High Dietary Sugar Reshapes Sweet Taste to Promote Feeding Behavior in *Drosophila melanogaster*

Highlight:
- A high sugar diet decreases sweet taste sensation
- Blunted sweet taste promotes overconsumption and obesity
- Excess dietary sugar impairs taste function via the enzyme O-GlcNAc Transferase
- Correcting fruit flies' sweet taste function protects them from diet-induced obesity

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In Brief
May et al. discover that excess dietary sugar promotes overfeeding by dulling sweet taste sensation in *Drosophila melanogaster*. Deficits in taste function occur independently of obesity and, instead, develop because of higher glucose utilization inside the gustatory neurons. Correcting sweet taste function prevents overconsumption and obesity in animals fed a high sugar diet.
High Dietary Sugar Reshapes Sweet Taste to Promote Feeding Behavior in Drosophila melanogaster

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https://doi.org/10.1016/j.celrep.2019.04.027

SUMMARY

Recent studies find that sugar tastes less intense to humans with obesity, but whether this sensory change is a cause or a consequence of obesity is unclear. To tackle this question, we study the effects of a high sugar diet on sweet sensation and feeding behavior in Drosophila melanogaster. On this diet, fruit flies have lower taste responses to sweet stimuli, overconsume food, and develop obesity. Excess dietary sugar, but not obesity or dietary sweetness alone, caused taste deficits and overeating via the cell-autonomous action of the sugar sensor O-linked N-Acetylglucosamine (O-GlcNAc) transferase (OGT) in the sweet-sensing neurons. Correcting taste deficits by manipulating the excitability of the sweet gustatory neurons or the levels of OGT protected animals from diet-induced obesity. Our work demonstrates that the reshaping of sweet taste sensation by excess dietary sugar drives obesity and highlights the role of glucose metabolism in neural activity and behavior.

INTRODUCTION

Many arguments about the underlying cause in the rise of obesity point toward the increased availability of highly palatable foods. Such foods are thought to alter the activity of reward pathways at least partly via their taste properties, and this leads to overconsumption and weight gain (Small, 2009; Volkow et al., 2011). No doubt the perception of palatable food qualities such as sweetness plays a key role in eating behaviors. However, this hypothesis does not fit with a growing body of evidence that associates obesity with reduced taste perceptions (Bartoshuk et al., 2006; Berthoud and Zheng, 2012; Rodin et al., 1976). Specifically, obesity has been associated with lower sweetness intensity (Bartoshuk et al., 2006; Overberg et al., 2012; Sartor et al., 2011) and sensitivity to sweet (Proserpio et al., 2016), umami (Pepino et al., 2010), monosodium glutamate (MSG; Donaldson et al., 2009), and salt (Simchen et al., 2006; Skrandies and Zschieschang, 2015). However, other studies reported no or opposite associations between BMI and taste sensitivity (Donaldson et al., 2009; Grinker, 1978; Hardikar et al., 2017; Thompson et al., 1977). Recently, research on rodents found that animals genetically prone to obesity or fed high energy diets have decreased behavioral (Berthoud and Zheng, 2012; Chevrot et al., 2013; Robinson et al., 2015) and physiological responses of the taste buds to sweet or fatty stimuli (Malphlou et al., 2013; Ozdener et al., 2014), changes in the number of taste buds (Kaufman et al., 2018), and lower expression of the sweet taste receptors (Chen et al., 2010), but these changes were not causally linked to taste function and feeding behavior. Thus, while there is accumulating evidence that taste signals are dulled in obese mammals, the picture is complex, and studies in model organisms with a simpler taste system and conserved metabolism would be greatly beneficial in probing the connection between taste function, feeding behavior, and obesity. Here, we exploited the relative simplicity of the Drosophila taste system, where the sweet-sensing cells are neurons that project directly to the brain, to tackle a number of important questions. First, do changes in taste sensation occur with diet-induced obesity? Are these changes a consequence of the altered physiology of the obese state or do they result from chronic exposure to a high nutrient diet? And, finally, if changes in taste function occur, what role do they play in the etiology of obesity?

Using behavioral assays and in vivo imaging, we found that fruit flies fed a high sugar diet show a dulled sense of sweet taste, and that this occurs because of lower responses of the sweet
taste neurons to sugar. This deficit is caused by excess dietary sugar, not obesity, and is mediated by the increased activity of the conserved sugar sensor O-linked N-Acetylglucosamine (O-GlcNAc) Transferase (OGT) (Hanover et al., 2010; Hardivillé and Hart, 2014) in the sweet taste cells. By monitoring feeding behavior at high resolution and using neuro- and optogenetic manipulations of sweet taste cell excitability, we show the dulling of sweet taste leads to overfeeding and obesity. Preventing a decrease in sweet taste sensation rescues feeding and obesity in animals exposed to the high sugar diet. Together, our results implicate deficits in sweet taste as drivers of obesity and begin to map the molecular underpinnings through which exposure to excess dietary sugar reshapes taste function and behavior.

RESULTS

A Sugar Diet Promotes a Reduction in Sweet Taste Responses Independently of Obesity

Recent reports found that humans with obesity and rodents fed highly palatable diets have a dulled sense of sweet taste (Bar-toshuk et al., 2006; Berthoud and Zheng, 2012; Overberg et al., 2012; Pasquet et al., 2007; Proserpio et al., 2016; Sartor et al., 2011). However, it is unclear whether this reduction is a metabolic consequence of obesity or an effect of diet. To address this question, we fed Drosophila melanogaster fruit flies an established model of a high sugar diet (Musselman et al., 2011; Musselman and Kühnlein, 2018) and assessed their taste responses to sweet stimuli (see STAR Methods for dietary manipulations). Fruit flies fed a 30% sucrose diet for several weeks develop obesity, metabolic syndrome, peripheral insulin resistance, and recapitulate the hallmarks of kidney and heart disease in their corresponding organs (Musselman et al., 2011; Musselman and Kühnlein, 2018; Na et al., 2013). In contrast, short, up to 1-week exposures to the high sugar diet (SD; 1.4 calories/gram), lead to fat accumulation compared to animals on a control diet (CD; 0.58 calories/gram), (Figures 1A and S1A), but have no effect on Drosophila insulin-like peptide (dilp) transcript levels (Figures S2A and S2B). The 30% sugar concentration in the diet is similar to that found in many cookies available at grocery stores; for comparison, mango and banana contain about ~15% sucrose, while the majority of children’s cereal in US grocery stores has 45%–60% sugar content (Ng et al., 2012).

In Drosophila, taste cells are neurons that sense the environment through taste hairs located on the labellum at the tip of the proboscis, the main taste organ in the fly (see Figure S3B for a schematic of the anatomy). Taste neurons, which express receptors for only one taste modality, send their projections to the fly brain, where taste modalities remain segregated (Harris et al., 2015; Marella et al., 2006; Scott, 2018). We examined fly taste responses using the Proboscis Extension Response (PER) assay, a behavioral measure of taste that records the magnitude of proboscis extension in response to stimulation of the taste hairs with a sweet stimulus (Shira-iwa and Carlson, 2007). Age-matched flies fed a SD showed a rapid and progressive decrease in taste responses to supra-threshold (30%, 5%, and 1%) concentrations of sucrose with time (Figure 1B). In flies, gustatory receptor neurons are also located in the legs and wings, and a SD also reduced proboscis responses induced by the stimulation of the leg sensory cells (Figure S1B). The decrease in taste responses was not due to motor defects because proboscis responses to the fatty acid octanoic acid (Masek and Keene, 2013) were unchanged between flies on the two diets (Figure S1C). Furthermore, taste deficits occurred regardless of fasting time (Figure S1D), ruling out the possibility that they are a consequence of higher energy stores in flies fed a SD. Thus, flies fed a SD have lower behavioral responses to supra-threshold concentrations of sucrose.

Figure 1. A High Sugar Diet Decreases Sweet Taste Sensation

(A) Triglyceride levels normalized to protein in age-matched male w1118CS flies on a control (salmon) or 30% sucrose diet (burgundy) for 2, 5, 7, or 10 days. n = 24, one-way ANOVA with Dunnett’s test, comparisons to control diet.

(B) Taste responses measured by the Proboscis Extension Response (PER) to the stimulation of the labellum with 1%, 5%, and 30% sucrose (right y axis, shades of blue) in age-matched male w1118CS flies fed a control (circles) or 30% sugar (squares) diet over 10 days. n = 24–61, Kruskal-Wallis with Dunn’s test, comparisons to control diet.

(C) Taste responses to 1%, 5%, and 30% sucrose stimulation (x axis) of the labellum in w1118CS flies fed a control, sucrose, lard, or saccharose diet for 7 days. n = 22–28, Wilcoxon matched-pairs signed rank test, comparisons to control diet response.

(D) Taste responses to 1%, 5%, and 30% sucrose stimulation (x axis) of the labellum in w1118CS flies fed diets supplemented with 30% fructose, 30% glucose, or a control diet for 7 days. n = 24–28, two-way ANOVA with Fisher’s LSD test, comparisons to control diet for each concentration.

All data shown as mean ± SEM, ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 for all panels unless indicated.

See also Figure S1.
To determine if a SD diet also alters the thresholds for detection of sweetness, we counted the percent of animals able to detect the non-caloric sweetener L-glucose at different concentrations in the range of 10–90 mM. We used L-glucose instead of other sweet sugars to eliminate any potential post-ingestive effects on food detection, since animals with impaired taste can still detect the presence of nutritious sugars (de Araujo et al., 2008; Dus et al., 2011; Stafford et al., 2012); the taste of L-glucose is transduced through the same cellular and molecular machinery as sucrose (Dus et al., 2013; Fujita and Tanimura, 2011). Compared to flies on a CD, animals fed a SD had a taste detection curve shifted to higher concentrations of the sweetener, suggesting that their sensitivity to sweetness was also lower (Figure S1E).

To probe whether taste deficits were due to high dietary sweetness, we examined the taste responses of animals fed a sweet, non-caloric sucralose diet. However, taste responses to sucrose remained unchanged in these flies (Figure 1C, dark green) and there was no fat accumulation (Figure S1F). Similarly, flies fed a calorically dense (1.4 calories/gram as the 30% high sugar diet), but not sweet, lard-supplemented diet accumulated fat (Figure S1G, lime green), but had normal taste responses (Figure 1C), indicating that sweetness or excessive calories alone are insufficient to lower sweet taste sensation. In contrast, only sweet and nutritious diets such as those supplemented with D-fructose, D-glucose, and sucrose promoted a decrease in sweet taste responses (Figure 1D).

In mammals, the molecular mechanisms through which diet-induced obesity lowers taste sensation are unknown. To test whether there is a connection between taste deficits and obesity, we set out to genetically uncouple excess body fat from dietary sugar exposure. First, we tested the taste responses of fly mutants for the adipose triglyceride lipase brummer (bmm), which is involved in the breakdown of fat (Grönke et al., 2005) (Figure 2A). The bmm mutants have as much body fat on a control diet as wild-type flies on a SD (Figure 2B), but their taste responses as measured by PER were normal on a CD and reduced on a SD (Figure 2C), suggesting that obesity alone is not sufficient to promote a reduction in sweet taste. This is consistent with our observation that a lard diet had no effect on sweet taste (Figures 1Ca and S1G). Next, we tested genetically lean flies to ask if a decrease in taste responses was linked to high dietary sugar, instead of obesity. perilipin2 (plin2) is a gene essential for fat mobilization (Beller et al., 2010) (Figure 2A); despite remaining lean (Figure 2B) and maintaining normal Drosophila insulin-like peptide transcript levels on a SD (Figures S2A and S2B), plin2 mutants experienced a comparable decrease in taste responses relative to that of control and bmm mutant flies (Figure 2C). These results suggest that obesity is neither necessary nor sufficient for the reduction in sweet taste, and that, instead, excess dietary sugar—but not just dietary sweetness, since a sweet sucralose diet did not dull sweet taste—may alter taste directly.

**Figure 2. A Sugar Diet Decreases Taste Sensation Independently of Obesity**

(A) Overview of the function of the ATG-lipase brummer (bmm) and perilipin2 (plin2) in lipid homeostasis.

(B) Triglyceride levels normalized to protein in age-matched male w^{11CS} (control), bmm^{+/--}, and plin2^{+/--} flies on control or 30% sugar diet for 7 days. n = 8–16, two-way ANOVA with Sidak’s test, comparisons to control diet.

(C) Taste responses to 1%, 5%, and 30% sucrose (x axis) of age-matched male w^{11CS}, bmm^{+/--}, and plin2^{+/--} flies on control (circles) or sugar (squares) diet for 7 days. n = 26–56, multiple t tests, comparisons to control diet.

All data shown as mean ± SEM, ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 for all panels unless indicated. See also Figure S2.
flies fed a control diet (day 0) or a sugar diet for 1, 3, and 7 days, before and after (arrow) stimulation of the proboscis with 30% sucrose. n = 26 brains, Kruskal-Wallis with uncorrected Dunn’s, comparisons to control diet (day 0). All data shown as mean ± SEM, ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 for all panels unless indicated.

See also Figure S3.

e.t al., 2006) driving GFP in the labellum of flies on a SD (Figure S3A), we reasoned that a high sugar diet may instead alter the response of these neurons to sugar. To test this possibility, we measured the in vivo, real-time responses of the sweet Gustatory receptor 64f (Gr64f+) (Dahanukar et al., 2007) neurons to stimulation of the labellum with 30% sucrose using the genetically encoded, presynaptic calcium sensor GCaMP6s-Bruchpilot-mCherry (Kiragasi et al., 2017) (Figure 3A). Presynaptic responses to 30% sucrose stimulation of the proboscis were identical after 1-day exposure to the SD, but decreased gradually with longer exposures (Figures 3B and 3C), which matched the magnitude and progression of sweet taste deficits as measured by PER (Figure 1B). In addition, we also found that animals had fewer sugar-induced action potentials after both short- and long-term exposure to the SD (Figures S3B and S3C). Thus, exposure to high dietary sugar decreases the responsiveness of the taste neurons to sugar; while we measured both changes in presynaptic calcium responses and action potentials, defects in synaptic activity more faithfully track the decrease in PER.

A Sugar Diet Promotes Feeding by Increasing the Size and Duration of Meals

To analyze the effects of changes in sweet taste function on feeding behavior, we first examined the effects of excess dietary sugar on feeding behavior using modifications to the Fly-to-Liquid-Food-Interaction Counter (FLIC) (Ro et al., 2014), an assay that measures feeding behaviors by detecting electronic signals, “licks,” when the fly proboscis touches the food. By attaching food reservoirs to the FLIC apparatus, we were able to record the feeding patterns of individual flies continuously and at a high temporal resolution (5 Hz) for days without disturbance or fasting. Because 30% sucrose was viscous, we conducted the experiments with 20% sucrose, which also promotes obesity and taste deficits (Figures S4A and S4B). Control w1118CS flies fed a 5% sucrose control diet while on the FLIC showed a stable number of licks per day (Figure 4A, salmon). In contrast, flies fed a sweeter diet of 20% sucrose, showed a progressive increase in licks over time; by days 3–5 these flies licked more per day than those fed 5% sucrose (Figure 4A, burgundy). Flies fed a CD or SD in standard fly food vials for 10 days and placed on the FLIC for a single day also showed an increase in licks on 20% sucrose (Figure S4C). To better characterize the temporal dynamics and investigate the effect of diet on meal patterns, we binned licks in 30-min intervals over many days for individual flies (Figure 4B) and as a group average (Figure 4C). We found that flies, like mammals, eat in discrete patterns, here termed “meals,” that closely follow circadian activity (Figures 4B and 4C). Flies on 20% sucrose still consumed only two meals per day, but the peaks became higher and wider with more time on the diet (Figure 4C). Furthermore, while the onset of each morning (a.m.) and evening (p.m.) meal was similar to that of flies on 5% sucrose, the offset changed with more days on diet, suggesting that meals became longer while flies are eating 20% sucrose (Figure 4C). To quantify meal duration, we measured the time of meal start and end for a.m. and p.m. meals for each fly (Figure 4D). The duration of the a.m. and p.m. meals of flies on 5% sucrose remained the same over 7 days (Figure 4E). In contrast, that of flies eating 20% sucrose became longer with more days on diet. By day 7 the meal duration of flies eating 20% sucrose was twice as long as that of flies eating 5% sucrose (Figure 4E). We next quantified the size of each meal by calculating the area under each a.m. and p.m. meal peak (Figure 4D, gray). Like with duration, the size of each a.m. and p.m. meal increased with more days on a 20% diet, while it stayed unchanged in flies eating 5% sucrose (Figure 4F). Overall, we observed a strong relationship between meal size and duration (Figures 4E and 4F), indicating that longer meals may contribute to increased meal size. Together, our high-resolution analysis of meal patterns suggests that a high sugar diet alters feeding by extending the duration and size of each meal, rather than by increasing the number of meals per day, which points to potential changes in satiety rather than hunger.

To investigate if these alterations in feeding behavior are a consequence of diet-induced obesity (Figures 1A and 1B) or possibly a result of high dietary sugar, we tested the feeding patterns of genetically obese bmm and genetically lean plin2 mutant flies (Grönke et al., 2005; Beller et al., 2010). The bmm mutants, despite being as obese as control flies on a SD (Figure 2B), showed similar patterns of feeding behavior with diets as control flies (Figures 5A, 5C, 5E, and S5A–S5C for controls). In contrast, 20% sucrose led to an increase in meal size and duration even in the absence of obesity in the plin2 mutant flies (Figures 5B, 5D, 5F, and S5A–S5C for controls). These data suggest that obesity alone does not drive the observed changes in feeding and open the possibility...
that, instead, these are a direct consequence of dietary sugar and linked to changes in sweet taste sensation.

**A Deficit in Sweet Taste Sensation Promotes Feeding Behavior**
Consumption of a high sugar diet promotes a decrease in the responses of sweet taste neurons to sugar and an increase in feeding. Are these phenomena linked and does a dulling of sweet taste sensation contribute to overfeeding? If diet-dependent deficits in sweet taste drive higher feeding behavior, then preventing animals from experiencing these should rescue overeating and obesity. To test this possibility, we expressed the bacterial voltage-gated sodium channel NaChBac, which is used to activate neurons in *Drosophila* (Nitabach et al., 2006), exclusively in the sweet taste neurons using Gr64f-GAL4 (Fujii et al., 2015) and assayed taste responses and feeding behavior. The taste responses of Gr64f>NaChBac flies were identical to those of genetic controls on a CD (Figure 6A).

Figure 4. Flies Fed a High Sugar Diet Show Increased Feeding Behavior, Meal Size, and Duration
(A) Average licks per day of age-matched w1118CS male flies feeding continuously on 5% (salmon) or 20% (burgundy) sucrose on the FLIC. n = 26–72, two-way ANOVA with Fisher’s LSD, compared to same-day 5% sucrose licks.
(B) Heatmap of the licks binned by 30 minutes of individual flies (left y axis) feeding continuously on 5% or 20% sucrose on the FLIC at days 1, 2, 5, and 7. The x axis represents time in 24 h, time 0 indicates midnight (Zeitgeber time 17 [ZT17]).
(C) Meal patterns quantified as average licks binned by 30 min for flies feeding on 5% or 20% sucrose on selected days 1, 2, 5, or 7 (from A). The x axis is as in (B).
(D) Schematic for how meal duration and size were determined for morning (a.m.) and evening (p.m.) meals.
(E and F) The meal (E) duration (h) and (F) size in licks of the morning (a.m.) and evening (p.m.) meals of flies feeding on 5% or 20% sucrose on day 1 (solid bars), day 5 (spotted bars), and day 7 (hatched bars). n = 23–65, two-way ANOVA with Fisher’s LSD, comparisons to same-diet day-1 meal-time duration or size. All data shown as mean ± SEM, ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 for all panels unless indicated.
See also Figure S4.
However, while control animals experienced a decrease in sweet taste when fed the SD, Gr64f>NaChBac flies retained the same taste responses to sucrose on both a CD and a SD (Figure 6A; 20% and 30% sucrose stimuli). Since expression of UAS-NaChBac in the Gr64f+ neurons corrected taste deficits so that Gr64f>NaChBac animals do not experience a sugar-diet-dependent decrease in taste function, we next measured their feeding patterns. Gr64f>NaChBac flies fed 20% sucrose showed little to no increase in feeding interactions compared to controls (Figure 6B). In addition, while the meals of control flies became longer, those of Gr64f>NaChBac animals stayed the same (Figure 6C). Consistent with these data, Gr64f>NaChBac flies also remained lean on the SD, while control flies accumulated fat (Figures 6D and S5 for adult-specific NaChBac expression). Thus, preventing animals from experiencing a diet-dependent decrease in sweet taste sensation rescued feeding behavior and obesity.

To further test the hypothesis that a decrease in the activity of the sweet taste neurons drives overeating, we used optogenetics to acutely activate the Gr64f+ neurons by expressing the light-activated channel csChrimson (Klapoetke et al., 2014). To ensure that the sweet taste neurons were activated only during feeding, we developed a closed-loop system so that the animals received light stimulation only upon eating. Feeding-initiated light stimulation of the sweet taste neurons in Gr64f>csChrimson animals fed retinal prevented overconsumption compared to Gr64f>csChrimson flies without retinal treatment (Figure 6E) or csChrimson flies without the GAL4 (Figure S5E). Thus, acute, feeding-dependent activation of the sweet-tasting cells prevented overeating. Together, these experiments argue that a sugar-diet-dependent decrease in sweet taste function increases feeding behavior and promotes obesity.

**The Sugar Sensor OGT Mediates the Effects of Sugar on Sweet Taste Sensation**

We next probed how dietary sugar alters sweet taste sensation. Our observations that a diet supplemented with the non-caloric sweetener sucralose or a non-sweet, high-fat diet did not lower sweet taste sensation (Figure 1C), while diets supplemented with 30% sucrose, D-fructose, or D-glucose decreased sweet taste responses, suggest that glucose metabolism plays a role in reducing sweet taste sensation (Figure 1D). The hexosamine biosynthesis pathway (HBP) is a conserved nutrient-sensing signaling pathway that mediates the deleterious effects of dietary sugar on cell physiology and has been implicated in most diseases caused by high-nutrient diets, such as diabetes, kidney, heart, and liver diseases (Hanover et al., 2010; Hardivillé and Hart, 2014). The levels of the metabolic end product of HBP, uridine diphosphate N-acetylglucosamine (UDP-GlcNac), are increased by high nutrient diets; a single enzyme, OGT (also known as super sex combs in Drosophila), adds the O-GlcNAc moiety from UDP-GlcNAc onto the serine and threonine residues of proteins to modify their activity, competing with protein phosphorylation (Hanover et al., 2010; Hardivillé and...
Hart, 2014) (see Figure S6A for schematic of the HBP). Recent data suggest that OGT may also play a role in the brain: OGT activity is high in neurons and regulates metabolism and synaptic maturation (Lagerlöf et al., 2016, 2017; Ruan et al., 2014). In fly heads, the levels of the first committed HBP metabolite glucosamine-6-phosphate were increased with a SD (Figure S6B). To test if OGT mediates the molecular effects of a SD on sweet taste, we used a previously characterized OGT RNAi transgene (Radermacher et al., 2014) to knock it down in the Gr64f+ neurons (50% knockdown efficiency; Figure S6C).

Gr64f>OGT knockdown (KD) animals had normal PER on a CD (Figure S6D), but this manipulation rescued sweet taste responses on a SD compared to controls (Figures 7A and S6E for a second independent OGT RNAi transgene).

Since correcting a decrease in sweet taste sensation by expression of NaChBac and activating the sweet taste cells with csChrimson prevented increased feeding and obesity (Figure 6), we asked if OGT KD could also restore feeding behavior. Indeed, KD of OGT in the Gr64f+ cells prevented feeding on 20% sucrose (Figure 7B); consistent with its effect on feeding, Gr64f+ > OGT KD animals remained lean compared to genetic control flies (Figure 7C). Thus, decreasing OGT activity solely in the sweet taste neurons blocked the effects of a sugar diet on taste responses, feeding behavior, and obesity.

OGT integrates cell physiology and nutrient environment by altering transcriptional and signaling pathways (Hanover et al., 2010; Hardiville and Hart, 2014). To identify the cellular processes through which excess dietary sugar decreases sweet taste function, we measured changes in RNA abundance in the labellum of flies fed a control (CD, circles) or sugar (SD, squares) diet for 7 days. n = 35–54, multiple t tests, comparisons to control diet.

Figure 6. Restoring Sweet Taste Sensation and Optogenetic Activation of the Gr64f+ Neurons Protects Animals from Diet-Induced Obesity

(A) Taste responses to 5%, 20%, and 30% sucrose stimulation (x axis) of the labellum in Gr64f->NaChBac (teal) and parental transgenic controls flies (gray, crossed to w1118CS) fed a control (CD, circles) or sugar (SD, squares) diet for 7 days. n = 35–54, multiple t tests, comparisons to control diet.

(B) Representative average licks per day of Gr64f->NaChBac (teal) and parental transgenic controls (gray) flies feeding continuously on 20% sucrose on the FLIC. n = 10–17, two-way ANOVA with uncorrected Fisher’s LSD, comparisons to each control genotype per day.

(C) Quantification of the average meal duration (both a.m. and p.m.) of Gr64f->NaChBac (teal) and parental transgenic controls (gray, crossed to w1118CS) flies feeding on 20% sucrose on day 1 (solid bars) and day 7 (hatched bars) on the FLIC. n = 56–90, two-way ANOVA with uncorrected Fisher’s LSD, comparisons to day-1 duration.

(D) Triglyceride levels normalized to protein in Gr64f->NaChBac (teal) and parental transgenic controls (gray, crossed to w1118CS) flies after feeding on a control (lighter-colored bars) or sugar (darker-colored bars) diet for 7 days. n = 15–16, two-way ANOVA with Sidak’s test, comparisons to control diet per each genotype.

(E) Average licks per day of Gr64f->csChrimson flies feeding on 20% sucrose with (fuchsia) or without (gray) all-trans-retinal pretreatment during closed loop, feeding-initiated 60-Hz red light pulse stimulation. n = 6–9, multiple t test with Holm-Sidak correction, comparisons per day to no-retinal condition.

All data shown as mean ± SEM, ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05 for all panels unless indicated.

See also Figure S5.
only the set of genes showing significant changes in labellar expression between CD and SD (q < 0.2), and then conditioning on membership in that set, calculated FDR-corrected p values for the significance of changes in transcript level for the same genes between the OGT KD animals and their corresponding GAL4 control. Genes showing significant expression changes in both RNA-seq experiments were classified based on the signs of the observed log2-fold changes. Using this approach, we found about ~150 genes changed by diet and “reverted” in Gr64f>OGT KD (Figure 7D). We used iPAGE, a pathway discovery program (Goodarzi et al., 2009), to identify Gene Ontology (GO) terms showing significant mutual information with the expression status of genes as being in either one of the two oppositely regulated categories (up or down or “reverting”: Figure 7E). GO terms altered by a SD and reversed by OGT KD were enriched in processes involved in neural function (regulation of membrane potential, phototransduction, neurotransmitter transporter activity) and metabolism (glutaminase activity, chitin bolic process, carbonate dehydratase activity). Combining these results on gene expression with the targeted behavioral experiments above, we propose a model where excess dietary sugar, through the cell-autonomous action of OGT, leads to a decrease in the responses of the sweet taste cells to sugar, which lowers sweet taste sensation (Figure 7F). This weakening of sweet taste alters feeding patterns to promote obesity, providing a mechanism for how excess dietary sugar functions as a driver of obesity.

**DISCUSSION**

Obesity has been linked to the high availability of affordable, tasty foods that contain sugar as a food additive (Small, 2009; Volkow et al., 2011). The increased eating in the presence of these palatable foods seems to be an evolutionarily conserved
behavior from flies to rodents and humans (Avena et al., 2008; Small, 2009). How these foods promote eating is still an open question with obvious public health implications. Changes in taste sensation with dietary sugar or obesity have been examined in humans, but no consensus has been reached on their role in feeding behavior and obesity (Bartoshuk et al., 2006; Berthoud and Zheng, 2012; Grinker, 1978; Hardikar et al., 2017; Overberg et al., 2012; Pasquet et al., 2007; Proserpio et al., 2016; Sartor et al., 2011; Thompson et al., 1977). Studies in rodent models found changes in behavior and physiology consistent with a decrease in taste function with diet-induced obesity, but did not draw a causal connection between the two (Chevrot et al., 2013; Kaufman et al., 2018; Maliphol et al., 2013; Ozdener et al., 2014; Robinson et al., 2015). Here, we show in fruit flies that excess dietary sugar, independently of obesity, causes a decrease in sweet taste function because of lower responses of the taste cells to sugar stimuli—similar to what was observed in the isolated taste buds of mice fed a high-fat diet (Maliphol et al., 2013). This dulling, in turn, promotes eating and obesity by increasing the duration and size of meals. Correcting taste deficits by activating the sweet-sensing cells so that the animals do not experience a lowering of their sweet taste world prevented overeating and obesity, drawing a causal link between diet-induced changes in taste function and obesity.

Diet composition is well known to change sensory perceptions (Hill, 2004). For example, high dietary sodium alters the intensity for salt perception in humans (Bertino et al., 1982; Huggins et al., 1992) and rodents (Contreras and Frank, 1979; Hill et al., 1986) and this promotes higher sodium intake (Bertino et al., 1982; Huggins et al., 1992). Exposure to savory or bitter foods in development or adulthood also alters taste preference across species, from humans (Mennella and Trabulsi, 2012), to mice (Ackroff et al., 2012), to invertebrates like Manduca and Drosophila (Glendinning et al., 2001; Zhang et al., 2013). Here, we show that, as with high dietary sodium, in flies excess dietary sugar decreases sweet taste sensation and promotes overconsumption. How this occurs remains an open question, and raises the intriguing possibility that the dulling of sweet taste may contribute to changes in the central reward processing of food observed in humans with obesity (Kroemer and Small, 2016).

An exciting finding from our work is the role of glucose metabolism in altering neural activity and behavior. While our experiments do not exclude the possibility that peripheral insulin resistance may also play a role in taste changes, they highlight the role of the enzyme OGT as a potential modulator of sweet taste neuron function. OGT activity was recently reported to modulate synapse maturation and behavior (Lagerlöf et al., 2016, 2017; Ruan et al., 2014). There are a few examples where metabolic sensors have been implicated in the modulation of neural activity, such as target of rapamycin (TOR) and eukaryotic translation elongation factor 2 (eEF2) (Davis, 2013), raising the interesting question of whether OGT may also function in a similar manner in the taste neurons. While the exact molecular mechanisms by which OGT mediates the effects of excess dietary sugar on sweet taste neuron physiology remain to be understood, our analysis indicates that OGT alters the expression of genes involved in neural function and metabolism. Given the conservation of OGT function from flies to humans, and the role of OGT in the etiology of obesity and diabetes (Hanover et al., 2010; Hardivillé and Hart, 2014), our findings raise the exciting possibility that increased OGT activity may act to dull taste function in response to excess dietary sugar in mammals. Finally, our work also brings to light the broader question of how diet may impact brain physiology and behavior through its action on metabolic pathways and their by-products.

Together, the identification of the neural and molecular underpinnings of diet-induced alterations in taste promises an avenue of investigation that is broadly relevant to understanding the etiology of obesity in humans. Based on our work and available human and rodent studies, the development of public health or therapeutic solutions that seek to correct dietary sweetness and the weakening of taste sensation may help curb the spread of obesity and reduce the risk of chronic disease.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2019.04.027.
ACKNOWLEDGMENTS

We thank Josie Clowney, Marco Gallio, Leslie Vossahl, and Randy Seeley for comments and suggestions on the manuscript. We also thank all the researchers that shared protocols and fly lines (listed in the STAR Methods) with us. We thank Caleb Vogt and Katty Wu for their help with some QAGs experiments and Jenna Persons for collecting samples for metabolomics. Katherine Hoffman wrote the FLIC meal patterns code. Julia Kuhn designed the graphical abstract and some of the graphics for the manuscript. This work was funded by NIH R01 DK-97141 and NIH 1DP2DK-113750, an NARSAD Young Investigator Award, the Klingenstein-Simons Fellowship in the Neurosciences, and the Rita Allen Foundation (to M.D.), by Training Grant T32-GM008322 (to C.E.M.), and NSF GRFP DGE 1256260 (to K.J.H.).

AUTHOR CONTRIBUTIONS

C.E.M. performed FLIC, TAGs, Nile red, taste sensitivity, and calcium imaging experiments. A.V. performed PER, qPCR, and the OGT RNA-seq experiments. M.D. performed PER experiments. O.G. quantified taste cells in the proboscis and collected the RNA-seq data from control and sugar diet proboscis. P.L.F. and M.K. analyzed the RNA-seq data. Y.Q.L. and Q.-P.W. performed the sensilla recordings. C.E.M., K.J.H., and S.D.P. developed the optoFLIC. C.E.M., A.V., and M.D. designed the experiments, wrote the manuscript, and prepared the figures with input from the other authors. M.D. supervised the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 16, 2018
Revised: March 14, 2019
Accepted: April 3, 2019
Published: May 7, 2019

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| L-glucose | Carbosynth | MG05247, CAS: 921-60-8 |
| all-trans-retinal | Sigma-Aldrich | R2500-100MG, CAS: 116-31-4 |
| **Critical Commercial Assays** | | |
| Pierce BCA Protein Assay Kit | Thermo Scientific | Cat. #23225 |
| Triglyceride LiquiColor Test (Enzymatic) | Stanbio | Ref. #2100-430 |
| **Deposited Data** | | |
| RNA sequencing this paper | | Accession #GSE113159 |
| **Experimental Models: Organisms/Strains** | | |
| Drosophila, w[1118]CS | A. Simon | n/a |
| Drosophila, y[plin2][51]/FM7i;P[w+mC] = ActGFPIJM3 or hom or Dp(1;Y) y[+] | R. Kühnlein | RKF610 |
| Drosophila, w[;bnm][1]/TM3,Stb[1] float | R. Kühnlein | SGF529 |
| Drosophila, w[;]: P[w+mC] = Gr64f-GAL4.9.7][S/CyO | H. Amrein | BDSC #57669; Dahanukar et al., 2007 |
| Drosophila, w[;]: P[w+mC] = Gr5a-GAL4.8.5][Dr[1]/TM3, Stb[1] | K. Scott | BDSC #57592; Chyb et al., 2003 |
| Drosophila, w[1118]; P[w+mC] = UAS-GFP.nls]14 | Bloomington | BDSC #4775 |
| Drosophila, y[1] w[;]: P[w+mC] = UAS-NaChBac]2 | M. Nitabach | BDSC #9469; Nitabach et al., 2006 |
| Drosophila, UAST-sxc(Ogt)RNAiCLb38 | C. Lehner | Radermacher et al., 2014 |
| Drosophila, y[1] v[1]; P[y+7.7] v[+1.8] = TRIP.HM03131]attP40 | Bloomington | BDSC #50909 |
| Drosophila, w[1118]; P[w+mC] = UAS-GCaMP6s.brpS.mCherry]2 | Bloomington | BDSC #77131 |
| Drosophila, w[1118]; P[y+17.7] w+mC = 20XUAS-IVS-CsChrimson.mVenus]attP40 | Bloomington | BDSC #55135 |
| **Oligonucleotides** | | |
| Rp49 Forward, ATGCTAAGCTGCGACAAAA | Integrated DNA Technologies | N/A |
| Rp49 Reverse, ACTTTCTGAAACTCCGTG GCC | Integrated DNA Technologies | N/A |
| OGT Forward, CGTCGCGGCGCCCATATATTA | Integrated DNA Technologies | N/A |
| OGT Reverse, CCAATCTAGTAAACCGACTGA | Integrated DNA Technologies | N/A |
| DiPl2 Forward, TCAATCCCTCGAGGTTGTC | Integrated DNA Technologies | N/A |
| DiPl2 Reverse, TGGATGTCACACCCCAAAGATA | Integrated DNA Technologies | N/A |
| DiPl5 Forward, TCCTGATCCGCTCTGCTA | Integrated DNA Technologies | N/A |
| DiPl5 Reverse, TGCTCGTTTGGCGAACATT | Integrated DNA Technologies | N/A |
| **Software and Algorithms** | | |
| Olympus Fluoview FV1200-ASW 4.2 | Olympus Life Science | N/A |
| FLIC Monitor | https://sites.google.com/a/ umich.edu/pletcher-lab/fly-tech | Ro et al., 2014 |
| RStudio | RStudio, Inc. | N/A |
| FLIC analysis R code | https://sites.google.com/a/ umich.edu/pletcher-lab/fly-tech | Ro et al., 2014, this paper |
| Axon pCLAMP 9 Clampfit | Molecular Devices | N/A |
| Imaris | Bitplane | N/A |
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Monica Dus (mdus@umich.edu).

There are no restrictions on reagent sharing to disclose.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

*Drosophila melanogaster*

Flies were grown and maintained on cornmeal food (Bloomington Food B recipe) at 25°C and 45%–55% humidity under a 12:12 hour light-dark cycle (ZT0 at 7 AM) for all experiments. We collected male flies under CO2 anesthesia at day 1-3 after eclosion. After collection, flies were aged for an additional 1-2 days before starting experiments, except in the case of lines carrying RNAi constructs, which were allowed to age with parental controls for an additional 5-6 days to promote expression of the RNAi transgene and knockdown of the target gene. For dietary manipulations, age-matched male flies were placed on either Bloomington Food B or Bloomington Food B supplemented with different sugars (See Dietary manipulations in Method Details) in groups of 30-35 flies. In experiments where flies were fasted (e.g., PER), all flies were food-deprived for 18-24 h. For optogenetic manipulations, flies were maintained on Bloomington cornmeal food supplemented with 200 μM all-trans-retinal for 6 days in the dark. For all manipulations, flies were changed to new food vials every other day.

The GAL4-UAS system was used to express the transgenes of interest in specific neuron subtypes. For each GAL4/UAS cross, transgenic controls were made by crossing the *w1118CS* (gift from A. Simon) to GAL4 or UAS flies, sex-matched to those used in the GAL4/UAS cross. For the complete genotypes of fly lines used in our manuscript, see the Key Resources Table.

GAL4 was expressed in sweet taste neurons by using the *Gr64f* (gift from H. Amrein) or *Gr5a* (gift from K. Scott) promoters. For neuron visualization and cell counting, we used *UAS-nls-GFP* (Bloomington #4775). *UAS-GCaMP6s-Brp-mCherry* (Bloomington #77131) was used for visualization of calcium transients in axon terminals. We used *UAS-NaChBac* (gift from M. Nitabach) and *UAS-csChrimson* (Bloomington #55135) to increase the excitability of sweet taste neurons. Two RNAi lines (RNAi-1 from C. Lehner; RNAi-2 from Bloomington, #50909) were used to knock down expression of *O-GlcNAc transferase (OGT)* in the sweet taste neurons. To uncouple taste deficits from fat accumulation, we compared mutants for *perilipin2* (RKF610) and *brummer* (SGF529) (both gifts from R. Kühnlein) to *w1118CS* flies.

METHOD DETAILS

**Dietary manipulations**

For each diet, the following compounds were mixed into standard cornmeal food (Bloomington Food B recipe) (0.58 calories per gram) by melting, mixing, and pouring new vials as in Musselman et al. (2011) and Na et al. (2013).

- Sugar diet\(^{20}\) = 20% Domino granulated sugar w/v (1.15 calories per gram)
- Sugar diet / Sugar diet\(^{30}\) = 30% Domino granulated sugar w/v (1.41 calories per gram)
- Lard = 10% lard w/v (1.42 calories per gram)
- Sucralose = 0.02% sucralose w/v (this is the concentration found in diet soda and Dus et al. (2011)).
- FLIC diets were made with 5, 20, or 30% w/v D-sucrose (Fisher Scientific) dissolved in milliQ-filtered deionized water with 4 mg/L MgCl\(_2\) (Sigma-Aldrich).

Age-matching of flies on the different diets occurred as in the table below:

| Experimental group | Day (post-eclosion) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|-------------------|---------------------|---|---|---|---|---|---|---|---|---|----|----|
| Control Diet      | eclosion            |   |   |   |   |   |   |   |   |   |    |    |
|                   | collect             |   |   |   |   |   |   |   |   |   |    |    |
|                   | test                |   |   |   |   |   |   |   |   |   |    |    |
| Sugar Diet 2 days | eclosion            |   |   |   |   |   |   |   |   |   |    |    |
|                   | collect             |   |   |   |   |   |   |   |   |   |    |    |
|                   | SD                  |   |   |   |   |   |   |   |   |   |    |    |
|                   | SD                  |   |   |   |   |   |   |   |   |   |    |    |
|                   | test                |   |   |   |   |   |   |   |   |   |    |    |
| Sugar Diet 7 days | eclosion            |   |   |   |   |   |   |   |   |   |    |    |
|                   | collect             |   |   |   |   |   |   |   |   |   |    |    |
|                   | SD                  |   |   |   |   |   |   |   |   |   |    |    |
|                   | SD                  |   |   |   |   |   |   |   |   |   |    |    |
|                   | test                |   |   |   |   |   |   |   |   |   |    |    |

**Triacylglyceride (TAG) Assay**

We assayed total TAGs normalized to total protein in whole flies (described in Tennessen et al. (2014). Following dietary manipulation, male flies were CO\(_2\)-anesthetized and flash frozen. Two flies per biological replicate were homogenized in lysis buffer (140 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.1% Triton-X) containing protease inhibitor (Thermo Scientific) and centrifuged, and the supernatants were frozen at −20°C. For total protein, supernatant and standards were reacted with protein reagent (Thermo Scientific Pierce BCA
Protein Assay) for 30 min at 37°C and the absorbance of 562 nm measured by a Tecan Plate Reader Infinite 200. For TAGs, supernatant and standards were reacted with TAG reagent (Stanbio Triglycerides LiquiColor Test) for 5 min at 37°C, and the absorbance of 500 nm measured by the Tecan Plate Reader.

**Nile Red Staining**
We stained abdominal fat body in male flies following dietary manipulation as described in Tennessen et al. (2014). Nile red stain stock was 10% in DMSO, and the mounting solution diluted stock 1:1000 in 1xPBS with 30% glycerol. Fly abdomens were dissected in 1xPBS by removing intestines and other internal organs, then separating the abdomen and transecting it along the ventral side to expose the subcuticular fat body. This was flattened and transferred to the Nile red mounting solution on slides with coverslips for confocal imaging 30 minutes later. Images were acquired on an Olympus FV1200 with a 543-nm laser.

**Proboscis Extension Response**
On the seventh day of dietary manipulation, vials of 35-40 flies were fasted between ZT8 and ZT10 (ZT0 is 7 AM) in a vial with a Kimwipe dampened with 2 mL of milliQ-filtered deionized (milliQ DI) water. 18-24 hours later, proboscis extension response (PER) testing was performed as in Shiraiwa and Carlson (2007). Flies were anesthetized on ice then placed in the narrow end of a P200 pipette tip. The tip was cut such that the fly could be gently pushed (with the round end of a melted glass capillary tube) to the end of the tip until the fly head showed through, with the legs still trapped within the tip. Once the fly was awake, it was presented with milliQ water on the labellum and allowed to drink. Water and all tastants were delivered manually via a small solution-soaked Kimwipe piece held in a clean pair of forceps. Sucrose solutions were dissolved in milliQ water and presented in order of descending concentration. Each concentration presentation consisted of three successive touches to the proboscis, and the response to each touch was scored. The touches were brief to ensure the fly did not drink any of the sucrose solution. After the three touches of a sucrose solution, the fly was again allowed to drink water, before progressing to the next concentration presentation. Groups of 7-10 flies were tested simultaneously.

**Fly-to-Liquid-food Interaction Counter (FLIC)**
We used the FLIC, described previously in Ro et al. (2014), to measure fly-to-food interactions as an estimate of feeding behavior over many days without fasting or interruption. The FLIC consists of a *Drosophila* Feeding Monitor (DFM, made by the Pletcher laboratory) which communicates fly-to-food interactions to computer software called FLICMonitor via a Master Control Unit (MCU, made by the Pletcher laboratory). For FLIC experiments, flies were CO₂-anesthetized and males were collected 1-3 days after eclosion. They were then allowed to recover on Bloomington Food B for at least one day before starting the FLIC. For a single experiment, all fly ages were within 3 days of each other. To load flies onto the FLIC, we briefly ice-anesthetized them and rapidly aspirated individual flies into arenas with a single food well. Each DFM has 12 food wells in two rows of six, and each row of six wells is supplied by a single food reservoir (cell culture flasks, Biofil). Once all flies for an experiment were loaded into the FLIC, we began the recording of their food interactions.

A fly-to-food interaction on the FLIC occurs when the fly stands on the capacitance pad surrounding the food well and contacts the food with its proboscis, as during feeding, or with its leg, as during tasting. The liquid food is a solution of sucrose (5% for normal diet and 20% for high sugar diet) and 4 mg/L MgCl₂ in milliQ water, and so can carry electrical current. The fly’s connection of the food to the capacitance pad with its body closes a circuit and changes the voltage readout for that well. The signal intensity is sufficient to distinguish tasting from food-intake-related contacts, a.k.a. “licks.” Signal threshold for licks was set to 40 units above calculated baseline, while tasting occurred between 10 and 40. The MCU samples the voltage from all wells every 200 ms.

Long-term FLIC was run in an incubator with a 12-hour light cycle (ZT0 is 7 AM), at 25°C and 35%–50% humidity. (Humidity greater than 60% can affect the baseline signal of the wells.) To correct sugar concentrations as water was lost to evaporation over many days, we added a small volume of fresh milliQ water daily to each reservoir.

**Optogenetic Stimulation for FLIC (optoFLIC)**
We developed a closed-loop optoFLIC setup to time a pulsed light stimulation to a fly-to-food interaction in order to augment sweet taste neuron activity in a behaviorally relevant way. This apparatus was a collaboration between the Dus and Pletcher labs. Stimulation programs were written by SP and modified by CEM and KH, and can be found on Github. The FLIC lids were customized to allow placement of one high-intensity LED (627nm SinkPAD-II, Luxeon) on the ceiling of each of the twelve single-well chambers. Modified “optolids” were constructed from black polyoxymethylene (e.g., Delrin), which prohibited the leakage of light among chambers. To control LED illumination, each modified lid was connected to its corresponding DFM through the existing expansion port. LEDs were individually controlled cooperatively by each DFM and the FLIC Master Control Unit (MCU) using custom firmware. Customization details, including firmware updates and electrical specifications, are available from the authors upon request.

OptoFLIC is technically similar to FLIC, but for a few exceptions: Unlike FLIC, optoFLIC requires a stimulation program for the MCU to deliver to the optolids. For our data, the light pulse frequency (0 or 60 Hz) and duration of pulsing (100 ms) were chosen based on our sensillar electrophysiology data and optimized to have no influence on feeding on control diet of 5% sucrose. OptoFLIC has a range of parameters associated with near instantaneous LED illumination in response to the behavior of the animal. These parameters include: signal activation threshold to control illumination in response to the intensity of a feeding event; Illumination frequency
and pulse width to control the intensity of neuronal stimulation or inhibition; and duration of illumination following the termination of feeding to maintain stimulation after the behavior has ended. For all experiments, signal threshold was set to 10; pulse width was set to 8 ms; duration of illumination was set to 100 ms. Also, the delay from onset of feeding signal to onset of light stimulus was set to 0 ms; however, because the response rate of the system was tuned to ensure feeding signals were distinct from background, the realtime delay in light onset was 200 ms after the initial feeding signal. Flies were maintained in the dark and fed on Bloomington cornmeal food supplemented with 200 μM all-trans-retinal for 6 days prior to experiments for the proper functioning of Chrimson. At the time of the optoFLIC experiments, the MCU had been upgraded to perform all the data collection independent of a computer and the FLICMonitor software.

**Taste Sensitivity Assay**

Following dietary manipulation for 7 days, male flies were fasted for 22 hours (ZT9 until ZT7 next day) and then placed for 30 minutes on 1% agar containing non-caloric L-glucose (CarboSynth) at the concentrations indicated and colored with 0.5% blue dye (McCormick Culinary). Flies were kept at 25℃ for the entirety of the experiment.

**Sensillar Electrophysiology**

Electrophysiological recordings were made at labellar sensilla of flies fed sugar or control diet for 1 or 7 days, following a protocol similar to those described previously (Hiroi et al., 2002; Wang et al., 2016). Briefly, three to five L-type labellar bristles were recorded on each fly. The recording electrode (tip diameter, 12–15 μm) was filled with designed experimental tastants. Each chosen L-type bristle was stimulated by different concentrations of sucrose (in text) in 30mM tricholine citrate (TCC, Sigma-Aldrich, as electrolyte). To avoid adaptation, each labellar taste sensilla was stimulated up to 4 s and allowed to recover for > 2 minutes before applying another stimulus. Signals were acquired using an AxonClamp 900A amplifier and digitized with a 1400A D-A converter and AxoScope 10 software (Molecular Devices) at sampling rate of 10 kHz, filtered at 3 kHz. Electric signals were further amplified and filtered by a second amplifier (CyberAmp 320, Axon Instrument, Inc., USA, with gain X 100, Lowpass filter 1600 Hz).

**Metabolomics**

Glucosamine-6-phosphate measurements were performed by Metabolon, Inc., using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy. The measurements were conducted on samples of 100 heads from age-matched male flies fed a control or high-sugar diet for 7 days. On the seventh day they were food-deprived for 24h, then refed 400 mM D-glucose (“fed”) or 1% agar (“fasted”) for 1 hour. Animals were then flash-frozen in liquid nitrogen, and their heads collected with a sieve.

**Calcium Imaging**

The brains of awake, behaving male flies expressing GCaMP6s-Brp-mCherry (Kiragasi et al., 2017) in the Gr64f+ neurons were prepared for imaging similarly to the preparation of LeDue et al. (2016), following 18-24 hours food deprivation. Briefly, each fly was fixed to a custom-printed plastic slide with paraffin wax. The fly’s distal leg segments were removed to prevent tarsal interference with labellar stimulation and response. The proboscis was wax-fixed in an extended position prevent retraction, minimizing brain movement during imaging and aiding in accuracy of stimulus delivery. Each fly was tested with milliQ water before stimulating with 30% sucrose dissolved in milliQ water. To image the SEZ, the well surrounding the head was filled with sugarless artificial brain movement during imaging and aiding in accuracy of stimulus delivery. Each fly was tested with milliQ water before stimulating

**RNA Extraction**

The proboscis of 20 Gr5a-GAL4/UAS-OGT-RNAi flies were dissected into Trizol (Ambion) and homogenized with plastic pestles and 4-5 biological replicates collected over two days. RNA was extracted by acid phenol chloroform (Ambion), and precipitated with isopropanol with Glycoblue Coprecipitant (Invitrogen). RNA pellet was washed as needed with 75% ethanol. RNA was eluted in nuclease free water and treated by DNase I, following manufacturer’s instructions (Turbo DNA-free DNA removal kit, Ambion). Gr5a-GAL4 was used instead of Gr64f-GAL4 because the Gr64f transgene is a 10kb fragment that includes the coding regions for the Gr64a-e genes, which increases the RNA abundance of these gustatory receptors and interferes with quantification of possible changes in the abundance of these transcripts. For Gr5a-GAL4/UAS-OGT-RNAi experiment flies were first tested according to their taste responses to 20, 10 and 5% sucrose using the proboscis extension response: Gr5a-GAL4/UAS-OGT-RNAi flies with PER < 0.5 and Gr5a-GAL4/+ flies with PER > 0.5 were selected. For the SD and CD libraries, 200 proboscises were dissected in 1xPBS and homogenized in Trizol (Ambion). RNA was extracted by chloroform followed by RNA clean up using RNasy MiniElute Clean Up Kit (QIAGEN), and on column DNA digestion by DNase I (QIAGEN). The concentration and integrity of RNA was validated using the Agilent Bio-analyzer system and Qubit RNA High Sensitivity Assay (Invitrogen). All steps were carried out in RNase free conditions, and RNA was stored at −80C until library preparation.
RNA-seq library preparation
Sequencing libraries were generated using the Ovation RNA-Seq System for Model Organisms (Nugen, 0350-32) for CD versus SD experiments, and Ovation SoLo RNA-Seq System for Drosophila (Nugen, 0502-96) for Gr5a-GAL4>UAS-OGT-RNAi SD experiments. All reactions included integrated HL-dsDNase treatment (ArcticZymes, Cat. #70800-201). All libraries were sequenced on the Illumina NextSeq platform (paired read, High-output kit v2 75 cycles) using 38x37 bp paired end reads.

Proboscis Immunofluorescence
Proboscis from Gr5a>nls-GFP flies were dissected in 1xPBS and fixed in 4% PFA, mounted in FocusClear (CelExplorer) on cover-slips, and the cell bodies imaged using a FV1200 Olympus confocal with a 40x objective.

Quantitative RT-PCR
RNA was extracted from 10 heads per group with 4-5 biological replicates in the OGT experiment (Figure S7) and from 10 heads per group with 3 biological replicates in the dilp2 and dilp5 experiment (Figure S2). Complementary DNA was synthesized by Superscript III (Invitrogen) reverse transcriptase, and iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad Laboratories) with the addition of Ribolock RNase inhibitor (Thermo Fisher Scientific). qPCR reactions were carried out using Power SYBR Green PCR master mix (Applied Biosystems) based on manufacturer’s instructions. Primers were added at a 2.5uM concentration. All reactions were run on a 96-well plate on the StepOnePlus Real-Time PCR System (Applied Biosystems) and quantifications were made relative to the reference gene Ribosomal protein 49 (Rp49). The following primers were used:

| Primer   | Sequence          |
|----------|-------------------|
| Rp49 Forward | ATGCTAAGCTGTCGCACAAA |
| Rp49 Reverse  | ACTTCTTGAATCCGGTGCC  |
| OGT Forward    | CGTCCGCGGCCCATATATTA |
| OGT Reverse     | CCAACTCGAGTAAACCGACTGA |
| Dilp2 Forward   | TCAATCCCCTGCAGTTGTC |
| Dilp2 Reverse    | TTGAGTACACCCCCAAGATA |
| Dilp5 Forward   | TCCTGATCCCGCTCTGCTA |
| Dilp5 Reverse    | TGCCCTGTGGCGGACATT |

QUANTIFICATION AND STATISTICAL ANALYSIS

Triacylglyceride (TAG) Assay
Data as presented are averages of the triglyceride:protein concentration ratio for each biological replicate per genotype and dietary manipulation. Each experiment had 8 biological replicates per group, and each experiment was replicated at least once. Figure panels and statistical tests were made in GraphPad Prism.

Nile Red Staining
Quantification of droplet surface area was performed using Imaris (Bitplane). Figure panels and statistical tests were made in GraphPad Prism.

Proboscis Extension Response
The fly’s response to a sugar stimulus was scored as follows: a full extension given a score of 1, a partial extension a score of 0.5, and no extension a score of 0. Each fly’s average response to a sucrose concentration was used to create the mean response per genotype for that concentration.

Flies that neither kicked their legs nor responded to water or any of the sucrose solutions were removed from analysis as they had likely been killed or compromised in the course of the prep. Figure panels and statistical tests were made in GraphPad Prism.

FLIC and optoFLIC
All analysis and visualization code for the FLIC is in R, and can be found on Github. Raw data collected by FLICMonitor was analyzed in RStudio to calculate a moving baseline and to count licks. Once licks were calculated, we used RStudio code to sum the number of licks in 30-minute bins. With this, we could calculate total daily licks or produce heatmaps of fly-to-food interaction intensity per 30-minute bin. We also calculated meal duration by finding feeding maxima for each fly, then acquiring the times of the last 30-minute bin with zero or minimal licks before each maximum and the first 30-minute bin with zero or minimal licks after each maximum.
Duration was then calculated as \((\text{meal end}) - (\text{meal start})\) per meal per fly. Meal size was calculated from meal duration as the number of licks occurring between meal start and meal end. Per meal per day, these were averaged for genotype and concentration of sucrose in FLIC food. Figure panels and statistical tests were made in GraphPad Prism or in RStudio.

**Taste Sensitivity Assay**

Flies were scored for ingestion of the blue food by visual inspection of their abdomens. Figure panels and statistical tests were made in GraphPad Prism.

**Sensillar Electrophysiology**

Data were analyzed using the Clampfit 10 software (Molecular Devices). Spikes between 0 and 2 s after initiation of stimuli were counted as firing frequency evoked by the tastant. The mean value of spikes was calculated on 3-5 bristles recorded on each fly as one statistical sample. Figure panels and statistical tests were made in GraphPad Prism.

**Metabolomics**

Raw data was extracted, peak-identified and QC processed using Metabolon’s hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI), mass to charge ratio \((m/z)\), and chromatographic data (including MS/MS spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library spectral data) on all molecules present in the library. Metabolite peaks were quantified using area-under-the-curve and their amounts normalized by the total protein present in each sample. Figure panels and statistical tests were made in GraphPad Prism.

**Calcium Imaging**

Areas of interest were drawn around the two neuropil regions apparent in the images taken. Data analysis was done in Microsoft Excel by calculating \(ΔF/F_0\) for each channel, subtracting the mCherry signal (red) from the GCaMP6s signal (green) to correct for movement, and then calculating the area under the curve after sugar stimulation. Figure panels and statistical tests were made in GraphPad Prism.

**Analysis of high throughput RNA sequencing**

Because we observed high rates of likely PCR duplicates among the reads for most samples, the raw reads were de-duplicated using ParDRe (Gonzalez-Dominguez and Schmidt, 2016), allowing one mismatch and using an 18 bp prefix. Testing on internal controls using the ERCC spike-in mix showed that de-duplication improved the correlation of transcript abundances with known relative values (data not shown). Surviving reads that had any recognized fragment of the Nugen sequencing adaptor were removed using cutadapt 1.8.1 (Martin, 2011) and low quality ends were removed using Trimmomatic 0.22 (Bolger et al., 2014) to remove all terminal bases with quality scores below three, and then requiring that for surviving bases, their average quality score over a 4 bp window was at least 15. Reads with fewer than 20 surviving bases were subsequently dropped. Preprocessed reads were aligned to the *Drosophila melanogaster* Flybase release 6.08 transcriptome, augmented with Gal4 and EGFP transcript sequences, using kallisto 0.43.0 (Bray et al., 2016) with a k-mer size of 21 and 200 bootstrap replicates. We used sleuth (Pimentel et al., 2017) for further post-processing of the RNA-seq data; in particular, all significance tests for differential expression on RNA-seq data use p values or q-values (as noted) from sleuth for a Wald test on the coefficient distinguishing the groups in question. While we initially obtained three biological replicates for each of the CD and SD cases, we noted that one replicate from each condition was a substantial outlier from all other points (across both conditions) based on the Jensen-Shannon divergence between samples; we excluded that outlier pair from all described analysis. Similar pruning was applied to other sample sets. The final numbers of biological replicates for analyzed sequencing data are given in Table S1. For the pathway analysis in Figure S7, we used iPAGE (Goodarzi et al., 2009) to find gene ontology (GO) terms showing significant mutual information with the profile of fitted gene-level effect sizes from sleuth. Note that due to the several tests incorporated into the iPAGE pipeline (many of which are not shown), the overall false discovery rate of the procedure on expression profiles has been empirically been shown to be less than 0.05 (Goodarzi et al., 2009). To classify genes for the Venn diagram in Figure 7D, we first selected only the set of genes showing significant changes in expression between CD and SD (q < 0.1), and then conditioning on membership in that set, calculated FDR-corrected p values for the significance of changes in transcript level for the same genes between the GrSa-GAL4/UAS-OGT RNAi flies and the corresponding GrSa-GAL4/+ controls, (using a threshold of an FDR-corrected p value < 0.2). Genes showing significant expression changes in both experiments were classified based on the signs of the observed log fold changes. For the pathway analysis shown in Figure 7E, we used iPAGE (Goodarzi et al., 2009) to identify GO terms showing significant mutual information with the status of genes as being
in any of the oppositely-regulated categories of Figure 7D, or among the set of all other genes (a ‘background’ set that is not shown). iPAGE calculations used GO term annotations from the dmel_r6.08 Flybase release. Data was uploaded to GEO as submission # GSE113159.

**qPCR analysis**
Primer efficiency was calculated by serial dilution of primers and only primers with efficiencies greater than 90% were selected. Relative fold changes in transcript abundance was determined with the Livak method using the Ribosomal protein 49 (Rp49) transcript as a housekeeping control.

**Statistics**
Statistical tests, sample size, and \( p \) or \( q \) values are listed in each figure legend. Data were evaluated for normality and appropriate statistical tests applied if data were not normally distributed. All data are shown as Mean ± SEM, **** \( p < 0.0001 \), *** \( p < 0.001 \), ** \( p < 0.01 \), * \( p < 0.05 \) for all figures unless otherwise indicated.

**DATA AND SOFTWARE AVAILABILITY**
Firmware for FLIC and optoFLIC is available upon request of the authors. Software for FLIC and optoFLIC data analysis and visualization in RStudio for this paper can be found on Github (https://github.com/chrismayumich/May-et-al-FLIC-Analysis/branches). The accession number for the RNA sequencing datasets reported in this paper is Gene Expression Omnibus: GSE113159.