, each bee is restrained in an individual harness such that it builds a bridge between two metallic plates through which an electric shock can be delivered (Fig. 1d). Bees stimulated in this way exhibit an unconditioned, defensive reaction which is the extension of the sting (sting extension reflex or SER) (Núñez et al. 1997). Using odorants paired with electric shocks, it is possible to condition the SER so that bees learn to extend their sting in response to the odorants previously punished (Vergoz et al. 2007). Because no appetitive responses are involved in this protocol, true aversive learning can be characterized in this way.

Contrarily to other cases of aversive learning, however, the consequence of SER conditioning is neither an avoidance response towards the stimulus (here a given odorant) predicting the noxious unconditioned stimulus (here the shock) nor the inhibition of a response. In traditional protocols of

aversive learning, individuals learn to actively avoid a noxious stimulus (e.g. fruit flies trained to associate an odorant with an electric shock in a T-maze actively avoid the arm of the maze presenting the odorant, which was negatively reinforced) (Tully and Quinn 1985) or to inhibit a response when confronted with a potentially harmful situation (e.g. mice trained to associate a given context with an electric shock exhibit a freezing response when replaced in the same context) (Bolles 1970; Fanselow 1980). In SER conditioning, bees learn to redirect an active response, stinging, towards an originally neutral stimulus that predicts shock delivery. This difference does not invalidate the term ‘aversive’ used to characterize this protocol but underlines the importance of relating responses to their biological background. In a natural context, bees facing a potential danger are not supposed to escape but to attack. In that sense the natural response to an aversive stimulus is precisely what is recorded in this protocol. Thus, independently of the fact that bees actively produce a response instead of inhibiting it, SER conditioning is a case of true aversive learning as it relies on the relevant, natural response to an aversive stimulus.

As in appetitive olfactory conditioning of PER, the experimenter controls the stimulus contingency and can therefore vary the interstimulus interval and/or the intertrial interval in order to study the impact of these variations on aversive olfactory memory. Responses recorded are also dichotomous (1 or 0) but continuous measures can be obtained by recording SER duration. To quantify learning, responses to the CS (the odorant) have to be measured *before* shock delivery in each acquisition trial. Quantifying responses to the shock is also important to control for the presence of the unconditioned reaction and thus for the aversive motivation of the bee throughout the experiment. As for PER conditioning, the integrity of SER has to be checked before and after the experiment to ensure the reliability of the data recorded.

Accessibility of the central nervous system

The brain of a honeybee has a volume of approximately 1 mm^3 and contains around 960,000 neurons (Fig. 2). Despite this apparent simplicity, the bee brain is capable of supporting learning and memory under simplified and restrictive conditions as those described above. Accessing it in order to understand how neural architecture relates to cognitive processing is therefore possible and several approaches can be employed to this end.

Although the free-flying visual conditioning protocol offers the obvious advantage of keeping the bee free and thus allows visualizing the richness of its natural learning abilities, it is not helpful to uncover the neural bases of such abilities. Appetitive and aversive olfactory PER and SER

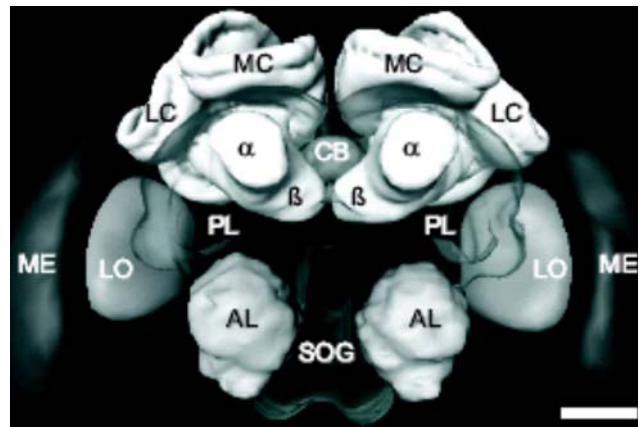


Fig. 2 Three-dimensional reconstruction of a honeybee brain in frontal view based on confocal microscopy (from Malun et al. 2002). *ME* medulla (a small portion of the medulla is shown); *LO* lobula. Visual processing occurs both in the *ME* and the *LO*. *AL* antennal lobe, the primary olfactory neuropile; *PL* protocerebral lobe (lateral horn), a higher-order center of the olfactory circuit; *SOG* suboesophageal ganglion, a region of the brain related to gustatory input; the *SOG* also controls motor outputs of the mouthparts. *CB* central body; the two prominent, lighter symmetric structures in the middle of the brain are the mushroom bodies. Each mushroom body consists of two subunits, the calyces, lateral (*LC*) and median (*MC*) that constitute the input region of the mushroom bodies. Two lobes α and β constitute their output. Bar = 200 μm

conditioning, respectively, have the advantage of being controlled learning protocols precluding the bees' movement so that they can be easily combined with physiological approaches allowing the study *in vivo* of cellular and molecular substrates of learning and memory. This is a considerable advantage offered by bees and by other invertebrate models with respect to some vertebrates, namely the possibility of an ‘on-line’ access to the nervous system of a non-anaesthetized animal while it learns and memorizes. It is possible to expose the bee brain through a small window cut in the cuticle of the head and to employ several invasive methods to study the bases of learning and memory. So far, these methods have been essentially used for the study of olfactory learning. Less is known about the neural bases of mechanosensory learning in bees, although in the different variants employed to study this learning form bees are harnessed and their nervous system accessible.

Physiological correlates of olfactory learning can be found at different levels, ranging from specific molecules that act as key signals and whose function can be manipulated through pharmacological treatments, to single identified neurons and neuronal ensembles whose activity can be visualized using electrophysiological or optophysiological techniques (Menzel 1999, 2001). Neuropharmacological approaches based on the injection or uncaging into the brain of agonists or antagonists of neurotransmitters or receptors can also be employed. Furthermore, RNAi antisense

can also be injected into the bee brain to characterize functionally the role of certain receptors and other proteins (Fiala et al. 1999; Farooqui et al. 2003).

Experimental access to the bee brain has allowed characterizing its basic architectural principles (Menzel and Giurfa 2001). It comprises (1) *dedicated neuropiles*, i.e. brain regions devoted to the processing of specific sensory information (vision, olfaction, etc.), (2) *dedicated neurons*, i.e. neurons that can be recurrently identified from bee to bee and within the same bee due to their unique morphology and because they accomplish specific functions in sensory-motor routines, and (3) *higher-order integration centers*, i.e. centers in which different sensory pathways converge such that multimodal integration takes place in them. Examples of these elements will be provided below when discussing the neural substrates of elemental and non-elemental learning in bees.

Elemental appetitive learning in bees

Having described the main protocols used for the study of learning in honeybees, I will outline in this section the findings that allowed characterizing elemental forms of learning at the behavioral and the cellular level.

Elemental color learning and memory in free-flying honeybees

The first pioneer study on honeybee learning and memory that used controlled protocols for characterizing individual acquisition and retention employed colors as rewarding stimuli (Menzel 1967). Free-flying bees were trained to choose a rewarded monochromatic light and were then presented in dual choice situations with the rewarded light vs. an alternative color. This study reported acquisition curves for different wavelengths and showed that bees learned all wavelengths after few (generally three) learning trials (Fig. 1d). Performance was independent of the alternative, non-rewarded wavelength presented in the test. Moreover, some wavelengths, particularly 413 nm, were learned faster than others, especially after a first acquisition trial (Menzel 1967). This result argued in favor of innate biases in color learning, probably reflecting the intrinsic biological relevance of the color signals that are learned faster (Menzel 1985). Indeed, color-naïve honeybees in their first foraging flight prefer those colors that experienced bees learn faster (Giurfa et al. 1995) and preliminary findings indicate that those colors could correspond to floral colors that are highly associated with a profitable nectar reward (Giurfa et al. 1995).

The experiments of Menzel (1968) allowed determining that one learning trial leads to a memory trace that fades a

few days after learning if the animal is not allowed to learn anything else during this time, while three learning trials lead to a life-long color memory. This was the very basis for discovering the existence of different memory phases in honeybees, some of which are short-term memories susceptible to interferences from additional color trials while others are long-term memories which are resistant to such interferences. Short-term memories which allow keeping memory active during shorter periods of time are dominated by non-associative processes such as sensitization. It was shown that 24 h after conditioning memory formation is not protein-synthesis dependent, thus leading to the conclusion that long-term visual memories were not dependent on such a synthesis (Wittstock and Menzel 1994). However, this conclusion is only valid for early components of long-term memory as bees in these experiments were not tested at intervals longer than 24 hours after conditioning. Results from olfactory conditioning (see below) have shown that olfactory memories older than 4 days are indeed dependent on protein-synthesis and gene transcription (Wüstenberg et al. 1998; Schwaerzel and Müller 2006). A similar dependency of color memory is expected given the parallels in dynamics between olfactory and visual memories. However, the demonstration that later components of long-term color memory do indeed depend on protein synthesis is still pending.

Color conditioning of free-flying bees was used to characterize other elemental learning phenomena such as *overshadowing*, the fact that after learning a color compound a bee responds significantly more to one color at the expense of the other (Couvillon and Bittermann 1980, 1989; Couvillon et al. 1983), and *blocking*, the fact that after learning a single color and being then trained with a color compound made of the previously rewarded color and a new color, a bee may not learn the new color despite its tight association with reward in the second training phase (Couvillon et al. 1997). Often, studies addressing these phenomena did not consider important stimulus characteristics that could bias performance such as chromatic salience and detectability. They had nevertheless the merit of underlining the fact that general principles of learning studied in vertebrates could be also found in honeybees.

In the 1980s and 1990s visual learning was mainly used as a tool to answer questions on orientation close to the goal and visual perception and discrimination. The questions raised by these works (see Lehrer 1997; Srinivasan and Zhang 1997 for reviews) focused on visual capabilities like visual spatial resolution, shape discrimination, orientation detection, movement perception and parallax, among others, and were not directly concerned by learning itself. Not surprisingly, individual acquisition curves were usually absent as learning was not the main focus of these works. Recent results have shown, however, that the visual

strategies used by bees to solve a visual discrimination may be affected by the amount of accumulated experience at the moment of a test (Giurfa et al. 2003; Stach and Giurfa 2006). Quantifying learning is therefore crucial even for the simplest visual discrimination experiment that involves training to a single visual target.

Due to obvious limitations, color learning in honeybees was never amenable to the cellular level because of the free-flying activity of the bees under study. Stages of central color processing such as color-opponent neurons (Kien and Menzel 1977; Yang et al. 2004) are known in the bee brain (Menzel and Backhaus 1991) but there is no evidence about possible interactions between the known neural elements of the color processing circuit and a reward-processing pathway. Recently, a protocol for color conditioning of the proboscis extension reflex has been proposed (Hori et al. 2006), based on pioneer findings by Kuwabara (1957). This protocol consists in training harnessed bees to extend the proboscis to color signals paired with sucrose solution.

Elemental mechanosensory learning and memory in harnessed honeybees

Because different variants of mechanosensory learning exist, I will focus here on tactile learning (Erber et al. 1998), the conditioning form which has been so far mostly studied. In tactile learning, bees learn the surface of tactile objects which are associated with a sucrose reward (Erber et al. 1998, Scheiner et al. 1999). Using such a protocol it was shown that bees distinguish between objects presented to the right or left antenna (Scheiner et al. 2001a) and that bees discriminate between different sizes of objects, different forms and different surface structures (Erber et al. 1998). Acquisition is very fast so that bees reach a plateau after few trials (Fig. 1g) and leads to different memory phases in the short-term, medium-term and long-term range (Dacher et al. 2005).

Tactile learning has been so far a tool to study questions related to genetic foraging specialization and division of labor (the fact that bees may be predetermined genetically to forage for pollen or nectar) rather than an object of study per se. Practically, no study has tackled in detail its neural bases, thus identifying the neural pathways underlying mechanosensory stimulus representation in the bee brain. Recently, the involvement of nicotinic pathways in tactile memory formation and retrieval processes was studied by injecting into the bee brain various nicotinic antagonists (Dacher et al. 2005). It was shown that nicotinic receptors are involved in tactile memory formation and retrieval. Nicotinic antagonists had different effects depending on the injection period, thus suggesting different pharmacological bases underlying different tactile memory phases. These results parallel findings in olfactory learning (see below).

Elemental olfactory learning and memory in harnessed bees

The first study on olfactory PER conditioning was performed by Takeda (1961) and was inspired by Kuwabara (1957) who reported PER conditioning using colors as CS. Olfactory PER conditioning was first used to assess olfactory discrimination capabilities in bees. These were typically trained with one rewarded odor and then tested for their choice of different odors which differed from the rewarded one in structural terms (Vareschi 1971). Even now, the protocol continues to serve to this end and has allowed characterizing a putative olfactory space for the honeybee (Guerrieri et al. 2005a). This space has been established by quantifying similarity relationships between several odorants through olfactory PER conditioning. However, the protocol turned to be the most powerful tool to characterize elemental olfactory learning after Bitterman et al. (1983) characterized it as a case of classical conditioning in which bees learn a CS (odorant)–US (sucrose solution) association. Forward pairing of the CS and the US, but not unpaired presentations of these stimuli, results in acquisition and learning of the trained odorant (Fig. 1e). The memory trace initiated by a single CS–US pairing follows a biphasic memory function similar to that found for color learning of free-flying bees. The initial high response level (< 3min) is dominated by a non-associative, sensitization component, because a single US alone also arouses the animal for a short period of time, leading to a transient increase of response to many stimuli, including the CS.

A single learning trial results in a medium-term memory that fades away after some days while three learning trials lead to a stable long-term memory that is resistant to different forms of interference. Olfactory memory and its different phases (short-term, early and late; medium-term, early and late and long-term, early and late; see Menzel 1999) have been characterized accurately in terms of their dynamics (Menzel 1999). The molecular bases of these phases are currently either known or being explored (Menzel 2001) but this subject will not be reviewed here. The fact that similar dynamics underlie color and olfactory memory can be related to the natural lifestyle of the honeybee. Indeed, olfactory memory phases correspond to the temporal dynamics of foraging activities in the field (Menzel 1999) such that early components of memory can be related to the fast succession of experiences that a bee gathers while foraging within a patch or when moving between close patches whilst medium-term memory corresponds, because of its intrinsic dynamic, to the intervals occurring between foraging bouts. Long-term memory relates to foraging bouts that are spaced in time and which may occur on different days or after long-interrupted bouts (Menzel 1999).

As in other classical (Pavlovian) protocols, olfactory memory acquired through PER conditioning is dependent on variables such as the kind of CS, US intensity (i.e. the amount and/or quality of sucrose solution received during conditioning), the number of conditioning trials and the intertrial interval (Menzel et al. 2001). Trial spacing is the dominant factor both for acquisition and retention. Generally, massed trials (i.e. trials succeeding each other in a fast sequence) lead to impaired memory performances compared to spaced trials (i.e. trials separated in time). Longer intertrial intervals lead to better acquisition and higher retention. Several studies on olfactory memory dynamics (reviewed in Menzel 1999) showed that memories in bees pass through an early consolidation phase, and that memories are fragile before consolidation is completed. Transfer from short-term memory to long-term memory via medium-term memory is not a purely sequential process but also includes parallel processes (Menzel 1999). As in color learning, olfactory short-term memories are dominated by non-associative sensitization component and long-term memory at its latest phase depends on gene transcription (Wüstenberg et al. 1998). Early long-term memory may depend also on protein synthesis, but from already available mRNA, without de novo transcription (see Schwaerzel and Müller 2006). The main conclusion arising from studies on honeybee olfactory memory (Menzel 1999, 2001) is that behavioral performance reflecting memory storage and retrieval is guided by multiple and discrete memory traces rather than by a single, continuously decaying memory trace.

Olfactory conditioning of PER allowed studying in a more controlled way associative phenomena such as overshadowing (Smith 1998), blocking (Smith and Cobey 1994; Gerber and Ullrich 1999; Hosler and Smith 2000; Guerrieri et al. 2005b) and other forms of compound conditioning (e.g. sensory preconditioning, Müller et al. 2000; backward blocking, Blaser et al. 2004). In some cases clear effects were found (e.g. overshadowing, sensory preconditioning) while in others (e.g. blocking) the responses were rather inconsistent (Guerrieri et al. 2005b).

Cellular bases of appetitive olfactory PER conditioning

Apart from behavioral studies, the significant advance yielded by olfactory PER conditioning was the possibility of tracing CS and US pathways in the honeybee brain and studying in an integrative way the neural circuits underlying elemental associative learning (Fig. 3). Odorants are processed in a neural pathway (*the CS processing pathway*) characterized by different processing stages (Fig. 3a). Olfactory perception starts at the level of the antennae where olfactory receptor neurons are located within specialized hairs called sensilla. Sensory neurons endowed with molecular olfactory receptors convey information on

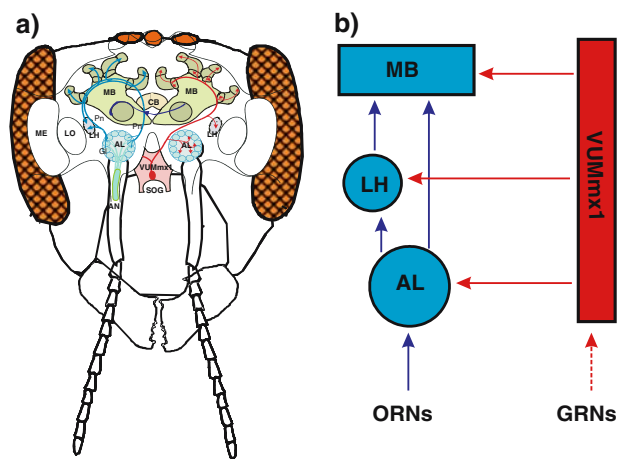


Fig. 3 CS–US associations in the honeybee brain (adapted from a scheme provided by courtesy of B. Gruenewald). **a** Scheme of a frontal view of the bee brain showing the olfactory (CS; in blue on the left) and gustatory (US, in red on the right) central pathways. *The CS pathway:* Olfactory receptor neurons send information to the brain via the antennal nerve (AN). In the antennal lobe (AL), these neurons synapse at the level of glomeruli (Gl) onto local interneurons (not shown) and projection neurons (Pn) conveying the olfactory information to higher-order centers, the lateral horn (LH) and the mushroom bodies (MB). MBs are interconnected through commissural tracts (in violet). *The US pathway:* this circuit is partially represented by the VUM_{mx1} neuron, which converges with the CS pathway at three main sites: the AL, the LH and the MB. *CB* central body, *SOG* subesophageal ganglion. **b** Scheme of the localization and distribution of CS–US associations in the bee brain. *ORNs* olfactory receptor neurons; *GRNs* gustatory receptor neurons. The dashed line between *GRNs* and VUM_{mx1} indicates that this part of the circuit is actually unknown

odorants to the antennal lobe. This paired structure is a good example of ‘dedicated neuropile’ (see above) as it is the primary olfactory centre of the bee brain. Antennal lobes are constituted by globular structures called glomeruli. Glomeruli are synaptic interaction sites between olfactory receptors, local inhibitory interneurons connecting glomeruli laterally and projection neurons conveying processed olfactory information to higher order centers such as the lateral horn or the mushroom bodies. The latter are ‘higher-order integration centers’ as they receive input from visual and mechanosensory pathways apart from the olfactory pathway.

Optophysiological studies based on recording calcium activity at the level of the antennal lobe following olfactory stimulation have shown that in naïve (i.e. non-trained) honeybees, odors are represented in terms of glomerular activity patterns (Joerges et al 1997; Galizia and Menzel 2000). Activity patterns for a given odor are symmetric between brain hemispheres and are conserved between individuals (Galizia et al. 1998, 1999). The pattern of active glomeruli ‘tells’ the brain the identity of the odorant processed. When two odorants are presented in a mixture, the glomerular

representation resembles the sum of the responses to the components or the response of the strongest component (Deisig et al. 2006). As more components are added, the picture changes and inhibitory interactions become apparent (Joerges et al. 1997; Deisig et al. 2006). This across-fiber pattern coding is maintained upstream and can be recorded both at the level of projection neurons, conveying information from the antennal lobe to higher order centers (Sachse and Galizia 2002), and of the mushroom bodies (Faber and Menzel 2001) but there are differences in odor coding between the antennal lobes and the mushroom bodies. Indeed, odors evoke combinatorial activity patterns also at the level of the calyces, the input region of the mushroom bodies, but these are significantly sparser (Szyszka et al. 2005). Moreover, the Kenyon cells, the neurons that constitute the mushroom bodies, exhibit a temporal sharpening of responses in response to odorants.

Besides coding through an across-fiber pattern, information about odor identity is also contained in the timing of action potentials in an oscillatory population response (Laurent et al. 1996). More specifically, each odorant is encoded through a specific oscillatory synchronization of assemblies of projection neurons. This temporal coding strategy is employed by the olfactory circuit of the locust (Wehr and Laurent 1996) and seems also to be present in the honeybee (Stopfer et al. 1997). In honeybees, the oscillatory synchronization of assemblies of projection neurons is abolished by picrotoxin, an antagonist of the GABA_A (γ -aminobutyric acid) receptor (Stopfer et al. 1997). After this treatment, honeybees trained using the olfactory conditioning of PER are impaired for discriminating the trained odorant from a molecularly similar odorant, but not from a dissimilar odorant. It was, therefore, suggested, that oscillatory synchronization and the kind of temporal encoding it affords provide an additional dimension by which the brain could segment spatially overlapping stimulus representations (Stopfer et al. 1997). These results are appealing as spatial (across-fiber pattern) and temporal (oscillatory synchrony) might coexist in the nervous system and could encode different odorant properties or be engaged for resolving different olfactory demands.

Although some parts of the CS pathway are still superficially characterized (for instance, the lateral horn), and although we only start to understand dynamic aspects of odorant processing in several of the stages of this pathway (Szyszka et al. 2005), an integrative view of the CS circuit is already available. How does learning modify neural activity in this circuit? Faber et al. (1999) found that olfactory differential conditioning induces an increase in the intensity of the glomerular activation pattern for a rewarded odorant but not a qualitative change in its global nature. The non-rewarded odorant induced no change in glomerular activity. This conclusion has been recently challenged

by experiments that showed no change in activity in a sub-population of uniglomerular output neurons (projection neurons) after different elemental olfactory conditioning protocols (Peele et al. 2006). How olfactory representations are modified by associative learning has therefore still to be determined. In doing this, changes at the different stages of the olfactory pathway have to be studied at different intervals post conditioning. Moreover, the effect of different conditioning protocols of varying complexity should also be considered as a possible determinant of the olfactory representation achieved.

In the case of the *US processing pathway*, our knowledge is only partial, at least in neuroanatomical terms, as it is so far restricted to a unique neuron which is necessary and sufficient to substitute for the sucrose reward in the honeybee brain. This neuron, which constitutes a good example of ‘dedicated neuron’ (see above) is called VUM_{mx1} (abbreviation for “ventral unpaired median neuron of the maxillary neuromere 1”) and responds with long-lasting spike activity to sucrose solution delivered both at the antennae and the proboscis (Hammer 1993). The dendrites of VUM_{mx1} arborize symmetrically in the brain and converge with the olfactory pathway at three sites: the antennal lobes, the calyces, which are the olfactory input areas of the mushroom bodies, and the lateral horns (Fig. 3a). Such a convergence is particularly remarkable in the case of a neuron coding for sucrose solution as it provides the structural basis for CS–US associations. That VUM_{mx1} constitutes indeed the neural representation of the US in olfactory PER conditioning was shown through an elegant substitution experiment performed by Hammer (1993). He showed that behavioral learning of an olfactory stimulus can be induced by substituting the sucrose reward in PER conditioning by an artificial depolarization of VUM_{mx1} immediately after olfactory stimulation (forward pairing). If depolarization, and thus spike activity, precedes olfactory stimulation (backward pairing), no learning was observed. The same forward-backward effect was seen when sucrose was used as the reward under similar experimental conditions. These results thus showed that VUM_{mx1} activity constitutes the neural correlate of the US in associative olfactory learning.

This conclusion was reaffirmed by neuropharmacological experiments aimed at discovering the neurotransmitter acting as appetitive reinforcement in olfactory PER conditioning. As VUM_{mx1} belongs to a group of octopamine-immunoreactive neurons (Kreissl et al. 1994), it was hypothesized that octopamine, a biogenic amine usually associated with increased levels of arousal and behavioral facilitation in invertebrates (Libersat and Pflüger 2004; Huber 2005), acts as the neurotransmitter necessary and sufficient to substitute for the sucrose reward (Hammer and Menzel 1998). Indeed, pairing an odorant with injections of

octopamine as a substitute for sucrose into the mushroom bodies or the antennal lobes (but not the lateral horn) lobe produced a lasting, learning-dependent enhancement of proboscis extension (Hammer and Menzel 1998). Thus, octopamine signaling via VUM_{mx1} is sufficient to substitute for sugar reinforcement in honeybees. This conclusion was confirmed by silencing octopaminergic receptor expression in the honeybee antennal lobe using double-stranded RNA (Farooqui et al. 2003). This treatment inhibited olfactory acquisition and recall, but did not disrupt odorant discrimination. This result underlines the fact that appetitive reinforcer function in the invertebrate brain is subserved by specific neurons and associated biogenic amines (here octopamine), which act as value systems in associative learning phenomena, i.e. as systems allowing ordering, prioritizing and assigning ‘good’ or ‘bad’ labels to odorants (Giurfa 2006).

A more integrative view of the US pathway is still missing. Although gustatory receptor neurons tuned to sucrose have been located on specialized sensilla on the antennae, mouth parts and tarsi (Whitehead and Larsen 1976; Whitehead 1978; Haupt 2004; de Brito Sanchez et al. 2005), less is known about the circuit allowing these receptors to convey US information to the central level, more specifically to VUM_{mx1} . This circuit is probably localized in the subesophageal ganglion, which is the first synaptic relay in the gustatory pathway (Altman and Kien 1987). Despite this incomplete view of the US pathway, an important principle of classical conditioning was verified by studying VUM_{mx1} activity, namely *stimulus substitution*. Classical conditioning relies on the fact that a CS acquires the capacity of replacing the US as it becomes a reliable predictor of reinforcement. This was evident in recordings of VUM_{mx1} activity after olfactory conditioning (Hammer 1993). After training a bee to discriminate a rewarded (CS1) from a non-rewarded odorant (CS2), it was found that VUM_{mx1} fired to CS1 and not to CS2 (Hammer 1993). Thus, CS1, the odorant that reliably predicted the US, acquired the capacity of activating VUM_{mx1} . At the same time, VUM_{mx1} continues to respond to the US when it is presented unexpectedly, i.e. not preceded by a predictive odorant (Menzel and Giurfa 2001). Thus, the VUM_{mx1} neuron has the characteristic properties of a system that provides reinforcement prediction error information that is critical to associative learning (e.g. Schultz and Dickinson 2000). In other words, it provides information on the discrepancy between the expected and delivered values of a reinforcing event (the prediction error), which determines the effective reinforcement value of that event (Rescorla and Wagner 1972). How and where does VUM_{mx1} get information from the olfactory pathway in order to be able to respond to an odorant after successful pairing with sucrose? So far, the answer to this question remains unknown.

The picture emerging from these and other studies is one in which elemental, associative, olfactory learning can be accurately characterized both at the behavioral and cellular levels in order to understand the mechanisms of this simple form of learning. The honeybee offers the unique chance of dissecting elemental olfactory learning and identifying its building blocks. From this dissection it appears that learning relies on distributed, but localized interactions between CS and US pathways throughout the brain (Fig. 3b). Distribution is reflected by the fact that interactions between these pathways occur in at least three different regions of the brain, the antennal lobes, the mushroom bodies and the lateral horns (see Fig. 3b). Localization is reflected by the fact that these interactions are spatially delimited. Redundancy could also be enounced as a principle due to the repetition of synaptic interactions between CS and US pathways, but so far it is unknown whether the interactions occurring at one of these three sites are equivalent to those occurring in another site, i.e. whether different memory contents are formed and stored in the antennal lobes, mushroom bodies and lateral horns. Instead, it appears that these different brain structures intervene in different forms of learning (see below) so that the concept of redundancy would not be appropriated.

Elemental aversive learning in bees

The previous sections underline that, for almost a century, research on honeybees has made significant contributions to our general understanding of learning and memory but that such an understanding is restricted to appetitive learning. As mentioned above, olfactory conditioning of PER has been used for 45 years (beginning in 1961; Takeda 1961) as the unique tool to access the neural and molecular bases of learning in honeybees.

Recently, aversive learning could be studied in honeybees in such a way that both behavioral and neural levels were made accessible to experimentation (Vergoz et al. 2007). Pairing an odorant with an electric shock resulted in associative learning in which bees learned to extend their sting (sting extension reflex or SER) in response to the odorant previously punished (Fig. 1f). They could also learn to master simultaneously appetitive and aversive associations and exhibited the appropriate response, PER or SER, to the appropriate odorant. Moreover, neuropharmacological experiments addressed the question of modularity of appetitive and aversive learning and the possible dependency of this modularity on two different biogenic amines subserving appetitive and aversive reinforcement. Indeed, while octopamine has been shown to substitute for appetitive reinforcement (Hammer and Menzel 1998; see above), it was found that blocking of dopaminergic, but not

octopaminergic, receptors suppresses aversive olfactory learning (Vergoz et al. 2007). Thus, octopamine and dopamine subserve appetitive and aversive reinforcement in the honeybee, respectively. Again, this finding underlines the conclusion that dedicated biogenic amines act as value systems in the invertebrate brain and that they fulfill different reinforcing roles in different forms of learning.

This finding brought the honeybee closer to other insect models such as the fruit fly *Drosophila melanogaster* and the cricket *Gryllus bimaculatus*. In crickets, pharmacological experiments showed that octopamine and dopamine subserve the appetitive and aversive reinforcing functions, respectively (Unoki et al. 2005). In fruit flies, the same result was previously found using mutants with inactivated dopaminergic or octopaminergic neurons (Schwaerzel et al. 2003). Recently Schroll et al (2006) showed that octopamine and dopamine are necessary and sufficient to substitute for appetitive and aversive reinforcement in *Drosophila* larvae. Furthermore, neurons capable of mediating and predicting aversive reinforcement have been found in the *Drosophila* brain (Riemensperger et al. 2005). These neurons may be a general feature of the insect brain and dopamine may underlie other forms of aversive learning involving stimuli of different sensory modalities (e.g. visual stimuli associated with aversive gustatory reinforcements; Unoki et al. 2006). Interestingly, dopaminergic neurons in the fly brain exhibit the same functional principle as the VUM_{mx1} neuron in the bee brain, namely stimulus substitution. Here too, dopaminergic neurons did not respond to the odorant used for aversive conditioning before conditioning and acquired this property after conditioning (Riemensperger et al. 2005). Similarly to the VUM_{mx1} neuron, dopaminergic neurons in the fly brain provide, therefore, reinforcement prediction error information that is critical to associative learning.

The study of aversive learning in honeybees is just starting. As this form of conditioning uses odorants as CS, it is possible to ask how odorant representations are modified by aversive experiences compared to appetitive experiences. An important goal will be to identify dopaminergic neurons in the bee brain whose morphology and functional properties make them candidates for integrating the aversive US pathway. Furthermore, characterizing aversive olfactory memory both at the cellular and molecular levels is crucial for a comparative analysis of appetitive and aversive learning and memory in bees, which can now be performed.

Non-elemental learning in bees

Elemental appetitive and aversive learning, as discussed above, rely on the establishment of elemental associative links connecting two specific and unambiguous events in

the bee's world. What has been learned for a given color in terms of its outcome is valid for that color but not for a different one. The sucrose reward that follows a given behavior, like contacting a given surface with the left antenna, is valid for that behavior but not for a different one like contacting the same surface with the right antenna. However, in the forms of associative learning that we will discuss here, unique links connecting specific events are not useful because the events under consideration are ambiguous in terms of their outcome. A typical case introduced above is negative patterning in which the subject needs to learn to discriminate a non-reinforced compound from its components (A+, B+, AB−). This problem does not admit elemental solutions because the animal has to learn that AB is necessarily different from the linear sum of A and B. In *biconditional discrimination*, the subject has to learn to respond to the compounds AB and CD and not to the compounds AC and BD (AB+, CD+, AC−, BD−). As in negative patterning, each element, A, B, C and D, appears as often reinforced as non-reinforced so that it is impossible to rely on the associative strength of a given stimulus to solve the task. These examples show that more elaborated computational strategies are necessary in the case of non-elemental discrimination problems.

Treating compound stimuli as entities different from the simple sum of their components (e.g. $AB = X \neq A + B$) constitutes the basis of *the configural learning theory* proposed to account for the solving of these non-linear discrimination problems (Pearce 1994). For this account, animals trained with AB can respond to A or B only to a low extent. Another theory, the *unique-cue theory*, proposes that a mixture is processed as the linear sum of its components plus a stimulus (u) that is unique to the joint presentation of the elements in the mixture (e.g. $AB = A + B + u$) (Whitlow and Wagner 1972). The unique cue is what gives a unique signature to a compound and which differentiates it from the linear sum of its components. For this account, animals trained with AB can respond to A or B to a relatively high extent.

Probably because of their inherent complexity, these problems have been rarely studied in invertebrates. However, several recent studies have addressed the issue of elemental vs. non-elemental learning in honeybees, using visual conditioning of free-flying animals and olfactory PER conditioning (Giurfa 2003). In both experimental protocols, bees were shown to solve biconditional *discrimination* (AB+, CD+, AC−, BD−). In the visual modality, free flying bees had to discriminate complex patterns that were arranged to fulfill the principles of this discrimination problem (Schubert et al. 2005). In the olfactory modality, olfactory compounds were used (Hellstern et al. 1995; Chandra and Smith 1998) and bees learned to respond appropriately to each compound, independently of the ambiguity inherent

to the components. This capacity demonstrates that under certain circumstances both visual and olfactory compounds are learned as entities different from the simple sum of their components.

This conclusion is underlined by studies showing that bees can solve a *negative patterning* discrimination (A+, B+, AB−) both in the visual (Schubert et al. 2005) and the olfactory modality (Deisig et al. 2001, 2002, 2003). Solving this problem is possible if the compound AB is treated as being different from the simple sum of its elements. In the case of olfactory compound learning, experiments were conceived to discern between the two non-elemental theories mentioned above, the configural and the unique-cue theory. It was shown (Deisig et al. 2003) that the bees' performance was consistent with the unique cue theory, i.e. when bees perceive an olfactory compound they detect the presence of the components in it but they also assign a unique identity to the compound, resulting from the interaction of its components.

Another study used an original protocol, the side-specific olfactory PER conditioning, which posed a non-linear discrimination problem (Sandoz and Menzel 2001). In this case, a thin plastic wall separates the honeybee's antennae during olfactory stimulation (Fig. 4a). Bees were differentially conditioned using two odors (A and B). When odorants were delivered to one antenna, the contingency was A+ vs. B− while it was reversed (A− vs. B+) when they were delivered to the other antenna. This discrimination resembles a form of contextual learning as the context of each antennal side (left vs. right) determines the contingency of the stimuli. Bees learned to respond appropriately to the rewarded odor and to inhibit their reaction to the non-rewarded odor on each side (Sandoz and Menzel 2001). They thus solved this side-specific, non-elemental discrimination and remembered the contingencies learned 24 h later (Fig. 4b). In this case, insight into the neural bases of such a non-elemental problem solving was obtained by combining this protocol with *in vivo* calcium imaging recordings of glomerular activity at the level of *both* antennal lobes (Fig. 4c). It was found that in naïve bees, odor response patterns were highly symmetrical, i.e. before conditioning, the same odorant elicited the same activation pattern in both antennal lobes. In conditioned bees, topical differences between sides were found. After side-specific conditioning, the left and right representations of the same odorant became slightly different, thus allowing differentiation between sides (Sandoz et al. 2003). Thus, this form of non-elemental learning resulted in a decorrelation of the representations of the conditioning odors between sides (Fig. 4d). This result emphasizes the fact that bees may form odor/side associations of the type AS1+/AS2− and BS1−/BS2+ (S1: side 1/S2: side 2). It is thus conceivable that structures situated upstream the antennal lobes (e.g. the

mushroom bodies or the lateral horn) are crucial for decoding differences in neural representations such as those generated in side-specific conditioning.

Komischke et al. (2003) showed that bilateral olfactory input is required for solving a negative patterning discrimination. Given that the olfactory circuit remains practically unconnected between hemispheres until the mushroom bodies, this result suggests that the reading of a unique cue, arising from odorant interaction within the mixture, occurs upstream the antennal lobes, i.e. at the level of the mushroom bodies. Komischke et al. (2005) used mushroom body-ablated honeybees to determine whether intact mushroom bodies are necessary to solve non-elemental olfactory discriminations. Bees were treated with hydroxyurea, which suppresses partially or totally the calyces (the input region to the mushroom bodies) (Malun 1998). In previous works, Scheiner et al. (2001b) and Malun et al. (2002) showed that such ablations do not affect elemental forms of learning. Scheiner et al. (2001b) showed that tactile learning, a form of elemental learning in which bees learn to associate an object within the range of one antenna with sucrose solution, and discriminate it from an object presented to the opposite side, was unaffected in ablated bees. Malun et al. (2002) studied olfactory learning and showed that the presence of ablations did not impair acquisition of an elemental olfactory discrimination in which one odor was rewarded and another odor was non-rewarded (A+ versus B−).

In the experiments of Komischke et al. (2005), bees with unilateral lesions of the mushroom bodies (a median calyx was usually absent) were trained in different olfactory discrimination problems. When odorants were delivered in a side-specific manner, bees with mushroom body lesions could not solve an unambiguous double discrimination (Problem 1: A+ vs. B− on one antenna, C+ vs. D− on the other; A+B−/C+D−) despite the fact that each of the four odorants had an unambiguous outcome. When confronted with the ambiguous side-specific discrimination (Problem 2: A+ vs. B− on one antenna, A− vs. B+ on the other; see above and Fig. 4), bees were also impaired because they could only learn the discrimination proposed to their intact brain side. Non-ablated bees could master both side-specific discriminations. When odorants were delivered simultaneously to *both* antennae (Problem 3: A+B−C+D−), ablated bees learned slower than normal bees.

Thus, in all three cases, the unilateral loss of a median calyx affected olfactory learning (Komischke et al. 2005). It was proposed that mushroom bodies are required for solving non-elemental discriminations but also elemental tasks whose complexity is enhanced by virtue of the number of stimuli involved (Problems 1 and 3: 4 stimuli). To solve an A+B−/A−B+ discrimination, information exchange between brain hemispheres has to be inhibited such that A on the right side is not generalized to A on the left side and

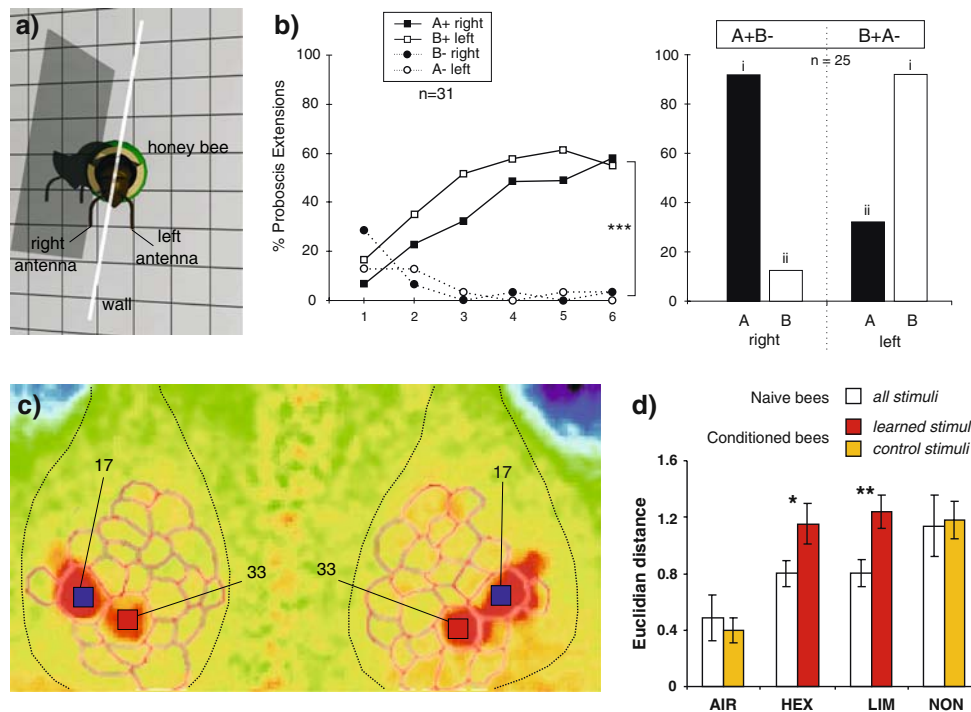


Fig. 4 Side-specific olfactory conditioning of harnessed bees (adapted from Sandoz and Menzel 2001 and Sandoz et al. 2003; courtesy of J.C. Sandoz). **a** A harnessed bee is conditioned to discriminate two odors A and B. When odors are delivered to the right antenna, the contingency is A+ versus B−, while it is A− versus B+ if they are delivered to the left antenna. Left and right antennae are separated by a thin wall glued along the bee body. **b** The acquisition curves display the responses (% PER) to the rewarded odors (A+ right and B+ left) and to the non-rewarded odors (B− right and A− left); the bars show the retention performances of the same bees when tested with the same odors on the left and right side 24 h after the training. *n*: number of bees trained or tested. **c** Simultaneous calcium imaging recording of

both antennal lobes (delimited by the dashed lines). The response of naïve (non-trained) bees to Nonanol is shown. Glomeruli 17 and 33 are activated by this odor and this activation is symmetric between sides. **d** The effect of side-specific conditioning on odor representation. The perceptual distance between the left and right representations of the same odorant was measured in a putative olfactory space, calculated for the honeybee. For Hexanol and Limonene, the two odors used in the side-specific conditioning, the distance between left and right representations increased significantly as a consequence of training (*red bars*) thus showing that left and right representations of the same odorant became different. For a control odorant, Nonanol, and for a clean-air control, the responses on the right and left antennal lobes were the same

vice versa. Mushroom body ablations could have an effect on this inhibition; in normal bees conditioning would result in inhibition of interhemispheric transfer. Ablations would restore the transfer from the intact side thus creating confusion on the ablated side.

Cumulative experience seems to play a critical role for adopting elemental or non-elemental learning strategies (Giurfa et al. 2003). When free-flying bees are trained to fly into a Y-maze to collect sucrose solution on a rewarded visual target presented in one of the arms of the maze, the strategy underlying the choice of visual compounds changes along training. Bees were trained with color stimuli that were color disks violet (V), green (G) or yellow (Y) and which were of equal salience for honeybees. Training followed an A+, BC+ design, followed by an AC vs. BC test. Training consisted of 6 (3 A+ and 3 BC+), 20 (10 A+ and 10 BC+) or 40 (20 A+ and 20 BC+) acquisition trials, thus increasing the amount of experience on the same problem. Elemental models of compound processing predict that in the test (AC vs. BC), a preference for the non-trained

stimulus AC should occur while configural models predict a preference for the trained stimulus BC (Giurfa et al. 2003). After six training trials, bees favored an elemental strategy and preferred AC to BC during the tests. Increasing the number of training trials resulted in an increase of the choice of BC. Thus, short training favored processing the compound as the sum of its elements (elemental theory) while long training favored its processing as being different from the sum of its elements (configural theory). It was also observed that the change in stimulus processing was influenced by stimulus similarity. Color similarity favored configural processing with increasing experience (Giurfa et al. 2003), a result that was consistent with the results of honeybee olfactory compound conditioning (Deisig et al. 2002). Further factors favoring non-elemental compound processing and learning could be the spatial and temporal proximity of elements and the animals' previous experience.

There is, however, a limitation in these studies that has to be overcome in future research, namely that all compound stimuli used were of the same modality, either visual

or olfactory. It would be important to verify that similar rules apply for intermodal compounds. This goal is particularly important in the framework of searching for the neural substrates of non-elemental learning forms. If indeed mushroom bodies, which are multimodal sensory integration structures, are an important center for achieving non-linear processing, affecting their normal function could have more dramatic consequences in the case of bimodal than in unimodal compounds. In studying non-elemental learning with stimuli of different modalities, one should guarantee comparable salencies between stimuli as differences at this level may lead to overshadowing or blocking.

Positive transfer of learning in honeybees

In this section, I will focus on problem solving in which 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 reinforcers. In the cases considered in this section, the response can attain levels in which it becomes independent of the physical nature of the stimuli presented so that it acts as a *rule* guiding the animal's behavior (like, for instance, relational rules such as 'on top of' or 'larger than' which can be applied irrespectively of the similarity of the stimuli considered).

Categorization of visual stimuli

Positive transfer of learning is a distinctive characteristic of categorization performances. Categorization refers to the classification of perceptual input 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 these 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 present or not 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 exhibits therefore positive transfer of learning.

Using this basic design in which procedural modifications can be introduced, several studies have shown recently the ability of visual categorization in free-flying honeybees trained to discriminate different patterns and shapes. For instance, van Hateren et al. (1990) trained bees

to discriminate two given gratings presented vertically and differently oriented (e.g. 45° vs. 135°) by rewarding one of these gratings with sucrose solution and the other not. 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 also a common orientation, perpendicular to the rewarded one. Under these circumstances, the bees had to extract and learn the orientation that was 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 were never exposed to before, which were all non-rewarded, but which exhibited 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.

They 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 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. 2006 for review) and even photographs belonging to a given class (e.g. radial flower, landscape, plant stem) (Zhang et al. 2004).

How could bees classify appropriately different photographs of radial flowers 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 to show that different coexisting orientations can be considered at a time, and integrated in a global stimulus representation that is the basis for the category (Stach et al. 2004). Thus, a radial flower would be, in fact, 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. 5). Honeybees also transferred their response to patterns with fewer correct orientations, depending on their match with the trained layout. These results show that honeybees extract regularities in their visual environment and establish

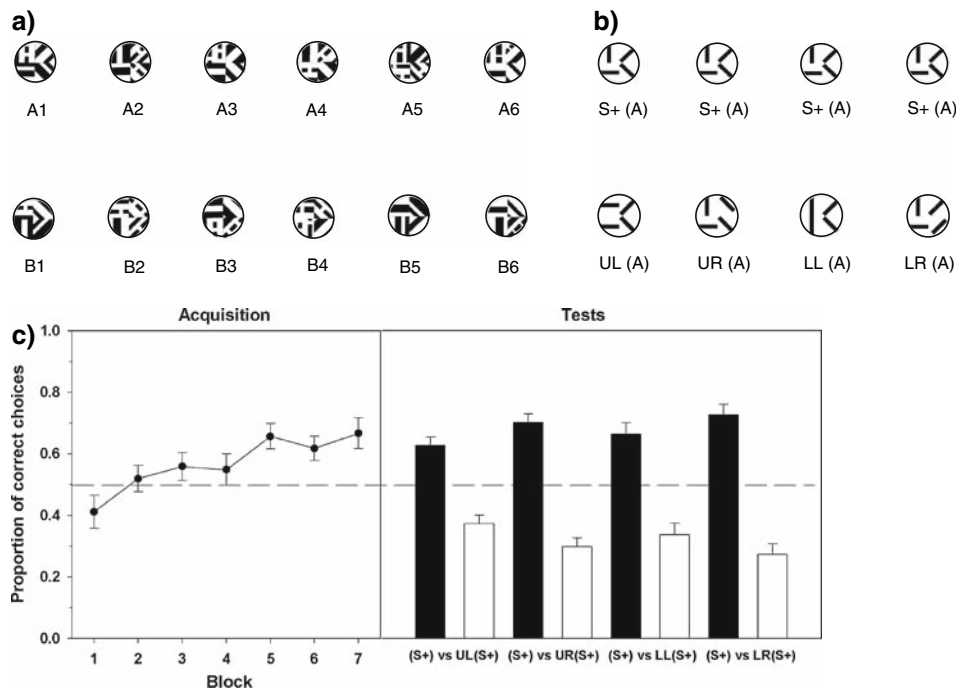


Fig. 5 Categorization of visual patterns based on sets of multiple features (adapted from Stach et al. 2004). **a** Training stimuli used in Stach et al.’s experiments (2004). Bees were trained to discriminate A from B patterns during a random succession of A vs. B patterns. A patterns (A1 to A6) differed 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 if 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 the rewarding patterns (A or B) and improved significantly their correct choices along 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

correspondences among correlated features such that they generate a large set of object descriptions from a finite set of elements.

Thus, honeybees show 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 prerogative of certain vertebrates. However, this result might not be surprising as it admits an elemental learning interpretation. To explain this interpretation, the possible neural mechanisms underlying categorization should 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 activate specific neuronal detectors in the optic lobes, the visual areas of the bee brain. Examples of such feature detectors are the orientation detectors whose orientation and tuning have been already characterized by means of electrophysiological recordings in the honeybee optic lobes (Yang and Maddess 1997). Thus, responding to different gratings having a common orientation of, say, 60°, is simple as all these gratings will elicit the same neural activation in the same

set of orientation detectors despite their different structural quality. In the case of category learning, the activation of an additional neural element is needed. Such element would be a reinforcement neuron equivalent to VUM_{mx1} (Hammer 1993; see above) but contacting the visual circuits at its relevant processing stages. Other VUM neurons whose function is still unknown are present in the bee brain (Schroter et al. 2007). It could be conceived that one of them (or more than one) acts as the neural basis of reinforcement in associative visual learning. Category learning could be thus reduced to the progressive reinforcement of an associative neural circuit relating visual-coding and reinforcement-coding neurons, similar to that underlying simple associative (e.g. Pavlovian) conditioning. From this perspective, even if categorization is viewed as a non-elemental learning form because it involves positive transfer of learning, it may simply rely on elemental links between conditioned and unconditioned stimuli.

Rule learning

This argument is not applicable to rule learning where positive transfer occurs independently of the physical natural of

the stimuli considered. In this case, the animal learns relations between objects and not the objects themselves. Typical examples are the so-called rules of *sameness* and of *difference*. These rules 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 and which is reinforced. 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). Bees were trained in a DMTS problem 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. 6). 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 have not experienced before solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained 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 kinds of modalities (pattern vs. color) within the visual domain, but could also operate between drastically different domains such as olfaction and vision (Giurfa et al. 2001). Furthermore, bees also mastered a DNMTS task, thus showing that they also learn a rule of difference between stimuli (Giurfa et al. 2001). These results document that bees learn rules relating 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. 2004, 2005). It was found that the working memory for the sample underlying the solving of DMTS lasts approximately 5 s (Zhang et al. 2005) and thus 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 learning of this sequence weight 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

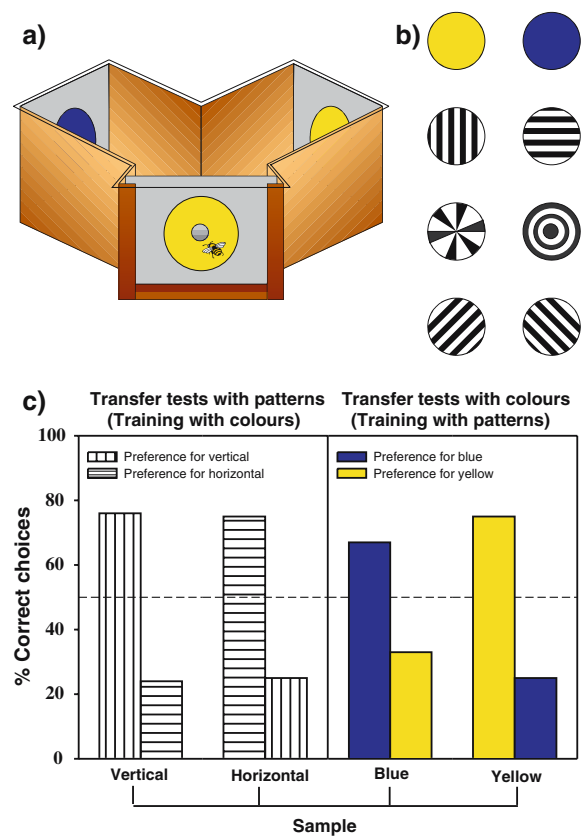


Fig. 6 Rule learning in honeybees (adapted from Giurfa et al. 2001). Honeybees trained to collect sugar solution in a Y-maze (a) on a series of different patterns (b) learn a rule of sameness. Learning and transfer performance of bees in a delayed matching-to-sample task in which they were trained to colors (Experiment 1) or to black-and-white, vertical and horizontal gratings (Experiment 2). c Transfer tests with novel stimuli. In Experiment 1 (left panel), bees trained on the colors were tested on the gratings. In Experiment 2 (right panel), bees trained on the gratings were tested on the colors. In both cases bees chose the novel stimuli corresponding to the sample although they had no experience with such test stimuli

for which rule extraction 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 by two strategies: (1) deductive reasoning (Fersen et al. 1990) in which the experimental subjects construct and manipulate a unitary and linear representation of the implicit hierarchy $A > B > C$; or (2) responding as a function of reinforced and not reinforced experiences (Terrace and McGonigle 1994), in which case animals choose among stimuli based on their associative strength, i.e. on the effective number of reinforced and non-reinforced experiences with the stimuli (A is always reinforced while C is always non-reinforced).

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 overlapping of adjacent premise pairs ($A > B$, $B > C$, $C > D$, $D > E$), which underlie a linear hierarchy $A > B > C > D > E$. After training, bees were tested with 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 absence of it during training. Thus, if bees were guided by the stimulus' associative strength, they should choose randomly between B and D. If, however, 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). But if shorter and interspersed blocks of trials were used, such that bees had to master all pairs practically simultaneously, performance collapsed and 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 result agrees with an evaluation of stimuli in terms of their associative strength (see above), during training bees visited more B when it was rewarding than D, such that a preference for B should have been expected if only the associative strength were guiding the bees' choices. It was then 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, equal choice of B and D in the tests could be explained (Benard and Giurfa 2004). In any case, memory constraints (in this case the fact that simultaneous mastering of the different premise pairs was not possible and the fact that the last excitatory memory seems to predominate over previous memories) impeded learning the transitive rule. Recently, Chen and Wignall (2006) demonstrated that failure to master several consecutive visual discriminations 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.

Distributed cognition in honeybees

So far, we have concentrated on individual cognitive capabilities but bees live in societies and face therefore problems that require coordination, task-sharing, and collective decision making. For instance, bumblebees are influenced

by other conspecifics when sampling unfamiliar flowers such that they land on unknown flowers where other bees can be seen (Leadbeater and Chittka 2005). Presumably, this strategy allows learning more rapidly and efficiently about rewarding flowers. From this perspective, it is legitimate to ask whether collective behaviors reflect or even surpass individual plasticity, due, for instance, to the possible additive effect of individual cognitive capacities.

This question has been the subject of debate in the case of social insects in which colonies were considered "superorganisms" (Southwick 1983; Seeley 1989). It has been argued that the 'superorganism' protects and constitutes itself thanks to colony recognition systems based on cuticular hydrocarbons which are transferred between individuals within the colony, thus obscuring, in theory, individual identity. The metaphor of the 'superorganism' may be in a sense misleading because an individual, behaving organism is made from cells and structures tightly interconnected by complex neuronal, circulatory and regulatory networks, and has a central brain that commands and produces behavior. The 'superorganism', on the other hand, is made up of individuals which may be interconnected by complex chemical interactions but which are rather autonomous and can be hardly compared to constituent cells. The essential difference, however, is that although an insect colony produces collective behavior, it does not have a central brain to command and control such behavior. On the contrary, studies on collective decision-making in social insects show that collective behavioral patterns can arise from simple interactions between individuals, without any central control and without memory (Theraulaz et al. 2003).

From this point of view, the sophisticated cognitive capacities that honeybees exhibit in individual tests are not required for the close coordination of the social group. Differences in individual thresholds for reacting to environmental sensory stimuli seem to be a critical factor for the emergence of collective behaviors based on task partitioning. This may account, for instance, for the collective behavior of nest choice by a honeybee swarm. Group decision-making in honeybee swarms has been studied (see Seeley and Vischer 2004) in order to determine the rules underlying collective choice. It was found that the essence of a swarm's decision making relies on sensing a *quorum* (a sufficient number of scouts) at one of the nest sites rather than sensing a *consensus* (agreement of dancing scouts) at the swarm cluster. By this quorum-sensing hypothesis, a scout bee "votes" for a site by spending time at it. Somehow the scouts act and interact so that their numbers rise faster at superior sites, and somehow the bees at each site monitor their numbers there so that they know whether they've reached the threshold number (quorum) and can proceed to initiating the swarm's move to this site. Exactly how scout bees sense a quorum remains an enigma (Seeley

and Visscher 2004). They may use visual, olfactory or even tactile information to assess the number of fellow scouts at a site. But the complex migration pattern involving the coordinate displacement of thousands of bees does not require sophisticated mechanisms such as dance comparisons or verifying the reliability of information conveyed by a hive-mate. In short, the bees appear to begin preparations for liftoff as soon as enough scout bees, but not all of them, have approved of one of the potential nest sites.

The interesting conclusion emerging from studies on social insect collective behavior is that individuals, who may be viewed as extremely sophisticated at the cognitive level when performing some individual tasks, appear as automatons with limited cognitive capacities when performing collective tasks. This difference may seem puzzling and could be due to cognitive richness being lost or at least temporally inhibited in a social context. However, a possible explanation is that in an individual and in a social context, the animal will adopt the behavioral strategies leading to adaptive solutions, either boosting or sacrificing what researchers would view as cognitive sophistication. Whenever simple behaviors may lead to adaptive solutions, they are adopted. When, on the contrary, cognitive abilities are required, they are used. The critical question in this context is therefore what determines the adoption of one or the other level of cognitive complexity? Which factors are responsible for the fact that an ant or a bee that can learn and memorize several cues while foraging, solve complex discriminations and generate novel behaviors leading to adaptive solutions, behaves like an automaton following a reduced set of repetitive patterns and simple rules in a social context? Which physiological changes, if any, determine the passage from one state to the other? Do social regulation pheromones intervene in the expression or inhibition of behavioral autonomy in a social context by acting on neurotransmitter levels (e.g. biogenic amines) in the insect nervous system? Do social pheromones determine changes in immediate early gene (IEG) expression in the brain affecting cognitive processing? So far, we have no answers to these questions, but they can be approached experimentally. Studying whether or not individual learning and memory are modified by exposure to social pheromones or by chemosensory cues within a group and whether or not biogenic amine and neurotransmitter levels and IEG expression are changed in the presence of a group of co-specifics are just some of the questions that need to be considered.

Conclusion

This review intends to underline the enormous richness of experience-dependent behavior in honeybees, its high flexibility, and the fact that it is possible to formalize and char-

acterize in controlled laboratory protocols some forms of cognitive processing. Adopting rigorous definitions from elemental and non-elemental learning frameworks is useful to determine the extent to which honeybees can go beyond simple forms of associative learning. Such an experimental approach is possible as illustrated by the numerous examples reviewed here and has allowed to appreciate the sophistication of cognitive processing in an insect, which, as most insects, was traditionally considered as being limited in terms of its cognitive capabilities.

Contrarily to simple forms of associative learning for which specific neural circuits have been identified, more work is needed to relate complex problem solving to neural structures of the honeybee brain. The evidence existing points towards the mushroom bodies, a central structure in the insect brain that has been repeatedly associated with learning and memory capabilities (Menzel 1999). It has been shown that some elemental discriminations can be achieved without the necessity of mushroom bodies (Malun et al. 2002) but this does not seem to be so obvious in the case of non-elemental ones. Although specific substrates or circuits for complex problem solving in the bee brain are still unknown, it is possible to be optimistic with respect to their future identification. In this case, what is retarding our understanding of cognitive brain processing is not the technical level but rather the fact that up to now researchers did not dare to raise questions on complex cognitive processing to an insect.

What are the specific limitations of the bee brain when compared to bigger brains and what might be the structural/functional basis for these? To address such a question one would need to know more about its deficiencies, an area which has so far been barely investigated (but see Benard and Giurfa 2004). Due to obvious limitation in space, we have not discussed the role of different forms of learning in natural contexts such as navigation and communication. These contexts also offer promising frameworks for the study of cognitive processing. Questions such as the nature of space representation and the flexibility of communication strategies are important to characterize the potential of the bee brain. They need to be related, when possible, to underlying neural circuits and structures, a task that has been impossible up to now.

Last but not least, studies on honeybee cognition should not obviate the ecological and evolutionary dimensions. These perspectives are rarely present in studies in which the main focus is the neurobiology of learning and memory and the honeybee is not an exception to this caveat. Although studies on ecological and evolutionary aspects of bee learning exist (see, for instance, Raine et al. 2006), we still need to bring together this kind of vision with that of cognitive neuroscience. Arguments like the interpretation of memory phases and their molecular substrates in terms of the ecol-

ogy and dynamics of bee foraging activities in nature (Menzel 1999; see above) are rare and need to inspire neurobiological analyses. Finally, although I have focused on a single species in this article, the honeybee *A. mellifera*, other bee species are also extremely attractive as a model study and deserve to be investigated as well in order to promote a comparative approach necessary to understand factors like the role of sociality in the emergence of cognitive abilities. Research on bumblebees is well developed (Raine et al. 2006) but other species like stingless bees, wasps and carpenter bees, among others, should also be considered.

Studies on honeybee behavior allow researchers to have an optimistic attitude in facing these questions. Moreover, as learning in honeybees can be compared to that of vertebrates in many senses, the honeybee may serve as a model system for understanding intermediate levels of complexity of cognitive functions and their neural substrates. The mini brain of the honeybee, with its 960,000 neurons has not yet revealed all its potential. Our review thus proves that Karl von Frisch was right when he called the honeybee a magic well—no matter how much is discovered, more remains to be uncovered.

Acknowledgments I thank JM Devaud, JC Sandoz and R Menzel for helpful criticisms on previous versions of this manuscript. I also thank all the members of my research team at the University of Toulouse for providing a stimulating and productive environment. Thanks are also due to the CNRS, the University of Toulouse and the Institut Universitaire de France for much support.

References

- Abel R, Rybak J, Menzel R (2001) Structure and response patterns of olfactory interneurons in the honeybee, *Apis mellifera*. *J Comp Neurol* 437:363–383
- Alvarado MC, Bachevalier J (2005) Selective neurotoxic damage to the hippocampal formation impairs performance of the transverse patterning and location memory tasks in rhesus macaques. *Hippocampus* 15:118–131
- Altman JS, Kien J (1987) Functional organization of the subesophageal ganglion in arthropods. In: Gupta AP (ed) *Arthropod brain: its evolution, development, structure and function*. Wiley, New York, pp 265–301
- Benard J, Giurfa M (2004) A test of transitive inferences in free-flying honeybees: unsuccessful performance due to memory constraints. *Learn Mem* 11:328–336
- Benard J, Stach S, Giurfa M (2006) Categorization of visual stimuli in the honeybee *Apis mellifera*. *Anim Cogn* 9:257–270
- Bitterman ME, Menzel R, Fietz A, Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J Comp Psychol* 97:107–119
- Blaser RE, Couvillon PA, Bitterman ME (2004) Backward blocking in honeybees. *Q J Exp Psychol B* 57:349–360
- Bolles RC (1970) Specifics-specific defense reactions and avoidance learning. *Psychol Rev* 77:32–48
- Borlikova GG, Elbers NA, Stephens DN (2006). Repeated withdrawal from ethanol spares contextual fear conditioning and spatial learning but impairs negative patterning and induces over-
- responding: evidence for effect on frontal cortical but not hippocampal function? *Eur J Neurosci* 24:205–216
- Bucci DJ, Saddoris MP, Burwell RD (2002) Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. *Behav Neurosci* 116:479–488
- Buffon (Leclerc G.L.a) (1749a) *Histoire naturelle générale et particulière: avec la description du cabinet du Roy*. Imprimerie Royale, Paris, vol II
- Buffon (Leclerc G.L.b) (1749b) *Histoire naturelle générale et particulière: avec la description du cabinet du Roy*. Imprimerie Royale, Paris, vol IV
- Chandra S, Smith BH (1998) An analysis of synthetic processing of odor mixtures in the honeybee. *J Exp Biol* 201:3113–3121
- Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* 86:361–377
- Cheng K, Wignall AE (2006) Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. *Anim Cogn* 9:141–150
- Collett TS, Collett M (2002) Memory use in insect visual navigation. *Nat Rev Neurosci* 3:542–552
- Collett TS, Graham P, Durier V (2003) Route learning by insects. *Curr Opin Neurobiol* 13:718–725
- Couvillon PA, Bitterman ME (1980) Some phenomena of associative learning in honey bees. *J Comp Physiol Psychol* 94:878–885
- Couvillon PA, Klosterhalfen S, Bitterman ME (1983) Analysis of overshadowing in honeybees. *J Comp Psychol* 97:154–166
- Couvillon PA, Arakaki L, Bitterman ME (1997) Intramodal blocking in honeybees. *Anim Learn Behav* 25:277–282
- Dacher M, Lagarrigue A, Gauthier M (2005) Antennal tactile learning in the honeybee: effect of nicotinic antagonists on memory dynamics. *Neuroscience* 130:37–50
- de Brito Sanchez MG, Giurfa M, de Paula Mota TR, Gauthier M (2005) Electrophysiological and behavioural characterization of gustatory responses to antennal ‘bitter’ taste in honeybees. *Eur J Neurosci* 22:3161–3170
- Deisig N, Lachnit H, Hellstern F, Giurfa M (2001) Configural olfactory learning in honeybees: negative and positive patterning discrimination. *Learn Mem* 8:70–78
- Deisig N, Lachnit H, Giurfa M (2002) The effect of similarity between elemental stimuli and compounds in olfactory patterning discriminations. *Learn Mem* 9:112–121
- Deisig N, Lachnit H, Sandoz JC, Lober K, Giurfa M (2003) A modified version of the unique cue theory accounts for olfactory compound processing in honeybees. *Learn Mem* 10:199–208
- Deisig N, Giurfa M, Lachnit H, Sandoz JC (2006) Neural representation of olfactory mixtures in the honeybee antennal lobe. *Eur J Neurosci* 24:1161–1174
- Delius JD, Jitsumori M, Siemann M (2000) Stimulus equivalences through discrimination reversals. In: Heyes C, Huber L (eds) *The evolution of cognition*. MIT Press, Cambridge, pp 103–122
- Erber J, Kierzek S, Sander E, Grandy K (1998) Tactile learning in the honeybee. *J Comp Physiol A* 183:737–744
- Faber T, Joerges J, Menzel R (1999) Associative learning modifies neural representations of odors in the insect brain. *Nature Neurosci* 2:74–78
- Faber T, Menzel R (2001) Visualizing mushroom body response to a conditioned odor in honeybees. *Naturwissenschaften* 88:472–476
- Fanselow MS (1980) Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci* 15:177–182
- Farina W, Gruter C, Diaz PC (2005) Social learning of floral odours inside the honeybee hive. *Proc Biol Sci* 272:1923–1928
- Farina W, Gruter C, Acosta L, Mc Cabe S (2006) Honeybees learn floral odors while receiving nectar from foragers within the hive. *Naturwissenschaften* 94:55–60

- Farooqui T, Robinson K, Vaessin H, Smith BH (2003) Modulation of early olfactory processing by an octopaminergic reinforcement pathway in the honeybee. *J Neurosci* 23:5370–5380
- Fersen LV, Wynne CDL, Delius JD (1990) Deductive reasoning in pigeons. *Naturwissenschaften* 77:548–549
- Fiala A, Müller U, Menzel R (1999) Reversible down regulation of protein kinase A during olfactory learning using antisense technique impairs long-term memory formation in the honeybee *Apis mellifera*. *J Neurosci* 19:10125–10134
- Frisch Kv (1914) Der Farbensinn und Formensinn der Biene. *Zool Jb Physiol* 37:1–238
- Frisch Kv (1962) Dialects in the language of the bees. *Sci Amer* 207:78–87
- Frisch Kv (1967) The dance language and orientation of bees. Belknap Press, Cambridge
- Galizia CG, Menzel R (2000) Odour perception in honeybees: coding information in glomerular patterns. *Curr Opin Neurobiol* 10:504–510
- Galizia CG, Nägler K, Hölldobler B, Menzel R (1998) Odour coding is bilaterally symmetrical in the antennal lobes of honeybees (*Apis mellifera*). *Eur J Neurosci* 10:2964–2974
- Galizia CG, Sachse S, Rappert A, Menzel R (1999) The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nat Neurosci* 2:473–478
- Gerber B, Ullrich J (1999) No evidence for olfactory blocking in honeybee classical conditioning. *J Exp Biol* 202:1839–1854
- Gil M, de Marco RJ (2005) Olfactory learning by means of trophallaxis in *Apis mellifera*. *J Exp Biol* 208:671–680
- Gil M, de Marco RJ (2006) *Apis mellifera* bees acquire long-term olfactory memories within the colony. *Biol Lett* 2:98–100
- Giurfa M (2003) Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. *Curr Opin Neurobiol* 13:726–735
- Giurfa M (2006) Associative learning: the instructive function of biogenic amines. *Curr Biol* 16:R892–R895
- Giurfa M, Malun D (2004) Associative mechanosensory conditioning of the proboscis extension reflex in honeybees. *Learn Mem* 11:294–302
- Giurfa M, Menzel R (1997) Insect visual perception: complex abilities of simple nervous systems. *Curr Opin Neurobiol* 7:505–513
- Giurfa M, Lehrer M (2001) Honeybee vision and floral displays: from detection to close-up recognition. In: Chittka L, Thomson J (eds) *Cognitive ecology of pollination*. Cambridge University Press, Cambridge, pp 61–82
- Giurfa M, Eichmann B, Menzel R (1996) Symmetry perception in an insect. *Nature* 382:458–461
- Giurfa M, Núñez JA, Chittka L, Menzel R (1995) Colour preferences of flower-naïve honeybees. *J Comp Physiol A* 177:247–259
- Giurfa M, Hammer M, Stach S, Stollhoff N, Müller-Deisig N, Mizrycki C (1999) Pattern learning by honeybees: conditioning procedure and recognition strategy. *Anim Behav* 57:315–324
- Giurfa M, Zhang S, Jenett A, Menzel R, Srinivasan MV (2001) The concepts of ‘sameness’ and ‘difference’ in an insect. *Nature* 410:930–933
- Giurfa M, Schubert M, Reisenman C, Gerber B, Lachnit H (2003) The effect of cumulative experience on the use of elemental and configural visual discrimination strategies in honeybees. *Behav Brain Res* 145:161–169
- Grant V (1951) The fertilization of flowers. *Sci Amer* 12:1–6
- Guerrieri F, Schubert M, Sandoz JC, Giurfa M (2005a) Perceptual and neural olfactory similarity in honeybees. *PLoS Biol* 3(4):e60
- Guerrieri F, Lachnit H, Gerber B, Giurfa M (2005b) Olfactory blocking and odorant similarity in the honeybee. *Learn Mem* 12:86–95
- Hammer M (1993) An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* 366:59–63
- Hammer M, Menzel R (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn Mem* 5:146–156
- Harnard S (1987) *Categorical perception. The groundwork of cognition*. Cambridge University Press, Cambridge
- Hateren JH v, Srinivasan MV, Wait PB (1990) Pattern recognition in bees: orientation discrimination. *J Comp Physiol A* 197:649–654
- Haupt SS (2004) Antennal sucrose perception in the honey bee (*Apis mellifera* L.): behaviour and electrophysiology. *J Comp Physiol A* 190:735–745
- Hellstern F, Wüstenberg D, Hammer M (1995) Contextual learning in honeybees under laboratory conditions. In: Elsner N, Menzel R (eds) *Proceedings of the 23rd Göttingen Neurobiology Conference on Learning and Memory*. Georg Thieme Verlag, Stuttgart, p. 30
- Hori S, Takeuchi H, Arikawa K, Kinoshita M, Ichikawa N, Sasaki M, Kubo T (2006) Associative visual learning, color discrimination, and chromatic adaptation in the harnessed honeybee *Apis mellifera* L. *J Comp Physiol A* 192:691–700
- Hosler JS, Smith BH (2000) Blocking and the detection of odor components in blends. *J Exp Biol* 203:2797–2806
- Huber R (2005) Amines and motivated behaviors: a simpler systems approach to complex behavioral phenomena. *J Comp Physiol A* 191:231–239
- Jacobs LF (2006) From movement to transitivity: the role of hippocampal parallel maps in configural learning. *Rev Neurosci* 17:99–109
- Joerges J, Küttner A, Galizia CG, Menzel R (1997) Representation of odours and odour mixtures visualized in the honeybee brain. *Nature* 387:285–288
- Kien J, Menzel R (1977) 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
- Kisch J, Erber J (1999) Operant conditioning of antennal movements in the honey bee. *Behav Brain Res* 99:93–102
- Komischke B, Sandoz JC, Lachnit H, Giurfa M (2003) Non-elemental processing in olfactory discrimination tasks needs bilateral input in honeybees. *Behav Brain Res* 145:135–143
- Komischke B, Sandoz JC, Malun D, Giurfa M (2005) Partial unilateral lesions of the mushroom bodies affect olfactory learning in honeybees *Apis mellifera* L. *Eur J Neurosci* 21:477–485
- Kreissl S, Eichmüller S, Bicker G, Rapus J, Eckert M (1994) Octopamine-like immunoreactivity in the brain and suboesophageal ganglion of the honeybee. *J Comp Neurol* 348:583–595
- Kuwabara M (1957) Bildung des bedingten Reflexes von Pavlovs Typus bei der Honigbiene, *Apis mellifica*. *J Fac Sci Hokkaido Univ Ser VI Zool* 13:458–464
- Laurent G, Wehr M, Davidowitz H (1996) Temporal representations of odors in an olfactory network. *J Neurosci* 16:3837–3847
- Leadbeater E, Chittka L (2005) A new mode of information transfer in foraging bumblebees? *Curr Biol* 15:R447–R448
- Lehrer M (1997) Honeybee’s visual orientation at the feeding site. In: Lehrer M (eds) *Orientation and communication in arthropods*. Birkhäuser, Basel, pp 115–144
- Libersat F, Pflüger HJ (2004) Monoamines and the orchestration of behavior. *Bioscience* 54:17–25
- Malun D (1998) Early development of mushroom bodies in the brain of the honeybee *Apis mellifera* as revealed by BrdU incorporation and ablation experiments. *Learn Mem* 5:90–101
- Malun D, Giurfa M, Galizia CG, Plath N, Brandt R, Gerber B, Eissermann B (2002) Hydroxyurea-induced partial mushroom body ablation does not affect acquisition and retention of olfactory differential conditioning in honeybees. *J Neurobiol* 53:343–360
- Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Z vergl Physiol* 56:22–62

- Menzel R (1968) Das Gedächtnis der Honigbiene für Spektralfarben. I. Kurzzeitiges und langzeitiges Behalten. *Z vergl Physiol* 60:82–102
- Menzel R (1985) Learning in honey bees in an ecological and behavioral context. In: Hölldobler B, Lindauer M (eds) *Experimental behavioral ecology and sociobiology*. Fischer, Stuttgart, pp 55–74
- Menzel R (1999) Memory dynamics in the honeybee. *J Comp Physiol A* 185:323–340
- Menzel R (2001) Searching for the memory trace in a mini-brain, the honeybee. *Learn Mem* 8:53–62
- Menzel R, Backhaus W (1991) Colour vision in insects. In: Gouras P (ed) *Vision and visual dysfunction. The perception of colour*. MacMillan Press, London, pp 262–288
- Menzel R, Erber J (1978) Learning and memory in bees. *Sci Amer* 239:80–87
- Menzel R, Giurfa M (2001) Cognitive architecture of a mini-brain: the honeybee. *Trends Cogn Sci* 5:62–71
- Menzel R, Manz G, Menzel R, Greggers U (2001) Massed and spaced learning in honeybees: the role of CS, US, the intertrial interval, and the test interval. *Learn Mem* 8:198–208
- Menzel R, Greggers U, Hammer M (1993) Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In: Papaj D, Lewis AC (eds) *Insect learning: ecological and evolutionary perspectives*. Chapman and Hall, New York, pp 79–125
- Moses SN, Cole C, Driscoll I, Ryan J (2005) Differential contributions of hippocampus, amygdala and perirhinal cortex to recognition of novel objects, contextual stimuli and stimulus relationships. *Brain Res Bull* 67:62–76
- Müller D, Gerber B, Hammer M, Menzel R (2000) Sensory preconditioning in honeybees. *J Exp Biol* 203:1351–1356
- Müller D, Abel R, Brandt R, Zöckler M, Menzel R (2002) Differential parallel processing of olfactory information in the honeybee, *Apis mellifera* L. *J Comp Physiol A* 188:359–370
- Núñez JA, Almeida L, Balderrama N, Giurfa M (1997) Alarm pheromone induces stress analgesia via an opioid system in the honeybee. *Physiol Behav* 63:75–80
- Núñez JA (1982) Honeybee foraging strategies at a food source in relation to its distance from the hive and the rate of sugar flow. *J Apicult Res* 21:139–150
- O'Reilly RC, Rudy JW (2001) *Conjunctive representations in learning and memory: Principles of cortical and hippocampal function*. Psychol Rev 108:311–345
- Pavlov IP (1927) *Lectures on conditioned reflexes*. International publishers, New York
- Pearce JM (1994) Similarity and discrimination: a selective review and a connectionist model. *Psychol Rev* 101:587–607
- Peele P, Ditzen M, Menzel R, Galizia G (2006) Appetitive odor learning does not change olfactory coding in a subpopulation of honeybee antennal lobe neurons. *J Comp Physiol A* 192:1083–1103
- Raine NE, Ings T, Dornhaus A, Saleh N, Chittka L (2006) Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. *Adv Study Behav* 36:305–354
- Rescorla RA, Wagner AR (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black AH, Prokasy WF (eds) *Classical conditioning II*. Appleton-Century-Crofts, New York, pp 64–99
- Riemensperger T, Völler T, Stock P, Buchner E, Fiala (2005). Punishment prediction by dopaminergic neurons in *Drosophila*. *Curr Biol* 15:1953–1960
- Robertson I (2001) *Problem solving*. Psychology Press, Hove
- Rudy JW, Sutherland RJ (1992) Configural and elemental associations and the memory coherence problem. *J Cognit Neurosci* 4:208–216
- Rudy JW, Sutherland RJ (1995) Configural association theory and the hippocampal formation: an appraisal and reconfiguration. *Hippocampus* 5:375–389
- Sachse S, Galizia CG (2002) The role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. *J Neurophysiol* 87:1106–1117
- Sandoz JC, Menzel R (2001) Side-specificity of olfactory learning in the honeybee: generalization between odors and sides. *Learn Mem* 8:286–294
- Sandoz JC, Galizia CG, Menzel R (2003) Side-specific olfactory conditioning leads to more specific odor representations between sides but not within sides in the honeybee antennal lobes. *Neuroscience* 120:1137–1148
- Scheiner R, Erber J, Page RE Jr (1999) Tactile learning and the individual evaluation of the reward in honey bees (*Apis mellifera* L.). *J Comp Physiol A* 185:1–10
- Scheiner R, Page RE Jr, Erber J (2001a) The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Learn Mem* 76:138–150
- Scheiner R, Weiß A, Malun D, Erber J (2001b) Learning in honey bees with brain lesions: how partial mushroom-body ablations affect sucrose responsiveness and tactile antennal learning. *Anim Cogn* 4:227–235
- Schroll C, Riemensperger T, Bucher D, Ehmer J, Völler T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, Fiala A (2006) Light-induced activation of distinct modulatory neurons substitutes for appetitive or aversive reinforcement during associative learning in larval *Drosophila*. *Curr Biol* 16:1741–1747
- Schroter U, Malun D, Menzel R (2007) Innervation pattern of subesophageal ventral unpaired median neurones in the honeybee brain. *Cell Tissue Res* 327:647–667
- Schubert M, Francucci S, Lachnit H, Giurfa M (2005) Nonelemental visual learning in honeybees. *Anim Behav* 64:175–184
- Schultz W, Dickinson A (2000) Neuronal coding of prediction errors. *Annu Rev Neurosci* 23:473–500
- Schwaerzel M, Müller U (2006) Dynamic memory networks: dissecting molecular mechanisms underlying associative memory in the temporal domain. *Cell Mol Life Sci* 63:989–998
- Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M (2003) Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J Neurosci* 23:10495–10502
- Seeley TD (1989) The honey bee colony as a superorganism. *Amer Sci* 77:546–553
- Seeley TD (1995) *The wisdom of the hive—the social physiology of honey bee colonies*. Harvard University Press, London
- Seeley TD, Visscher K (2004) Quorum sensing during nest site selection by honeybee swarms. *Behav Ecol Sociobiol* 56:594–601
- Skinner BF (1938) *The behavior of organisms*. Appleton, New York
- Smith BH (1998) Analysis of interaction in binary odorant mixtures. *Physiol Behav* 65:397–407
- Smith BH, Cobey S (1994) The olfactory memory of the honeybee *Apis mellifera*. II. Blocking between odorants in binary mixtures. *J Exp Biol* 195:91–108
- Southwick EE (1983) The honey bee cluster as a homeothermic superorganism. *Comp Biochem Physiol* 75:641–645
- Srinivasan MV (1994) Pattern recognition in the honeybee: recent progress. *J Insect Physiol* 40:183–194
- Srinivasan MV, Zhang SW (1997) Visual control of honeybee flight. In: Lehrer M (ed) *Orientation and communication in arthropods*. Birkhäuser, Basel, pp 95–114
- Srinivasan MV, Poteser M, Kral K (1999) Motion detection in insect orientation and navigation. *Vision Res* 39:2749–2766
- Stach S, Giurfa M (2005) The influence of training length on generalization of visual feature assemblies in honeybees. *Behav Brain Res* 161:8–17
- Stach S, Benard J, Giurfa M (2004) Local-feature assembling in visual pattern recognition and generalization in honeybees. *Nature* 429:758–761

- Stopfer M, Bhagavan S, Smith BH, Laurent G (1997) Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* 390:70–74
- Szyszkka P, Ditzgen M, Galkin A, Galizia G, Menzel R (2005) Sparsening and temporal sharpening of olfactory representations in the honeybee mushroom bodies. *J Neurophysiol* 94:3303–3313
- Takeda K (1961) Classical conditioned response in the honey bee. *J Insect Physiol* 6:168–179
- Terrace HS, McGonigle B (1994) Memory and representation of serial order by children, monkeys and pigeons. *Curr Dir Psychol Sci* 3:180–185
- Theraulaz G, Gautrais J, Camazine S, Deneubourg JL (2003) The formation of spatial patterns in social insects: from simple behaviours to complex structures. *Philos Trans R Soc Lond A* 361:1263–1282
- Troje F, Huber L, Loidolt M, Aust U, Fieder M (1999) Categorical learning in pigeons: the role of texture and shape in complex static stimuli. *Vis Res* 39:353–366
- Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J Comp Physiol Psychol* 156:263–277
- Unoki S, Matsumoto Y, Mizunami M (2005) Participation of octopaminergic reward system and dopaminergic punishment system in insect olfactory learning revealed by pharmacological study. *Eur J Neurosci* 22:1409–1416
- Unoki S, Matsumoto Y, Mizunami M (2006) Roles of octopaminergic and dopaminergic neurons in mediating reward and punishment signals in insect visual learning. *Eur J Neurosci* 24:2031–2038
- Vareschi E (1971) Duftunterscheidung bei der Honigbiene - Einzelzell-Ableitungen und Verhaltensreaktionen. *Z vergl Physiol* 75:143–173
- Vergoz V, Roussel E, Sandoz JC, Giurfa M (2007) Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. *PLoS One* 2(3):e288
- Wehner R. (1981) Spatial vision in arthropods. In: Autrum HJ (ed) *Handbook of sensory physiology VIc*. Springer, Berlin, pp 287–616
- Wehr M, Laurent G (1996) Temporal combinatorial encoding of odours with oscillations. *Nature* 384:162–166
- Whitehead AT (1978) Electrophysiological response of honey bee labial palp contact chemoreceptors to sugars and electrolytes. *Physiol Entomol* 3:241–248
- Whitehead AT, Larsen JR (1976) Electrophysiological responses of galeal contact chemoreceptors of *Apis mellifera* to selected sugars and electrolytes. *J Insect Physiol* 22:1609–1616
- Whitlow JW, Wagner AR (1972) Negative patterning in classical conditioning: summation of response tendencies to isolable and configural components. *Psychon Sci* 27:299–301
- Wittstock S, Menzel R (1994) Color learning and memory in honey bees are not affected by protein synthesis inhibition. *Behav Neural Biol* 62:224–229
- Wüstenberg D, Gerber B, Menzel R (1998) Long- but not medium-term retention of olfactory memories in honeybees is impaired by actinomycin D and anisomycin. *Eur J Neurosci* 10:2742–2745
- Yang EC, Maddess T (1997) Orientation-sensitive neurons in the brain of the honey bee (*Apis mellifera*). *J Insect Physiol* 43:329–336
- Yang EC, Lin HC, Hung YS (2004) Patterns of chromatic information processing in the lobula of the honeybee, *Apis mellifera* L. *J Insect Physiol* 50:913–925
- Zentall TR, Galizio M, Critchfield TS (2002) Categorization, concept learning and behavior analysis: an introduction. *J Exp Anal Behav* 78:237–248
- Zhang SW, Srinivasan MV, Zhu H, Wong J (2004) Grouping of visual objects by honeybees. *J Exp Biol* 207:3289–3298
- Zhang S, Bock F, Si A, Tautz J, Srinivasan M (2005) Visual working memory in decision making by honey bees. *Proc Natl Acad Sci USA* 102:5250–5255