Taste-independent detection of the caloric content of sugar in *Drosophila*

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Feeding behavior is influenced primarily by two factors: nutritional needs and food palatability. However, the role of food deprivation and metabolic needs in the selection of appropriate food is poorly understood. Here, we show that the fruit fly, *Drosophila melanogaster*, selects calorie-rich foods following prolonged food deprivation in the absence of taste-receptor signaling. Flies mutant for the sugar receptors Gr5a and Gr64a cannot detect the taste of sugar, but still consumed sugar over plain agar after 15 h of starvation. Similarly, *pox-neuro* mutants that are insensitive to the taste of sugar preferentially consumed sugar over plain agar upon starvation. Moreover, when given a choice between metabolizable sugar (sucrose or D-glucose) and nonmetabolizable (zero-calorie) sugar (sucralose or L-glucose), starved *Gr5a; Gr64a* double mutants preferred metabolizable sugars. These findings suggest the existence of a taste-independent metabolic sensor that functions in food selection. The preference for calorie-rich food correlates with a decrease in the two main hemolymph sugars, trehalose and glucose, and in glycogen stores, indicating that this sensor is triggered when the internal energy sources are depleted. Thus, the need to replenish depleted energy stores during periods of starvation may be met through the activity of a taste-independent metabolic sensing pathway.

Food quantity and quality can vary greatly in natural habitats. To survive such variations, animals must be able to search for and detect appropriate food sources under all conditions, especially during times of food scarcity. Peripheral chemosensory neurons, such as sugar taste neurons, allow animals to detect palatable foods (1–5). Additional mechanisms may be necessary for the detection of foods to meet acute nutritional needs. Indeed, animals learn to positively associate a flavor paired with intragastric sugar infusion (6). Recently, studies of *Trpm5*$^{-/-}$ mice, which are insensitive to the taste of sugar, also have revealed that these animals develop a preference for a sugar solution on the basis of its caloric content even in the absence of gustatory input (7). Unfortunately, the nature of such mechanisms is currently unknown. It is also not clear whether they function under starvation conditions.

To search for mechanisms by which animals can respond to the caloric content of food independently of orosensory cues, we studied the effect of starvation on food choice in *Drosophila* mutants that are unable to taste sugar. Specifically, we sought to determine whether food-deprived flies carrying mutations in *Gr5a* and *Gr64a* (3–5), the sugar receptor genes, and in *pox-neuro* (*poxn*) (8–10), a gene that specifies chemosensory neurons, develop a preference for the caloric content of sugars in the absence of taste perception. We found that these mutant flies demonstrated a preference for caloric food upon starvation and that this preference correlated with the energy needs of the fly. Furthermore, wild-type (WT) flies showed a shift in preference to metabolizable sugars following prolonged periods of starvation even though nonmetabolizable sugars induced similar taste responses. Our findings suggest that starvation activates a previously uncharacterized pathway that allows animals to make feeding choices based on nutritional needs rather than palatability.

Results

Flies Mutant for the Sugar Taste Receptors Gr5a and Gr64a Exhibit a Preference for Sugars After Prolonged Periods of Starvation. Flies mutant for *Gr5a* and *Gr64a* are unable to taste most sugars, including sucrose, glucose, and trehalose (3–5). To determine whether flies have a taste-independent pathway that enables the detection of calorie-rich food after periods of food deprivation, we asked whether starved *Gr5a; Gr64a* mutants develop a preference for the sugar when given a choice between sucrose and plain agar in two-choice feeding assays. In this assay, flies were presented with two food substrates, each colored with a different dye, and feeding preference was scored by dye accumulation in the abdomen of individual flies (11) (Fig. S1 *A* and *B*). Thus, this is a qualitative measurement of food choice that reflects the preference for one food substrate over another. We tested *Gr5a; Gr64a* mutants (*Gr5a; Gr64a*^{1} and *Gr5a; Gr64a*^{2} alleles) that had been starved for 22 h. For controls, we used flies lightly food-deprived for 5 h. WT *Canton-S* (*CS*) and flies carrying single mutations for *Gr5a* or *Gr64a* (*Gr64a*^{1} and *Gr64a*^{2} alleles) were attracted to sucrose after either 5 or 22 h of starvation. However, the majority of *Gr5a; Gr64a* double mutants did not eat when they were lightly food-deprived for 5 h (Fig. 1*A*), and no dye accumulation was observed in their crop and gut after dissection (Fig. 1*B*). In contrast, most of the *Gr5a; Gr64a* mutants developed a strong preference for sucrose after 22 h of starvation, as shown by the presence of dye in their abdomens (Fig.1 *C* and *D*). This feeding choice happened despite the inability of *Gr5a; Gr64a* mutants to taste sugar. We asked whether *Gr5a; Gr64a* mutant flies did not eat after 5 h of food deprivation because they have abnormal responses to starvation. To test this, we measured circulating glucose levels, glycogen stores, and starvation-induced sleep suppression (12), a physiological manifestation of hunger, but we did not detect any differences between *Gr5a; Gr64a* mutants and WT flies (Fig. S1 *C–G*). *Gr5a; Gr64a* mutants therefore showed the behavioral changes that normally accompany starvation.

To determine whether the preference of *Gr5a; Gr64a* mutants for sucrose after prolonged starvation reflected the activity of an unknown sugar taste receptor, we measured their proboscis extension reflex (PER) (13) to a 100-mM sucrose solution after 22 h of starvation. *Gr5a; Gr64a* mutants failed to respond to the taste of sucrose after starvation (Fig. 1*E* and Fig. S2), indicating that they did not acquire gustatory responses to sucrose even after periods of prolonged starvation.

To validate the existence of a taste-independent mechanism for detecting sugar and to ensure that the preference for sucrose over plain agar was not influenced by any other gustatory neurons in the fly, we tested mutants for the *poxn* gene, which

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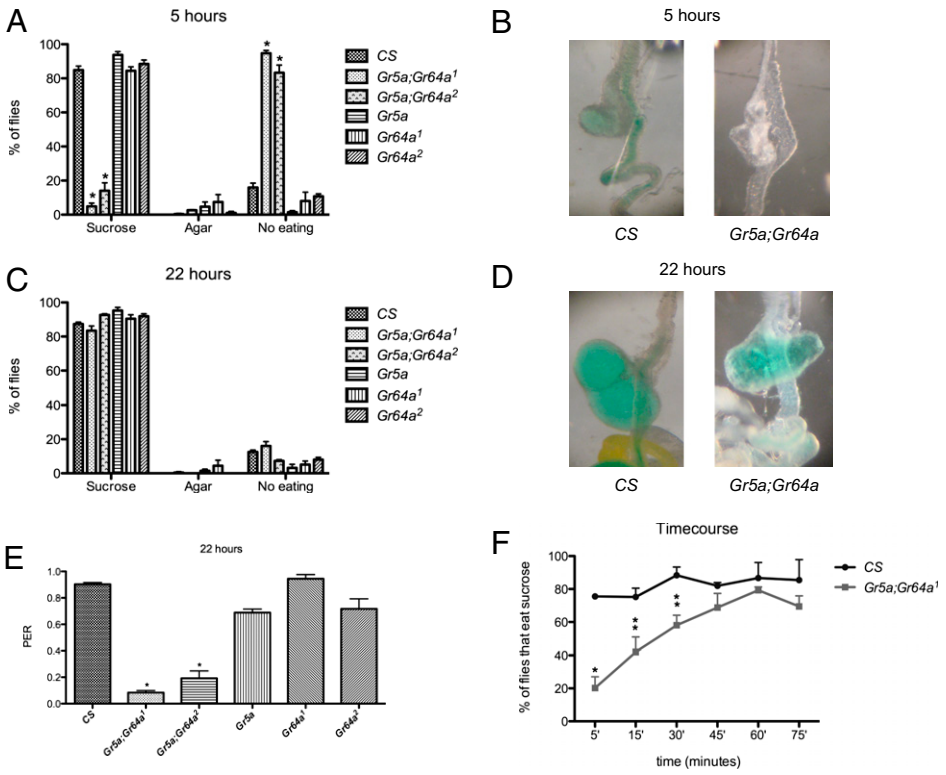


Fig. 1. *Gr5a; Gr64a* mutants develop a preference for sucrose after prolonged periods of food deprivation. (*A* and *C*) Two-choice preference assay with sucrose versus agar after 5 h (*A*) and after 22 h (*C*) of starvation. Flies of different genotypes were given a choice between agar containing 100 mM sucrose versus plain agar. The *y* axis shows the average percentage of flies that ate sucrose, plain agar, or did not eat. n = 4–8 with each trial comprising 50 flies. Error bars: SEM, $*P < 0.001$, two-way ANOVA with Bonferroni post hoc test in this figure and all subsequent figures unless indicated otherwise. (*B* and *D*) Dissected crop and gut from 5-h (*B*) and 22-h (*D*) starved flies that were given a choice between agar containing 100 mM sucrose mixed with green dye versus plain agar mixed with red dye (*A* and *C*). (*E*) PER of 22-h starved flies when their labellum was stimulated by 100 mM sucrose. A full extension was given a score of 1 and a partial extension was given a score of 0.5. n = 24–37. (*F*) Time course for the development of sucrose preference. Flies were starved for 22 h and then given a choice between 100 mM sucrose versus agar for different durations of time (*x* axis). n = 4. $*P < 0.001$ and $**P < 0.05$.

have no chemosensory neurons in any of their gustatory organs (8–10). We used two different alleles, *poxnΔM22B5* and *poxnΔM22B5; full152* (a transgene that rescues *poxnΔM22B5* CNS defects), as taste mutants and mutant animals bearing a full genomic rescue construct, *poxnΔM22B5; SuperA158*, as controls. The *poxn* mutations did not exhibit a PER response to sucrose after 22 h of starvation, whereas the controls had the same responses as WT flies (Figs. S2 and S3*A*). As was observed in *Gr5a; Gr64a* mutant flies, the majority of 5-h food-deprived *poxnΔM22B5* and *poxnΔM22B5;full152* flies did not eat, whereas the controls, *poxnΔM22B5; SuperA 158*, chose sucrose over agar (Fig. S3*C*). After 22 h of starvation, both *poxn* mutants and the controls chose sucrose (Fig. S3*D*). Thus, *poxn* mutants, like *Gr5a; Gr64a* mutants, are able to detect sucrose in the absence of gustatory input. As a final control, we tested whether taste-blind flies use olfactory cues to distinguish sucrose from agar. However, *Gr5a; Gr64a* mutant flies still chose sucrose in the absence of the antennae and maxillary palps, which house the olfactory receptor neurons (Fig. S4). Taken together, these results suggest the existence of a taste- and olfactory-independent pathway that mediates a preference to sugar upon starvation.

A taste-independent metabolic mechanism would be expected to take longer than direct peripheral sensory input to detect the presence of food. If such a mechanism does direct *Gr5a; Gr64a* mutants to select sucrose after 22 h starvation, we would expect it to take longer for the sugar blind mutants to develop a preference for sugar compared with WT flies. To test this, we gave flies a choice of 100 mM sucrose versus plain agar after depriving them of food for 22 h and then assessed their food choices at different time points thereafter (Fig. 1*F*). Within 5 min after the food was presented, the majority of WT flies had already chosen sucrose, whereas most of the *Gr5a; Gr64a* mutants did not. After 45 min, the percentage of *Gr5a; Gr64a* mutant flies that consumed sucrose was comparable to that of the WT flies (Fig. 1*F*). *poxnΔM22B5* mutants behaved similarly to *Gr5a; Gr64a* mutants (Fig. S3*B*). Therefore, the selection of an appropriate food choice is delayed, but not abolished, in the absence of gustatory input. This delay is consistent with assessment of the food nutritional value by a mechanism independent of direct peripheral gustatory input, and instead dependent on metabolic evaluation.

Glucose and trehalose are the two main sugars in the *Drosophila* hemolymph (14). We tested whether these sugars induce a taste-independent feeding behavior in starved flies. As was observed for sucrose, *Gr5a; Gr64a* mutants did not eat glucose or trehalose after 5 h of starvation (Fig. 2 *A* and *C*), but developed a robust preference for both sugars after 22 h of starvation (Fig. 2 *B* and *D*). GR5a is the only known receptor for trehalose, and *Gr5a* mutants lack behavioral and electrophysiological responses to trehalose (15, 16). Unlike the WT and *Gr64a* controls, and similar to *Gr5a; Gr64a* flies, *Gr5a* mutants did not eat after 5 h of food deprivation, but preferred trehalose over plain agar after 22 h of starvation (Fig. 2 *C* and *D*). We tested a nonhemolymph sugar, galactose, and observed that *Gr5a; Gr64a* mutants also developed a preference to the sugar after prolonged starvation (Fig. 2 *E* and *F*).

If taste plays a primary role in food-choice behavior of animals that are sated or food-deprived for short times, then *Gr5a; Gr64a* mutants should eat sugars that they can taste. *Gr5a* and *Gr64a* are not required for detection of fructose (4). As expected, *Gr5a; Gr64a* mutants showed robust PER responses to this sugar (Fig. S5) and chose fructose over agar after 5 h of food deprivation (Fig. 2*G*). To determine whether fructose can trigger the taste-independent food choice, we used *poxn* mutants, which lack a PER response to fructose (Fig. S3*A*). After 5 h of light food deprivation, the majority of *poxnΔM22B5* and *poxnΔM22B5; full 152* mutants did not eat fructose, whereas most of the controls did (Fig. S3*E*). After 22 h of starvation, both *poxn* mutants chose fructose over plain agar (Fig. S3*F*).

Taste-Independent Pathway Detects the Nutritional Content of Sugars. The taste-independent food choice is triggered by different sugars with diverse structures. Once ingested, these sugars are soon metabolized. We asked whether this pathway indeed responds to the nutritional value of sugars. To address this question, we examined whether *Gr5a; Gr64a* mutants prefer the non-metabolizable sweetener sucralose to agar (Fig. 3*A*, *Upper*). WT flies exhibited a robust PER to sucralose, but *Gr5a; Gr64a*^{1} mutants

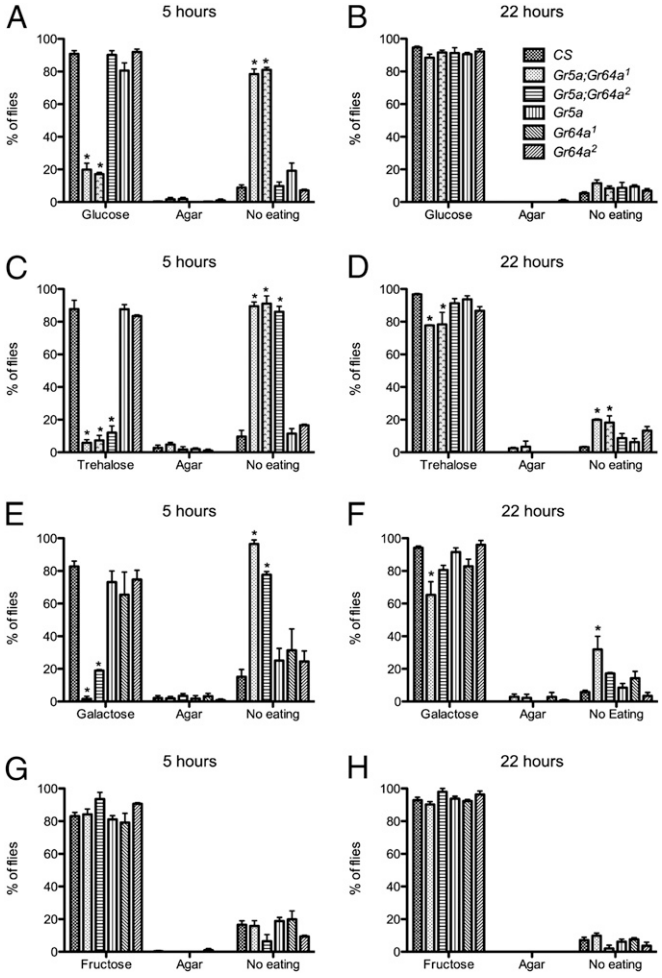


Fig. 2. The taste-independent sensor responds to different sugars. (*A–H*) Two-choice preference assay with agar containing 200 mM glucose (*A* and *B*), 200 mM trehalose (*C* and *D*), 400 mM galactose (*E* and *F*), or 200 mM fructose (*G* and *H*) versus plain agar. *Gr5a;Gr64a*^{1} and *Gr5a;Gr64a*^{2} double mutants and control flies—*CS* WT, *Gr5a*, *Gr64a*^{1}, and *Gr64a*^{2} single mutants—were tested after 5 h (*A*, *C*, *E*, and *G*) and after 22 h of starvation (*B*, *D*, *F*, and *H*). $n = 3–6$. $^*P < 0.001$.

did not (Fig. 3*B*). In two-choice feeding assay, WT flies and single-mutant controls preferred sucralose to plain agar after 22 h of starvation (Fig. 3*C*), which suggests that sucralose is palatable to flies, consistent with a recent report (12, 17). By contrast, the majority of *Gr5a; Gr64a* mutant flies failed to choose either sucralose or agar (Fig. 3*C*), but chose sucrose when given a choice between sucrose and sucralose (Fig. 3*D*). We obtained the same result with *poxn* mutant flies (Fig. S3 *G* and *H*). These results support our hypothesis that the preference for a calorically rich sugar develops independently of sensory cues.

To further investigate the possibility that the taste-independent pathway detects the nutritional value of sugars, we carried out two-choice assays with L-glucose versus D-glucose (Fig. 3*A*, *Lower*). L- and D-glucose are stereoisomers, but only D-glucose can be metabolized and can thus generate energy. WT flies exhibited a robust PER to both glucose stereoisomers (Fig. 3*E*), whereas *Gr5a; Gr64a*^{1} mutant flies responded to neither. In a two-choice assay with L-glucose versus plain agar, WT and control flies chose L-glucose over agar, whereas *Gr5a; Gr64a* mutants chose neither (Fig. 3*F*). When given a choice between D-glucose and L-glucose, all of the flies, including *Gr5a; Gr64a* mutants, selected D-glucose over L-glucose after starvation (Fig. 3*G*). These findings provide further support to our hypothesis that the taste-independent pathway responds to the caloric content of sugars.

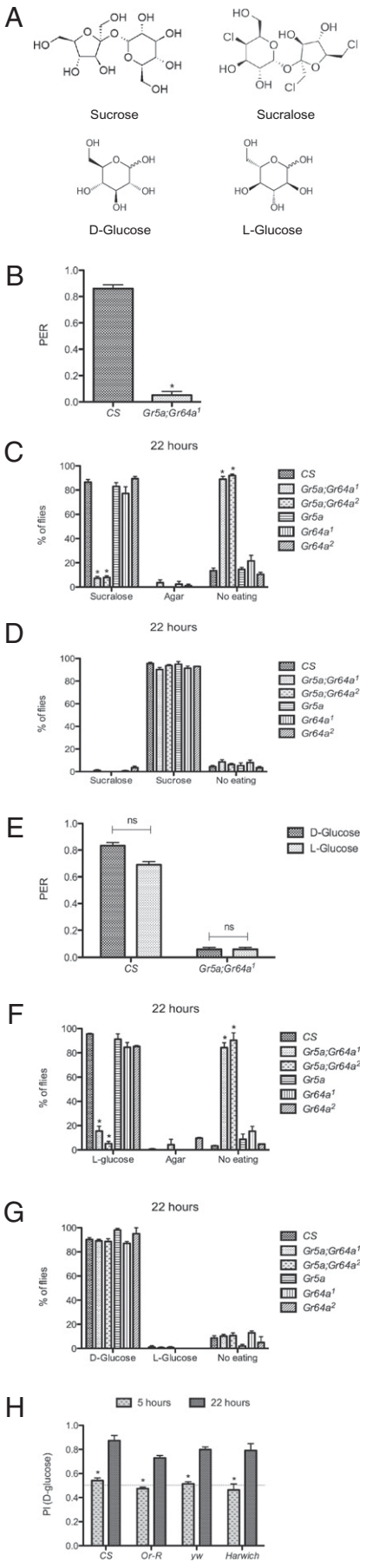
If the nutritional value of food can be detected independently of orosensory cues, it would be expected to take priority over food palatability to ensure that the animal's metabolic needs are met, especially during periods of food scarcity. To test this, we identified a concentration of L- and D-glucose at which four different fly lines—*CS*, *Oregon-R* (*Or-R*), *yw*, and *Harwich*—showed no preference for either sugar after 5 h of food deprivation in a two-choice feeding assay (Fig. 3*H*, light stippled bars). We calculated the preference index (PI) for D-glucose to determine whether the food preference shifted to the metabolizable enantiomer during starvation. All four *Drosophila* lines exhibited a marked preference for D-glucose over the more concentrated L-glucose after 22 h of starvation (Fig. 3*H*, dark stippled bars). This shift in preference to the metabolizable compound during starvation indicates that the mechanisms directing the choice for caloric versus noncaloric foods are triggered when internal energy reserves are depleted.

Taste-Independent Food Choice Correlates with a Decrease in Hemolymph Sugar Levels. We next sought to determine whether the metabolism of carbohydrates influences the preference for metabolizable over nonmetabolizable sugars during starvation. We measured circulating sugar levels and glycogen stores in WT flies after 5, 10, 15, and 22 h of starvation. We found that a drop in the hemolymph glucose and trehalose levels occurs between 10 and 15 h of starvation (Fig. 4*A*). Glycogen stores also declined, but before 10 h of starvation (Fig. 4*B*).

To characterize the timing of the induction of the taste-independent food choice in more detail, we observed the choice made by *Gr5a; Gr64a* mutants and controls when given a choice between sucrose and plain agar after 5, 10, 15, and 22 h of starvation. There was no significant difference in the percentage of *Gr5a; Gr64a* mutants that ate sucrose between 5 and 10 h of starvation. However, more than half of the *Gr5a; Gr64a* mutants chose sucrose by 15 h of food deprivation (Fig. 4*C*), suggesting that the taste-independent food choice in *Gr5a; Gr64a* flies occurs between 10 and 15 h of starvation. These data indicate a strong relationship between the levels of sugar in the hemolymph and the metabolic decision to choose metabolizable sugars.

If glucose and trehalose levels in the hemolymph are sensed by a metabolic sensing pathway, we would expect that manipulating their levels would alter the timing of the shift in preference to metabolizable sugars. To test this prediction, we fed flies the glucoprivic reagent 2-deoxyglucose (2DG), an inhibitor of glucose metabolism, to decrease total hemolymph sugar levels and determined whether this induces an early shift to a preference for D-glucose. We selected 2DG because, like glucose, it is phosphorylated by hexokinase, but unlike glucose, the phosphorylated compound cannot undergo glycolysis (18). Thus, 2DG obstructs the glycolytic pathway and disrupts sugar metabolism. After WT flies were fed with D-glucose (no starvation), 2DG or starved on agar for 10 and 15 h, their hemolymph glycemia was measured. We found no significant differences in the levels of circulating glucose and trehalose in flies fed with D-glucose or flies starved on agar for 10 h (Fig. 4*D*). By contrast, flies fed with 2DG for 10 h had lower levels of circulating glucose and trehalose (Fig. 4*D*). At 15 h, both 2DG-fed and agar-starved flies showed low circulating sugar levels, whereas flies fed with D-glucose maintained high hemolymph glycemia (Fig. 4*E*). These results demonstrated that in flies, as in mammals, 2DG accelerates the depletion of circulating sugar levels.

We then examined whether 2DG influences the timing of the preference shift to D-glucose. WT flies fed with D-glucose or starved on agar for 10 h showed equal preference for D- and L-glucose (Fig. 4*F*). This is consistent with the findings that *Gr5a; Gr64a* mutants do not develop a behavioral preference to sugars and that hemolymph glycemia does not decrease after 10 h of starvation (Fig. 4 *A* and *C*). By contrast, flies fed 2DG for 10 h preferentially consumed D-glucose over L-glucose, suggesting that 2DG accelerates the ability of flies to select food on the basis of its caloric content (Fig. 4*F*). Flies fed with 2DG for 15 h demonstrated



a strong preference for D-glucose, but were not significantly different from flies starved for 15 h (Fig. 4*G*), which already preferred the metabolizable sugar. In starved flies and flies treated with 2DG, the shift in behavior correlated with a measurable decrease in hemolymph glucose and trehalose levels. This observation suggests that circulating glucose and trehalose levels control the functioning of the metabolic sensing pathway.

Discussion

We have shown that fruit flies can develop a preference for the caloric content of sugars. This is accomplished even in the absence of sugar taste receptors or any known peripheral chemosensory systems. This taste-independent sugar-sensing pathway has several distinctive characteristics. First, this pathway is specifically associated with a starved state; taste-blind flies execute food-choice behavior after prolonged food deprivation of between 10 and 15 h of starvation. This time frame coincides with the onset of starvation-induced sleep suppression (12), indicating that these two behaviors might share a common metabolic trigger. Second, the taste-independent pathway operates on a different timescale from the gustatory pathway. Whereas WT flies made a food choice almost instantly, taste-blind flies chose sugars only after the ingestion of food. Third, this pathway responds to the nutritional content of sugars, but not to their orosensory value. Taste-blind flies chose metabolizable sugars over nonmetabolizable sugars and never consumed nonmetabolizable sugars. Furthermore, the fact that WT flies failed to distinguish a metabolizable sugar from a nonmetabolizable sugar, but shifted their preference to the metabolizable sugar after starvation, indicates that the taste-independent pathway is not an artifact associated with taste-blind flies, but functions in WT flies. Finally, the ability to detect the caloric content of sugars correlated under multiple experimental conditions with drops in hemolymph glycemia.

These results demonstrate that starvation directs the selection of nutrient-rich foods in the fly in the absence of the gustatory cues. Thus, as previously suggested in mice, postingestive cues can drive feeding behavior independently of gustatory information (6, 7). The physiological factors that triggered the taste-independent food choices in mice are, however, unknown. In *Drosophila*, the internal energy state and carbohydrate metabolism play crucial roles in the metabolic sensing of food according to our results. A possible evolutionary purpose of taste-independent metabolic sensing is to ensure that animals select calorie-rich foods to quickly replenish energy, especially in times of food shortage.

How do starved sugar-blind flies preferentially ingest metabolizable sugar over nonmetabolizable sugar? It is plausible that sugar-blind flies are equally attracted to and feed on both sugars,

Fig. 3. The taste-independent sensor responds to metabolizable sugars, but not to nonmetabolizable sugars. (*A*) Molecular structures of sucrose and sucralose (*Upper* panels) and D-glucose and L-glucose (*Lower* panels). (*B*) PERs of *Gr5a; Gr64a*^{1} mutants and *CS* WT flies to 0.3 mM sucralose. *$P < 0.001$ with one-way ANOVA. $n = 37–40$. (*C* and *D*) Two-choice preference assay with (*C*) 0.3 mM sucralose versus plain agar and (*D*) 0.3 mM sucralose versus 100 mM sucrose after 22 h of starvation. An amount of 0.3 mM sucralose induces comparable PER responses to 100 mM sucrose (Fig. 1*E*). $n = 6–8$. (*E*) PERs of *Gr5a; Gr64a*^{1} mutants and *CS* WT flies to 200 mM D-glucose and 200 mM L-glucose. The same concentration of D-glucose and L-glucose leads to similar PER responses in WT flies. *$P < 0.001$ with one-way ANOVA. $n = 22–36$. (*F* and *G*) Two-choice preference assay with (*F*) 200 mM L-glucose versus plain agar and (*G*) 200 mM L-glucose versus 200 mM D-glucose after 22 h of starvation. $n = 4$. (*H*) PI for D-glucose in 5-h (light stippled bars) and 22-h (dark stippled bars) starved flies, which were given a choice between 200 mM L-glucose versus 50 mM D-glucose. Four independent WT strains—*CS*, *Or-R*, *yellow white* (*yw*), and *Harwich*—were used. A PI value of 0.5 indicates that flies equally prefer D- and L-glucose whereas a PI value of 0.5–1 indicates a preference for D-glucose. For the detailed calculation of PI (D-glucose), see *Materials and Methods*. *$P < 0.001$, Student's *t* test within each genotype; $n = 4$ for each trial comprising 50 flies.

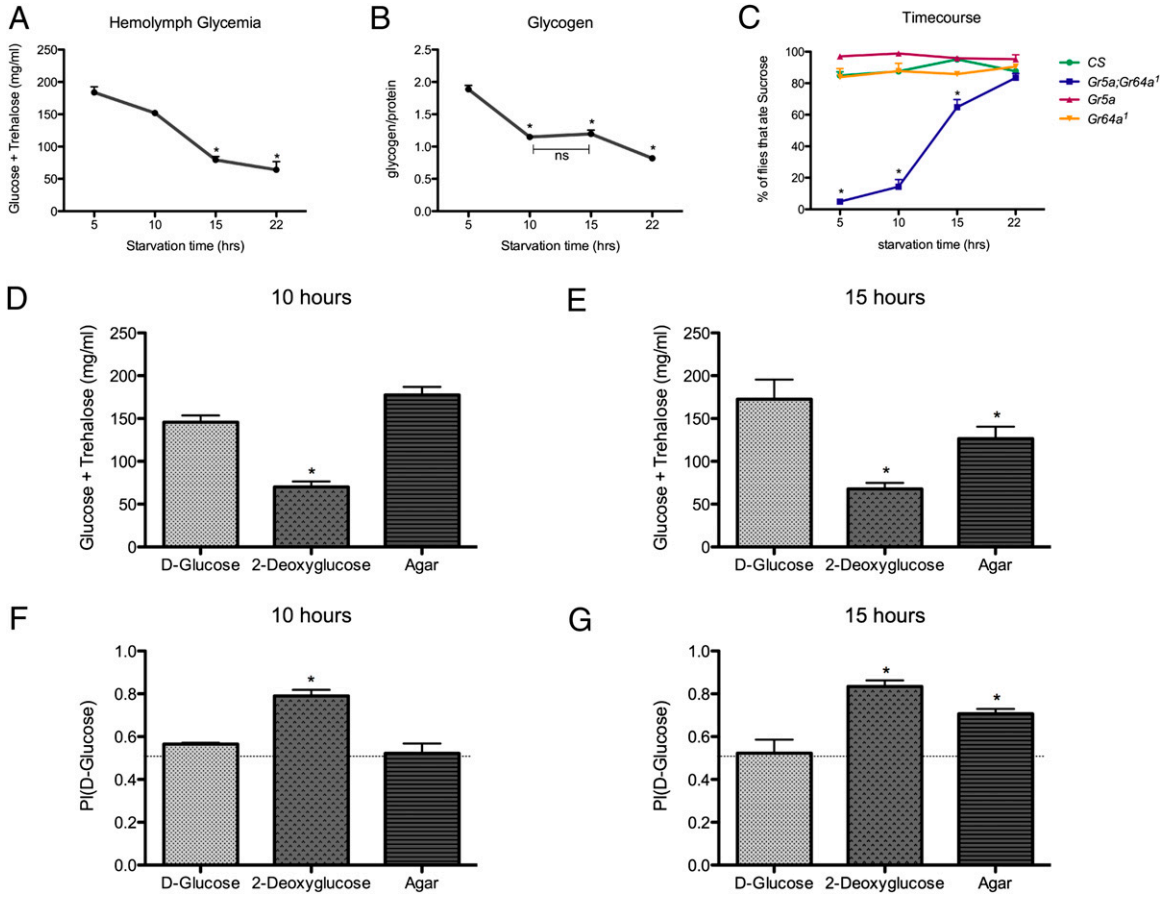


Fig. 4. Changes in the hemolymph glycemia correlate with the timing of the behavioral switch to metabolizable sugars. (*A* and *B*) The levels of (*A*) hemolymph glucose and trehalose and (*B*) glycogen as a function of starvation time. *$P < 0.001$ in comparison with 5-h starvation, one-way ANOVA, Bonferroni post hoc test; $n = 7–10$ for hemolymph glycemia and $n = 9$ for glycogen. (*C*) Time course for the induction of the taste-independent food choice behavior. *Gr5a;Gr64a*^{1}, and controls were given a choice between agar containing 100 mM sucrose versus plain agar after 5, 10, 15, and 22 h of starvation. *Gr5a; Gr64a*^{1} mutants showed a preference for sucrose between 10 and 15 h of starvation. $n = 4–6$. (*D* and *E*) Concentrations of glucose and trehalose in the hemolymph of flies fed 400 mM glucose, 400 mM 2DG, or plain agar after (*D*) 10 h or (*E*) 15 h of starvation. *$P < 0.001$ with one-way ANOVA. $n = 10–12$ for 10 h of starvation and $n = 9–10$ for 15 h of starvation. (*F* and *G*) PI for D-glucose in flies fed 400 mM D-glucose, 400 mM 2DG, and plain agar for (*F*) 10 h and (*G*) 15 h. These flies were then given a choice between D-glucose versus L-glucose. *$P < 0.001$ with one-way ANOVA. $n = 4$.

but those on nonmetabolizable sugar resume foraging because of the lack of nutritional value in this sugar. These foraging flies are again equally attracted to both sugars, but those on nonmetabolizable sugar continue to forage until they find the correct food substrate. Food choice in this model is mediated by random selection and "trapping" of the flies on the metabolizable sugar. Alternatively, sugar-blind flies might readily detect the metabolizable sugar without ingesting a large amount of food because nutrient information is rapidly conveyed to the brain within minutes of ingesting food. In this model, the flies select for metabolizable sugar over nonmetabolizable sugar by a metabolic sensor that operates on a fast timescale to mediate discrimination between the two sugar substrates. Tracking and monitoring the locomotor activity and feeding behavior that generates a preference for metabolizable sugar will address this question.

It is intriguing to speculate on the molecular nature of the metabolic sensor. This sensor could be expressed in a subset of neural, digestive, or other tissues. Among the organs and cells that have been proposed for their involvement in feeding regulation in the fly are the fat body (19–21), the *i*nsulin-*p*roducing *c*ells (IPC) (22), and the corpora cardiaca/allata complex (23). These cells may respond to the metabolic value of sugars in circulation, as seen with the glucose-excited and glucose-inhibited neuropeptide neurons in the arcuate nucleus of the mammalian hypothalamus (24, 25). A model that explains how changes in circulating glucose levels alter the electrical and secretory properties of the hypothalamic glucose-responsive neurons could also describe how metabolizable sugars trigger the metabolic sensor. In mammals, glucose-sensitive cells detect glucose availability by responding to metabolites of glycolytic enzymes such as hexokinase (26, 27) or the energy-sensing AMP-activated protein kinase (28).

Almost all crucial metabolic functions in mammals are also conserved in *Drosophila* (22, 23, 29). During the past decade, researchers using the fruit fly as a model system for studying feeding behaviors and feeding-related disorders, including obesity, have shed much light on the molecular mechanisms of metabolism (29, 30). By revealing the possibility of a metabolic sensing pathway in *Drosophila*, we have introduced the possibility of understanding the molecular mechanism underlying this pathway. Identification of the cellular and genetic nature of this sensor might reveal the identity of the master switch that regulates many hunger-driven behaviors.

Materials and Methods

Feeding Assays. Flies were reared in standard cornmeal medium. Male flies (0–2 d old) in groups of 50 were collected under anesthesia and allowed to recover in standard cornmeal food vials for at least 2 d before experiments. After 4–8 d the male flies were then starved for 5, 10, 15, or 22 h in vials containing 2 mL of milliQ water soaked in Kim-wipe tissue. For two-choice preference assays, the groups of 50 male flies were cold-anesthetized, transferred into 60-well microtiter plates (MicroWell MiniTrays with lids,

Nunc), and allowed to feed for 120 min. The flies were then scored by examining the color of their abdomen. All feeding experiments were conducted at the same time of the day, around zeitgeber time 6–7.

The agar substrate containing sugars was made of 1% agar and was color-labeled with 0.5–0.6% of green and red McCormick tasteless food dyes. Color-labeled 1% agar without sugar did not produce a PER response and did not generate a preference to either dye in WT flies. Preference index for D-glucose was calculated as [(# flies ate D-glucose) + (0.5 × # flies ate both D- and L-glucose)]/(total # flies that fed). Thus, a PI of 0.5 indicates no preference whereas a PI of 0.5–1 indicates a preference. All tested sugars—sucrose, sucralose, D-glucose, L-glucose, fructose, trehalose, galactose, and 2-deoxyglucose at 99% purity—were purchased from Sigma-Aldrich.

Crop and Gut Dissections. Flies subjected to the two-choice preference assay with sucrose versus plain agar were sorted into two groups, "sucrose eating" and "no eating," according to the color of their abdomen. Each group was then dissected. Briefly, a fly was immobilized on a silicon plate using insect pins (Fine Science Tools; #26002–10) and its legs and wings were removed under a dissecting microscope. The cuticle of the thorax and abdomen was peeled off in PBS using fine tweezers (Fine Science Tools; #11251–20) to expose the crop and gut. Images were taken with a digital camera (Canon; Powershot A450).

PER. PER assay was performed according to the protocol of ref. 13 with some modifications. Flies starved for 22 h (in the presence of water) were tested with water before the experiment and only flies that did not respond to water were used. The taste bristles on the labellum or the legs were stimulated by a Kim-wipe thread soaked in tastant solution. PER responses were scored as follows: no extension = 0, half-extension = 0.5, and full extension = 1.

Hemolymph Glycemia Measurement. Hemolymph glucose and trehalose concentrations were measured as previously described (20). Briefly, 10 flies starved on agar or fed 2DG or D-glucose for 5, 10, 15, or 22 h were decapitated and their hemolymph was drawn with a capillary pipette. A total of 0.5 μL of hemolymph was mixed with 100 μL of the Glucose (HK) Assay Kit (Sigma; GAHK20) adjusted to pH 6.8. A total of 10 μL of pig kidney trehalase (Sigma) per 5 mL of glucose reagent was added to the mixture, incubated at 37 °C for 16 h, and measured with a fluorescent plate reader using quantitative NADH fluorescence (excitation wavelength of 375 nm and emission wavelength of 465 nm). Standard curves were generated from D-glucose and trehalose (0–1,000 mg/mL) standards for each trial.

Glycogen Measurement. Total glycogen was measured as previously reported (19). Briefly, 10 male flies were homogenized in 250 μL of lysis buffer [10 mM KH_2PO_4, 1 mM EDTA (pH 7.4)] and centrifuged at 2,000 × *g* for 2 min. A total of 1.5 μL of the supernatant was mixed with 2.5 μL of 0.1 unit/μL amyloglucosidase (Sigma; A1602-25MG) and 221 μL of the peroxidase/glucose oxidase (PGO) enzymes reaction solution. The mixture was incubated for 30 min at 37 °C, and its absorbance was measured at 450 nm. G0885-1G glycogen (Sigma) standard was used to plot a standard curve.

Sleep Analysis. Three- to four-day-old males were placed in *Drosophila* Activity Monitors (Trikinetics) in tubes containing fly food. Following 24 h of baseline recording, flies were transferred to agar containing 100 mM sucrose (or 5% sucrose for Fig. S1 *F* and *G*), agar containing 0.3 mM sucralose, plain agar, or left in regular fly food (Fig. S1 *F* and *G*) (12). Sleep was monitored continuously for 2–5 d and analyzed using an Excel-based macro as previously described (31).

Statistical Analysis. The results from the two-choice preference assay were analyzed by using two-way ANOVA that compared genotype and food choice for parametrically distributed data. For the results from the PER assay and for the glycemia and glycogen measurements, one-way ANOVA was used. Following ANOVA analysis, we used the Bonferroni post hoc test to determine significances. When only two groups were compared, we performed Student's *t* test.

Note. While our manuscript was under revision, two papers claiming that *Drosophila* can learn to associate an odorant paired with nutritious food were published in *Current Biology* (32, 33).

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