Interactions between taste modalities in *D. melanogaster*

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Abstract

Efficient neural control of feeding is essential for an animal's survival. However, how different taste modalities (e.g., sugar, water, bitter, and salt) are integrated to make feeding decisions is not yet well understood. To understand how different taste modalities are integrated in the fruit fly *Drosophila melanogaster*, I reconstructed neurons involved in feeding, and analyzed the effects of activation and silencing of different populations of gustatory receptor neurons (GRNs). I used the annotation platform CATMAID to reconstruct Roundup, a neuron that is sufficient for proboscis extension and responds to sugar. My tracing identified Roundup as a premotor neuron and directly postsynaptic to three second-order sugar neurons. Remarkably, Roundup is also directly postsynaptic to a second-order bitter neuron, Scapula, indicating how sugar and bitter are integrated in the central brain. Reconstruction of sugar and water GRNs indicated they may share downstream second-order neurons, so we tested if silencing sugar-sensing neurons might influence proboscis extension to water. I found that silencing sugar GRNs inhibits water consumption in both starved and desiccated flies; these results are consistent between silencing sugar neurons by hyperpolarization with GtACR1 or blocking synaptic transmission with tetanus toxin light chain (TNT). To further understand how different taste modalities influence feeding, I optogenetically activated distinct taste modalities (sugar, water, bitter, low salt, and high salt) to see if they are sufficient for proboscis extension or if activation inhibits proboscis extension. Our preliminary results suggest that, in fed flies, Gr64f (sugar) and Ir94e (low salt) neurons cause proboscis extension, while Ppk23 (high salt), Ppk28 (water) and Gr66a (bitter) do not. Activation of Gr64f, Ir94e and Gr66a inhibited proboscis extension in response to water and 100 mM sucrose. Our results suggest that Ir94e neurons may play a role in inhibiting feeding initiation, consistent with their synaptic connectivity onto second-order bitter neurons. In total, my research uses neural connectivity and behavioral assays to identify mechanisms through which *Drosophila* can integrate different taste modalities.

Introduction

Efficient neural control of feeding is essential for an organism's survival. A critical cue guiding feeding decisions is taste. Sugars and sweet taste generally promote feeding behavior, signaling essential nutrients that an organism needs. Bitter compounds inhibit feeding behavior, signaling potentially harmful substances that an organism should avoid. However, how different taste modalities are integrated is poorly understood. The fruit fly, *Drosophila melanogaster*, is a powerful model organism to study taste processing because of its relatively simple brain circuitry and the ease in quantifying feeding behavior as proboscis extension. *D. melanogaster* is similar to mammals, because it uses common taste modalities to make feeding decisions (e.g., sugar, salt and bitter), which suggests that taste coding may be similar across organisms (Scott, 2018; Montell, 2020).

Following the activation of sensory neurons on the legs, the fly stops walking and either extends its proboscis to appetitive compounds or retracts its proboscis to aversive compounds. Distinct neurons have been identified that are directly activated by different taste compounds, the gustatory receptor neurons (GRNs). Each set of GRNs is thought to have an inherent valence; activation of sugar-sensing GRNs promotes feeding, while activation of bitter GRNs inhibits consumption. GRNs are divided into five categories based on taste response including sugar, water, bitter, high salt, and low salt; each GRN category expresses specific receptors that directly respond to tastants (Scott, 2018; Montell, 2020). Gr64f expressing GRNs respond to sugar, while Gr66a neurons respond to bitter; they are involved in proboscis extension and retraction respectively. Ppk28 has been shown to be involved in responding to water and can function as an osmosensor (Cameron et al., 2010; Chen et al., 2010). More recently, populations of neurons that respond to high salt and low salt have been identified. Ir94e neurons have been suggested to be "low salt" neurons and are suggested to promote feeding, while a subset of Ppk23 neurons are thought to respond to "high salt" and be aversive (Jaeger et al., 2018).

However, how these taste modalities are integrated in higher order neurons is poorly understood. Specifically whether different taste modalities, like sugar and water, use shared or distinct pathways to inform response is unknown. While sugar and bitter GRNs project to distinct regions of the feeding region of the fly brain, the subesophageal zone, sugar and water project to a common region, raising the possibility that they may share common downstream pathways. Likewise, we also don't know how, or if, sugar and bitter are integrated in the central brain. In the periphery, bitter compounds may directly suppress sugar-sensing sensory neuron activity (Scott, 2018; Jeong et al., 2013). How a single compound, salt, can promote both attractive and aversive responses based on concentration is also unknown. Here, I examined how different taste modalities are integrated to make feeding decisions. I found that sugar and bitter are integrated at the level of premotor neurons, that sugar-sensing neurons are required for proboscis extension to water, and that Ir94e neurons may be aversive, rather than attractive.

Materials and Methods

Electron microscopy tracing of neurons in the female adult fly brain (FAFB)

The FAFB was imaged by researchers (Zheng et al., 2018) at synaptic resolution to support mapping of brain-spanning circuits. I used the annotation platform CATMAID to trace along the length of the neuron of interest. Additionally, presynaptic and postsynaptic connections to the neuron of interest were marked. Synaptic connections were annotated based on three markers: a T-shaped bar, synaptic vesicles, and a synaptic cleft. The full neuron of interest was visualized using the 3D viewer tool in CATMAID. To determine the level of connectivity between two neurons, synaptic connections between them were quantified and compared.

Measuring proboscis extension response (PER) in fruit flies
Fruit flies were anesthetized with CO_2 gas then mounted on glass slides using nail polish in groups of 10 flies per slide. The experiments were performed blind to the genotype of each set of flies. The flies were allowed to recover in a humid chamber, or in a desiccation chamber, for 2 hours. Each fly was presented with stimuli one at a time and observed under the microscope for PER. Stimuli was presented as a droplet at the end of a syringe with a pipette tip attached. Stimuli was either water, 50mM sucrose, 100mM sucrose, or polyethylene glycol (PEG).

Creating split Gal4-lines
Members of the Scott lab created a library of 400 split Gal4 lines that specifically labeled 216 cell types in the SEZ (Sterne et al., 2021).

GFP reconstitution across synaptic partners (GRASP)
Second-order neurons were identified using GFP reconstitution across synaptic partners (GRASP), as in Kim, Kirkhart, and Scott, 2017. The following primary antibodies were used: 1:500 Living Colors rabbit anti-RFP: 632496, 1:200 Sigma monoclonal mouse anti-GFP: G6539 The following secondary antibodies were used: 1:100 Invitrogen 488 goat anti-mouse, 1:100 Invitrogen 568 goat anti-rabbit.

Activation screen of neurons required for PER
The neurons labeled by the split Gal4 lines and the second-order neurons were optogenetically activated and screened to identify which ones were required for proboscis extension response (PER). Flies were activated with CsChrimson or silenced with GtAcr1 (Klapoetke et al., 2014; Mohammed et al., 2017).

Statistical analysis
Fisher's exact test was used to calculate statistical significance for the PER experiments.

Results

Reconstruction of the premotor neuron Roundup
To understand how different taste modalities might elicit or inhibit proboscis extension, we began by reconstructing the proboscis extension circuit using an electron microscopy volume, the Full Adult Female Brain (FAFB; Zheng et al., 2018). In particular, I reconstructed a neuron directly presynaptic to the proboscis motor neuron 9 (MN9). MN9 is required for proboscis extension and feeding initiation. I reconstructed Roundup in the FAFB EM dataset using CATMAID. We found that Roundup is a large neuron with dendrites on one side of the brain, and axons that project onto MN9 on the other side of the brain (**Figure 1A and B)**. My reconstruction confirmed that Roundup is presynaptic to MN9 and that Roundup is postsynaptic

to three second-order sugar neurons. Remarkably, Roundup is presynaptic to two motor neurons: MN6 and MN9. Roundup synapses onto MN9 337 times, and MN6 39 times. Likewise, two other premotor neurons I partially reconstructed, Rounddown and Roundtree, both synapse strongly onto MN9, and less strongly onto MN6. MN9 and MN6 are involved in extending different portions of the proboscis, the rostrum and labellum, respectively; we speculate that the fact Roundup and other premotor neurons strongly synapse onto MN9 and weakly onto MN6 may partially reveal how these two different proboscis muscles are coordinated.

We also found Roundup as directly postsynaptic to a second-order bitter neuron, Scapula. This is interesting because previous research has identified that sugar and bitter are integrated in the periphery, but my tracing shows that they are also integrated in the central brain at the premotor level. Consistent with this connectivity, members of the Scott lab have found that Roundup responds to sugar, but is inhibited by optogenetic bitter activation, while upstream neurons are not inhibited by bitter (Shiu, Sterne et al., 2022). Thus, I have identified a central mechanism by which sugar and bitter tastes are integrated.

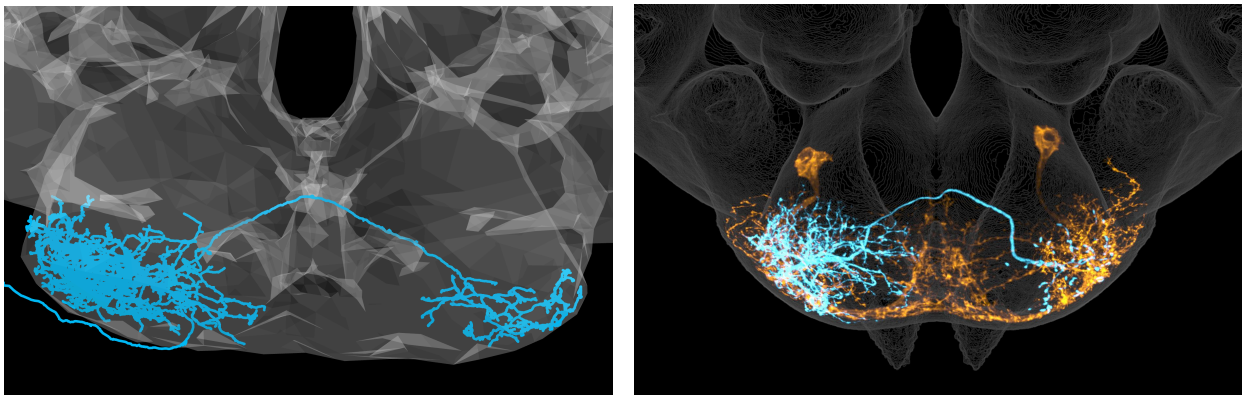

Figure 1. CATMAID tracing of Roundup and light microscopy image of Roundup and Motor Neuron 9. Fig. 1A (left): Electron microscopy (EM) reconstruction of the premotor neuron Roundup using the annotation platform, CATMAID. Fig. 1B (right): Light microscopy image of Roundup (blue) and MN9 (orange).

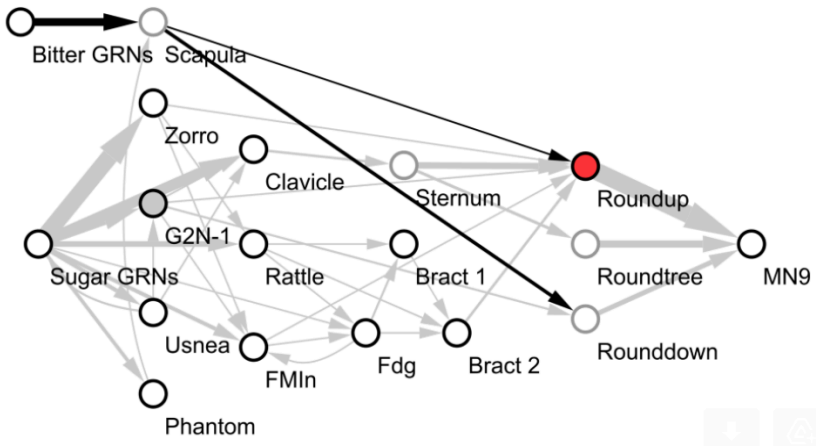

Figure 1C. An overall connectivity of the sugar sensorimotor circuit (Shiu, Sterne et al., 2022), showing the site of integration between sugar and bitter taste information.

GRASP analysis of a putative second-order taste neuron

To examine how different taste modalities are integrated, we examined their projections in the SEZ. Previous members of the lab have observed that sugar and water GRNs project to a similar region, raising the possibility that these neurons might share common downstream neurons and form a shared attractive pathway (Engert et al., 2021; Scott, 2018; Cameron et al., 2010). To identify connectivity between GRNs and second-order neurons, we performed GFP reconstitution across synaptic partners (GRASP; Gordon and Scott, 2009). In GRASP, two "halves" of GFP are expressed in different cell populations, and only where the two are in close physical proximity is GFP reconstituted. The second-order taste neuron, Usnea, was found by a lab member to GRASP with sugar GRNs; later EM reconstruction of Usnea confirmed this connectivity (Shiu, Sterne et al., 2022).

I sought to examine whether Usnea might also GRASP with water GRNs. Indeed, I found a significant GRASP signal between Usnea and water GRNs (**Figure 2**). Later EM reconstruction found that Usnea does indeed get synaptic input from putative sugar and water GRNs. Furthermore, Usnea responds to water in calcium imaging experiments (Shiu, Sterne et al., 2022). Thus, I identify a second-order neuron that receives both sugar and water GRN input, and responds to water.

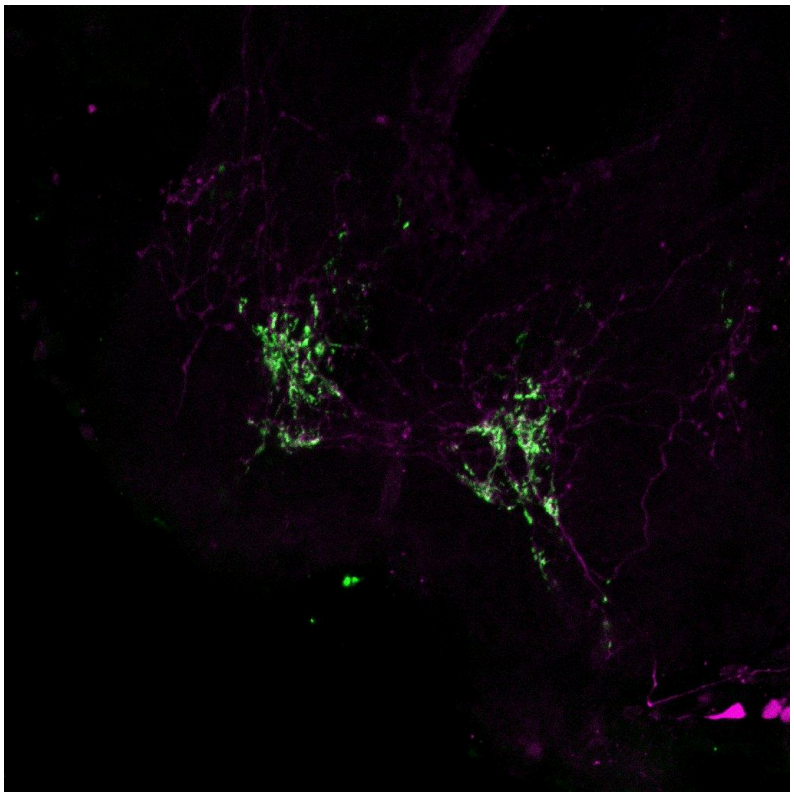

Figure 2. GRASP fluorescence of Usnea with water (Ppk28) GRNs. GRASP fluorescence is in green; Usnea is in purple.

The integration of water and sugar

Because sugar and water project to a similar region of the brain, and may synapse onto a common set of second-order neurons including Usnea, we decided to test their integration using behavioral assays. To do this, we silenced GRN populations using the green-light activated anion channelrhodopsin GtAcr1 (Mohammad et al. 2017). Consistent with their known roles, silencing Gr64f sugar-sensing neurons inhibited proboscis extension to sugar (**Figure 3**), while silencing Ppk28 water-sensing neurons inhibited proboscis extension to water. The Gr5a/+ control had low rates of proboscis extension to sugar, so we did not find a difference between Gr5a/+ and Gr5a > GtAcr1 in proboscis extension to sucrose.

Remarkably, we found that inactivation of either Gr64f or Gr5a neurons lead to decreased responses to water (**Figure 3**). These results raised the possibility that sugar-sensing neurons not only may be important in proboscis extension to sugar, but surprisingly, to water as well. That sugar-sensing neurons may contribute to water consumption is somewhat surprising because sugar-sensing neurons do not respond strongly to water (Marella et al., 2006; Jeong et al., 2018). We therefore decided to test the role of sugar-sensing neurons in water consumption in a variety of conditions to exclude the possibility that these results may be an artifact of our experimental conditions.

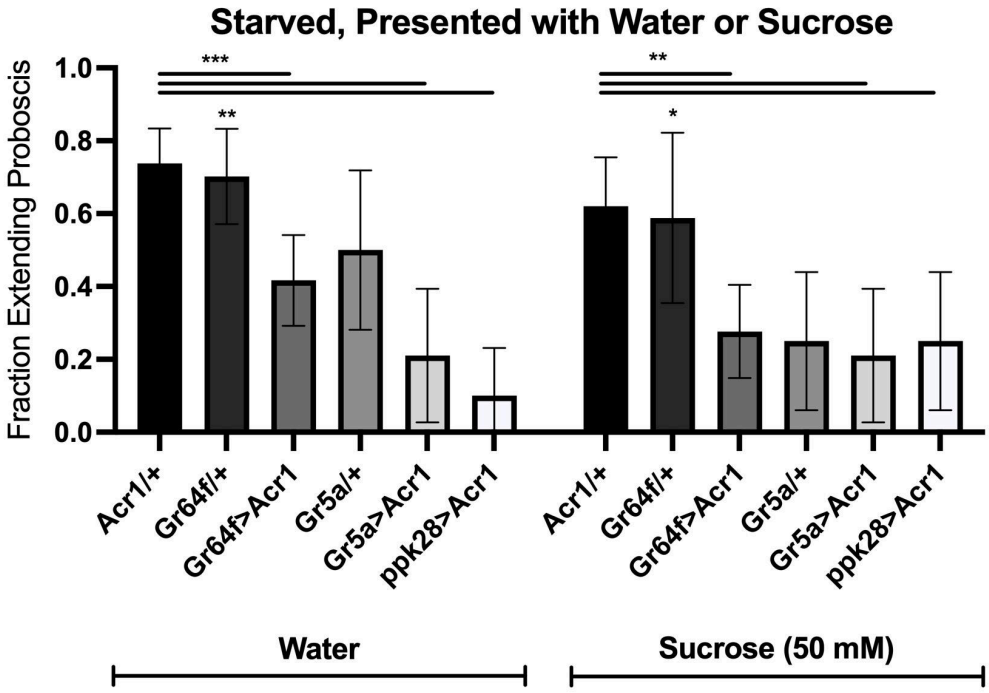

Figure 3. Inactivating the sugar-sensing neuron Gr64f with GtACR1 shows a decreased response to water and sugar. Inactivating the sugar-sensing neuron Gr5a shows a decreased response to water, but not sugar. Inactivating the water-sensing neuron ppk28 shows a decreased response to water, but not sugar. (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) n = 10 per genotype.

First, we reproduced these results using two other methods of inactivation. We used the inwardly rectifying potassium channel Kir2.1, and found that silencing Gr64f neurons using Kir2.1 inhibited proboscis extension to both sugar and water (**Figure 4A**). We also tested blocking chemical synaptic transmission by expressing tetanus toxin light chain in Gr64f neurons. This also decreased proboscis extension to both sugar and water (**Figure 4B**).

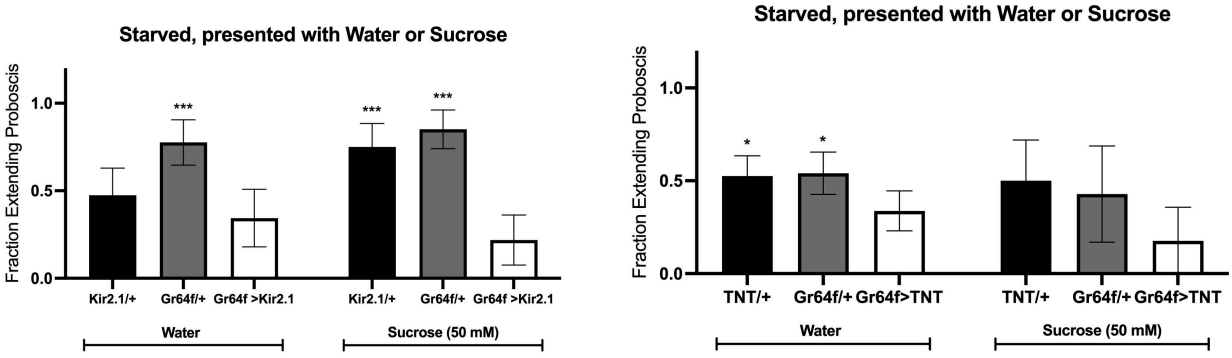

Figure 4. Inactivating the sugar-sensing neuron Gr64f with Kir2.1 or TNT. Fig. 4A (left): Inactivating the sugar-sensing neuron Gr64f with the inward rectifying potassium channel Kir2.1 shows decreased response to sugar. Fig. 4B (right): Inactivating the sugar-sensing neuron Gr64f with tetanus toxin (TNT) also causes a decreased response to sugar. (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) n = 10 per genotype.

Additionally, because this assay was originally designed to detect decreases in consumption of sugar, the previous experiments were performed in starved flies. To test if this effect is specific to starved flies, we instead performed these experiments in dessicated flies, and indeed found that dessicated flies also require Gr64f neurons for proboscis extension (Table 1). Finally, we also wanted to test that the response to water is based on sensing of low osmolarity, so we measured response to 20% polyethylene glycol (PEG), a tasteless high osmolarity solution (by itself or with 50mM sucrose). The flies did not extend their proboscis to PEG by itself across genotypes (**Figure 4C**), demonstrating that proboscis extension to water is not based on mechanosensation but based on sensing the low osmolarity content of the stimuli.

Table 1: Silencing PER Data (Non-starved, Desiccated Flies)

	FRACTION EXTENDING PROBOSCIS	FRACTION EXTENDING PROBOSCIS
GENOTYPE	Water	50 mM Sucrose
Acr1/+	27/30	23/30
Gr64f/+	27/30	25/30
Gr64f>Acr1	12/25	10/25

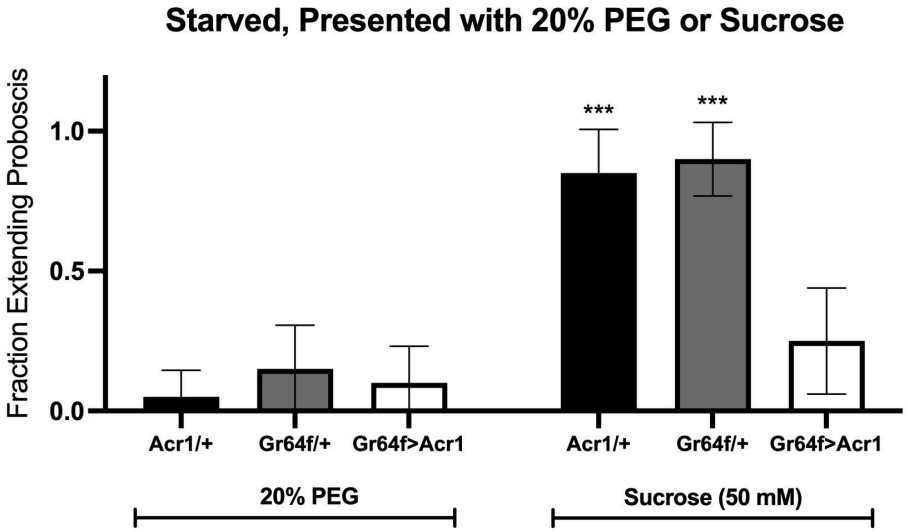

Figure 4C. Presenting flies with PEG lead to no proboscis extension across genotypes. Inactivating the sugar-sensing neuron with Acr1 and presenting flies with PEG containing 50mM sucrose lead to decreased response. (* = P < 0.05; ** = P < 0.01; *** = P < 0.001) n = 10 per genotype.

Activating different taste modalities to water and sugar

Next we wanted to get a broad understanding of how different taste modalities contribute to promoting or inhibiting proboscis extension. To do this, we activated different populations of GRNs, and measured whether this was sufficient for proboscis extension, or if activating these populations of neurons inhibited proboscis extension. We found that, in fed flies, activating sugar-sensing neurons caused proboscis extension in roughly half of the flies, consistent with our previous observations (Shiu, Sterne et al., 2022). Remarkably, activation of Gr66a and Ir94e also caused proboscis extension (**Figure 5**). Ir94e neurons have previously been suggested to be attractive "low salt" neurons. Activation of Gr66a has previously been shown to cause proboscis extension; this proboscis extension is distinct from proboscis extension to attractive foods because the rostrum does not appear to extend (Joie Zhou thesis, 2020). Optogenetic activation of Ir94e neurons caused proboscis extension similar to that of Gr66a.

Activation of known attractive populations of neurons appeared to decrease proboscis extension, which we interpret as the result of saturating these neurons, resulting in an inability of these neurons to cause proboscis extension. The sugar-sensing neuron Gr64f, when activated, decreased proboscis extension rates to water or sugar. Activation of Ppk28, a water-sensing neuron, leads to decreased response when presented with water and sugar. Remarkably, activation of Ir94e inhibited proboscis extension. We interpret these results to indicate that sugar and low salt-sensing neurons cause proboscis extension while bitter and putative low-salt sensing neurons also inhibit proboscis extension, suggesting that Ir94e neurons likely are aversive, rather than attractive.

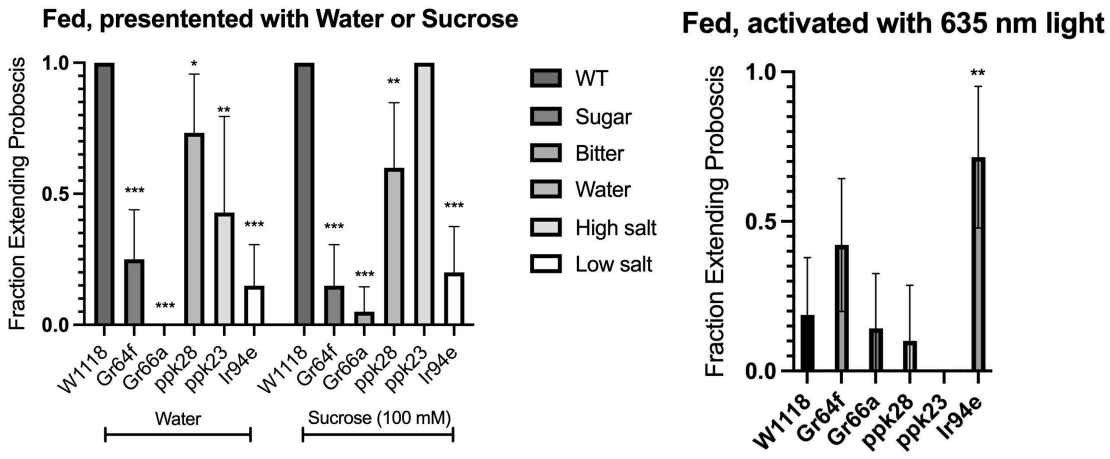

Figure 5. Split Gal4 lines for Gr64f (sugar), Gr66a (bitter), water (ppk28), high salt (ppk23), and low salt (Ir94e) were crossed to UAS-csChrimson. The offspring of this cross were exposed to 635 nm light to optogenetically activate each specific cell line and presented with water or 100 mM sucrose. The fraction of flies that extended their proboscis was recorded. (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) n = 10 per genotype. Figure 4A (left): When activated and presented with water, sugar-, bitter-, water-, low-salt-, and high-salt-sensing neurons showed decreased PER. When activated and presented with sugar, sugar-, bitter-, water-, and low-salt-sensing neurons showed decreased PER. Figure 4B (right)**:** High-salt-sensing Ir94e neurons demonstrated a statistically significant PER when activated.

Discussion

In total, my research uses neural connectivity and behavioral assays to identify mechanisms through which *Drosophila* can integrate different taste modalities. I found that sugar and bitter taste information is integrated at the premotor level by tracing the premotor neuron Roundup. This was suggested by the level of post and presynaptic connections between Roundup and both sugar GRNs and bitter GRNs. Consistent with this proposed connectivity, members of the lab found that optogenetic bitter activation inhibited the activity of Roundup, but not neurons directly upstream of Roundup (Shiu, Sterne et al., 2022). Prior to my research, we were unsure on which level sugar and bitter taste information are integrated, and we now know it is on the premotor level. We can continue to trace neurons to come up with complete neural circuits and determine relationships between other taste modalities.

Integration of aversive bitter, and potentially Ir94e, with attractive sugar information at the level of premotor neurons

It is possible that integration at the level of premotor neurons may be a common mechanism for the integration between aversive and attractive tastes. I found that activation of Ir94e neurons, thought to be attractive (Jaeger et al., 2018), instead appears to inhibit proboscis extension. Two neurons downstream of these Ir94e neurons directly synapse onto Roundup, and computational modeling suggests that these neurons are required for the inhibition of motor neuron activity due to activation of Ir94e (Philip Shiu, personal communication). Thus, it is

possible that both bitter and Ir94e integrate with the attractive sugar/water pathways at the level of premotor neurons.

Integration of sugar and water

I found silencing sugar inhibits the consumption of water based on behavioral assays. This suggests that sugar and water taste modalities interact as well, perhaps through a shared attractive pathway. The integration of taste neurons in the central brain suggests a medium between the labeled line hypothesis and the population coding model. Rather than completely separate circuits, parts of the pathways involved in sensing and responding to different attractive taste modalities seem to overlap.

Why might inhibiting sugar prevent proboscis extension to water?

An electron microscopy analysis of different populations of second-order neurons suggests that water GRNs synapse onto second-order neurons that get input from both sugar GRNs and water GRNs. For example, the second-order neuron Clavicle gets roughly 50% of its synaptic input from sugar GRNs (**Figure 6**), while the rest of its GRN synaptic input is from the high-salt-sensing Ppk23 neurons and water GRNs. Clavicle responds to water in thirsty flies (Shiu, Sterne et al., 2020). Thus, one explanation from my result that silencing sugar GRNs inhibits water consumption, is that sugar GRNs directly synapse onto second-order water neurons, and that the activity of these sugar GRNs is required to elicit activity of second-order water neurons.

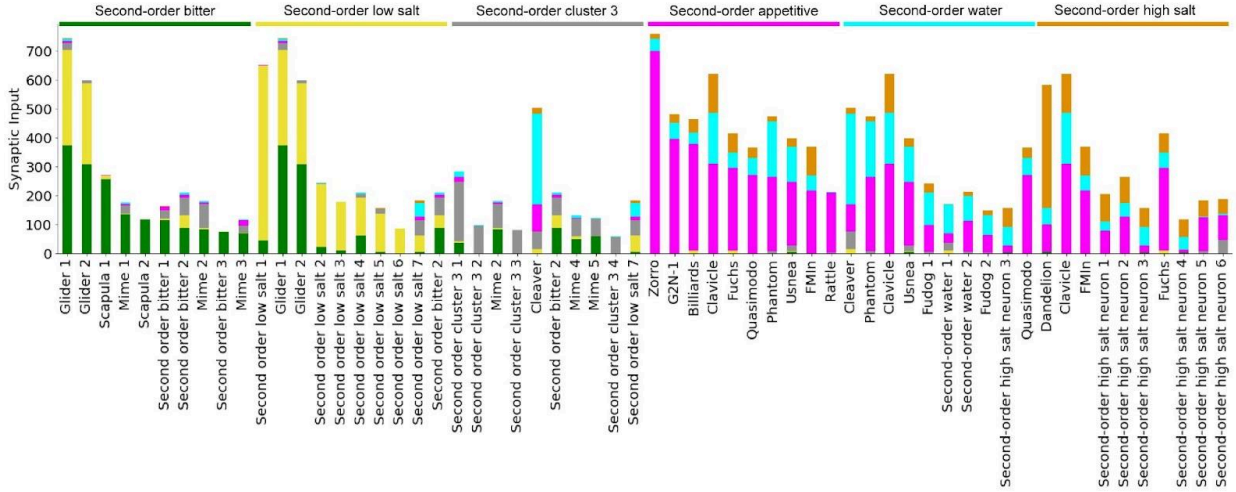

Figure 6. Synaptic input from GRNs onto different putative second-order neurons, generated from Flywire connectivity. (From Philip Shiu) Green = connectivity from bitter GRNs, yellow = Ir94e, gray = unknown, pink = sugar GRNs, blue = water GRNs, orange = high salt GRNs.

Are Ir94e neurons aversive?

Ir94e neurons were previously suggested to be attractive, "low salt" neurons (Jaeger et al., 2018). However, my results here suggest they may instead be aversive. Jaeger et al. suggested that Ir94e neurons may be attractive for two reasons. First, "low salt" neurons have

previously been speculated to be attractive; that Ir94e responds to low salt suggests that Ir94e may be these attractive "low salt" neurons. Furthermore, while silencing Ir94e with Kir2.1 did not inhibit attraction to 50 mM NaCl, silencing Ir94e with TNT did (Jaeger et al., 2018). However, differences between this assay, a binary two-choice assay, and our experiments, in which proboscis extension in fixed flies is measured, may account for differences in the interpretation of our results. Jaeger et al. never examined activation phenotypes of Ir94e neurons.

Future directions

The results from Figure 4 need to be further examined and reproduced. Gr64f has been strongly characterized as a sugar-sensing neuron and should promote PER but seemed to inhibit PER in my experiment. This is likely due to saturating Gr64f activity. We also thought sensing low salt via the Ir94e neuron promotes an attractive response but based on the results from Figure 4, we think it promotes an aversive response. Further experiments may help resolve this discrepancy.

Overall, I learned more about the neural circuitry that facilitates feeding decisions in the fly brain. The integration of sugar and bitter taste GRNs at the level of premotor neurons is now understood based on connectivity. Future research includes analyzing the downstream connectivity of other GRNs to develop a full understanding of the neural circuitry involved in feeding. The behavioral assays involving activation of the five taste modalities should be repeated to strengthen the reliability of our results. My behavioral and connectivity results found that sugar and water may be integrated; specifically that the two taste modalities are related based on both behavioral assays and connectivity analysis. Further analysis into the response of second-order taste neurons to water and sugar will help resolve how shared or distinct the attractive sugar and water pathways are.

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References

Chen, Yu-Chieh David, and Anupama Dahanukar. "Recent Advances in the Genetic Basis of Taste Detection in Drosophila." SpringerLink, Cellular and Molecular Life Sciences, 9 Oct. 2019, https://link.springer.com/article/10.1007/s00018-019-03320-0.

Engert, Stefanie, et al. “Drosophila Gustatory Projections Are Segregated by Taste Modality and Connectivity.” BioRxiv, Cold Spring Harbor Laboratory, 1 Jan. 2022, https://www.biorxiv.org/content/10.1101/2021.12.08.471796v2.full.

Gordon, M.D., and Scott, K. (2009). Motor Control in a Drosophila Taste Circuit. Neuron 61, 373–384.

Jaeger AH, Stanley M, Weiss ZF, Musso PY, Chan RC, Zhang H, Feldman-Kiss D, Gordon MD (2018) A complex peripheral code for salt taste in Drosophila. eLife 7:e37167.

Jeong YT, Shim J, Oh SR, Yoon HI, Kim CH, et al. 2013. An odorant-binding protein required for suppression of sweet taste by bitter chemicals. Neuron 79(4):725–37

Mohammad, F., Stewart, J.C., Ott, S., Chlebikova, K., Chua, J.Y., Koh, T.-W., Ho, J., and Claridge-Chang, A. (2017). Optogenetic inhibition of behavior with anion channelrhodopsins. Nat Methods 14, 271–274.

Montell, Craig. “Drosophila Sensory Receptors-a Set of Molecular Swiss Army Knives.” Genetics, Oxford University Press, 3 Mar. 2021, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8045702/.

Scott, Kristin. “Gustatory Processing in Drosophila Melanogaster.” Annual Reviews, Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, Jan. 2018, https://www.annualreviews.org/doi/10.1146/annurev-ento-020117-043331.

Shiu, Philip K., et al. “Taste Quality Interactions and Transformations in a Sensorimotor Circuit.” BioRxiv, Cold Spring Harbor Laboratory, 1 Jan. 2022, https://www.biorxiv.org/content/10.1101/2022.03.06.483180v1.

Sterne, Gabriella R, et al. “Classification and Genetic Targeting of Cell Types in the Primary Taste and Premotor Center of the Adult Drosophila Brain.” ELife, ELife Sciences Publications, Ltd, 2 Sept. 2021, https://elifesciences.org/articles/71679.

Zheng Z; Lauritzen JS; Perlman E; Robinson CG; Nichols M; Milkie D; Torrens O; Price J; Fisher CB; Sharifi N; Calle-Schuler SA; Kmecova L; Ali IJ; Karsh B; Trautman ET; Bogovic JA; Hanslovsky P; Jefferis GSXE; Kazhdan M; Khairy K; Saalfeld S; Fetter RD; Bock DD; “A Complete Electron Microscopy Volume of the Brain of Adult Drosophila Melanogaster.” Cell, U.S. National Library of Medicine, 19 July 2018, https://pubmed.ncbi.nlm.nih.gov/30033368/.

Zhou, Joie. A Quantitative Analysis of Drosophila Proboscis Extension Phenotypes Reveals Behavioral Subprograms. 2020 UC Berkeley MCB Honors Thesis.