Two Antagonistic Gustatory Receptor Neurons Responding to Sweet-Salty and Bitter Taste in *Drosophila*

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ABSTRACT: In *Drosophila*, gustatory receptor neurons (GRNs) occur within hair-like structures called sensilla. Most taste sensilla house four GRNs, which have been named according to their preferred sensitivity to basic stimuli: water (W cell), sugars (S cell), salt at low concentration (L1 cell), and salt at high concentration (L2 cell). Labellar taste sensilla are classified into three types, l-, s-, and i-type, according to their length and location. Of these, l- and s-type labellar sensilla possess these four cells, but most i-type sensilla house only two GRNs. In i-type sensilla, we demonstrate here that the first GRN responds to sugar and to low concentrations of salt (10–50 m*M* NaCl). The second GRN detects a range of bitter compounds, among which strychnine is the most potent; and also to salt at high concentrations (over 400 m*M* NaCl). Neither type of GRN responds to water. The detection of feeding stimulants in i-type sensilla appears to be performed by one GRN with the combined properties of S + L1 cells, while the other GRN detects feeding inhibitors in a similar manner to bitter-sensitive L2 cells on the legs. These sensilla thus house two GRNs having an antagonistic effect on behavior, suggesting that the expression of taste receptors is segregated across them accordingly. © 2004 Wiley Periodicals, Inc. J Neurobiol 00: 000–000, 2004

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INTRODUCTION

For most animals, taste and smell are essential for detecting food, predators, mates, and noxious stimuli in their environment. Although olfaction allows them to discriminate between a large number of different odors and in different combinations, taste is a more elementary sense. Psychophysical studies on humans have indicated that taste sensations belong to five categories, sweet, bitter, sour, salty, and umami. This is difficult to reconcile with electrophysiological studies on mammals that show individual taste receptor cells respond to multiple taste qualities (Kimura and Beidler, 1961; Caicedo and Roper, 2001; Gilbertson et al., 2001; Caicedo et al., 2002). Recent molecular studies, however, suggest that receptors for sweet and bitter taste are expressed in different cells (Hoon et al., 1999; Adler et al., 2000; Nelson et al., 2001; Zhang et al., 2003) but the link between central neural coding and the peripheral sensitivity of individual taste cells remains elusive.

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Insects provide a simple and attractive model in which to study the neural coding mechanism of taste (for reviews, see Chapman, 2003; Rogers and Newland, 2003; Ishimoto and Tanimura, 2004). Unlike with vertebrates, taste transduction is performed by bipolar nerve cells called gustatory receptor neurons (GRNs). In insects, several GRNs are held within bristles called taste sensilla, which have a pore on the tip and are located on the mouth parts, the legs, the wing margins, and some parts of the thorax and the abdomen (for review, see Chapman, 2003). This feature enables us to easily record the neural activity originating from a single GRN (Hodgson et al., 1955). The GRNs are usually designated according to the type of compounds that stimulates them, that is, sugars, amino acids, salts, or bitter compounds, although it has been shown that many insects partly hold GRNs responding to more than one class of compounds (Chapman, 2003).

In *Drosophila*, four GRNs have been described according to their sensitivity to basic stimuli (hereafter called "qualities"), i.e. water (W cell), sugars (S cell), salts at low concentration (L1 cell), or salts at high concentration (L2 cell) (Siddiqi and Rodrigues, 1980; Fujishiro et al., 1984; Wieczoreck and Wolff, 1988). These sensitivity profiles, however, are not shared among all taste sensilla, as has recently been shown for leg taste sensilla lacking sensitivity to one of those stimuli (Meunier et al., 2000), or housing L2 cells sensitive to bitter compounds as well as to salts at high concentration (Meunier et al., 2003b). A putative pheromone receptor expressed in some male-specific sensilla of the legs has also been reported (Bray and Amrein, 2003). Furthermore, putative gustatory receptors (*Gr*s) are expressed in single GRN within small subsets of taste sensilla (Clyne et al., 2000; Dunipace et al., 2001; Scott et al., 2001). This means that each GRN might express one of different sensitivities according to its situation within a sensillum and its location on the body. Further knowledge of peripheral coding of *Drosophila* is therefore needed to take advantage of the genetic tools available.

On the labellum, taste sensilla are classified into three types, l-, s-, and i-type, according to their length and location. Five out of 10 *Gr* genes are mainly expressed in s-type or i-type sensilla but not in l-type sensilla as monitored by the GAL4/UAS system (Hiroi et al., 2002). As in larger flies, most electrophysiological experiments in *Drosophila* have been done on l-type sensilla, and no difference in sensitivity has been reported between them. To fill this gap, we examined these taste sensilla, focusing on i-type sensilla. Although most taste sensilla in *Drosophila* house four GRNs, i-type sensilla are unique in that they house only two GRNs (Stocker, 1994; Shanbhag et al., 2001). Given the reduced numbers of neurons in these sensilla, we asked if the GRN in i-type sensilla would express a subset of the four qualities (W, S, L1, and L2), found in other sensilla or express a new combination of them.

Our results on i-type sensilla indicate that one GRN responds to sugar and salt at low concentration, and the other responds to bitter compounds and salt at high concentration. Thus, i-type sensilla house two antagonistic GRNs: one sensitive to phagostimulatory compounds while the other is sensitive to deterrent compounds.

METHODS

Fly Stocks

Strains of *Drosophila melanogaster* were maintained on a standard cornmeal-glucose agar medium at 25°C. One-day-old flies were fed on a fresh medium for 1 day before experiments.

Chemicals

KCl, NaCl, sucrose, strychnine nitrate, salicin, and berberine sulfate trihydrate were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Trehalose, glucose, fructose, caffeine, aristolochic acid, and denatonium benzoate were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Quinine hydrochloride was purchased from Tokyo Kasei Chemicals Co. (Tokyo, Japan). All compounds were dissolved in a 1-m*M* KCl solution prepared using distilled water and stored at −20°C. Solutions for stimulation were stored at 4°C for less than 1 week.

Electrophysiology

Canton-S was used as a wild-type for electrophysiology. A fly was secured at the tip of an electrically grounded glass capillary filled with *Drosophila* Ringer solution inserted from the abdomen through to the head. The proboscis was fixed at the base of a labellum using lanolin (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). Nerve responses from labellar chemosensilla were recorded using the tip-recording method (Hodgson et al., 1955). Labellar taste sensilla were stimulated up to 2 s by a recording electrode with a 20-μm tip diameter. The electrolyte (1 m*M* KCl) does not elicit spikes from the L1 cell but it elicits spikes from the W cell.

The recording electrode was connected to a preamplifier (TastePROBE) (Marion-Poll and Van der Pers, 1996) and electric signals were further amplified and filtered by a second amplifier (CyberAmp 320, Axon Instrument, Inc.,

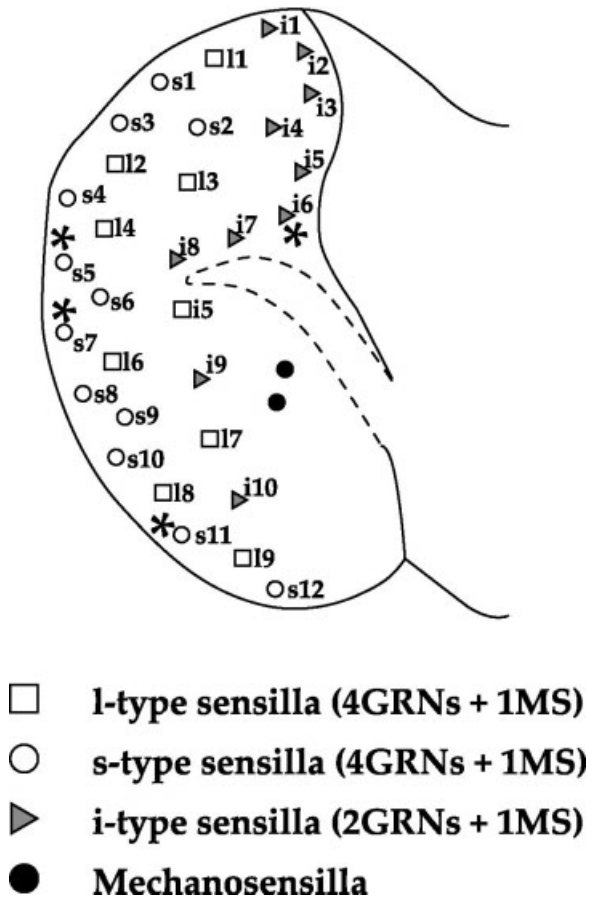

Figure 1 Schematic diagram of the labellum (lateral view) and location of the 31 chemosensilla identified. Anterior is top and dorsal to the right. Asterisks indicate sensilla that have a variable number (2–4) of taste neurons (Stocker, 1994; Shanbhag et al., 2001). GRN: Gustatory receptor neuron, MS: Mechanosensory neuron.

USA, gain = 100, eighth order Bessel pass-band filter = 1–2800 Hz). The recorded signals were digitized (DT2821, Data Translation, USA, sampling rate = 10 kHz, 12 bits), stored on computer and analyzed using custom software, Awave (Marion-Poll, 1995, 1996).

The sensilla recorded in this work were labeled according to the notation in Figure 1. We sampled systematically the responses from i-type sensilla. Recordings performed on sensilla i6 (Fig. 1) were not used, because they contain a variable number (2–4) of GRNs (Stocker, 1994; Shanbhag et al., 2001). For comparison, recordings were also performed on two s-type (s2 and s6), and on all l-type sensilla. The other s-type sensilla were not accessible to the recording electrode.

Data Analysis

Action potentials were detected by a visually adjusted threshold set across the digitally filtered signal (Fiore et al., 1996). They were sorted on the basis of shapes with the aid of interactive software procedures. The identification of the active cell in a given recording was performed after analyzing a series of recordings obtained on the same sensilla at different concentrations. This was necessary because in *Drosophila*, the amplitude of spikes from S, L1, and L2 cells, increase with the concentration of the stimulus while the amplitude of spikes elicited from W cells become smaller with the osmolarity of the stimulating solution (Fujishiro et al., 1984). The intensity of the responses was measured by counting the number of spikes occurring within the first second of each recording.

Statistical Analysis

The Wilcoxon's signed rank sum test and a normal *t* test were performed in the cross-adaptation test and the feeding test, respectively. All samples obtained here were tested for normal distribution of the data. We also evaluated if sugar and salts interfere when presented as a mixture. Because several statistical analysis have been reported to examine additive effect between two stimulating compounds (Nelson and Kursar, 1999), we performed a modified two-tail *t* test, which is founded upon assumptions of a normality and equal variance of samples (Greco et al., 1995). We analyzed *t* value for delta $= R_{\text{sucrose}} + R_{\text{NaCl}} - R_{\text{sucrose+NaCl}}$ at each concentrations (null hypothesis H_0: $R_{\text{sucrose}} + R_{\text{NaCl}} = R_{\text{sucrose+NaCl}}$, where R = response to corresponding molecules).

Cross-adaptation Test

To verify that one GRN within i-type sensilla responds both to low concentration of salt and to sugar, we performed cross-adaptation tests as follow. The response (R1) to a first stimulus (50 mM NaCl or 1 m*M* strychnine) was evaluated over a group of i-type sensilla; 15–20 min later, these sensilla were adapted to sugar with 100 m*M* sucrose for 30 s. Then, we recorded the response (R2) of these sensilla to the initial stimulus at 3, 5, and 10 min after the adaptation to sucrose. This experiment was performed on sensilla i1–i10 (except i6) and on all l-type sensilla. The resulting responses were expressed as the ratio (R2/R1). We obtained two to four samples per sensillum for 50 m*M* NaCl (i-type and l-type sensilla) and for 1 m*M* strychnine (i-type sensilla only).

Feeding Test

Flies were fed with a 100 m*M* glucose solution soaked in Kimwipe paper for about 2 h. They were then transferred into glass vials that were wrapped with porous paper and the vials introduced into a desiccator over silica gel (relative humidity: approximately 20%) for 2 h.

A pair of 20-mm rectangular filter papers (Whatman 3MN) was placed in a Petri-dish (90 mm) and soaked with 220 μL of a test solution colored with a blue food dye (0.5

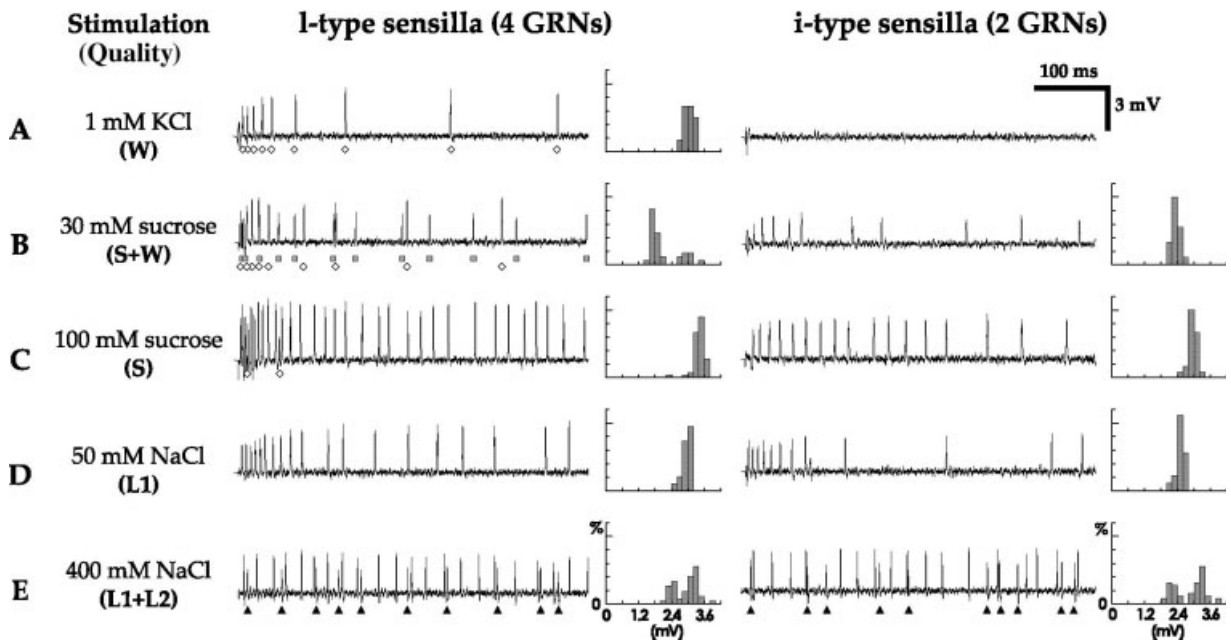

Figure 2 Typical recordings of l- and i-type sensilla in response to 1 m*M* KCl, 30,100 m*M* sucrose, 50,400 m*M* NaCl, and their corresponding spike-amplitude histograms (on the right side of recordings, bin size = 2 mV). Horizontal axis indicates classes of spike amplitude (mV), and vertical axis indicates percentage of classified spikes (1 DIV = 10%). Spikes originating from different GRNs can be discriminated according to their temporal pattern and to the distribution of their amplitudes. (A) 1 m*M* KCl: at low osmolarity, the W cell is active in l-type sensilla (open lozenge) but not in i-type sensilla. (B–C) 30, 100 m*M* sucrose: At higher osmolarity, the W cell is inhibited. Sugar activates the S cell (gray square) in both l- and i-type sensilla. (D–E) 50, 400 m*M* NaCl: one cell (named L1) is active at low concentration (50 m*M* NaCl). A second cell (named L2, closed triangle) becomes active at higher concentration (400 m*M* NaCl). As i-type sensilla house only 2 cells, one of the two GRNs in i-type sensilla responds to two taste qualities; either to sugar and salt at low concentration or to sugar and salts at high concentration.

mg/mL). This concentration of food dye does not affect the feeding behavior (Tanimura et al., 1982). For each concentration tested, about 100 flies were each introduced into three dishes and allowed to feed for 20 min in the dark. Flies were then killed by freezing. Female or male flies were homogenized in an Eppendorf tube with 300 μL of 50% ethanol (20 flies per tube). After centrifugation, the relative absorbance at 630 nm was measured with a microplate reader (Nalge Nunc International, Denmark). To avoid the absorbance originating from the eye pigments of the wild-type, we used white-eyed flies, w^{1118}, in this experiment. We did at least three repetitions for one concentration.

RESULTS

i-type Sensilla Respond to Sugar and Salt

Responses to 1 m*M* KCl, 30, 100 m*M* sucrose, and to 50, 400 m*M* NaCl were recorded from i-type sensilla (Fig. 2). To determine if one or more cells was active in our recordings, we examined the spike-amplitude distribution as shown on the right side of each trace (Fig 2). This was possible because each cell was firing spikes with different amplitudes for the range of concentrations used here. An unimodal distribution displayed in the spike-amplitude histogram indicated that a single cell was active in the recording. In addition, if the spike shapes were too similar to be accurately sorted, we analyzed the frequency of spike doublets, which occur when more than one cell is active [Fig. 3(A–B); Meunier et al., 2003a].

In contrast to l-type sensilla, 1 m*M* KCl did not elicit any spikes in i-type sensilla [Fig. 2(A)]. A single class of spikes was observed in response to stimulation by 10 to 100 m*M* sucrose in i-type sensilla, with spiking activity increasing as a function of sucrose concentration (mean: 12.1 spikes/s at 10 m*M*, 23.2 at 30 m*M*, and 33.3 at 100 m*M*) [Figs. 2(B–C)]. In a similar manner, spiking activity increased in response to growing concentrations of NaCl (mean: 8.9 at 10

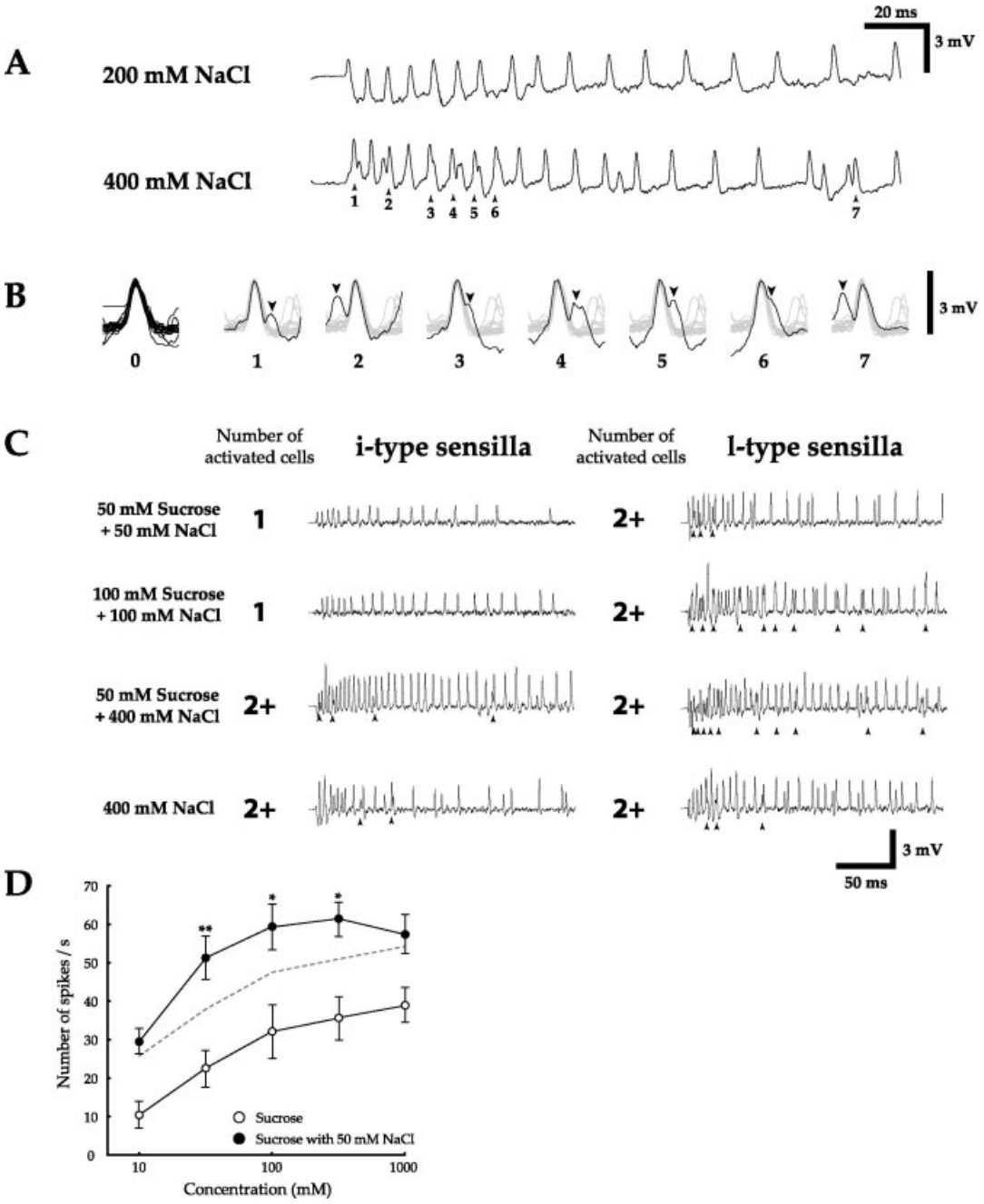

Figure 3 (A) Sample recordings in response to 200 m*M* and to 400 m*M* NaCl on l-type sensilla; 200 m*M* NaCl elicits spikes of a single class (upper trace) and 400 m*M* NaCl elicits spikes of several classes (lower trace). Arrows indicate the spikes occurring as doublets, i.e., within less than 2 ms. (B) Regular spikes (B0) and doublets (B1–7). Each doublet spike found in trace A is displayed in a 4 ms window (black line) superimposed on all non-doublet spikes (gray lines). Spikes elicited with 200 m*M* NaCl are displayed in B0. The presence of doublets reflects that at least two cells are active. (C) Comparison of the responses to sugar–salt mixtures in l-type (right column) and in i-type sensilla (left column). Recordings from i-type sensilla show only one class of spikes in response to sugars and low concentrations of salts. Recordings from l-type sensilla stimulated with the same compounds exhibit two classes of spikes. All traces are shown after the onset of the stimulation. (D) Response of i-type sensilla to a mixture of 10–1000 m*M* sucrose plus 50 m*M* NaCl (closed circles) or to sucrose alone (open circles). Each data point was calculated from at least six recordings using 37 flies [error bars indicate standard error of the mean (S.E.M.)]. A dashed line indicates the result obtained by simply adding the firing frequency in response to 50 m*M* NaCl and sucrose applied individually. $*p < 0.05$, $**p < 0.001$ (H_0: $R_{\text{sucrose+NaCl}} = R_{\text{sucrose}} + R_{\text{NaCl}}$, for abbreviations see Methods in this text).

m*M*, 18.6 at 50 m*M*, and 28.5 at 100 m*M*) [Fig. 2(D)]. Although only one class of spike was observed at NaCl concentrations below about 200 m*M*, an additional class of spikes with smaller amplitudes was found at higher concentrations [Fig. 2(E)].

Because each i-type sensillum houses only two GRNs, these results indicate that one GRN responds to two taste qualities: either sugar *and* salt at low concentration or sugar *and* salt at high concentration. We tested the first hypothesis using a mixture of sugar and salt at low concentration (50 m*M* NaCl). If it were true, then only one cell would be active. In l-type sensilla, two classes of spikes were active (S and L1 cell) whereas in i-type sensilla only one class of spikes was present [Fig. 3(C)]. The same experiment was performed with a mixture of sugar (50–100 m*M* sucrose) and a higher concentration of salt (400 m*M* NaCl). The mixture elicited spikes belonging to three classes in l-type sensilla and only two spike classes in i-type sensilla. This indicates that i-type sensilla house one GRN that can be activated by sugar and by salt at low concentration.

Because the detection of salts and sugars is generally considered to involve separate transduction pathways, we checked possible crosstalks between these qualities by stimulating i-type sensilla with increasing concentrations of sucrose with or without 50 m*M* NaCl. For concentrations ranging from 30 to 300 m*M* sucrose, there was a larger difference ($p < 0.05$) of the spike activities between sucrose–NaCl mixture and the simple addition of both stimuli than for concentrations of 10 and 1000 m*M* sucrose [Fig. 3(D)]. The number of samples (N) at 10, 30, 100, 300, and 1000 m*M* are: $N = 8, 16, 14, 14, 9$ (the mixture), $N = 12, 15, 13, 15, 12$ (sucrose), respectively. $N = 16$ (50 m*M* NaCl).

i-type Sensilla Respond to Bitter Compounds

GRN activity in all l-type and i-type sensilla was examined in response to each of seven bitter compounds: berberine, caffeine, quinine, strychnine, denatonium, aristolochic acid, and salicin, used at concentrations ranging from 0.01 to 10 m*M*. Among them, all of the i-type sensilla and the two s-type sensilla responded to berberine, caffeine, quinine, and strychnine at lower thresholds than those seen for sugars or salts. None of the l-type sensilla responded to these bitter compounds. The time course of the responses to the bitter compounds in the i-type sensilla was similar to the responses previously observed in the tarsal sensilla (Meunier et al., 2003b). A latency was always observed between the onset of the stimulation and the beginning of the discharge activity depending on the stimulus concentration, that is, 100–150 ms with 0.1 m*M* strychnine and 50 ms with 10 m*M* strychnine [Fig. 4(A)]. The spike train of i-type sensilla following stimulation with bitter compounds was always very regular and we did not observe any doublets of spikes [Fig. 4(A); and see Meunier et al., 2003a]. This indicates that bitter compounds are activating only one GRN in i-type sensilla.

To test if these bitter-sensitive GRNs are sensitive to another quality (e.g., to sugars and salt at low concentration or to salts at high concentration), we stimulated i-type sensilla with a mixture of 50 m*M* sucrose and 1 m*M* strychnine. This mixture elicited two classes of spikes, indicating that sucrose and bitter compounds activate different GRNs in i-type sensilla [Fig. 4(A)]. Because i-type sensilla house only two GRNs and one GRN is sensitive to sugars and salts at low concentration, the bitter-sensitive GRN is the one that responds to high concentrations of NaCl.

Among i-type sensilla, the relative sensitivity to strychnine, berberine, quinine, and caffeine was similar [Fig. 4(B)]. These bitter-sensitive GRNs, however, exhibit different sensitivities from ones in the tarsal sensilla. For example, strychnine elicited the highest frequency of spike activity in labellar sensilla, while berberine was the most active on tarsal sensilla.

A Cross-adaptation Test Using Sugar, Salt, and Bitter Compound

Cross-adaptation tests were performed in i-type sensilla to confirm that sugar and salt at low concentration are detected by the same GRN while bitter compounds are detected by the other GRN. Responses of each i-type sensillum to 50 m*M* NaCl were recorded 3, 5, and 10 min after stimulation with 100 m*M* sucrose for 30 s [Fig. 5(A)]. Responses of i-type sensilla to 1 m*M* strychnine and responses of l-type sensilla to 50 m*M* NaCl were examined in the same way. In i-type sensilla, the responses to 50 m*M* NaCl decreased by 53% 3 min after adaptation to sucrose and completely recovered within 10 min [Fig. 5(B)]. The response to 1 m*M* strychnine was not affected. Control experiments on the l-type sensilla showed no such adaptation.

Feeding Test for Salty Preference

To clarify which concentrations of salt represent an attractive stimulus for *Drosophila*, we performed a feeding test with NaCl at concentrations ranging from 25 to 400 m*M*. After flies were fed on water (control)

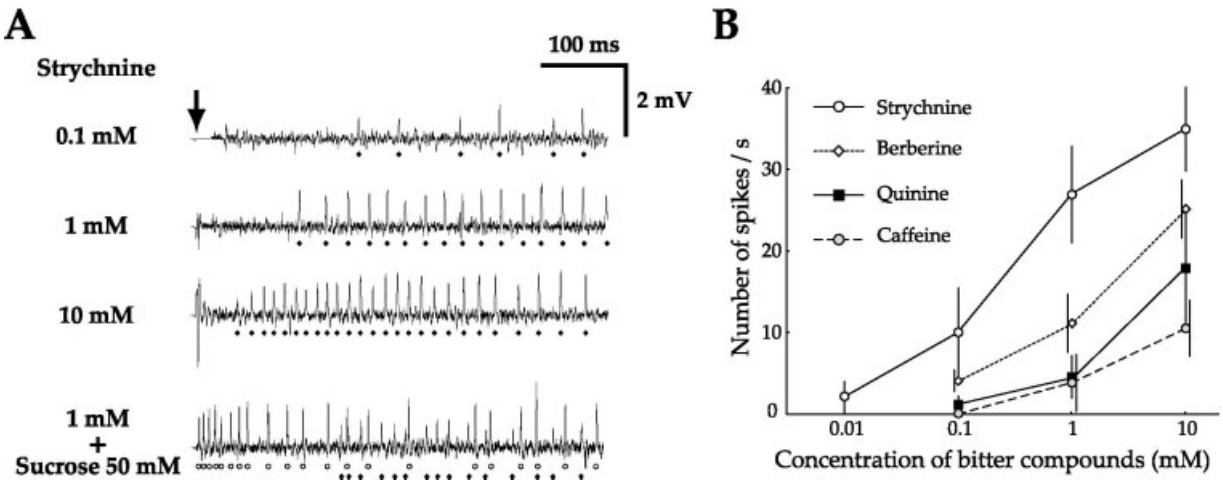

Figure 4 Responses of i-type sensilla to bitter compounds. (A) Stimulation with strychnine (0.1 m*M*, 1 m*M*, and 10 m*M*) or with a mixture of 1 m*M* strychnine plus 50 m*M* sucrose. Arrow indicates the onset of stimulation. Spikes elicited by strychnine are underscored by closed diamonds. Initial spike activity always commenced after a latency, which decreased with increasing concentration of the bitter compound used. The bottom trace shows a response to a mixture of sucrose and strychnine, demonstrating that these compounds activate different GRNs. (B) Dose–response curves to strychnine (open circles), berberine (open diamonds), quinine (closed squares), and caffeine (gray circles). All spikes elicited in the first 500 ms after the onset of stimulation were counted. Each data point represents at least six recordings made from 25 flies in total. Error bars indicate S.E.M.

or on NaCl solutions, we evaluated the amount of intake of the solutions with absorbance of a food dye. Means of the absorbance were 0.110 (water:control), 0.216 (25 m*M* NaCl), 0.250 (50 m*M*), 0.243 (100 m*M*), 0.144 (200 m*M*), 0.024 (400 m*M*) in females, and 0.030 (water), 0.055 (25 m*M*), 0.073 (50 m*M*), 0.075 (100 m*M*), 0.025 (200 m*M*), 0.007 (400 m*M*) in males. Figure 6 shows the difference of NaCl intake to control water for both sexes. For concentrations of salts under 200 m*M*, flies were significantly feeding more on salty solutions than on water.

DISCUSSION

A GRN Encodes a Combination of Two Taste Qualities

In *Drosophila* most taste sensilla contain four GRNs, and each GRN was considered to respond to different qualities accordingly called W, S, L1, or L2 cell. This is the case for l-type sensilla of the labellum where each sensillum houses these four GRN types (Siddiqi and Rodrigues, 1980; Fujishiro et al., 1984; Wieczoreck and Wolff, 1988). Next to them, and at the periphery of the labellum, i-type sensilla house only two GRNs (except i6, which house two to four GRNs: Shanbhag et al., 2001).

Our results indicate that in i-type sensilla, one GRN expresses a combination of sensitivities that are expressed separately in S and L1 cells of l-type sensilla. In flies, a general consensus has been that one GRN responds to only one taste quality. However, a number of observations performed on different insect species challenged this hypothesis. For example, in the blowfly, the water cell also responds to fructose at a high concentration (Wieczorek and Koppl, 1978). In phytophagous insects numerous examples exist where one taste cell responds to chemicals belonging to different qualities (Blaney, 1974, 1975; White and Chapman, 1990; Bernays et al., 2000).

To our understanding, this is the first time in *Drosophila* that the same GRN has been found to be sensitive to sugar and to salt. This observation qualitatively differs from an earlier report on the blowfly, that some sugars enhance the sensitivity to salt of the salt receptor cell (Schnuch and Hansen, 1990) in so far as sugars did not activate salt receptor cells by themselves. Even if we cannot exclude similar mixture interactions in i-type sensilla, we find that sugars or salts alone activate the same GRN in i-type sensilla. This suggests that one GRN coexpresses receptor proteins responsible for the detection of salt at low concentration and of sugars. For salts and especially Na^+, the proteins encoded by DEG/ENaC genes, *ppk11/19*, are likely candidates (Liu et al., 2003). As for sugars, *Gr5a* and *Tre1* that encode G-protein cou-

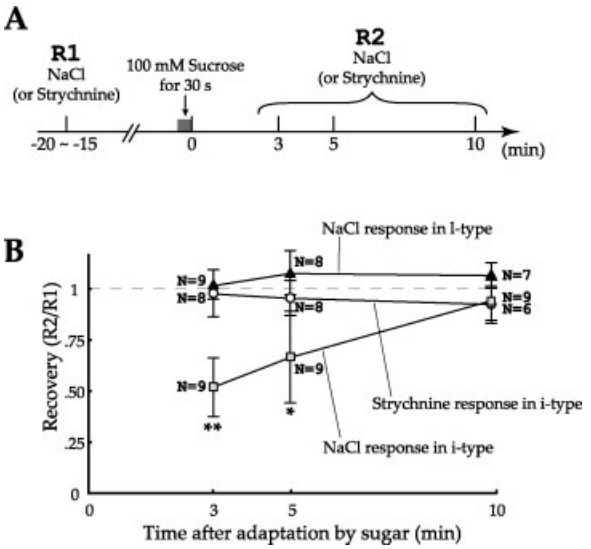

Figure 5 Crossadaptation tests between sucrose, NaCl, and strychnine on i-type sensilla. (A) Stimulation protocol. R1: first response to 50 m*M* NaCl (or 1 m*M* strychnine). R2: second response to the same stimuli in R1. (B) Results of crossadaptation tests using 100 m*M* sucrose, 50 m*M* NaCl and 1 m*M* strychnine. The vertical axis represents the ratio (R2/R1) as "recovery." The horizontal axis represents the time after the adaptation stimulus (100 m*M* sucrose for 30 s). Numbers of recordings for calculation of data points are shown in the figure (error bars indicate S.E.M.). The adaptation by sugar is only effective on the GRN sensitive to salts in i-type sensilla indicating that the same GRN is sensitive to sugars and salts at low concentration. $*p < 0.05$, $**p < 0.0001$ (significantly below chance level).

pled receptors have been identified as receptors to the sugar trehalose. Because we reported a new response property in addition to the four basic types (W, S, L1, and L2), it would be interesting to examine how the receptors for sugars and salts are expressed in i-type sensilla.

Bitter Recognition on the Labellum

In i-type sensilla, the second GRN responds to bitter compounds and to salts at a high concentration. This response pattern matches the responses of L2 cells recently found in three tarsal sensilla of each prothoracic leg (Meunier et al., 2003b). Such cells were initially described as sensitive to salts at high concentration. In the two s-type sensilla evaluated here, one GRN also responds to these bitter compounds and to salt. This is not the case for l-type sensilla, where none of the GRNs respond to the bitter compounds tested. The detection threshold of L2 cells to NaCl is above 100 m*M* in l-type sensilla, and above 200 m*M* in i-type sensilla and in tarsal sensilla. Such a high threshold and the weak intensity of the responses suggest that NaCl may not be the optimum stimulus for all L2 cells. Considering the results obtained here for bitter compounds, it is likely that bitter compounds represent the optimal stimuli for L2 cells housed in i-type and s-type sensilla.

The sensitivity to bitter compounds differs between sensilla located on the labellum and on the tarsi. On the tarsal sensilla, bitter-sensitive L2 cells respond preferentially to quinine or to berberine (Meunier et al., 2003b). These sensitivity profiles adequately explain the behavioral responses of *Drosophila* to bitter compounds, except for strychnine. Strychnine elicited less spike activity in the tarsal sensilla than quinine, while the two compounds were equally deterrent in binary choice feeding tests. In this work, we have found that L2 cells in i-type sensilla of the labellum are more sensitive to strychnine. These results suggest that information from both the labellum and the tarsal taste sensilla could complement each other and inhibit the feeding behavior equally. In this way, strychnine can be detected by labellar sensilla even if tarsal sensilla are unable to detect it. Unless other labellar sensilla detect strychnine, this demonstrates that i-type sensilla contribute to regulate feeding, despite of their position at the periphery of the labellum.

One question concerning the putative *Gr*s is how their expression patterns match the sensitivity of the GRNs. There was no obvious difference of sensitivity between the bitter-sensitive GRNs to the set of bitter compounds tested. We thus expect that one *Gr* would

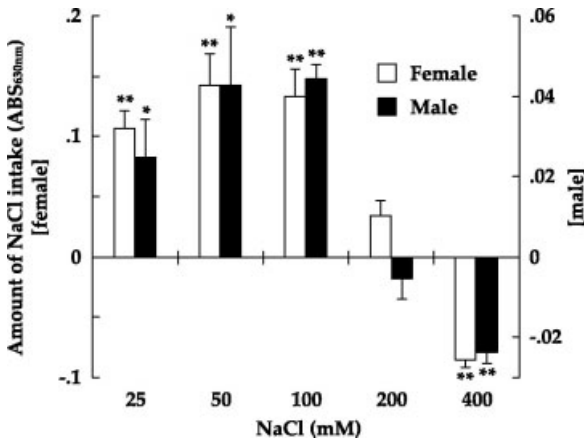

Figure 6 Feeding preference for NaCl ranging from 25 m*M* to 400 m*M*. The vertical axis indicates the difference of NaCl intake versus water intake. Both females and males preferred NaCl at under 100 m*M*, and they avoided NaCl at 400 m*M*. $*p < 0.001$, $**P < 0.0001$ (significantly above or below chance level).

be expressed in all i-type sensilla. Whereas we previously mapped the expression pattern of several *Gr* genes (*Gr22c*, *Gr22e*, *Gr22f*, *Gr32a*, *Gr59b*, and *Gr66a*) on the labellum (Hiroi et al., 2002), none of the expression patterns of the *Gr*s matches the response profiles of the i-type sensilla examined so far. A detailed analysis of expression patterns of other *Gr* genes is needed to determine the relationship between ligands and *Gr* receptors.

Antagonistic Taste Neurons in a Sensillum

In i-type sensilla, whereas one GRN is sensitive to deterrent compounds and salts at high concentrations, the other is sensitive to both sugars and salts at low concentration. Salt is known to be a nutrient essential for electrolyte homeostasis (Lindemann, 1996; Contreras and Lundy, 2000) and to be phagostimulatory for many insects (Smedley and Eisner, 1995). Our behavioral test showed that *Drosophila* preferred NaCl at low concentration (~50 m*M*) to water, but avoided NaCl at higher concentrations starting from 200 m*M*. This threshold reflects the electrolyte homeostasis of the fly as the hemolymph osmolarity is about 390 mO (Pierce et al., 1999). In this way, one of the two GRNs in the i-type sensilla may act as a receptor cell sending out an acceptance signal, while the other may act as a receptor cell sending out a rejection signal, which allows flies to elicit stereotyped behaviors— involving either feeding or its inhibition.

Antagonistic taste neurons have been described many times in *Lepidoptera* (Schoonhoven and van Loon, 2002) but it has not been described in sensilla housing only two GRNs. Our results indicate that i-type sensilla contains two antagonistic neurons, encoding the presence of positive or aversive stimuli. Further anatomical and physiological studies on the i-type sensilla might provide information on how these signals are segregated and processed in the central nervous system. These observations are compatible with the working hypothesis proposed by Chapman (2003) that phagostimulatory and deterrent neurons could be considered the basic labeled lines of the insect taste receptor system.

Coupled with the genetics tools available in *Drosophila*, these two GRNs would provide a useful model to elucidate how taste receptors and their corresponding molecules are respectively expressed and segregated across neurons, and how the signals from two different taste qualities are integrated into a common transduction pathway.

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