How Sweet It Is: The Role of Sweet Taste Perception in Diet-Induced Obesity

by

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Dedication

I dedicate this dissertation to my family: to my parents, for lighting the science spark in me when I was so small, and for sheltering and feeding that little flame my whole life, especially in times when I could not; and to my sister, Hannah, for her unwavering faith in me, for her memes, and for reminding me that a love of science can be found in every person.

Acknowledgments

I generated this dissertation with data and input from members of the Dus lab and collaborators far and wide. Each of Chapters 2 through 4 was on its own a huge team effort. The people I list in this section were instrumental in the formation of these chapters, for technique and analysis development, and for feedback on experimental design and narrative logic.

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For Chapter 4, I thank Katherine Hoffman and Abby Roelofs for their essential contributions to the FLIC analyses; Zane Dunnings for his assistance in developing an early optoFLIC prototype; and Kristy Weaver and Dr. Scott Pletcher who created the optoFLIC as it exists today and who helped me thoroughly troubleshoot the system.

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Preface

This dissertation contains original work by Christina E. May and members of the Dus laboratory.

Chapter 2 was published in the journal Cell Reports in May of 2019. Its purpose is to describe and explain how dietary sugar impairs sweet taste perception and the effect this has in promoting overeating and weight gain. Christina E. May performed FLIC, TAGs, Nile red, taste sensitivity, and calcium imaging experiments. Anoumid Vaziri performed PER, qPCR, and the OGT RNA-seq experiments. Dr. Monica Dus performed PER experiments. Dr. Olga Grushko quantified taste cells in the proboscis and collected the RNA-seq data from control and sugar diet probosces. Dr. Peter Freddolino and Morteza Khabiri analyzed the RNA-seq data. Yong Qi Lin and Qiao-Ping Wang performed the sensilla recordings. Christina E. May, Kristy Weaver, and Dr. Scott Pletcher developed the optoFLIC. Christina E. May, Anoumid Vaziri, and Dr. Monica Dus designed the experiments, wrote the manuscript, and prepared the figures with input from the other authors. Dr. Monica Dus supervised the project.

At the time of writing this dissertation, Chapter 3 is under revision for acceptance to eLife. Its purpose is to elucidate the circuit by which sweet taste perception controls feeding behavior, particularly on a high sugar diet. Christina E. May conducted all the experiments, with the exception of PER. Dr. Monica Dus carried out the PER experiments and supervised the project. Julia Rosander, Jen Gottfried, and Evan Dennis helped with the TAG measurements. Dr. Monica Dus and Christina E. May wrote the manuscript together.

Chapter 4 describes new methods for manipulating, recording, and analyzing fly feeding behavior using a closed-loop optogenetic apparatus called the optoFLIC. The system was

developed by Dr. Scott Pletcher with input from Christina E. May, Kristy Weaver, and Dr. Monica Dus. FLIC analyses were developed as a collaboration between Christina E. May and Katherine Hoffman, Abby Roelofs, and Dr. Scott Pletcher. The manuscript in its chapter form here was written by Christina E. May and edited by Dr. Monica Dus.

All other dissertation content was generated by Christina E. May and edited by Dr. Monica Dus.

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Abstract

Our ability to taste sugar evolved to tell us that certain foods are good to eat because they provide us with energy. However, in the modern food environment of ubiquitous “added sugars”, this good taste can be hijacked in ways that cause overeating. This dissertation details the discovery of a means by which dietary sugar causes overeating in the fruit fly *Drosophila melanogaster*, via impairment of peripheral sweet taste sensitivity through increased intracellular glucose metabolic signaling by a conserved protein modifier. This in turn affects central value/motivational processing of the sweet sensation to alter feeding. We also present a new fly feeding assay, the optoFLIC, that is optimized for the monitoring and the closed-loop, optogenetic manipulation of long-term, steady-state feeding behaviors. Together, these findings depict a theory overeating that links excess dietary sugar with peripheral sensation and central reward, and they guide future research into the etiology and treatment of diet-induced obesity.

Chapter 1: Introduction

Why is Food that Tastes Good "Bad" for Me?:

On the Obesity Epidemic and Hyperphagia of Palatable Food

Humanity's relationship with food is complicated and often perplexing. Food serves multiple cultural roles, as well as providing the necessary ingredients for an organism to maintain its physical form. But at the center of the modern world's relationship to food is a piece of contradictory wisdom: foods that taste good are "bad" to eat (Fischler 1987; Liem, Mars, and de Graaf 2004; Taubes 2012). The pleasure response to foods containing high-energy compounds like sugars and fats developed during long periods of evolutionary history when such food was scarce and valuable. Yet in the modern food environment, high-energy foods are often more abundant than any other kind of food. The availability of energy-dense foods has tracked with an increase in consumption of such foods (USDHHS, USDA 2015) and with development of the obesity epidemic (Hill and McCutcheon 1984; NHANES, CDC 2010), encompassing increased prevalence of obesity-linked comorbidities like type-2 diabetes, metabolic syndrome, some cancers, and cardiovascular disease. These diseases account for an estimated 200,000 obesity-related deaths in the U.S. per year (Flegal et al. 2005), and $147 billion per year in

obesity-related health care costs (Finkelstein et al. 2009). Despite the knowledge that eating too much high-energy food has negative health consequences, the tendency to eat more than is healthy persists. Research into what aspects of the modern food environment are obesogenic, and what happens to food perception and feeding motivation when these foods are consumed, can help curb unhealthy eating habits and these costs to modern life.

Human beings have daily feeding rhythms, a trait we share with many animal species. Most human cultures observe three meals a day plus snacking, or non-meal feeding. Eating rates during meals generally follow the same pattern: increase in rate until a peak, after which eating slows down until eventually it stops. Obesity research focuses on the factors that control feeding frequency, meal size, and meal duration – factors like hunger/satiety, emotional state, and food familiarity, availability, and sensory appeal (Stice and Yokum 2016; Berthoud and Klein 2017; Strien 2018; van der Valk et al. 2019). Altered perception of the food environment, in particular of its tastes, smells, and appearance, has been scrutinized as a major cause of hyperphagia of palatable, energy-dense foods (Liu et al. 2019); yet the full etiology of diet-induced obesity is not understood. A relatively understudied topic is that of added sugars, which are sugars added to foods that do not traditionally contain them, and that are often consumed beyond caloric need.

Sugars, BMI, and Taste

During the middle of the twentieth century, it became easier for food production companies to add sugars to many processed foods – like breads, yogurts, cereals,

condiments, and sauces – and to create ultraprocessed snack foods packed with sugars (see: Little Debbie®, Hostess®) that quickly packed the shelves of convenience stores, grocery stores, and gas stations. Ubiquity of added sugars has coincided with an increase in carbohydrate consumption and a rise in the prevalence of obesity (Gross et al. 2004), raising the question of whether exposure to excess sugars could induce overeating and obesity. It has been shown that adding sugar to the diet leads to increased caloric intake in animal models of obesity (Avena et al. 2008; Johnson and Kenny 2010; Soto et al. 2015), supporting the idea that the addition of sugar to the food environment could be causing hyperphagia. Interestingly, studies of people and animals with higher body mass indices (BMI, a metric of body fat using a ratio of body weight and height) also report increased feeding during meals and greater caloric intake, in addition to other physiological effects. Among these is an impairment in their ability to taste sweetness.

The search for a causal relationship between sweet taste perception and overeating and obesity has been underway for many years. Multiple recent studies have noted lower sweet taste sensitivity in obese individuals (Bartoshuk et al. 2006; Sartor et al. 2011; Berthoud and Zheng 2012; Proserpio et al. 2016; Vignini et al. 2019) compared to lean controls, though other published reports contradict this finding (Pasquet et al. 2007; Guido et al. 2016; Low et al. 2016; Hardikar et al. 2017), likely owing to the heterogeneous nature of obesity etiology. But another clue about sweet taste's relationship to obesity comes from a study of sweet taste inhibition by pharmacology – a research group found that normal-weight humans who had partial inhibition of their sweet taste receptors after drinking *Gymnema sylvestre* tea preferred

higher concentrations of sucrose in a flavored beverage (Noel et al. 2017). This suggests that diminished sweet taste can link dietary added sugars with overeating and obesity.

Taste is a plastic sensory system. In addition to pharmacological manipulation, increased circulating insulin, gastric bypass surgery, and certain cancer treatments also diminish taste sensitivity (Giza and Scott 1987; Nance et al. 2019; Pugnaloni et al. 2019). Diet too can induce changes to taste. One of the earliest clear demonstrations of diet-induced taste plasticity manipulated dietary salt levels and recorded both behavioral and neuronal responses to salt taste. Preferred salt concentrations are higher following dietary sodium depletion in rats (Berridge et al. 1984); these data are matched by corresponding experiments in humans (Bertino et al. 1982; Bertino et al. 1986; Beauchamp et al. 1990; Pangborn and Pecore 1982). Taste nerve recordings in rats also exhibit plasticity following sodium depletion (Contreras and Frank 1979; Hill et al. 1986). Sweet taste may be similarly influenced by the level of dietary sugar. Comparisons between peoples with differing levels of sugar in their diets delineate an inverse relationship between dietary sugar and sweet taste sensitivity (Macdonald et al. 1993; Jamel et al. 1996; Kim et al. 2019). Perhaps a theory linking “added sugars”, sweetness perception, and food choice could help explain the modern obesity epidemic.

But taste’s role in the etiology of obesity has been elusive for many years, for a few reasons. Heterogeneity of known and speculated causes of obesity adds uncontrolled variability to cross-sectional datasets, and even to some prospective studies (Stice and Yokum 2016). Furthermore, some known causes of obesity, like genetic mutations that cause a predisposition to being overweight or obese (particularly of leptin, its receptor,

or mutations in dopamine signaling genes like dopamine receptor D2 (D2R)), may affect feeding and taste independently. To hone in on the molecular, cellular, and circuit principles that govern diet-altered-taste-induced obesity, it is best to use cell-specific manipulations in a model organism.

Advantages of the Fly

The fruit fly *Drosophila melanogaster* has a long, storied history as a successful animal model helping us understand how genes influence development and behavior. It has been the source of many critical discoveries, such as genetic inheritance, Homeobox segmentation, and the molecular clock (Morgan 1910; McGinnis et al. 1984; Reddy et al. 1984). Using the fly as a model organism for taste neuroscience affords many advantages, such as the wealth of available transgenic lines, metabolic and transcriptional network tools (Davie et al. 2018; Wilinski et al. 2019), simpler taste circuitry (Wang et al. 2004; Fujii et al. 2015), elucidated neuronal connectivity (Xu et al. 2020), rapid proliferation, straightforward control of their diet, and multiple convergent methods to measure both taste ability and feeding behavior. Importantly, fruit flies also retain many similarities to humans: they eat two meals per day, with snacking between; they sleep at night and are awake (and feeding) during the day; they seek out the taste of sugar, and find it rewarding; and their molecular pathways, particularly for neurotransmitter synthesis and sugar metabolism, are largely conserved with ours. These traits make them ideal as a model to tease apart the interplay of dietary sugar, sweet taste, and obesity.

Flies are Just Like Us

The identification of the molecular clock in fruit flies was awarded the 2017 Nobel Prize in Physiology and Medicine because, as with 75% of disease-related genes, circadian molecular biology is largely conserved between flies and humans. Thus understanding the molecular mechanisms underlying fly behavior is relevant to humans as well. Some of the disease-related genes shared between our species are obesogenic when mutated (Beshel et al. 2017), and fly metabolic regulation critical for appropriate feeding, including sensing of internal energy state and external environment, is similar to humans (Trinh and Boulianne 2013). Unsurprisingly, flies become obese on energy-dense diets like humans do. Both high-fat and high-sugar diets have been shown to produce obesity and associated comorbidity phenotypes, like cardiovascular disease and metabolic syndrome (Birse et al. 2010; Musselman et al. 2011; Diop and Bodmer 2012; Rovenko et al. 2015). Some of this research has shown that increased dietary sugar specifically shortens fly lifespan (Skorupa et al. 2008). Fascinatingly, there are narrow populations of neurons in the fly that not only regulate precise aspects of feeding behavior (Flood et al. 2013; Youn et al. 2018), but also that control metabolic memory (Senapati et al. 2019) and lipid metabolism (Al-Anzi et al. 2009). The fly has five orders of magnitude fewer neurons than a human and an array of genetic means that allow researchers to access these neurons and to discover neuronal and molecular mechanisms of our common behaviors.

Fly Tools

One main reason flies are prime model organisms for neuroscience research starts with their famously straightforward genetics. Because tricks for mutating and inserting genes in fly genomes were discovered decades ago, large collections of transgenic and mutant lines have been made. Notable among the transgenic lines are those which use expression systems like Gal4/UAS. This system affords researchers the ability to express proteins or interference RNAs (RNAi) into neuron populations of their choice. This vastly improves the cellular and circuit resolution of neuronal manipulations. Because the system was invented for flies nearly 30 years ago (Brand and Perrimon 1993), multiple expression libraries now exist, which has made possible many remarkable findings on the power of a single or pair of neurons to control fly behavior.

The Gal4/UAS system in flies works by breeding flies to contain two transgenes: one a “Gal4” and the other a “UAS”. Gal4 encodes a transcription factor from yeast, which recognizes a particular upstream activating sequence, the UAS. In flies, Gal4 can be expressed under the control of a cloned promoter region, for example, the promoter for tyrosine hydroxylase; similarly, another gene of interest can be placed under the control of the UAS. A fly with both transgenes will express the gene of interest in tyrosine-hydroxylase-positive cells. Other systems like Gal4/UAS (i.e., QF/QUAS, LexA/LexAop) can be used in parallel with it, allowing for activity manipulation in one set of cells with readout of a response in another. This is extremely advantageous for systems neuroscience research, to bridge understanding of molecular mechanism with cellular and circuit function.

Because flies have been used in research for so long, they are also one of the first organisms with multiple thorough functional visualization tools, like FlyScape, for converging transcriptional and metabolic data (Wilinski et al. 2019); SCope, a single-cell transcriptomic brain atlas (Davie et al. 2018); and the Virtual Fly Brain, for driver line (e.g., Gal4) neuroanatomical overlay (Milyaev et al. 2012). Recently, a collaboration between Janelia Research Campus (FlyEM) and Google, Inc., has made great strides toward completing a brain-wide connectome for flies, which will guide studies of synaptic structure and information transformation across neuronal populations (Xu et al. 2020). Also, because fly genetic mutation is straightforward, molecular tools for probing neuronal function (i.e., genetically encoded indicators of membrane potential, intracellular calcium level, cAMP level, glucose level) are under constant development and improvement in flies. Combining these various toolsets makes the fly an excellent model for satisfactorily answering systems neuroscience questions.

Fly Feeding Behavior

Human and fly shared daily meal patterns include a breakfast, occurring after the fasting during sleep, and a dinner, occurring just before sleep. Forces like circadian rhythms, appetite, hunger, and food sensory appeal drive the start of a meal (Xu et al. 2008; Krashes et al. 2011; Denis et al. 2015). These forces weaken or become overpowered by other forces, such as energy or sensory satiation and the desire to perform other biological functions once caloric need is met, to induce meal termination. After a meal, the hypothalamus in the mammalian brain and the homologous structures

in the fly brain use information about the internal energy state to prevent further eating until caloric need surpasses a threshold: this is satiety.

In the fly brain, meal start is strongly cued by the circadian rhythm through activity of neurons in the pars intercerebralis, which is a structure with homologous function to the hypothalamus (Xu et al. 2008; Ro et al. 2014; Zhang 2016; Dreyer et al. 2019). A meal on standard food will last approximately four hours, and then the meal terminates. Meal termination occurs in mammals due to palatability habituation (see: Section 1.7.2: Sensory-specific satiety), sensory feedback for feeling full (e.g., gastric pressure), and circulating sugars activating energy-sensing neurons in the brain (e.g., *cupcake+* (Dus et al. 2013)), but these processes are poorly understood in fruit flies, as little attention has been given to their steady-state meal structure. Feeding has been largely studied in the context of fasting (Edgecomb, Harth, and Schneiderman 1994; Farhadian et al. 2012; Dus et al. 2013), and the commonly used CAFÉ assay gives data best on the order of hours or days (Ja et al. 2007). Generally speaking, some mutations are known to cause overall hyperphagia in the fly (Meunier et al. 2007; Söderberg et al. 2012) and these may play into meal termination directly. Recent developments in feeding assay technology have made it possible to probe the drivers of meal structure and in particular meal termination in the fly. Fly feeding can be recorded with high temporal resolution using the Fly-to-Liquid-food Interaction Counter (FLIC) (Ro et al. 2014). When a fly feeds in the FLIC arena, it closes a circuit connecting the slightly electrolytic food to a capacitance pad under its feet. The current flow (change in potential) is recorded by a connected computer, and the computer can be set to record data as often as 2000 Hz. At a recording rate of 5 Hz, the FLIC can be run for many days, though it requires daily

maintenance to refresh the water content of the liquid food. Thresholding FLIC data reveals two main behaviors, tasting and feeding. When flies engage with the food lightly – i.e., by touching it with their leg – it is not considered feeding, but it is a distinct behavior that can be quantified from the raw data. Feeding interactions, which are counted as single recording instances of signal above the feeding threshold (see Methods of Chapters 2-4), can be both timed and summed in measures of feeding behavior. Feeding interactions can also be combined into feeding events, which are consecutive sequences of feeding interactions that indicate the duration of one bout of proboscis contact with the food. This means events approach a closer estimation of the size of the fly's "sips".

In addition to the FLIC, one can measure the levels of glycogen or fat, as triglycerides, in whole flies as a convergent method for gauging caloric intake and storage. Alternatives to the FLIC for directly measuring intake include the Capillary Feeder (CAFE, (Ja et al. 2007)) and Expresso (Yapici et al. 2016) assays, the fly Proboscis and Activity Detector (flyPAD, (Itskov et al. 2014)), and feeding the flies food supplemented with radioisotopes or dye. Each comes with its own pros and cons, but notably none are as effective for monitoring steady-state feeding over many days as the FLIC (for more detail, see Chapter 4).

Sweetness: It's on the Tip of my ~~Tongue~~ Proboscis

How do we taste sugar? From insects to humans, the taste organ contains dedicated cells expressing chemoreceptors in or around the oral cavity for perception of sweet compounds within food. The sweet taste information from these cells is

processed in the brain to influence food preference. There are six main taste modalities including sweetness – the others are umami, fat, salt, sour, and bitter (Breslin 2013). However, there is also substantial evidence for oral sensing of calcium and carbonation (Chandrashekar et al. 2009; Mattes 2011; Tordoff et al. 2012; Lee et al. 2018). Segregation of taste modalities in the oral cavity is conserved across species (Marella et al. 2006; Chen et al. 2011). Taste organs are also sites of mechanosensation; food texture (Koç et al. 2013) and viscosity (Yeomans et al. 2014) play a role in taste preference and regulate food choice and intake. In addition to oral tasting, other food qualities such as energy content are processed post-ingestively in the brain and gut (Ochoa et al. 2015).

Anatomic sweet taste coding is remarkably similar across insects, rodents, and humans. For all, taste receptors are located on taste cells in or around the oral cavity, and sugar binding to its receptor activates the cell. In mammals, taste cells are clustered into units on the tongue called taste buds and innervate taste nerves that project to the brain. Three types of taste receptor cells are present within the taste bud, and the sense of sweetness initiates in Type II taste cells; other Type II taste cells sense bitter or umami, while Type I cells sense salt and Type III sense sour, though recently some Type III cells have been found to respond to bitter, umami, and sweet (Kemp et al. 2019). Mammalian sugar receptors, sensitive also to non-caloric sweeteners, are G-protein-coupled heterodimers of Taste Receptor type 1 member 2 (T1R2) and Taste Receptor type 1 member 3 (T1R3) subunits. The T1R3 subunit also forms the receptor for umami (together with Taste Receptor type 1 member 1), and there is some evidence for non-T1R3-mediated sugar sensing (Damak et al. 2003).

When bound, sugar receptors activate PLC-β-2, which triggers IP3-mediated calcium release from intracellular storage. This calcium activates transient receptor potential cation channel subfamily M member 5 (TRPM5) channels in the cell membrane, leading to membrane depolarization and release of ATP onto the local taste nerve (Iwata et al. 2014). Intriguingly, taste cells also release hormones in a paracrine fashion, and sweet-sensitive Type II cells in particular express receptors for many hormones involved in energy balance, such as GLP-1 and leptin (Shigemura et al. 2004; Calvo and Egan 2015 for review).

Once sweet taste information is transferred from primary sweet taste cells to the local afferent nerve fiber within a taste bud, it travels via the Chorda Tympani (CT) of the facial nerve (CN VII) to make its first synaptic connection in the nucleus of the solitary tract (NTS) in the brainstem. The taste signal then relays again in the gustatory thalamus, on its way to the gustatory cortex, the insula. Taste information from the NTS also enters the canonical reward circuit by projecting to the ventral tegmental area (VTA) by way of the parabrachial nucleus of the pons (PBN).

In lieu of taste buds, insects have sensilla, or taste hairs, surrounding the oral cavity at the distal end of the proboscis. Each sensillum contains chemosensory nerve endings for four taste neurons, including one sweet-sensitive neuron (Fujii et al. 2015). Taste neurons can also be found in the legs, pharynx, and wing edges (Scott 2018). In the fruit fly each sweet taste neuron expresses a subset of the fly's eight sweet taste receptors (Gustatory receptor (Gr) 5a, 61a, and 64a-f) (Fujii et al. 2015). The fly Grs are not structurally related to the mammalian TRs, and though their predicted structures are like G-protein-coupled receptors, studies on the fly olfactory coreceptor Orco suggest

that Grs could form ligand-gated ion channels (Butterwick et al. 2018). However, the necessity of G-proteins in the function of sweet and bitter taste neurons has been established (Ueno et al. 2006; Devambez et al. 2013). Like mammalian TRs, fly Grs also recognize non-caloric sweeteners like sucralose and arabinose (Gordesky-Gold et al. 2008; Fujita and Tanimura 2011). Interestingly, the taste of certain fatty acids, which are abundant in fruits, is also transduced through sweet taste neurons (Masek and Keene 2013), but mediated via different receptors (Tauber et al. 2017; Chen and Dahanukar 2019 for review). While the exact details of how the sweet signal is transduced inside the gustatory neurons are also unclear, stimulation of proboscis or leg sensilla with sugar solutions results in calcium influx in the presynaptic terminals of the sweet sensing cells, which precedes vesicular release. Like paracrine signaling across mammalian taste cells, fly taste neurons can influence the activity of their cross-modal neighbors: bitter neuron activity inhibits sweet neuron activity (Chu et al. 2014). The taste neurons in the labellum and legs project to the subesophageal zone (SEZ) of the fly brain for their first synaptic relay in taste processing, and here, as in the periphery, taste modalities remain segregated to create a sensory map (Harris et al. 2015). This is a “labeled lines” scheme, which holds true for mammalian taste coding in the brain as well. Some second-order sweet taste neurons have been identified (Kain and Dahanukar 2015; Miyazaki et al. 2015; Talay et al. 2017), but further delineation of these circuits is yet to be done. From the SEZ, the sweet taste signal is relayed and activates a subset of dopaminergic neurons in the PAM (Harris et al. 2015; Huetteroth et al. 2015), which can reinforce associations between food odors and sweet taste.

Central processing of oral tastes by the brain assigns valence to the taste: sweetness, fat and umami are innately attractive, while bitter and sour are innately aversive (Drewnowski 1997). Increasing concentrations of attractive tastants in a food increases consumption of the food; conversely, increasing concentrations of aversive tastants will decrease consumption. Central neural responses during tasting or intake can thus reveal the value assigned to a taste. Preference for tastes integrates the intensity of the taste with motivational circuits that drive feeding behavior. Because the strength of the taste intensity-preference relationship is dependent upon the sensitivity of each taste modality system, consumption can be affected when peripheral taste sensitivity is changed. Research into excessive feeding of palatable foods has therefore dedicated attention to measuring the taste responses in both peripheral and central sites along the taste circuit.

Accounting for Taste

Methods for measuring taste vary depending on the research subfield and the subject species (human, rodent, or insect). Direct reports of the taste experience by humans subjects distinguish taste perception sensitivity from taste preference. Taste perception refers to the ability to detect tastes of varying intensity by the peripheral taste organ. Using the forced-choice method, human subjects report whether they can detect a tastant dissolved in water at a particular concentration (detection threshold), and whether they can identify the tastant (recognition threshold). At suprathreshold concentrations, the general Labeled Magnitude Scale (gLMS, Bartoshuk et al. 2006) is commonly held as the gold standard to control for subjective reporting of tastant

intensity (compared to the Visual Analog Scale and Labeled Magnitude Scale). Unlike its predecessors, the gLMS more effectively normalizes subjective experience between subjects by asking its participants to score taste intensities against the ‘most intense experience imaginable’. This diminishes the different-baseline problem derived from asking for the rating of tastes against the ‘most intense taste ever experienced’, thus resulting variability should come from a lack (or surfeit) of imagination, and not constitutive differences in the ability to taste. Measurements are taken after the subjects have engaged in the sip-and-spit method of tastant delivery or with taste strips, which is critical to prevent any confound of satiety or ingestion reward from influencing the reporting of peripheral perception. In animal models, perception can be gauged with brief-access lick tests in rodents or PER in flies; however these methods incorporate a degree of food motivation that may bias interpretation.

The degree of preference, on the other hand, is a result of taste perception ability combined with the motivational and hedonic response to the tastant. Unlike perception, preference is measured in both human and animal models with similar methods; because of this, it is a valuable assay to understand the effects of diet on taste experiences across species. Preference is driven by the separable processes of liking and wanting. Assays that measure tastant consumption indicate wanting, while assays of hedonic behaviors indicate liking. Consumption is measured with single-option or two-choice assays. Measuring consumption in humans is done through the use of food frequency and addiction surveys, such as the Harvard Food Frequency Questionnaire; food given to lab rodents individually can be carefully weighed before and after presentation; and in flies the CAFE and Expresso assays (Ja et al. 2007; Yapici et al.

2016) are used to control and measure the volume of food consumed by small groups of or individual flies. Two-bottle preference tests, in which relative consumption of solution in the bottles is compared after a period of time, are often used in rodents. For two-choice assays in flies, distinct nontoxic dyes can be added to two foods; after feeding, flies are sorted based on the color of the food in their abdomen, which is visible through their cuticle. Automated food-interaction systems, such as lickometers for rodents and the FLIC for fruit flies, allow for higher throughput assays of feeding interactions as a proxy for consumption.

On the other hand, involuntary hedonic taste responses as readouts of liking are tested with taste reactivity assays, which quantify facial expressions following presentation of a tastant in human babies and in rodents (Berridge 1991). Similarly, in insects, the proboscis extension response (PER) measures the ability of flies to detect different concentration of tastants, while also assaying their hedonic and motivational quality; this assay can be used as a measure of perception or preference depending on its design (Dethier 1976; Masek and Scott 2010). In humans, preference is measured with implicit association test (IAT) questionnaires to determine the bias of a person towards a particular food type, which may probe either liking or wanting for that food.

In addition to self-reporting and behavioral assays, electrophysiological and imaging techniques are also used to measure the neuronal correlates of perception and preference in sensory and reward circuits. In human subjects, perception is assessed via visual analysis of tongue anatomy (i.e., taste bud counting) or brain imaging (i.e., fMRI, PET) of the regions involved in processing the primary taste information from the oral cavity (e.g., nucleus of the solitary tract (NTS), thalamus, insula). In animals, taste

perception is measured by recording the electrophysiological activity of the chorda tympani, NTS, and parabrachial nucleus (PBN) neurons in rodents and the sensilla in insects, or with calcium imaging of dissociated taste buds in rodents and in vivo imaging of the insect taste neuron presynaptic terminals in insects. Regions of the mammalian brain governing preference, like the striatum, ventral pallidum, ventral tegmental area (VTA), basolateral amygdala, and orbitofrontal cortex (Tindell et al. 2006; Stice and Yokum 2016; de Araujo et al. 2017), have functional homologs in the fruit fly brain: the pars intercerebralis, the PAM and PPL clusters, the mushroom body, and the Fdg neurons (Flood et al. 2013). In both mammals and insects, neurons in these regions can be imaged for calcium transients or their activity recorded electrophysiologically. Convergence of the variety of methods described here can provide a picture of how different aspects of taste function, from the behavioral to the neural, are affected by dietary manipulations or BMI.

Diet-induced Taste Plasticity

How does dietary sugar affect sweet taste? In Section 1.1, I discussed the evidence supporting relationships between obesity and sugar consumption and between obesity and sweet taste impairment. To link sugar consumption and sweet taste impairment, I first turn to studies that manipulate the level of sugar in the diet and measure the changes to sweet taste perception.

Reducing Dietary Sugar

A common therapeutic angle to treat obesity is reduced consumption of palatable, high-energy (and often high-sugar) foods. In the short-term, this can be an effective way to induce weight loss, but once the treatment has ended, feeding habits often return to pre-treatment levels (Colombo et al. 2014). It is therefore critical to understand the mechanism by which the treatment changes feeding behavior, then to discover what persists at treatment's end to push patients back into old habits. There are few studies of taste sensitivity after dietary restriction from which to build our understanding. Hanci and Altun (2015) find that acute hunger amplifies sweet taste sensitivity compared to the sated state in normal-weight humans (Hanci and Altun 2016), while others have also found this to be true of rodents (Berridge 1991; Chen et al. 2010) and fruit flies (Inagaki et al. 2014). Three groups report that sweet taste detection thresholds in obese patients are lowered by fasting or a restricted-diet weight loss treatment (Glöckner et al. 1986; Umabiki et al. 2010; Nielsen et al. 2019), yet this is contrary to an earlier finding (Rodin et al. 1976) as well as a recent study in obese children (Sauer et al. 2017). In such cases careful attention must be made to methodology. In reviewing research on taste changes following weight loss by bariatric surgery, (Nance et al. 2019) conclude that findings based on subjective recall of sweetness intensity are rarely supported by studies using validated sensory techniques.

Other methods of weight loss address the role of weight status in altered taste, independently of diet. Bariatric surgery is an increasingly prevalent method of weight loss, and post-op patients do report altered taste experiences. By validated metrics,

however, the direction and magnitude of sensitivity changes are inconsistent, seemingly dependent upon the type of surgery performed as well as the taste test used (Pepino et al. 2014; Makaronidis et al. 2016; Shoar et al. 2019; Hubert et al. 2019). Weight loss can also be induced hormonally in obese patients, by treatment with GLP-1 receptor agonists like exenatide and liraglutide, and this also decreases the sweetness detection threshold (Zhang et al. 2013; Brindisi et al. 2019), though whether this is because these drugs influence dietary choices elsewhere in the brain or because they act directly on taste cells (Martin et al. 2009; Takai et al. 2015) is unclear.

A single study has specifically reduced sugar consumption in normal weight human subjects to determine the effects on sweet taste sensitivity. Using the gLMS, (Wise et al. 2016) tested sweet taste sensitivity in normal-weight human subjects before, during, and after a low-sugar diet in which sugar calories were replaced with fat, protein and complex carbohydrates. The group found that after 2 months on the low-sugar diet, participants gave higher intensity ratings to sucrose-sweetened food than did controls. Interestingly, similar findings for the taste of fat were reported by (Newman et al. 2016) in obese human subjects following a reduced-fat diet. In Wise et al.'s study, they noted that after the dietary restrictions were lifted, sugar consumption returned to pre-diet levels, as did taste intensity. It would be beneficial to repeat the experiment in an animal model in order to investigate the cellular and molecular mechanisms underlying both the altered taste and the persistence of eating habits from before the study began.

Adding Sugar

Given that reducing dietary sugar improves sweet taste sensitivity, it follows that increasing dietary sugar may impair sweet taste. This is supported by evidence that people with obesity – who overall tend to consume more sugary food than people of normal weight – have impaired sweet taste, but certain research groups have set out to test this hypothesis in the specific context of increased sugar consumption. A negative correlation between sugary food consumption and sweet taste sensitivity has been repeatedly shown using food histories from adults (Kato and Roth 2012; Jayasinghe et al. 2017) and children (Feeney et al. 2017), as well as with recorded buffet meal choices in an experimental setting (Han et al. 2017). This correlation can be food- rather than tastant-specific, and is especially strong with sugar-sweetened beverages (Cornelis et al. 2017; Appleton et al. 2018). Likewise, daily soft drink consumption alters both sweetness intensity and pleasantness (Sartor et al. 2011).

Studies in animal models reveal that this correlation between perceived sweetness intensity and sugary food consumption may be causal. Last year, our group described a set of studies in fruit flies fed a high-sugar diet ((May et al. 2019); also, Chapter 2). In these flies, peripheral sweet taste neuron responses and behavioral taste responses (PER) were decreased regardless of weight gain, while interaction with the food increased. We further showed that correcting the activity of the sweet taste neurons with optogenetics was sufficient to prevent overeating on the sugar diet. It was remarkable to find that sweet taste could control feeding behavior, though its relevance and conserved status from insects to mammals was in question. Connecting mammalian and fly sugar

hyperphagia depends upon understanding the mechanisms governing sweet taste and feeding behavior.

Mechanisms of Diet-altered Taste

In the sugar diet flies from (May et al. 2019), peripheral sweet taste neuron sensitivity was impaired through glucose metabolism resulting in increased protein modification with O-GlcNAc. Importantly, the O-GlcNAcylating enzyme, OGT, is highly conserved from fruit flies to mammals. OGT acts as a glucose metabolic sensor to modify activity of hypothalamic neurons (Raun et al. 2007; Lagerlöf et al. 2016), which govern feeding behavior in mammals, and its activity in adipocytes has been linked to fat-diet-induced hyperphagia (though this has not been tested on a high-sugar diet) (Li et al. 2018). OGT is also necessary for sensory neuron branch development and maintenance (Su and Schwarz 2017). Its potential involvement in mammalian sweet taste impairment on a sugary diet is yet to be explored.

Diminished sweet taste sensitivity from a sugary diet occurs in rodent models as well. Two groups studying sugary diets in rats measured decreased neuron responsiveness to sweet taste at different points in the mammalian peripheral taste circuit: the CT (McCluskey et al. 2020) and the NTS (Weiss et al. 2019). However, a third group reported that CT responses in rats increase following a short-term sugary diet (Treesukosol et al. 2018). The mechanism by which taste changes in mammals remains elusive. (Han et al. 2017) found that certain polymorphisms of sweet taste receptor subunit T1R2 are related to sweet food intake in humans, but whether the polymorphisms increase or decrease sweet taste sensitivity was not directly tested.

(Togo et al. 2019) report that a sugary diet upregulates transcripts of both T1R2 and T1R3 in mice, which is counter to what might be expected in a situation of reduced sugar-stimulated activity (and is indeed opposite to what (Chen et al. 2010) reported of rats that had become obese on a high-fat diet). Nutriepigenetic regulation of T1R2 and TrpM5 transcription is another means by which diet can alter taste, and it relates to BMI and carbohydrate intake in humans (Ramos-Lopez et al. 2018).

Another branch of diet-induced taste change research derives from the fact that mammalian taste cells are known to be targets of hormones regulated by nutritional status: leptin and GLP-1. Sweet taste cells express the receptors for these hormones, resulting in hormonal modulation of sweet taste cell activity (Kawai et al. 2000; Shigemura et al. 2004). Unfortunately, there have yet to be studies of leptin or GLP-1 modulation of taste cell activity that specifically manipulate dietary sugar levels.

The variety of potential mechanisms demonstrates that diet-induced changes to taste sensitivity may depend upon the diet to which sugars are added. Most of the aforementioned studies focused on adding sugars to “normal” or control diets, but sugar overconsumption in humans rarely happens without concurrent overeating of other palatable foods containing high concentrations of fat and salt. High fat is another singular dietary manipulation commonly used to induce obesity in animal models. It has been shown to have its own effects on sweet taste sensitivity (Maliphol et al. 2013; Kaufman et al. 2018), some of which are independent of the obese state (Ahart et al. 2020). The interaction of high fat and high sugar, both of which are common components in human obesogenic diets, has stronger effects on feeding than either component alone (Soto et al. 2015; Oliva et al. 2017). In 2012, a research group

reported that eating a high-fat diet initially causes rats to increase their food intake, but after a week they decrease their feeding to better match their energetic needs (Sampey et al. 2011). In contrast, the same group found that rats fed an equally energy-dense but highly palatable "cafeteria" diet, containing a variety of processed foods with added sugars, develop persistent hyperphagia. This raises the question of what interactive effects added sugar taste has on feeding behaviors – and whether taste changes induced by sugar are causal to the obesity epidemic.

Diet-altered Reward

Reward for food promotes its consumption, and sugar intake has been shown to recruit reward regions with greater effectiveness than even fat intake (Stice et al. 2013). Sugar activates reward regions in the brain in two main ways: through calorie content, which requires energy to be absorbed from ingested sugary food in the gut (Zhang et al. 2018), and by taste, even in the absence of ingestion (Hajnal et al. 2004; Burke et al. 2012; Huetteroth et al. 2015; Yamagata et al. 2015; Thanarajah et al. 2019). Reward for sugar can be translated as a signal of "liking" (hedonic value, or pleasure) or of "wanting" (incentive value, or motivation). Hedonic value of sweet taste (often reported as pleasantness) is equivocally associated with taste sensitivity (Thompson et al. 1976; Puputti et al. 2019; Mouillot et al. 2020), while intake of food is more strongly associated with taste sensitivity (Puputti et al. 2019). This suggests that alterations to sweet taste should focus on central brain processes of "wanting" over "liking" in order to reveal taste's influences on motivation to feed. To understand more deeply the relationship

between sugar-altered taste and sweet taste reward, the next section will cover the literature examining diet-correlated changes in reward to sweet taste.

Sweet Taste Reward

Sweet taste can influence feeding decisions by stimulating reward centers in the brain. Because energy-dense food was rare but valuable during the evolutionary development of taste, sweet taste now has a fast neuronal link to food motivation and reward brain regions, to promote feeding on sugar whenever the opportunity presents itself. The sweetness of sugar produces a reinforcing dopamine response in the mammalian striatum (Hajnal et al. 2004; Thanarajah et al. 2019) and in the fly mushroom body (Burke et al. 2012; Huetteroth et al. 2015; Yamagata et al. 2015). In rodents and flies, the strength and direction of the striatal dopamine signal has been shown to dictate the motivating power of coincident cues associated with a source of sugar. A drastic change in the food environment, such as sudden ubiquity of added sugars and processed foods, upsets the balance between these taste-triggered, food-motivating signals and metabolic requirement – perhaps by altering taste perception. If sweet taste sensitivity is impaired by diet, then the reward signal for the taste of sugar will be decreased, with consequences for food intake.

The neural correlates of the two aspects of sugar reward in the mammalian brain segregate the striatum, with the dorsal striatum attending to the calorie-induced dopaminergic signals, and the ventral striatum receiving taste-associated dopamine release (Thanarajah et al. 2019). Evidence of impaired sweet taste reward in obesity is equivocal, with some studies indicating that obese mammals have depressed reward

dopamine responses to palatable tastes (Stice et al. 2010; Green et al. 2011; Babbs et al. 2013)or have reduced dopamine receptor 2 (D2R) levels (Thanos et al. 2008; Johnson and Kenny 2010; Winterdahl et al. 2019) while others demonstrate the opposite: increased reward region responses to sucrose predict future weight gain (Nolan-Poupart et al. 2013; Geha et al. 2013; Winter et al. 2017). On the other hand, the hypothesis that overeating, and exposure to energy-dense food, specifically results in lowered reward region responsivity to palatable tastes has more consistent support. Multiple studies have demonstrated in rats that reward dopamine responsivity decreases following chronic high-energy diets(Bello et al. 2002; Kelley et al. 2003; Rada et al. 2005; Geiger et al. 2009; Alsiö et al. 2010; Johnson and Kenny 2010). Similarly, a report from (Burger and Stice 2012) showed that striatal responses to milkshake taste were reduced in people with frequent ice cream consumption. Importantly, this study also showed that responses in the insula (the primary gustatory cortex) to the milkshake were also decreased. Together, these animal and human studies support the hypothesis that palatable diets may alter taste reward independently of the obese state. However, the change in sweet taste reward may in turn cause an increase in weight gain (Stice and Yokum 2016).

How does the reduction in sweet taste reward influence food choice? The relationship between these is not trivial. All things being equal, the sweeter a food is, the greater its reward response and the more it is preferred. However, a study by (Vickers et al. 2001) clearly demonstrated that stronger tastes can correlate with less intake, not more. The group presented human subjects with undersweetened and oversweetened isocaloric yogurts and asked them to report how much they liked each one, and then

they allowed them to eat each one unto satiety. Though the undersweetened yogurt was less liked than the oversweetened yogurt, more of it was consumed. As both yogurts contained the same number of calories, the difference in intake cannot be explained by energy need.

Another way to relate chronic exposure to sugar with reward and intake is to borrow from addiction research. In the case of drug abuse, chronic use coincides with both a decrease in drug-stimulated mesolimbic dopamine and a behavioral sensitization wherein drug users seek and consume more and more drug. Given that dopamine responsivity to palatable foods is decreased following high energy diets, it is reasonable to hypothesize that another behavioral sensitization process may occur with added sugar consumption. Consequently, an increase in preference for higher concentrations of sugar may also indicate that diet-altered taste reward is at play. One report demonstrates that rats fed a high-fat diet have increased liking for higher concentrations of sucrose (Shin et al. 2011), as though the concentration-preference curve is shifted right. This is remarkably similar to findings from an aforementioned research group showing that weakening sweet taste in human subjects using a pharmacological block increased preferences for higher sugar concentrations, resulting in more sugar consumed (Noel et al. 2017). In this way, decreased sweet taste sensitivity and reward may underlie hyperphagia and weight gain on sugary food.

Sugar Reward in Drosophila

The fruit fly reward system is constructed by the input of dopaminergic PAM cluster neurons to the mushroom body (MB), the site of associative learning in the fly (Aso et

al. 2014). The PAM neurons are functionally homologous to the mesolimbic dopamine neurons in mammalian brains. Activation of the PAM neurons at the same time as another sensory input to the MB will assign value to that sensation. The taste of sugar is sufficient to induce some PAM neurons to fire, while other PAM neurons respond to the energy content of consumed food. These valuation signals are often used to study learning and memory in the fly (Krashes et al. 2009). Importantly, recent research has also started to use this system in the fly to further understand the development and maintenance of addiction-like behaviors (Scaplen et al. 2019). Therefore, it is possible and advantageous to use this system to probe the similarity of sugar consumption to drug abuse.

The PAM neurons are considered to be rewarding because when they are engaged by a sweet taste, their activation assigns enough value to coincident stimuli to create short-term appetitive memories of those stimuli (Burke et al. 2012; Huetteroth et al. 2015; Yamagata et al. 2015). I hypothesize that sugar-impairment of sweet taste is causally associated with reduction in PAM neuron activation to sweet taste, and that these lead to overeating and weight gain (see Chapter 3).

Sweet Control of Feeding Behavior

Though reward signals are clearly linked to motivation, it seems contradictory that a decrease in taste reward signaling would lead to an increase in wanting and intake. It may help to return to what is known about the forces that control meal structure, then use those principles to determine the aspects of feeding sweet taste controls.

The acuity and sensitivity of the taste system allows for the creation of an estimate of the satiating power of a meal, which is critical for the control of meal duration given that actual nutrient sensing by absorption in the gut is too slow for appropriate meal termination. Experience and habituation of food palatability, particularly of taste, can give a much more rapid estimation of the energy content of a meal. The satiety cascade, first put forth by Blundell and colleagues in 1987, describes a model of meal termination whereby pre-absorptive sensory and cognitive engagement with food recruits neural satiation systems to slow down feeding (Blundell et al. 1987). The ability to sense sweetness, umami, fattiness, and saltiness initially promotes intake, as these are attractive food components. However, it has been shown that immediately upon experience of these food qualities (long before energy needs are met), activity of pro-feeding neurons in the mammalian brain decreases (Chen et al. 2015; Betley et al. 2015). Additionally, food palatability habituates over the course of a meal, which decreases reinforcement for intake until the meal ends (Rolls et al. 1981). It is therefore possible that sugar-induced taste impairment alters the effectiveness of taste to help terminate a meal.

There are two processes within taste-driven satiation that can contribute to incorrect meal termination that may lead to overeating and obesity. Sensory-specific satiety (SSS) occurs as a single type of food is consumed, decreasing preference specifically for the taste of the consumed food (Miller et al. 2000; Bell et al. 2003). Sensory-enhanced satiety (SES) can augment SSS through increased orosensory stimulation during eating, which promotes even earlier meal termination (Lavin et al. 2002; Weijzen et al. 2009; Yeomans et al. 2014). This section will describe what is

known about each of these processes with respect to food choice and how they may become dysregulated by a sugary diet.

Sensory-Specific Satiety

The lessening of a particular taste's pleasantness over the course of a meal was reported in human subjects as early as 1981 (Rolls et al. 1981). Following observations that neurons in the lateral hypothalamus of monkeys responded less to food the monkey had become satiated on than to other foods, this set of experiments showed that though eating reduced ratings of pleasantness to a certain degree for all foods, foods that had been consumed showed significantly greater pleasantness reductions than foods that had not. Studies demonstrating that this reduction in pleasantness was not dependent upon energy content of the food were published in the early 2000s (Miller et al. 2000; Bell et al. 2003). Interestingly, one paper reported that no changes in taste sensitivity could account for the reduction in pleasantness (Rolls et al. 1984); however, it was recently shown that human subjects with high sweet sensitivity displayed stronger sweet SSS (Han et al. 2017). Importantly, a mammalian neural correlate of SSS has been identified: activity of neurons in the orbitofrontal cortex (OFC) highly correlates with rated pleasantness of food as it is eaten to satiety (Kringelbach et al. 2003). Future studies investigating altered SSS could use this information to measure it in animal models.

One study reported that SSS can be attenuated by daily consumption of an energy-dense snack food, and they speculated that this might lead to greater intake of the snack (Tey et al. 2012). If an attenuation of SSS underlies longer (and therefore

larger) meals, it may be expected to correlate with obesity. Indeed, SSS seems to be a more potent factor for controlling meal termination than other sensory feedback predicting satiety from a meal, described as “feedforward anticipatory control of appetite” by (Andermann and Lowell 2017). Subjects given a food to eat ad libitum who stopped eating due to sensory fatigue consumed overall fewer calories than those who claimed they stopped eating due to gastric fullness (Hetherington 1996). But a study in obese humans (Snoek et al. 2004) and one in high-fat-diet-induced obese rats (Myers 2017) found no difference in SSS between obese and lean subjects. Interestingly, these studies focused on the satiating properties of fatty foods. Another study in obese women tested SSS to sweetness and found that obesity correlated with weakened SSS that could produce longer meals (Pepino and Mennella 2012). Recently, a group found that sweet SSS is generalizable across different kinds of sweeteners (Rogers et al. 2020). As of yet, none have looked at the potential of repeated exposure to sugar to lengthen meals and promote obesity development.

Sensory-specific satiety can also work against an organism trying to curb their feeding. Diets with a variety of tastes, like cafeteria and junk food diets that are common models of the human food environment, can allow an organism to overeat by giving them other energy-dense options that become more preferred as satiety for one is reached (Rolls et al. 1981; Brondel et al. 2009). Research into effective dietary methods to prevent this from happening explore the process of sensory-enhanced satiety, aiming to increase the orosensory feedback from food and thereby enhance its satiating effects.

Sensory-Enhanced Satiety

The main principle of SES is that heightened food texture or taste intensity reduces overall intake. By increasing the amount of time a food spends in the mouth or intraoral surface area it covers, one can increase its satiating power. This effect is, like SSS, largely independent of energy content. Let us return to (Vickers et al. 2001), which analyzed liking and intake of isocaloric undersweetened and oversweetened yogurts in human subjects. Since each yogurt had the same energy content, the sweetness of the yogurt was assumed to drive the satiation rate during the consumption phase of the experiment. The oversweetened yogurt, though it was more liked than the undersweetened yogurt, nevertheless resulted in less overall intake, demonstrating the satiating power of sweet taste. Similarly, other groups have shown that required chewing (Lavin et al. 2002), larger total volume to consume (Bell et al. 2003), and smaller sip size (and increased sip number) (Weijzen et al. 2009) all increase the satiating effect of orosensation.

Proposed Model of Sweet Taste Control of Sugar Feeding

On a sugar diet in flies, feeding is increased by extension of meal duration ((May et al. 2019), and Chapter 2). I propose that weaker sweet taste stimuli due to sugar-induced impairment allow the fly to poorly estimate the energy content of the food, thus extending a meal. This should be controlled by taste reward circuitry (especially those homologous to the OFC). The test of this hypothesis is described in Chapter 3. See model below (Figure 1-1).

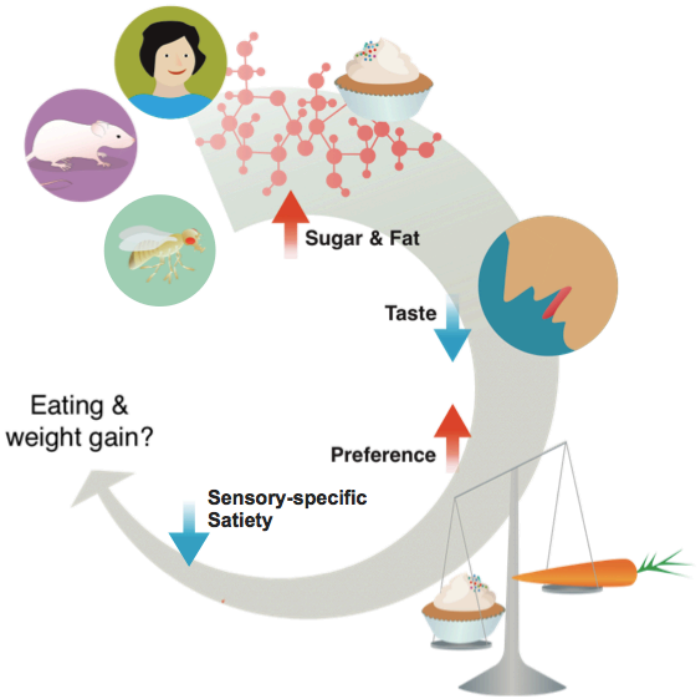

Figure 1- 1 Proposed model of sugar diet driven overeating.

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Chapter 2: High Dietary Sugar Diet Reshapes Sweet Taste to Promote Overconsumption in *Drosophila melanogaster*

Please see the Preface (p. iv) for contributor information.

Abstract

The sensation of pleasurable food qualities plays a crucial role in regulating eating. Recent studies found that humans with obesity have lower taste responses to sweet stimuli, but whether these sensory changes impact food intake is unclear. To tackle this question, we studied the effects of a high sugar diet on sweet taste sensation and feeding behavior in *Drosophila melanogaster.* Fruit flies on this diet had lower behavioral and physiological responses to sweet stimuli, overconsumed food, and developed obesity. Correcting taste deficits by manipulating the excitability of the sweet gustatory neurons, prevented overeating and fat accumulation in animals exposed to the high sugar diet. By using genetically obese and lean animals, we found that excess dietary sugar, but not obesity or dietary sweetness alone, promoted taste deficits and eating via the cell-autonomous action of the sugar sensor O-GlcNAc Transferase in the sweet-sensing neurons. Our work demonstrates that the reshaping of sweet taste

sensation by high dietary sugar is a driver of obesity and highlights the role of glucose metabolism in altering neural activity and behavior.

Introduction

Many arguments about the underlying cause in the rise of obesity point towards 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), 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 (Maliphol *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 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-GlcNAc-Transferase (OGT) (Hanover *et al.*, 2010; Hardiville 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 (Bartoshuk *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 high sugar diet (Musselman *et al.*, 2011; Musselman and Kuhnlein, 2018) and assessed their taste responses to sweet stimuli (see 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 Kuhnlein, 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), (Fig. 2-1A and Supplementary Information Fig. 2-S1A), but have no effect on *Drosophila insulin-like peptide (dilp)* transcript levels (Supplementary Information Fig. 2-S2A and B). 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 (Supplementary Information Fig. 2-S3B for a schematic of the anatomy). Taste neurons, which express receptors for only one taste modality, send their projections to the SubEsophageal Zone (SEZ), the taste processing center in 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 (Shiraiwa and Carlson, 2007). Ten-day-old flies fed a SD showed a rapid and progressive decrease in taste responses to supra-threshold (30, 5, 1%) concentrations of sucrose with time (Fig. 2-1B). 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 (Supplementary Information Fig. 2-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 (Supplementary Information Fig. 2-S1C). Furthermore, taste deficits occurred regardless of fasting time (Supplementary Information Fig. 2-S1D). Given that this fasting time reduces SD fly triglyceride levels whereby they match CD fly levels at 18-hours of starvation (data not shown), we ruled out the possibility that the decrease in PER is 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. 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 to 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 (Supplementary Information Fig. 2-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 (Fig. 2-1C, *dark green*) and there was no fat accumulation (Supplementary Information Fig. 2-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 (Supplementary Information. Fig. 2-S1G, *lime green*), but had normal taste responses (Fig. 2-1C), indicating that sweetness or excessive calories alone are insufficient to lower sweet taste sensation. In contrast, only sweet nutritious diets, such as those supplemented with D-fructose, D-glucose, and sucrose, promoted a decrease in sweet taste responses (Fig. 2-1D).

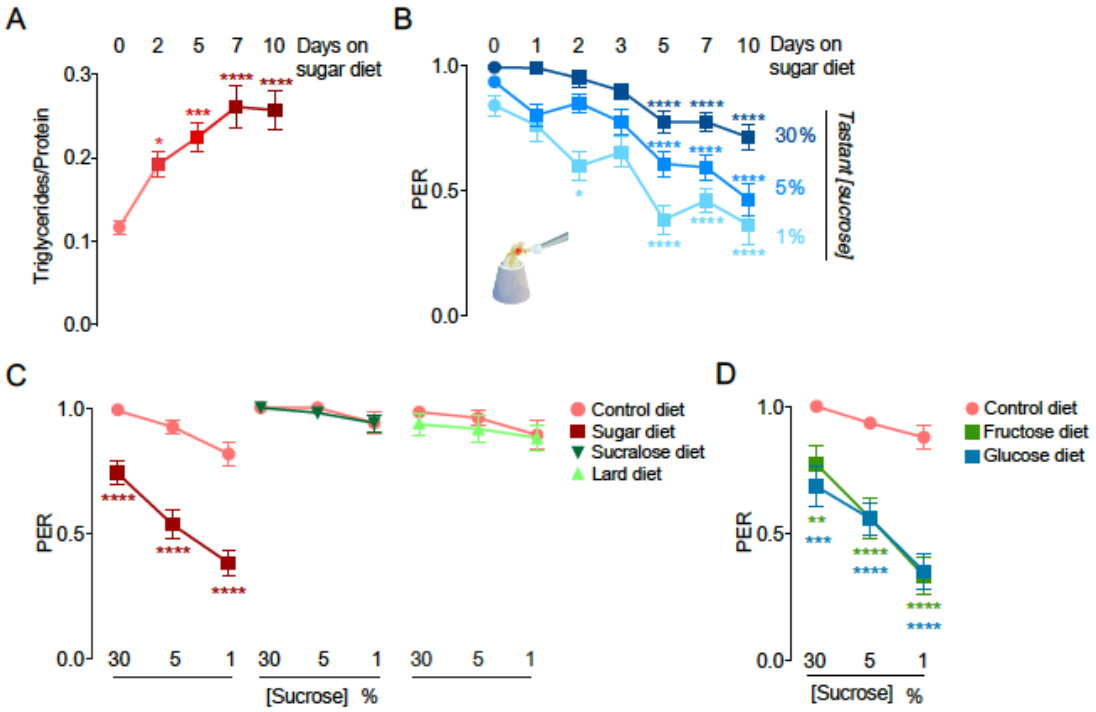


Figure 2- 1 A high sugar diet decreases sweet taste sensation. (See also Figure 2-S1.)

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

A) Triglyceride levels normalized to protein in age-matched male *w*^{1118}*CS* 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 *w*^{1118}*CS* 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 *w*^{1118}*CS* flies fed a control, sucrose, lard, or sucralose 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 w^{1118}*CS* 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.

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 (Gronke *et al.*, 2005) (Fig. 2-2A). *bmm* mutants have as much body fat on a control diet as wild-type flies on a SD (Fig. 2-2B), but their taste responses as measured by PER were normal on a CD and reduced on a SD (Fig. 2-2C), 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 (Fig. 2-1C and Supplementary Information Fig. 2-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) (Fig. 2-2A); despite remaining lean (Fig. 2-2B) and maintaining normal *Drosophila insulin-like peptide* transcript levels on a SD (Supplementary Information Fig. 2-S2A and B), *plin2* mutants experienced a comparable decrease in taste responses to that of control and *bmm* mutant flies (Fig. 2-2C). 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.

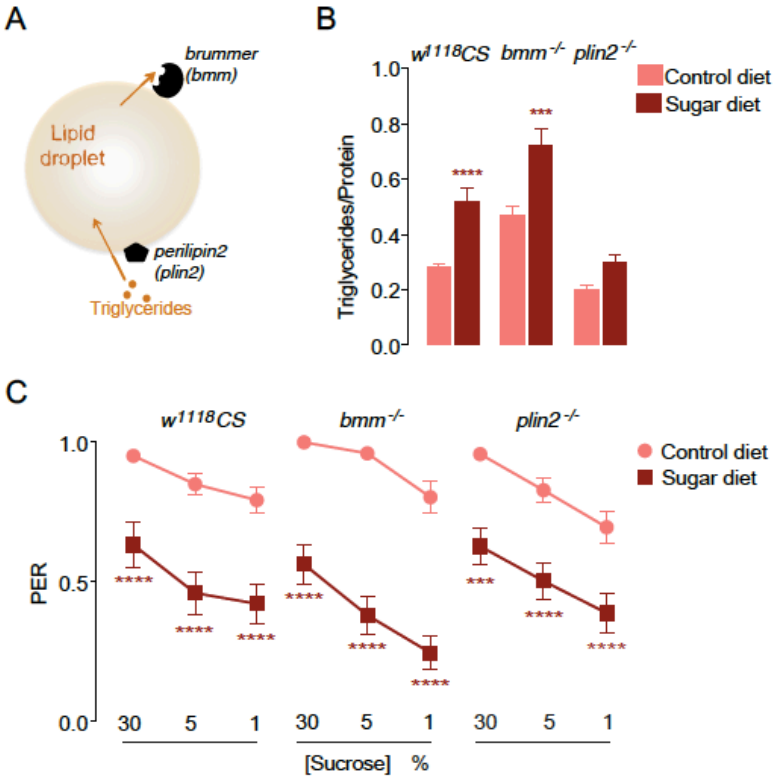


Figure 2- 2 A sugar diet decreases taste sensation independently of obesity. (See also Figure 2-S2.)

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

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 $w^{1118}CS$ (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 $w^{1118}CS$, $bmm^{-/-}$, and $plin2^{-/-}$ flies on control (*circles*) or sugar (*squares*) diet for 7 days. n=26-56, multiple *t* tests with Holm-Sidak correction for multiple comparisons, comparisons to control diet.

A high sugar diet decreases the responses of the sweet-sensing neurons to sugar

To better understand how a sugar diet decreases sweet taste sensation, we examined the physiology of the sweet taste neurons. Since, we observed no changes in the number of sweet taste neurons labeled by the sweet *Gustatory Receptor 5a-Gal4* transgene (Chyb *et al.*, 2003; Fujii *et al.*, 2015; Marella *et al.*, 2006) driving GFP in the

labellum of flies on a SD (Supplementary Information Fig. 2-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-Brp-mCherry* (Kiragasi *et al.*, 2017) (Fig. 2-3A). Presynaptic responses to 30% sucrose stimulation of the proboscis were identical after 1-day exposure to the SD, but decreased gradually with longer exposures (Fig. 2-3B and C), which matched the magnitude and progression of sweet taste deficits as measured by PER (Fig. 2-1B). In addition, we also found that animals had fewer sugar-induced action potentials after both short- and long-term exposure to the SD (Supplementary Information Fig. 2-S3B and C). 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.

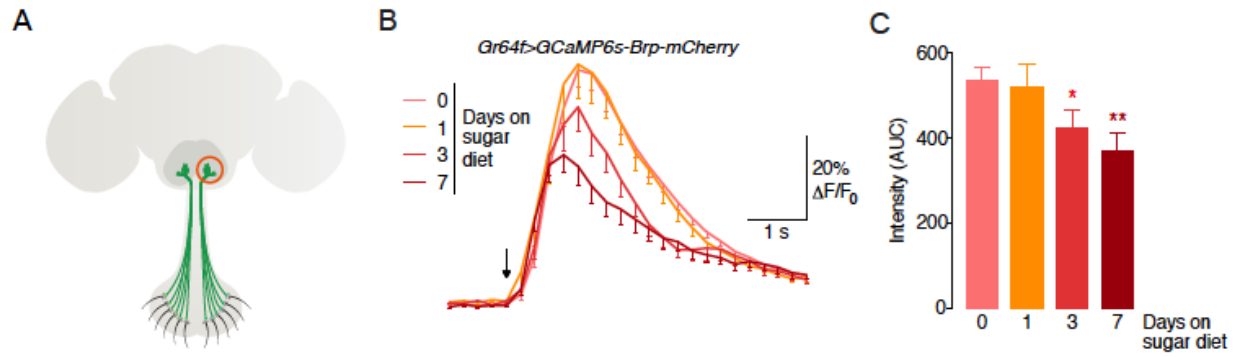


Figure 2- 3 A sugar diet decreases synaptic responses to a sugar stimulus in the sweet taste neurons (See also Figure 2-S3.)

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

A) The cell bodies of chemosensory neurons are in the labellum with dendrites protruding into the taste hair (sensillum, *black*) and the axons (*red* circle) terminating in the SubEsophageal Zone (SEZ, *darker grey*) of the brain. Sweet taste cells are in green.

B-C) B) Average $\Delta F/F_0$ calcium response traces and **C)** their area under the curve in the synaptic terminals of *Gr64f>GCaMP6s-Bruchpilot-mCherry* 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).

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 (Supplementary Information Fig. 2-S4A and B). Control $w^{1118}CS$ flies fed a 5% sucrose control diet while on the FLIC showed a stable number of licks per day (Fig. 2-4A, *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 (Fig. 2-4A, *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 (Supplementary Information Fig. 2-S4C). To better characterize the temporal dynamics and investigate the effect of diet on meal patterns, we binned licks in 30' intervals over many days for individual flies (Fig. 2-4B) and as a group average (Fig. 2-4C). We found that flies, like mammals, eat in discrete patterns, here termed "meals", that closely follow circadian activity (Fig. 2-4B and C). Flies on 20% sucrose still consumed only two meals per day, but the peaks became higher and wider with more time on the diet (Fig. 2-4C). Furthermore, while the onset of each morning (AM) and evening (PM) 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 (Fig. 2-4C). To quantify meal duration, we measured the time of meal start and end for AM and PM meals for each fly (Fig. 2-4D). The duration of the AM and PM meals of flies on 5% sucrose remained the same over 7 days (Fig. 2-4E). 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 (Fig. 2-4E). We next quantified the size of each meal by calculating the

area under each AM and PM meal peaks (Fig. 2-4D, *gray*). Like with duration, the size of each AM and PM meal increased with more days on a 20% diet, while it stayed unchanged in flies eating 5% sucrose (Fig. 2-4F). Overall, we observed a strong relationship between meal size and duration (Fig. 2-4E and F), 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.

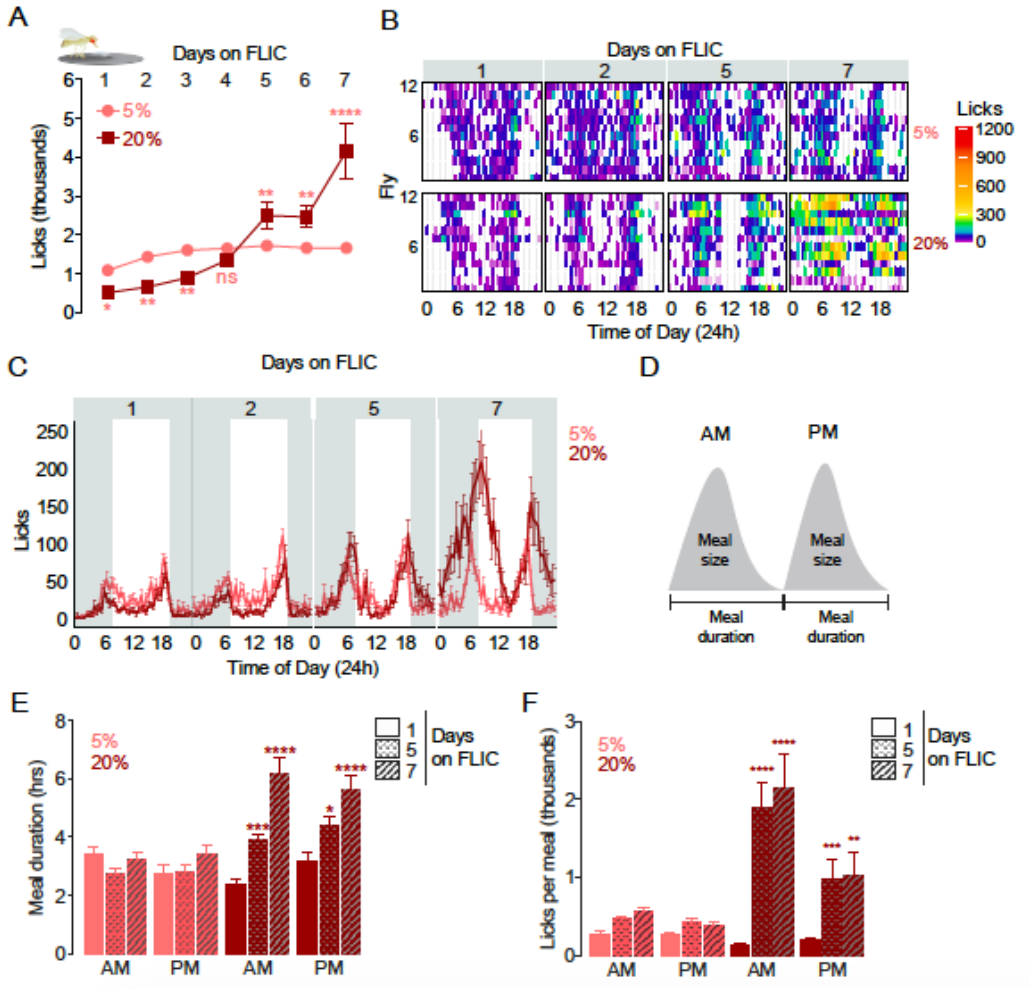


Figure 2- 4 Flies fed a high sugar diet show increased feeding behavior, meal size and duration. (See also Figure 2-S4.)

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

A) Average licks per day of age-matched w^{1118}*CS* 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 24h, time 0 indicates midnight (ZT17).

C) Meal patterns quantified as *average* licks binned by 30 minutes for flies feeding on 5 or 20% sucrose on selected days 1, 2, 5, or 7 (from **A)**. *x* axis as in **B**.

D) Schematic for how meal duration and size were determined for morning (AM) and evening (PM) meals.

E-F) The meal **E)** duration in hours (hrs) and **F)** size in licks of the morning (AM) and evening (PM) 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.

To investigate if these alterations in feeding behavior are a consequence of diet-induced obesity (Fig. 2-1A and B) or possibly a result of high dietary sugar, we tested the feeding patterns of genetically-obese *brummer* (*bmm*) and genetically-lean *perilipin2* (*plin2)* mutant flies (Gronke *et al.*, 2005; Ro *et al.*, 2014). *bmm* mutants, despite being as obese as control flies on a SD (Fig. 2-2B), showed similar patterns of feeding behavior with diets as control flies (Fig. 2-5A, C, E and Supplementary Information Fig. 2-S5A-C for controls). Therefore, obesity in the absence of high dietary sugar has no effect on meal size and duration. In contrast, 20% sucrose leads to an increase in meal

size and duration even in the absence of obesity in the *plin2* mutant flies (Fig. 2-5B, D, F and Supplementary Information Fig. 2-S5A-C for controls). These data suggest that obesity alone does not drive the observed changes in feeding patterns and open the possibility that, instead, these are a direct consequence of dietary sugar and linked to changes in sweet taste sensation.

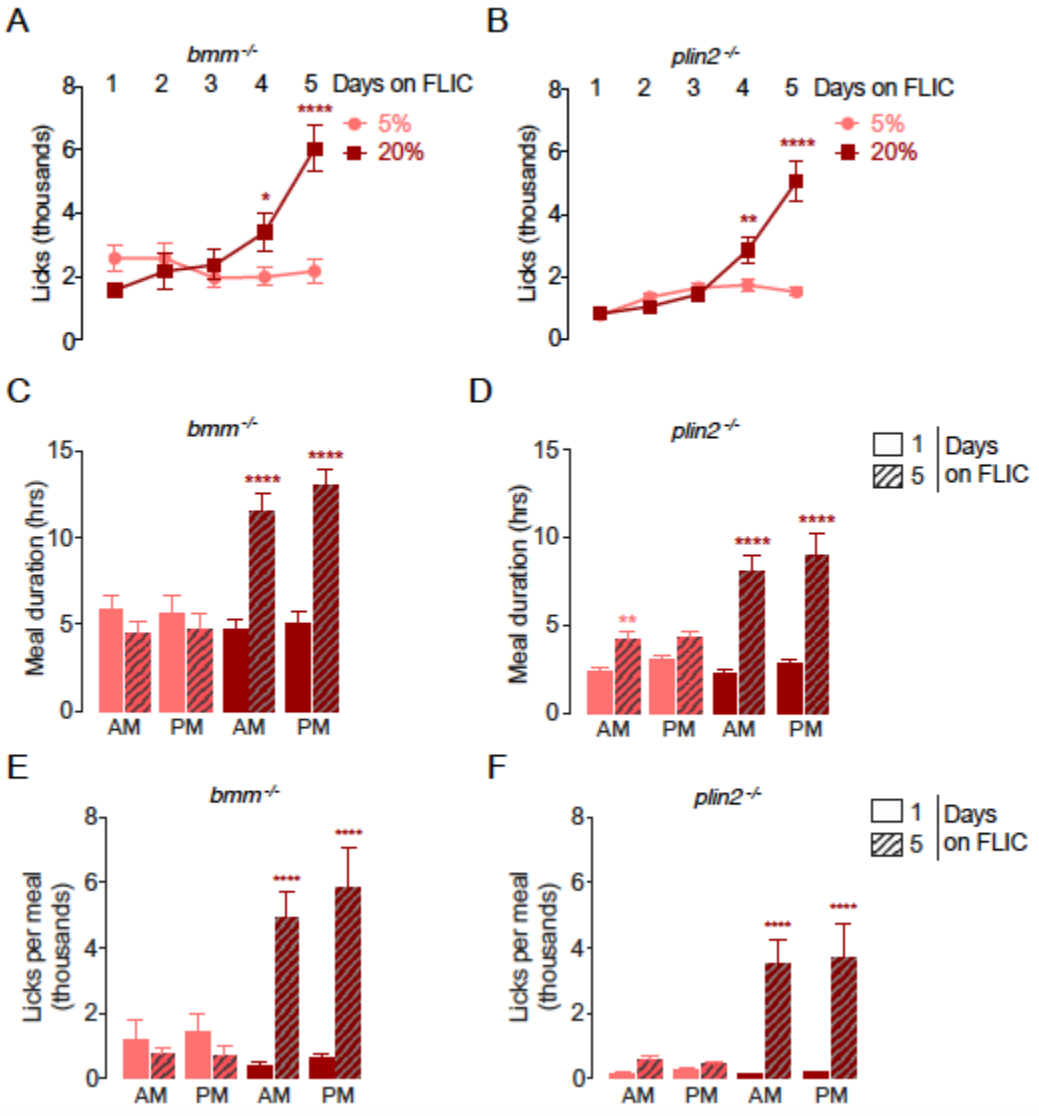


Figure 2- 5 A sugar diet promotes increased feeding behaviors independently of fat accumulation (See also Figure 2-S5.)

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

A-B) Average licks per day of age-matched male **A)** *bmm*$^{-/-}$ and **B)** *plin2*$^{-/-}$ mutant flies feeding on either 5 or 20% sucrose food on the FLIC. n=15-36, two-way ANOVA with Fisher's LSD, comparisons to same-day 5% sucrose licks.

C-D) The meal duration in hours (hrs) for the morning (AM) and evening (PM) meals of **C**) *bmm*$^{-/-}$ and **D)** *plin2*$^{-/-}$ mutant flies feeding on 5 or 20% sucrose on day 1 (*solid bars*) and day 5 (*hatched bars*). n=16-33 and n=18-30, two-way ANOVA with Fisher's LSD, comparisons to same-diet day 1 meal-time duration.

E-F) The size as licks per meal for the morning (AM) and evening (PM) meals of **E)** *bmm*$^{-/-}$ and **F)** *plin2*$^{-/-}$ mutant flies feeding on 5 or 20% sucrose on day 1 (*solid bars*) and day 5 (*hatched bars*). n=11-29 and n=16-30, two-way ANOVA with Fisher's LSD, comparisons to same-diet day 1 meal size.

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 (Fig. 2-6A). 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 SD (Fig. 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 (Fig. 2-6B). In addition, while the meals of control flies became longer, those of *Gr64f>NaChBac* animals stayed the same (Fig. 2-6C). Consistent with these data, *Gr64f>NaChBac* flies also remained lean on 20% sucrose, while control flies accumulated fat (Fig. 2-6D and Supplementary Fig. 2-S5D 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 overfeeding, 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 (Fig. 2-6E) or *csChrimson* flies without the *Gal4* (Supplementary Fig. 2-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.

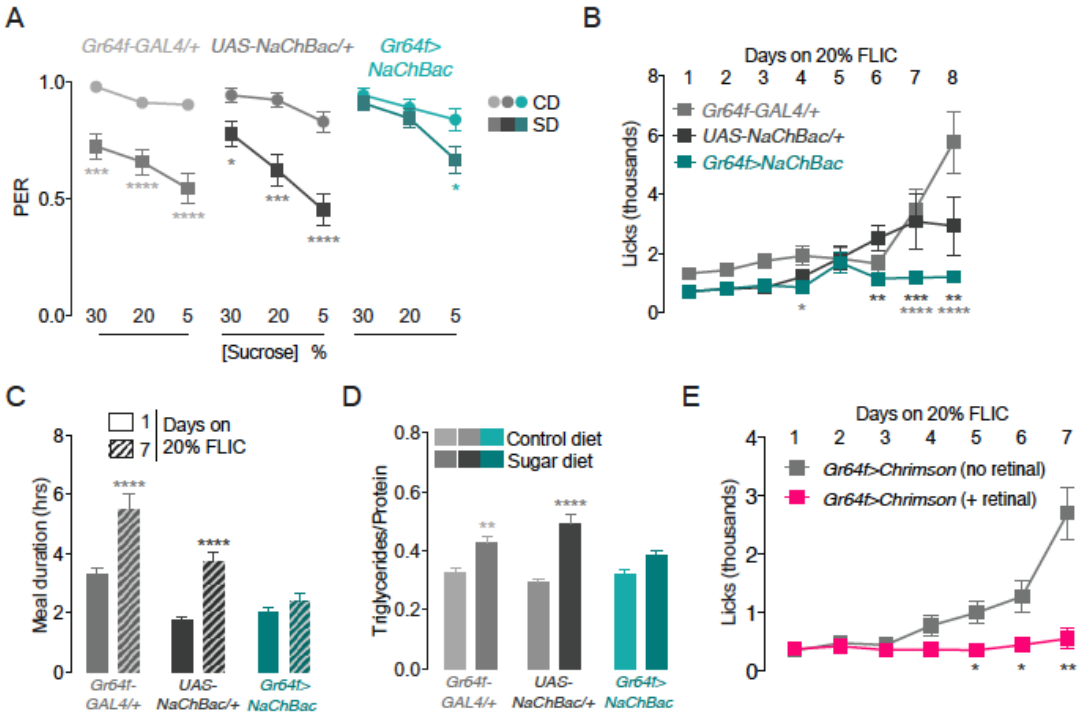


Figure 2- 6 Restoring sweet taste sensation and optogenetic activation of the Gr64f+ neurons protects animals from diet-induced obesity. (See also Figure 2-S5.)

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

A) Taste responses to 5, 20, 30% sucrose stimulation (*x* axis) of the labellum in *Gr64f>NaChBac* (*teal*) and parental transgenic controls flies (*grey*, crossed to w^{1118}*CS*) fed a control (CD, *circles*) or sugar (SD, *squares*) diet for 7 days. n=35-54, multiple *t* tests with Holm-Sidak correction, comparisons to control diet.

B) Representative average licks per day of *Gr64f>NaChBac* (*teal*) and parental transgenic controls (*grey*) 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 AM and PM) of *Gr64f>NaChBac* (*teal*) and parental transgenic controls (*grey,* crossed to w^{1118}*CS*) 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 (*grey,* crossed to w^{1118}*CS*) 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 (*grey*) 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.

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 (Fig. 2-1C), 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 (Fig. 2-1D). 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; Hardiville and Hart, 2014). The levels of the metabolic end product of HBP, UDP-GlcNAc, are increased by high nutrient diets; a single enzyme, *O-GlcNAc Transferase* (*OGT*, also known as *super sex combs* in *Drosophila*), adds the O-linked N-Acetylglucosamine (O-GlcNAc) moiety from

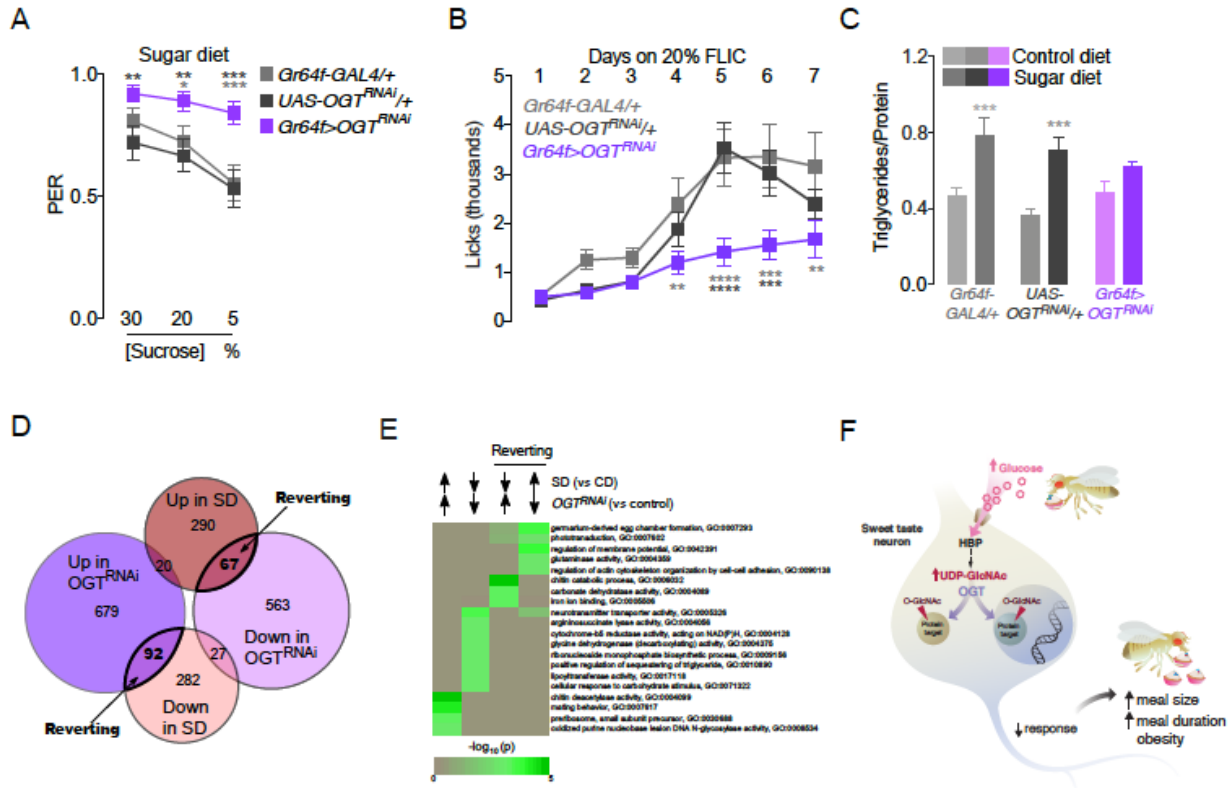
UDP-GlcNAc onto the serine and threonine residues of proteins to modify their activity, competing with protein phosphorylation (Hanover *et al.*, 2010; Hardiville and Hart, 2014) (*see* Supplementary Information Fig. 2-S6A for schematic of the HBP). Recent data suggests that OGT may also play a role in the brain: OGT activity is high in neurons and regulates metabolism and synaptic maturation (Lagerlof *et al.*, 2017; Lagerlof *et al.*, 2016; Ruan *et al.*, 2014). In fly heads, the levels of the first committed HBP metabolite glucosamine-6-phosphate were increased with a SD (Fig. 2-S6B). To test if OGT mediates the molecular effects of a SD on sweet taste, we used a previously characterized OGT RNA interference (RNAi) transgene (Radermacher *et al.*, 2014) to knock it down in the *Gr64f*+ neurons (50% knock down efficiency, Supplementary Information Fig. 2-S6C). *Gr64f>OGT* knockdown (KD) animals had normal PER on a CD (Supplementary Information Fig. 2-S6D), but this manipulation rescued sweet taste responses on a SD compared to controls (Fig. 2-7A and Supplementary Information Fig. 2-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 (Fig. 2-6), we asked if *OGT* KD could also restore feeding behavior. Indeed, KD of *OGT* in the *Gr64f*+ cells prevented feeding on 20% sucrose (Fig. 2-7B); consistent with its effect on feeding, *Gr64f*+*>OGT* KD animals remained lean compared to genetic control flies (Fig. 2-7C). Thus, decreasing OGT activity solely in the sweet taste neurons blocked the effects of 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 labella of flies fed a CD and SD for 7 days and with *OGT* KD (Supplementary Table 2-1 and Supplementary Fig. 2-S7). Since knockdown of *OGT* in the sweet-sensing cells restores taste function and prevents overfeeding and obesity on a high sugar diet, we reasoned that the genes altered on a sugar diet *and* important for taste function would show an opposite expression trend when *OGT* is knocked down (i.e., up in SD/down in *OGT* knockdown; down in SD/up in *OGT* knockdown). To carry out this analysis, we first selected 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 RNAseq experiments were classified based on the signs of the observed $\log_2$-fold changes. Using this approach, we found about ~150 genes changed by diet and "reverted" in *Gr64f>OGT* KD (Fig. 2-7D). We used iPAGE, a pathway discovery program (Goodarzi *et al.*, 2009) to identify GO terms showing significant mutual information with the expression status of genes as being in either one of the two oppositely-regulated categories (up/down or "reverting," Fig. 2-7E). 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 catabolic 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 (Fig. 2-7F). 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.



For GO term clarity in Fig. 7E, see below legend.

Figure 2- 7 OGT mediates the effects of sugar diet on sweet taste, feeding behavior, and obesity. (See also Figures 2-S6 and 2-S7.)

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

A) Taste responses to 5, 20, or 30% sucrose stimulation (*x* axis) of the labellum in *Gr64f>OGT* ^{RNAi} (*purple*) and parental transgenic control (*grey,* crossed to w^{1118}*CS*) flies fed a sugar diet for 7 days. n=36-60, multiple *t*-tests with Holm-Sidak correction, comparisons to genetic controls.

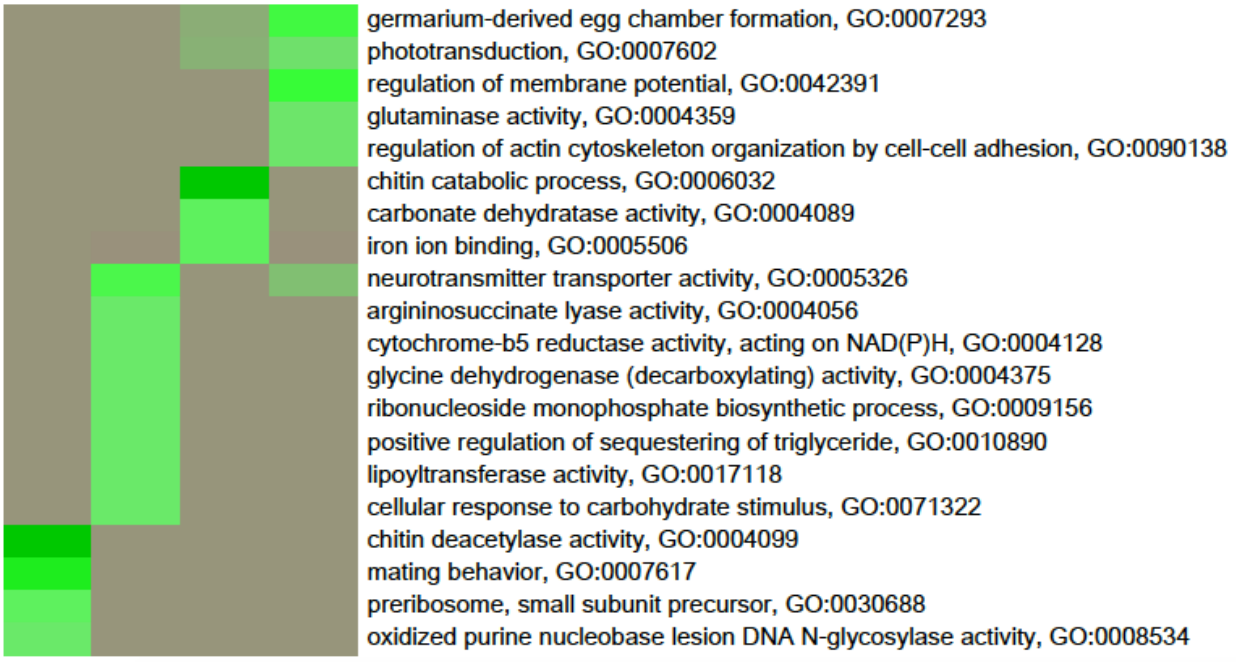
B) Average licks per day of *Gr64f>OGT* ^{RNAi} (*purple*) and parental transgenic control (*grey,* crossed to w^{1118}*CS*) flies fed on 20% sucrose. n=11-44, two-way ANOVA with Fisher's LSD, comparisons to control genotypes.

C) Triglyceride levels normalized to protein in *Gr64f>OGT* ^{RNAi} (*purple*) and parental transgenic control (*grey,* crossed to w^{1118}*CS*) flies on control (*lighter shades*) or sugar (*darker shades*) diet for 7 days. n=8, two-way ANOVA with Sidak's test, comparisons to CD.

D) Overlap of genes showing differential expression in SD vs. CD labella, compared with those showing significant changes with OGT knockdown compared to *Gal4/+* control alone. Genes with opposite expression (see text for explanation of analysis) are marked as "reverting".

E) iPAGE-based identification of pathways enriched in the set of genes from **D)** showing opposing transcription changes in flies fed a SD with or without *OGT* knockdown. Coloration shows hypergeometric *p* values from iPAGE. (*see below for clearer text*)

F) Model of physiological and metabolic changes in sweet taste neurons in flies fed a high sugar diet. HPB, hexosamine biosynthesis pathway; OGT, *O-GlcNAc transferase.*



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 mouse (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 do 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 (Lagerlof *et al.*, 2017; Lagerlof *et al.*, 2016; Ruan *et al.*, 2014). There are a few examples where metabolic sensors have been implicated in the modulation of neural activity, such as TOR and 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; Hardiville 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 byproducts.

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.

Supplemental Tables and Figures

Supplemental Table 2-S 1 Tab 1

Sample Name	Diet	Genotype	Response status	Status	Reads (preprocessed)	Reads (pseudoaligned)
CD7d_1	Control diet	WT	Not assessed	Excluded (outlier)	58043879	20263924
CD7d_2	Control diet	WT	Not assessed	Normal	47642520	7563260
CD7d_3	Control diet	WT	Not assessed	Normal	48951503	8109905
SD7d_1	Sugar diet	WT	Not assessed	Excluded (outlier)	60134948	16301613
SD7d_3	Sugar diet	WT	Not assessed	Normal	45995514	5635089
SD7d_5	Sugar diet	WT	Not assessed	Normal	55183354	6484129
SD_5agal_NR 1	Sugar diet	Gr5a-Gal4/+	Non-responder	Normal	6798919	4337603
SD_5agal_NR 2	Sugar diet	Gr5a-Gal4/+	Non-responder	Normal	4837288	2917731
SD_5agal_NR 3	Sugar diet	Gr5a-Gal4/+	Non-responder	Normal	5278428	3239341
SD_5agal_NR 4	Sugar diet	Gr5a-Gal4/+	Non-responder	Normal	5080562	3026423

SD_5aogt_R1	Sugar diet	Gr5a-Gal4/PGal4→ OGT RNAi	Responder	Normal	6048289	3868457
SD_5aogt_R1	Sugar diet	Gr5a-Gal4/PGal4→ OGT RNAi	Responder	Normal	5277202	3301130
SD_5aogt_R1	Sugar diet	Gr5a-Gal4/PGal4→ OGT RNAi	Responder	Normal	5505008	3463351
SD_5aogt_R1	Sugar diet	Gr5a-Gal4/PGal4→ OGT RNAi	Responder	Normal	3624817	2213999
SD_5aogt_R1	Sugar diet	Gr5a-Gal4/PGal4→ OGT RNAi	Responder	Normal	5899915	3634774

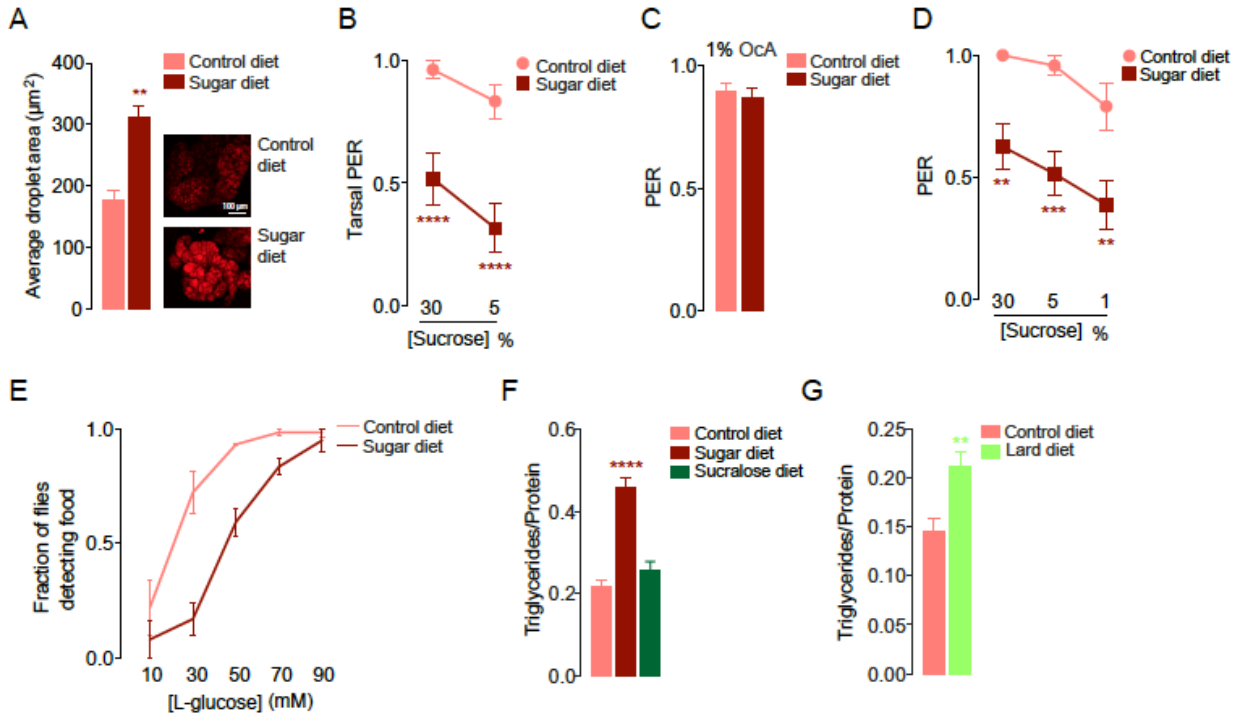
Supplemental Table 2-S 2 Tab 2

Test Condition	**Reference Condition**	**# Genes significantly up (q<0.2)**	**# Genes significantly up (q<0.05)**	**# Genes significantly down (q<0.2)**	**# Genes significantly down (q<0.05)**
SD	CD	377	210	401	244
SD, OGT RNAi	SD, Gr5a-Gal4/+	166	69	112	36

Table 2-S1. Statistics, RNA-seq reads, and differential expression calling. (Related for Figure 2-7 and Supplemental Figure 2-S7).

The first tab of the supplementary table (“Read Stats”) gives information on all RNA-seq samples used in the present study. “Response status” refers to whether or not flies showed normal (responder) or abnormal (non-responder) sugar responses, as described in the Methods. Read counts are given after cutadapt/trimmomatic (preprocessed) and again after kallisto alignment (pseudoaligned); note that the ERCC spike-in mix was not included in the reference transcriptome used for final alignment. In the “Analysis Stats” tab, we indicate the numbers of genes called as significantly up- or

down-regulated in the indicated comparisons of conditions, as assessed using our analysis with the sleuth package (see Methods for details).



Supplemental Figure 2-S 1 Changes in sweet taste sensation and obesity with different diets. Related to Figure 2-1.

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

A) Quantification of lipid droplet size in the adipose tissue of flies fed a control or sugar diet for 7 days. n=1939-4007 droplets in the visceral fat of 3 animals per dietary condition, Wilcoxon signed rank test, comparison to control diet. (Right, Nile Red staining of the fat body on a control or 7-day sugar diet fly, scale bar = 100 μm.)

B) Measurement of taste responses by PER to 30 or 5 % sucrose (x axis) stimulation to the tarsi in age-matched male $w^{1118}CS$ flies fed a control (circles) or sugar (squares) diet for 7 days. n=19-27, two-way ANOVA with Fisher's LSD test, comparisons to control diet per concentration.

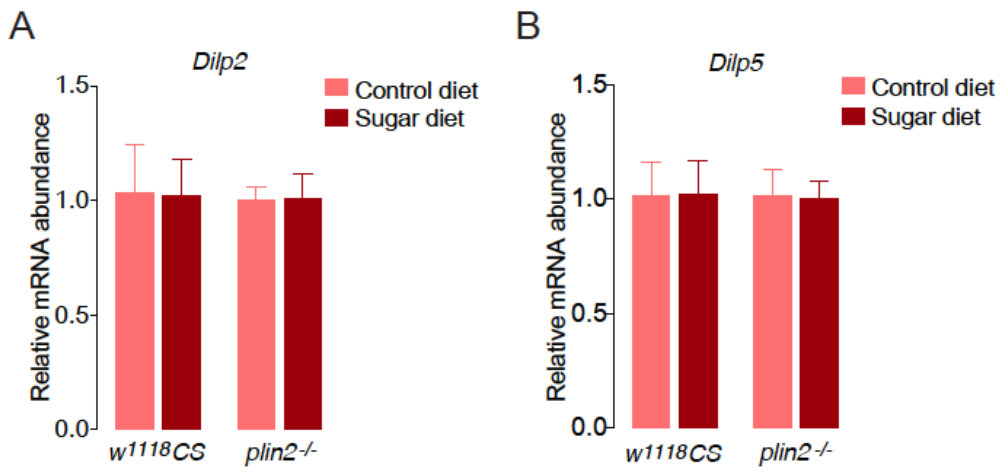
C) Taste responses to stimulation of the proboscis with 1% octanoic acid in w^{1118}*CS* flies fed a control or sugar diet for 7 days. n=43-48, no significance, Mann-Whitney test, comparison to control diet.

D) Taste responses to proboscis stimulation with sucrose in w^{1118}*CS* flies fed a control or sugar diet for 7 days and fasted for 32-34 hours. n=16-21, two-way ANOVA with Fisher's LSD test, comparisons to control diet per concentration.

E) Fraction of flies feeding on different L-glucose concentrations (x axis) following 22 hours fasting. n=50 flies, two-way ANOVA for diet effect.

F) Triglyceride levels normalized to protein in w^{1118}*CS* flies fed a control diet or diets supplemented with 0.02% sucralose or 30% sucrose for 7 days. n=16, one-way ANOVA with Fisher's LSD test, comparisons to control diet.

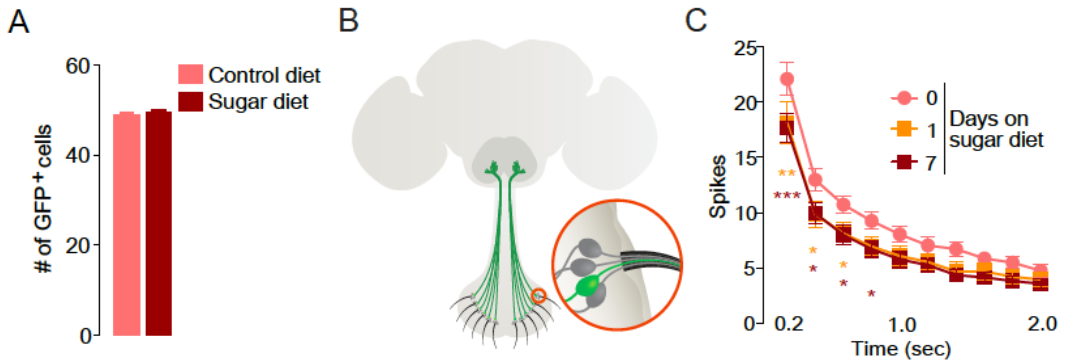
G) Triglyceride levels normalized to protein in flies fed a control or 10% lard diet for 7 days. n=10, unpaired t test, comparison to control diet.



Supplemental Figure 2-S 2 The mRNA levels of Drosophila insulin-like peptides *Dilp2* and *Dilp5* are unchanged in control and *plin2* mutant flies on a sugar diet. Related to Figure 2-2.

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

A-B) Changes in the relative mRNA abundance of A) *Dilp2* and B) *Dilp5* transcripts measured by qPCR in w^{1118}*CS* or *plin2* mutant flies fed a control (*salmon*) or sugar (*burgundy*) diet for 7 days. n=3, two-way ANOVA with Sidak's test, comparisons to control diet.



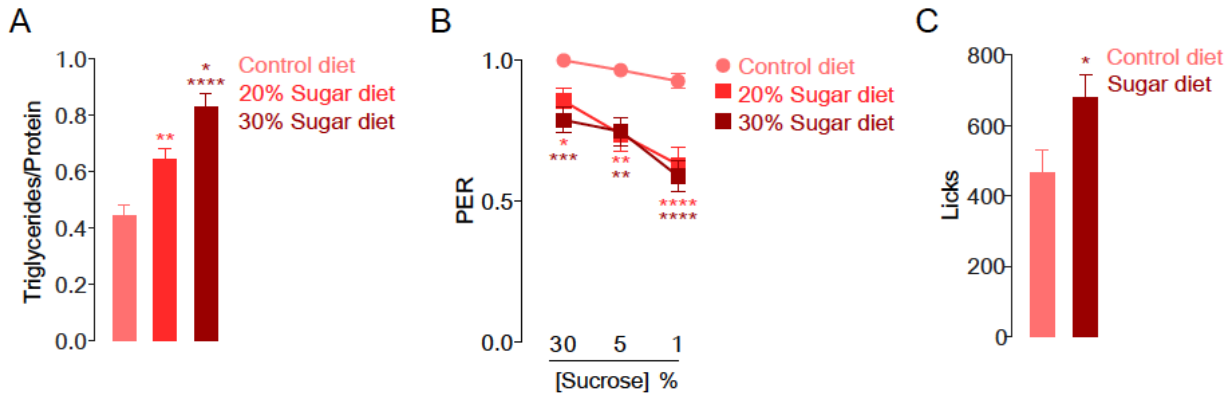
Supplemental Figure 2-S 3 A sugar diet has no effect on the number of sweet taste cells in the proboscis. Related to Figure 2-3.

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

A) Quantification of the number of GFP-labeled cells in the labella of *Gr5a>nls-GFP* flies fed a control or sugar diet for 10 days. n=17-19 probosces, no significance, unpaired t test, comparison to control diet.

B) The cell bodies of chemosensory neurons are in the labellum with dendrites protruding into the taste hair (sensillum, black) and the axons terminating in the SubEsophageal Zone (SEZ, dark grey) of the brain. Sweet taste cells are in green.

C) Quantification of average spike frequency per 200 ms from electrophysiological recordings of the labellar taste sensilla stimulated with 25-mM sugar, in age-matched w^{1118}*CS* flies fed a control or sugar diet for 1 or 7 days. n=10, two-way ANOVA with uncorrected Fisher's LSD, comparisons to control diet per day.



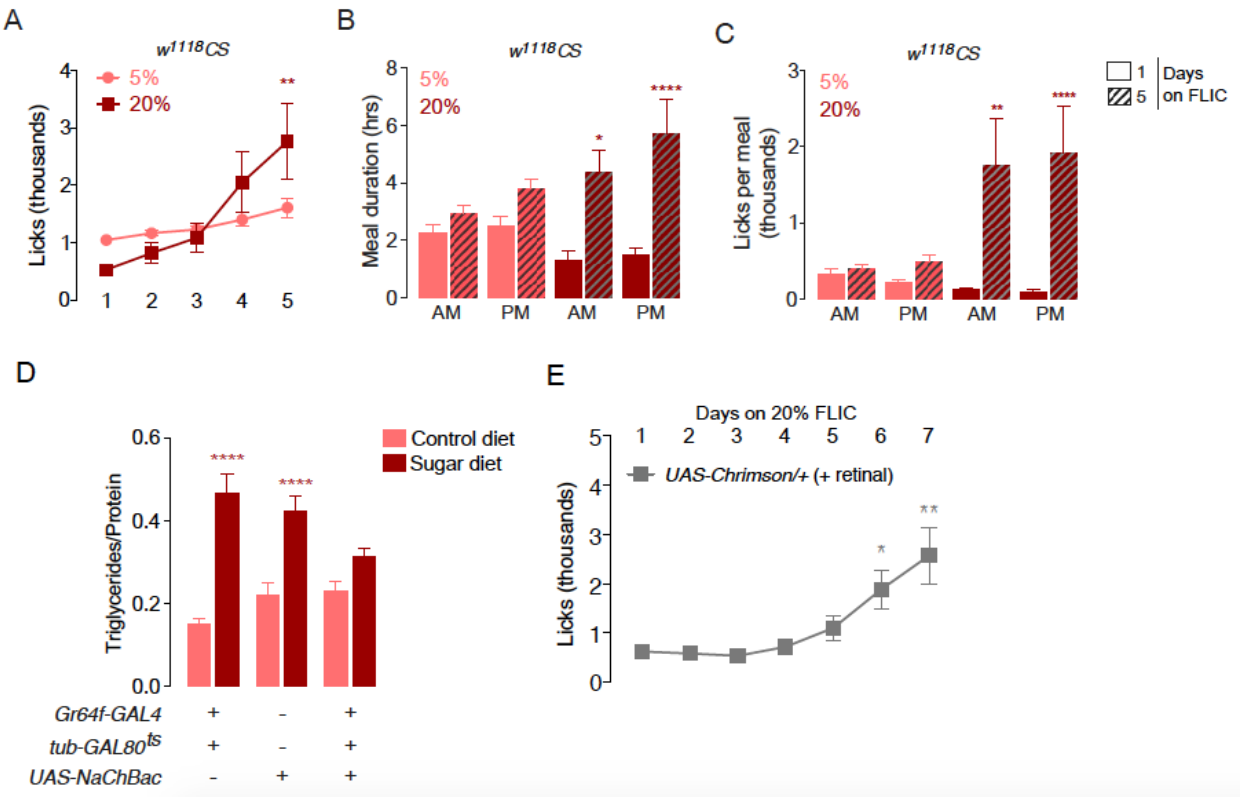
Supplemental Figure 2-S 4 A 20% sucrose diet leads to a decrease in sweet taste responses and obesity. Related to Figure 2-4.

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

A) Triglyceride levels normalized to protein in *w*^{1118}*CS* flies fed a control, 20% or 30% sucrose diet for 7 days. n=8, one-way ANOVA with Tukey's test, comparisons to control and 20% sucrose diet.

B) Taste responses to 30, 5, and 1% sucrose stimulation (x axis) of the labellum in *w*^{1118}*CS* flies fed control, 20% or 30% sucrose diet for 7 days. n=42-52, two-way ANOVA with Fisher's LSD test, comparisons to control and 30% sucrose diet.

C) The feeding behaviors of w1118CS flies fed for 10 days a control or sucrose diet on standard fly vials and assayed for a single day on the FLIC on 20% sucrose. n=24-32, one-way ANOVA with Fisher's LSD test, comparisons to control diet.



Supplemental Figure 2-S 5 Control flies for *bmm*, *plin2* mutant, NaChBac and *csChrimson* FLIC experiments. Related to Figures 2-5 and 2-6.

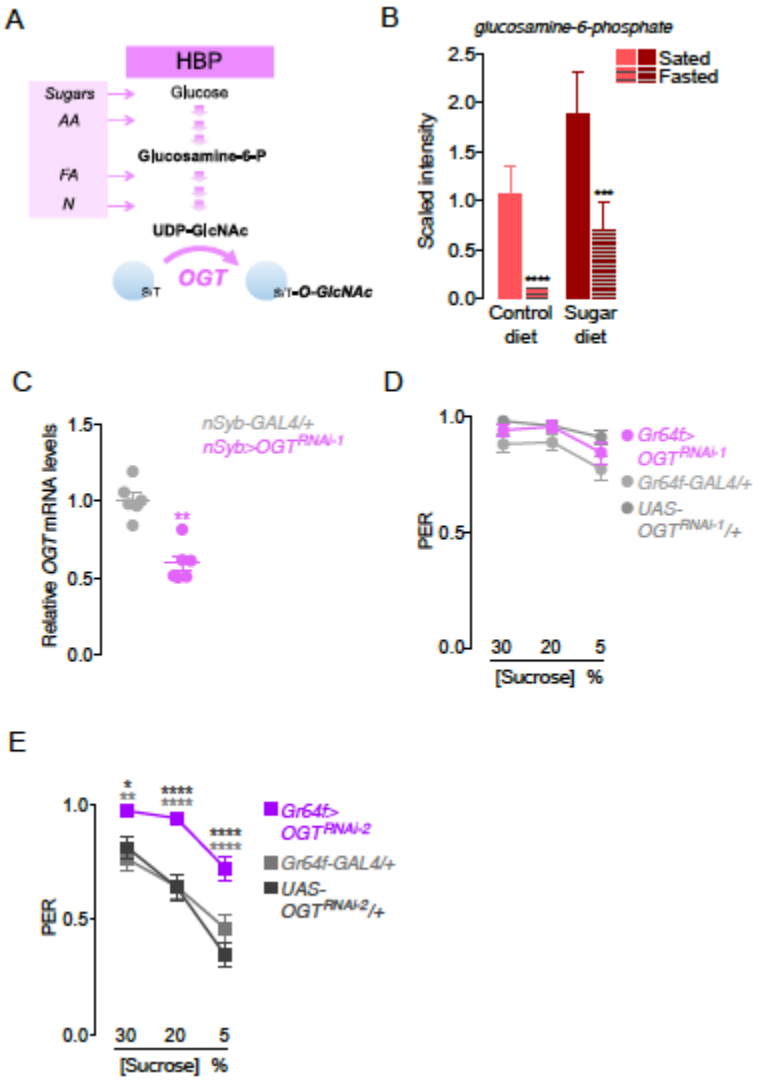
All data shown as Mean ±SEM, **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 for all panels unless indicated.

A) Average daily licks of *w*^{1118}*CS* flies fed 5% and 20% sucrose on the FLIC. n=18-30, two-way ANOVA with uncorrected Fisher's LSD, comparisons to 5% sucrose licks each day.

B-C) Quantification of meal B) duration in hours (hrs) and C) size of the morning (AM) and evening (PM) meals of flies feeding on 5 or 20% sucrose on day 1 (solid bars) and day 5 (hatched bars) on the FLIC. n=17-30 and n=18-30, two-way ANOVA with Fisher's LSD, comparisons to same-diet day 1 meal.

D) Triglyceride levels normalized to protein in *Gr64f>NaChBac, tubulin-GAL80ts* and parental transgenic control flies (crossed to *w*^{1118}*CS*) fed a control or sugar diet for 7 days. n=15-16, two-way ANOVA with Sidak's test, comparisons to control diet per genotype.

E) Average licks of adult *UAS-csChrimson/+* flies (treated with 200 µM all-*trans*-retinal), on 20% sucrose FLIC, without red light stimulation. n=12, one-way ANOVA with Kruskal-Wallis test, comparisons to Day 1.



Supplemental Figure 2-S 6 The HBP mediates changes in sweet taste sensation. Related to Figure 2-7.

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

A) Overview of the Hexosamine Biosynthesis Pathway (HBP): sugars, amino acids (AA), fatty acids (FA), and nucleotide (N) metabolism feed into the HBP. Addition of the N-acetylglucosamine moiety (O-GlcNAc) O-linked to the serine (S) and threonine (T) residues of proteins is catalyzed by the enzyme OGT.

B) The levels of the HBP metabolite glucosamine-6-phosphate in the heads of sated (solid bars) or 24-hour fasted (striped bars) w^{1118}*CS* flies fed a control or sugar diet for 7 days. n=4-5, Diet main effect, **** p=0.001, *** p=0.0289, two-way ANOVA, comparisons to control diet.

C) Fold change of OGT mRNA levels in the heads of flies with and without pan-neuronal OGT knockdown (lavender and grey, respectively) measured by qPCR. n=6, Mann-Whitney test, compared to Gal4 transgenic control (crossed to $w^{1118}CS$).

D) Taste responses to 5, 20, or 30% sucrose stimulation (x axis) of the proboscis in Gr64f>OGT RNAi-1(purple) and parental transgenic control (grey, crossed to $w^{1118}CS$) flies fed a control diet. n=36-60, multiple t tests, comparisons to genetic controls.

E) Taste responses to 30, 20, 5 % sucrose stimulation (x axis) of the labellum in Gr64f>OGT RNAi-2 (purple) and parental transgenic control (grey, crossed to $w^{1118}CS$) flies fed a sugar diet for 7 days. n=51-58, two-way ANOVA with Fisher's LSD test, comparisons to transgenic controls per tastant.

Supplemental Figure 2-S 7 iPAGE profile of differential gene expression changes in the labella of flies fed a sugar diet with or without OGT knockdown. Related to Figure 2-7. **A-B**) iPAGE-based identification of GO pathways enriched in genes showing log2 fold changes in the labella of **A**) w1118CS flies fed a SD for 7 days and **B**) Gr5a>OGT RNAi-1 and Gr5a-Gal4/+ flies, compared to those in the whole transcriptome. Coloration shows hypergeometric p values from iPAGE. The numbers on the horizontal arrow indicate the magnitude of log2 fold changes in each of the 7 bins. In each bin, genes within a GO term may be over or under-represented compared to the transcriptome background.

Methods

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 CO_2 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 $w^{1118}CS$ (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 w^{1118}*CS* flies.

Method Details

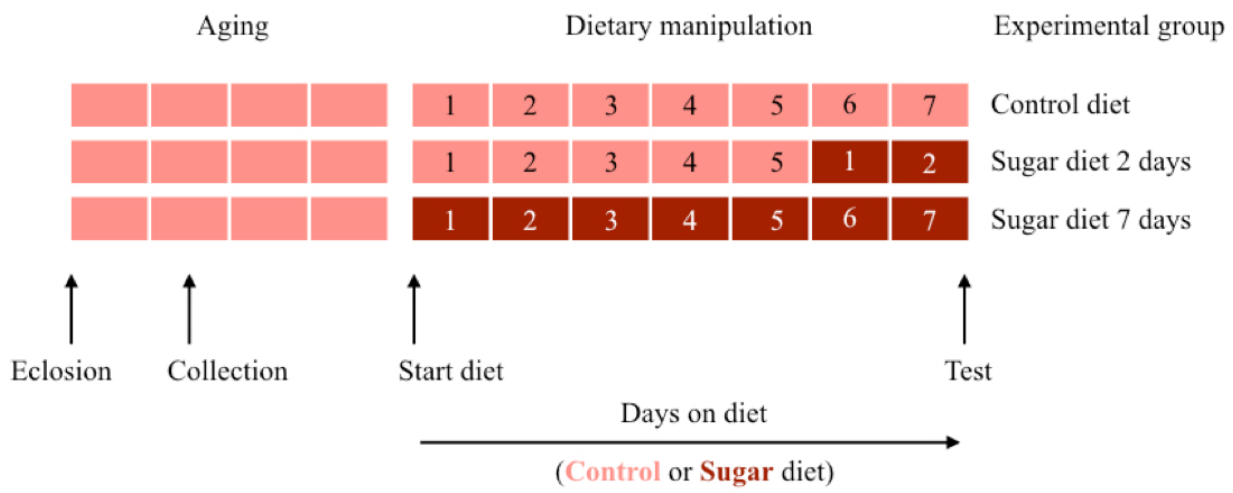
Dietary manipulations

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; 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 schematic below:



Methods Table 2-M 1 Dietary scheme for age-matching.

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 -20ºC. For total protein, supernatant and standards were reacted with protein reagent (Thermo Scientific Pierce™ BCA Protein Assay) for 30 min at 37ºC 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 37ºC, 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) that communicates fly-to-food interactions to computer software called FLICMonitor via a Master Control Unit (MCU). For FLIC experiments, flies were CO_2-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 $MgCl_2$ 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 25ºC 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 real-time 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 25ºC 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 seconds 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 to 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 hemolymph solution (recipe in mM: 108 NaCl, 8.2 $MgCl_2$, 4 $NaHCO_3$, 1 NaH_2PO_4, 2 $CaCl_2$, 5 KCl, 5 HEPES, pH 7.4) and the dorsal cuticle between the eyes was removed by microdissection. Stimulus (a piece of Kimwipe soaked in tastant and held in a clean pair of forceps) delivery to the proboscis was manual and timed to coincide with the 100^{th} recording sample of each time series. Imaging was done with an upright confocal microscope (Olympus, FluoView 1200 BX61WI) with a 20x water-immersion objective and laser excitation at 488 and 543 nm. Images were recorded at 4 Hz (512 x 512 pixels). The plane of interest was kept to the most ventral neuropil regions innervated by the *Gr64f+* neurons.

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 by 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 probosces were dissected in 1xPBS and homogenized in Trizol (Ambion). RNA was extracted by chloroform followed by RNA clean up using Rneasy MinElute 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 vs 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

Probosces from *Gr5a>nls-GFP* flies were dissected in 1xPBS and fixed in 4% PFA, mounted in FocusClear (CelExplorer) on coverslips, 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 (Supplementary Fig. 7) and from 10 heads per group with 3 biological replicates in the dilp2 and dilp5 experiment (Supplementary Fig. 2). 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:

Rp49 Forward	ATGCTAAGCTGTCGCACAAA
Rp49 Reverse	ACTTCTTGAATCCGGTGGGC
OGT Forward	CGTCCGCGGCCCATATATTA
OGT Reverse	CCAACTCGAGTAAACCGACTGA

Dilp2 Forward	TCAATCCCCTGCAGTTTGTC
Dilp2 Reverse	TTGAGTACACCCCCAAGATA
Dilp5 Forward	TCCTGATCCCGCTCCTGCTA
Dilp5 Reverse	TGCCTCGTTTGGCGAACATT

Methods Table 2-M 2 Primer sequences for dILP qPCR.

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 *[(meal end) - (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 +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. 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 $\Delta F/F_0$ 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 recognizable fragment of the Nugen sequencing adapter 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 postprocessing 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 Supplementary Table 1. For the pathway analysis in Supplementary 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 Fig. 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 *Gr5a-Gal4/UAS-OGT RNAi* flies and the corresponding *Gr5a-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 Fig. 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 Fig. 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.

For PER experiments, we were interested in testing whether a sugar diet impaired responsiveness. We performed multiple t-tests and not ANOVA because PER data is inherently ceilinged and floored, and homogeneity of variance cannot be guaranteed, which renders ANOVA invalid.

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).

RNA sequencing data sets are available at the Gene Expression Omnibus under Accession #GSE113159.

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Chapter 3: Dietary Sugar Inhibits Satiation by Decreasing Central Processing of Sweet Taste

Please see the Preface (p. iv) for contributor information.

Abstract

From humans to flies, exposure to diets rich in sugar and fat lowers taste sensation, changes food choices, and promotes feeding. However, how these peripheral alterations influence eating is unknown. Here we used the genetically tractable organism *D. melanogaster* to define the neural mechanisms through which this occurs. We characterized a population of protocerebral anterior medial dopaminergic neurons (PAM DANs) that innervates the β'2 compartment of the mushroom body and responds to sweet taste. In animals fed a high sugar diet, the response of PAM-β'2 to sweet stimuli was reduced and delayed, and sensitive to the strength of the signal transmission out of the sensory neurons. We found that PAM-β'2 DANs activity controls feeding rate and satiation: closed-loop optogenetic activation of β'2 DANs restored normal eating in animals fed high sucrose. These data argue that diet-dependent alterations in taste weaken satiation by impairing the central processing of sensory signals.

Introduction

Consumption of diets high in sugar and fat decreases the perception of taste stimuli, influencing food preference and promoting food intake (Bartoshuk et al. 2006; Sartor et al. 2011; Kaufman et al. 2018; Ahart et al. 2019; May et al. 2019; Weiss et al. 2019). Recent studies have examined the effects of these diets on the sensitivity of the peripheral taste system and the intensity of taste experience (Maliphol, Garth, and Medler 2013; Kaufman et al. 2018; May et al. 2019; Weiss et al. 2019), but how exactly taste deficits increase feeding behavior is not known. Orosensory signals determine the palatability or "liking" for foods (Berridge and Kringelbach 2015), but they also promote meal termination via a process called "sensory-enhanced (or mediated) satiety" (Chambers, McCrickerd, and Yeomans 2015). Indeed, foods that provide longer and more intense sensory exposure are more satiating, reducing hunger and subsequent test-meal intake in humans (Cecil, Francis, and Read 1998; Bolhuis et al. 2011; Viskaal-van Dongen, Kok, and de Graaf 2011; Yeomans and Chambers 2011; Forde et al. 2013; Ramaekers et al. 2014). Specifically, sensory signals are thought to function early in the satiety cascade (J. E. Blundell, Rogers, and Hill 1987) by promoting satiation and bringing the on-going eating episode to an end (J. Blundell et al. 2010; Bellisle and Blundell 2013). This is in contrast to nutrient-derived signals, which develop more slowly and consolidate satiety by inhibiting further eating after the end of a meal (J. Blundell et al. 2010; Bellisle and Blundell 2013). We reasoned that if orosensory attributes like taste intensity are important to curtail a feeding event, then diet-dependent changes in taste sensation could promote feeding by impairing sensory-enhanced satiation. Here we

investigated the relationship between diet composition – specifically high dietary sugar – the central processing of sweet taste signals, and satiation by exploiting the simple taste system and the conserved neurochemistry of the fruit fly *D. melanogaster*.

Like humans and rodents, fruit flies exposed to palatable diets rich in sugar or fat overconsume, gain weight, and become at-risk for obesity and metabolic syndrome (Musselman and Kühnlein 2018). We recently showed that, in addition to promoting feeding by increasing meal size, consumption of high dietary sugar decreased the electrophysiological and calcium responses of the *Gr64f+* sweet sensing neurons to sweet stimuli, independently of weight gain (May et al. 2019). These physiological changes in the *Gr64f+* cells reduced the fruit flies' taste sensitivity and response intensity. Opto- and neurogenetics manipulations to correct the responses of the *Gr64f+* neurons to sugar prevented animals exposed to high dietary sugar from overfeeding and restored normal meal size (May et al. 2019). Thus, the diet-dependent dulling in sweet taste causes higher feeding in flies, but how does this happen? How do alterations in the peripheral sensory neurons modulate a behavior as complex as feeding? To better understand how this occurs, we decided to examine the effects of high dietary sugar and taste changes in the central processing of sweet stimuli by dopaminergic neurons (DANs) in the Protocerebral Anterior Medial (PAM) cluster, which respond to the sweet sensory properties to signal sugar reward (Burke et al. 2012; Liu et al. 2012) and reinforce short term appetitive memories (Yamagata et al. 2015; Huetteroth et al. 2015). We hypothesized that impairments in the peripheral responses to sugar could influence the way sweet taste information is transduced through PAM-DANs to affect feeding.

We found that in flies fed a high sugar diet the presynaptic responses of a specific subset of PAM DANs to sweet taste are decreased and delayed. These changes are specific to sweet stimuli and mediated by high dietary sugar. Further, we show that the reduction in the central processing of sweet taste information increases the duration and size of meals: closed-loop optogenetic stimulation of a specific set of PAM DANs corrected meal size, duration, and feeding rate. Together, our results argue that diet-dependent alterations in the central processing of sweet sensory responses delay meal termination by impairing the process of sensory-enhanced satiation.

Results

Consumption of a high sugar diet decreases and delays the central processing of the sweet taste signal

In the absence of mapped II-order labellar sweet taste neurons, we used the genetically encoded vesicular release sensor *synaptobrevin-pHluorin* (*syb-pHluorin)* (Poskanzer *et al.* 2003) to ask if the transmission of the sweet taste signal out of the *Gr64f+* sensory neurons was decreased. We measured the *in vivo* fluorescence from the *Gr64f+* presynaptic terminals in the Sub Esophageal Zone (SEZ) in response to 30% sugar stimulation of the proboscis. We found that the *syb-pHluorin* fluorescent changes upon sugar presentation were markedly decreased when flies were fed a high sugar diet (SD, 30% sucrose) for 7 days, compared to age-matched flies fed a control diet (CD, ~8% sucrose) (Figure 3-1A, B). These data suggest that both the responses of the sweet sensing *Gr64f+* neurons to sugar and the transmission of the sweet taste signal are impaired by exposure to the SD.

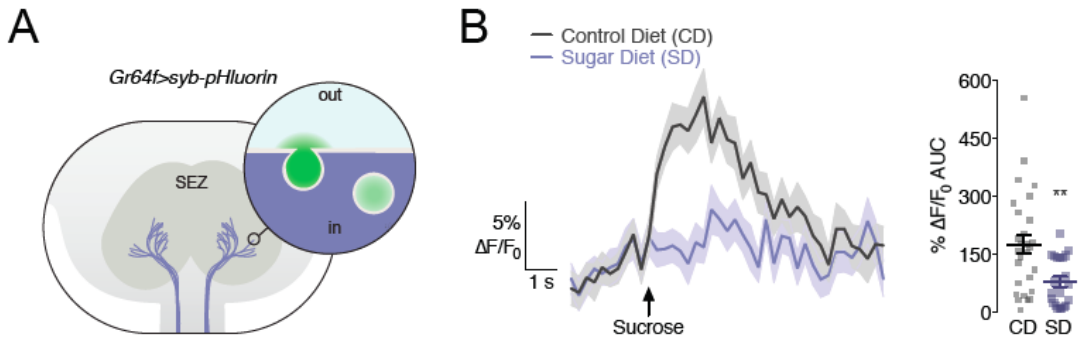


Figure 3- 1 Vesicular release from the *Gr64f+* taste neurons in response to a sucrose stimulus is decreased in flies fed a high sugar diet.

A) Schematic of the subesophageal zone (SEZ), highlighting the *Gr64f+* neuron terminals in lavender. Popout bubble demonstrates increased fluorescence upon vesicular release.

B) *Left,* Mean %$\Delta F/F_0$ response traces and *Right*, Area-under-the-curve (AUC) value of %$\Delta F/F_0$ responses when *Gr64f>syb-pHluorin* flies fed a control (CD, *grey*) or sugar diet (SD, *lavender*) were stimulated with 30% sucrose on the labellum. n=22-28; shading and error bars depict the standard error of the mean. Mann-Whitney test; *** p<0.001.

While the neural pathways that bring sensory information from the periphery to the higher order brain regions are unique across organisms, dopaminergic circuits dedicated to the central processing of sweet taste information exists in humans, rodents, and fruit flies; interestingly, the taste and nutrient properties of sugar are relayed via distinct pathways in these organisms (Yamagata et al. 2015; Huetteroth et al. 2015; Tellez et al. 2016; Thanarajah et al. 2019). Since the involvement of DANs in feeding behavior and in central processing of sensory information is a homologous feature, we decided to center on this circuit as a possible link between diet-dependent changes in sweet responses, higher feeding, and weight gain. In flies, DANs in the Protocerebral Anterior Medial (PAM) cluster that are labeled by the *R48B04-Gal4*

transgene and innervate the β’2 and γ4 compartments of the Mushroom Body (MB), respond to sweet sensory properties (Huetteroth et al. 2015; Yamagata et al. 2015); neurons of this population also reinforce water taste (Lin et al. 2014). Here we focused on the β’2 compartment because of its role in processing of the taste properties alone, compared to γ4, which is modulated by both taste and additional factors, such as internal state (Lin et al. 2014; Yamagata et al. 2015). In addition to labeling ~60 DANs in each PAM cluster, *R48B04* is expressed in other neurons, including in the SEZ. To avoid potential confounding effects of its expression in the SEZ, we used FlyLight to visually identify *Gal4* lines that label subsets of PAM-β’2, but do not label neurons in the SEZ (Aso and Rubin 2016), and identified the split-*Gal4* line *MB301B,* which labels ~12 TH+ neurons in PAM-β2β'2a (Figure 3-2A and Supplemental Figure 3-S1A). We then used the presynaptically targeted *GCaMP6s::Bruchpilot::mCherry* (Kiragasi et al. 2017) to record the response of *MB301B* neurons to stimulation of the labellum with 30% sucrose. We observed an increase in signal in the β’2 compartment, showing that these PAM-β’2 neurons process sweet sensory information (Figure 3-2B, grey lines). Next we measured the responses of *MB301B* neurons to sucrose taste in flies fed a SD for 7 days and we found a nearly 50% decrease (Figure 3-2B, rose lines). Furthermore, when we looked at both the average and individual traces, we saw a ~600 millisecond delay in the peak responses to the sucrose stimulus delivery to the labellum (Figure 3-2C). No sugar taste responses were recorded in β2, consistent with the idea that it is not involved in taste processing (Figure 3-2D). Thus, the central processing of sweet stimuli in PAM-β’2 *MB301B* neurons is both decreased and delayed by exposure to a high sugar diet.

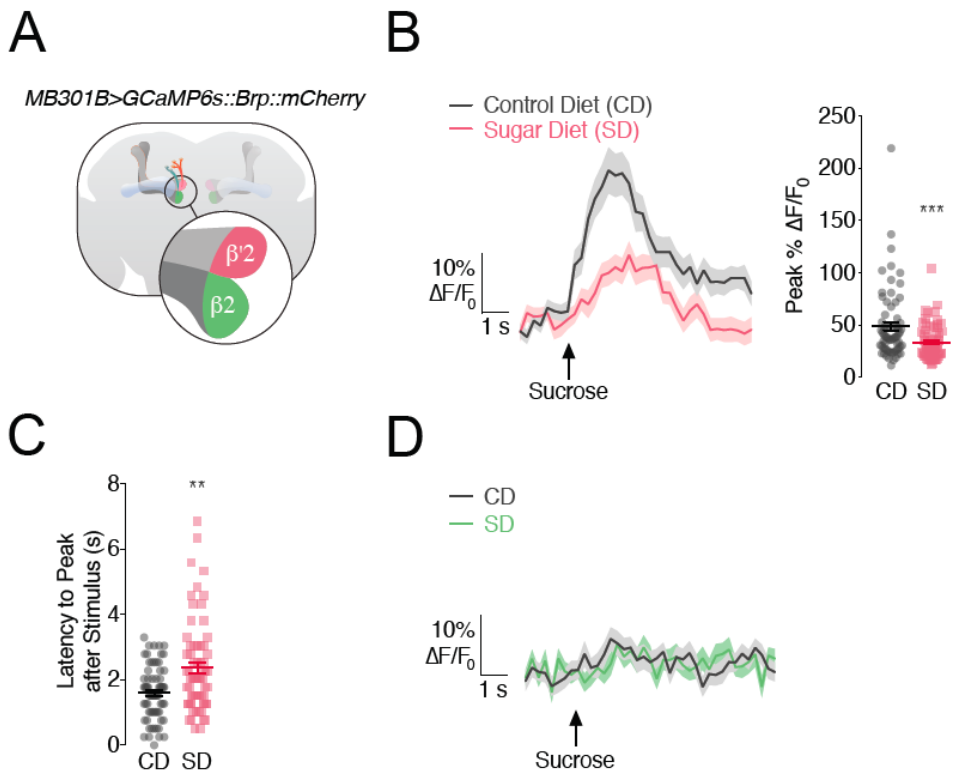


Figure 3- 2 The responses of PAM-β'2 neurons to sweet stimuli change in flies fed a high sugar diet

A) Anatomy of the Mushroom Body (MB) of the *Drosophila melanogaster* brain, with α/β, α'/β' lobes in greys, γ in translucent blue, and *MB301B* neurons in rose and green; *popout bubble*, schematic showing the β'2 (*rose*) and β2 (*green*) compartments in their respective MB lobes.

B) *Left,* Mean %$\Delta F/F_0$ traces and *Right,* quantification of the maximum peak %$\Delta F/F_0$ responses to 30% sucrose stimulation of the labellum in the β'2 compartment of *MB301B>GCaMP6s::Brp::mCherry* flies fed a control (CD, *grey*) and sugar diet (SD, *rose*). n=67-70; Shading and error bars are standard error of the mean. n=67-70; Mann-Whitney test; *** p<0.001.

C) The delay in the calcium responses quantified as latency in seconds (s) to maximum peak $\Delta F/F_0$ from the animals in B. n=67-70; Mann-Whitney test; ** p<0.01.

D) Mean $\%\Delta F/F_0$ traces for the responses to 30% sucrose stimulation of the labellum in the β2 compartment of *MB301B>GCaMP6s::Brp::mCherry* flies fed a control (CD, *grey*) and sugar diet (SD, *green*). n=67-70; shading is standard error of the mean.

Alterations in PAM-β'2 responses are specific to high dietary sugar and sweet stimuli

The reduction and delay in central responses to sugar taste in PAM-β'2 DANs on a SD could be due either to the lower transmission of the sensory signal out of the peripheral sweet taste neurons (Figure 3-1) or to the metabolic side effects of the high nutrient diet. To differentiate between these possibilities, we took multiple approaches. In addition to sweet stimuli, PAM-β'2 neurons also respond to water (Lin et al. 2014); we reasoned that if high dietary sugar unspecifically changed the activity of the PAM-β'2, we would expect flies on the SD to also exhibit impaired central responses to water. However, the magnitude and timing of the β'2 response to water stimulation of the labellum was unchanged between flies on a CD or SD (Figure 3-3A, B, water stimulation was delivered in the same flies as in Figure 3-2). Thus, the decrease in PAM-β'2 responses in flies fed a SD is specific to the sweet sensory stimulus. This argues that the overall ability of these DANs to respond to stimuli is not generally affected, and the reduction observed on a SD could occur because of the diet-dependent changes in the sweet taste neurons in the periphery ((May et al. 2019) and Figure 3-1).

To further probe this question, we fed flies a high fat diet (FD), which has the same caloric content of the high sugar diet (SD) and promotes fat accumulation, but does not decrease the responses of the *Gr64f*+ sensory neurons to sugar stimuli (May et al.

2019). If changes in PAM-β'2 responses to sugar taste occur because of the metabolic side-effects of high nutrient density (i.e, fat accumulation) – rather than via changes in the sweet sensory neurons output – we would expect a FD to also induce PAM-β'2 dysfunction. However, a FD diet had no effect on the PAM-β'2 responses to sucrose or water stimulation of the labellum in *MB301B>GCaMP6s::Bruchpilot::mCherry* flies (Figure 3-3C, D and Supplemental Figure 3-S2A, B). Together, these two lines of evidence argue that the dysfunction in the processing of sweet taste stimuli in the PAM-β'2 neurons of flies on a SD is linked to alterations in the peripheral sensory processing of sugar taste caused by high dietary sugar.

To test this hypothesis directly, we examined the effect of correcting sweet taste sensation on the responses of the PAM-β'2 *MB301B* neurons to sugar. To rescue the sweet taste deficits caused by a high sugar diet we fed flies an inhibitor of the metabolic-signalling enzyme O-GlcNAc-Transferase (OGT), which we previously found to be responsible for decreasing sweet taste on a SD (May et al. 2019). In accordance with our previous findings on OGT (May et al. 2019), supplementing the flies' diet with 75 μM of OSMI-1 (OGT-small molecule inhibitor 1) resulted in no changes in PER between a CD and SD (Supplemental Figure 3-S2C). In these flies, the calcium responses of PAM-β'2 neurons to sucrose stimulation of the labellum were identical in SD+OSMI and CD+OSMI flies, consistent with the idea that deficits in the peripheral responses drive impairments in the central processing of sweetness (Figure 3-3E, F). Together, these orthogonal lines of evidence show that the impairments in the central processing of sweet sensory information by DANs are mediated by deficits in peripheral sweet taste responses.

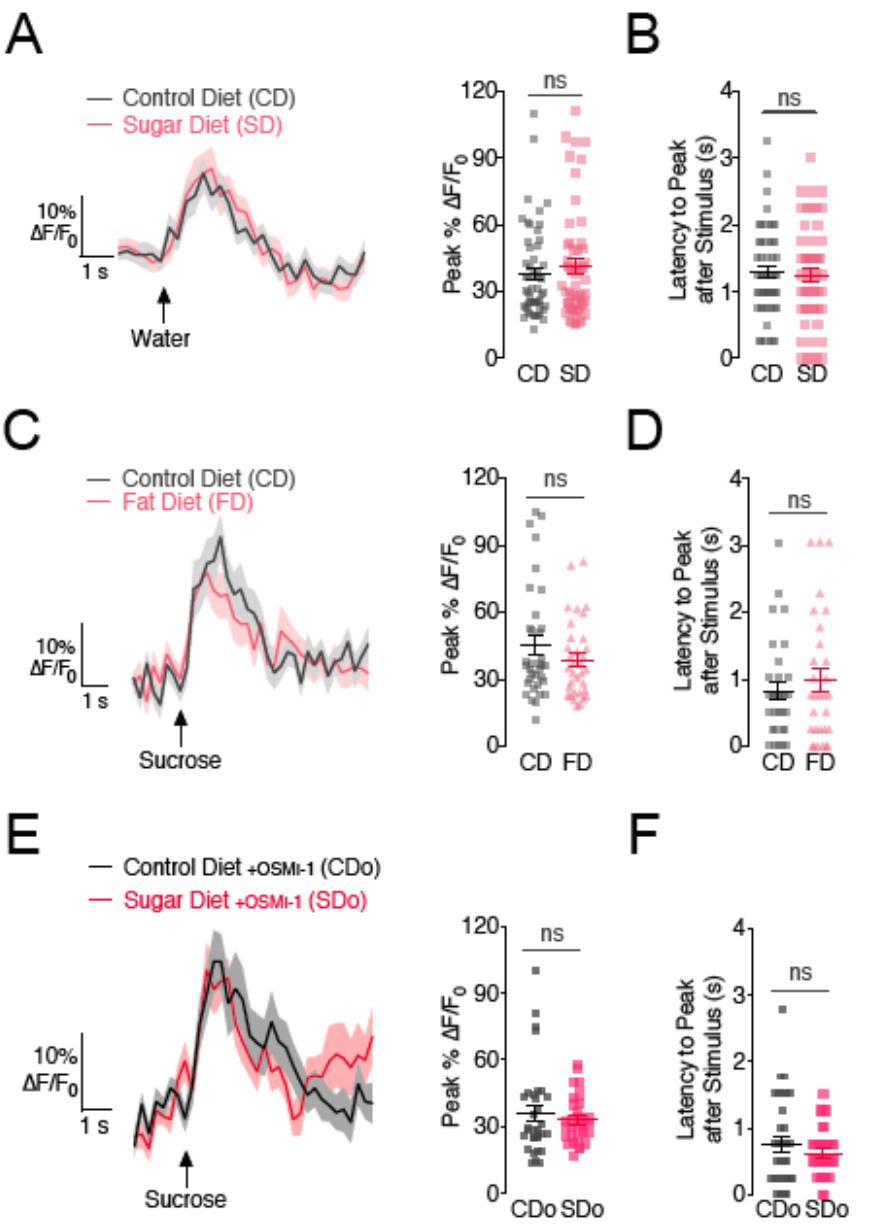


Figure 3- 3 The changes in PAM-β'2 activity are specific to sugar stimuli and caused by deficits in sweet sensation.

A) *Left,* Mean %$\Delta F/F_0$ traces and *Right,* quantification of the maximum peak %$\Delta F/F_0$ responses to water stimulation of the labellum in the β'2 compartment of *MB301B> GCaMP6s::Brp::mCherry* flies fed a control (CD, *grey*) and sugar diet (SD, *rose*), same animals as in Figure 2. n=67-70; shading and error bars are standard error of the mean. Mann-Whitney test; no significance.

B) The delay in the calcium responses quantified as latency in seconds (s) to maximum peak $\Delta F/F_0$ response from the animals in A. n=67-70; error bars are standard error of the mean. Mann-Whitney test; no significance.

C) *Left:* Mean %$\Delta F/F_0$ response traces and *Right,* quantification of the maximum peak %$\Delta F/F_0$ responses to 30% sucrose stimulation of the labellum in the β'2 compartment of *MB301B>GCaMP6s::Brp::mCherry* flies fed a control (CD, *grey*) or high fat diet (FD, *rose*)

n=31-32; shading and error bars are standard error of the mean. Mann-Whitney test; no significance.

D) Latency-to-peak response times for the animals in C. n=31-32; error bars are standard error of the mean. Mann-Whitney test; no significance.

E) *Left,* Mean $\%\Delta F/F_0$ traces and *Right,* quantification of the maximum peak $\%\Delta F/F_0$ responses to sucrose stimulation of the labellum in the β'2 compartment of *MB301B>GCaMP6s::Brp::mCherry* flies fed a control (CD, *charcoal*) and sugar diet (SD, *red*) supplemented with 75 μM OSMI-1. n=30-32; shading and error bars are standard error of the mean. Mann-Whitney test; no significance.

F) The delay in the calcium responses quantified as latency in seconds (s) to maximum peak $\Delta F/F_0$ response from the animals in E. n=30-32; error bars are standard error of the mean. Mann-Whitney test; no significance.

Correcting the activity of PAM DANs rescues feeding behavior

We previously showed that a diet-dependent dulling of sweet taste drives higher feeding behavior and weight gain by increasing the size and duration of meals (May et al. 2019). Since sweet taste deficits underlie the changes in PAM-β'2 activity, we reasoned that impairments in the central processing of orosensory signals may also play a role in promoting higher feeding in animals fed a high sugar diet. Specifically, if PAM-β'2 neurons were critical for integrating sweet taste information into feeding decisions, then correcting their activity may also prevent increased eating and weight gain when flies are exposed to a SD. To test this possibility we expressed the light-activated cation channel *ReaChR* (Inagaki et al. 2014) in the *MB301B* neurons, and used the optoFLIC, a feeding frequency assay (Ro, Harvanek, and Pletcher 2014)

modified for closed-loop optogenetic stimulation (May et al. 2019), to stimulate the activity of PAM-β’2 neurons only when the flies were interacting with the food starting at day 3. *MB301B>ReaChR* flies that did not receive retinal supplementation (ATR, all-*trans*-retinal is required to form a functional light-sensitive opsin) exhibited the characteristic increase in feeding behavior on 20% sucrose (Figure 3-4A, *rose line*); however, *MB301B>ReaChR* +ATR animals, which were activated by light, had stable feeding for 10 days (Figure 3-4A, *peach line*). Control animals on 20% sucrose had more feeding interactions per meal and longer meal duration with more days on the SD (Figure 3-4B and C, *rose lines*), consistent with our previous data (May et al. 2019). In particular, we found that a SD induced a lengthening of the peak-to-end of the meal by ~4 hours, suggesting that the satiation process is delayed in these animals (Figure 3-4D, *rose line*). However, feeding-paired stimulation of PAM-β’2 neurons stabilized the size and duration of the meal, as well as the time to satiation, over the entire duration of the experiment (Figure 3-4B, C, and D, *peach lines*). Interestingly, activation of the *Gr64f+* sweet taste neurons also corrected these two aspects of meal structure (May et al. 2019). Importantly, flies in which these PAM-β’2 DANs were activated still developed taste deficits on a SD (Supplemental Figure 3-S3A), arguing against the possibility that PAM-β’2 DANs activation prevents increased feeding by rescuing the taste changes in the *Gr64f+* neurons. Instead, our data suggest that PAM-β’2 DANs modulate meal structure and feeding behavior by integrating the sensory signal from the periphery. In accordance with the stable feeding patterns recorded on the optoFLIC, we found that activation of PAM-β’2 DANs also prevented diet-induced obesity in animals fed high dietary sugar (Supplemental Figure 3-S3B). Interestingly, PAM-β’2 DANs labeled by

MB301B seem to play a unique role in this process. Activation of different subpopulations of PAM-β'2 with 8 distinct *Gal4* transgenes (Aso and Rubin 2016) (*MB056B*, *MB109B*, *MB042B*, *MB032B*, *MB312B*, *MB196B*, *MB316B*, some of these also express in γ4) failed to rescue diet-induced obesity (Supplemental Figure 3-S3C). Further, flies with activation of nutrient-responsive PAM DANs (Yamagata et al. 2015; Huetteroth et al. 2015), which express in β2, still accumulated fat as controls when fed high dietary sugar, suggesting that effects of *MB301B* neuron activation come from the β'2 compartment alone (Supplemental Figure 3-S3D).

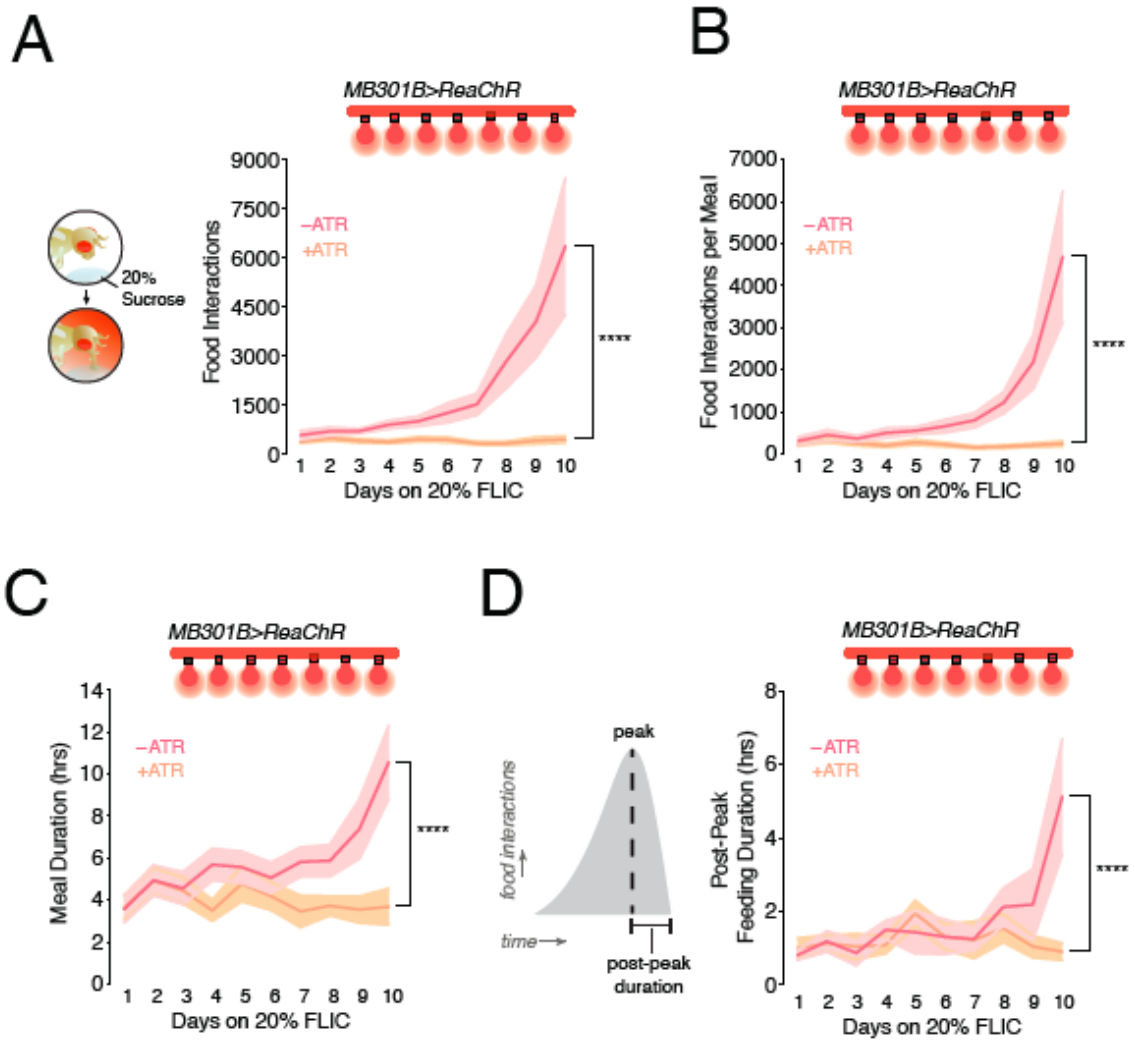


Figure 3- 4 Closed-loop optogenetic activation of PAM-β'2 neurons corrects meal size and duration in flies fed a high sugar diet.

A) *Left:* Conceptual schematic for the closed-loop optogenetic FLIC (optoFLIC), wherein a fly feeding on the 20% sucrose food triggers delivery of the red light during the food interaction. *Right:* Mean number of food interactions per day for *MB301B>ReaChR* flies fed 20% sucrose on the optoFLIC . Closed-loop light delivery was started on day 3 (indicated with *red light bulbs*). Control flies were not fed retinal (-ATR, *rose*), while experimental animals were fed retinal food before starting the experiment on the optoFLIC (+ATR, *peach*). n=8-11; shading is standard error of the mean. Two-way Repeated Measure (RM) ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

B) The size of the evening meal measured as the number of food interactions per meal for animals in **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

C) The duration of the evening meal for animals in **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

D) *Left*, schematic of an evening meal, and *Right*, mean duration of the portion of the evening meal after the peak (satiation) in animals from **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

PAM-β'2 activity modulates the feeding rate during a meal

Since the FLIC records feeding interactions every 200 milliseconds (Ro, Harvanek, and Pletcher 2014), we used this information to look at how feeding rate changed during a meal, as this has been linked to the process of satiation. To do this, we first calculated the number of feeding events per meal, where a feeding event is defined as a succession of consecutive feeding interactions above an established signal threshold,

(see Methods, and (Ro, Harvanek, and Pletcher 2014)). We next divided the number of events per meal by the duration of each meal per day to obtain a feeding rate and to control for the fact that meals last longer on a SD. We found that both the feeding events per meal and the feeding rate increased with chronic exposures to high dietary sugar (Figure 3-5A and B). However, optogenetic stimulation of PAM-β'2 prevented these increases and maintained a stable number of events and a constant feeding rate per meal over the duration of the experiment. We next examined whether the feeding rate changed during the course of the meal, by calculating it *before* and *after* the peak of meal feeding (Figure 3-5C, diagram). The feeding rate past the peak of the meal increased with time in animals fed 20% sucrose, but stayed the same in flies with activation of PAM-β'2 neurons (Figure 3-5C). Interestingly, the pre-peak eating also increased gradually with exposure to high dietary sugar (Figure 3-5D). Together, these data suggest that diet-dependent impairments in PAM-β'2 neurons promote overfeeding by impairing satiation, and specifically by affecting the feeding rate during a meal. Since PAM-β'2 neurons process sensory experiences from the periphery, our experiments argue that this phenomenon is connected to sensory-enhanced satiation. Together we propose that the central processing of sensory experiences during a meal by PAM-β'2 DANs, controls feeding rate and sensory-enhanced satiation. This process is altered by high dietary sugar, leading to an attenuated satiation process and higher feeding (Figure 3-5E).

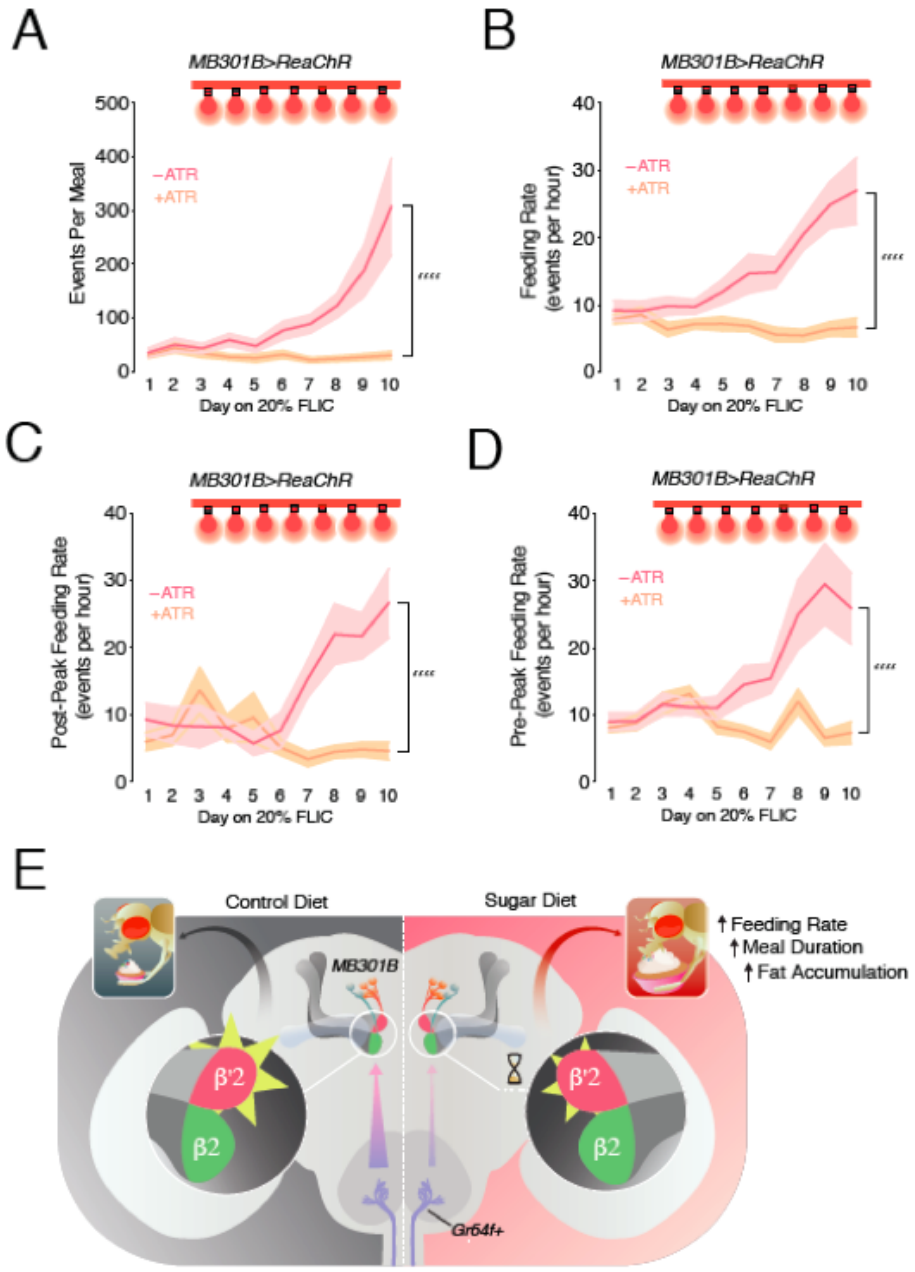


Figure 3- 5 Feeding rate is modulated by a high sugar diet and controlled by the activity of PAM-β'2 neurons.

A) The mean of total feeding events per meal in *MB301B>ReaChR* flies with (- ATR, *rose*) or without (+ATR, *peach*) retinal pretreatment. A feeding event is calculated as the number of consecutive licks above and below the signal threshold (see Methods). n=8-11; shading is

standard error of the mean. Two-way Repeated Measures (RM) ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

B) The feeding rate per meal, calculated as the mean number of events per hour of mealtime in the animals from **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

C) Quantification of the mean feeding rate *after* the peak of the meal in animals from **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

D) Quantification of the mean feeding rate *before and including* the peak of the meal from flies in **A**. n=8-11; shading is standard error of the mean. Two-way RM ANOVA; ****p<0.0001, Time by Retinal-treatment interaction.

E) Model of the sweet taste and PAM DAN circuit changes when flies are fed a control (*left*) or high sugar diet (*right*): a decrease in the output of the *Gr64f+* neurons (*lavender axons, arrows*) contributes to a decrease (*yellow rays*) and a delay (*hourglass*) in the central processing of sweet taste information in the *PAM-β'2* terminals (*rose*), promoting higher feeding.

Discussion

In this study we found that diet-dependent changes in sensory perception promote feeding and weight gain by impairing the central dopaminergic processing of sweet taste information. When animals consume a high sugar diet, the responses to sweet taste of a distinct population of PAM DANs innervating the β'2 compartment of the MB are decreased and delayed. These alterations in dopaminergic processing increase the eating rate and extend the duration of meals, leading to attenuated satiation, higher feeding and weight gain (Figure 3-5E). Interestingly, we observed a reduction in PAM DAN responses only when flies ate diets that resulted in sweet taste deficits;

consumption of an equal calorically-rich lard diet that did not impact taste had no effect on the PAM DANs responses. Similarly, animals fed high dietary sugar exhibited differences in PAM-β'2 responses to sweet, but not water taste stimuli, reinforcing the idea that PAM DAN alterations occur because of lower signal transmission from the sensory neurons (Figure 3-5E). Indeed, correcting sweet taste deficits also prevented impairments in PAM-β'2 responses. Thus, we propose a model where diet-dependent changes in taste intensity and sensitivity reduce the central processing of sensory stimuli to cause weaker and attenuated satiation. A weakness of the current study is that we were unable to follow the transmission of the taste signal from the primary sensory neurons through the different circuits that eventually communicate with PAM. Studies that will identify taste projection neurons genetically will allow us to further probe this point in the future.

Studies in rodents and humans have delineated the importance of sensory signals to modulate satiation and terminate meals. This process, termed sensory-enhanced satiation (Chambers, McCrickerd, and Yeomans 2015), plays an early role in the satiety cascade before post-oral nutrient-derived signals consolidate satiety (J. E. Blundell, Rogers, and Hill 1987; Bellisle and Blundell 2013). Studies show that higher sensory intensity and oral exposure promote stronger satiation (Bolhuis et al. 2011; Ramaekers et al. 2014). For example, high sensory characteristics, such as saltiness and sweetness, enhanced the satiating effect of both low and high energy test drinks (Yeomans and Chambers 2011; Yeomans et al. 2014), and decreased consumption of pasta sauce (Yeomans 1998, 1996), yoghurt (Vickers, Holton, and Wang 2001) and tea (Vickers and Holton 1998). However, the neural basis for this phenomenon is unknown.

Here we characterized the circuit-based mechanisms of sensory-enhanced satiation by exploiting the simplicity of the fruit fly system. We show that sensory-enhanced satiation involves the central dopaminergic processing of peripheral sweet taste stimuli by a dedicated group of PAM-β'2 neurons. Given the role of PAM DANs transmission in reinforcing appetitive memories (Burke et al. 2012; Liu et al. 2012), this discovery is significant because it suggests that satiation may involve a learning or rewarding component and that diet composition may direct food intake by influencing this aspect. Indeed, sensory cues function as a predictor of nutrient density and set expectations for how filling different types of foods should be (Chambers, McCrickerd, and Yeomans 2015; McCrickerd and Forde 2016; Yeomans 2017). This information could be used to modulate the feeding rate during the meal and initiate the process of meal termination without relying uniquely on nutrient-derived cues, which arrive later (Bellisle and Blundell 2013; J. E. Blundell, Rogers, and Hill 1987).

The idea that sensory cues could set cognitive expectations about the fullness of future meals is also in line with the known roles of DA in promoting the formation of appetitive memories. In fruit flies, PAM DANs promote the formation of short-term associative memories based on taste and long-term associative memories based on nutrient density by modulating plasticity of the postsynaptic Mushroom Body Output Neurons (MBONs) (Cohn, Morantte, and Ruta 2015; Owald et al. 2015). MBONs are, in turn, connected to pre-motor areas like the Central Complex (Aso et al. 2014) – the fly genetic and functional analog of the basal ganglia (Strausfeld and Hirth 2013) – providing an anatomical route to modulate aspects of feeding such as proboscis extension (Chia and Scott 2019), the analogue of licking or chewing rate. Interestingly,

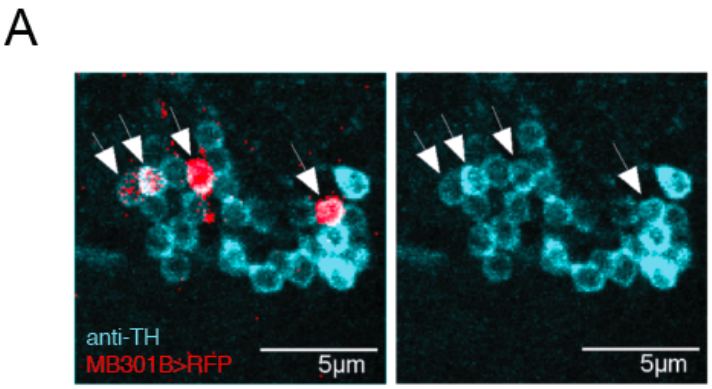
some MBONs receive input from both the taste (β'2) and nutrient (γ5) compartments, raising the possibility that sensory and nutrient memories may be integrated in the same cells to regulate different aspects of the satiety cascade (satiation vs. satiety). In flies, the mode and timing of DA delivery onto the MBONs is critical to establish the strength and valence of the associations (Handler et al. 2019). The delay and decrease we measured in animals on a high sugar diet could impair MBON synaptic plasticity and the formation of new appetitive memories (Cohn, Morantte, and Ruta 2015; Owald et al. 2015). If this is the case, we would expect that flies on this diet may be insensitive to new learning, use old food memories to predict the filling effects of the meal, and thus overshoot their food intake. This is consistent with the idea elegantly espoused by (Kroemer and Small 2016) who explain the decrease in DA transmission with diet or obesity in a reinforcement learning framework.

Another possibility, however, is that alterations in PAM DAN processing are not related to reinforcement learning per se, but instead to a decrease in overall reward receipt. In this light, sensory signals would cue reward not learning, and the pleasure experienced during eating would promote satiation and curb food intake. The idea that decreases in the sensitivity of the reward system increases food intake has been described as the "reward deficit" theory of obesity (Wang, Volkow, and Fowler 2002), which also draws a parallel between the effects of drugs of abuse and that of sugar on the brain. Our results are consistent with both reinforcement learning and reward deficit scenarios, as well as with other integrated theories of obesity (Stice and Yokum 2016); future experiments examining the role of circuits downstream of PAM DANs, and especially the involvement of MBONs, will differentiate between these possibilities. Our

study also adds to the current body of evidence connecting diet with DA alterations in mammals (Geiger et al. 2009; van de Giessen et al. 2013; Kroemer and Small 2016; Friend et al. 2017; DiFeliceantonio and Small 2019). In particular, we speculate that some of the changes in DA transmission observed with diet exposure in rodents and humans may be due to impairments in sensory processing, since humans and rodents also process the taste and nutritive properties of sugar separately (Tellez et al. 2016; Thanarajah et al. 2019).

In conclusion, our experiments demonstrate that by reducing peripheral taste sensation, a high sugar diet impairs the central DA processing of sensory signals and weakens satiation. These studies forge a causal link between sugar – a key component of processed foods – taste sensation, and weakened satiation, consistent with the fact that humans consume more calories when their diets consist of processed foods (Hall et al. 2019). Given the importance of sensory changes in initiating this cascade of circuit dysfunction, understanding how diet composition mechanistically affects taste is imperative to understand how the food environment directs feeding behavior and metabolic disease.

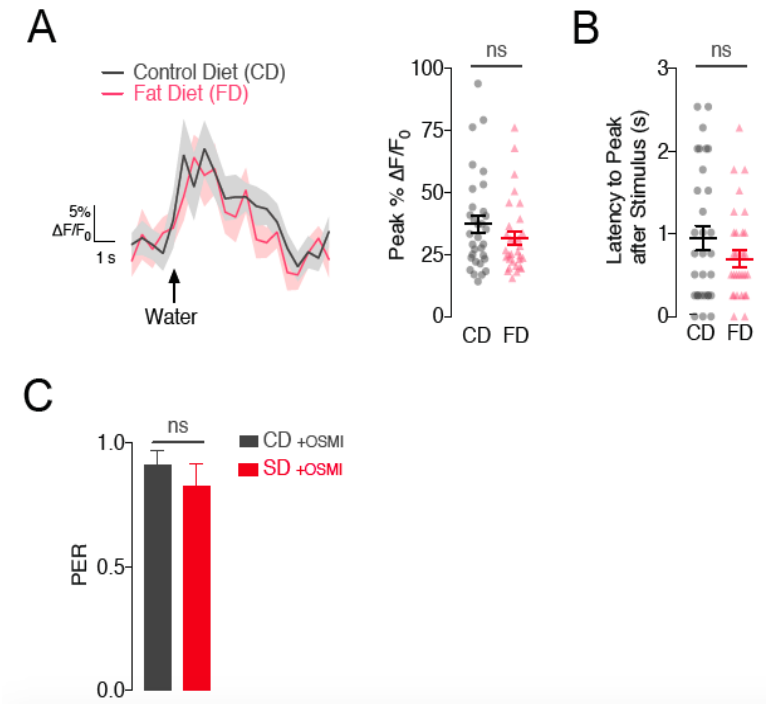
Supplemental Figures



Supplemental Figure 3-S 1

Colocalization of MB301B neurons with TH+ neurons.

A) Confocal fluorescence image of the PAM cluster neurons stained with an antibody against tyrosine hydroxylase (TH, *cyan*) in *MB301B>RFP* flies. Arrows indicate *MB301B* cell bodies (*red*), which are also positive for TH. Scale bar = 5 µm.



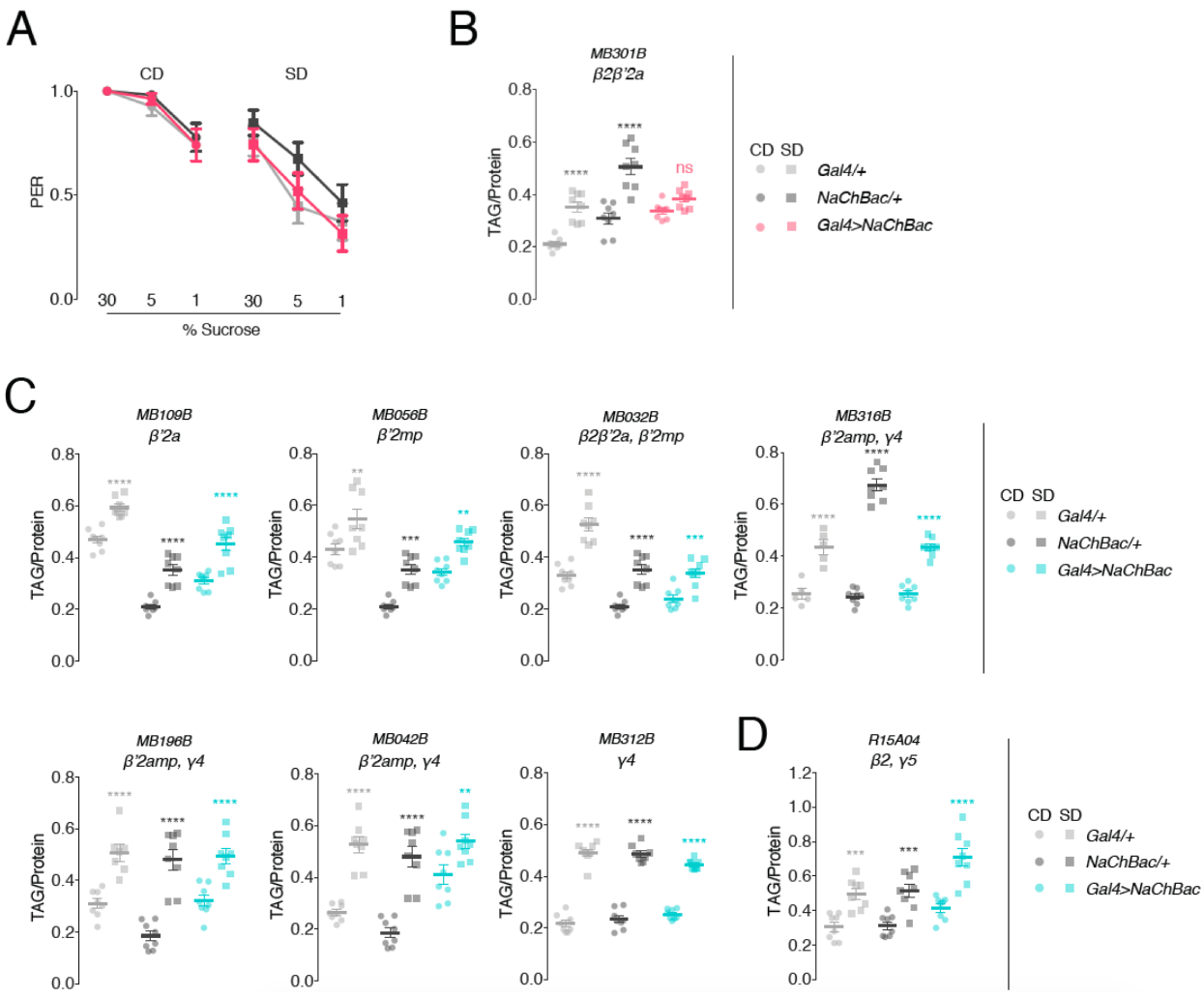
Supplemental Figure 3-S 2

Responses of MB301B neurons on FD, and PER with OGT inhibitor.

A) *Left,* Mean %$\Delta F/F_0$ traces and *Right,* quantification of the maximum peak %$\Delta F/F_0$ responses to stimulation of the labellum with water in the β'2 compartment of *MB301B>GCaMP6s::Brp::mCherry* flies fed a control (CD, *grey*) and high fat diet (FD, *rose*). n=31-32; shading and error bars are standard error of the mean. Mann-Whitney test; no significance.

B) Latency-to-peak response times for the animals in A. n=31-32; error bars are standard error of the mean. Mann-Whitney test; no significance.

C) Mean score of Proboscis Extension Response (PER) to stimulation of the labellum with 30% sucrose in male *w*^{1118}*CS* flies, fed a control (CD, *charcoal*) and high sugar (SD, *red*), supplemented with 75 µM OSMI-1 (OSMI-1). n=18 per group; error bars are standard error of the mean. Mann-Whitney test; no significance.



Supplemental Figure 3-S 3 Fat accumulation with constitutive activation of other PAM neurons.

A) Mean proboscis extension response (PER) to sucrose stimulation of the labellum (30%, 5%, and 1%) in *MB301B>NaChBac* flies following 7 days of exposure to a control (CD, *left*) or sugar diet (SD, *right*). n=27 per condition; error bars are standard error of the mean. Kruskal-Wallis with Dunn's multiple comparisons, no significance.

B) Mean triacylglyceride (TAG) content normalized to protein of *MB301B>NaChBac* flies and single transgenic control male flies fed a CD or SD for 7 days. n=8 per condition; error bars are standard error of the mean. Two-way ANOVA with Sidak's multiple comparisons test; ****p<0.0001, comparison to CD within genotype.

C) Mean TAG content normalized to protein of male flies with expression of *UAS-NaChBac* in different subsets of PAM neurons innervating β'2 or γ4 regions of the mushroom body. n=5-8; error bars are standard error of the mean. Two-way ANOVA with Sidak's multiple comparisons test; **p<0.01, ***p<0.001, ****p<0.0001, comparison to CD within genotype. Legends are on the right of the figure.

D) Mean TAG content normalized to protein of male flies with expression of *UAS-NaChBac* in nutrient-reward PAM neurons, which innervate the β2 compartment of the mushroom body. n=8 per condition; error bar is standard error of the mean. Two-way ANOVA with Sidak's multiple comparisons test; no significance, comparison to CD within genotype.

Methods

Fly Lines and Preparation

All flies were maintained at 25ºC in a humidity-controlled incubator with a 12:12 hours light/dark cycle. For all experiments, males were collected under CO_2 anesthesia, 2-4 days following eclosion, and housed in groups of 20-30 within culture vials. The *Gal4*/*UAS* system was used for cell-type specific expression of transgenes. Stocks used are listed in the table below; w^{1118}*Canton-S* was used as control.

Gr64f-Gal4	gift from H. Amrein
UAS-sybA (syb-pHluorin)	gift from B. Ye
MB301B-Gal4	BDSC #68311
UAS-GCaMP6s::Brp::mCherry	BDSC #77131
UAS-ReaChR	BDSC #53741
UAS-NaChBac	gift from M. Nitabach
MB032B-Gal4	BDSC #68302
MB042B-Gal4	BDSC #68303
MB056B-Gal4	BDSC #68276
MB109B-Gal4	BDSC #68261
MB196B-Gal4	BDSC #68271
MB312B-Gal4	BDSC #68314
MB316B-Gal4	BDSC #68317
UAS-RFP ,LexAop-GFP	BDSC #32229

Methods Table 3-M 1 *Drosophila melanogaster* lines.

Dietary Manipulations

Flies were transferred to each diet 2-4 days after eclosion in groups of 30 animals per vial and fed on experimental diets (SD or FD) for 7 days with age-matched controls on CD.

The composition and caloric amount of each diet was as below:

- “Control Diet/CD” was a standard cornmeal food (Bloomington Food B recipe), with approx. 0.6 cal/g.

- “Sugar Diet/SD” was 30 g of table sugar added to 89 g Control Diet for 100 mL final volume of 30% sugar w/v, with approx. 1.4 cal/g.
- “Fat Diet/FD” was 10 mL of melted lard added to 90 mL of liquid Control Diet for 100 mL final volume of 10% lard v/v, with approx 1.4 cal/g.
- For diets supplemented with OSMI-1, the inhibitor was dissolved in 55% DMSO for a stock concentration of 500 μM, and then diluted 3:20 in liquid Control or Sugar Diet for a final concentration of 75 μM in food.
- For diets supplemented with all-*trans*-retinal, retinal was dissolved in 95% EtOH for a stock concentration of 20 mM, then diluted 1:100 in liquid Control Diet for a final concentration of 200 μM in food.
- Diets on the FLIC were 5% and 20% w/v D-sucrose in 4 mg/L $MgCl_2$.

In vivo Imaging

Adult age-matched male flies, following 7 days of CD or SD, were fasted on a wet Kimwipe for 18-24 hours before prepping for *in vivo* confocal laser imaging. As previously described (May et al. 2019; LeDue et al. 2015), the preparation consisted of a fly affixed to a 3D-printed slide with melted wax around the head and on the dorsal part of the thorax. Distal tarsal segments were removed to prevent interference of the proboscis stimulus, and the proboscis was wax-fixed fully extended with the labellum functional and clear of wax so that proboscis contraction and extension could not perturb the brain’s position. A glass coverslip was placed such that artificial hemolymph (108 mM NaCl, 8.2 mM $MgCl_2$, 4 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2 mM $CaCl_2$, 5 mM KCl, 5 mM HEPES) placed over the head did not touch the proboscis. Data were

acquired with a FV1200 Olympus confocal microscope, a 20x water immersion objective, and a rate of 0.254 s per frame. Stimuli consisted of a brief touch of a small Kimwipe soaked in milliQ water or 30% sucrose solution to the labellum.

Optogenetic Stimulation for Fly-to-Liquid-food Interaction Counter (optoFLIC)

optoFLIC was run as previously described (Chapter 2; May et al. 2019). Briefly, adult flies 3-5 days past eclosion were placed on ATR food and kept in the dark for 3 days until starting the optoFLIC. optoFLIC experiments were run in an incubator with consistent 25ºC and 30-40% humidity, on a dark/dark light cycle to prevent ambient-light activation of the ReaChR. Following two days recording of feeding activity on the FLIC food without LED activation, a protocol for closed-loop feeding-triggered LED activation was begun. The LED activation protocols were as follows:

For experiments with MB301B>ReaChR, 200 ms of red (~627 nm) light pulsing at frequency 60 Hz and with a pulse width of 4 ms was triggered by every food interaction signal over 10.

Immunofluorescence Staining

Immunofluorescence protocol was performed as described in (Dus et al. 2015). Briefly, brains were dissected in 1xPBS from male *MB301B>RFP* flies 3-5 days post-eclosion, then fixed in 4% paraformaldehyde in 1xPBS for 20 min, blocked in blocking buffer (10% normal goat serum, 2% Triton X-100 in 1xPBS), and incubated overnight at RT in anti-TH (rabbit polyclonal Ab from Novus Bio) 1:250 in dilution buffer (1% normal goat serum, 0.25 Triton X-100 in 1xPBS). Secondary antibody was goat anti-rabbit

Alexa Fluor 488 diluted 1:500 in dilution buffer, and brains were washed then incubated with secondary antibody overnight at RT. Brains were mounted in FocusClear between two coverslips and imaged within 24 hours.

Triacylglyceride (TAG) Assay

Following the protocol in (Tennessen et al. 2014), we assayed total TAG levels normalized to total protein in whole male flies. To assay, flies were CO_2-anesthetized and flash frozen. Pairs of flies were homogenized in lysis buffer (140 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.1% Triton-X) containing protease inhibitor (Thermo Scientific). Separation by centrifugation produced a supernatant containing total protein and TAGs. Protein reagent (Thermo Scientific Pierce™ BCA Protein assay) was added to the supernatant and the standards and incubated for 30 min at 37ºC, then tested for absorbance at 562 nm on a Tecan Plate Reader Infinite 200. TAG reagent (Stanbio Triglycerides LiquiColor Test) was added to supernatant and standards, incubated for 5 min at 37ºC, then tested for absorbance at 500 nm.

Proboscis Extension Response

Flies were fasted for 24h in a vial with a Kimwipe dampened with 2 mL of milliQ-filtered deionized (milliQ DI) water and tested for the proboscis extension response (PER) (Shiraiwa and Carlson 2007). Water and all tastants were tested manually via a solution-soaked Kimwipe. Sucrose solutions were dissolved in milliQ water and presented in descending order by concentration. Groups of 10-15 flies were tested simultaneously.

Imaging Data Analysis

For each fly, $\Delta F/F_0$ was calculated from a baseline of 10 samples recorded just prior to the stimulus (sucrose or water). Area under the curve (AUC) was calculated by summing the $\Delta F/F_0$ values from the initiation of the response to its end. Peak $\Delta F/F_0$ is the single maximum acquired within a response, and latency to peak was calculated by determining the time between the stimulus delivery and the peak response.

optoFLIC Data Analysis

OptoFLIC analysis of daily food interactions, meal size, and meal duration was performed as previously described (Chapter 2; May et al. 2019). R code used can be found on Github. Briefly, food interactions were determined by calculating a moving baseline on the raw data and selecting signals that surpassed threshold above baseline. These signals were then summed in 30-minute bins. From the binned data, daily food interactions and the start and end of meals were calculated. The evening meal was used for all meal-based calculations to control for variability in meal shape. Meal size and duration were derived using meal start and end. Post-peak feeding duration was quantified as *[(time of meal end) - (time of meal peak)]*.

An event is defined as a string of consecutive food interactions. R code used to extract event information can also be found on Github. To calculate events per meal, the number of events between the meal start and meal end per meal were summed for each fly. Feeding rate was quantified as *[(events per meal) / (meal duration)]* per meal per fly. Pre- and post-peak feeding rates were quantified, using the time of the meal

peak determined by food interactions, also used to calculate post-peak feeding duration, as *[(number of events pre- or post-peak) / (pre- or post-peak feeding duration)]*. Pre-peak feeding duration was quantified as *[(time of meal peak) - (time of meal start)]*.

Statistics

Data were visually examined and found to be non-normal; in the absence of normalcy t-tests were not used. For imaging, each point represents one ROI in a fly and each fly had 2 ROIs drawn that responded to a single stimulus, but measurements from different flies were independent. For the triglyceride measurements, every point represents a homogenate of two flies, thus these measurements are independent. Triglyceride data represent a single trial but findings were replicated at least once more (data not shown). For optoFLIC experiments, two-way repeated measures ANOVA were used to compare the rate of increased feeding across different diets and fly transgenic lines. For all data, multiple comparison corrections were performed as recommended by GraphPad Prism, in which all statistical tests were performed, for each test based on distribution and variance parameters assigned by the experimenter.

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Chapter 4: optoFLIC: A Closed-Loop Stimulation Apparatus for Long-Term Recording and Manipulation of Steady-State Fly Feeding Behavior

Please see the Preface (p. iv) for contributor information.

Abstract

A formidable obstacle in systems neuroscience research is accurate connection of cause to effect during neuronal manipulation experiments. Closed-loop optogenetic stimulation apparatus, like the Sip-Triggered Optogenetic Behavior Enclosure (STROBE, (Musso et al., 2019)) and the optogenetic Proboscis and Activity Detector (optoPAD, (Moreira et al., 2019)), have advanced our ability to link precise neuronal activity to feeding behavior outputs in the fruit fly. Yet these are limited in their ability to record and manipulate long-term, steady-state feeding. In this manuscript we present the optoFLIC, a closed-loop optogenetic stimulation system based on the Fly-to-Liquid-food Interaction Counter, which can be used to monitor feeding behavior with high temporal resolution for many days. We show that the optoFLIC can be used to modulate steady-state feeding behavior through feeding-locked or constitutive activation of sweet taste neurons. Additionally, we describe analyses of FLIC and optoFLIC data to develop metrics of steady-state fly feeding behaviors, like meal size, meal duration, and feeding

bout (“event”) duration. Together these demonstrate the value of using the optoFLIC to understand causal neuronal control of steady-state feeding behavior in the fruit fly.

Introduction

Precise understanding of neuronal control of behavior relies upon the spatial and temporal resolution of the experimental manipulation and the acquisition of data. To this end, optogenetics has moved to the forefront for precise spatial and temporal manipulation of neuronal activity. Behavior- or activity-triggered optogenetic manipulation of the activity of large neuronal populations in behaving mice have been essential to modern understandings of learning and memory (Rangel Guerrero et al., 2018; Rickgauer et al., 2014), because using such closed-loop systems increases certainty regarding cause and effect. A drawback of these examples is the complexity inherent in mammalian brain circuitry. Spatial resolution improves using the brains of simpler model organisms, like the fruit fly *Drosophila melanogaster*, which allows for genetic manipulation of narrowly defined neuronal populations using targeted expression systems like Gal4/UAS. Expression of light-sensitive channelrhodopsins can be limited to even a single neuron in the fly brain, and many neurons in the fly brain can receive light stimulation through the fly’s cuticle, removing the need for any invasive procedure. By combining the spatial definition available in the fly with the temporal resolution of closed-loop optogenetic systems, much can be learned about neuronal control of behavior.

Many aspects of steady-state feeding behavior are shared between insects and mammals. Like humans, fruit flies sleep at night and eat a meal upon waking in the

morning. They continue to feed in smaller sessions throughout the day, often referred to as snacking, and then eat another meal before sleeping (Ro et al., 2014; Zhang, 2016). The internal and external signals that drive feeding initiation and termination are also broadly conserved between flies and humans. Internal start feeding signals come from the fly's circadian clock and hormones secreted as levels of circulating sugars fall (Dus et al., 2013; Martelli et al., 2017; Xu et al., 2008). Encounters with high-energy food prompt feeding: the taste of sugar in particular is effective at inducing proboscis extension, the motor pattern that initiates feeding (Dus et al., 2015; Flood et al., 2013; Murata et al., 2017). The fly intuits when to stop feeding from sensory feedback about food palatability and gastric expansion, as well as neuronal sensing of rising circulating sugar levels that indicate energy needs are being met (Hergarden et al., 2012; Miyamoto et al., 2012; Olds & Xu, 2014; Pool et al., 2014; Söderberg et al., 2012).

The fly's taste experiences and food acceptance criteria also mirror that of a human, as nutrient-associated tastes like sweet, salt, fat, and umami are attractive, and bitter and sour are aversive. Sweet taste in particular has been shown to control sugar feeding (the most common substrate for automated measurements of feeding in flies) (Chapters 2 and 3). Orosensory sweetness detection in the fly requires dedicated gustatory receptors 5a and members of the gene cluster 64 (Gr5a, and Gr64a-f). When bound, these receptors activate their gustatory neurons, which project to the fly brain. Sweet taste processing in the fly's central brain produces rewarding signals as it does in humans, resulting in attractive behaviors and memory formation of any associated stimuli. Discovering that sweet taste was impaired by sugary diets ((May et al., 2019), see also Chapter 2) prompted our group to ask whether correction of sweet taste

neuron activity could affect the hyperphagia the flies exhibited on these diets. Because sweet taste neurons are peripheral sensory neurons, they are not active in the absence of a stimulus. Thus any corrective activation paradigm we employed needed to be locked to relevant taste stimuli, so we pursued the development of a closed-loop stimulation system that would allow us to manipulate taste experience only when the fly was engaged with its food.

Among the sundry ways to measure fly feeding, two systems are available that allow for automated recording of feeding data. The flyPAD, first reported in 2014, uses a solid, agar-based food and measures changing capacitance signals as a fly interacts with the food while standing on a capacitance pad (Itskov et al., 2014). The FLIC works in a similar way, but the food medium is liquid, and the number of behavior arenas per unit is increased relative to the flyPAD (Ro et al., 2014). The value of the liquid food is that it can be replenished easily, allowing the FLIC to run for many days without disturbing the flies. The flyPAD has been independently developed twice to perform closed-loop optogenetic manipulations (Moreira et al., 2019; Musso et al., 2019). To fulfill the need for a closed-loop optogenetic feeding assay that can run for many days, we developed the optoFLIC. Here, we follow up on a previous report of its use (May et al., 2019) to detail more completely its hardware and programming, and to demonstrate additional support for the control of sugar diet feeding by sweet taste. We also explain in more depth certain analyses of fly feeding data acquired from the FLIC and the optoFLIC, which we used to add to the growing body of knowledge on steady-state feeding behaviors in the common model organism *Drosophila melanogaster*.

Results

Design of the optoFLIC

FLIC hardware consists of two main components, a Drosophila Feeding Monitor (DFM) and a FLIC Master Control Unit (MCU), the firmware of each were modified to create the optoFLIC, but their hardware was largely unaltered except for the DFM lid. In the original FLIC design, the lid covered the food wells of the DFM to create arenas containing one or two wells. For the optoFLIC, this lid was redesigned such that 1) there was a LED above each well of food, 2) a heat sink projected above the LEDs away from the arena, 3) a connection could be made between the lid and the DFM for food-interaction-triggered LED illumination, and 4) a power source could be connected to the lid independently of the DFM (Figure 4-1A). The optolid material was opaque black to prevent bleed of the LED light between arenas. To run long-term (more than one day) FLIC, excess-food reservoirs made of cell-culture flasks were connected to the main reservoirs supplying the FLIC food wells within the DFMs in order to minimize the rate of evaporation from the food.

The capacitance of the pad surrounding the well of food is recorded at a rate predetermined by the experimenter. Realtime data can be sampled by the MCU as frequently as 2000 Hz; for the experiments detailed in this dissertation, data sampling was 500 Hz, and every 100 samples were averaged for data output at a frequency of 5 Hz. This temporal resolution has been empirically determined to be high enough to distinguish single feeding bouts, while keeping the system efficient with respect to data writing and storage (Ro et al., 2014). When a fly-to-food interaction occurs, the circuit across the capacitance pad is closed, and the change in voltage is recorded by the

MCU. In the optoFLIC, this signal is checked against an experimenter-set threshold and passed back to the optolid, which will turn on the corresponding LED (Figure 4-1B).

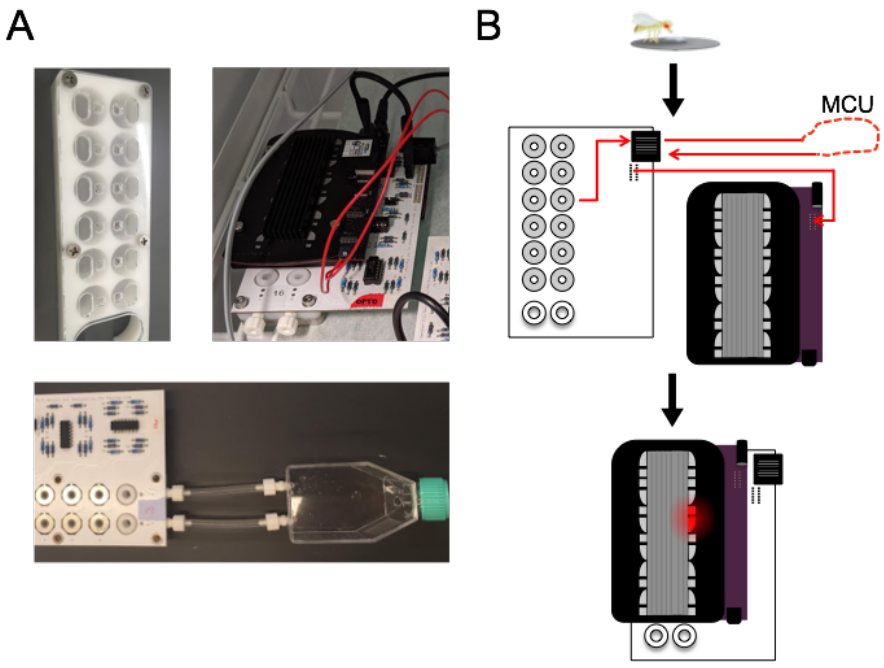

Figure 4- 1 optoFLIC is designed to use fly-to-food interactions to trigger LED stimulation. (**A**) Top left and right, original FLIC lid and optolid; below, long-term FLIC reservoir, attached to DFM. (**B**) Schematic of the optoFLIC triggering of light. Top, a fly interacts with its food. Middle, the interaction sends a signal to the computer for recording (dashed black line) and to the optolid (solid red line) to trigger the LED. Bottom, if the signal surpasses the experimenter-determined threshold, the LED comes on.

Chronic Stimulation of Sweet Taste Neurons With or Without Food Interaction Differentially Influences Feeding

Correcting neuronal activity requires meaningful stimulation strength and patterning (e.g., distinct action potentials, bursting). Sweet taste neurons have a particular context and pattern to their firing: they do not fire in the absence of a stimulus, and they exhibit rapid response adaptation across the first second of sweet exposure (Figure 2-S3C). Because sweet taste neuron activity in the first 500 ms of sugar exposure was decreased by a 7-day sugar diet (Figure 2-S3C), we posited that this was

causative to sugar-diet hyperphagia, and that correcting their activity would restore stable feeding.

Table 4- 1 Coded optoFLIC parameters and their definitions.

Parameter Name	Range	Definition
ExpDurationMin	>0	experiment duration in minutes
OptoFrequency	>0	frequency of pulse, max determined by OptoPW
OptoPW	>0	duration of a single light pulse, in milliseconds; limits OptoFrequency
OptoDelay	>0	time in milliseconds between decision to turn LED on and actual LED-on
OptoDecay	>0	time in milliseconds after end of LED stimulus before a new LED stimulus can be triggered
MaxTimeOn	>0	maximum duration of LED pulsing to a feeding signal
OptoLid	“Twelve”, “Six”	indicates the number of arenas in use; “Twelve” for single-choice and “Six” for two-choice
Interval	>-1	threshold setting for LED to come on; “-1” keeps the LED always off, “0” keeps the LED always on, “10” is threshold used for experiments in this dissertation

optoFLIC stimulation is a pulsed light, and the frequency, pulse width, duration of, and delay to the stimulation can all be encoded in the optoFLIC program (for a list of coded parameters, see Table 2-1). Using electrophysiology data recorded from the sweet taste neurons during exposure to different concentrations of sugar, we hypothesized that a pulse frequency of at least 20 Hz lasting less than 200 ms would appropriately increase the taste neurons’ activity (Figure 2-S3C, data not shown). Interestingly, while feeding-locked 20-Hz pulses on the closed-loop optoFLIC system did not affect sugar diet (20% sucrose) hyperphagia, 40- or 60-Hz stimuli progressively reduced hyperphagia (Figure 4-2A), suggesting that these stimuli were restoring commensurate neuronal firing to higher sucrose concentrations. Remarkably, a 60-Hz

stimulus had minimal discernable effect on feeding on the control diet (5% sucrose) (Figure 4-2B). It appears to mildly increase feeding interactions over time, which might be expected if the fly is perceiving the food to be a higher concentration of sucrose than it actually is. Importantly, giving the weaker 20-Hz stimulus for much longer periods of time without linking it to food interaction (LED on for 30 seconds every 4 minutes) resulted in severe reduction in feeding interactions on a control diet compared to transgenic controls (Figure 4-2C, red shading). We conclude from this that the impaired activity of the sweet taste neurons on a sugar diet is necessary for hyperphagia to occur, and that the optoFLIC has the versatility to probe the distinct roles of various neuronal firing patterns and contexts that are critical for long-term, natural feeding behaviors.

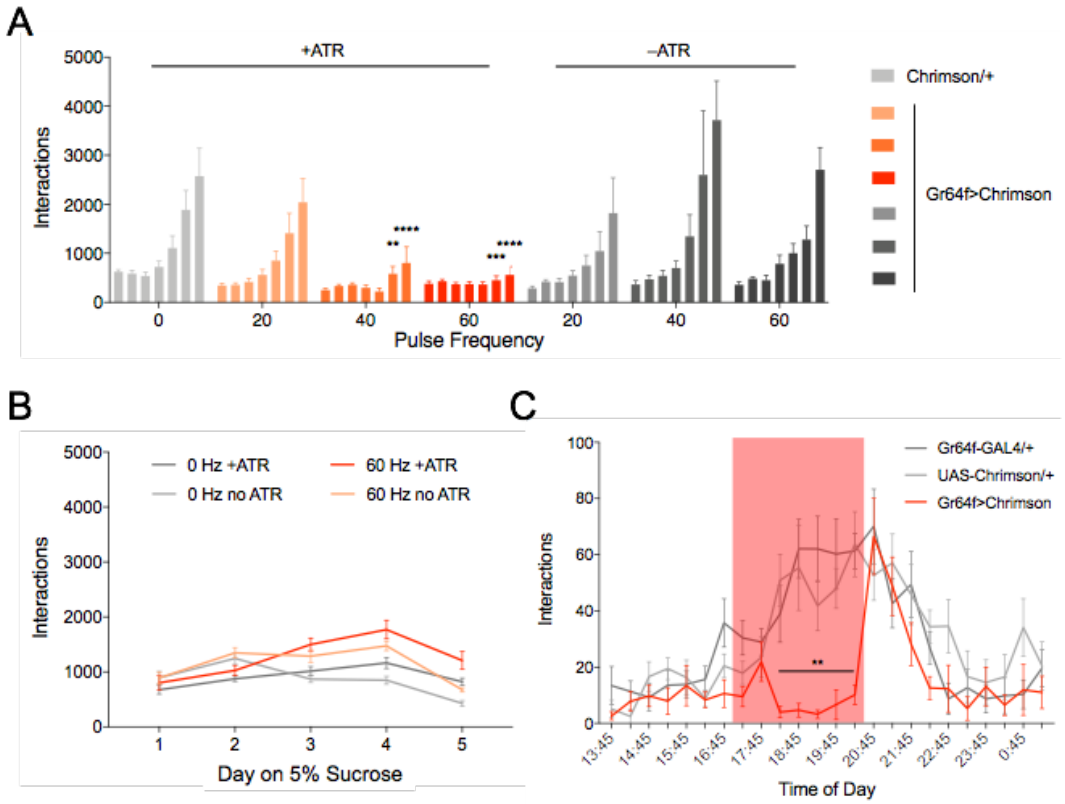

Figure 4- 2 Constitutive and closed-loop optogenetic stimulation paradigms of the taste neurons differentially affect feeding behavior.

(A) Expression of sugar-diet (20% sucrose) hyperphagia in *Gr64f>Chrimson* flies or transgenic control that have been fed all-trans-retinal (ATR), with feeding-locked 100-ms light stimuli pulsing at the denoted frequencies. Cumulative daily interactions shown as bars, mean ± SEM. n=6-12; two-way repeated measures ANOVA indicated a Time by Genotype/ATR interaction, $p<0.0001$, which was entirely explained by the *Gr64f>Chrimson* +ATR 40- and 60-Hz stimulation groups (orange and red bars, respectively). Tukey's multiple comparisons test across genotype/ATR per day; **p<0.01, ***p<0.001, ****p<0.0001.

(B) Feeding of *Gr64f>Chrimson* flies on control diet (5% sucrose) with stimulation of the sweet taste neurons at the highest frequency used in (**A**). n=10-12; two-way repeated measures ANOVA indicated a Time by Genotype/ATR interaction, $p<0.0001$, which was not explained by the *Gr64f>Chrimson* +ATR group.

(C) Feeding of *Gr64f>Chrimson* flies with non-food-interaction-tethered 20 Hz stimulation on the 5% sucrose control diet . n=11-12; two-way repeated measures ANOVA indicated a Time by Genotype interaction, $p<0.0001$, which was entirely explained by the *Gr64f>Chrimson* group. Dunnett's multiple comparisons test across genotype over time; **p<0.01.

Meal Structure on the FLIC

Fly daily feeding structure consists of many feeding bouts – wherein the fly approaches food, engages with it for a series of consummatory interactions, and then disengages, hormonal control of which has been admirably reviewed in (Pool & Scott, 2014) – which are clustered particularly around dawn and dusk. On the FLIC, one of these bouts is called an "event", defined as a consecutive series of fly-to-food

interactions. The crepuscular clusters of feeding events are referred to in this dissertation as “meals”. The timing and relationship of these meals to the circadian rhythm was first described in (Ro et al., 2014), who showed that in 12-hr light/dark cycles, meal start anticipates the transition between light states, and that in the absence of light cues, morning meal structure becomes broader and flatter, while the evening meal structure is maintained. This discovery was made possible by the FLIC’s high temporal resolution of fly feeding behavior. There is much yet to learn about the neuronal control of meal timing and duration. Given the importance of accurate sweet tasting to the maintenance of normal feeding on a sugar diet, we asked what aspects of the meal structure are governed particularly by sugary diets and the activity of primary sweet taste neurons.

Augmenting the activity of Gr64f+ neurons of flies feeding on a sugar diet stabilized feeding and prevented hyperphagia compared to control flies (Figure 4-2A, 4-3A). To extract information regarding meal structure, meals were defined after the interaction data were binned into half-hour periods. This bin size is common to circadian rhythm research; since circadian rhythms are powerful drivers of feeding behavior, it is sensible to use the same data visualization structure. Given that the experiment had to be run in dark/dark and evening meal structure is more stable in dark/dark conditions (Ro et al., 2014; Zhang, 2016), we used the evening meal for our analyses. Evening meals occurred close to the flies’ previously entrained 12-hr light/dark cycle, with lights on at 7 AM and off at 7 PM. Bins of peak feeding were identified, from which the start and end of meals could be determined and then meal size and duration calculated.

Evening meal size and duration increased with time on a sugar diet in control flies, but was stable in retinal-treated flies on the closed-loop optoFLIC (Figure 4-3B, C).

For a deeper analysis of meal structure that could suggest changes to the underlying neuronal control of feeding, we analyzed the duration of feeding before and after the peak bin of feeding. Meal initiation and pre-peak feeding is governed by hunger, appetite, and food appeal. We found that pre-peak feeding duration was increased in control flies with time on the sugar diet, but stable in the retinal-treated flies (Figure 4-3D), suggesting that the increase in duration of pre-peak feeding was controlled by sweet taste neuron responsivity to sucrose. Interestingly, post-peak feeding was increased in flies with or without retinal treatment, suggesting that this sugar-diet-induced lengthening of feeding was not controlled by sweet taste neuron activity (Figure 4-3E).

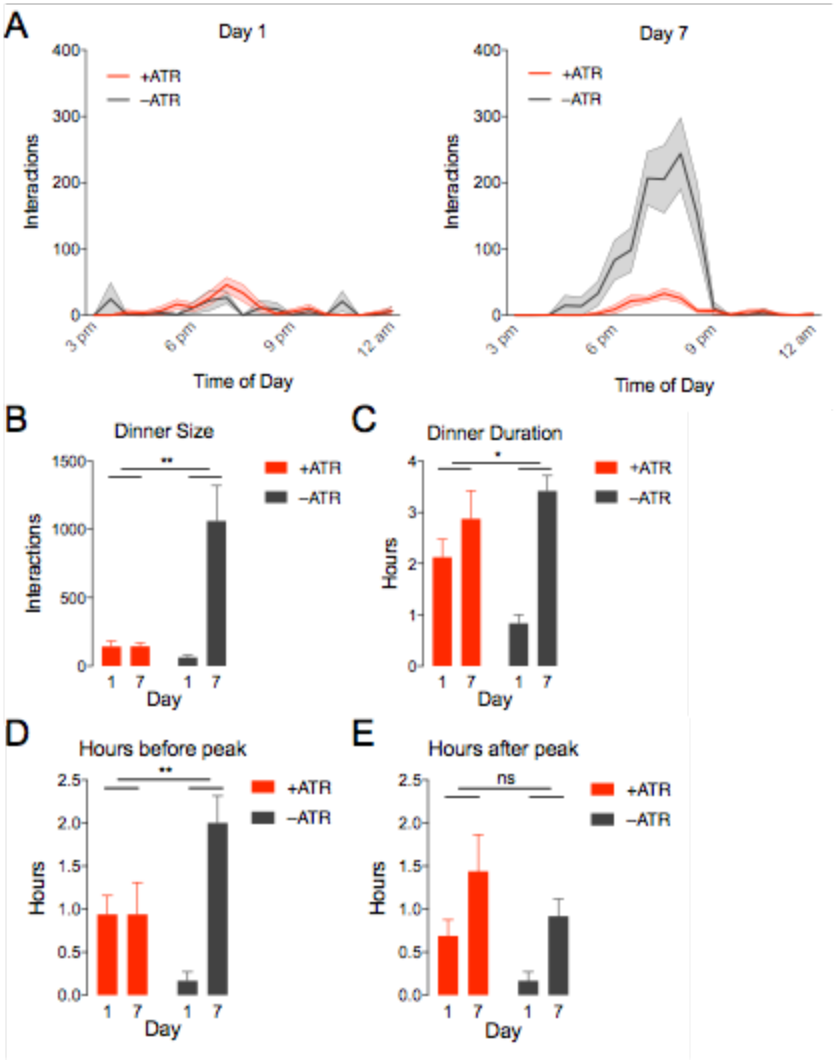

Figure 4- 3 Sweet taste neuron activity governs sugar diet meal size and duration.

(A) Feeding patterns across the first (left) and seventh (right) evening meals of *Gr64f>Chrimson* flies in the closed-loop optoFLIC.

(B) Total number of interactions in the first and seventh evening meals of *Gr64f>Chrimson* flies in the closed-loop optoFLIC. n=6-9, bars represent mean ± SEM; two-way repeated measures ANOVA reveals a Time-by-Retinal-treatment interaction, **p<0.01.

(C) Duration of first and seventh evening meals of *Gr64f>Chrimson* flies in the closed-loop optoFLIC. n=6-9, bars represent mean ± SEM; two-way repeated measures ANOVA reveals a Time-by-Retinal-treatment interaction, *p<0.05.

(D) Duration of feeding before meal peak in the first and seventh evening meals of *Gr64f>Chrimson* flies in the closed-loop optoFLIC. n=6-9, bars represent mean ± SEM; two-way repeated measures ANOVA reveals a Time-by-Retinal-treatment interaction, **p<0.01. **(E)** Duration of feeding after meal peak in the first and seventh evening meals of *Gr64f>Chrimson* flies in the closed-loop optoFLIC. n=6-9, bars represent mean ± SEM; two-way repeated measures ANOVA reveals no significant Time-by-Retinal-treatment interaction.

Duration of Feeding Events is Under Sweet Taste Control

To elucidate the effect of sugar diet and sweet taste neuron activity on the microstructure of fly feeding behavior, we next wanted to ask whether the duration of feeding events were altered in our optoFLIC experiments. We could probe the event durations on the optoFLIC using a separate analysis of feeding event duration. Published reports of this aspect of feeding largely refer to event duration as “meal size”, referring to the volume consumed during a single event or the time between the extension and retraction of the proboscis. Because in this dissertation I use the term “meal” to refer to the circadian clusters of feeding events occuring at the beginning and the end of the light cycle, I will use the FLIC term “event” for these bouts of feeding occurring across a single proboscis extension.

In Figure 4-4A, we demonstrate from an older dataset that flies of a control strain exhibit longer event durations on control (5% sucrose) diet than a sugar (20% sucrose) diet on the light/dark FLIC, in keeping with what is known about how flies compensate their feeding structure for the energy content of the food (Al-Anzi et al., 2010; Edgecomb et al., 1994; Williams et al., 2014). There is also a marked progressive decrease in event duration for both diets over the course of the week, possibly due to

less-than-ideal control of humidity conditions. Intriguingly, the last day of the experiment actually shows the average event duration of flies on the sugar diet increasing rather than decreasing. This may indicate that high sugar diets cause an increase in event duration. Given that sweet taste is impaired by a sugar diet, we hypothesized that such an increase in event duration is a sign that the fly is perceiving a lower concentration of sugar than it is ingesting, and that correcting the sweet taste neuron activity should prevent the increase in event duration.

Using the same analysis on the closed-loop optoFLIC (run in dark/dark conditions) revealed that correcting sweet taste neuron activity kept the event duration steady over the course of a week (Figure 4-4B). We also note that, for both experiments, there is a strong effect of first-day-on-the-FLIC that results in a long average event duration, and that is diminished by the second day. Interestingly, the sugar diet flies in both experiments exhibit a stronger first-day effect than either of the other groups of flies; indeed, this effect is entirely absent in the flies whose sweet taste neuron activity is augmented by the optoFLIC. However, as these flies do not receive light stimulation until their second day on the FLIC, it is possible that this is a nonspecific effect due to the retinal treatment, the dark/dark condition, the flies' genetic background, or a combination thereof.

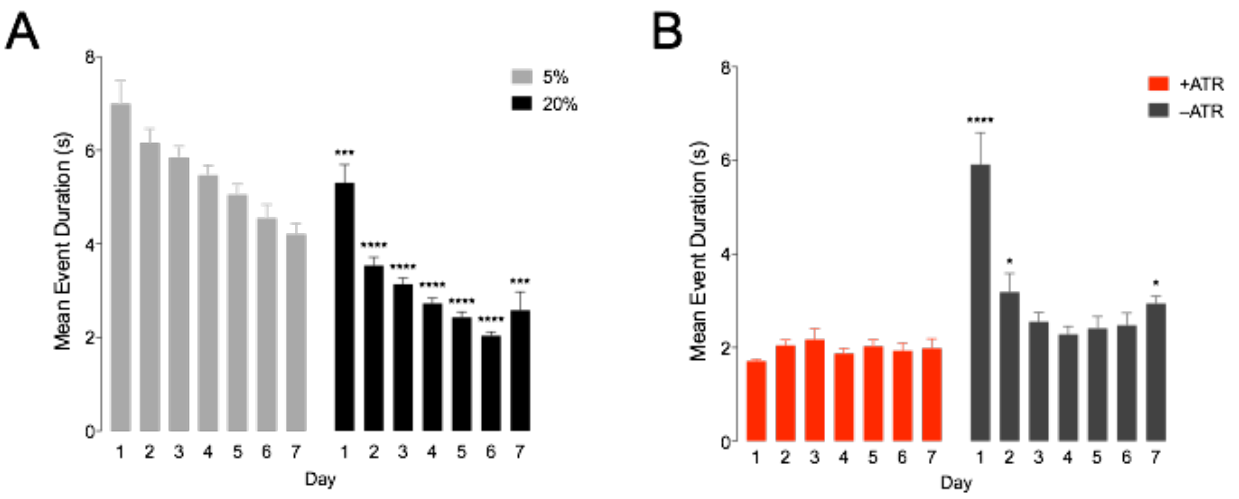

Figure 4- 4 Feeding event duration depends on activity of peripheral sweet taste neurons.

(A) Average event durations per day of *w*^{1118}*-CantonS* flies on the original FLIC. n=20-23; bars are Mean ±SEM; two-way repeated measures ANOVA revealed a Diet effect, ****p<0.0001, and a Time-by-Diet interaction, *p<0.05; Sidak's multiple comparisons test per day across diet conditions, ***p<0.001, ****p<0.0001.

(B) Average event durations per day of *Gr64f>Chrimson* flies on the optoFLIC. n=6-9; bars are Mean ±SEM; two-way repeated measures ANOVA revealed a Retinal-treatment effect, ***p<0.001, Time-by-Retinal-treatment interaction, ****p<0.0001; Sidak's multiple comparisons test per day across retinal conditions, *p<0.05, ****p<0.0001.

Discussion

In this manuscript we present a fuller account of the use of the closed-loop optoFLIC, a new feeding behavior apparatus that vastly improves the temporal resolution of neuronal manipulations leading to deeper understanding of long-term, steady-state feeding. We build off of a previously published dataset and conclusions (see Figure 2-6E; (May et al., 2019)) to show that sweet taste responsivity affects specific aspects of feeding, particularly the duration of feeding before the peak of the

meal. Sugar-induced impairment of sweet taste extends the time before flies begin to slow their feeding in response to satiation signals; correction of this impairment with optogenetic activation stabilizes the pre-peak feeding duration. This suggests that sweetness intensity contributes to the fly's perception of the energy it will receive from a meal to set its early eating rate.

Taste is critical for any organism to determine the acceptability of a food source. Aversive compounds activating bitter and sour senses indicate toxicity and induce avoidance, while appetitive compounds like sugar, amino acids, fat, and salt promote feeding. Importantly, flies like other animals can change their level of intake to compensate for caloric content: they feed more on lower-energy foods than high-energy foods in order to meet energy needs (Edgecomb et al., 1994). With time on a sugary diet, however, fruit flies drastically increase their high-energy feeding, and this can be controlled through the activity of the sweet taste neurons, whose responsivity is dulled by the sugar diet (Chapter 2; (May et al., 2019)). In Chapter 3, we concluded that processing of the sweet taste by a subset of reward neurons was critical for increased post-peak meal feeding on the sugar diet, but this processing was also important for early meal (pre-peak) feeding (Figure 3-5D). It is surprising that direct correction of the peripheral taste neurons does not fully restore the sugar-diet post-peak feeding (Figure 4-3E), given our findings detailed in Chapter 3. Future experiments should use electrophysiology to validate the patterns of activation in both the Gr64f+ and MB301B neurons to more accurately interpret the effects of the manipulations described here. Another direction for future experiments would be to find other sweet taste processing centers in the brain, like PAM-B'2, which specifically control event duration. Neuronal

populations that control proboscis extension and consummatory pumping have been identified (Flood et al., 2013; Marella et al., 2012; Scheiner et al., 2014; Williams et al., 2014; Youn et al., 2018), but how these neurons change in response to changing sweetness intensity or sugary diets is unknown.

The experiments listed here just begin to scratch the surface of the many uses of the optoFLIC to delineate neuronal functions that affect feeding behaviors. We introduce the ability of the FLIC to define particularly nuanced behaviors like event duration and pre- and post-peak meal duration, which give researchers access to these differentially driven aspects of feeding. With the right tools it is possible to tease apart the many neuromodulators, hormonal systems, and circuits that create normal as well as dysregulated feeding structures.

Methods

Fly Stocks

The following stocks were used in the above experiments: *w^{1118}-CantonS* (gift of A. Simon), *UAS-csChrimson* (Bloomington #55135), and *Gr64f-Gal4* (gift of H. Amrein). The data presented in Figures 4-2A, 4-3, and 4-4 were collected from the same flies used for Figures 2-4 and 2-6E. Flies were grown and maintained on solid cornmeal medium (Bloomington Food B), and housed in incubators kept at 25ºC and 30-50% humidity before and during the FLIC experiments. Flies experienced twelve-hour light/dark cycles with lights on at 7 AM except where otherwise noted. For activation of

csChrimson, flies fed on their standard food supplemented with 200 μM all-*trans*-retinal for 3 days in the dark before being loaded into the optoFLIC.

optoFLIC

optoFLIC experiments were performed as previously described (Chapters 2 and 3, Methods sections). Briefly: flies were ice-anesthetized and then aspirated into the single-well optoFLIC arenas where food was already loaded. Once all flies for an experiment were in place, recording began. Daily FLIC watering added 2-5 mL of milliQ filtered DI water to each food reservoir. FLIC food was molecular grade sucrose dissolved in 4 mg/L $MgCl_2$.

The following optoFLIC settings were used:

For Figures 4-2A, 4-3, and 4-4B:

ExpDurationMin: 11520
OptoFrequency: 60
OptoDelay: 0
OptoDecay: 0
MaxTimeOn: 100
OptoLid: Twelve
Interval: 10

For Figure 4-2B:

ExpDurationMin: 1440
OptoFrequency: 60
OptoDelay: 0
OptoDecay: 0
MaxTimeOn: 100
OptoLid: Twelve
Interval: 10

Figure 4-2C had a unique program that turned on the 20-Hz LEDs for 30 seconds every four minutes, but only during predicted mealtimes (from two hours before to two hours after light/dark transitions, for a total of 4 hours).

optoFLIC Data Analyses

Analyses of daily feeding interactions, 30-min binning, meal size and duration were performed as previously described (refer to Chapter 2 and 3, Methods). Event duration was calculated as number of interactions determined by R code (which can be found on Github: https://github.com/chrismayumich/May-et-al-FLIC-Analysis/branches), then divided by 5 to calculate the number of seconds, given that each interaction represents 200 ms. Statistical comparisons (of Mean ± SEM unless otherwise noted) were run in GraphPad Prism.

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Chapter 5: Discussion and Future Directions

Accurate tasting is critical for appropriate consumption of food. It assists in detection of valuable substances, such as those containing calories, essential amino acids, and ionic compounds; and of harmful substances, such as food that is rotten or toxic (Scott 2005). This dissertation presents two main findings, that diets high in nutritive sugar impair sweet taste perception and that this has unhealthy consequences on food decisions, as well as a new closed-loop steady-state-feeding assay that can be used to probe nuanced relationships between diet and feeding. However, there is much still unknown about how diet contributes to the obesity epidemic; some avenues of future research are suggested in the following section.

Effect of Sugar Diet on Other Taste Modalities

This dissertation primarily focuses on the interaction between dietary sugars and the sensing and processing of sweet tastes. However, in Chapter 1 we began with the understanding that sweetness is not the only taste modality that exhibits diet-induced plasticity (citing the case of salt), and in Chapter 2 we reveal that the mechanism of sugar-induced sweet taste impairment in flies relies upon the abnormal influx of glucose. Logically, this could affect every neuron in the organism, not only the peripheral sweet taste neurons. Chapter 3 addresses this with respect to the sweetness reward neurons by knocking down the critical sugar-impairment enzyme OGT using the

Gal4/UAS system, showing that, while these neurons' activity could control feeding behavior on a sugar diet, OGT did not govern their responses to sweet tastes. This suggests either (1) that glucose flux through the Hexosamine Biosynthesis Pathway (HBP) was not uniform across all neurons, or (2) that O-GlcNAcylation did not perform the same functions in the peripheral and central sweet taste responsive neuronal populations.

The question remains as to whether there are other neuronal subsets that are targeted by a sugary diet, whose functions were not tested here but that may contribute to hyperphagia. While analyzing every neuron population for sugar-induced changes to responsivity is somewhat outside the scope of this dissertation, a few populations – particularly of other peripheral taste neurons – do stand out as likely targets that could also affect feeding. Given that the sweet taste neurons are vulnerable to fluctuations in glucose levels (May et al. 2019; also Chapter 2), it is possible that other peripheral taste neurons could be as well. Indeed, published literature reports correlations between overeating of palatable foods and sensitivity or preference for two other taste modalities: bitter and fat. Children's genetically encoded sensitivity to propylthiouracil (PROP), a bitter compound, is predictive of intake of sweets at a buffet and of preference for dressing served with broccoli, indicating that children with greater sensitivity to bitter seek to avoid it (Fisher et al., 2012; Keller et al., 2014). Studies of insects have shown that dietary exposure to bitter compounds reduces bitter sensitivity and increases interaction with bitter food (Glendinning et al., 1999; Zhang et al., 2013). Furthermore, bitter sensation modulates sweet taste perception in flies (Chu et al., 2014; Jeong et al., 2013), and sweet can similarly modulate bitter sensitivity (Inagaki et al., 2014). Even

should the increased glucose metabolism leave bitter sensing neurons intrinsically unaffected, the cellular interaction between bitter and sugar modalities could contribute to an alteration in bitter taste that affects feeding behavior.

Similarly, decreased sensitivity to the taste of fat correlates with BMI and with exposure to fatty foods (reviewed by (D. Liu et al., 2016)). As the taste of fat is attractive, like sugar, it is possible that the central processing of these two tastes are similar, and may respond similarly to peripheral taste impairment. Genetic variation of the putative fat taste receptor, CD36, correlates with taste sensitivity and feeding behavior as well (Pioltine et al., 2016). As mentioned in Chapter 1, the combination of excess sugar and fat is common in modern obesogenic diets and has also been shown to have synergistic effects on hyperphagia (Oliva et al., 2017; Soto et al., 2015). In flies, fatty acid and sugar tastes are transduced through the same neuronal population (Masek & Keene, 2013), but whether they segregate within this population is uncertain. Nevertheless, this evidence indicates that both cell-intrinsic and cell-extrinsic mechanisms could cause alterations to fat tasting on sugar diets, with consequences for feeding behavior and weight gain.

Reversing the Diet, and Persistence of Taste Impairment

One question that the research presented here leaves unanswered is, given that sugar in the diet has such profound effects on taste and feeding, can changing the diet back to “normal” also fix the taste impairment? Preliminary investigation from our lab indicates that it does not. Seven days on sugar diet followed by seven days on the control diet reduces fat accumulation, but taste impairment measured by PER does not return sweet responsivity to pre-sugar levels (data not shown). This is in stark contrast

to diet-induced changes to other taste modalities, such as salt, which is highly malleable in both directions regardless of previous experience (Bertino et al., 1982, 1986; Contreras & Frank, 1979). The persistence of sugar-induced taste loss points to different underlying mechanisms, which may be “locked in” by the presence of sugar. Current efforts in the lab are investigating these mechanisms, particularly how O-GlcNAcylation may influence them and what allows them to persist after sugar is removed from the diet. One hypothesis that follows from the persistence of taste is that sugar hyperphagia may develop more rapidly in flies with prior history of a sugary diet. Results from our initial attempts to determine the validity of this hypothesis are in Appendix 1.

Sweet Taste Processing Circuitry

A number of publications guided us instead toward the study of dopaminergic neurons in the PAM cluster (PAM DANs) after our study of the primary sweet taste neurons. Brain-wide imaging of neurons activated by sucrose on the proboscis showed two clearly responsive cell body populations: one in the SEZ, and the other in the PAM cluster (Harris et al., 2015). Though analysis of the projections from both populations does not immediately reveal a connection between the two, this activation pattern was nevertheless highly consistent across brains. Reports of lines targeting putative second-order neurons from the SEZ encouraged us to test their activity following a sugar diet; however, in our hands sweet taste responses were elusive ((Kain & Dahanukar, 2015; Miyazaki et al., 2015); data not shown). Encouragingly, a series of papers detail the role of the PAM DAN population in encoding the unconditioned appetitive values of sucrose in olfactory learning paradigms (Burke et al., 2012; Huetteroth et al., 2015; C. Liu et al.,

2012; Yamagata et al., 2015). Two of these in particular demonstrated that sweet taste and energy content of sugar were separable both anatomically and in the learning paradigm, with sweet taste sufficient to form short-term odor memories and energy content sufficient to form long-term odor memories (Huetteroth et al., 2015; Yamagata et al., 2015). PAM neurons are broadly dopaminergic, but sweet taste short-term memory requires octopaminergic input to a subset of the PAM neurons (Burke et al., 2012; Huetteroth et al., 2015), which can be accessed using the R48B04-Gal4 line (R48B04 is a sequence derived from the promoter region of the octopamine receptor Oamb).

Dopaminergic and octopaminergic systems are not only downstream of the primary sweet taste neurons. It has been shown that both play a role in modulating sweet taste as a function of starvation state (Inagaki et al., 2012; Marella et al., 2012; Youn et al., 2018). As a fly is starved, the likelihood it will display a PER to sucrose increases. Inputs from the TH-VUM (dopaminergic) and OA-VPM4 (octopaminergic) neurons onto sweet taste neurons in the SEZ are necessary for this to occur, though they do not themselves respond to sweet tastes. We initially explored the idea that dopaminergic signaling may be involved in sugar diet taste sensitivity by feeding flies L-DOPA, the precursor to dopamine. We found that this reduced fat accumulation on a sugar diet, like correction of sweet taste neuron activity (see Appendix 2). From this result we hypothesized that the rewarding value of sweet taste carried by the PAM DANs was decreased by a sugar diet, correctable by feeding the fly L-DOPA to increase its dopaminergic tone, and this led to our investigations in Chapter 3. However, the alternative hypothesis, that L-DOPA augmented sweet taste sensitivity directly through

the TH-VUM or other unidentified populations, remains untested. Future work may focus on understanding how dopaminergic, and perhaps also octopaminergic, modulation of sweet taste is affected by sugary diets.

Similarly, sweet activation of other neurons may curb feeding independently of sensory satiation or energy feedback circuits. Two recently published works point to ionotropic receptors (Irs), which are expressed in neurons that sense sweetness and whose activation reduces sweet taste behaviors (Chen et al., 2019; Joseph et al., 2017). Joseph and colleagues discovered that *Ir60b* is expressed in a single pharyngeal neuron that requires it in order to be activated by sugar, but its activation promotes shorter feeding events. The authors propose that this is a mechanism by which flies can rapidly inhibit feeding on high-calorie food so as not to overeat. In the same vein, Chen et al. showed that *Ir76b* expressed in sweet taste (*Gr64f+*) neurons limits their calcium responses to sucrose, which is proposed to guide animals away from foods with extremely low sugar content in search of more useful, higher-calorie foods. The roles of these neurons in sugar diet hyperphagia was beyond the scope of this dissertation, but may prove to be interesting avenues of research.

Principles of Taste and Feeding

There is little doubt that taste matters to feeding decisions, and that sweet taste can be affected by diet. A controversy arises, however, when we try to specify the nature of the relationship between sweet taste and feeding. In mammalian literature, which encompasses nearly all of the research on the subject, we see disagreement as to whether sweet taste is less intense following excessive sugar consumption (Cicerale

et al., 2012; Jayasinghe et al., 2017; Low et al., 2016; May et al., 2019; Sartor et al., 2011). Furthermore, even if peripheral taste is conclusively decreased (as we have demonstrated in our fruit flies in Chapter 2), how this is processed in the brain is difficult to test and interpret. Does a decrease in sugar 'pleasantness', a common metric for self-reporting of sugar reward, indicate a desire for higher sugar concentrations – such as would result from a rightward shift in the concentration-pleasantness curve – or for less sugar altogether? What do either of these possibilities mean for actual feeding? Moreover, are there addiction-like mechanisms operating in the reward system that create tolerance-like behaviors toward sugar, in addition to attenuation of sensory-induced satiety coming from the weakening of sweet taste intensity (as we claim in Chapter 3)?

To derive new hypotheses from our data about sugar diet feeding behavior, we may benefit from placing our findings into an existing framework for food decision-making, such as addiction. My attempts to integrate the data presented in this dissertation into theories of overeating and obesity are below.

Sweet Taste Reward Deficit Theory

The Reward Deficit Theory of Obesity, first proposed by Wang, Volkow, and Fowler, states that a loss of reward region responsivity promotes compensatory overeating to achieve desired reward (Wang et al., 2002). On its face, this framework for overeating and obesity matches our data from Chapter 3 very well: a decrease in reward neuron responsivity to sweet taste is matched by an increase in feeding on sugary food. However, a thorough review of this and alternative theories that predict

weight gain concluded that the evidence for reward deficiency driving obesogenic behavior was weaker than the evidence for other theories (Stice & Yokum, 2016). They cite that while many published findings demonstrate a correlation between obesity and decreased dopaminergic reward responsivity, it does not on its own predict future weight gain, weakening the argument that it causes obesity. Among the alternative theories they covered were Incentive Sensitization, which is more representative of the reviewed data. Nevertheless, reward deficit theory may yet prove useful, if properly integrated into newer theories of obesogenic feeding. We do not claim here that sweet taste reward deficit is the only path to obesity; merely that its contributions to hyperphagia have been inconsistently observed and thus overlooked.

Sugar is not only rewarding by its taste. Research interest in changes to energy reward from sugar, rather than taste, as a driver of obesogenic feeding has grown in recent years. Food energy is detected even in the absence of orosensory taste in mammals and in flies (Dus et al., 2013; Sclafani, 2004), and is a rewarding stimulus (de Araujo et al., 2008; Huetteroth et al., 2015; Yamagata et al., 2015). Interestingly, energy reward is slower to signal than taste reward, as the energetic molecules must reach internal energy sensing neurons in the gut or brain in order for dopamine to release (de Araujo et al., 2017; Dus et al., 2013; Thanarajah et al., 2019). From this we may craft a theory of sugar-reward-driven hyperphagia that treats sweet taste as a constantly relearned, rather than hardwired, cue for energy reward.

Incentive Sensitization and Reinforcement Learning:

Sweet Taste as a Food Cue

Certain properties of sweet taste are hardwired, however its reinforcing power may not be so. While it is true that infants have innate hedonic responses to sweet taste (Berridge, 1991), these hedonic properties are processed through opioidergic circuits, rather than the reinforcing dopaminergic circuitry we have discussed here (Kroemer & Small, 2016). Furthermore, dopaminergic signaling to food cues (e.g., sight or smell) is well established under the theory of incentive sensitization. This theory explains that central-brain dopamine responses can shift from an innately rewarding experience to its cue following chronic exposure to both, and that this process leads to addiction (Robinson & Berridge, 1993). In this model of addiction, the dopamine signals denote increased importance of cues for an addictive substance, promoting cue fixation and drug seeking, which indicate wanting. Importantly, peripheral sweet taste perception intensity is more closely linked to consumption over reported pleasure, suggesting that peripheral taste drives wanting over liking (Puputti et al. 2019). Consumption of sugar in early life may induce a form of incentive sensitization by repeatedly pairing sweet taste – now acting as a sensory cue for food – with energetic reward, and this may increase sweet taste's potency as an independent reinforcer. Sweet taste's prediction of sugar's energy content through dopamine signaling would thus be established early in life but could still be malleable and independently critical for accurate feeding behaviors in the adult. A recent publication demonstrated that a "matching" of sweet taste with caloric value was necessary for appropriate reinforcement of a sweet beverage (Veldhuizen et al., 2017). Similarly, when flies have been given a sweet but noncaloric meal following

starvation, the reinforcing properties of sweet taste to nutritive sugars become attenuated in a phenomenon termed ‘caloric frustration memory’ (Musso et al., 2017). This indicates that sweet taste can be constantly reupdated to accurately cue for food energy.

If we hypothesize that sweet taste acts as a sensory cue for food reward much like the food’s visual or olfactory properties, we may predict that changing the strength of the dopaminergic signal to sweet taste through diminishing peripheral taste sensitivity may change the desire to feed – but does it make sense that this would increase feeding behavior? (Kroemer & Small, 2016) provide a framework for reinforcement learning via positive outcomes that clarifies how diminishing cue intensity (via sugar-impaired sweet taste) in our data from adult flies might drive overeating because sweet taste has been established through incentive sensitization as a potent cue for imminent food consumption. As stated before, sweet taste activates the PAM DANs in the fly brain, which, with their downstream MB output neurons, form the substrate for associative learning. As the signal for sweet taste decreases over time on a sugar diet, so too does the activation of these neurons (see Chapter 3). If sweet taste is a cue for energy content, then the fly is experiencing a greater energy-induced reward than it expects from its cued signal, and the sweet taste becomes a stronger cue for energy reward from future meals. This sets the stage for positive outcome learning to occur, whereby the fly becomes behaviorally sensitized to the taste of sugar, prompting increased feeding.

However, the fact that sweet taste processing is decreased also leads to the hypothesis that new memory formation using sweet taste as an unconditioned stimulus

will be less powerful, and this relies on the trans-synaptic communication between the PAM DANs and their downstream connections, the MBONs. The synaptic plasticity changes likely occurring across the connection between the PAM DANs and the MBONs is the focus of future projects from our lab.

Concluding Remarks

In conclusion, I propose that nutritive sugar (i.e., sucrose, glucose, fructose) is unique among tastants, in that it directly acts as a substrate to an organism's energy harvesting and utilization pathways while simultaneously acting as a signaling molecule, as in the case of O-GlcNAcylation, which uses a glucose metabolite to protein modification. This duality of sugar has myriad effects on metabolic and neuronal plasticity systems, some of which have been elucidated, while many are yet to be revealed. I have shown here that among these effects on neurons, the sensitivity of primary sweet taste neurons to sugar is impaired, and feeding behavior is increased. Control of feeding through changes to sweet taste-responsive neurons by sugar can be explained both with sensory-induced satiation and with a reinforcement learning framework whereby the fly's perception of its meal's energy is underestimated, leading to overeating and obesity. The molecular and circuit underpinnings of the fly's sweet taste perception and behavior are largely conserved with mammals; thus, the information presented in this dissertation may contribute to humanity's understanding of obesity etiology, with the hope of mitigating its detrimental effects to individuals and to society as a whole.

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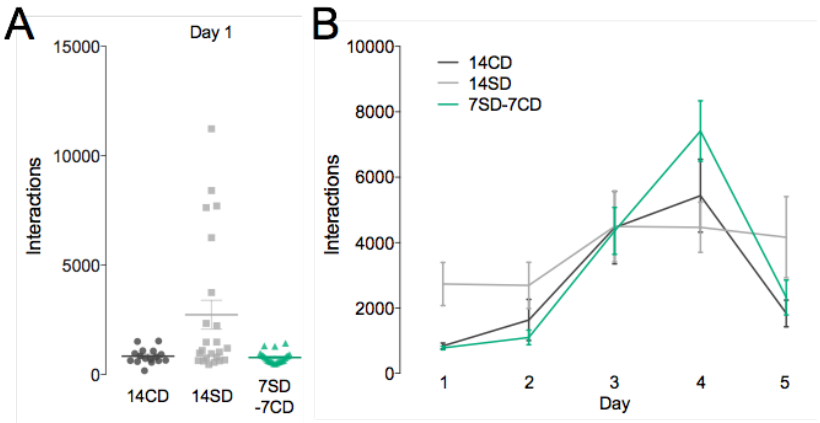
Appendices

Appendix 1: Persistence of Sweet Taste Impairment

Exposure to a sugar diet impairs taste and sweet taste controls feeding (see Chapters 2-4). Some preliminary evidence from our lab indicates that sweet taste impairment persists even after excess sugar is removed from the diet. Thus, we hypothesized that flies that have fed for 7 days on a sugar diet (SD) followed by 7 days on a control diet (CD) will exhibit hyperphagia earlier than control flies fed CD.



Appendix Figure 1 Effect of a reversal diet on feeding behavior.

A) Mean number of interactions on the first day for *w*^{1118}*CS* flies on 20% sucrose FLIC.

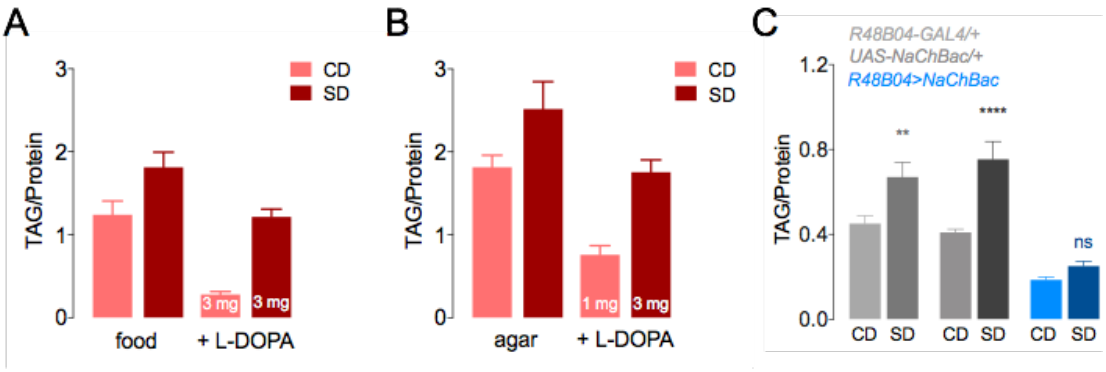
B) Mean number of interactions per day for *w*^{1118}*CS* flies on 20% sucrose FLIC.

As expected, the flies fed the sugar diet displayed hyperphagia even on the first day of the FLIC experiment. However, both the control diet and the “reversal” diet flies exhibited hyperphagia at the same rate, though it was earlier than expected for both (as

early as the second day, whereas previous experiments demonstrated it between days 4 and 9, see Chapters 2-4). We suspect that this is due to the extreme age of the flies when they start the FLIC (15-18 days old), as taste acuity is impaired with age. The hypothesis that persistence of taste loss will promote hyperphagia upon reintroduction to sugar requires further testing.

Appendix 2: Increasing Dopaminergic Tone Decreases Hyperphagia

Initial investigations into central processing of sweet taste focused on dopamine, particularly from neurons in the PAM cluster. Other labs had shown that dopamine inputs to the sweet taste neurons could increase PER, and that PAM DANs were activated by sugar (Marella et al. 2012; Inagaki et al. 2012; Harris et al. 2015). Given that sweet taste neuron activity and sweet taste response behavior (PER) was impaired, we hypothesized that dopaminergic tone was decreased in the fly brain on a sugar diet. To test this hypothesis, we supplemented their food with the dopamine precursor L-DOPA, and constitutively activated sweet-taste, short-term memory PAM neurons (R48B04-Gal4).



Appendix Figure 2 Effect of feeding L-DOPA on sugar-diet fat accumulation.

A) Mean whole-fly triglycerides normalized to protein levels in w^{1118}*CS* flies fed 7-day control (salmon) or sugar (red) cornmeal-based diets, supplemented with 3mg/mL L-DOPA.
B) Mean whole-fly triglycerides normalized to protein levels in w^{1118}*CS* flies fed 7-day control (salmon) or sugar (red) 1% agar diets, supplemented with 1 or 3 mg/mL L-DOPA.
C) Mean whole-fly triglycerides normalized to protein levels in flies with constitutively activated sweet-taste PAM-DANs fed 7-day control (blue) or sugar (dark blue) cornmeal-based diets, with transgenic controls (greys). n=8 per group, two-way ANOVA with Sidak's multiple comparisons test, **p<0.01, ****p<0.0001, comparisons between diets within genotypes.

We concluded from this data that dopaminergic tone in the PAM/MB region was decreased, and that specifically activating sweet-taste PAM neurons would correct feeding behavior downstream of the peripheral sweet taste neurons.