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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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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.

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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

![Chemical structures of tastants.](A) D-fructose, Caffeine, D-glucose, Methyl α-D-glucoside, Methyl β-D-glucoside, 3-0-methyl-D-glucose.

![Behavioral responses to six 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).
reported 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 in six strains (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 and aversive behavior (Fig. 1C and fig. S3). We therefore suggest that glucose and related compounds by bitter-GRNs, 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.

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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. 3. Glucose aversion is elicited by stimulation of bitter-GRNs and inhibition of sugar-GRNs.](image)

(A) Cockroaches were tested with fructose alone (Fru), fructose mixed with 30 or 300 mmol liter⁻¹ glucose (F30G and F300G), and fructose 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.
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 structural-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 insects.

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 (EC50) 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).

References and Notes

4. Materials and methods are available as supplementary materials on Science Online.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S5
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