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Differential Inputs from Chemosensory Appendages Mediate Feeding Responses to Glucose in Wild-Type and Glucose-Averse German Cockroaches, *Blattella germanica*

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Abstract

Glucose is a universal phagostimulant in many animal species, including the cockroach *Blattella germanica*. However, some natural populations of *B. germanica* have been found that are behaviorally deterred from eating glucose. In dose–response studies, glucose was a powerful phagostimulant for wild-type cockroaches, but it strongly deterred feeding in a glucose-averse strain. Both strains, however, exhibited identical dose–response curves to other phagostimulants and deterrents. As a lead to electrophysiological and molecular genetics studies to investigate the mechanisms that underlie glucose-aversion, we used 2 assay paradigms to delineate which chemosensory appendages on the head contribute to the reception of various phagostimulatory and deterrent chemicals. Both simultaneous dual stimulation of the antenna and mouthparts of the insects and 2-choice preference tests in surgically manipulated insects showed that the glucose-averse behavior could be elicited through the gustatory systems of the antennae and mouthparts. The paraglossae alone were sufficient for maximum sensitivity to both phagostimulants and deterrents, including glucose as a deterrent in the glucose-averse strain. In addition to the paraglossae, the labial palps were more important than the maxillary palps in the reception of deterrents (caffeine in both strains and glucose in the glucose-averse strain). The maxillary palps, on the other hand, played a more important role in the reception of phagostimulants (fructose in both strains and glucose in the wild-type strain). Our results suggest that distinct inputs from the chemosensory system mediate opposite feeding responses to glucose in the wild-type and glucose-averse strains.

Key words: avoidance behavior, Blattella germanica, chemosensory appendages, gustation, glucose aversion, sugar

Introduction

Gustation is an essential sensory modality for food selection, reproduction, and avoiding toxic substances. Most likely, the ability to discriminate phagostimulants evolved as a screening mechanism for potentially nutritious foods and the ability to discriminate deterrents evolved as a screening mechanism for toxic compounds (Chapman 1998; Ozaki et al. 2003; Wyatt 2003). In cockroaches, sugars such as glucose, fructose, sucrose, maltose, and maltotriose act as phagostimulants (Tsuji 1965; Nojima et al. 1996; Gore and Schal 2004). Nutritional requirement studies indicate that cockroaches develop optimally on diets with more than 50% carbohydrate (Forgash 1958; Cohen et al. 1987), and defined diets for the German cockroach (*Blattella germanica*) have included high levels of glucose or related sugars (House 1949; Gordon 1959, 1968).

Silverman and Bieman (1993) discovered a field population of glucose-averse German cockroaches (T164) that rejected D-glucose and consumed less of a glucose-supplemented diet in all its life stages; however, other sugars (e.g., D-fructose, D-mannose, sucrose, and maltose) stimulated feeding in these glucose-averse cockroaches (Silverman and Bieman 1993; Silverman and Ross 1994; Silverman 1995; Silverman and Selbach 1998). These assays explored sugar preferences of wild-type and glucose-averse cockroaches in behavioral whole-animal arena tests and also examined the inheritance pattern of glucose aversion with genetic crosses of the 2 strains. Glucose-averse cockroaches also develop slower and have lower survival than wild-type cockroaches, suggesting that they would be at a selective disadvantage when naturally foraging, especially for glucose-containing foods

(Silverman 1995; Wang, Scharf, and Bennett 2004). Conversely, the glucose-averse trait would turn out to be a significant advantage in avoiding insecticide baits that contain glucose. Because hemocoelic injections of glucose have no apparent detrimental effects on weight and mortality of the glucose-averse cockroaches (see Results), it appears that glucose does not act as a toxic substance in the glucose-averse strain. Rather, it appears that glucose inhibits feeding through the chemosensory system. However, the mechanism(s) that underlie glucose aversion has not been studied, and the chemosensory systems involved in transduction of the aversive stimuli have not been experimentally established.

Gustatory sensilla of insects are usually localized on several external appendages including antennae, mouthparts, tarsi, and ovipositor, but they also may be widely distributed on the body and wing edges (Chapman 1998; Ozaki and Tominaga 1999; Newland et al. 2009). Each gustatory sensillum houses gustatory receptor neurons (GRNs), each of which is thought to be specific for tastants within a single taste modality, resulting in distinct populations of GRNs for sugars, salts, water, and deterrents (Hodgson et al. 1955; Dethier 1976; Chapman 1998; Ozaki and Tominaga 1999; Vosshall 2007). The selective activation of GRNs that respond to phagostimulants and deterrents results in appetitive and aversive feeding behavior, respectively, in many insects, including *Drosophila* (Ozaki and Tominaga 1999; Vosshall 2007; Montell 2009). Each chemosensory organ (antenna, maxillary, and labial palps, proboscis, etc.) has a specific localization and topology of gustatory sensilla, resulting in its ability to elicit adaptive behavioral and physiological responses. The GRNs on the proboscis, for instance, are involved in the proboscis extension and recoiling responses, as well as food consumption in several insect species (Dethier 1976; Chapman 1998; Amakawa 2001), whereas those on the antennae are involved in orientation toward or away from the stimulus, proboscis extension, and salivation (Ramaswamy 1987; Haupt 2004; de Brito Sanchez et al. 2005; Jørgensen, Almaas, et al. 2007; Watanabe et al. 2008). The GRNs on the tarsi are also involved in proboscis extension in addition to courtship and egg laying behaviors (Ramaswamy 1987).

The German cockroach has 4 major paired external sensory appendages on the head, namely antennae, maxillary palps, labial palps, and paraglossae (Figure 1). Although little is known about their chemosensitivities for phagostimulants and deterrents, morphologically defined gustatory sensilla have been observed on the antennae and maxillary and labial palps (Ramaswamy and Gupta 1981), as in the American cockroach *Periplaneta americana* (Seelinger and Tobin 1981; Hansen-Delkeskamp 1992; Nishino et al. 2005). Gustatory sensilla were also identified on the paraglossae, whose GRNs respond to sucrose, maltotriose, NaCl, and the male's nuptial pheromone which is composed of oligosaccharides, lipids, and amino acids (Nojima, Nishida,

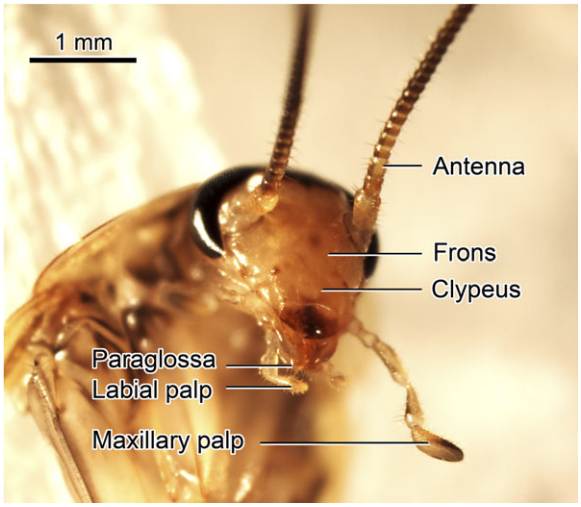

Figure 1 The 4 major external chemosensory paired appendages on the head of an adult male German cockroach. The translucent clypeus and frons are shown, through which ingested dyed solutions and agar could be observed. This figure appears in color in the online version of *Chemical Senses*.

et al. 1999; Nojima, Sakuma et al. 1999; Kugimiya et al. 2002; Nojima et al. 2002; Wada-Katsumata et al. 2009). Behavioral and electrophysiological studies of the paraglossae have suggested that phagostimulants, including glucose, stimulate GRNs that mediate appetitive feeding behavior in the German cockroach (Nojima et al. 1996; Wada-Katsumata et al. 2009). Normally, the German cockroach has the opportunity to contact chemical stimuli first with their long antennae, then with the maxillary palps and labial palps, and ultimately with the paraglossae, which are most proximal to the mouth. But food applied directly to the mouthparts stimulates feeding behavior (ingestion) without antennal stimulation. Moreover, paraglossa stimulation with the male's nuptial pheromone elicited feeding motion of the mandibles and galeae without maxillary palp stimulation (Wada-Katsumata et al. 2009).

In this study, we hypothesized that 4 major chemosensory appendages function as first-order peripheral detectors in food preference but that not all sensory appendages have the same chemospecificity for reception of phagostimulants and deterrents. In order to demonstrate the chemospecificity of various sensory organs in glucose aversion, we aimed to 1) identify which chemosensory appendages contribute to the appetitive and aversive responses to phagostimulants and deterrents in the wild-type and glucose-averse strains and 2) clarify the relative importance of these sensory appendages in glucose detection in the glucose-averse strain. We tested feeding responses to phagostimulants including fructose and glucose (Tsuji 1965) and deterrents such as caffeine and quinine, known to serve as powerful deterrents in *Drosophila* (Moon et al. 2006), after stimulating or ablating the 4 major external sensory appendages in the wild-type and glucose-averse strains.

Materials and methods

Insects

The wild-type strain (American Cyanamid) and glucose-averse strain (T164/Orlando normal) of *B. germanica* were reared on water and food pellets (Purina No. 5001 Rodent Diet, PMI Nutrition International) at 27 ± 1 °C, 40% relative humidity, and 12:12 h light:dark photoperiod. The glucose-averse strain was produced from 7 consecutive backcrosses between wild-type cockroaches (Orlando normal) and T164, which was collected in Florida in 1989 (Silverman and Bieman 1993). The progeny from each cross were then exposed to a glucose-toxicant (hydramethylnon) mixture, thus yielding glucose-averse survivors. Although assigned different names based on their recent acquisitions, wild-type American Cyanamid and Orlando normal originated from a common insecticide-susceptible strain colonized some 40 years ago. Newly emerged males were separated and kept in groups of 10–50 with water and food until use. Seven- to ten-day-old virgin males were tested. We did not use females to avoid effects of the ovarian cycle and associated hormonal changes on feeding behaviors.

Chemicals

β-D-Fructose, α-D-glucose, maltose, and maltotriose were tested as general phagostimulants that elicit appetitive behavior in many insect species. Caffeine and quinine hydrochloride dihydrate were tested as general deterrents that induce aversive behavior in many insect species. NaCl was tested as a general salt. Allura red AC (maximum absorbance at ~504 nm; 1 mmol L^{-1}) and erioglaucine (Brilliant Blue, maximum absorbance at ~625 nm; 0.5 and 1 mmol L^{-1}) were used for coloring the stimulus solutions and/or agar discs. These food colorings at these concentrations had no effect on feeding behavior and were not toxic to the German cockroach. Glucose, maltotriose, caffeine, quinine hydrochloride dihydrate, Allura red, and erioglaucine were purchased from Sigma-Aldrich Co. Fructose was purchased from ICN Biochemicals, Inc. Maltose and NaCl were purchased from Fisher Scientific, Inc. Agar was purchased from Bioline USA, Inc.

Tests of glucose toxicity by hemocoelic injection

The glucose aversion trait might be associated with glucose toxicity and it might have evolved as a mechanism to avoid toxic compounds. To test for chronic and acute effects of glucose, we directly delivered glucose into the hemocoel of wild-type and glucose-averse cockroaches. Ten-day-old adult males were sedated by cooling on ice for 30 min and injected 1 μL of 3 mol L^{-1} glucose, 3 mol L^{-1} fructose, or distilled water between the fourth and fifth segments (sternites) of the ventral abdomen. After 7 days with water and food, mortality was recorded and live cockroaches were weighed. From 13–26 cockroaches were tested in each of the 3 treatment groups. Body mass of cockroaches in different treatment groups was compared with analysis of variance (ANOVA) and Tukey's test ($P < 0.05$).

Involvement of the antennae in glucose aversion

In order to test the effect of antennal chemosensory input on ingestion of phagostimulants and deterrents, a dual stimulation bioassay was performed, simultaneously stimulating an antenna and the mouthparts. Cockroaches were deprived of food for 24 h, but supplied with water. Each cockroach was placed in a plastic pipette tip with only its head protruding. Before the test, cockroaches were satiated with distilled water. Two drops of stimulus solution colored with blue dye (1 mmol L^{-1} erioglaucine) were tested. The antenna and mouthparts, which consist of the maxillary palps, labial palps, and paraglossae, were stimulated as follows. A 0.3 μL drop of stimulus solution was applied to the dorsal-middle section of the right antenna and constant contact between the solution and antenna was maintained. As soon as possible (within 0.5 s), a second 0.3 μL drop of stimulus solution was applied for less than 1 s to the mouthparts. The tested cockroaches ingested <0.01 μL of the stimulus solution before the solution was withdrawn. Because satiation of starved cockroaches requires about 2 μL of 1 mol L^{-1} fructose solution, none of the cockroaches in the dual stimulation assays were considered satiated, and they were highly motivated to feed. As the cockroach ingested the stimulus solution, blue color could be seen through the clypeus and frons, the translucent front-middle area of the head capsule (Figure 1). We recorded if stimulation of the mouthparts elicited ingestion. To minimize adaptation, intervals between stimulus applications were >3 min because the GRNs on the paraglossa fully recovered within 3 min after they showed maximum electrophysiological responses to 1 s stimulation with sugar (data not shown). At the end of the test series, we tested the motivation of cockroaches to eat sugar by stimulating the antenna and mouthparts with 1 mol L^{-1} fructose solution. The German cockroach is well adapted to eat various foods in the human environment, including high concentrations of sugars such as fructose and glucose. Moreover, insecticidal baits often contain high concentrations of various phagostimulants, including sugars, often to inhibit microbial growth. Therefore, we tested high concentrations of sugars in this study, also because they yield robust differentiation between the wild-type and glucose-averse strains. The stimulus solutions applied on the antenna were fructose (300, 1000 and 3000 mmol L^{-1}), glucose (300, 1000, 3000 mmol L^{-1}), and caffeine (3, 10, 30 mmol L^{-1}). Distilled water was also applied on the antenna of all cockroaches. The second stimulus, applied to the mouthparts, consisted of 1000 mmol L^{-1} fructose, 1000 mmol L^{-1} glucose, 10 mmol L^{-1} caffeine, or distilled water. For example, when 1000 mmol L^{-1} fructose solution was applied

to the mouthparts, the antenna received 1000 mmol L^{-1} fructose solution and distilled water. Then, fructose, glucose, or caffeine was applied to the antenna in an experimental series from the lowest to the highest concentration. The test was performed with 10 cockroaches and repeated 3 times. The percentage of cockroaches showing feeding response was subjected to arcsine square root transformation and treatments were compared (ANOVA, Dunnett's test, $P < 0.05$).

Involvement of various mouthpart appendages in glucose aversion

Two-choice bioassays were performed to determine the chemospecific profiles of 3 mouthpart appendages for phagostimulants and deterrents. Adult male cockroaches were deprived of food and water for 2 days and then divided into 7 treatment groups: (I) intact cockroaches; (LP) cockroaches with intact labial palps and paraglossae, but with maxillary palps ablated; (MP) cockroaches with intact maxillary palps and paraglossae, but labial palps ablated; (ML) cockroaches with maxillary and labial palps intact, but paraglossae ablated; (M) cockroaches with intact maxillary palps, but labial palps and paraglossae ablated; (L) cockroaches with intact labial palps, but maxillary palps and paraglossae ablated; and (P) cockroaches with intact paraglossae, but maxillary and labial palps ablated.

For surgical manipulations, insects were immobilized on ice for ~30 min. The chemosensory appendages were cut at the base using fine scissors under a binocular microscope, and hemolymph was allowed to coagulate at the cut surface. After 30 min recovery, 10 cockroaches were placed in a large petri dish (2.5 cm height, 14 cm ID) containing 2 agar discs (6 mm height, 25 mm ID): one disc contained 1% agar and 1 mmol L^{-1} allura red, whereas the second disc contained a mixture of 1% agar, a test stimulus, and 0.5 mmol L^{-1} erioglaucine. The assay duration was 2 h during the dark phase of the insects' light:dark cycle. After feeding, individuals were dissected, and the foregut and midgut of each cockroach were homogenized in 50 µL distilled water. Each homogenate was centrifuged at 11 750 *g* for 8 min. The absorbance of the supernatant was measured at 504 nm (red) and 625 nm (blue) with a microplate scanning spectrophotometer (PowerWave-X; Bio-Tek Instruments). The calibration curve for measurement of red and blue colors was $\text{Abs.} = (A - D)/(1 + (x/C)^B + D)$; where $A = 12.92$, $B = -0.96$, $C = 7.9$, $D = 0.0031$ for measurement of red color; $A = 7.53$, $B = 1.12$, $C = 0.73$, $D = 0.0075$ for measurement of blue color; x is concentration. The amounts of red and blue colors ingested by each cockroach ([Red] and [Blue]) were obtained from the calibration curve. The percentage of red and blue color intake as a preference response was calculated by [Red]/([Red] + [Blue]) and [Blue]/([Red] + [Blue]). Thus, 50% indicates that cockroaches fed equally on both discs.

Fructose, glucose, and caffeine were tested at concentrations ranging from 1 to 300 mmol L^{-1}, depending on the compound. These concentrations are lower than the effective concentrations tested in the dual stimulation test. The numbers of tested cockroaches ranged from 10 to 70 per treatment.

About 24% of 1980 cockroaches failed to feed (had empty foregut and midgut), and they were eliminated from data analysis. To test for significant differences in preference between red and blue color intake in each treatment group, the percentage was arcsine square root transformed and compared using paired Student's *t*-test. In order to discern how the chemosensory appendage ablations alter the feeding responses, a preference index was obtained by [Blue – Red]/[Blue + Red] in each treatment group. Thus, a preference index of 0 indicates that cockroaches fed equally on both discs, 1 indicates a preference for the supplemented agar and –1 indicates preference for plain agar over supplemented agar. Differences in the preference index were tested among different concentrations of each stimulus within each treatment group and among different treatments of each stimulus within each concentration using ANOVA and Tukey's test ($P < 0.05$).

Paraglossae-mediated feeding responses to phagostimulants and deterrents

To conduct dose–response studies with phagostimulants and deterrents, adult male cockroaches were deprived of food for 24 h, but supplied with water. When testing deterrents, the cockroaches were deprived of both food and water for 24 h to increase their thirst. The maxillary and labial palps were ablated in the same manner as in the 2-choice bioassays. After 30 min recovery, each cockroach was placed in a plastic pipette tip with only its head protruding. Before each test, the cockroaches were satiated with water except when they were tested with deterrents. The paraglossae of the cockroach were carefully touched with a 0.3 µL drop of stimulus solution colored with 1 mmol L^{-1} erioglaucine in a sequence from the lowest to the highest concentration. Fructose, glucose, maltose, and maltotriose were tested at 0.001, 0.01, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, and 3000 mmol L^{-1}. Caffeine and quinine were tested at 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 mmol L^{-1}. NaCl was tested at 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 mmol L^{-1}. Each concentration of each stimulus was tested with 36–236 cockroaches. The duration of stimulation, intervals between stimulus applications, and observation details of the feeding response were the same as in the dual stimulation bioassays. The percentages of cockroaches showing feeding responses were plotted against the logarithmically scaled concentration of each stimulus to obtain dose–response curves. A median effective concentration (EC_{50}) of each test solution was obtained by using a probit-analysis program, where the significance was tested by a χ^2 test of heterogeneity (Sakuma 1998).

Results

Lack of glucose toxicity in injected cockroaches

All cockroaches survived the hemocoelic injections. There were no significant differences in body mass among treatment groups: for wild-type cockroaches: glucose-injected 50.6 ± 3.7 mg (mean ± standard deviation [SD]), $n = 22$; fructose-injected 50.5 ± 3.6 mg, $n = 26$; distilled water-injected 50.6 ± 2.6 mg, $n = 10$; for glucose-averse cockroaches: glucose-injected 51.0 ± 2.5 mg, $n = 20$; fructose-injected 50.1 ± 2.7 mg, $n = 20$; distilled water-injected 50.6 ± 3.2 mg, $n = 10$. Furthermore, we did not observe any differences in the behavior or activity of cockroaches in the various treatment groups. Although it is not known whether the 2 strains differ in their metabolism of injected glucose or fructose, these results indicate that glucose does not have toxic or lethal effects on either the wild-type or glucose-averse strain. Therefore, the glucose aversion trait is not likely an evolutionary response to glucose toxicity.

Modulation of feeding responses by antennal gustation

To test whether or not phagostimulants and deterrents are detected at the antennal level in the wild-type and glucose-averse cockroaches, we performed assays in which we simultaneously stimulated an antenna and the mouthparts with either the same or different stimuli. When an antenna and the mouthparts were stimulated by the same stimulus solution, all wild-type and glucose-averse cockroaches accepted 1000 mmol L^{-1} fructose (Figure 2A,E) but not 10 mmol L^{-1} caffeine (Figure 2C,G) or distilled water (Figure 2D,H). Glucose applied to the antenna and mouthparts induced feeding responses from 83% of the wild-type cockroaches (Figure 2B). However, all the glucose-averse cockroaches, though starved for 24 h, refused to ingest 1000 mmol L^{-1} glucose applied simultaneously to one antenna and the mouthparts (Figure 2F).

Next we examined the interaction of gustatory inputs from the antenna and mouthparts when one stimulus solution was applied to the antenna, whereas another stimulus solution was applied to the mouthparts. In wild-type cockroaches, application of water to the antenna (negative control) did not alter any of the feeding responses when fructose, glucose, caffeine, or water was applied to the mouthparts. Application of fructose or glucose to the antenna did not significantly affect the feeding responses when fructose or glucose was applied to the mouthparts, but this is because nearly 100% of the cockroaches already responded to the negative controls (water applied to the antenna and fructose or glucose applied to the mouthparts). However, 300 and 3000 mmol L^{-1} fructose applied on the antenna significantly decreased the feeding inhibition of 10 mmol L^{-1} caffeine applied to the mouthparts, resulting in greater ingestion of the caffeine solution (13.3% and 30%, respectively, compared with 0% ingestion when water was applied to the antenna and caffeine to the mouthparts) (Figure 2C). The application of 3000 mmol L^{-1} fructose to the antenna also increased the feeding response to distilled water applied to the mouthparts (13.3%) (Figure 2D). Glucose applied to the antenna of wild-type cockroaches, like fructose, also stimulated some males to ingest caffeine that was applied to the mouthparts. On the other hand, 30 mmol L^{-1} caffeine applied to the antenna significantly reduced the feeding responses to either 1000 mmol L^{-1} fructose (40% responded) or glucose (30%) applied to the mouthparts (Figure 2A,B).

The responses to combinations of fructose, water, and caffeine in the glucose-averse strain were similar to the same combinations in the wild-type strain. However, 3000 mmol L^{-1} glucose or 30 mmol L^{-1} caffeine applied to the antenna significantly reduced the feeding responses to 1000 mmol L^{-1} fructose applied to the mouthparts (Figure 2E). Although glucose or caffeine applied to the mouthparts completely inhibited ingestion in this strain, the application of 3000 mmol L^{-1} fructose to the antenna significantly increased the feeding responses of the cockroaches when 1000 mmol L^{-1} glucose (30.0%), 10 mmol L^{-1} caffeine (26.7%), or water (20.0%) was applied to the mouthparts (Figure 2F–H). Glucose applied to the antenna, unlike fructose, further inhibited ingestion of glucose, caffeine, or water applied to the mouthparts.

These results indicate that antennal gustatory neuronal inputs were integrated in the brain with gustatory inputs from the mouthparts and affected the preference and aversive feeding responses. The results clearly show that glucose acts as a deterrent in the glucose-averse strain not only through gustatory inputs from the mouthparts but also through gustatory antennal input.

Differential involvement of various mouthpart gustatory appendages in feeding behavior

In the 2-choice bioassay, we hypothesized that surgical ablation of various sensory appendages of the mouthparts would result in differential loss of the ability and acuity of the cockroach to show preference responses to various tastants. Because there were no significant differences in the preference responses between intact cockroaches (I) and cockroaches with one pair of mouthpart appendages ablated (treatment LP, MP, and ML) (data not shown), in our design, we ablated 2 pairs of appendages, leaving only one pair intact. Figure 3 shows the percentage intake of 2 food choices for wild-type and glucose-averse cockroaches. All wild-type and glucose-averse cockroaches in all treatments failed to discriminate between plain agar and agar supplemented with a low concentration (30 mmol L^{-1}) of fructose or glucose or 1 mmol L^{-1} caffeine (Figure 3). Intact cockroaches (I) of both strains discriminated between plain agar and agar supplemented with a high concentration (100 and 300 mmol L^{-1}) of fructose or glucose or 3 and 10 mmol L^{-1} caffeine. The wild-type cockroaches preferred the

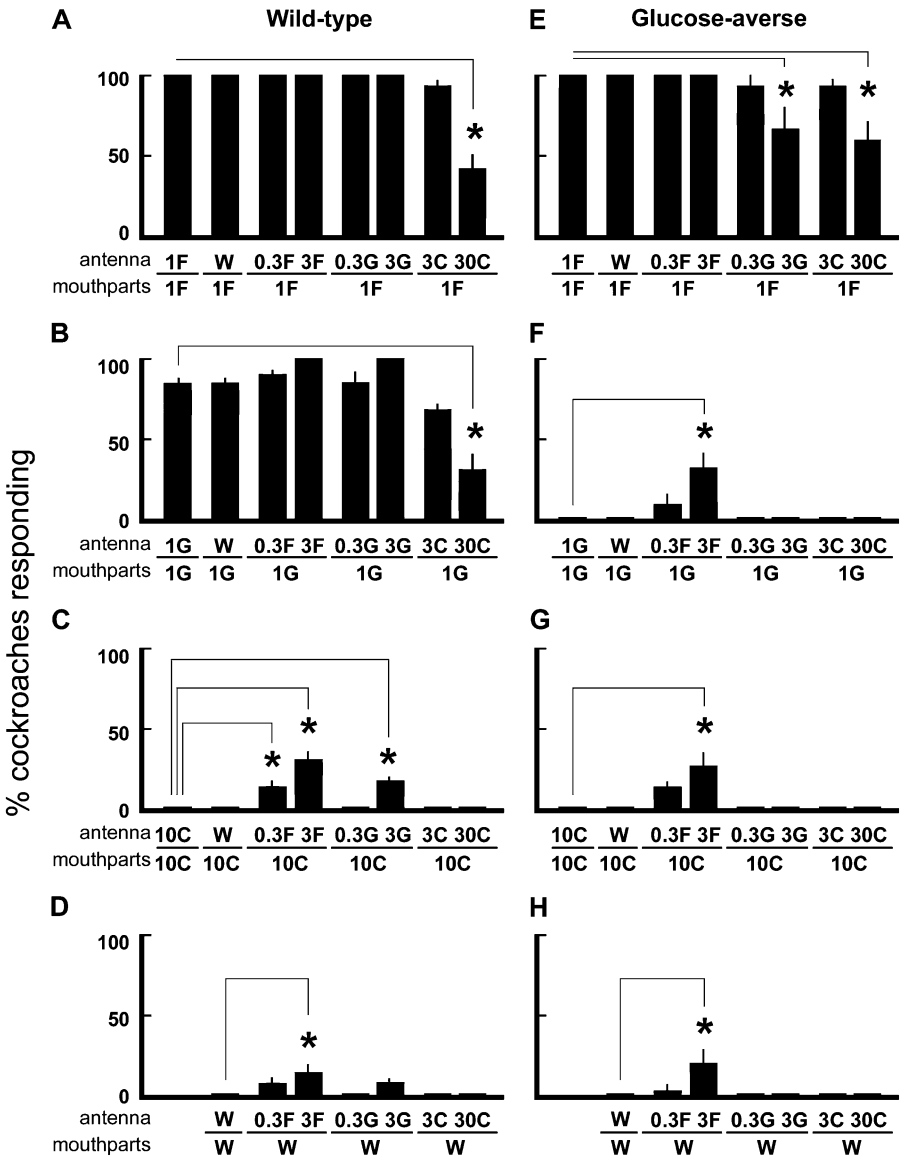

Figure 2 Mean percentages of cockroaches showing feeding responses in the dual stimulation bioassay. A stimulus solution was applied to the mouthparts, whereas the same or a different stimulus solution was applied to an antenna (error bars represent standard error of the mean). **(A–D)** Responses of wild-type cockroaches. **(E–H)** Responses of glucose-averse cockroaches. Antenna, stimulus solutions applied on the antenna; Mouthparts, stimulus solutions applied on the mouthparts. The stimulus solutions are shown as follows: W, distilled water; F, fructose; G, glucose; C, caffeine. Values preceding a stimulus are expressed in moles per liter for fructose and glucose and in millimoles per liter for caffeine, so 0.3F is 300 mmol l^{-1} fructose and 30C is 30 mmol l^{-1} caffeine. *Indicates a significant difference from the control, where both the antenna and mouthparts received the same stimulus (ANOVA, Dunnett's test, $P < 0.05$).

sugar-supplemented agar over plain agar; they avoided eating the caffeine-supplemented agar (Figure 3A–C). The glucose-averse cockroaches preferred the fructose-supplemented agar over plain agar (Figure 3D); they avoided eating agar containing either glucose or caffeine (Figure 3E,F). All wild-type cockroaches, except one treatment group (treatment L, 100 mmol L^{-1}), regardless of surgical manipulation, significantly preferred agar containing 100 or 300 mmol L^{-1} fructose or glucose over plain agar (Figure 3A,B). Wild-type cockroaches generally avoided agar containing 3 or 10 mmol L^{-1} caffeine but failed to discriminate 3 mmol L^{-1} of caffeine from plain agar in treatment M (Figure 3C). As in the wild type strain, all glucose-averse cockroaches, regardless of surgical manipulation, significantly preferred agar containing 100 or 300 mmol L^{-1} fructose over plain agar (Figure 3D). They avoided agar containing 3 or 10 mmol L^{-1} caffeine, but treatment M insects failed to discriminate caffeine from plain agar at both 3 and 10 mol L^{-1} (Figure 3F). The glucose-averse cockroaches ingested much more plain agar than agar supplemented with 100 or 300 mmol L^{-1} glucose, but treatment M insects failed to discriminate 300 mmol L^{-1} glucose from plain agar (Figure 3E).

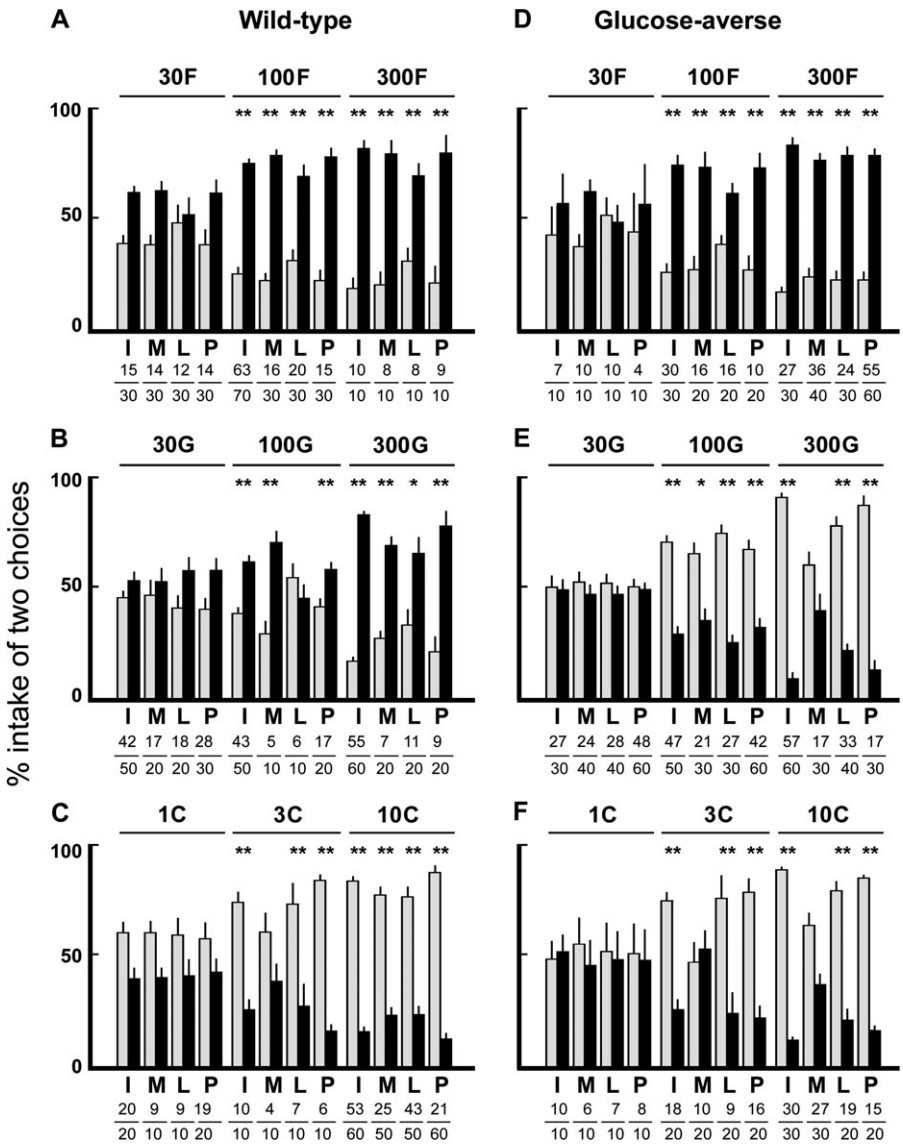

Figure 3 Feeding sensitivities of cockroaches with various mouthpart appendages ablated in 2-choice bioassays. The mean proportion of plain agar (red) and supplemented agar (blue) ingested are shown as grey and black columns that add up to 100% (error bars represent standard error of the mean). **(A–C)** Agar ingested by wild-type cockroaches. **(D–F)** Agar ingested by glucose-averse cockroaches. The treatment types are shown as follows: I, intact cockroaches; M, cockroaches with intact maxillary palps but with labial palps and paraglossae ablated; L, cockroaches with intact labial palps but with maxillary palps and paraglossae ablated; P, cockroaches with intact paraglossae but maxillary and labial palps ablated. The stimuli in the blue agar are shown as follows: F, fructose; G, glucose; C, caffeine. All values preceding a stimulus are expressed in millimoles per liter, so 300F is 300 mmol l^{-1} fructose. The number of cockroaches that responded and the total number of tested cockroaches are shown under each treatment group as a numerator and denominator, respectively. * and ** indicate significant differences between ingestion of plain and supplemented agar in each treatment group at $P < 0.05$ and $P < 0.01$, respectively (paired Student's *t*-test).

To compare the responses of cockroaches across various concentrations of tastants and across various ablation treatments, we generated a preference index as a ratio of ingested supplemented and plain agar. ANOVA of the preference index for wild-type cockroaches showed that for both fructose and glucose, all treatments except treatment L (ablated maxillary palps and paraglossae) exhibited significant dose–response patterns, cockroaches readily discriminating high sugar concentrations from plain agar (Supplementary Table 1). The preference index of cockroaches with ablated maxillary palps and paraglossae did not increase significantly with higher sugar concentration. On the other hand, these cockroaches responded appropriately to increasing concentrations of caffeine, whereas cockroaches with intact maxillary palps but ablated labial palps and paraglossae (treatment M) required high concentrations of caffeine to be deterred (Supplementary Table 1). Thus, it appears that the maxillary palps and paraglossae are more important than the labial palps in sugar

reception, and the labial palps and paraglossae are more important than the maxillary palps in reception of aversive tastants. Although the glucose-averse cockroaches exhibited a significant dose–response to fructose in all treatments, they were least discriminating in treatment L, as were the wild-type cockroaches (Supplementary Table 2). Moreover, like the wild-type cockroaches, they exhibited a significant dose–response to caffeine in all treatments, except treatment M (Supplementary Table 2). Notably, as did both wild-type and glucose-averse cockroaches in response to caffeine, glucose-averse cockroaches with ablated labial palps and paraglossae (treatment M) failed to discriminate glucose from plain agar at both 3 and 10 mol L^{-1} (Supplementary Table 2).

In a comparison of the wild-type strain across various ablation treatments, with 300 mmol L^{-1} glucose, there were no significant differences in the preference index between intact cockroaches and either treatment M or P cockroaches (Supplementary Table 1). The preference index of treatment L was significantly lower than that of the intact group (Supplementary Table 1). In the glucose-averse strain, treatment L cockroaches chose agar containing 100 mmol L^{-1} fructose less than the other groups (Supplementary Table 2). The preference index of treatment M cockroaches was significantly lower than in the other treatment groups with 300 mmol L^{-1} glucose as well as with 3 and 10 mmol L^{-1} caffeine (Supplementary Table 2).

To reiterate, in both strains, the finest discrimination between phagostimulants and plain agar and between deterrents and plain agar was by intact cockroaches (I). Although the 3 paired mouthpart appendages are complementary detectors for phagostimulants and deterrents, the maxillary palps and paraglossae appear more important than the labial palps for appetitive behavior. The labial palps and paragossae, however, are more important than the maxillary palps for aversive behavior. The results indicate that differential inputs from chemosensory appendages mediate feeding responses to glucose in the wild-type and glucose-averse strains. Most importantly, cockroaches with ablated maxillary and labial palps (intact paraglossae, P) exhibited similar discriminatory preference responses to phagostimulants and deterrents as intact cockroaches, and therefore we focused the following studies on the paraglossae.

Role of the paraglossae in gustatory reception

Cockroaches with surgically ablated maxillary and labial palps were restrained in a pipette tip and the paraglossae offered various gustatory stimuli. The feeding responses to fructose, maltose, and maltotriose increased sigmoidally with concentration in both the wild-type and glucose-averse strains (Figure 4A,B). However, while glucose elicited feeding responses in wild-type cockroaches, it reduced the feeding responses to water in the glucose-averse cockroaches in a dose-dependent manner. The EC_{50} of the wild-type strain was 3.78 (95% fiducial limits 2.26, 6.31) mmol L^{-1} for maltotriose, 8.34 (5.36, 12.95) mmol L^{-1} for maltose, 24.7 (20.0, 30.5) mmol L^{-1} for fructose, and 56.0 (40.6, 77.3) mmol L^{-1} for glucose. In the glucose-averse strain, the EC_{50} was 2.98 (1.89, 4.69) mmol L^{-1} for maltotriose, 6.95 (4.23, 11.38) mmol L^{-1} for maltose, 33.45 (27.66, 40.44) mmol L^{-1} for fructose, and 46.44 (30.10, 69.60) mmol L^{-1} for glucose. There were no significant differences in the EC_{50} values for each stimulus between the 2 strains, but we did not compare the EC_{50} for glucose in the 2 strains because of their opposite dose–response curves.

Caffeine, quinine, and NaCl reduced the feeding responses to water in a dose-dependent manner similarly in the 2 strains (Figure 4C,D). The EC_{50} of the wild-type cockroaches was 0.73 (0.57, 1.07) mmol L^{-1} for caffeine, 1.46 (1.05, 2.03) mmol L^{-1} for quinine, and 49.6 (25.7, 96.2) mmol L^{-1} for NaCl. The corresponding EC_{50} values of the glucose-averse strain were 1.67 (1.23, 2.27) mmol L^{-1} for caffeine, 2.39 (1.77, 3.21) mmol L^{-1} for quinine, and 29.5 (28.2, 48.1) mmol L^{-1} for NaCl. There were no significant differences in the EC_{50} values for each stimulus between the 2 strains.

Thus, our dose–response studies indicate that the 2 strains of cockroaches have similar gustatory responses to phagostimulants and deterrents, but with one notable exception: Although the paraglossae of wild-type cockroaches receive glucose as a phagostimulant, glucose is received as a deterrent by the paraglossae of glucose-averse cockroaches.

Discussion

Complementary roles of head chemosensory appendages in feeding behaviors

To test whether or not phagostimulants and deterrents are detected at the antennal level in wild-type and glucose-averse cockroaches, we performed assays in which we simultaneously stimulated an antenna and the mouthparts with either the same or different stimuli. Fructose and glucose applied to the mouthparts acted as phagostimulants in water-satiated wild-type cockroaches. On the other hand, both caffeine and distilled water did not elicit feeding responses when applied to the mouthparts of water-satiated cockroaches. Application of fructose or glucose to the antenna elevated the feeding responses to both caffeine and water that were applied to the mouthparts. When the antenna was stimulated with caffeine, the feeding response to fructose and glucose applied to the mouthparts was significantly reduced. These results indicate that fructose and glucose acted as phagostimulants, whereas caffeine acted as a deterrent in gustatory reception not only by the mouthparts but also by the antennae.

In the glucose-averse strain, fructose also acted as a phagostimulant on the mouthparts and antennae. However, the effects of applying glucose to either the mouthparts or the antenna were more similar to stimulation with caffeine than

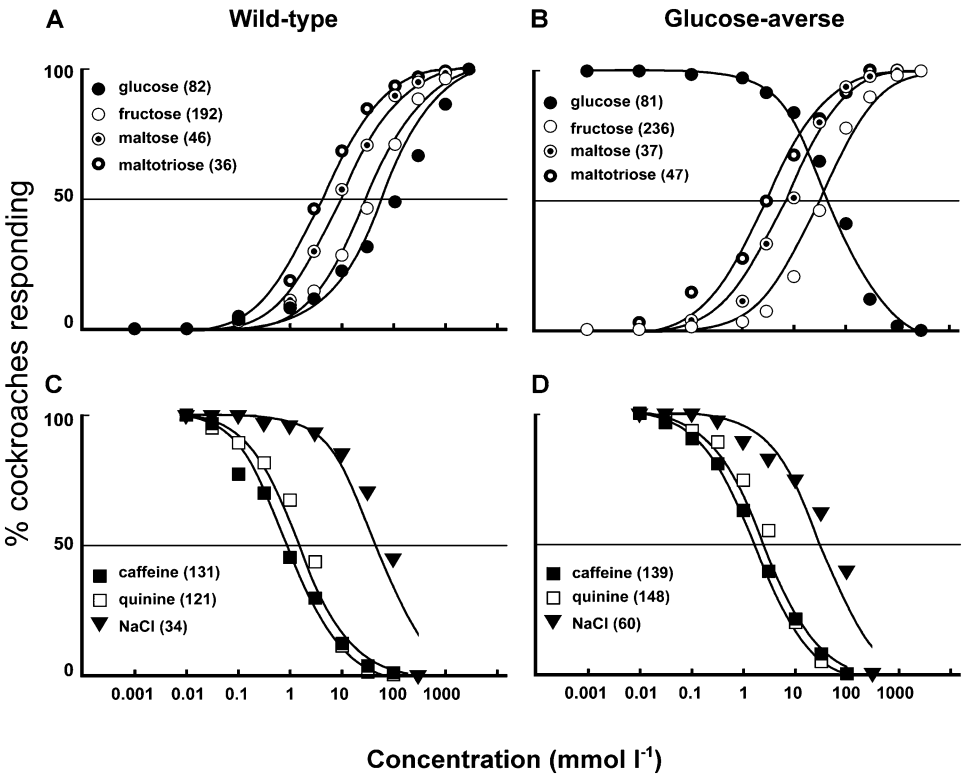

Figure 4 Dose–response bioassays with phagostimulants and deterrents in wild-type and glucose-averse cockroaches with intact paraglossae but maxillary and labial palps ablated. The maxillary and labial palps were surgically removed from 24-h starved cockroaches, each cockroach was placed in a pipette tip with only its head protruding, and the paraglossae were stimulated with a series of tastants in 0.3 μL water. **(A, B)** Responses to sugars in the 2 strains. **(C, D)** Responses to deterrents in the 2 strains. Each symbol indicates the stimulus and the number of cockroaches tested. There were no significant differences in EC_{50} values for sugars (except glucose) and deterrents between the 2 strains (χ^2 test, $P < 0.05$).

with fructose. Thus, with caffeine or distilled water applied to the mouthparts, stimulating the antenna with glucose did not elicit any feeding responses in the glucose-averse strain, as it did in the wild-type cockroaches. Instead, stimulating the antenna with glucose reduced the feeding responses to fructose. The results indicate that the antenna detects phagostimulants and deterrents. We suggest that the antennal neuronal inputs for phagostimulants and deterrents are integrated in the brain with the inputs from the mouthparts and affect the nature and magnitude of the feeding response, even if there are differences in the numbers, distribution, and morphology of the chemosensilla on the antennae and mouthparts. Thus, when both antennal and mouthpart chemosensilla detect glucose, the feeding response is heightened in wild-type cockroaches. But in the glucose-averse cockroaches, where glucose acts as a deterrent, glucose detection by the antennae diminishes the feeding response to all gustatory stimuli at the mouthparts.

In the 2-choice preference tests, as in the dual stimulation tests, fructose and caffeine acted as phagostimulant and deterrent, respectively, in cockroaches of both strains. Although glucose was received as a phagostimulant in all wild-type cockroaches, it strongly deterred feeding in all cockroaches of the glucose-averse strain. Through systematic surgical ablation of head chemosensory appendages, we found significant differences in the ingestion of phagostimulants and deterrents among the treatment groups. Cockroaches with intact paraglossae (maxillary and labial palps ablated) exhibited identical responses as intact unoperated cockroaches to both phagostimulants and deterrents, suggesting that the paraglossae play a pivotal role in gustatory discrimination. Ablation of the paraglossae along with the maxillary palps (L group) tended to reduce the preference for and ingestion of phagostimulants (fructose and glucose for the wild-type strain, fructose for the glucose-averse strain) compared with the other treatment groups. But the L treatment (only labial palps intact) did not interfere with the ability of the cockroaches to discriminate deterrents (caffeine for the wild-type strain, caffeine and glucose for the glucose-averse strain), suggesting that beside the paraglossae, the maxillary palps also serve as important sensory appendages for discriminating phagostimulants. The labial palps, on the other hand, appear to play a more prominent role in discriminating deterrents because cockroaches without labial palps (M group; only the maxillary palps intact, labial palps, and paraglossae ablated) tended to ingest more deterrents (caffeine for the wild-type strain, caffeine and glucose for the glucose-averse strain) than did the other treatment

groups. Furthermore, when ablating only one pair of mouthpart appendages and leaving the other 2 pairs intact (treatment LP, MP and ML), there were no significant differences in the respective intakes among the treatment groups for both phagostimulants and deterrents. These results indicate that while the paraglossae alone can effectively discriminate both phagostimulants and deterrents, the maxillary palps are more responsible for phagostimulant reception and the labial palps are more responsible for deterrent reception. It is also interesting to note that the assignment of priority to reception of deterrents increases with proximity to the mouth, whereas priority for reception of phagostimulants extends outward with the longer maxillary palps. This sensillar organization on head chemosensory appendages may be adaptive in facilitating the detection of phagostimulants from some distance but preventing the ingestion of deterrents. Nevertheless, there is obviously great overlap in the chemosensory spectra of mouthpart sensory appendages to various tastants.

Glucose reception in wild-type and glucose-averse cockroaches

In the dose–response studies of cockroaches with intact paraglossae but ablated maxillary and labial palps, there were no significant differences between the wild-type and glucose-averse strains in the respective EC_{50} values for each tastant, except glucose. Although maltose and maltotriose, which consist of 2 and 3 glucose molecules, respectively, acted as phagostimulants, glucose was received as a deterrent through the paraglossae in the glucose-averse cockroach. These results support previous observations that this glucose-averse cockroach strain was behaviorally deterred by glucose but not by other sugars such as sucrose (which also contains a glucose unit) and mannose that generally stimulate feeding in insects (Silverman and Bieman 1993). Additionally, we demonstrated that the aversive responses to caffeine, quinine, and NaCl in the glucose-averse strain were similar to those in the wild-type strain. The results indicate that the glucose-averse strain has a normal wild type–like GRN network for reception of sugars (except glucose) and deterrents. However, it appears that glucose is misinterpreted as a deterrent, apparently during the peripheral processing of the glucose stimulus. Generally, GRNs are localized on the antennae and mouthparts of insects (Chapman 1998; Ozaki and Tominaga 1999; Newland et al. 2009). Each GRN receives specific tastants within a single taste modality (Hodgson et al. 1955; Dethier 1976; Chapman 1998; Ozaki and Tominaga 1999; Vosshall 2007; Newland et al. 2009). In *Drosophila*, different types of receptor proteins within a particular gustatory sense (e.g., sweet), such as Gr5a and Gr64a for different types of sugars, exist on the same GRN. It is thought that Gr5a and Gr64a are involved in glucose and fructose reception, respectively (Dahanukar et al. 2007). Also in the blowfly, it was suggested that different types of receptor sites for glucose and fructose coexist on the same GRN (Ozaki and Tominaga 1999; Newland et al. 2009).

Additionally, in *Drosophila*, the neural basis for discrimination between appetitive and aversive tastants is apparent at the peripheral level (Thorne et al. 2004; Wang, Singhvi, et al. 2004; Montell 2009). The GRNs of the mouthparts and legs that respond to phagostimulants and those that respond to deterrents project to different regions in the subesophageal ganglion (SEG) (Thorne et al. 2004; Wang, Singhvi, et al. 2004; Montell 2009). Information about various tastants sent from the GRNs is integrated in the SEG, which in turn regulates the behavior and the appropriate physiological response (Melcher and Pankratz 2005). The GRNs of the moth *Heliothis virescens* are also tuned to phagostimulants, like sucrose, and deterrents, like quinine (Jørgensen et al. 2006; Jørgensen, Almaas, et al. 2007; Jørgensen, Kvello, et al. 2007). The appetitive and aversive information is further processed at the central gustatory neurons (CGNs) of the SEG (Jørgensen et al. 2006; Jørgensen, Almaas, et al. 2007; Kvello et al. 2010). The CGNs respond to sucrose and quinine applied to the antenna, proboscis, or tarsus but with varying tuning breadths for each sensory appendage. It was suggested that the integrated neural information from GRNs evokes appetitive and aversive behaviors (Kvello et al. 2010).

In *P. americana*, the mouthparts chemosensory system could discriminate between 500 mmol L^{-1} sucrose and 5 mol L^{-1} NaCl, resulting in salivation (appetitive behavior) or aversive feeding behavior (Watanabe et al. 2003; Sato et al. 2006; Decker et al. 2007; Watanabe and Mizunami 2007). Antennal stimulation using 500 mmol L^{-1} sucrose and 5 mol L^{-1} NaCl also affected the salivation level, but the effect of antennal stimulation on feeding behavior is not known (Watanabe et al. 2008). The antennal gustatory sensilla of the cockroaches *P. americana* and *P. brunnea* contain 3 or 4 GRNs (Seelinger and Tobin 1981; Nishino et al. 2005), one of which is a salt receptor neuron and the other is a sugar receptor neuron (Ruth 1976; Hansen-Delkeskamp 1992; Hansen-Delkeskamp and Hansen 1995; Hansen-Delkeskamp 1998). The individual axons of presumptive antennal GRNs project into the ventro-medial region of the dorsal lobe in the deutocerebrum and the anterior-ventral region of the SEG, which has been implicated in gustatory signal transduction and feeding responses in different insects (Nishino et al. 2005).

These and other studies lead to our expectation that a similar GRN system for mediating appetitive and aversive information exists in the German cockroach. Both the antennae and mouthpart appendages of the German cockroach contain gustatory sensilla (Ramaswamy and Gupta 1981; Wada-Katsumata et al. 2009). It has been suggested that excitation of GRNs of the paraglossae that are tuned to phagostimulants leads to appetitive feeding behavior (Wada-Katsumata et al. 2009), as in other insect species

(Ozaki and Tominaga 1999; Vosshall 2007). There are at least 4 types of GRNs in each gustatory sensillum on the paraglossae, one of which responds to sucrose and maltotriose and another responds to NaCl at high concentrations, which is a deterrent (Wada-Katsumata et al. 2009). Little is known about the sensilla and GRN organization in each sensillum on the paraglossae that transduce ''appetitive'' and ''aversive'' stimuli in the German cockroach or about the roles of GRNs in the antennae and mouthparts in discriminating phagostimulants and deterrents. However, we hypothesized that phagostimulants, such as fructose and glucose, and deterrents, such as caffeine, are received separately by GRNs that mediate appetitive and aversive feeding behaviors, respectively (i.e., labeled line signal processing).

These observations, coupled with the results of the present study and ongoing electrophysiological investigations, lead to us to suggest the following model for glucose signaling in the glucose-averse strain of *B. germanica*. In the German cockroach, glucose-sensitive neurons are organizationally independent from GRNs that receive other sugars and deterrents. The projection patterns of the glucose-sensitive neurons of the glucose-averse strain may be different from those of the wild-type strain. The glucose receptors, which are normally expressed on GRNs for appetitive response in the wild-type strain, also may be expressed on other GRNs for deterrent response in the glucose-averse strain, resulting in a robust aversive response. Preliminary electrophysiological studies show that glucose-aversion can be detected in sensillar recordings as an aberrant response to glucose that matches the normal neuronal response to bitter compounds. Therefore, we suspect that the glucose-averse trait has arisen through misexpression of glucose receptors on GRNs which normally express bitter receptor molecules and elicit avoidance behavior.

Genetic studies of the glucose-averse cockroaches are in their infancy. The glucose-averse phenotype is apparently controlled by a single semidominant autosomal gene on chromosome 9 (Silverman and Bieman 1993; Ross and Silverman 1995). However, no genomic resources are currently available for *B. germanica*, and the GRs of this or any other cockroach species remain unknown. Nevertheless, this system offers a fascinating window into the rapid evolution of the peripheral nervous system in support of adaptive behaviors. The glucose-averse trait occurs naturally and has been isolated from several populations from diverse geographic localities (Silverman and Ross 1994). It appears to be a recent adaptive response to the extensive use of pest control baits that pair glucose with insecticides. Thus, cockroaches with the glucose-aversion trait enjoy a selective advantage under these conditions. This model can accommodate similar genetic changes that impart behavioral aversion to other normally phagostimulatory tastants such as fructose, maltose, etc. To test this model, we are now collecting electrophysiological data and sequencing GRs from *B. germanica* to enable their localization on GRNs and various sensory appendages.

Supplementary material

Supplementary materialcan be found at http://www.chemse.oxfordjournals.org/.

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