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Taste Perception in Honey Bees

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Abstract

Taste is crucial for honeybees for choosing profitable food sources, resins, water sources, and for nestmate recognition. Peripheral taste detection occurs within cuticular hairs, the chaetic and basiconic sensilla, which host gustatory receptor cells and, usually a mechanoreceptor cell. Gustatory sensilla are mostly located on the distal segment of the antennae, on the mouthparts, and on the tarsi of the forelegs. These sensilla respond with varying sensitivity to sugars, salts, and possibly amino acids, proteins, and water. So far, no responses of receptor cells to bitter substances were found although inhibitory effects of these substances on sucrose receptor cells could be recorded. When bees are free to express avoidance behaviors, they reject highly concentrated bitter and saline solutions. However, such avoidance disappears when bees are immobilized in the laboratory. In this case, they ingest these solutions, even if they suffer afterward a malaise-like state or even die from such ingestion. Central processing of taste occurs mainly in the subesophageal ganglion, but the nature of this processing remains unknown. We suggest that coding tastants in terms of their hedonic value, thus classifying them in terms of their palatability, is a basic strategy that a central processing of taste should achieve for survival.

Key words: central processing of taste, gustation, gustatory receptors, honeybee, insect, peripheral taste detection, subesophageal ganglion, taste

Introduction

Since the pioneer work of von Frisch (1967), the honeybee *Apis mellifera* has emerged as an important insect model for the study of problems as diverse as perception, learning, memory, communication, navigation, and social organization. Although the processing of olfactory and visual information by honey bees has been intensively studied in the last decades in the context of their interaction with flowers (vision: Menzel and Backhaus 1991; Giurfa and Menzel 1997; Wakakuwa et al. 2005; olfaction: Galizia and Menzel 2000; Deisig et al. 2002, 2006; Guerrieri et al. 2005), less is known about the processing of gustatory stimuli by honey bees. Taste, the sense that distinguishes between chemical compounds and the sensations they produce based on contact with chemoreceptors, allows discriminating edible from nonedible items and is, therefore, crucial for survival. Here I will review fundamental aspects of the biology of taste of the honeybee, indicating thereby what is known and what requires further investigations. I will focus on "taste" in a natural context in the life of a honeybee and highlight characteristics of taste receptor cells and the peripheral processing of taste via the main gustatory appendages. I will afterward present newer characterizations of gustatory molecular receptors present in gustatory cells using a comparative approach and discuss whether or not honey bees possess a limited taste perception. This question will be analyzed through a special focus on the perception of substances that taste bitter to humans (henceforth bitter substances). Finally, I will analyze the central processing of taste using again a comparative approach. The conclusion will underline open questions that need to be answered to achieve a better understanding of the taste biology of the honeybee.

Honey bee taste in a natural context

Gustatory stimuli play a fundamental role in a honeybee's life. In a foraging context, honeybee foragers collect nectar and pollen, which respectively provide carbohydrates and proteins that are necessary for survival. Nectar presents not only different types of sugars such as sucrose, glucose, and/or fructose but also organic acids, lipids, minerals, vitamins, and aromatic compounds, even if these substances constitute a low

percentage of nectar contents (Harborne 1994). Pollen contains proteins but also lipids, mineral salts, albumin, zvitamins, amino acids, growth regulator factors, folic acid, and enzymes among others (Harborne 1994). Furthermore, besides foraging for nectar and pollen, bees collect water, and in this context, they respond to salts. Additionally, bees collect resin for elaborating propolis and should then taste several compounds such as prenylated and nonprenylated phenylpropanoids, terpenoids, and anthracene derivatives, which have been identified in the resin loads transported in the corbiculae of the posterior legs (Weinstein Texeira et al. 2005). Finally, bees chew and process wax with their mouthparts and, thus, may taste and react to the chemicals contained in it.

Taste stimuli may play further vital roles in the life of honeybees. Although the examples provided above refer essentially to adult bees that engage in different foraging activities outside the hive, younger bees within the hive may also use their gustatory senses for different purposes. Besides olfaction, taste may allow intracolonial recognition within the dark world of a hive. It has been repeatedly shown that cuticular hydrocarbons confer a chemical signature allowing nestmate recognition (e.g., Châline et al. 2005; Dani et al. 2005). So far, it is not clear whether such recognition occurs via olfactory or gustatory input. In the fruit fly *Drosophila melanogaster*, olfactory and gustatory inputs are involved in sensing cuticular hydrocarbons (Ferveur 2005). Cuticular hydrocarbons are usually high-molecular weight compounds so that airborne detection may not be the primary detection channel; contact chemoreceptors may be involved and gustatory detection may be the privileged channel for nestmate recognition. A tight interaction between wax comb and cuticular hydrocarbons has been shown (Breed et al. 1988) so that both may constitute a continuous medium for any hydrocarbon-soluble substances used by honeybees in nestmate recognition.

Peripheral processing of taste

Searching for the gustatory receptors

In the honeybee, the antennae, mouthparts, and distal segments of the forelegs constitute the main chemosensory organs (Goodman 2003; see Figure 1a). On these appendages, gustatory but also hygro, thermo, mechanosensory, and olfactory receptor cells are located within specialized cuticular structures called sensilla. Different sensillum types can be distinguished on the basis of their particular cuticular structure (Esslen and Kaissling 1976). Taste receptors are located within hair-like sensilla. Already in the 19th Century, the "blunt hairs" found on the antennae (Briant and Jackson 1884) and on the glossa (Will 1885) were described as taste receptors The discovery that certain hairs on the tarsi of butterflies and the proboscis of the blowfly initiate feeding responses when touched by sugar solutions (Minnich 1921, 1926) led to numerous investigations of contact chemoreceptors in a number of insects (reviewed by Frings and Frings 1949). In the case of the honeybee, behavioral approaches were first used to characterize its gustatory responses. Kunze (1933) and Minnich (1932) stimulated body appendages with sugars to elicit the appetitive reflex of proboscis extension (proboscis extension reflex [PER]) and determined that taste receptors, whose stimulation elicits PER, were not only on the antennae but also on the front tarsi but not on the hind tarsi. Frings H and Frings N (1949) confirmed later the presence of gustatory receptors on the antennae and distal segments of the first pair of legs, whereas they found no evidence for gustatory receptors on the mid- and hind legs.

von Frisch (1934) trained free-flying honey bees to sugar solutions of different quality and determined that bees are responsive to only 7 of 30 sugars tested, 5 of which occur naturally in nectar or honeydew (sucrose, glucose and fructose in nectar, and melezitose and trehalose in honeydew). Bees were also attracted to maltose and α-methyl glucoside even if these compounds play no part in their natural food as far as is known (von Frisch 1967). It was concluded that gustatory receptors located on the mouthparts were responsible for the specificity of honeybee responses to these sugars (von Frisch 1934).

The gustatory sensilla

Gustatory sensilla take the form of hairs (chaetic sensilla; Figure 1b: ch) or pegs (basiconic sensilla; Figure 1b: bs) (Esslen and Kaissling 1976). In agreement with previous behavioral accounts (see above), these sensilla can be found essentially on the antennae, mouthparts, and forelegs of a honeybee. The morphology of gustatory sensilla found on the mouthparts was described by Galic (1971) using light microscopy; later, Whitehead and Larsen (1976a) used light and electron microscopy to describe sensilla located on the mouthparts, antennae, and distal segments of the forelegs. They found chaetic sensilla of different sizes on the glossa, labial palps, galea, antennae, and tarsi of honeybee workers. Basiconic sensilla were also found on these structures, except on the antennae and glossa (Whitehead and Larsen 1976a).

Gustatory sensilla have a characteristic aperture at the apex (a pore or a papilla) through which gustatory substances can penetrate after contacting the hair or peg. Usually 3–5 gustatory receptor cells innervate each sensilla and bath in a sensillum lymph (Mitchell et al. 1999) (Figure 1c). An exception is provided by sensilla on the mandibles that present only one sensory neuron, but the gustatory role of these sensilla is unclear. Each gustatory receptor neuron projects a dendritic branch up the shaft of the hair or peg to the apex. Such a branch bears the molecular gustatory receptors, which are thought to be G-protein–coupled proteins (Clyne et al. 2000) and which bind specific tastants depending on their molecular structure. Gustatory receptor cells are thought to convey the message to postsynaptic neurons by means of acetylcholine as at least in the fruit fly *D. melanogaster* choline acetyltransferase, the enzyme responsible for the formation of acetylcholine, can be found in gustatory receptor afferences (Python and Stocker

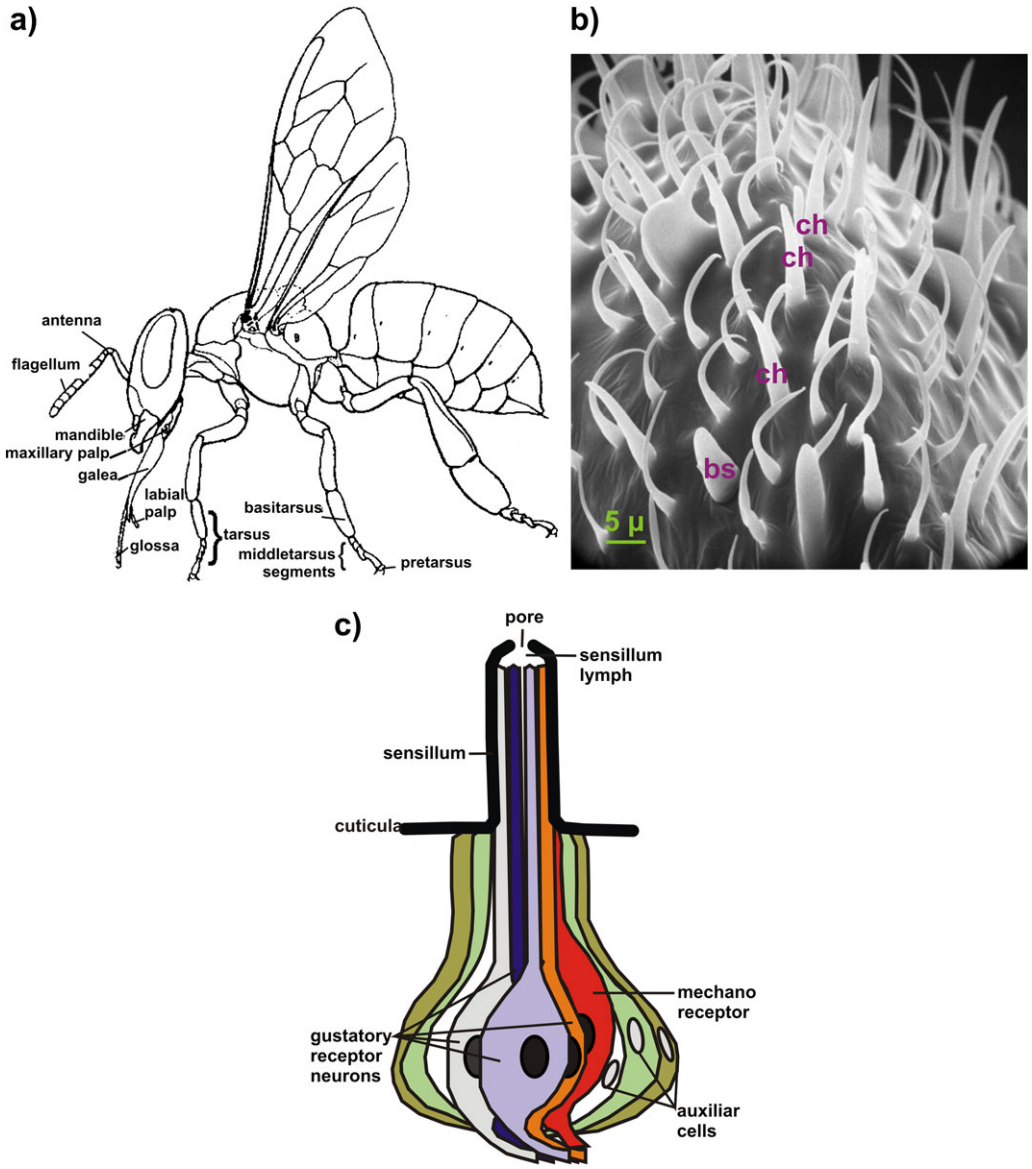

Figure 1 **(a)** Anatomy of the honeybee. The main chemosensory organs involved in taste perception (antennae, mouthparts, and tarsal regions of the legs) are indicated. **(b)** Scanning electron microscope picture of the antennal tip surface of the honeybee showing chaetic (ch) and basiconic (bs) sensilla. **(c)** Schematic drawing of a chaetic sensillum. Four gustatory receptor cells bathing in a cavity defined by auxiliary sensillar cells and filled with sensillum lymph extend their dendrites toward the apex of the cuticular hair. A mechanoreceptor cell is attached to the basal wall of the hair. Tastants penetrate into the sensillum through a pore at the apex. This figure appears in color in the online version of *Chemical Senses*.

2002). In most cases, besides gustatory receptor neurons, a mechanoreceptor cell terminating at the base of the shaft can also be found within gustatory sensilla (Figure 1c). This neuron is stimulated by the movement experienced by the sensilla and allows evaluating the position and density of the food.

Gustatory sensilla on the antennae

Gustatory antennal perception plays a role in appetitive food sensing as shown by the fact that stimulation of the antennae with sucrose solution elicits PER (Takeda 1961; Bitterman et al. 1983). Approximately 300 chaetic sensilla were found distributed over the antennal flagellum (Esslen and Kaissling 1976). An important concentration of these sensilla was found on the ventral surface of the distal segment of the antennae, which constitutes the primary antennal contact region with tastants. About half of the chaetic sensilla observed on the antennae are innervated by 6 gustatory receptor neurons and 1 mechanoreceptor neuron; the other half has 5 gustatory receptor neurons and 1 mechanoreceptor (Whitehead and Larsen 1976a).

Electrophysiological, extracellular recordings of single sensilla were used to characterize the gustatory sensitivity of receptor neurons hosted in antennal sensilla located on the tip of the antennae. Haupt (2004) showed that antennal chaetic sensilla (which he termed "trichoid") are very

sensitive to sucrose stimulation. The response threshold of these sensilla was below 0.1% as they responded to a sucrose concentration of 0.1% w/w (2.9 mM). Their sensitivity is higher than that of sensilla on the proboscis that exhibit thresholds of about 0.34% (10 mM) (Whitehead and Larsen 1976b; Whitehead 1978; see below). This high sensitivity highlights the fundamental role of antennal gustatory receptors in locating a potential food source.

Sucrose responses of antennal sensilla are dose dependent (Haupt 2004; de Brito Sanchez et al. 2005) (Figure 2a). It seems that, in most cases, only a single cell type is activated by sucrose stimulation although relying on spike amplitude is not always a consistent criterion in the case of taste cells. Indeed, it is a common observation that electrophysiological responses of gustatory receptor cells are not always regular and may even vary in spike amplitude or interspike intervals (Hiroi et al. 2002). Sucrose responses between different hairs on the same antenna show a high degree of variability in spike frequency (Haupt 2004; see Figure 2b). Such variability allows extending the dynamic range of sucrose perception in an individual bee (Haupt 2004). The fact that bees within a hive may drastically differ in their sucrose sensitivity and thus in their responsiveness to sucrose solutions of different concentrations is a well-established fact (Page et al. 2006), which accounts for task specializations and has a genetic basis. Such differences may rely on interindividual differences in the proportions of taste hairs of different sensitivity.

Antennal chaetic sensilla recorded in 2 different studies (Haupt 2004; de Brito Sanchez et al. 2005) did not respond to a diluted solution of KCl (10 mM), suggesting that these sensilla do not have a cell responding to water, which has been found in other insects (e.g., Hiroi et al. 2004). On the other hand, it has been shown that very sensitive bees respond with PER to water vapor (Kuwabara 1957). It has to be assumed that these responses are elicited by antennal hygroreceptors (Lacher 1964; Yokohari et al. 1982; Yokohari 1983). Responses to a solution of Nacl 50 mM were recorded at the level of antennal chaetic sensilla, thus indicating that receptor cells tuned to salts exist on the antennae (de Brito Sanchez et al. 2005).

Interestingly, stimulation with bitter substances such as quinine and salicine did not allow recording any action potential (de Brito Sanchez et al. 2005) at the level of antennal chaetic sensilla despite using different concentrations. However, responses of these sensilla to sucrose solution 15 mM were inhibited upon stimulation with a mixture of sucrose 15 mM and quinine 0.1 mM but not with a mixture of sucrose 15 mM and salicine 0.1 mM (de Brito Sanchez et al. 2005). Such an effect can be explained by considering that amphiphilic molecules such as quinine cross the membrane of the taste cell thus producing inhibition (Koyama and Kurihara 1972). The simplest explanation for this inhibition is that quinine modifies the membrane properties of taste neurons unspecifically (Koyama and Kurihara 1972). This conclusion is reaffirmed by the finding that quinine also inhibits the response of sensilla responding to NaCl 50 mM when delivered in a mixture with NaCl 50 mM (de Brito Sanchez et al. 2005).

In spite of not having found so far bitter receptors at the level of the antennae, there may be other receptor types present thereon, but the number of electrophysiological studies having focused on the sensitivity of antennal sensilla in honeybees is small.

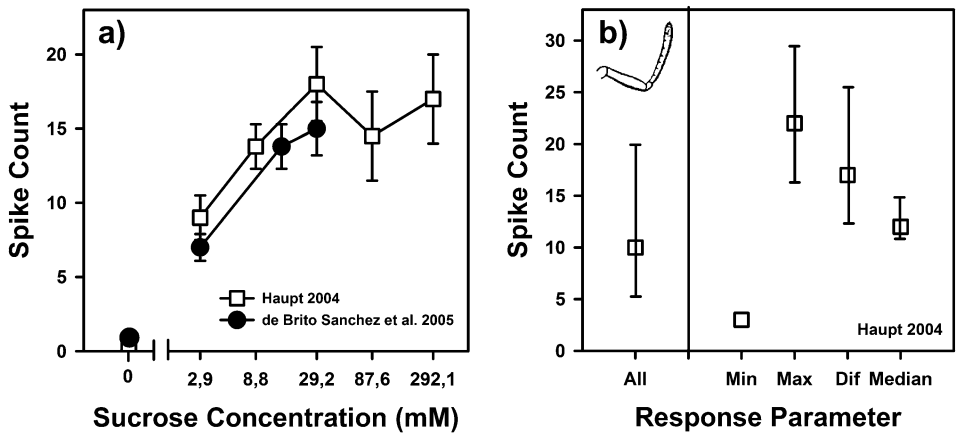

Figure 2 **(a)** Dependency of electrophysiological responses of chaetic sensilla on the antennae on sucrose concentration. The 2 curves correspond to 2 independent experiments (Haupt 2004: 10 sensillae from 6 animals and de Brito Sanchez et al. 2005: 8 sensillae from 4 animals); curves represent the number of spikes in the first 500 ms of stimulation (spike count) as a function of sucrose concentration of the stimulus solution. Error bars represent standard errors of the means. **(b)** Properties of the antennal taste hair population analyzed (225 taste hairs from 45 bees) using the variability of taste hair responses in terms of spike counts in the first 500 ms after stimulus onset measured in different bees during stimulation on a given antenna with 0.1% sucrose (from Haupt 2004). Medians and quartiles are shown. "All" represents responses recorded in the sample of 225 taste hairs from 45 bees. In this sample, it was difficult to determine whether variability was intraindividual or interindividual. Thus, to determine between these options, a different sample was studied in which at least 3 sensilla were recorded on a single antenna in 34 bees. A total number of 161 sensilla were recorded. In this case, "min" and "max" represents the minimum and the maximum number of spikes of a taste hair recorded from each of the 34 antennae, respectively; "diff" the response range, that is, the difference between min and max, "median" the median number of spikes measured in all sensilla recorded from each of the 34 antennae.

Gustatory sensilla on the mouthparts

The mouthparts are the mandibles, maxillae, and the labium (Figure 3a). The maxillae and the labium form the proboscis. Each maxilla is constituted by a broad, flat plate, the stipe, and by an elongated lobe, the galea. A small maxillary palp and a membranous lobe, the inner lacinia, are also present. The labium is made from a small plate, the postmentum, a broad plate, the prementum (together they form the mentum), and a glossa made from inner glossal lobes that have become fused and extended to form the tongue, terminated in a labellum. Small paraglossal lobes surround the base of the tongue; labial palps, together with the galea, surround the tongue to form a food canal groove through which liquids can be sucked up into the mouth. The whole structure is folded against the head when not in use. When extended, ingestion of liquids through the food canal is inversely proportional to their viscosity following Poiseuille's equation (Farina and Núñez 1991). An important consequence of this is that extremely concentrated sucrose solutions—sometimes used to train bees—are not necessarily attractive to foragers due to their high viscosity.

At the base of the mouthparts, the preoral cavity forms a sac where the food is first ingested (Figure 3b). This cavity is divided into frontal and posterior sacs by the central hypopharingeal lobe. Salivary glands open into the posterior sac or salivarium. The preoral cavity is prolonged into the cibarium, a cavity whose muscles in its walls form a suction pump, which facilitates food ingestion through the proboscis. The cibarium continues into the pharynx. At the intersection of both lies the true mouth; from there the food passes into the pharyngeal tube, then into an esophagus, which leads to a crop, whose capacity can reach 60 µl (Núñez 1982).

As mentioned above, sensilla on the mandibles have a unique receptor cell besides a mechanosensory cell. There are no studies implicating these sensilla in taste detection. The proboscis presents many sensilla that have been related to gustatory processes. Electrophysiological studies have focused on the galea of the maxilla (Whitehead and Larsen 1976b). Single-sensilla recordings showed that chaetic sensilla on the galea respond linearly to the log of solute concentrations of sucrose, glucose, fructose, NaCl, KCl, and LiCl but not to CaCl2 or MgCl2, which fail to give consistent

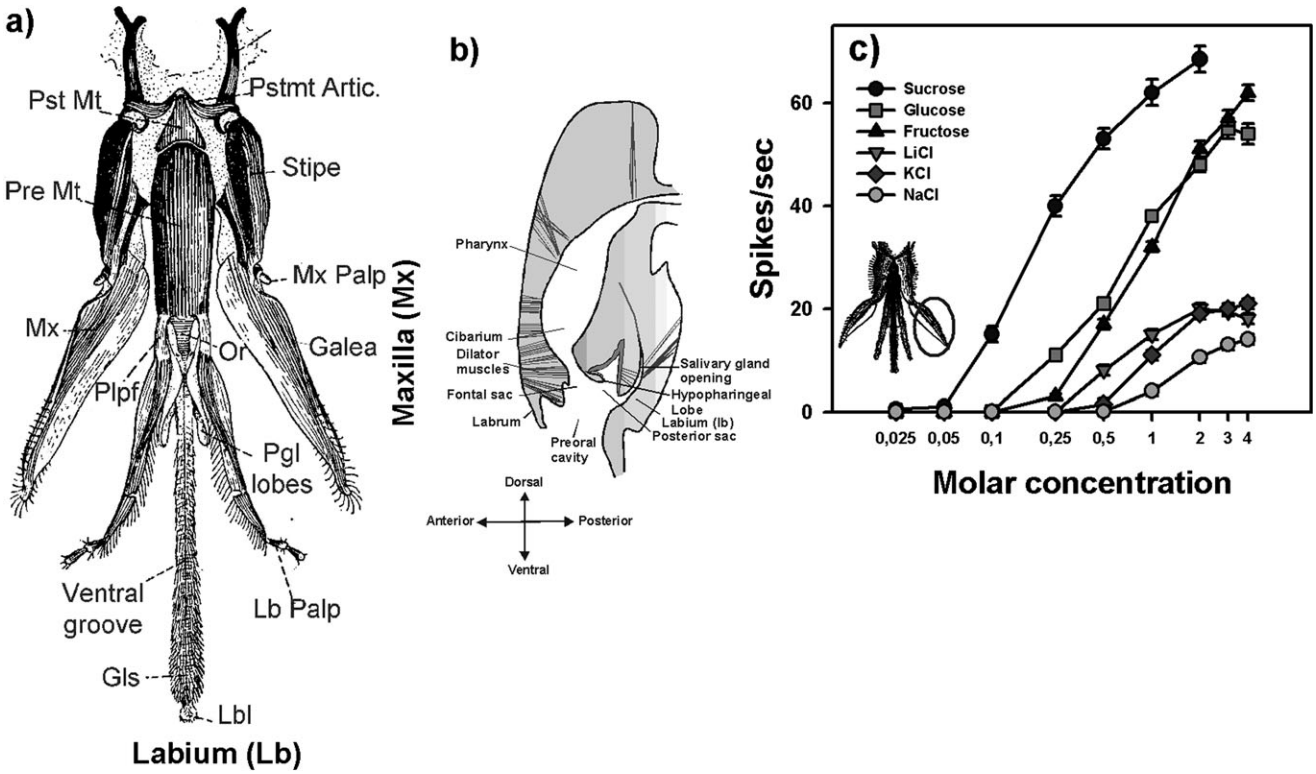

Figure 3 Mouth parts of the honey bee worker. **(a)** Parts forming the proboscis, labium in middle and maxillae at sides, flattened out, ventral view (adapted from Snodgrass 1956). Gls, glossa; Lbl, labellum; Lb Palp, labial palp; Mx, maxilla; Or, salivarium opening; Pgl lobes, paraglossal lobes; Plpf, palpiger; Pre Mt, prementum; Pst Mt, postmentum; Pstmt Artic, postmental articulation. **(b)** Longitudinal section through head of a worker honeybee showing the oral cavities. The food first enters the preoral cavity formed from the labrum and the bases of the mouthparts (e.g., labium); the cavity is divided into a frontal and a posterior sac by the hypopharingeal lobe. Salivary glands open into the posterior sac or salivarium. The preoral cavity continues into the cibarium and then into the pharynx. **(c)** Chaetic sensilla on the galea respond linearly to the solute concentrations of sucrose, glucose, fructose, NaCl, KCl, and LiCl when these are expressed in a logarithmic scale. Points represent the means of the responses from an average of 8 hairs per 10 bees with 2 applications per hair (=160 responses point). Error bars represent 2× standard error of the mean. The inset shows the proboscis; the circle shows the galea where these recordings were made (from Whitehead and Larsen 1976b).

responses (Figure 3c). These sensilla exhibit much higher firing rates for sugar than for salt solutions. Four different spike types can be seen. The first type has the highest amplitude and results from sugar stimulation. The second type has a lower height and occurred in the first 30 s of salt stimulation. A third type with the lowest height appears with those of the second type after prolonged stimulation with KCl. A fourth type with a high amplitude results from mechanical stimulation. It was concluded that from the 5 neurons present in each galeal chaetic sensilla, one is mechanosensory, and the other 4 respond to tastants, one definitely to sugars, and 2 to electrolytes. The gustatory tuning of the fourth cell remains unknown. Whitehead and Larsen (1976b) suggested that this cell may be responsive to proteins (Dethier 1961), amino acids (Shiraishi and Kuwabara 1970; Goldrich 1973; Shimada 1975), "natural foods" (Dethier 1974), or simply glandular secretions. Responses to mechanical stimulation show phasic–tonic characteristics. None of the sensilla tested by Whitehead and Larsen (1976b) exhibited action potentials to water.

At the level of the labium, chaetic sensilla are concentrated on the glossa (see Figure 3a). Each of these sensilla also presents 4 gustatory receptor cells and a mechanosensory cell. Other taste sensilla are located on the distal segments of the labial palps. Chaetic sensilla on these segments were investigated electrophysiologically (Whitehead 1978). Their spike responses correlate with the log of the concentrations of sucrose, glucose, fructose, NaCl, KCl, and LiCl, but not with CaCl2 or MgCl2. The firing rates are higher and thresholds to sugars lower than to electrolytes. None of the sensilla tested exhibited action potentials to water.

Sensilla are also present in the oral cavity. Food entering this cavity contacts approximately 50 to 60 hypopharingeal sensilla, which are located on the basis of the cibarium (Figure 3b). Light microscope observations suggest that these sensilla are innervated by 4 neurons (Galic 1971). Although functional studies on these sensilla have not been performed, they resemble cibarial contact chemoreceptors known from other insects (e.g., blowflies *Calliphora erythrocephala*: Rice 1973; cabbage looper moths *Trichoplusia ni*: Eaton 1979; rice brown planthoppers *Nilapavarta lugens*: Foster et al. 1983). Thus, gustatory receptors in these sensilla would process food before it passes on into the esophagus. These receptors could also sample brood food and solutions regurgitated by worker bees (Goodman 2003).

Gustatory sensilla on the forelegs

Taste sensilla are located on the tarsus and pretarsus of the forelegs (Figure 4a). Sensilla are mostly chaetic and are distributed evenly between the 5 subsegments of the tarsus, with a high concentration on the terminal claw-bearing pretarsus. Chaetic sensilla share similarities with those found in the mouthparts, with a mechanosensory cell ending at their base and 4 cells running to the tip of the shaft (Whitehead and Larsen 1976a). Until recently, practically nothing was known about gustatory sensitivity of these sensilla.

PER can be elicited upon sucrose stimulation of the tarsi, thus indicating that sugar receptors have to be present within tarsal gustatory sensilla. Marshall (1935) found that bees exhibited PER at a concentration of 2.85% when stimulated at the antennae but that a concentration of 34% was required to elicit PER when the tarsi were stimulated. Similar results were found by de Brito Sanchez et al (2008) as they showed that over a wide range of sucrose concentrations sucrose responsiveness is always significantly higher for antennal than for tarsal stimulation. A mechanistic basis for this difference could be found at the level of taste sensilla, existing on the

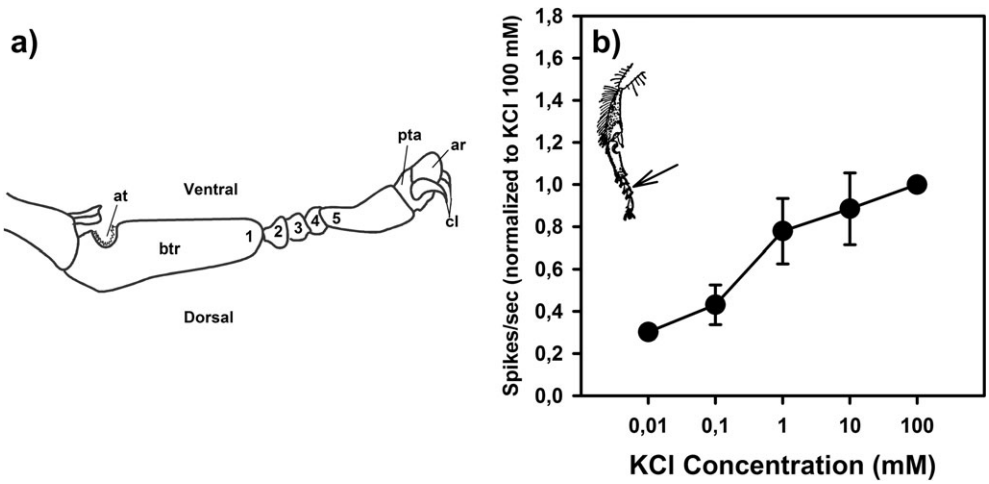

Figure 4 **(a)** The tarsus of a honeybee worker. It consists of 5 tarsomeres (1–5): the longer basitarsus (btr), 3 small tarsomeres, and a larger (5th) tarsomere. The distal pretarsus (pta) presents a pair of claws (cl) on either side of a soft lobe, the arolium (ar). **(b)** Electrophysiological responses (spikes/s) of chaetic sensilla located on the small tarsomeres (6 sensilla from 5 bees; inset: see arrow) stimulated with different concentrations of KCl (mM). Responses were normalized to the response recorded upon stimulation with a solution of KCl 100 mM. Error bars represent standard errors of the means.

antennae and the tarsi. Whitehead and Larsen (1976a) reported 318 chaetic sensilla but no basiconic sensilla on the antennae and 10–20 chaetic sensilla and 0–6 basiconic sensilla per tarsomere of the forelegs. Thus, a simple numeric comparison shows that, at least for chaetic sensilla, the antennae are equipped with 15–30 times more receptors than the tarsi, a fact that could be related to the higher responsiveness for sucrose evinced upon antennal sucrose stimulation. Such a comparison is, however, senseless without an accurate functional characterization of the specificity and sensitivity of tarsal taste receptor cells by means of electrophysiological recordings.

Electrophysiological studies on tarsal sensilla were recently performed by Lorenzo (2009). Dose-dependent responses for sucrose were found, which correspond to the known sucrose sensitivity recorded in behavioral experiments (see above). Contrarily to antennal chaetic sensilla (see above), responses were found for extremely low concentrations of KCl (0.1 mM), thus suggesting that a water cell may exist within chaetic sensilla of the tarsi. Besides, a dose–response curve was obtained for KCl, thus demonstrating the presence of a cell responding to electrolytes (Figure 4b). As for the antennae (de Brito Sanchez et al. 2005), no action potentials to quinine were found.

Molecular studies on honeybee gustation

Since the decoding and publication of the genome of the honey bee (The Honeybee Genome Sequencing Consortium 2006), researchers interested in different aspects of the biology of the honey bee have access to bioinformatics tools that allow performing comparative research using as a model the other insect for which most is known in terms of genetic architecture, the fruit fly *D. melanogaster*. In this way, it was possible to search for similarities and differences at the genomic level in order to understand functional principles of the bee biology. Although the value of the comparison between fruit flies and honey bees is relative due to the absence of genomic information for other hymenopterans or even other primarily nectar-feeding holometabolous insects, no other comparison with more closely related or ecologically similar insects was available in the last years.

Bioinformatic identification of gustatory receptor genes (*Grs*) in the honeybee genome taking as reference the genome of the fruit fly was undertaken as this task was considered straightforward after having previously identified the *Grs* of the fruit fly. Grs are responsible for encoding the molecular gustatory receptors located in the membrane of the gustatory receptor neurons and confer the specificity for a given tastant. In the fruit fly, 68 gustatory receptors encoded by 60 genes through alternative splicing have been identified (Dunipace et al. 2001; Scott et al. 2001; Scott 2005). These encode putative heptahelical 7-transmembrane proteins, but it is not clear whether the resulting gustatory receptors signal through G-protein–dependent second messenger cascades or operate as ligand-gated ion channels (Silbering and Benton 2010). Some of the fruit fly's Grs have been linked to specific gustatory stimuli. For instance, DmGr5a has been associated with sweet taste as it responds to a subset of sugars among which is trehalose and is expressed in most sugar-responsive gustatory receptor neurons (Dahanukar et al. 2001; Ueno et al. 2001; Chyb et al. 2003; Marella et al. 2006; Dahanukar et al. 2007). Similarly, DmGr64a is involved in the detection of a different subset of sugars including sucrose, glucose, and maltose (Dahanukar et al. 2007; Jiao et al. 2007). Both receptor types are capable of mediating response to a subset of sugars independently of the other, and together, they allow identifying sweet food sources.

DmGr66a, on the other hand, has been associated with "taste sensations" that are bitter to humans as it responds to caffeine and its mutation eliminates caffeine-avoidance behavior (Marella et al. 2006; Moon et al. 2006). Similar results (inability to respond to caffeine and to theophyline) were obtained upon mutations in *DmGr93a*, which is coexpressed with *DmGr66a*. Using neurogenetic methods available in *Drosophila*, it has been possible to determine that gustatory receptor neurons expressing DmGr5a respond to a broad spectrum of sweet substances, whereas gustatory receptor neurons expressing DmGr66a respond to a broad spectrum of bitter substances (Marella et al. 2006). Furthermore, other gustatory receptor neurons expressing different Grs exhibit practically the same profile of responses to a variety of sweet or bitter substances. The DmGr5a molecular receptor, reported as a trehalose receptor (Dahanukar et al. 2001; Chyb et al. 2003), is coexpressed with another molecular receptor, DmGr64f, which is broadly required for the detection of most sugars. DmGr64f may also be coexpressed with DmGr64a which appears to be tuned to detect other sugars such as sucrose, glucose, and maltose (Jiao et al. 2008). Thus, combinations of DmGr5a/DmGr64f and DmGr64a/DmGr64f may enhance the spectrum of responsiveness to sugars of a single gustatory receptor neuron. Flies also possess a taste for carbonated water. A population of neurons was identified which detects CO_2 in water and mediates taste acceptance behavior (Fischler et al. 2007).

Bioinformatic identification of gustatory receptor genes in the honeybee genome taking as reference the Drosophila genome yielded a surprising result: only 10 gustatory receptor genes were found (Robertson and Wanner 2006; Figure 5), which was taken as a proof of a rather limited taste repertoire, at least compared with that of fruit flies (see above) and mosquitoes (76 gustatory receptors encoded by 52 genes; Hill et al. 2002). Yet, it is unclear whether all fly or mosquito Grs code for functional gustatory receptors. In any case, from the 10 gustatory receptor genes found in the honey bee, 2 (*AmGr1* and *AmGr2*) seem to correspond to the 8 candidate sugar receptors identified in the fly, based on the role of DmGr5a as a trehalose receptor (see Chyb et al. 2003). The specificity of the other 8 remains to be determined.

The explanation provided by Robertson and Wanner (2006) to account for such a limited number of gustatory receptor genes mentions that bees have little need for gustatory receptors to locate and recognize food because flowering plants have evolved mechanisms to attract and reward bees for pollination services. They argued, in addition, that bees do not require the ability to detect and discriminate between the numerous plant secondary chemicals and toxins usually deployed in the chemical ecological arms races between most plants and many insect herbivores so that there is no need for the bees to develop additional taste receptors. Several additional explanations, other than the one offered by Robertson and Wanner (2006), could be provided to account for the difference in the number of receptor orthologues identified in the honeybee genome from comparison with fruit flies. One hypothesis for why fruit flies and mosquitoes have more gustatory receptors is phylogenetic and posits that their common dipteran ancestor may have undergone gene duplication for several receptors. Yet, as mentioned above, it remains to be determined whether all of them are functional. The chemosensory protein gene families have very different histories in the Diptera, Lepidoptera, and Hymenoptera (Hallem et al. 2006). Another hypothesis is functional; fruit flies regularly assess the degree of substrate fermentation as well as sugar meals so that they may need to track more diverse gustatory stimulants than most hymenopterans. Interestingly, the sequencing of the genome of 2 ant species, the carpenter ant *Camponotus floridanus* and the jumping ant *Harpegnathos saltator*, yielded also a reduced set of *Grs* (Bonasio et al. 2010). For carpenter ants, which forage on nectar sources and other insects, only 11 *Grs* were found, whereas for jumping ants, which are strictly carnivorous and prey on other insects, only 6 *Grs* were reported. Both species differ in their feeding biology from the honey bee. It seems, therefore, that Hymenoptera exhibit in general a reduced set of *Grs* without an obvious link to their feeding habits.

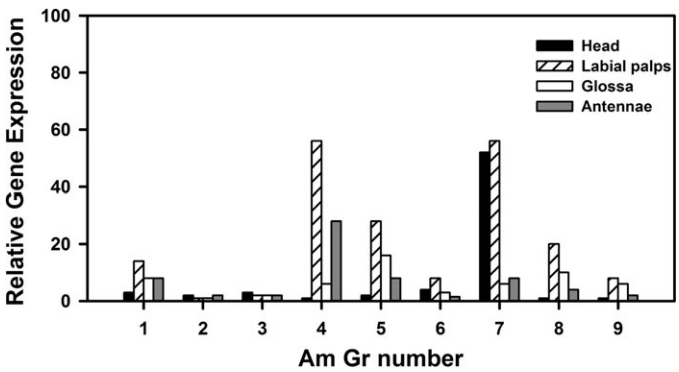

Figure 5 Gustatory gene (*AmGr*) expression in the head, labial palps, glossa, and antennae of honeybees. Expression levels were calculated relative to the body levels. RT-qPCR was used to determine gene expression levels which were normalized to levels of a ribosomal protein S8 found in honeybees (adapted from Robertson and Wanner 2006). *AmGr1* and *AmGr2* have been related to the 8 candidate sugar receptors identified in the fly, based on the role of DmGr5a as a trehalose receptor (see Chyb et al. 2003).

Although no functional study is so far available to determine the tastant specificity of any of the 10 *Grs* of the honey bee, Real-time quantitative polymerase chain reaction (RT-qPCR) and in situ hybridization studies, combined with electrophysiological analyses of receptor sensitivity in heterologous systems could soon provide some answers about their functional value. In this way, a fundamental step toward understanding the gustatory world of honeybees would be achieved.

A limited taste repertoire in honeybees?

The arguments stating that the honeybee gustatory repertoire is very limited could be questioned along several lines. First, a same Gr may encode for different receptor proteins through alternative splicing, thus enhancing the gustatory repertoire of an organism. In other words, although 10 *Grs* have been characterized, these may in fact encode more than just 10 molecular receptors. In particular, it might be that the 2 *Grs* which have been attributed to sweet taste (*AmGr1* and *AmGr2*) may have in fact several splicing forms, which could relate to the bees' capacity to respond behaviorally and electrophysiologically to different kinds of sugars such as sucrose, fructose, maltose, and glucose (von Frisch 1934; Wykes 1952; Whitehead and Larsen 1976b; Whitehead, 1978).

Second, having 10 *Grs* does not necessarily imply an impoverished perceptual world as perceptual richness can be built with relatively few input channels. An example would be the case of color vision where, in the case of trichromats, 3 photoreceptor types allow perceiving an impressive variety of colors. Studying the central coding of gustatory substances at the level of the subesophageal ganglion (SEG) of the honeybee (see below) is therefore crucial to determine whether the simultaneous excitation of few taste receptors generates a complex and rich pattern of taste perceptual sensations.

Third, the expression patterns of 9 of the 10 gustatory receptor genes reported by Robertson and Wanner (2006) are intriguing (Figure 5). Expression was measured through RT-qPCR in the head, the glossa, and the antennae. Other regions of the body that have been consistently associated with taste in bees (e.g., the tarsi; see Goodman 2003) were not included in the analyses. Also, AmGr1 and AmGr2, the sweet receptors that should be abundantly expressed following the arguments on the kind of relationship that bees have developed with plants, are barely expressed in the body parts where they should be definitely present (antennae and glossa, for instance). Other genes, whose specificity is currently unknown (e.g., *AmGr4* and *AmGr7*), are expressed 5–10 times more in the mouth parts, thus raising questions about their specificity.

Fourth, the biology of taste of honeybees is certainly much more complex than just gathering sugars. We have mentioned

above the variety of chemicals that bees experience in gustatory terms along the different phases of their lives: salts, organic acids, lipids, minerals, vitamins, aromatic compounds, proteins, lipids, mineral salts, albumin, vitamins, amino acids, growth regulator factors, folic acid, and enzymes are some of the substances that may be perceived via gustatory input (Harborne 1994).

The case of "bitter" taste perception in honeybees

Probing bitter substances in an ecological context

The argument used to justify the scarceness of bee gustatory receptor genes, stating that bees do not have the ability to detect and discriminate between the numerous plant secondary chemicals and toxins usually employed as defense by some plants, contrasts with behavioral responses of foraging bees to natural nectars and pollens, which may contain phenolic compounds and other secondary compounds such as nicotine and caffeine (Liu et al. 2004, 2006, 2007; Singaravelan et al. 2005). Naturally occurring plants such as *Nicotiana* sp., *Citrus* spp., and *Amygdalus* spp., which present various alkaloids in their nectars, completely depend on bees for pollination (Detzel and Wink 1993; Kretschmar and Baumann 1999; London-Shafir et al. 2003). Concentration of deterrent compounds in nectar and pollen are, however, usually low. For instance, naturally occurring concentrations of amygdalin are between 4 and 10 ppm (London-Shafir et al. 2003), which correspond to 8.75×10^{-6} M and 2.19×10^{-5} M, respectively. Honeybees seem to cope efficiently with this natural range of concentrations. Whereas high concentrations of phenolic substances deter them (Hagler and Buchmann 1993), low concentrations are attractive to them (Liu et al. 2006). Some alkaloid-containing nectars attract bees in the field even when alternative nectar sources are available (Ish-Am and Eisikovitch 1998). For instance, honeybees prefer solutions with low concentrations of nicotine and caffeine over a control (20% sucrose) solution (Singaravelan et al. 2005). A similar but nonsignificant pattern was detected also for all concentrations of amygdalin (Singaravelan et al. 2005). It seems, therefore, that nectars containing substances that are considered deterrent due to their unpalatable taste are in fact preferred by honeybees although if concentrations of such substances are too high, nectars may be rejected. This finding shows that considering bitter substances as straightforward aversive unconditioned stimuli, eliciting spontaneous aversion, is incorrect. Preference or aversion may also depend on the resources that are effectively available to bees. Tan et al. (2007) investigated feeding preferences and mortality of worker bees supplied in cages with a diet of *Tripterygium hypoglaucum* honey. Honey could contain triptolide, a toxic compound, mixed with sugar powder or sugar powder only. Mortality induced by the former treatment within 6 days was high (68%), whereas it was significantly lower (16%) with the latter treatment. Freely flying bees preferred the feeders with normal honey to those with toxic honey. However, when the feeder of normal honey was removed, leaving only the toxic one, bees accepted it and increased their visiting frequency and drinking time until reaching values previously recorded for the normal honey. Toxic honey thus became acceptable to the bees in the absence of other nectar sources (Tan et al. 2007). This observation may be related to Karl von Frisch's statement on honeybee's reactions toward bitter substances (von Frisch 1967). He wrote that "bees are much less sensitive to bitter substances than we" and that "it is possible to contaminate sugar with a bitter substance that does not interfere with its being taken up by bees but that renders it unacceptable to man." As we will see, sensitivity or lack of it with respect to aversive compounds may depend not only on what is available to forager bees, as shown by field experiments reported above but also on the specific experimental context used to probe the bee's taste detection capabilities.

Probing bitter substances in the laboratory in restrained honey bees

The selective behavior exhibited by bees toward deterrent compounds, which may be of avoidance or of preference depending on the circumstance, suggests that contrarily to what has been argued to justify the reduced number of Grs in honey bees, these insects might able to taste the presence of these different secondary compounds in nectars in order to improve their foraging efficiency. Yet, experiments in the laboratory with harnessed honeybees as well as electrophysiological investigations on different body appendages could not so far support this conclusion (Ayestaran et al. 2010).

On one hand, electrophysiological recordings of taste sensilla performed at the level of the antennal tip (chaetic sensilla; de Brito Sanchez et al. 2005), mouth parts (chaetic and basiconic sensilla on the galea, labial palps and glossa; de Brito Sanchez, unpublished data), and distal segments of the forelegs (chaetic sensilla; Lorenzo 2009) could not reveal sensilla that respond specifically to the bitter substances quinine and salicine at the different concentrations tested. Depending on the appendages considered, other deterrent substances were also assayed with the same result. The fact that electrophysiological responses of chaetic sensilla to sucrose solution are inhibited by stimulation with a mixture of sucrose and quinine suggests that a honeybee could eventually detect the presence of quinine solution due to its peripheral, within-sensillum inhibitory effect on sugar receptor cells (de Brito Sanchez et al. 2005; see above). Yet, mixtures of sucrose with other bitter substances such as salicine did not yield the same inhibitory effect.

Behavioral experiments with harnessed bees in the laboratory could not show that substances whose taste is bitter to

humans have an unpalatable taste for bees. Neither quinine nor salicine inhibited the PER elicited by previous antennal stimulation with sucrose solution when delivered at the level of the antennae at different concentrations (de Brito Sanchez et al. 2005). Similar results were obtained when quinine, salicine, and caffeine when delivered at the level of the tarsi (Lorenzo 2009). Focusing on the mouth parts showed that harnessed bees that extended the proboscis when stimulated on the antennae with sucrose and that received different concentrations of quinine or salicine on the mouth parts upon PER retracted the proboscis only in few cases (20%) and only if a fully saturated bitter solution was used (e.g., quinine 100 mM) which is unnatural for bees and unacceptable to human taste (de Brito Sanchez et al. unpublished data). In these experiments, sucrose solution and bitter substance were not mixed but delivered separately. Thus, the above-mentioned inhibitory effect of quinine on sucrose receptors upon stimulation with mixtures of sucrose and quinine does not account for the responses observed. A massive retraction of the proboscis—which was never observed—would be predicted in the case of such an inhibitory effect.

Further behavioral experiments showed, nevertheless, that pairing aversive substances with an odor retards learning of this odor when it is subsequently paired with sucrose (Ayestaran et al. 2010). In other words, having associated an odor with quinine 100 mM, salicine 100 mM, or a highly concentrated saline solution such as NaCl 3 M affects negatively the bees' ability to associate afterward this odor with sucrose reward (Figure 6a). This result was intriguing because it indicated that deterrent compounds had yet an aversive effect despite the lack of obvious rejection evinced in previous behavioral experiments on harnessed bees. It was therefore suggested that such compounds do not exert an aversive effect via a distasteful sensory experience but rather through a postingestional malaise-like state (Ayestaran et al. 2010). Indeed, it was shown that harnessed honey bees in the laboratory ingest without reluctance a considerable volume (20 µl, i.e., one-third of their crop capacity; Núñez 1982) of various aversive substances, including concentrated saline solutions and substances that taste bitter to humans, even if some of them induce a high postingestional mortality and affect, therefore, their probability of survival (Figure 6b). These substances do not seem, therefore, to be unpalatable to harnessed bees, but they induce a malaise-like state that in some cases results in death (Ayestaran et al. 2010). Consistently with this finding, bees having learned that one odor is associated with sugar, and experiencing in a subsequent phase the malaise induced by the aversive substance (devaluation phase), exhibit reduced responsiveness to the odor and the sugar. Such stimulus devaluation can be accounted for by the malaise-like state induced by the aversive substances ingested and resembles conditioned taste aversion as shown in rodents (Reilly and Schachtman 2009). Taken together, these results indicate that bitter substances as well as concentrated saline solutions generate a postingestional malaise in harnessed bees, which do not seem to react in an obvious way to their unpalatable taste. At the sensory level, harnessed bees have exhibited so far, in different experiments, "a reduced ability" for sensing bitter substances. Postingestional malaise due to these substances can, on the other hand, exert a reinforcing effect and thus affect learning processes (Ayestaran et al. 2010). This

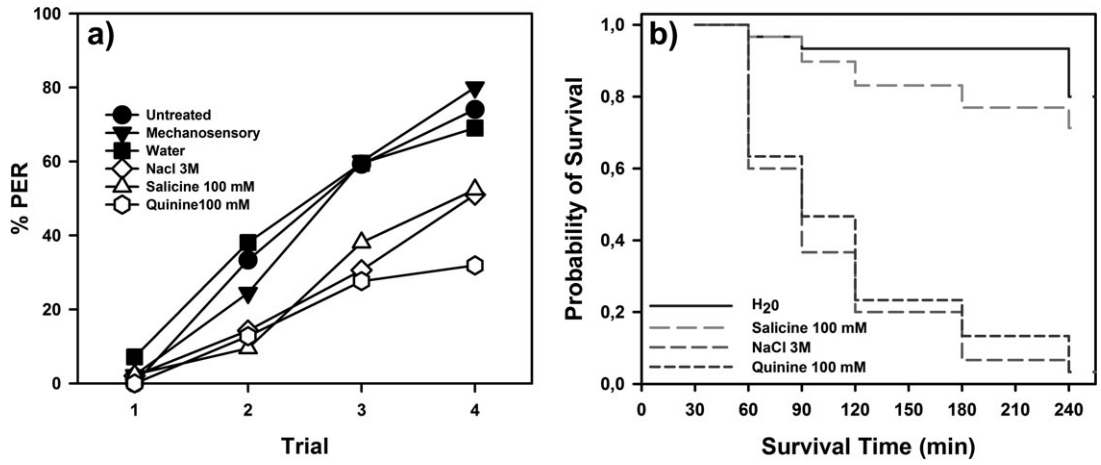

Figure 6 **(a)** Effect of preexposure to aversive substances on olfactory appetitive learning in harnessed honeybees. The graph shows the performance (% of proboscis extension responses or PER) of honey bees during 4 trials of appetitive olfactory conditioning in which the odor 1-nonanol was paired with sucrose 1 M. Prior to this conditioning phase, bees were pre-exposed to 1-nonanol paired either with a mechanosensory stimulus (n = 45), distilled water (n = 42), NaCl 3 M (n = 49), salicine 100 mM (n = 42), or quinine 100 mM (n = 47). The untreated group (n = 54) was not pre-exposed. Bees having experienced NaCl, salicine, and quinine showed lower acquisition than the other groups (water, mechanosensory, and untreated). No significant differences in acquisition were found between bees of the untreated, mechanosensory, and water group (adapted from Ayestaran et al. 2010). **(b)** Kaplan–Meier curves of survival for harnessed honeybees following feeding of aversive compounds. The probability of survival differed significantly between groups. The group of honeybees having ingested NaCl 3 M (n = 30) and quinine 100 mM (n = 30) exhibited a significant decrease of their survival probability compared with the distilled water group (n = 30). The group having ingested salicine 100 mM (n = 30) had intermediate mortality levels (adapted from Ayestaran et al. 2010).

difference underlines the necessity of distinguishing between the sensory effects of tastants (i.e., how do they affect feeding responses) and their reinforcing properties (i.e., how do they affect learning and memory processes) (Schipanski et al. 2008).

Probing bitter substances in the laboratory in freely-flying bees

Is this the whole story for bitter taste perception in honey bees? Certainly not. A new twist into this story has been introduced by recent behavioral experiments that, contrarily to the previous ones, used freely flying honey bees (Avarguès-Weber et al. 2010). In this case, it was studied whether discrimination of similar colors by freely flying honey bees trained to a Y-maze is improved by pairing the rewarded color (the target) with sucrose solution, as usual, and by associating the alternative color (the distracter) either with 60 mM quinine solution or with water. These experiments were based on previous reports showing that for freely flying bumblebees foraging on an artificial arena with feeders which presented sucrose solution or quinine (Chittka et al. 2003; Dyer and Chittka 2004a, 2004b), bees chose more efficiently the feeders rewarded with sucrose if the ''negative'' (nonrewarded) feeders presented quinine instead of plain water. In the recent experiments with honeybees, Avarguès-Weber et al. (2010) also showed that the presence of quinine solution on a visual distracter promoted its rejection, thus improving discrimination of perceptually similar stimuli. If plain water was associated to the distracter, however, discrimination was not possible given the high perceptual similarity between target and distracter. In other words, a difficult visual discrimination was rendered possible by the penalizing, aversive effect of the concentrated quinine solution (60 mM) experienced by freely flying bees (Avarguès-Weber et al. 2010). Interestingly, quinine had no effect if the colors were perceptually different. In this case, such a difference was sufficient for the bees to learn the discrimination without the contribution of the penalizing effect of quinine.

Freely flying bees did not use remote cues to detect the presence of quinine solution. Measuring drinking times showed that the aversive effect exerted by this substance was mediated via a gustatory input, that is, via a distasteful sensory experience, rather than via a postingestional malaise (Avarguès-Weber et al. 2010). Note, however, that the concentration of quinine solution used in these experiments (60 mM) is far from being ecologically relevant as it was 2–3 orders of magnitude higher than natural concentrations of deterrent substances in nectar (Singaravelan et al. 2005). Its experimental use was nevertheless justified as a tool to uncover the real visual discrimination abilities of honeybees.

The results of these experiments with freely flying bees show a surprising difference with the responses exhibited by harnessed bees in the laboratory for which the same quinine solution does not seem to have an unpalatable effect (Ayestaran et al. 2010; see above). It therefore appears that the critical aspect for uncovering the aversive nature of a bitter compound is the possibility of freely moving that was available in one case (Avarguès-Weber et al. 2010) but not in others (de Brito Sanchez et al. 2005; Lorenzo 2009; Ayestaran et al. 2010). In the laboratory, bees are harnessed in individual metal tubes, which is the common procedure to test their sucrose responsiveness and/or learning in olfactory conditioning of the PER (see Giurfa 2007 for review). In these experimental conditions, harnessed bees do not show an aversion for even higher concentrations of quinine solution than that used by Avarguès-Weber et al. (2010) (see above). They even imbibe large amounts (20 µl) of different aversive solutions even if the solutions drank turn to be toxic and induce postingestional mortality (Ayestaran et al. 2010).

A crucial difference between both experimental contexts is the possibility to express an active avoidance of the aversive reinforcement. When bees are in contention, the impossibility to move may induce important changes in acceptance or rejection thresholds for gustatory compounds making them more tolerant to substances that they would otherwise reject, even at the cost of the own death. This hypothesis is not farfetched given that harnessed and freely flying bees exhibit striking differences in other performances such as color learning and discrimination. Experiments with freely flying bees have shown that the $\Delta\lambda$ discrimination function (i.e., the function accounting for the bees' wavelength discrimination along their visual spectrum) varies depending on the region of the spectrum. It reaches extremely low values of 4.5 nm (i.e., very fine discrimination performances) for wavelengths at the intersection of photoreceptor sensitivity curves (von Helversen 1972). On the contrary, harnessed bees in the laboratory, which can be trained to associate a color with sucrose reward and which extend their proboscis to the learned color, have difficulties in learning this association and show very poor color discrimination abilities (Niggebrügge et al. 2009). This difference may be motivational as to learn colors in harnessed conditions it is necessary to cut the bees' antennae (Hori et al. 2006). This procedure substantially decreases the subjective value of sucrose as a reward (de Brito Sanchez et al. 2008), thus impairing learning. The important conclusion that can be derived from these experiments is that concluding that bees have extremely poor color discrimination capabilities based solely on the laboratory experiments with harnessed bees would be a mistake. Similarly, we need to contemplate the possibility that in another experimental scenario, with bees that freely express their choices and avoidance behaviors, the effect of aversive compounds may be different. If this were the case, the fundamental goal to reach would be to determine the kind of physiological switch changing acceptance or rejection thresholds for aversive substances once bees are immobilized.

Central processing of taste

In the honeybee, as in other insects (Mitchell et al. 1999), primary projections of taste neurons on head appendages reach the central nervous system mostly at the level of a structure called the SEG. (Figure 7a–c). Besides motor control of the mouthparts and mechanosensory information processing, gustatory processing is one of the major roles of the SEG. The SEG results from the fusion of the mandibular, maxillary, and labial neuromeres. These are arranged sequentially with the mandibular neuromere being anterior and the labial posterior (Figure 7c). The more anterior mandibular and maxillary neuromeres successively decrease in volume compared with the posterior labial neuromere. Eight longitudinal tracts run through each half of the ganglion. Dorsal and ventral commissures have been described for the 3 different neuromeres (Rehder 1988).

Axons of gustatory neurons and mechanosensory neurons hosted in gustatory sensilla project to the mandibular, maxillary, and labial neuromeres via the mandibular nerve, the labial nerve, and the maxillary nerve, respectively (Rehder 1988). Projections of gustatory and mechanosensory neurons hosted in gustatory sensilla on the antennae also project to the SEG (Pareto 1972; Suzuki 1975; Haupt 2007). Mechanosensory and gustatory neurons project to different regions of the SEG. Sensory projections from the proboscis are confined to the ventral portions of the maxillary and labial neuromeres of the SEG, overlapping with the arborizations of neurons of the subesophageal calycal tract (SCT). The SCT links the ventral SEG to the calyces of the mushroom bodies (Schröter and Menzel 2003), suggesting that these important structures in the bee brain receive also mechanosensory and/or gustatory input from the SEG.

The first-described ventral unpaired median neuron of the maxillary neuromere (VUMmx1; Figure 7d) has been characterized in great detail, both at the physiological and morphological levels (Hammer 1993, 1997). Its cell body lies in a median position within the ventral cell cluster of the SEG and its primary neurite innervates the antennal lobes, the lateral horn, and the lip and basal ring of the mushroom bodies, all key structures of the bee olfactory circuit (Figure 7d). Such a neural connectivity and the fact that VUMmx1 is activated upon sucrose stimulation of the antennae and proboscis led to the hypothesis that VUMmx1 mediates the rewarding properties of sucrose. VUMmx1 stimulation does not lead to proboscis extension. Yet, activity of this neuron has been found to be sufficient to mediate the reward in olfactory conditioning (Hammer 1993). In other words, pairing of an odorant with an artificial depolarization of VUMmx1 generated by injecting current into the neuron is the equivalent of having experienced an odorant followed by sucrose. As a consequence, a bee treated in this way learns to respond with a PER to the odorant even if it had never experienced real sucrose associated to it. How gustatory sucrose receptors convey information to VUMmx1 is still unknown, but it is thought that they project to the SEG where they would synapse directly or indirectly onto VUMmx1. Given that VUMmx1 is an octopaminergic neuron, in a further study, local octopamine injections into the antennal lobes or the mushroom body did also substitute for sucrose reward during olfactory conditioning (Hammer and Menzel 1998); accordingly, downregulation of the octopamine receptor through RNAi technique reduces olfactory learning (Farooqui et al. 2003).

In the central ventral portion of the SEG, Schröter et al. (2007) identified 10 different VUM neurons, 6 of which innervate neuropile regions of the brain and the SEG exclusively (central VUM neurons) and 4 with axons in peripheral nerves (peripheral VUM neurons). They are putatively octopaminergic and therefore might be involved in octopaminergic modulation of behavior. Central VUM neurons innervate the antennal lobes, the protocerebral lobes (including the lateral horn), and the mushroom body calyces. Among these neurons, a neuron termed VUMmd1 whose soma lies in the mandibular neuromere exhibits the same branching pattern in the brain as VUMmx1 and responds to sucrose and odors in a similar way. However, no experiment has been so far performed to show that, like VUMmx1, it can also substitute for reward in PER olfactory conditioning. Peripheral VUM neurons innervate the antennal and the mandibular nerves, thus suggesting that they receive gustatory and mechanosensory input from antennae and mandibles. VUM neurons as recorded by Schröter et al. (2007) not only responded to sucrose; in some cases, they responded to water and salt thus making the question of taste encoding in the bee brain even more complex. Interestingly, the anatomical pendant of VUMmx1 has been found in the brain of *Drosophila* larvae (Thum A, personal communication) and adults (Busch et al. 2009) as well as in moths *Manduca sexta* (Dacks et al. 2005). In both cases, the soma is located in the SEG, medioventrally at the midline, and the neuron innervates the antennal lobes, lateral horns, and calyces of the mushroom bodies. Functional studies are missing in these insects to determine whether or not these neurons also mediate the reinforcing properties of appetitive reward in olfactory learning as shown for the honeybee (Hammer 1993).

Other neurons in the central nervous system of honeybees exhibit significant responses upon antennal and proboscis stimulation with sucrose. For instance, the PE1 neuron (Mauelshagen 1993), a neuron arising from the peduncle of the mushroom bodies and which has extensive arborizations in the median and lateral protocerebrum, exhibits increased spiking activity upon sucrose stimulation; yet, this neuron also responds to odors and mechanical stimulations, and no other tastants have been assayed to determine its gustatory tuning so that its role in gustatory coding is unclear. The same applies to the so-called feedback neurons (Grünewald 1999), which connect the output regions of the mushroom bodies (alpha and beta lobes, and peduncle)

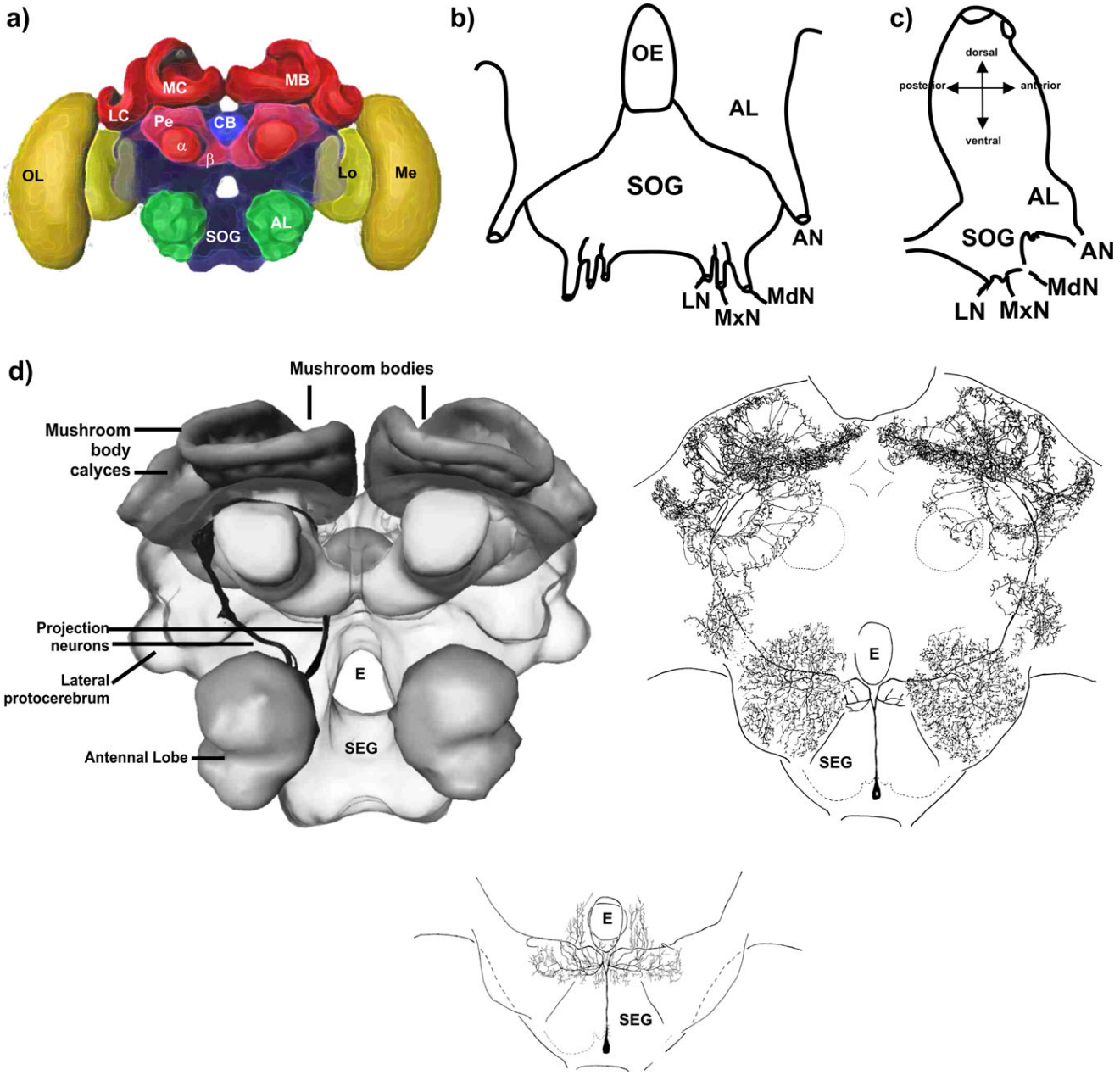

Figure 7 **(a)** The 3D reconstruction of the honeybee brain in frontal view showing the SEG. AL, antennal lobe; CB, central body; OL, optic lobes; Lo, lobula; Me, medulla; MB, mushroom bodies; MC, median calyx; LC, lateral calyx; Pe, peduncle; α,β, alpha and beta lobes (from Rybak et al. 2010). **(b)** Schematic frontal view of the SEG region showing the afferences of the labial nerves (LN), the mandibular nerves (MdN), and the maxillary nerves (MxN). AN, antennal nerve; OE, esophagus. **(c)** Side view of the brain showing the SEG. A transverse section is along the dorsoventral axis, a horizontal section along the anterior–posterior axis. **(d)** The VUMmx1 neuron (ventral unpaired median cell of the maxillary neuromere (courtesy of R. Menzel). Left: 3D reconstruction of the honeybee brain in frontal view without the optic lobes, showing the main stages of the olfactory circuit: antennal lobes, lateral protocerebrum, and mushroom bodies (via projection neurons). Right: Morphology of VUMmx1 showing the connectivity with the key stages of the olfactory circuit: antennal lobes, lateral protocerebrum, and lips and basal rings of the mushroom body calyces. Bottom: In the SEG, the primary neurite projects dorsally from the ventral median soma and bifurcates beyond the esophagus (E). Dendritic arborizations occur in the dorsal SEG and tritocerebrum. This figure appears in color in the online version of *Chemical Senses*.

with their ipsilateral input region (ipsilateral calyx). These neurons also respond to odors and sucrose stimulation, but as for the PE1 neuron, these responses reflect the multimodal and integrative nature of mushroom bodies, from which they take the information, rather than providing a precise gustatory code.

So far, no systematic study has tried to uncover whether there are organizational functional principles in the architecture of the honeybee SEG. Yet, a comparative analysis focusing on other animals may be enlightening. In mammals, recent studies performed on central processing of taste have provided a clearer picture in which an organized form

of taste representation seems to be present in the gustatory cortex (Accolla et al. 2007). Imaging this brain region upon gustatory stimulation in rats showed that the 4 different "taste qualities" tested (salty, sour, sweet and bitter) are represented by specific spatial patterns containing both distinct and overlapping regions. Quantifying the overlap between different taste representations allowed to see the emergence of 2 groups of stimuli, related to what can be defined as the hedonic (i.e., palatable vs. non-palatable) value of the stimulus itself. Higher overlap values were found between NaCl and sucrose, associated with good nutrients, or between quinine and citric acid, associated with noxious substances (Accolla et al. 2007). This suggests a possible representation of taste in terms of their hedonic value in the gustatory cortex as common activity patterns were shared by attractive stimuli, whereas different common patterns were shared by aversive stimuli (Accolla et al. 2007).

Interestingly, similar conclusions were reached in the fruit fly, in which projections of gustatory receptor neurons were identified at the level of the SEG (Marella et al. 2006). It was found that neurons expressing Gr5a, involved in sweet detection, project laterally and anterior to projections of neurons expressing Gr66a, involved in bitter detection. Marella et al. (2006) concluded that there is a spatial activity map of different taste modalities in the fly brain that corresponds to the anatomical projections of Gr5a and Gr66a receptor neurons, thus segregating taste sensations according to their palatable versus nonpalatable nature. This conclusion has to be considered with caution: although the spatial segregation of projections of receptor neurons seems to support the hedonic representation hypothesis, it has to be underlined that such a spatial segregation refers to the receptor neuron level but not to second-order neurons, which may impose different forms of gustatory processing.

In that sense, electrophysiological studies performed in the desert locus *Schistocerca migratoria* (Newland 1999; Rogers and Newland 2002) provide fundamental information as they reported how tastants detected by gustatory receptor neurons on the hind legs are encoded by a population of interneurons of the metathoracic ganglion (MG). Rogers and Newland (2002) focused on spiking interneurons located in the midline of the MG and analyzed their responses upon stimulation of gustatory receptor neurons of the locust hind leg with various tastants. These interneurons responded differently to various tastants such as NaCl, water, sucrose, and nicotine hydrogen tartrate (NHT) thus showing that there is convergence of a large number of taste qualities onto the same interneurons (Rogers and Newland 2002). Furthermore, the response durations of these interneurons were a function of chemical identity and concentration. The 7 interneurons recorded responded highly to the deterrent substances NHT and NaCl at a high concentration (250 mM) while showing low responding to attractive sucrose and water. Rogers and Newland (2002) proposed that rather than establishing chemical identity, the duration of response to different chemicals provides a direct measure of aversiveness because the relative size of the neuronal response of spiking local interneurons and motor neurons correlates strongly with behavioral withdrawal responses. Thus, local circuits in the MG mediate motor responses that differentiate between acceptable and unacceptable tastants, a conclusion which again underlines the idea of a central representation of taste in terms of the tastants' hedonic value.

This idea is, however, not so clear in the moth *Heliothis virescens* where intracellular recordings of single neurons in the SEG have revealed a large diversity of neurons responding with varying tuning breadth to sucrose, quinine, water, and mechanosensory stimuli applied to the antennae, proboscis, and right tarsus (Kvello et al. 2010). Responses recorded suggest a population coding mechanism in which information is represented by distinct activity patterns in partly overlapping populations of SEG neurons. With just one appetitive (sucrose 1 M) and one aversive stimulus (quinine hydrochloride 0.1M) tested, it is difficult to determine whether or not a spatial form of hedonic coding can be found in the SEG of the moth *H. virescens*. In this case, as for the other insect models discussed, including the honeybee, multielectrode recording techniques, allowing to measure populational codes upon gustatory stimulation, could represent an important endeavor to decipher the principles of central gustatory processing.

Conclusion/future directions

Research on honeybee gustation is still in its infancy compared with the impressive progress that has been done in the last decades to understand, for instance, honey bee vision and olfaction. Yet, important progress has been made in the last years even if these remain limited in number. A fundamental advance has been the sequencing of the honeybee genome which allowed determining that honey bees possess, in principle, 10 gustatory receptor genes (Robertson and Wanner 2006). Yet, the gustatory tuning of the molecular receptors encoded by these gustatory receptor genes remains unknown. Research should therefore concentrate on determining the natural ligands of these receptors in order to understand the gustatory world of a honeybee. In doing this, comparative analyses between workers, drones, and queens should be performed. Different casts may express different taste receptors as a consequence of their different gustatory environments. This argument can be extended to honey bee foragers, which can also exhibit important variation in gustatory receptors depending on their specialization (i.e., pollen vs. nectar foragers). Furthermore, exploring the gustatory world of honeybee larvae is also a pending task.

Furthermore, the presence and potential gustatory role of other classes of molecular receptors should also be explored. Recently, DmX, a gustatory receptor that has partially diverged from the metabotropic glutamate receptor family and is not related to the Gr family, has been characterized in the fruit fly

(Mitri et al. 2009). This receptor is tuned to detect a natural toxic molecule, L-canavanine, and is expressed in bitter-sensitive gustatory receptor neurons, where it triggers the premature retraction of the proboscis, thus leading to the end of food searching and food aversion. Also, another class of receptors has been recently discovered in the fruit fly, the ionotropic receptors (IRs) (Benton et al. 2009), which are expressed in appendages where olfactory but also gustatory receptor neurons are located. It has been proposed that IRs constitute a novel family of chemosensory receptors and their role in gustation cannot be excluded (Benton et al. 2009). Whether these receptor types (DmX and IR like) exist in the honeybee and whether they intervene in gustatory processes remains to be determined.

Molecular receptors are hosted by gustatory sensilla and even if there has been some electrophysiological works to characterize taste processing at the level of these sensilla, one has to admit that studies on peripheral processing are extremely scarce. It has to be underlined that from the 2 typical gustatory sensilla, chaetic and basiconic, electrophysiological research on honey bee gustation using single-sensilla recordings has only analyzed neuronal responses of receptors hosted in chaetic sensilla. Basiconic sensilla have not been recorded, probably due to their reduced size, which renders investigations more difficult than those that can be achieved on chaetic sensilla. Recording from basiconic sensilla should thus be achieved in a systematic way. Otherwise, peripheral analyses on honeybee gustation represent only a partial view of what honey bees could detect in gustatory terms.

An important endeavor will be to determine the kind of processing occurring at the central level as no perceptual phenomenon, in this case taste perception, can be directly derived from receptor responses. A combination of intracellular recordings of single neurons and populational recordings using, for instance, multielectrode techniques already applied successfully to analyze olfactory processing in honey bees (Denker et al. 2010) could open a promising research avenue toward understanding how the bee brain encodes and classifies taste.

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