Changes in Taste Neurons Support the Emergence of an Adaptive Behavior in Cockroaches

Ayako Wada-Katsumata, Jules Silverman, Coby Schal*

In response to the anthropogenic assault of toxic baits, populations of the German cockroach have rapidly evolved an adaptive behavioral aversion to glucose (a phagostimulant component of baits). We hypothesized that changes in the peripheral gustatory system are responsible for glucose aversion. In both wild-type and glucose-averse (GA) cockroaches, D-fructose and D-glucose stimulated sugar–gustatory receptor neurons (GRNs), whereas the deterrent caffeine stimulated bitter-GRNs. In contrast, in GA cockroaches, D-glucose also stimulated bitter-GRNs and suppressed the responses of sugar-GRNs. Thus, D-glucose is processed as both a phagostimulant and deterrent in GA cockroaches, and this newly acquired peripheral taste sensitivity underlies glucose aversion in multiple GA populations. The rapid emergence of this highly adaptive behavior underscores the plasticity of the sensory system to adapt to rapid environmental change.

Sensory systems guide the assessment of food, habitat, and potential mates, and prominently govern intra- and interspecific interactions. Although great progress has been made in our understanding of chemosensory processing, especially in insects (*1*, *2*), how chemosensory systems change in response to rapidly changing environments remains largely unknown. Cross-species divergence has been well investigated, particularly in olfactory processes (*2*–*4*). However, identifying the chemosensory mechanisms that underlie adaptive intraspecific polymorphisms has been challenging. Among the most important such polymorphisms are sensory adaptations that confer behavioral resistance to insecticides (*5*).

The German cockroach, *Blattella germanica*, offers a tractable system to explore mechanisms of sensory adaptation. Since the mid-1980s, control of this pest has increasingly shifted to baits that combine an insecticide with various phagostimulants, typically D-glucose (glucose henceforth) and D-fructose (fructose) (*6*). Within just several years, cockroach populations evolved a

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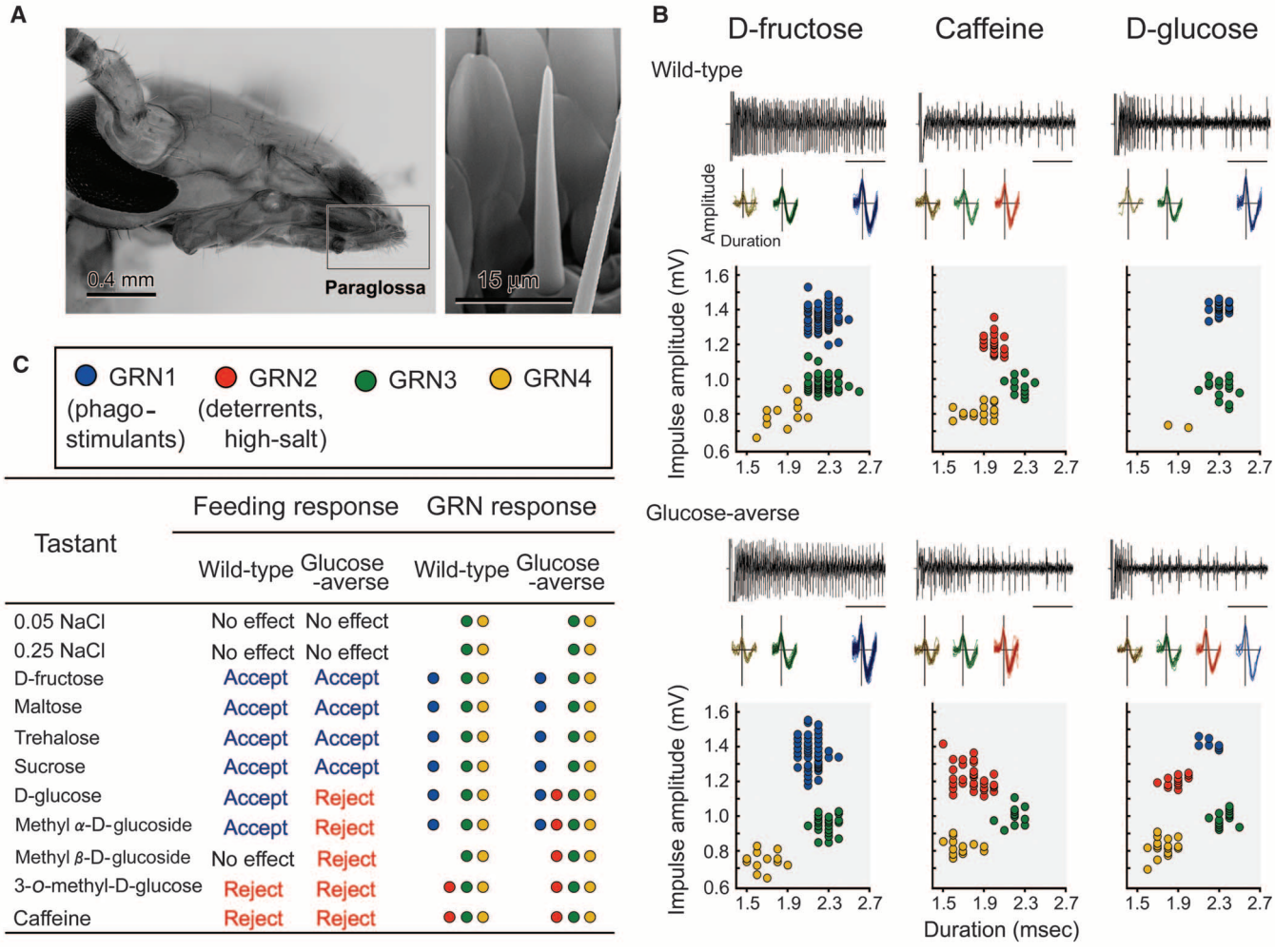

Tastant	Feeding response		GRN response	
	Wild-type	Glucose-averse	Wild-type	Glucose-averse
0.05 NaCl	No effect	No effect	●●●	●●●
0.25 NaCl	No effect	No effect	●●●	●●●
D-fructose	Accept	Accept	●●●	●●●
Maltose	Accept	Accept	●●●	●●●
Trehalose	Accept	Accept	●●●	●●●
Sucrose	Accept	Accept	●●●	●●●
D-glucose	Accept	Reject	●●●	●●●●●
Methyl α-D-glucoside	Accept	Reject	●●●	●●●●●
Methyl β-D-glucoside	No effect	Reject	●●●	●●●●●
3-O-methyl-D-glucose	Reject	Reject	●●●●●	●●●●●
Caffeine	Reject	Reject	●●●●●	●●●●●

Fig. 1. Sensitivities of the GRNs of wild-type (WT) and GA (T164-BC) cockroaches to various tastants. (**A**) Side view of the right paraglossa of a WT male cockroach (left, maxillary and labial palps were removed), and a taste sensillum used in electrophysiological recordings (right). (**B**) GRN responses, showing sample recordings (top) of the same sensillum stimulated sequentially with fructose, caffeine, and glucose (top); impulse sorting (middle); and hierarchical cluster analysis (bottom). The time bar under each recording indicates 200 ms. (**C**) Responsiveness of GRNs of WT and GA cockroaches (20 sensilla each) to 10 tastants. Feeding responses are from fig. S3. Fructose elicited impulses in GRN1, and caffeine elicited impulses in GRN2 in both strains. Glucose and related compounds stimulated GRN1 in WT cockroaches and both GRN1 and GRN2 in GA cockroaches.

new behavioral trait—glucose aversion. Glucose-averse (GA) cockroaches avoid eating glucose-containing baits (movies S1 to 4 and fig. S1), resulting in failure of otherwise highly effective baits (*7*). The GA trait is heritable (*7*, *8*), and the aversive response is robustly evoked by glucose alone (*7*, *9*). Although growth and reproduction are slower in GA than in wild-type cockroaches (*10*), GA cockroaches outcompete wild-type cockroaches under the strong selection pressure of glucose-containing baits (*7*, *11*).

We hypothesized that the GA trait could be encoded by changes in glucose detection. Tastant detection in insects occurs in peripheral gustatory receptor neurons (GRNs), which are housed within hairlike sensilla on the mouthparts (*12*, *13*). The GRNs have modal taste specificity, so in *Drosophila*, for example, four GRNs encode four taste classes: sugar-, bitter-, water- and salt-sensitive GRNs (*13*, *14*). Each GRN expresses multiple gustatory receptors (GRs) that recognize tastants and transduce information about their quality and strength into neuronal impulses that can be distinguished by their amplitude and duration (*15*, *16*). As in other animals, tastants that activate sugar-GRNs elicit appetitive behavior (*13*, *17*) and tastants that activate bitter-GRNs drive aversive behavior (*13*, *18*).

The organization and functions of GRNs in the German cockroach are poorly understood (*19*). We concentrated on glucose-sensitive sensilla on the paraglossae (Fig. 1A) because the paraglossae alone can drive glucose acceptance in wild-type cockroaches and its rejection in GA cockroaches (*9*). Analysis of impulse waveforms [Fig. 1B; also see (*20*)] and cross-adaptation experiments (fig. S2) in wild-type cockroaches demonstrated that glucose-sensitive sensilla contain four distinct GRNs. Fructose and glucose selectively stimulated GRN1, whereas caffeine selectively stimulated GRN2. GRN3 and GRN4 responded to both sugars and caffeine. Using a panel of tastants (Fig. 1C and fig. S3), we established that all tastants that stimulated feeding in wild-type cockroaches also stimulated GRN1 but not GRN2, and all deterrents stimulated GRN2 but not GRN1. The results indicate that the appetitive and aversive inputs in wild-type cockroaches segregate by the organization of GRN1 (sugar-GRN) and GRN2 (bitter-GRN) at the peripheral sensory level, as in other insect species (*12*, *13*, *19*).

The sugar- and bitter-GRN sensitivities of GA cockroaches (strain T164-BC) were considerably different from those of wild-type cockroaches. Glucose stimulated four rather than only three types of GRNs (Fig. 1B and fig. S2), corresponding to the sugar-GRN, bitter-GRN, GRN3, and GRN4 of wild-type cockroaches. Electrophysiological recordings from GA cockroaches with 10 tastants further demonstrated that the bitter-GRN

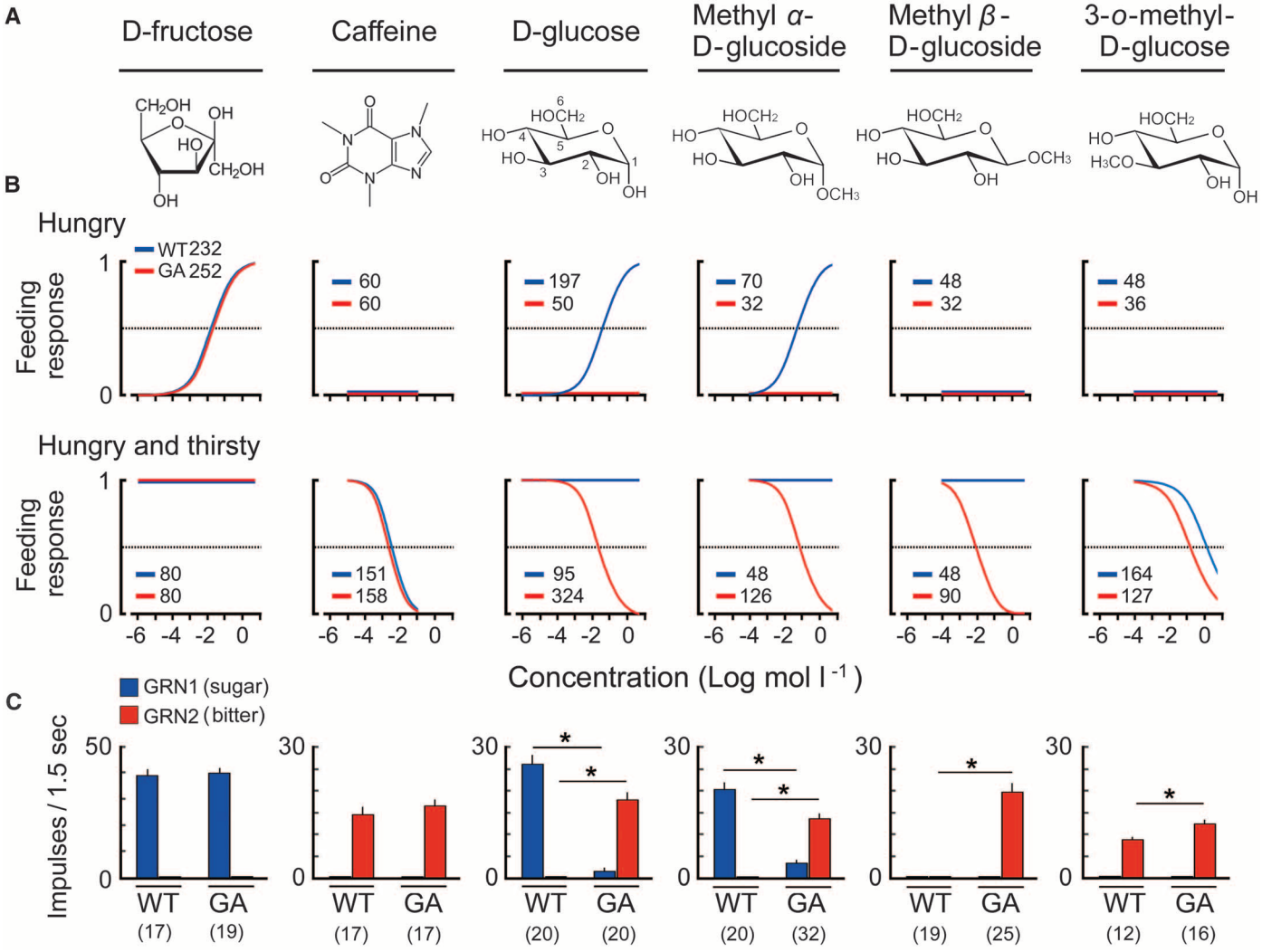

Fig. 2. Behavioral and electrophysiological responses to six tastants. (**A**) Chemical structures of tastants. (**B**) Dose-feeding responses in WT (blue) and GA (T164-BC, red) cockroaches motivated to accept phagostimulants but not water (Hungry), or to take both phagostimulants and water (Hungry and thirsty). Feeding response is the proportion of cockroaches ingesting the test solution, and the legends indicate sample size. GA cockroaches rejected glucose and related compounds. (**C**) The sugar- and bitter-GRNs of WT and GA cockroaches respond differentially to six tastants (mean ± SEM). Number of tested sensilla is in parentheses. (*P < 0.05, Student's t test).

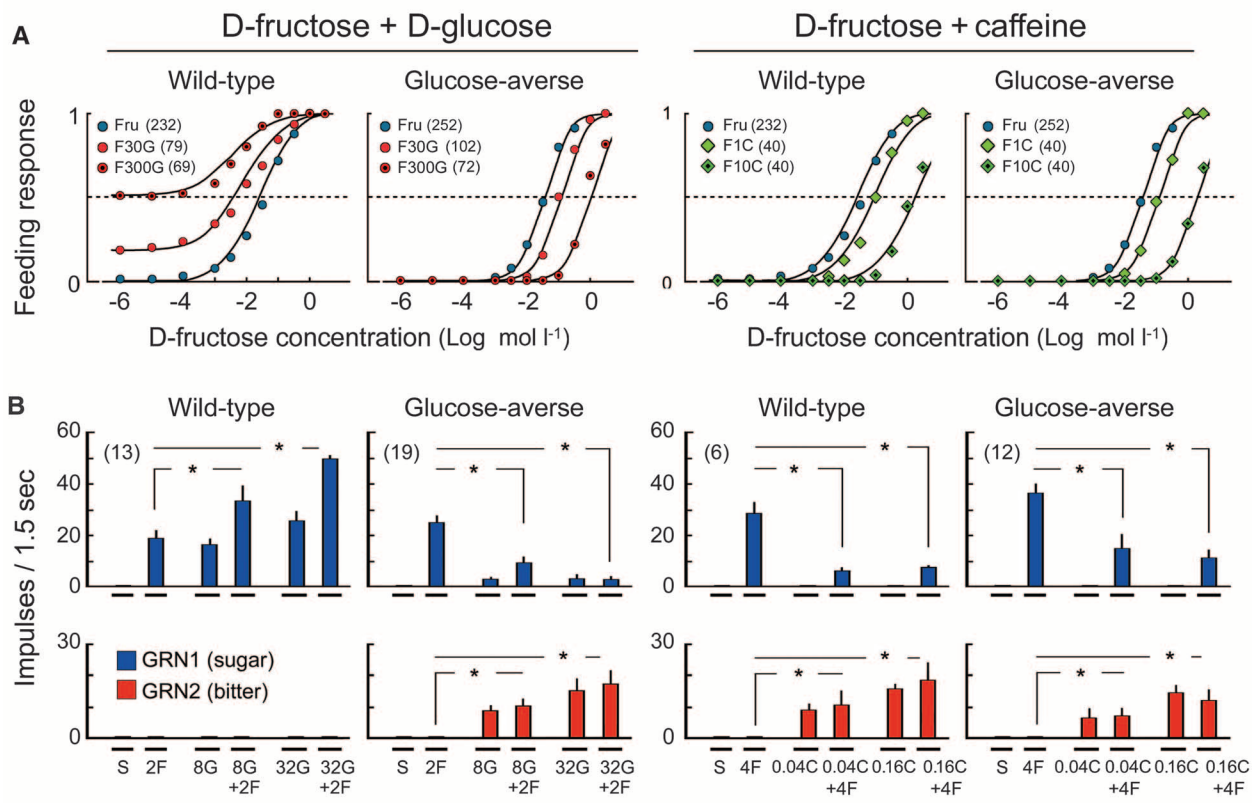

Fig. 3. Glucose aversion is elicited by stimulation of bitter-GRNs and inhibition of sugar-GRNs. (**A**) Cockroaches were tested with fructose alone (Fru), fructose mixed with 30 or 300 mmol liter^{-1} glucose (F30G and F300G), and fructose mixed with 1 or 10 mmol liter^{-1} caffeine (F1C and F10C). Numbers of tested WT and GA (T164-BC) cockroaches are in the legends (in parentheses). The response to fructose alone is also in Fig. 2B. (**B**) Sensitivity of sugar-GRN (top, blue) and bitter-GRN (bottom, red) to fructose alone and to binary mixtures (means ± SEM). S, 0.25 mmol liter^{-1} NaCl (control electrolyte); 2F and 4F, 2 and 4 mmol liter^{-1} fructose; 8G and 32G, 8 and 32 mmol liter^{-1} glucose; 0.04C and 0.16C, 0.04 and 0.16 mmol liter^{-1} caffeine. Number of tested sensilla is in parentheses. The GRN responses to fructose alone were compared to the responses to binary mixtures (analysis of variance, Dunnett's test, $*P < 0.05$). Glucose and caffeine attenuate the feeding response to fructose in GA cockroaches and depress the sugar-GRN responses.

responded to glucose and all the tastants that elicited aversive behavior (Fig. 1C and fig. S3). We therefore suggest that glucose and related compounds drive the aversive response in GA cockroaches by stimulating the bitter-GRN, the same GRN that is stimulated by caffeine in both cockroach strains (Fig. 1C). By contrast, GRN3 and GRN4 responded without any apparent discrimination among stimuli (Fig. 1C, fig. S4A, and table S1), suggesting that they do not contribute to the differential discrimination of appetitive and aversive tastants by the two cockroach strains.

We compared the sensitivities of the sugar- and bitter-GRNs in the wild-type and GA strains with dose-behavioral response studies with six tastants (Fig. 2A). The two cockroach strains showed similar behavioral and GRN responses to fructose and caffeine (Fig. 2, B and C), suggesting that wild-type and GA cockroaches have fundamentally similar gustatory neural networks for appetitive and aversive behaviors. However, glucose and two related compounds stimulated the bitter-GRN in GA cockroaches (Fig. 2, B and C), and 3-*o*-methyl-D-glucose, which was aversive to both strains, elicited significantly higher bitter-GRN responses in GA than in wild-type cockroaches. The results suggest that in wild-type cockroaches, glucose and related compounds are discriminated structurally by narrowly tuned receptors on sugar-GRNs, eliciting appetitive behavior. In GA cockroaches, by contrast, the expression of a broadly tuned receptor or multiple narrowly tuned receptors may contribute to the broad acceptance of glucose and related compounds by bitter-GRNs, driving aversive behavior.

Sugar-GRNs in GA cockroaches also exhibited a significantly lower response to glucose than in wild-type cockroaches (Fig. 2C). We tested whether the sugar-GRNs of GA cockroaches are less sensitive to glucose, or if their responses are depressed by the activities of adjacent GRNs. Complementary behavioral assays and electrophysiological recordings with mixtures of phagostimulants and deterrents revealed that in GA cockroaches, both glucose and caffeine attenuated the appetitive response to fructose (Fig. 3A and table S2) and significantly depressed the sugar-GRN responses relative to fructose alone (Fig. 3B). By contrast, in wild-type cockroaches, combining glucose with fructose increased both the appetitive response and the electrophysiological responses of sugar-GRNs compared to fructose alone (Fig. 3B). These results demonstrate that GA cockroaches detect glucose as a genuine deterrent, which also suppresses sugar-GRN responses, as alkaloids and glucosides do in other insect species (*21*–*23*).

How prevalent is this mechanism in glucose-averse field populations? We screened the feeding responses of 19 field-collected populations and found seven populations with GA cockroaches (Fig. 4A). Two of these strains were used in behavioral and GRN dose-response studies. Although both were less GA than the lab-selected strains (Fig. 4B and table S2), in both strains glucose stimulated the bitter-GRN (Fig. 4C) and depressed the sugar-GRN (table S1). In four GA strains, the behavioral feeding responses negatively correlated with bitter-GRN responses (Fig. 4D and table S3). The wild-type and field-collected strains did not differ in GRN sensitivities for both fructose and caffeine (fig. S5 and table S1), confirming that a similar mechanism gave rise to glucose aversion in multiple cockroach populations.

Most natural genetic polymorphisms in taste receptors modify behavioral responses over a finite range, from exquisite sensitivity to complete insensitivity to a particular tastant [e.g., (*24*)]. In bait-selected cockroach populations, however, the modal specificity of glucose has been dramatically

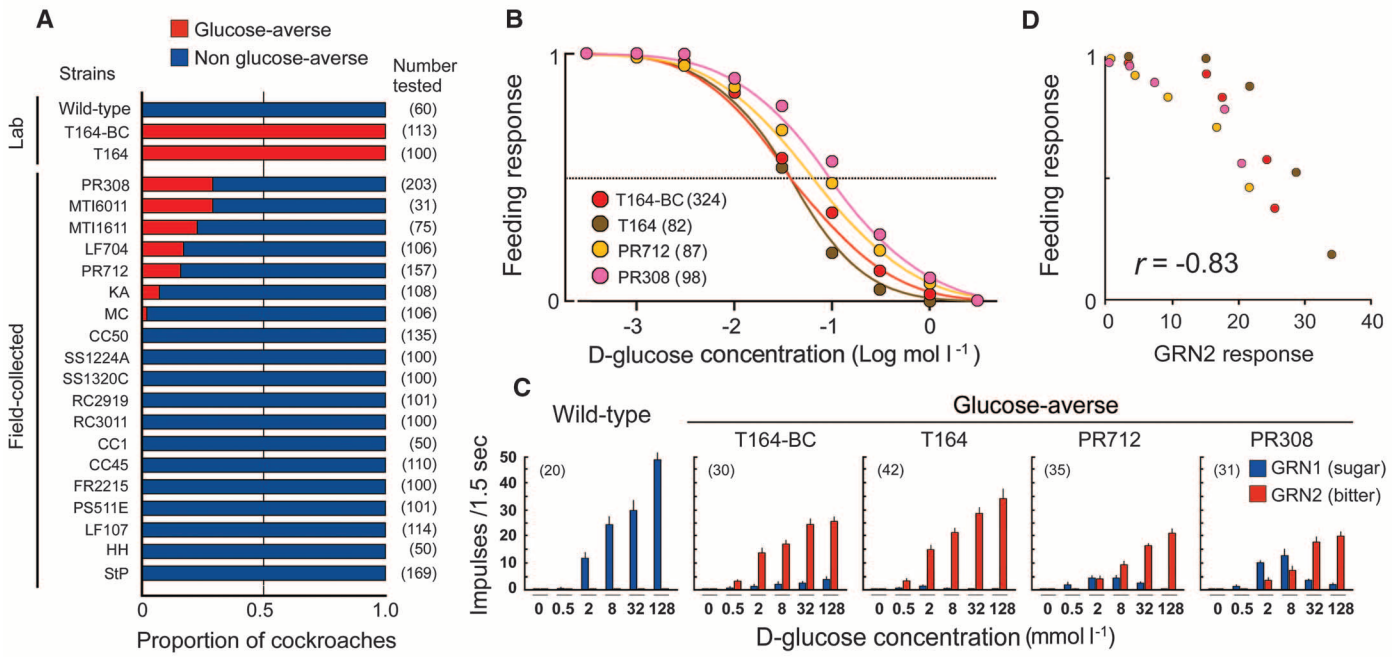

Fig. 4. Glucose stimulates bitter-GRNs in field-collected cockroaches. (**A**) Behavioral assays showing 7 of 19 field-collected populations with some GA cockroaches. (**B**) Dose-feeding responses to glucose in four GA strains, with the number of tested cockroaches in parentheses. T164-BC response to glucose is also shown in Fig. 2B, and the median effective concentration (EC_{50}) for each strain is in table S2. (**C**) Dose-GRN responses to glucose in WT and four GA strains (mean impulse frequency ± SEM, with number of tested sensilla in parentheses). (**D**) Feeding responses [from (B)] and GRN2 responses [from (C)] at similar glucose concentrations are negatively correlated (*r*, Pearson's correlation coefficient, $P < 0.001$, table S3).

transformed from "sweet" and highly phagostimulatory to "bitter" and highly deterrent. Generally, bitter-GRNs of insects coexpress a large number of GRs (*18*, *25*) and are therefore broadly tuned to respond to various deterrents (*18*, *21*, *22*). The coexpression patterns of GRs ultimately account for the unique sensitivity of bitter-GRNs and their capacity to selectively respond to specific deterrents (*18*). Our electrophysiological studies with GA cockroaches suggest two major hypotheses: One or more mutations have either (i) modified the structure of GRs on the bitter-GRN to accept glucose and/or (ii) caused the misexpression of native glucose GRs on the bitter-GRN. The structure-activity studies tentatively support the former hypothesis that the glucose-sensitive GRs on bitter-GRNs are differently tuned from the native glucose GRs on sugar-GRNs, because wild-type and GA cockroaches responded differently—both behaviorally and with GRN responses—to changes in the chemical structures of glucose and related compounds.

Our results show that by recruiting glucose and related sugars as bitter-GRN ligands, a gain-of-function adaptation has emerged, expressing glucose-aversion as a novel behavior that offers protection against toxic baits. The change in valence of glucose, without compromising the exquisite sensitivity of the gustatory system to glucose, highlights the specificity of this adaptive change. Moreover, the aversion to glucose is further amplified by a preexisting inhibition of sugar-GRN responses by deterrents. Glucose aversion is a clear example of a chemosensory gain-of-function adaptation that confers behavioral resistance to anthropogenic pressures, protecting the German cockroach from insecticides.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6135/972/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S3
References (*26*–*28*)
Movies S1 to S4

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Movies S1 to S4

Materials and Methods

Insects

The wild-type strain (WT = American Cyanamid = Orlando normal) has been maintained in the lab for >25 years. The T164 strain was collected in Florida as a glucose-averse strain (*7*), and selected in the laboratory with D-glucose-containing insecticide bait >15 years. The T164-BC strain was generated in 1995 from a WT x T164 cross followed by 8 generations of backcrossing to WT, and subsequently selected in the laboratory with D-glucose-containing insecticide bait >15 years. T164-BC was used in most experiments as a glucose-averse (GA) strain. Nineteen other *B. germanica* populations were collected in 2009-2010. PR308, PR712, MTI6011, MTI1611, LF704 and LF107 were collected in Puerto Rico. KA was obtained from Bayer Corporation. MC and StP were collected in Russia. CC50, SS1224A, SS1320C, RC2919, RC3011, CC1, CC45, FR2215, PS511E and HH were collected in Raleigh, NC.

Cockroaches were reared on water and food pellets (Purina No. 5001 Rodent Diet, PMI Nutrition International) at 27 ± 1 °C, 40–70% relative humidity, and L:D = 12:12 photoperiod. Newly emerged adult males were separated and kept in groups of 10–50 with water and food until use. Seven to 10 day old virgin males were tested. We did not use females to avoid effects of the ovarian cycle and associated hormonal changes on feeding behavior.

Chemicals

Six aldohexoses (D-glucose, L-glucose, 2-deoxy-D-glucose, 6-deoxy-D-glucose, D-galactose and L-galactose), one ketohexose (D-fructose), three aldopentoses (D-arabinose, L-arabinose and D-xylose), three monosaccharide derivatives (3-*O*-methyl-D-glucose, methyl α-D-glucoside and methyl β-D-glucoside), three disaccharides (maltose, trehalose and sucrose), an alcoholic β-glucoside (salicin), a purine-like alkaloid (caffeine), and a benzoic sulfilimine (saccharin) were used as tastants. Unless otherwise stated, glucose = D-glucose and fructose = D-fructose. Allura red AC (maximum absorbance ~504 nm; 1 mmol l^{-1}) and erioglaucine (Brilliant Blue, maximum absorbance ~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 (*9*). NaCl was used as an electrolyte for electrophysiological recordings. D-fructose was purchased from ICN Biochemicals, NaCl from Fisher Scientific, and all other chemicals from Sigma-Aldrich.

Behavioral assays

1. Two-choice preference tests for fig. S1

The two-choice test was performed with the WT and GA (T164-BC) cockroach strains essentially as described in (*9*) (see movies S1, S2). Ten cockroaches were placed in a large Petri dish containing two agar discs: one disc contained 1% agar, 1 mmol l^{-1} allura red and 100 mmol l^{-1} D-fructose, while the second disc contained 1% agar, 0.5 mmol l^{-1} erioglaucine, 100 mmol l^{-1} D-fructose and either 1 mol l^{-1} D-glucose or 3 mmol l^{-1} caffeine. These concentrations of sugars and caffeine are higher than the median effective concentrations (*9*) to maximize responsiveness of cockroaches. The assay duration was 2 hrs during the dark phase of the insects' L:D cycle. After each assay, the

foregut and midgut of each cockroach were dissected and the amounts of red and blue colors ingested ([Red] and [Blue]) were obtained from the absorbance of the homogenate of the foregut and midgut at 504 nm (red) and 625 nm (blue) in a microplate scanning spectrophotometer (PowerWave-X, Bio-Tek Instruments). About 14.6% of 130 cockroaches failed to feed (had empty foregut and midgut), and they were excluded from data analysis. A preference index was calculated as described in (*9*), where 0 indicates that cockroaches fed equally on both discs, +1 indicates a preference for the D-glucose- or caffeine-supplemented agar and –1 indicates preference for the D-fructose-only agar over the supplemented agar. Differences in the preference index were tested among different treatment groups in each strain using ANOVA and Dunnett's test ($P < 0.05$).

2. Feeding response tests with 19 tastants for fig. S3

Cockroaches were deprived of food but not water for 24 hrs. We concentrated on D-glucose-sensitive sensilla on the paraglossae because the paraglossae alone can drive D-glucose acceptance in WT cockroaches and its rejection in GA cockroaches (*9*). To avoid the effects of other sensory inputs on the feeding response, the maxillary and labial palps of each cockroach were ablated, leaving the paraglossae intact (Fig. 1A). After 30 min recovery, each cockroach was placed in a plastic pipette tip with only its head protruding and offered 0.3 µl of stimulus solution colored with 1 mmol l^{-1} erioglaucine. Test solutions consisted of 30 mmol l^{-1} D-fructose plus 500 mmol l^{-1} of either a monosaccharide, derivative of glucose, or disaccharide, a saturated solution of salicin, 100 mmol l^{-1} saccharin, or 10 mmol l^{-1} caffeine. Each cockroach received two types of stimulations: 30 mmol l^{-1} D-fructose alone and a mixture consisting of 30 mmol l^{-1} D-fructose and a test tastant. At the end of the test series, the motivation of cockroaches to drink 30 mmol l^{-1} D-fructose was tested and cockroaches that did not ingest D-fructose were excluded from the analysis. The number of cockroaches ingesting the solution was used as the feeding response. The effect of each tastant on the feeding response to D-fructose was evaluated by comparing the feeding response to D-fructose with the feeding response to a binary mixture using McNemar's test ($P < 0.05$). To compare the effects of test compounds, an effectiveness index (EI) was obtained for each tastant by the following formula: EI value = [proportion of cockroaches ingesting the mixture]-[proportion of cockroaches ingesting D-fructose alone]. The feeding responses of the WT and GA (T164-BC) strains were compared by χ^2 test ($P < 0.05$).

3. Dose-feeding response assays

Dose-response studies were carried out essentially as described in (*9*) and above (see movies S3, S4). Before each test the maxillary and labial palps of each cockroach were ablated, leaving the paraglossae intact, and the cockroach was allowed to recover for 30 min. The paraglossae were then stimulated with 0.3 µl of test solution colored with 1 mmol l^{-1} erioglaucine in a sequence from the lowest to the highest concentration of test solution. At the end of the test series, the motivation of cockroaches to eat sugar was tested by stimulating the paraglossae with 1000 mmol l^{-1} D-fructose, and cockroaches that failed to ingest were excluded from analysis. The proportion of cockroaches ingesting the solution was obtained as the feeding response. A median effective concentration (EC_{50}) of each test solution was obtained with probit-analysis, and the significance was tested by a χ^2 test of heterogeneity ($P < 0.05$) (*26*).

3-1. Dose-feeding response assays with six tastants for Fig. 2B and table S2

Two types of cockroach groups were prepared from WT and GA (T164-BC) cockroaches. One group was deprived of food for 24 hrs, but supplied with water and thus motivated to take phagostimulants but not water. Another group was deprived of both food and water for 24 hrs to increase their hunger and thirst. Six tastants were tested: D-fructose and D-glucose (0.001, 0.01, 0.1, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}); caffeine (0.001, 0.01, 0.1, 0.3, 1, 3, 10, 30 and 100 mmol l^{-1}); methyl α-D-glucoside, methyl β-D-glucoside and 3-*O*-methyl-D-glucose (0.1, 1, 10, 100, 1000 and 3000 mmol l^{-1}). Each cockroach received a concentration series of a single tastant. The EC_{50} values for the six tastants are shown in table S2.

3-2. Dose-feeding response assays with binary mixtures for Fig. 3A and table S2

In bioassays with binary mixtures of D-fructose and either caffeine or D-glucose, WT and GA (T164-BC) cockroaches were deprived of food but not water for 24 hrs. A dose-response curve for D-fructose was obtained for both strains as described in the dose-feeding response assay with six tastants. In order to test the effect of D-glucose on feeding responses to D-fructose, either 30 or 300 mmol l^{-1} D-glucose was added to each test concentration of D-fructose. For testing the effect of caffeine, either 1 or 10 mmol l^{-1} caffeine was added to each test concentration of D-fructose. Each cockroach received a concentration series of a single type of test solution. EC_{50} values are shown in table S2.

3-3. Dose-feeding response assays with field-collected glucose-averse strains for Fig. 4B and table S2

T164, T164-BC, PR308 and PR712 cockroaches were deprived of both food and water for 24 hrs to increase their hunger and thirst. Each cockroach received a concentration series of D-glucose (0.3, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}). A dose-response curve was obtained for each strain as described in the dose-feeding response assay with six tastants. EC_{50} values are shown in table S2.

4. Feeding assays with 19 field-collected cockroach populations for Fig. 4A

To examine the prevalence of glucose-averse cockroaches in the field, 19 populations were tested as described in the dose-feeding response assays. The cockroaches were deprived of both food and water for 24 hrs to increase their hunger and thirst. Each intact cockroach received 1000 and 3000 mmol l^{-1} of D-glucose, D-fructose, trehalose, sucrose and maltose. The proportion of cockroaches that rejected 3000 mmol l^{-1} of D-glucose and accepted the other sugars was determined.

Morphology of the mouthparts

Images of the cockroach head and mouthparts were obtained with a macro-microscope (Z16APO, Leica, Microsystems). The paraglossae from six WT cockroaches and six GA (T164-BC) cockroaches were prepared for SEM in an ethanol series (40, 50, 60, 70, 80, 85, 90, 95, 99 and 100%) and sputter coated with AuPd (Hammer 6.2 Sputtering System, Anatech). SEM images were obtained in high vacuum (JSM-5900LV JEOL). Electrophysiological recordings were conducted with chemosensilla of the same morphology and location on the paraglossa.

Electrophysiological recordings

1. Screening of cockroaches for electrophysiological recordings

To obtain cockroaches for electrophysiology recordings WT, T164-BC, T164, PR308 and PR712 adult males were screened with D-fructose, D-glucose and caffeine concentrations near the respective EC_{50}, as described in the dose-feeding response assays. Adult males were deprived of both food and water for 24 hrs to increase their hunger and thirst. At first screening, each intact cockroach was tested with 3 and 10 mmol l^{-1} caffeine, 30, 100 and 3000 mmol l^{-1} D-glucose, and 30 and 1000 mmol l^{-1} D-fructose. Subsequently, the cockroach was satiated with water and thus motivated to take phagostimulants but not water. The cockroach was tested with caffeine, D-glucose and D-fructose at the same concentrations as above. In the WT strain, the cockroaches that rejected 3 mmol l^{-1} caffeine and accepted 100 mmol l^{-1} D-glucose and 30 mmol l^{-1} D-fructose were used in electrophysiology.

There were no apparent topological differences in the distribution of taste sensilla in the two strains, and ~70% of the sensilla in both responded to D-glucose, D-fructose and caffeine.

In the four strains with glucose-averse cockroaches, cockroaches that rejected 3 mmol l^{-1} caffeine and 100 mmol l^{-1} D-glucose and accepted 30 mmol l^{-1} D-fructose were used in electrophysiology. Screened males were used for electrophysiological recordings within 2 days.

2. Recordings from gustatory GRNs

Electrophysiological responses were recorded from chemosensilla on the paraglossa by the tip-recording method (*15, 19*). One to four sensilla per cockroach were tested, except in the impulse classification analysis (Fig. 1B). A cockroach was briefly chilled on ice and its maxillary and labial palps and mandibles were removed with fine scissors. The whole body was placed in a plastic pipette tip with only the mouthparts exposed. The head capsule and antennae were fixed with sticky tape. A glass capillary (TW150-3, World Precision Instruments, Sarasota, Florida, USA), which contained a gold wire and saline was inserted into the esophagus of the cockroach and served as the reference electrode. Another glass capillary, containing a tungsten recording electrode and a test stimulus dissolved in 0.25 mmol l^{-1} NaCl, was slipped over the tip of a taste sensillum with the aid of a microscope (Labolux 11, Leitz, Wetzler, Germany). The duration of stimulation was 5 sec with an interval of 10 min between stimuli to avoid any effects of adaptation. The impulses generated in the sensillum were acquired with a preamplifier (Taste Probe, Syntech, Hilversum, Netherlands) connected to a data acquisition system (USB IDAC4, Syntech). To check for stability of GRN sensitivity, each sensillum was tested with 4 mmol l^{-1} D-fructose at the beginning and at the end of each test series.

The impulses generated were analyzed based on the impulse waveforms (amplitude and duration) using Auto Spike v. 4.0 (Syntech). When the signals appeared in an irregular waveform which did not fit the software analysis, visual analysis was employed. For classification of impulses, a hierarchical cluster analysis (Ward's method) was carried out (IBM SPSS Statistics). Only responses in which clear and consistent activity was seen until the end of the trial were used in data analysis.

2-1. Classification of impulses generated by GRNs for Fig. 1B

Ten to 15 sensilla in random locations on the paraglossa in WT and GA (T164-BC) cockroaches were stimulated with 8 mmol l^{-1} D-fructose, 0.16 mmol l^{-1} caffeine and 8 mmol l^{-1} D-glucose. Impulses generated from 0.02 to 2.02 sec after contact with the sensillum were analyzed and their impulse waveforms and results of a hierarchical cluster analysis were compared across the three tastants. In the WT strain, 675 of 952 chemosensilla (93 cockroaches) responded to all three tastants, and in the GA strain, 323 of 448 sensilla (62 cockroaches) responded to all three tastants.

2-2 Cross-adaptation tests for fig. S2

Cross-adaptation experiments were performed to discriminate which GRNs respond to phagostimulants and deterrents in WT and GA (T164-BC) cockroaches. Sensilla in the anterior region of the paraglossa (Fig. 1A) were adapted and stimulated with either 8 mmol l^{-1} D-fructose, 32 mmol l^{-1} D-glucose, or 0.16 mmol l^{-1} caffeine. Each sensillum was first stimulated with a test compound for 5 sec (#1), rested for 10 min, stimulated with the adapting stimulus for 4 min (#2 and 3), and then stimulated within 30 sec with the same test compound (#4). After a 10 min recovery period, the sensillum was again stimulated with the test compound for 5 sec (#5). Impulses generated from 0.1 to 1.6 sec after stimulation were analyzed in #1, 2, 4 and 5. In #3, impulses in the last 1.5 sec of the 4 min adaptation period were analyzed. The impulse frequency in the pre-adaptation stimulation (#1) was compared with the impulse frequency during cross-adaptation (#4) or post-adaptation (recovery, #5) by ANOVA (Dunnett's test, $P < 0.05$).

2-3. Modal specificities of GRNs for 10 tastants for Fig. 1C

To evaluate the taste modal specificities of GRNs, 20 sensilla in the same location on the paraglossa from either WT or GA (T164-BC) cockroaches (Fig. 1A) were stimulated with 10 tastants: NaCl (0.05 and 0.25 mmol l^{-1}), maltose (8 mmol l^{-1}), trehalose (8 mmol l^{-1}), sucrose (8 mmol l^{-1}), D-fructose (8 mmol l^{-1}), D-glucose (32 mmol l^{-1}), methyl α-D-glucoside (32 mmol l^{-1}), methyl β-D-glucoside (32 mmol l^{-1}), 3-*O*-methyl-D-glucose (32 mmol l^{-1}) and caffeine (3 mmol l^{-1}). Impulses generated from 0.1 to 1.6 sec after stimulation were analyzed.

2-4. Effect of chemical modification of D-glucose on sugar- and bitter-GRNs for Fig. 2C

In order to test the effect of differences in the chemical structures of D-glucose and related compounds on sugar- and bitter-GRNs, sensilla in the same location on the paraglossa in WT and GA (T164-BC) cockroaches (Fig. 1A) were tested with six tastants: D-fructose (8 mmol l^{-1}), D-glucose (32 mmol l^{-1}), methyl α-D-glucoside (32 mmol l^{-1}), methyl β-D-glucoside (32 mmol l^{-1}), 3-*O*-methyl-D-glucose (32 mmol l^{-1}) and caffeine (3 mmol l^{-1}). Impulses generated from 0.1 to 1.6 sec after stimulation were analyzed. Significant differences in impulse frequency between the WT and GA strains were tested by Student's *t*-test ($P < 0.05$).

2-5. Electrophysiological recordings with binary mixtures of D-fructose and either D-glucose or caffeine for Fig. 3B

To evaluate the effect of D-glucose on sugar- and bitter-GRN responses to D-fructose, sensilla in the same location on the paraglossa in WT and GA (T164-BC) cockroaches were tested with 2 mmol l^{-1} D-fructose alone or D-fructose mixed with 8 or 32 mmol l^{-1} D-glucose. To check for constant sensitivity of GRNs, the sensilla were stimulated again with 2 mmol l^{-1} D-fructose at the end of each test series. Similar assays were used to evaluate the effect of caffeine on GRN responses to D-fructose. Sensilla were stimulated with three types of stimuli: 4 mmol l^{-1} D-fructose, either 0.04 or 0.16 mmol l^{-1} caffeine and a mixture of 4 mmol l^{-1} D-fructose and 0.04 or 0.16 mmol l^{-1} caffeine. To check for constant sensitivity of GRNs, the sensilla were stimulated with 4 mmol l^{-1} D-fructose at the end of each test series. The numbers of impulses generated from 0.1 to 1.6 sec after contact with the sensillum were compared by ANOVA (Dunnett's test, $P < 0.05$).

2-6. Dose-GRN response tests for Fig. 4C, fig. S4, S5 and table S1

In order to determine the sensitivity of GRNs of WT, T164-BC, T164, PR308 and PR712, dose-response tests were performed with D-fructose (0.5, 2, 8, 32 and 128 mmol l^{-1}), D-glucose (0.5, 2, 8, 32 and 128 mmol l^{-1}), and caffeine (0.02, 0.04, 0.08, 0.16 and 0.32 mmol l^{-1}). Sensilla in the same location on the paraglossa were tested. A test compound was assayed at every concentration in random order on a sensillum; the same sensillum was tested only with a single test compound. Impulses generated from 0.1 to 1.6 sec after stimulation were analyzed. For comparison of the GRN sensitivities among different strains, impulse frequencies from four types of GRNs were compared at the same stimulus concentration by ANOVA (Tukey's HSD, $P < 0.05$) (table S1).

The behavioral feeding responses and bitter-GRN responses to D-glucose were compared at similar concentrations for all four glucose-averse strains with Pearson's correlation analysis (see table S3). Additionally, to determine the taste modal specificity of GRN3 and GRN4, the impulse frequencies of GRN3 and GRN4 in WT cockroaches were compared for three tastants and methyl β-D-glucoside, which did not elicit GRN1 and GRN2 responses in WT cockroaches (for fig. S4).

2-7. Taste modal specificities of GRN3 and GRN4 for fig. S4

Every test solution we tested, including the control NaCl electrolyte, stimulated GRN3 and GRN4. To evaluate the taste modal specificity of GRN3, we tested four tastants that differentially stimulated the four GRNs in WT cockroaches. Methyl β-D-glucoside (0.03, 0.12, 0.5, 2, 8, 32 and 128 mmol l^{-1}), which stimulated GRN3 and GRN4; D-fructose and D-glucose (0.5, 2, 8, 32 and 128 mmol l^{-1}), which stimulated GRN1, GRN3 and GRN4; and caffeine (0.02, 0.04, 0.08, 0.16 and 0.32 mmol l^{-1}), which stimulated GRN2, GRN3 and GRN4. Although these compounds have different physical properties such as solubility and viscosity, they have the same osmotic concentration at the same molar concentration and temperature. Therefore we expected that these compounds could be used to test the effect of osmotic concentration on GRN3 and GNR4. Sensilla of WT cockroaches were stimulated, as described in dose-GRN response tests. The numbers of impulses generated by GRN3 and GRN4 from 0.1 to 1.6 sec after contact with the sensillum were counted. The impulse frequency for methyl β-D-glucoside was compared with the impulse frequency for the other three tastants at each concentration

(the data for the three tastants were obtained from dose-GRN response tests) by either Student's *t*-test ($P < 0.05$) or ANOVA (Tukey's HSD, $P < 0.05$)

GRN4 of WT cockroaches responded equally to the four tastants. Therefore, the sensitivity of GRN4 was tested with two concentrations of the electrolyte, 0.05 and 0.25 mmol l^{-1} NaCl. Sensilla of WT cockroaches were stimulated as described in dose-GRN response tests. The numbers of impulses generated by GRN3 and GRN4 from 0.1 to 1.6 sec after contact with the sensillum were counted, and compared between the two different concentrations of NaCl (Student's paired *t*-test, $P < 0.01$).

Fig S1

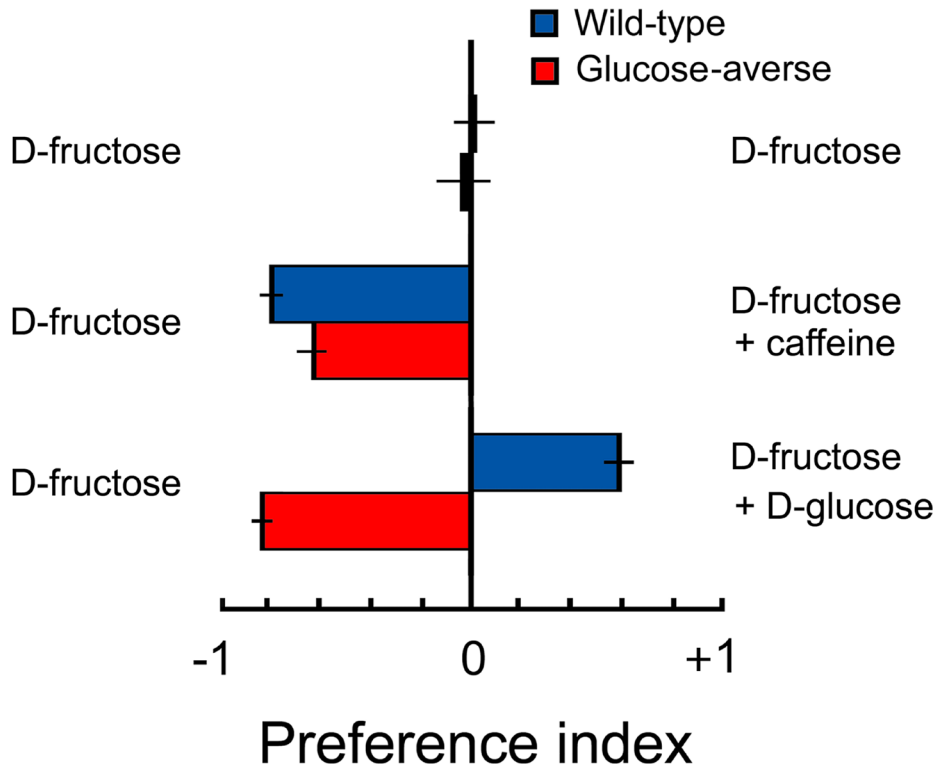


Fig. S1. Behavioral responses of WT and GA (T164-BC) cockroaches in two-choice preference tests.

A preference index of 0 indicates that cockroaches fed equally on both discs, +1 indicates a preference for one disc, and -1 indicates preference for the other disc. WT cockroaches significantly preferred a mixture of D-fructose and D-glucose over D-fructose alone, but they avoided eating a mixture of D-fructose and caffeine (ANOVA, Dunnett's test, $F_{2, 49}$ = 149.79, $P < 0.0001$). On the other hand, GA cockroaches preferred plain D-fructose over D-fructose supplemented with either caffeine or D-glucose ($F_{2, 56}$ = 149.79, $P < 0.0001$), showing not only that GA cockroaches discriminate D-glucose from D-fructose, but also that D-glucose had the same effect as a genuine deterrent, caffeine.

Fig S2

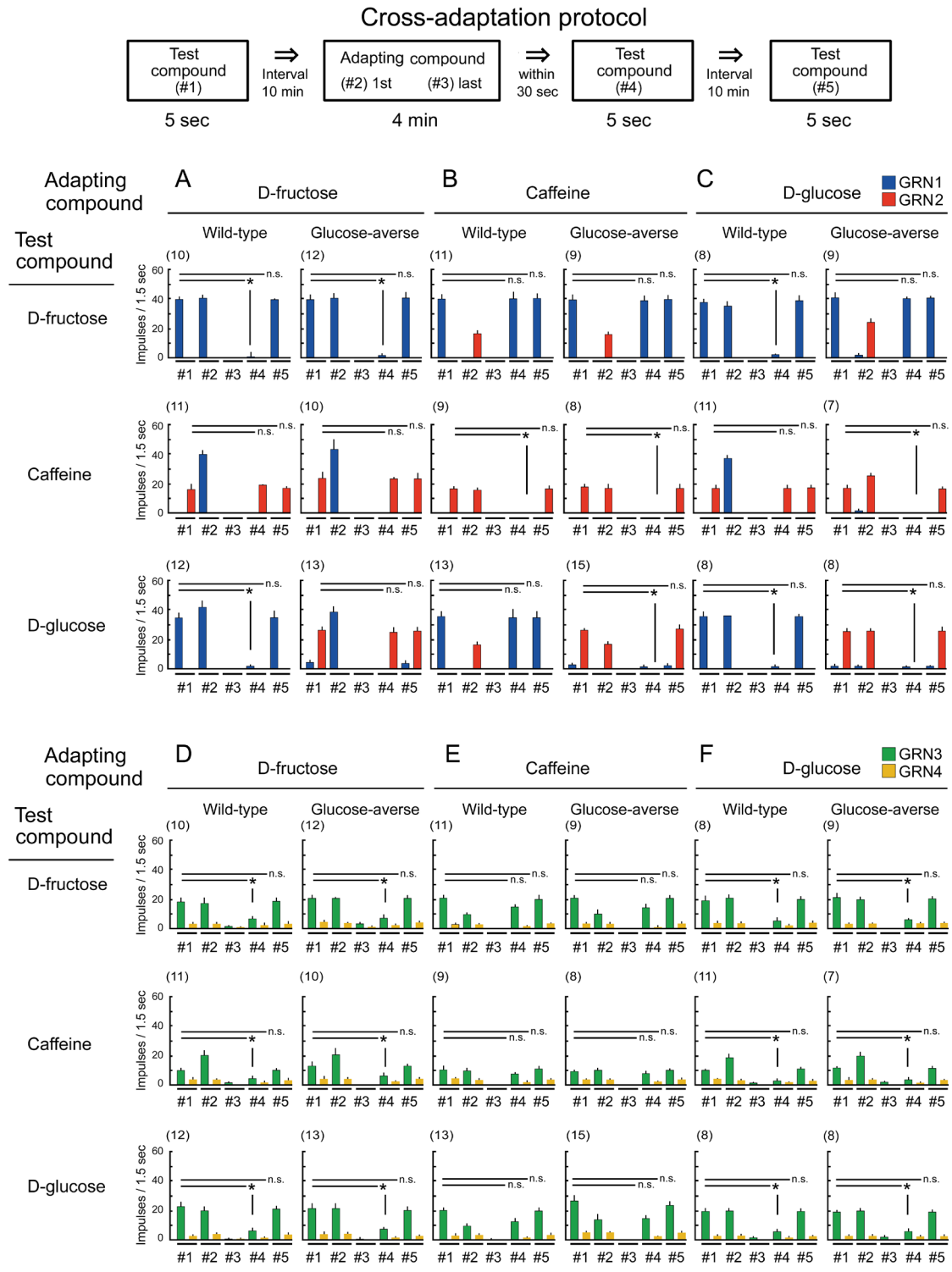

Cross-adaptation protocol
Test compound (#1)
5 sec
Interval 10 min
Adapting compound
(#2) 1st
(#3) last
4 min
within 30 sec
Test compound (#4)
5 sec
Interval 10 min
Test compound (#5)
5 sec
Adapting compound
Test compound
D-fructose
Caffeine
D-glucose
A
B
C
D
E
F
GRN1
GRN2
GRN3
GRN4
Wild-type
Glucose-averse
Impulses / 1.5 sec
n.s.
*
#1 #2 #3 #4 #5

Fig. S2. Cross-adaptation experiments to discriminate the response profiles of GRNs in two cockroach strains.

Experiments were performed with 8 mmol l^{-1} D-fructose, 0.16 mmol l^{-1} caffeine, and 32 mmol l^{-1} D-glucose. Impulse frequency (mean ± SEM) in the pre-adaptation stimulation (#1) was compared with the impulse frequency during cross-adaptation (#4) or post-adaptation (recovery, #5) by ANOVA and Dunnett's test (asterisks indicate significant differences, $P < 0.05$; n.s. = not significant). Number of tested sensilla is shown in parenthesis. In both strains, the adapting compound D-fructose adapted GRN1 to further stimulation (#4) with D-fructose (**A**, upper), but it did not affect the GRN2 response to caffeine (**A**, middle). The caffeine-adapted GRN2 of both strains did not respond to further stimulation (#4) with caffeine (**B**, middle), but caffeine as the adapting stimulus did not affect the GRN1 responses to D-fructose and D-glucose (**B**, upper). These results indicate that D-fructose selectively stimulated GRN1, whereas caffeine selectively stimulated GRN2. D-fructose-adaptation of GRN1 in WT cockroaches significantly decreased the GRN1 response to D-glucose (**A**, bottom), whereas adaptation of GRN2 with caffeine did not affect the GRN1 response to D-glucose (**B**, bottom). Finally, in WT cockroaches the reciprocal adaptation of GRN1 with D-glucose decreased the GRN1 response to both D-fructose and D-glucose (**C**, upper and bottom) but not the GRN2 response to caffeine (**C**, middle). In GA (T164-BC) cockroaches, the adapting compound D-fructose did not adapt the D-glucose-sensitive GRN2 (**A**, bottom), but adaptation of GRN2 with caffeine decreased its response to D-glucose (**B**, bottom). The reciprocal adaptation of GRN2 with D-glucose decreased its response to caffeine (**C**, middle) but not the GRN1 response to D-fructose (**C**, upper). These results indicate that whereas in the WT strain the D-glucose-sensitive GRN is GRN1, in the GA strain the D-glucose-sensitive GRN is GRN2. In all experiments with GA cockroaches and the test compound D-glucose, GRN1 responded with a low frequency of impulses (**A**-**C**, bottom). Therefore, no significant effects of adapting compounds on the GRN1 response to D-glucose could be detected because the GRN1 response to D-glucose was so low to start with.

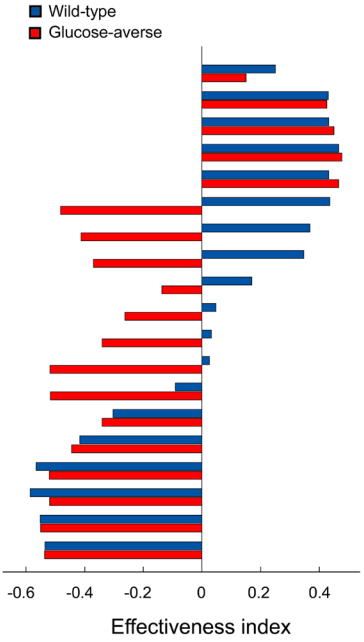
On the other hand, there were no significant differences in the effects of adapting compounds on GRN3 and GRN4 responses between WT and GA cockroaches with any of the test compounds (**D**-**F**). These results suggest that there are no differences in GRN3 and GRN4 sensitivities between WT and GA strains.

Fig S3

A

Tastant	Feeding response (n)	
	Wild-type	Glucose-averse
L-arabinose	**Accept** (80)	**Accept** (60)
D-Fructose	**Accept** (60)	**Accept** (140)
Maltose	**Accept** (60)	**Accept** (140)
Trehalose	**Accept** (60)	**Accept** (140)
Sucrose	**Accept** (60)	**Accept** (140)
D-glucose	**Accept** (80)	**Reject** (220)
6-deoxy-D-glucose	**Accept** (60)	**Reject** (80)
Methyl α-D-glucoside	**Accept** (80)	**Reject** (60)
D-arabinose	**Accept** (100)	**Reject** (200)
2-deoxy-D-glucose	No effect (100)	**Reject** (80)
D-xylose	No effect (60)	**Reject** (180)
Methyl β-D-glucoside	No effect (80)	**Reject** (60)
L-galactose	**Reject** (100)	**Reject** (60)
D-galactose	**Reject** (80)	**Reject** (140)
3-*O*-methyl-D-glucose	**Reject** (60)	**Reject** (140)
L-glucose	**Reject** (60)	**Reject** (140)
Salicin	**Reject** (60)	**Reject** (120)
Saccharin	**Reject** (60)	**Reject** (120)
Caffeine	**Reject** (60)	**Reject** (60)

B



Effectiveness index		χ^2 test (Wild-type vs Glucose-averse)
Wild-type	Glucose-averse	
0.25	0.15	$P = 0.89$
0.43	0.43	$P = 0.16$
0.43	0.46	Not tested
0.47	0.48	$P = 0.70$
0.43	0.46	Not tested
0.44	-0.48	$P < 0.001$
0.37	-0.41	$P < 0.001$
0.35	-0.37	$P < 0.001$
0.18	-0.13	$P < 0.001$
0.05	-0.26	$P < 0.001$
0.03	-0.34	$P < 0.001$
0.03	-0.52	$P < 0.001$
-0.09	-0.52	$P < 0.001$
-0.30	-0.34	$P = 0.16$
-0.42	-0.44	$P = 0.11$
-0.57	-0.52	Not tested
-0.58	-0.52	Not tested
-0.55	-0.55	Not tested
-0.53	-0.53	Not tested

Fig. S3. The chemical structures of D-glucose and related compounds drive the glucose-aversion behavior.

(A) The effect of tastants on feeding response to D-fructose alone. The number of cockroaches ingesting the binary mixture of D-fructose and another tastant and the number of cockroaches ingesting D-fructose alone were tested by McNemar's test ($P < 0.05$). Accept: a significantly greater response to the mixture, indicating that the test compound acts as a phagostimulant. No effect: no significant difference between the mixture and D-fructose alone, indicating that the test compound does not affect appetitive or aversive behavior. Reject: a significantly lower response to the mixture indicates that the test compound acts as a deterrent. The number of tested cockroaches is indicated in parenthesis. **(B)** Differences in the effectiveness index (EI) values of the two cockroach strains correlate with the molecular structures of 19 tastants. All hexoses, except D-fructose, were aversive to GA (T164-BC) cockroaches, whereas WT cockroaches showed diverse feeding responses to different hexoses. Effectiveness index is the difference between the proportion of cockroaches accepting the mixture and the proportion accepting D-fructose alone. The numbers of WT and GA cockroaches ingesting the solution were compared by χ^2 test ($P < 0.05$). In cases where all tested cockroaches showed either acceptance or rejection, a χ^2 test was not performed.

Fig S4

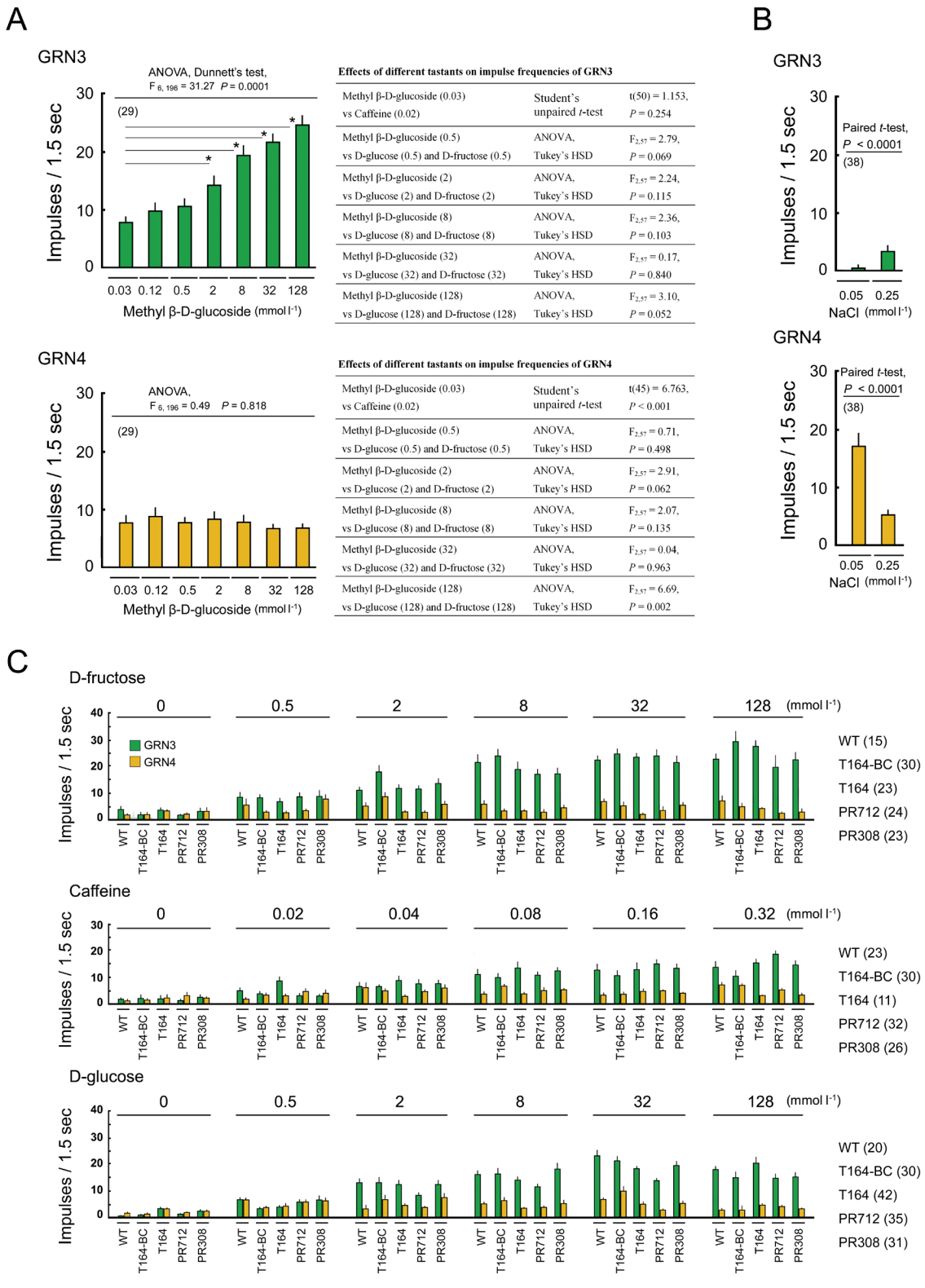

A
GRN3
ANOVA, Dunnett's test,
F 6, 196 = 31.27 P = 0.0001
(29)
Impulses / 1.5 sec
0.03 0.12 0.5 2 8 32 128
Methyl β-D-glucoside (mmol l-1)
Effects of different tastants on impulse frequencies of GRN3
Methyl β-D-glucoside (0.03) vs Caffeine (0.02) | Student's unpaired t-test | t(50) = 1.153, P = 0.254
Methyl β-D-glucoside (0.5) vs D-glucose (0.5) and D-fructose (0.5) | ANOVA, Tukey's HSD | F2,57 = 2.79, P = 0.069
Methyl β-D-glucoside (2) vs D-glucose (2) and D-fructose (2) | ANOVA, Tukey's HSD | F2,57 = 2.24, P = 0.115
Methyl β-D-glucoside (8) vs D-glucose (8) and D-fructose (8) | ANOVA, Tukey's HSD | F2,57 = 2.36, P = 0.103
Methyl β-D-glucoside (32) vs D-glucose (32) and D-fructose (32) | ANOVA, Tukey's HSD | F2,57 = 0.17, P = 0.840
Methyl β-D-glucoside (128) vs D-glucose (128) and D-fructose (128) | ANOVA, Tukey's HSD | F2,57 = 3.10, P = 0.052
GRN4
ANOVA,
F 6, 196 = 0.49 P = 0.818
(29)
Impulses / 1.5 sec
0.03 0.12 0.5 2 8 32 128
Methyl β-D-glucoside (mmol l-1)
Effects of different tastants on impulse frequencies of GRN4
Methyl β-D-glucoside (0.03) vs Caffeine (0.02) | Student's unpaired t-test | t(45) = 6.763, P < 0.001
Methyl β-D-glucoside (0.5) vs D-glucose (0.5) and D-fructose (0.5) | ANOVA, Tukey's HSD | F2,57 = 0.71, P = 0.498
Methyl β-D-glucoside (2) vs D-glucose (2) and D-fructose (2) | ANOVA, Tukey's HSD | F2,57 = 2.91, P = 0.062
Methyl β-D-glucoside (8) vs D-glucose (8) and D-fructose (8) | ANOVA, Tukey's HSD | F2,57 = 2.07, P = 0.135
Methyl β-D-glucoside (32) vs D-glucose (32) and D-fructose (32) | ANOVA, Tukey's HSD | F2,57 = 0.04, P = 0.963
Methyl β-D-glucoside (128) vs D-glucose (128) and D-fructose (128) | ANOVA, Tukey's HSD | F2,57 = 6.69, P = 0.002
B
GRN3
Paired t-test,
P < 0.0001
(38)
Impulses / 1.5 sec
0.05 0.25
NaCl (mmol l-1)
GRN4
Paired t-test,
P < 0.0001
(38)
Impulses / 1.5 sec
0.05 0.25
NaCl (mmol l-1)
C
D-fructose
0 0.5 2 8 32 128 (mmol l-1)
GRN3
GRN4
Impulses / 1.5 sec
WT T164-BC T164 PR712 PR308
WT (15)
T164-BC (30)
T164 (23)
PR712 (24)
PR308 (23)
Caffeine
0 0.02 0.04 0.08 0.16 0.32 (mmol l-1)
WT (23)
T164-BC (30)
T164 (11)
PR712 (32)
PR308 (26)
D-glucose
0 0.5 2 8 32 128 (mmol l-1)
WT (20)
T164-BC (30)
T164 (42)
PR712 (35)
PR308 (31)

Fig. S4. Taste modal specificities of GRN3 and GRN4 for four tastants and two electrolyte concentrations.

(A) Responses of GRN3 and GRN4 to various concentrations of methyl β-D-glucoside in the WT strain. The numbers of tested sensilla are shown in parenthesis. Sugar- and bitter-GRNs (GRN1 and GRN2, respectively) did not respond to this compound at the tested concentrations. Responses of GRN3 to methyl β-D-glucoside increased in a concentration-dependent manner, suggesting that it responds to solute osmotic concentration. Comparisons of GRN3 responses to methyl β-D-glucoside and GRN3 responses to D-fructose, caffeine and D-glucose at the same concentrations revealed no significant differences in impulse frequencies in WT cockroaches (Student's *t*-test, $P > 0.05$). The responses of GRN4 were not affected by the concentration of any of the test compounds. GRN4 responded with a constant low firing rate independent of the nature of the chemical. **(B)** Responses of GRN3 and GRN4 in WT cockroaches to two concentrations of the NaCl electrolyte. Sugar- and bitter-GRNs did not respond to NaCl at the tested concentrations. GRN3 responded more to the higher concentration of NaCl, in support of a role in sensing osmolarity. The lower concentration of NaCl elicited significantly more impulses in GRN4 than the higher NaCl concentration (Student's paired *t*-test, $P < 0.05$). Generally, insect water-receptor neurons have hypo-osmotic sensitivity, and they are inhibited by salt, sugar and amino acids in a concentration-dependent manner (*27*, *28*). However, because the response of GRN4 did not systematically decrease with osmolarity (**A**), we could not determine its taste modality in this study. **(C)** Responses of GRN3 and GRN4 to D-fructose, caffeine, and D-glucose in the WT, T164-BC, T164, PR712 and PR308 strains. The responses of the sugar- and bitter-GRNs to these three tastants are shown in Fig. 4C and fig. S5. There were no significant differences in the sensitivities of GRN3 and GRN4 among different strains (see table S1). Combined, these results suggest that GNR3 is sensitive to changes in osmotic concentration with no tastant specificity and therefore is an osmo-GRN, and that GRN4's responses are independent of the nature of the chemical stimulus. Because different cockroach strains did not differ in the sensitivities of these two GRNs, GRN3 and GRN4 appear to not be involved in discrimination of tastants and in glucose-aversion.

Fig S5

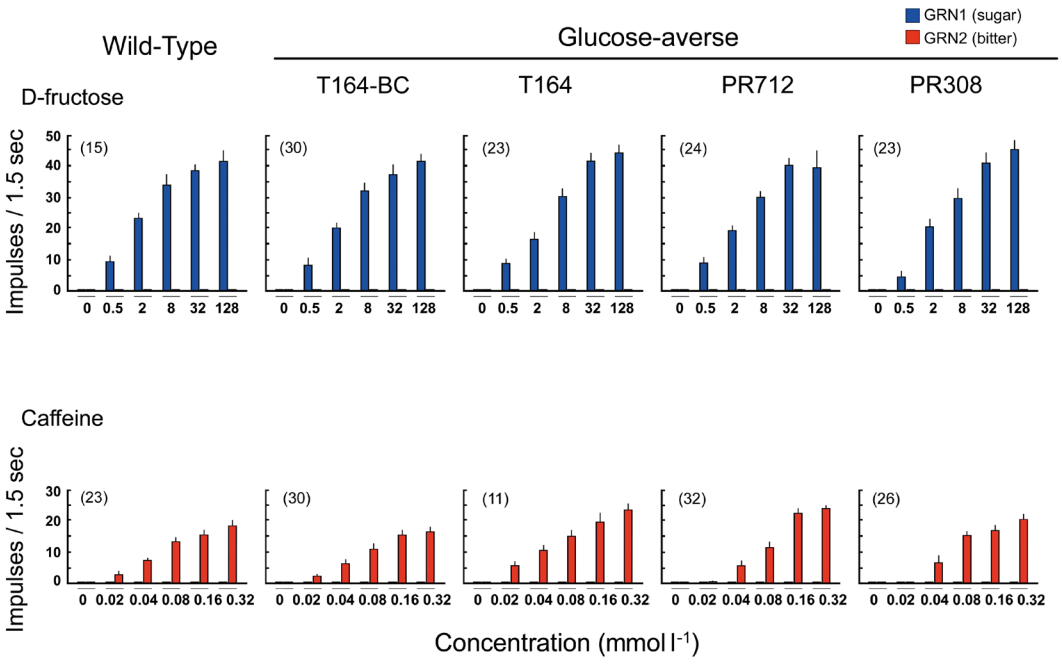


Fig. S5. Dose-GRN responses of cockroaches to D-fructose and caffeine.
Number shown in parenthesis indicates the number of tested sensilla. At each solute concentration, there were no significant differences among different cockroach strains in the responses of their sugar-GRNs or bitter-GRNs (ANOVA, $P > 0.05$), suggesting that WT and four glucose-averse cockroach strains have fundamentally similar taste processing neural mechanisms (see table S1).

Table S1. GRN responses to D-glucose, D-fructose and caffeine in five cockroach strains
There are no significant differences in the responses of any of the four GRNs of the five strains to either D-fructose or caffeine stimulation. D-glucose stimulation, however, elicited significant differences in the responses of GRN1 and GRN2 among different strains.

D-glucose (ANOVA and Tukey's HSD)						
	Concentration	0.5 mmol l^{-1}	2 mmol l^{-1}	8 mmol l^{-1}	32 mmol l^{-1}	128 mmol l^{-1}
GRN1 (sugar)	$F_{4, 153}$	1.38	16.20	33.32	137.1	454.8
	P value	0.245	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	Strains[1]		WT[a] T164-BC[b] T164[b] PR712[b] PR308[a]	WT[a] T164-BC[b] T164[b] PR712[b] PR308[c]	WT[a] T164-BC[bc] T164[b] PR712[bc] PR308[c]	WT[a] T164-BC[b] T164[c] PR712[bc] PR308[b]
GRN2 (bitter)	$F_{4, 153}$	8.55	23.13	32.74	24.97	27.87
	P value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	Strains[1]	WT[a] T164-BC[b] T164[b] PR712[a] PR308[a]	WT[a] T164-BC[b] T164[b] PR712[a] PR308[a]	WT[a] T164-BC[b] T164[b] PR712[b] PR308[b]	WT[a] T164-BC[b] T164[b] PR712[c] PR308[bc]	WT[a] T164-BC[b] T164[c] PR712[b] PR308[b]
GRN3 (osmo)	$F_{4, 153}$	2.16	1.06	2.06	1.78	1.56
	P value	0.076	0.380	0.088	0.136	0.188
	Strains[1]	No significant differences among the five strains				
GRN4	$F_{4, 153}$	1.60	2.83	1.38	1.57	2.12
	P value	0.178	0.126	0.242	0.186	0.081
	Strains[1]	No significant differences among the five strains				

D-fructose (ANOVA and Tukey's HSD)						
	Concentration	0.5 mmol l^{-1}	2 mmol l^{-1}	8 mmol l^{-1}	32 mmol l^{-1}	128 mmol l^{-1}
GRN1 (sugar)	$F_{4, 110}$	1.50	1.38	0.49	0.75	0.69
	P value	0.207	0.245	0.741	0.563	0.601
	Strains[1]	No significant differences among the five strains				
GRN2 (bitter)	Did not respond to D-fructose					
GRN3 (osmo)	$F_{4, 110}$	0.31	1.88	1.53	0.38	1.47
	P value	0.871	0.119	0.199	0.825	0.215
	Strains[1]	No significant differences among the five strains				
GRN4	$F_{4, 110}$	1.79	1.50	1.57	1.30	1.59
	P value	0.136	0.208	0.187	0.274	0.183
	Strains[1]	No significant differences among the five strains				

Caffeine (ANOVA and Tukey's HSD)						
	Concentration	0.02 mmol l^{-1}	0.04 mmol l^{-1}	0.08 mmol l^{-1}	0.16 mmol l^{-1}	0.32 mmol l^{-1}
GRN1 (sugar)	Did not respond to caffeine					
GRN2 (bitter)	$F_{4, 117}$	2.43	0.81	1.16	2.11	1.60
	P value	0.052	0.519	0.333	0.084	0.179
	Strains[1]	No significant differences among the five strains				
GRN3 (osmo)	$F_{4, 117}$	0.92	0.59	0.60	0.99	2.03
	P value	0.453	0.670	0.661	0.415	0.094
	Strains[1]	No significant differences among the five strains				
GRN4	$F_{4, 117}$	1.68	0.97	1.83	1.56	1.76
	P value	0.159	0.427	0.128	0.190	0.141
	Strains[1]	No significant differences among the five strains				

[1] Strains not sharing superscript letters are significantly different.

Table S2. Behavioral EC_{50} of wild-type and glucose-averse cockroaches in dose-feeding response assays

Behavioral EC_{50} for the six tastants tested in Fig. 2B:

There are no differences in EC_{50} with either D-fructose or caffeine between WT and GA (T164-BC) cockroaches. However, the two strains differ in their feeding responses to D-glucose and related compounds.

Treatment	Tastant[3]	Feeding response EC_{50} (mmol l^{-1})[4]		χ^2 test[6]
		Wild-type	Glucose-averse (T164-BC)[5]	
Hungry[1]	D-fructose	28.2 (23.00, 34.71)	31.8 (28.23, 35.91)	$P = 0.256$
	Caffeine	rejected by all cockroaches	rejected by all cockroaches	Not tested[7]
	D-glucose	66.6 (25.40, 186.59)	rejected by all cockroaches	Not tested[7]
	Methyl α-D-glucoside	95.2 (65.77, 136.42)	rejected by all cockroaches	Not tested[7]
	Methyl β-D-glucoside	rejected by all cockroaches	rejected by all cockroaches	Not tested[7]
	3-*O*-methyl-D-glucose	rejected by all cockroaches	rejected by all cockroaches	Not tested[7]
Hungry and thirsty[2]	D-fructose	accepted by all cockroaches	accepted by all cockroaches	Not tested[8]
	Caffeine	0.92 (0.771, 1.099)	1.34 (1.119, 1.596)	$P = 0.083$
	D-glucose	accepted by all cockroaches	43.2 (26.00, 72.14)	Not tested[9]
	Methyl α-D-glucoside	accepted by all cockroaches	127.4 (98.75, 163.35)	Not tested[9]
	Methyl β-D-glucoside	accepted by all cockroaches	18.0 (13.45, 24.05)	Not tested[9]
	3-*O*-methyl-D-glucose	1358.7 (983.20, 1973.70)	159.0 (116.48, 217.91)	$P < 0.001$

Behavioral EC_{50} for D-fructose and binary mixtures tested in Fig. 3A:

D-glucose increases the appetitive response to D-fructose in WT cockroaches. In GA (T164-BC) cockroaches, on the other hand, D-glucose decreases the feeding response to D-fructose, as caffeine does.

Strain[1]	Test solution[10]	Feeding response EC_{50} (mmol l^{-1})[11]		χ^2 test[12]
Wild-type	D-fructose	28.2	(23.00, 34.71)[13]	
	D-fructose + 30 mmol l^{-1} D-glucose	9.2	(6.72, 12.52)	Not tested[14]
	D-fructose + 300 mmol l^{-1} D-glucose	4.7	(3.00, 7.41)	Not tested[14]
	D-fructose + 1 mmol l^{-1} caffeine	86.8	(52.22, 144.7)	$P < 0.0001$
	D-fructose + 1 mmol l^{-1} caffeine	1674.7	(925.0, 33084.5)	$P < 0.0001$
Glucose-averse (T164-BC)[5]	D-fructose	31.8	(28.23, 35.91)	
	D-fructose + 30 mmol l^{-1} D-glucose	101.9	(83.71, 124.1)	$P < 0.0001$
	D-fructose + 300 mmol l^{-1} D-glucose	864.2	(672.8, 1112.8)	$P < 0.0001$
	D-fructose + 1 mmol l^{-1} caffeine	104. 9	(76.71, 143.8)	$P < 0.0001$
	D-fructose + 1 mmol l^{-1} caffeine	1729.5	(1209.5, 2483.9)	$P < 0.0001$

Behavioral EC_{50} for D-glucose in four glucose-averse strains tested in Fig. 4B:

The feeding responses to D-glucose in the field-collected PR308 and PR712 are significantly lower than in lab-selected glucose-averse cockroaches (T164 and T164-BC). However, all four strains reject D-glucose.

Strain[2]	Feeding response EC_{50} (mmol l^{-1})[15]		χ^2 test[16]
T164-BC (backcross)[5]	43.2	(26.00, 72.14)[17]	
T164 (parental of T164-BC)	38.4	(31.75, 46.41)	$P = 0.075$
PR308 (field collected)	98.5	(79.83, 121.9)	$P < 0.0001$
PR712 (field collected)	69.2	(55.14, 87.01)	$P < 0.0001$

[1] Cockroaches were starved without food, but with access to water for 24 hrs.

[2] Cockroaches were starved without food and water for 24 hrs.

[3] The concentrations tested for each tastant were: D-fructose and D-glucose, 0.001, 0.01, 0.1, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}; Caffeine, 0.001, 0.01, 0.1, 0.3, 1, 3, 10, 30 and 100 mmol l^{-1}; The other three compounds, 0.1, 1, 10, 100, 1000 and 3000 mmol l^{-1}.

[4] The EC_{50} (95% fiducial limits) for each tastant was obtained from probit analysis based on the dose-response curves in Fig. 2B.

[5] T164-BC cockroaches were obtained by crossing wild-type and T164, followed by 8 rounds of backcrossing to WT cockroaches. In this study, behavioral and GRN sensitivities were always compared between WT and T164-BC cockroaches.
[6] The behavioral EC_{50} values for each tastant were compared between the WT and T164-BC cockroaches by a χ^2 test of heterogeneity ($P < 0.05$).
[7] χ^2 test was not performed because all cockroaches rejected the tastant.
[8] χ^2 test was not performed because all cockroaches accepted the tastant.
[9] χ^2 test was not performed because of problems in parallelism and lack of fit with the probit model.
[10] The concentrations of D-fructose were 0.001, 0.01, 0.1, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}. For assays with D-glucose, either 30 or 300 mmol l^{-1} of D-glucose was added to each concentration of D-fructose. For assays with caffeine, either 1 or 10 mmol l^{-1} of caffeine was added to each concentration of D-fructose.
[11] The EC_{50} (95% fiducial limits) for each test solution was obtained from probit analysis based on the dose-response curves in Fig. 3A.
[12] The behavioral EC_{50} of each binary mixture was compared with the behavioral EC_{50} for D-fructose alone by a χ^2 test of heterogeneity ($P < 0.05$).
[13] The behavioral EC_{50} for D-fructose is the same as in Fig. 2B.
[14] In the WT strain the χ^2 test could not be performed for comparisons of D-fructose and mixtures of D-fructose and D-glucose because of problems in parallelism and lack of fit with the probit model.
[15] The EC_{50} (95% fiducial limits) for D-glucose was obtained from probit analysis based on the dose-response curves in Fig. 4B. D-glucose concentrations tested in each strain were: GA, 0.001, 0.01, 0.1, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}; other three strains, 0.3, 1, 3, 10, 30, 100, 300, 1000 and 3000 mmol l^{-1}.
[16] Behavioral EC_{50} for D-glucose in GA cockroaches was compared with other strains by a χ^2 test of heterogeneity ($P < 0.05$).
[17] The behavioral EC_{50} for D-glucose is the same as in Fig. 2B.

Table S3. Correlation between behavioral responses and GRN2 responses to D-glucose

Concentration of D-glucose (mmol l^{-1})[1]	Appetitive response[2] vs GRN2 impulse frequency[3]			
	T164-BC[4]	T164	PR712	PR308
1 and 0.5	0.98 vs 3.3	1.00 vs 3.4	1.00 vs 0.3	0.98 vs 0.1
3 and 2	0.93 vs 13.9	1.00 vs 15.1	0.93 vs 4.6	0.97 vs 3.5
10 and 8	0.83 vs 17.2	0.88 vs 21.5	0.84 vs 9.5	0.90 vs7.2
30 and 32	0.58 vs 24.6	0.54 vs 28.5	0.70 vs 16.5	0.80 vs 17.8
100 and 128	0.37 vs 25.9	0.20 vs 34.0	0.47 vs 21.3	0.56 vs 20.0

Pearson's correlation coefficient[5] = -0.83, $P < 0.01$,
A negative correlation was found between appetitive responses and GRN2 responses in four glucose-averse strains.

[1] For correlating the appetitive responses and GRN2 responses to D-glucose, similar but not identical concentrations of D-glucose were used in behavioral assays (listed first) and electrophysiological recordings.

[2] The proportions of cockroaches showing appetitive responses are from Fig. 4B.

[3] The averages of GRN2 impulse frequencies are from Fig. 4C.

[4] T164-BC cockroaches were obtained by crossing wild-type and T164, followed by 8 rounds of backcrossing to WT cockroaches.

[5] Pearson's correlation coefficient, *r*, was obtained from four glucose-averse strains. A significant negative correlation was found between the appetitive responses and GRN2 responses to D-glucose.

Movie S1. Example of a two-choice feeding assay with WT cockroaches.

Ten adult male cockroaches were given a choice of two agar discs: the red disc contains 1000 mmol l^{-1} D-fructose and the blue disc contains 1000 mmol l^{-1} D-glucose. WT cockroaches sample and eat both sugars.

Movie S2. Example of a two-choice feeding assay with glucose-averse T164-BC cockroaches.

Ten adult male cockroaches were given a choice of two agar discs: the red disc contains 1000 mmol l^{-1} D-fructose and the blue disc contains 1000 mmol l^{-1} D-glucose. The GA cockroaches sample and reject D-glucose and accept D-fructose.

Movie S3. Example of a feeding response test with a WT cockroach.

The cockroach was deprived of food but not water for 24 hr. Before each assay the antennae, maxillary palps and labial palps were ablated, leaving the paraglossae intact, and then the paraglossae-mediated feeding response was obtained. The cockroach is offered a dyed stimulus solution containing D-glucose, and ingestion is also monitored through the clypeus and frons, the translucent front-middle area of the head capsule. The cockroach readily accepts D-glucose.

Movie S4. Example of a feeding response test with a glucose-averse T164-BC cockroach.

The cockroach was deprived of food and water for 24 hrs, and screened. Before the assay the antennae, maxillary palps and labial palps were ablated, leaving the paraglossae intact, and then the paraglossae-mediated feeding response was obtained. The cockroach is offered a dyed stimulus solution containing D-glucose, and ingestion is also monitored through the clypeus and frons, the translucent front-middle area of the head capsule. The cockroach rejects D-glucose.

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