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Authors: Hiroi, Makoto, Marion-Poll, Frédéric, and Tanimura, Teiichi

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Differentiated Response to Sugars among Labellar Chemosensilla in *Drosophila*

Makoto Hiroi[1], Frédéric Marion-Poll[2] and Teiichi Tanimura[1]*

[1]*Department of Biology, Graduate School of Sciences, Kyushu University, Ropponmatsu, Fukuoka, 810-8560, Japan*
[2]*INRA Station de Phytopharmacie et Médiateurs Chimiques route de Saint Cyr, 78026 Versailles Cedex, France*

ABSTRACT—Recent findings have indicated that the *Gr* genes for putative gustatory receptors of *Drosophila melanogaster* are expressed in a spatially restricted pattern among chemosensilla on the labellum. However, evidence for a functional segregation among the chemosensilla is lacking. In this work, labellar chemosensilla were classified and numbered into three groups, L-, I- and S-type, based on their morphology. Electrophysiological responses to sugars and salt were recorded from all the accessible labellar chemosensilla by the tip-recording method. All the L-type sensilla gave good responses to sugars in terms of action potential firing rates, while the probability for successful recordings from the I-type and S-type sensilla was lower. No differences were found in the responses to sugars between chemosensilla belonging to the same type; however, dose-response curves for several different sugars varied among the sensilla types. The L-type sensilla gave the highest frequency of nerve responses to all the sugars. The I-type sensilla also responded to all the sugars but with a lower magnitude of firing rate than the L-type sensilla. The S-type sensilla gave a good response to sucrose, and lower responses to the other sugars. These results suggest that there might be variations in the expression level or pattern of multiple receptors for sugars among the three types of chemosensilla. The expression pattern of six *Gr* genes was examined using the Gal4/UAS-GFP system, and sensilla were identified according to the innervation pattern of each GFP-expressing taste cell. None of the spatial expression patterns of the six *Gr* genes corresponded to the sugar sensitivity differences we observed.

Key words: taste, receptor, sugar, electrophysiology, *Drosophila*

INTRODUCTION

Chemoreception is essential for all living organisms to perceive chemical information in their environment. Remarkable progress has been made toward the molecular identification of olfactory receptors in some vertebrates and in *Drosophila*. Olfactory receptors have been identified as G-protein coupled transmembrane receptors (GPCRs) (for review, see Firestein, 2001). In relation to gustation, only a few GPCRs have been identified as functional gustatory receptors in mammals (for review, see Lewcock and Reed, 2001) and *Drosophila* (Ishimoto *et al*., 2000; Ueno *et al*., 2001; Dahanukar *et al*., 2001).

In *Drosophila*, gustatory neurons are housed in a hair-like structure, called a sensillum, on the labellum and tarsi. A typical sensillum houses one mechanoreceptor and four gustatory neurons, each of which responds to either water (W cell), sugar (S cell), low salt concentration (L1 cell) or high salt concentration (L2 cell) (Dethier, 1976; Rodrigues and Siddiqi, 1978; Fujishiro *et al*., 1984; Wieczorek and Wolff, 1989). The response properties of several sensilla on the prothoracic tarsi have been reported and it was shown that contrasting responses existed between sensilla (Meunier *et al*., 2000). On a labellum of *Drosophila*, 62 chemosensilla are present which can be grouped by their length into three types - long, short and intermediate types (L-, S- and I-) (Shanbhag *et al*., 2001). All previous electrophysiological recordings on the labellar sensilla were made on L-type sensilla which were considered as functionally equivalent each other. This is in sharp contrast with the multiplicity of putative gustatory receptor (*Gr*) genes recently found in the *Drosophila* genome using a computer algorithm to probe a database of the *Drosophila* genome (Clyne *et al*., 2000; Scott *et al*., 2001; Dunipace *et al*., 2001). Since some of these genes show an expression that is restricted to a lim-

* Corresponding author: Tel. +81-092-726-4759;
FAX. +81-092-726-4625.
E-mail: tanimura@rc.kyushu-u.ac.jp

ited number of sensilla, the probability exists that the taste responsiveness might differ among the labellar sensilla.

Here we recorded, using the tip-recording method, nerve responses to several different sugars from all the three types of labellar chemosensilla. We classified sensilla according to their responsiveness of each sensillum to a range of sugars and salts. In order to test if the functional types found by this method matched *Gr* gene segregation, we examined the green fluorescent protein (GFP) expression pattern of six of the 65 *Gr* genes. This study thus provides us with a basic understanding of the physiology of the gustatory sense in *Drosophila*.

MATERIALS AND METHODS

Fly stocks

Strains of *Drosophila melanogaster* were maintained on a standard cornmeal-glucose agar medium at 25°C. Canton-S was used as wild type. One-day-old flies were fed on a fresh medium for one day before experiments. *Gr* promoter-Gal4 strains were provided by H. Amrein and R. Axel. The UAS-Gal4 strain, P{w^{+mC}=UAS-GFP.S65T}, was from the Bloomington *Drosophila* Stock Center. The nomenclature of the *Gr* genes described in Flybase (http://flybase.bio.indiana.edu/) was used.

Scanning electron microscopy

Flies were fixed, dehydrated in acetone, and dried. Mounted flies were sputter-coated with platinum and observed by a JEOL JSM-5600 LV scanning electron microscope.

Visualizing GAL4 expression patterns by GFP

To visualize the expression pattern of *Gr* genes, the *Gr* promoter-Gal4 strains were crossed to the strains carrying a UAS-GFP transgene on the second chromosome. Homozygous strains for both transgenes were established. Proboscises were dissected from 2 day-old flies, fixed in 4% formaldehyde (MERCK, Haar, Germany) in phosphate buffered saline (PBS), washed with PBS, and mounted in Antifade (SlowFade-Light, Molecular Probes, Inc., Eugene, USA). GFP images of a half-labellar lobe were captured at 2 μm intervals across a 30–40 μm thick section by a confocal laser-scanning microscope (LSM510, Carl Zeiss, Inc., Germany).

Chemicals

KCl, NaCl and sucrose were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). Trehalose, glucose and fructose were from Sigma-Aldrich Corp. (St. Louis, USA). All compounds were dissolved in a 1 mM KCl solution prepared using distilled water, and were stored at –20°C. Solutions for stimulation were stored at 4°C for less than one week.

Tip-recording method

The proboscis was fixed at the base of a labellum using lanolin (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). A glass capillary filled with *Drosophila* Ringer solution was inserted from the abdomen through to the head and served as an indifferent electrode. Nerve responses from labellar chemosensilla of female flies were recorded by the tip-recording method (Hodgson *et al*., 1955). Chemosensilla on the labellum were stimulated by a recording electrode with a 20 μm tip diameter. Sugar solutions for stimulation contain 1 mM KCl as electric substance. 1 mM KCl dose not elicit salt spikes but only water spikes. 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., USA, gain = 100, 8th order Bessel pass-band filter = 1 Hz - 2800 Hz). The recorded signals were digitized (DT2821, Data Translation, USA, sampling rate = 10 kHz, 12 bits), stored on computer and analyzed using a custom software, Awave (Marion-Poll, 1995, 1996) software. Action potentials were detected by a visually-adjusted threshold set across the digitally filtered signal. The action potentials were filtered by a running median algorithm spanning a 6 ms window (Fiore *et al*., 1996) and sorted on the basis of their amplitudes and shapes with the aid of interactive software procedures.

RESULTS

Arrangement of chemosensilla on the labellar surface

In *Drosophila*, 31 chemosensilla are consistently found on each side of the labellum. They are organized in four rows oriented in the anterior-posterior axis (Fig.1A). The 31 chemosensilla can be classified into three types; long (L), intermediate (I) and short (S) types, according to their length (Fig. 1B–D) (Shanbhag *et al*., 2001). Labellar chemosensilla

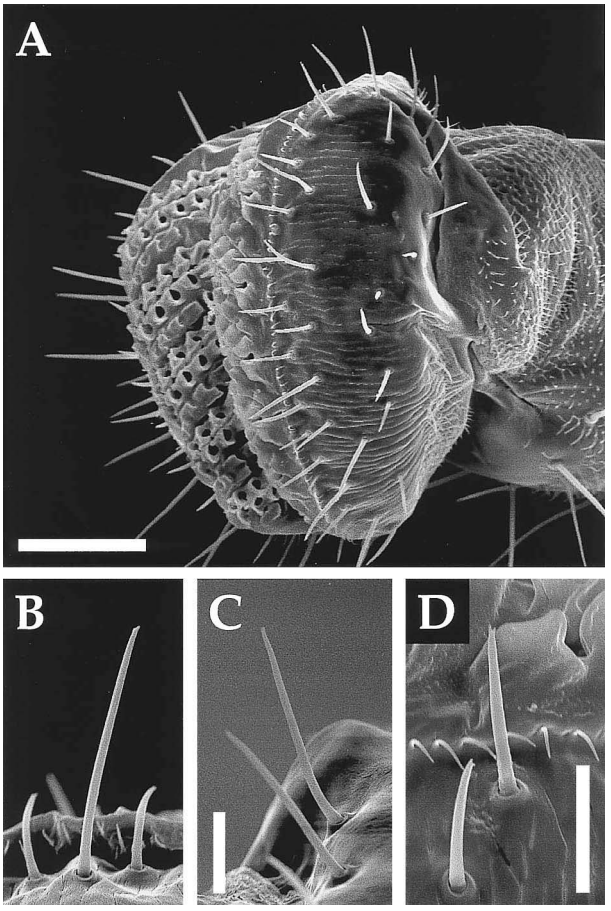

Fig. 1. Morphology of the labellar chemosensilla in *Drosophila*. Three types of sensillum, L-, I- and S-type, are visible on the labellum (Shanbhag *et al*., 2001). (A) Lateral view of the left lobe of a labellum. Anterior is top and dorsal to the right. Chemosensilla are arranged in four rows oriented in the anterior-posterior axis (Falk *et al*., 1976; Ray *et al*., 1993). The S-type sensilla are in the most ventral area and the I-type sensilla are located in the most dorsal area. Enlarged images of L-type (B), I-type (C) and S-type sensilla (D). B–C are shown in the same scale. Scale bars represent 40 μm (A) and 10 μm (C and D).

generally house four gustatory neurons and one mechanosensory neuron. The I-type sensilla, however, have just two gustatory neurons and one mechanosensory neuron (Falk *et al*., 1976; Nayak and Singh, 1983; Ray *et al*., 1993; Shanbhag *et al*., 2001). We numbered the chemosensilla in each class from the anterior to the posterior side of the labellum (Fig. 2B) and found no variation in the total number of the L-type sensilla among females of the Canton-S strain. There were small variations in the total number of the S-type and I-type sensilla (S: 12–13, I: 9–10).

Expression pattern of several *Gr* promoter-Gal4 strains

Over 60 *Gr* genes have been proposed to be candidate taste receptor genes in *Drosophila*. The expression of a number of *Gr* genes in chemosensory organs was confirmed by *in situ* hybridization and reverse transcription polymerase chain reaction (RT-PCR) (Scott *et al*., 2001; Dunipace *et al*., 2001). These authors have independently generated 23 transgenic strains in total expressing GAL4 under the control of a *Gr* promoter, for which 20 *Gr* genes were covered. In 12 out of 23 lines, which covered 10 *Gr* genes, expression of the transgene was reported. We obtained these *Gr* promoter-Gal4 strains and re-examined their expression pattern. Six homozygous lines were established that contained both *Gr* promoter-Gal4 (*Gr22c*, *Gr22e*, *Gr22f*, *Gr32a*, *Gr59b* and *Gr66a*) and UAS-GFP transgenes. Fig. 3A-F shows the location of GFP-expressing cells in the six lines.

It was possible to identify the sensillum innervated by a particular sensory cell expressing GFP by tracing the pathway of a dendrite extending from a single cell (Fig. 3G-I). GFP expression was always observed in a subset of labellar chemosensilla (Table 1). For most *Gr* promoter-Gal4 strains, the expression seemed to be in a single cell of the S-type sensilla. For *Gr22c*, *Gr22f* and *Gr59b*, expression was observed in the L-type sensilla, but not in all of them. For *Gr22c*, the GFP expression was observed in sensory cells associated with only three L-type sensilla (L4, L5 and L6). For *Gr22e* and *Gr66a*, GFP was expressed both in the S-type and I-type sensilla. In *Gr22f* and *Gr59b*, expression was observed in both S-type and L-type sensilla, while for *Gr32a*, GFP was expressed only in the S-type sensilla. In all lines we noticed two different levels of GFP expression (shown in Table 1 as '++' or '+'). All *Gr* genes except *Gr22c* showed strong expression only in the S-type sensilla ('++' in Table 1). The numbers of GFP-positive sensilla showing strong expression roughly agree with previous observations using the UAS-lacZ reporter gene (Scott *et al*., 2001; Dunipace *et al*., 2001).

Nerve response characteristics of three types of chemosensilla

In the present study, recordings were made from all labellar chemosensilla that were accessible by microelectrode. In this way, all the L- and I-type sensilla were accessible, while for the S-type sensilla, only two of them, S2 and

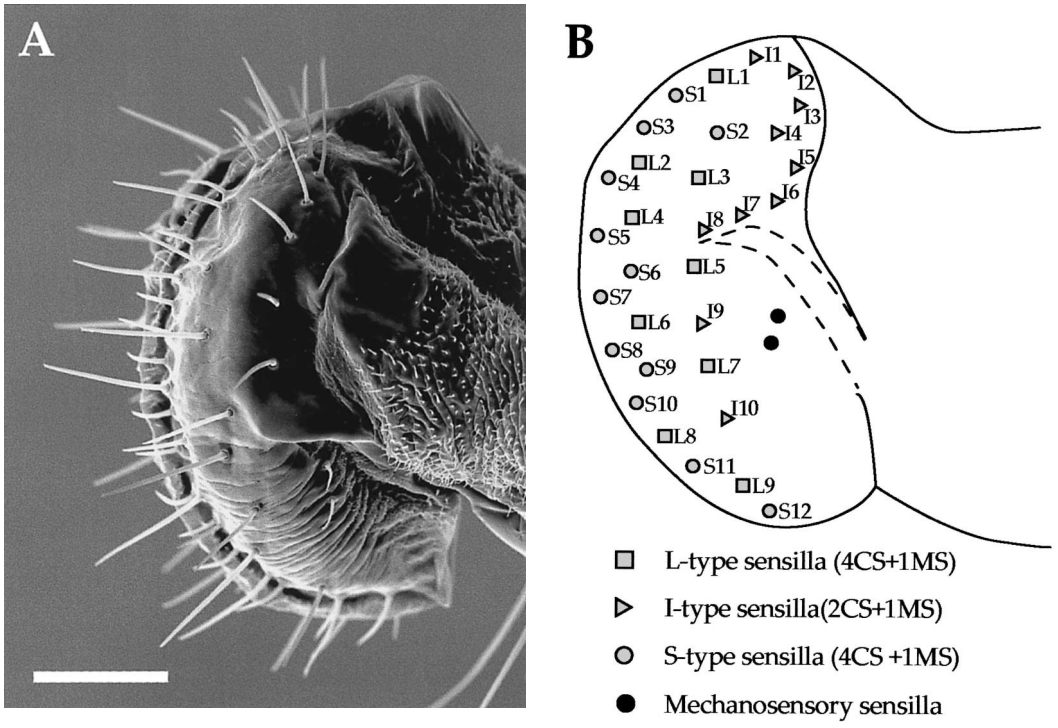

Fig. 2. Classification and numbering of chemosensilla on the labellum. Anterior is top and dorsal to the right. (A) Surface image of a left labellar lobe. Scale bar represents 30 μm. (B) Schematic diagram of sensilla arrangement modified from Shanbhag *et al*. (2001). Squares, triangles and open circles indicate, L-type, I-type and S-type sensilla, respectively. S5, S7, S11 and I6 sensilla are reported to have variable neuronal composition (Nayak and Singh, 1983; Ray *et al*., 1993; Shanbhag *et al*., 2001).

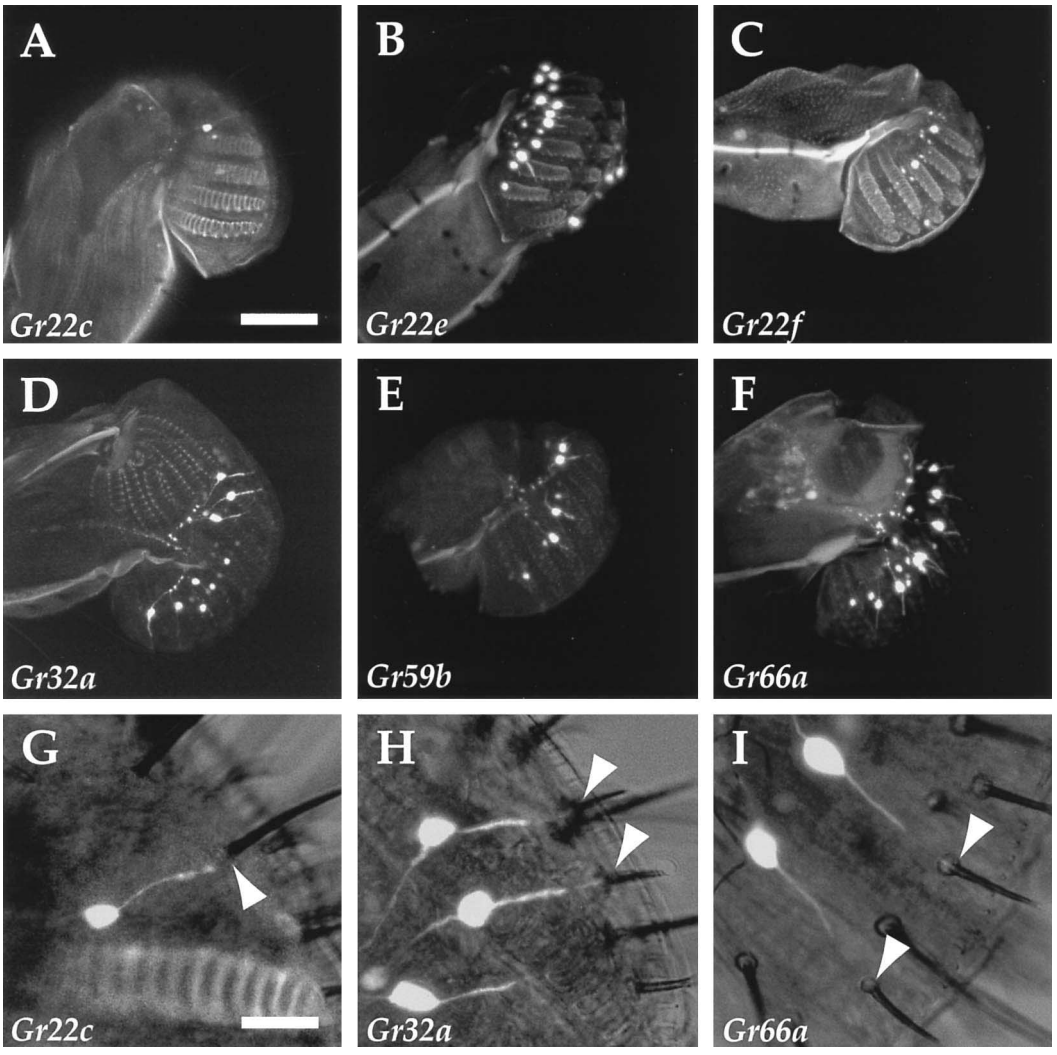

Fig. 3. Expression of *Gr* genes monitored by *Gr* promoter-Gal4/UAS-GFP. Images captured at 2 μm intervals for a 30–40 μm-thick section of a labellum were overlaid. (A–F) Each *Gr* promoter-Gal4 line shows a different expression pattern. In all lines, two levels of GFP expression were observed. A–F and G-I are each shown in the same scale. (G–I) Overlaid images of fluorescent and Nomarski images. Arrowheads show the identified sensilla. Scale bar in A is 40 μm and in G is 10 μm.

S6, could be accessed. The remaining S-type sensilla could not be touched with an electrode because the tips of these sensilla are bent and located very close to each other on the margin of the labellar lobes.

A typical sensillum has four gustatory neurons, each of which responds to sugar (S cell), water (W cell) and salts (L1 and L2 cells). Fig. 4A shows a typical example in which a 1 mM KCl solution in the electrode elicited W spikes, while low concentrations of sugar (*e.g.* 30 mM sucrose) elicited spikes from both the S cell and W cell (Fig. 4D). Because the activity of the W cell is inhibited by stimulating solutions of increased osmolarity, higher concentrations of sugars elicited almost solely S spikes (Fig. 4E). Low concentrations of NaCl elicited L1 spikes (Fig. 4F), while high NaCl concentrations (*e.g.* 400 mM) elicited not only L1 spikes but also L2 spikes (Fig. 4G).

The responses of W, L1, L2 and S cells were assessed using 1 mM KCl, 400 mM NaCl and four kinds of sugars (sucrose, trehalose, glucose and fructose) as stimulating solutions. Results shown in Table 2 are based on 6-10 recordings from each sensillum using 45 flies. The L-type sensilla responded to all compounds examined, while S-type sensilla showed W, L1, L2 and S cell activity. Trehalose and glucose gave noisy signals in S-type sensilla, and accordingly we could not confirm the responses of this sensillum-type to these two compounds. The I-type sensilla responded to 400 mM NaCl but not to 1 mM KCl (Fig. 4B). Stimulation of these sensilla with sugar elicited only S spikes (Fig. 4C).

We occasionally failed to record any responses from some sensilla. Even in such cases where we obtained no response to sugars, we are certain that an electrical contact was established. Non-responsive sensilla were more frequently observed for I- and S-type sensilla than for L-type sensilla where more than 85% of recordings were successful (Fig. 5). In I-type sensilla, the percentage differed

Table 1. Expression profiles of *Gr* promoter-Gal4

Sensilla	*Gr22c*	*Gr22e*	*Gr22f*	*Gr32a*	*Gr59b*	*Gr66a*
L1						
L2						
L3			+		+	
L4	++				+	
L5	+		+		+	
L6	+				+	
L7			+		+	
L8					+	
L9					+	
I1						+*
I2						+*
I3						+*
I4						+*
I5						+*
I6						+*
I7						+*
I8						+*
I9		+				+
I10		+				+
S1		++	+	++	++	++
S2		++	+	++	+	++
S3		++	+	++	++	++
S4		+	++	+	+	+
S5		++	+		+	++
S6		++	+	++	++	++
S7		++	+		+	++
S8		+	+	+	++	+
S9		++	++	++	+	+
S10		++	+	+	+	++
S11		++	+	++	+	++
S12		++	+	+		+
Number of '++'	1	10	2	6	4	8
Scott *et al.*†	–	–	3–4	6	–	6–8
Dunipace *et al.*†	0	~15	2	–	2	8

Higher level (++) and lower level (+) of GFP expression. *: Dendrites of neurons expressing GFP could not be precisely confirmed. Data not available (–). †: The total numbers of LacZ-positive sensilla per labellum previously described (Scott *et al.*, 2001; Dunipace *et al.*, 2001). Data from our observations of five to eight flies in each line were found to confirm these results.

depending on their location, with low success rates (<35%) for sensilla from I1 to I3.

Dose response curves for sugars

We recorded responses from sensilla L1-L9, I1-I10 and S2 and S6 (Fig. 2B), to four kinds of sugars (sucrose, trehalose, glucose and fructose) at five different concentrations ranging from 10 mM to 1000 mM. 5–13 recordings were obtained from each sensillum in response to stimulation by five concentration of sucrose. Similarly 5–10 recordings were made for each concentration of fructose, 5–9 recordings for glucose and 4–9 recordings for trehalose. Each sensillum belonging to the same type gave a similar dose-response curve, so results are shown as the average number of spikes per second of data obtained for each type of chemosensilla. The L-type sensilla responded to all sugars with a higher frequency than the other sensilla (Fig. 6). The I-type sensilla gave responses to all the sugars, but with a

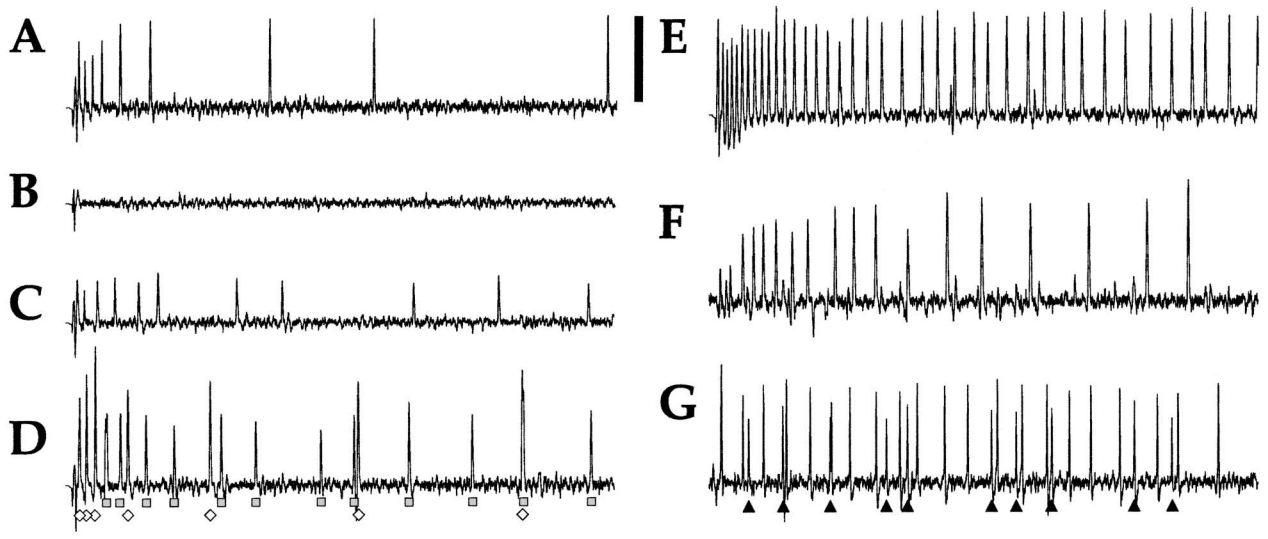

Fig. 4. Typical recordings from labellar chemosensilla. Traces show impulses during the first 500 ms after stimulation. Scale bar represents 3 mV. (A), (B) Stimulations with 1 mM KCl in L- (A) and I-type (B) sensilla. In the L-type sensilla W spikes can be seen. (C) Stimulation of an I-type sensillum (I7) with 50 mM sucrose. Only S spikes are observed. (D) Stimulation of an L-type sensillum (L3) with 30 mM sucrose. Open diamonds show W spikes, gray squares show S spikes. (E) Stimulation of an L-type sensillum (L3) with 100 mM sucrose. Most spikes are from the S cell. (F) Stimulation of an L-type sensillum (L7) with 50 mM NaCl. The spikes arise mainly from the L1 cell. (G) Stimulation of an L-type sensillum (L7) with 400 mM NaCl. With a high concentration of salt, spikes from the L2 cell are observed (shown as closed triangles).

Table 2. Response profile of labellar chemosensilla to water, sugars and salt

Sensilla	KCl (1 mM)	Sucrose	Glucose	Fructose	Trehalose	NaCl (400 mM)
L1	+	+	+	+	+	++
L2	+	+	+	+	+	++
L3	+	+	+	+	+	++
L4	+	+	+	+	+	++
L5	+	+	+	+	+	++
L6	+	+	+	+	+	++
L7	+	+	+	+	+	++
L8	+	+	+	+	+	++
L9	+	+	+	+	+	++
I1	–	+	+	+	+	++
I2	–	+	+	+	+	++
I3	–	+	+	+	+	++
I4	–	+	+	+	+	++
I5	–	+	+	+	+	++
I6	–	+	+	+	+	++
I7	–	+	+	+	+	++
I8	–	+	+	+	+	++
I9	–	+	+	+	+	++
I10	–	+	+	+	+	++
S2	+	+	*	+	*	++
S6	+	+	*	+	*	++

+: Positive response from a single cell to a particular stimulus. ++: Responses from L1 and L2 cells. *: Not yet determined.

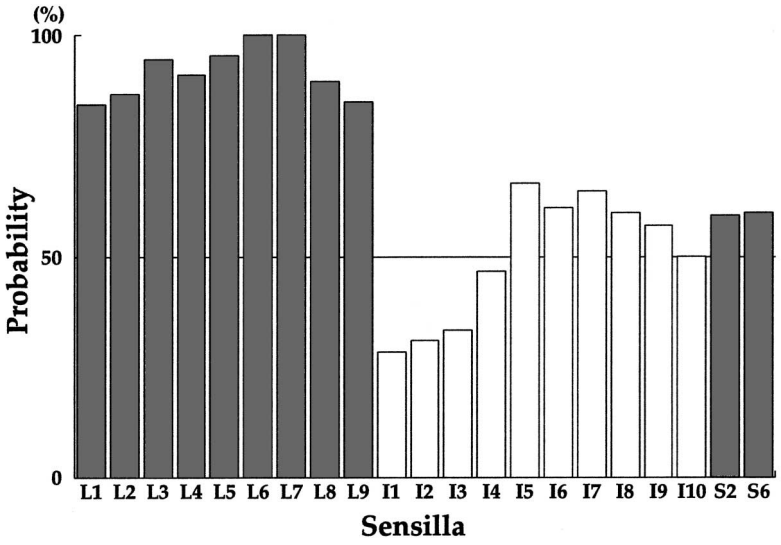

Fig. 5. Variation of successful recordings among sensilla. Mean values are shown, each from 15-22 recordings using 33 flies for stimulation with 100 mM sucrose.

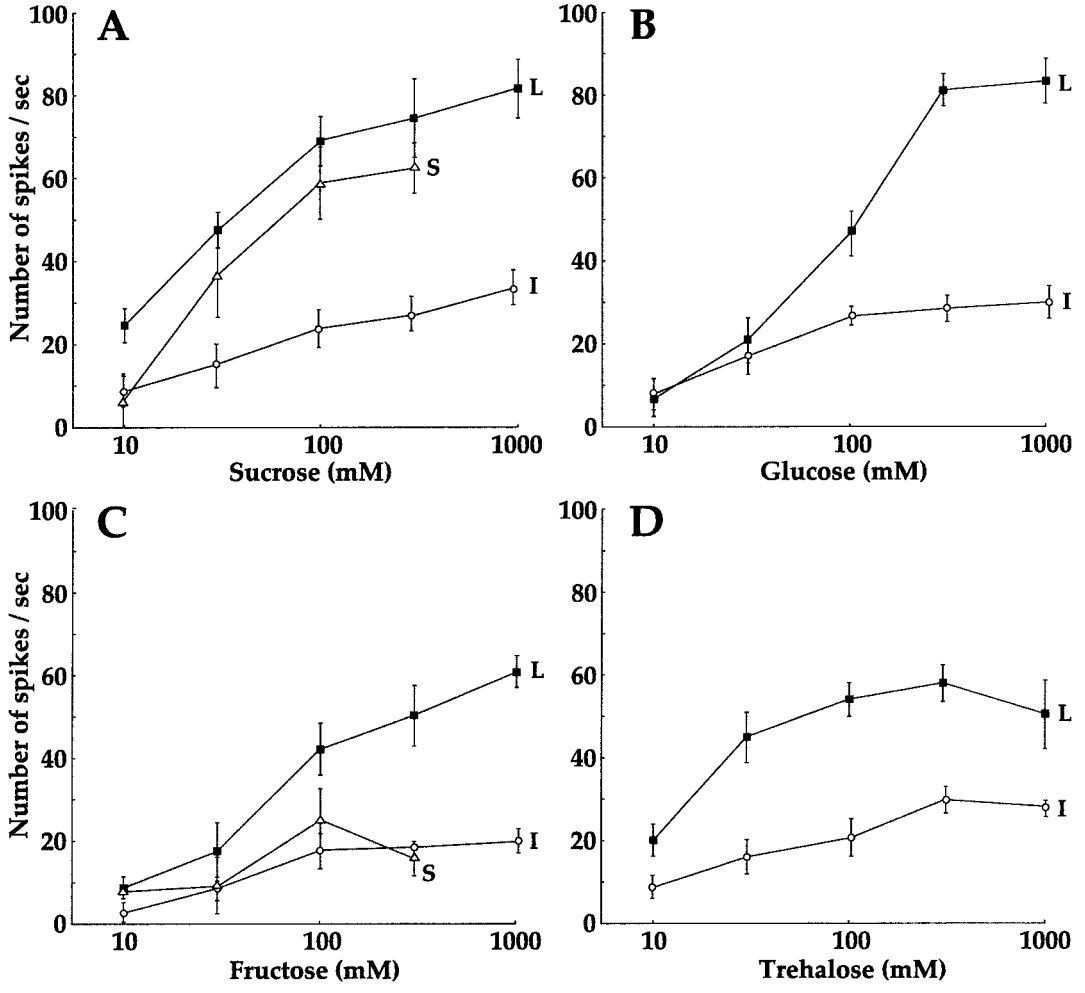

Fig. 6. Dose-response curves of the L-, S- and I-type sensilla to sucrose (A), glucose (B), fructose (C) and trehalose (D). Vertical bars represent standard errors. Responses of the L-, I- and S-type sensilla are shown as closed squares, open circles and open triangles, respectively. Each point was calculated from 40-52 recordings from L-type sensilla, 35-45 recordings from I-type sensilla and 10-21 recordings from S-type sensilla using 44 flies.

lower frequency. The S-type sensilla gave a good response to sucrose which was comparable to that of the L-type sensilla, but their responses to other sugars were weak. However, when recordings in response to stimulation by glucose and trehalose were obtained in the S-type sensilla, spike trains were noisy and spike identification was not possible. These results indicate that sugar response among the three types of chemosensilla differs and that responses of S-type sensilla to sugars are more difficult to obtain than are sugar-stimulated responses from the other types.

DISCUSSION

Variation of responsiveness among chemosensilla

In previous reports of electrophysiological recordings made on *Drosophila* taste sensilla, only L-type sensilla were examined (Tanimura and Shimada, 1981; Rodrigues and Siddiqi, 1981; Fujishiro *et al*., 1984; Wieczorek and Wolff, 1989). We presented here data on the basic electrophysiological responses of all the labellar chemosensilla. First we examined the rate of successful recordings from all sensilla. Results indicated that the I- and S-type sensilla gave a low response rate, with three sensilla of the I-type in particular having much lower success rates than the others on the labellum. These results explain why the previous studies used mainly the L-type marginal sensilla for recordings. The reason why some sensilla tend to fail to give responses to stimulants is not known. We occasionally observed that a sensillum gave responses to salt, but not to sugars, and *vice versa*. This may be caused by mechanical damage to a particular cell. However, in most cases a non-responding sensillum did not respond to any stimulus at all. We used only newly emerged flies and believe that mechanical damage and aging were unlikely to be the cause of the non-responsiveness. It has long been known that the nerve response of chemosensillum of flies is fairly variable, and depends on the fly being used and on each sensillum (Den Otter *et al*., 1972; Uehara and Morita, 1972). We have no sound explanation as to why particular groups of sensilla might give a poor response. One possible explanation for no responses is a contact failure, which can be caused by changes in conductivity at the tip of the chemosensilla (Maes and Den Otter, 1976). The involved structures are the viscous substance (Stürckow, 1967a), the pore in the dendrite-containing lumen and the opening mechanism in the dendrite-free lumen of the chemosensilla (Stürckow *et al*., 1967b, 1973).

The I-type sensilla lack water receptor cells

Typical chemosensilla have one mechanoreceptor and four gustatory neurons, each of which responds to water, sugar, and low or high concentrations of salt. An anatomical study by electron microscopy showed that only two gustatory neurons innervated the I-type sensilla (Falk *et al*., 1976; Shanbhag *et al*., 2001). In our experiments, W spikes were never observed in I-type sensilla when 1 mM KCl was used as the stimulus (which usually elicits only W spikes). The I-type sensilla responded to sugars and salts, apparently via two different cell types (Hiroi *et al*., in preparation) for which the developmental process to produce these two kinds of taste cells is probably different from that in the L- and S-type sensilla.

Differences in dose-response kinetics of sugars between the L-, I- and S-type sensilla

We found that the response to sugars differed among the three types of sensilla. The L-type sensilla showed the highest response to all sugars examined. The S-type sensilla responded to sucrose in a similar manner to that seen in the L-type sensilla, but responded to fructose with a firing rate of lower magnitude. The L-type and I-type sensilla both responded to the four sugars tested, whereas S-type sensilla did not produce good response for glucose and trehalose. The different sensilla types also differed in their maximal firing frequency, with the I-type sensilla firing at about one-third of the rate observed in the L-type sensilla. Such sensitivity differences to a sugar among different types of sensilla were also reported in blowfly (Liscia *et al*., 1998).

Our previous studies suggested the presence of at least three separate receptor sites, F, G and T, for fructose, glucose and trehalose, respectively, in the labellar sensilla (Tanimura and Shimada, 1981; Tanimura *et al*., 1982). If we consider the differences of excitability between the three sensilla types, it is possible that similar receptor proteins are expressed in cells of the L-type and I-type sensilla but that their expression level is lower in the I-type sensilla. Another possible explanation is that the signal transduction pathway may differ between the sensilla types. The S-type sensilla gave a good response to sucrose, but did not respond well to glucose and trehalose. If we assume the three receptor sites hypothesis, receptors for glucose and trehalose may not be properly expressed in the S-type sensilla. Previously, we postulated that the G site binds sucrose as well as glucose. The presence of cells exhibiting a good response to sucrose but a lower response to glucose suggests that separate receptor sites exist for these two sugars. Most of the S-type sensilla were not accessible with electrodes as described, but further studies are required to confirm these differentiated responses to sugars among sensilla types.

Possible functions of *Gr* genes

The 65 *Gr* genes belong to a large family of seven-transmembrane G-protein coupled receptors (Clyne *et al*., 2000; Dunipace *et al*., 2001; Scott *et al*., 2001; Robertson, personal communication to Flybase, 2001). *Gr* genes might code receptors for sugars, pheromones, bitter compounds, *etc*., if they function as taste receptors. So far only one *Gr* gene has been reported as a functional receptor (Ueno *et al*., 2001; Dahanukar *et al*., 2001). In our study we could not find any relationship between the pattern of *Gr* expression and variations of sugar sensitivities. Our data, obtained with six *Gr* promoter-Gal4 lines, suggest that these six genes are expressed mainly in the S-type sensilla. A limited number of

L-type sensilla expressed *Gr22c* and *Gr22f*. To the present time we have not found that these particular sensilla show any unique sensitivity to sugars. There still remains a possibility that these sensilla respond to compounds other than sugars. Preliminary recordings using amino acid mixtures did not reveal any differences either. Most of the *Gr* genes examined in this study were originally chosen for their expression as confirmed by *in situ* hybridization on labella (Scott *et al*., 2001; Dunipace *et al*., 2001). We cannot exclude the possibility that other *Gr* genes, not examined in our study, with low levels of expression that cannot be monitored by promoter-Gal4 may function as taste receptors.

In the olfactory system of *Drosophila*, a single olfactory receptor gene is expressed in one sensory neuron in antennae (Vosshall *et al*., 2000). Each sensory neuron projects to a specific glomerulus in the antennal lobe. In this manner, chemical information of odors will be represented in the brain. In order to discriminate between thousands of chemicals, olfactory receptor number might have increased as a result of an evolutionary process. In the gustatory system, however, it might not be an essential prerequisite to be able to discriminate between different sugar molecules. All the sugars stimulate sugar receptor cells and the information about a chemical identity may not be particularly important for flies. These considerations do not, however, coincide with the view that multiple *Gr* genes are expressed in a spatially restricted manner and each receptor binds to a specific ligand (Scott *et al*., 2001; Dunipace *et al*., 2001).

The electrophysiological and histological study presented here reveals that the labellar chemosensilla are differentiated in their response to sugars. Further physiological and molecular studies are required to elucidate the molecular mechanism of taste in *Drosophila*.

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