Interactions between taste modalities in *D. melanogaster*

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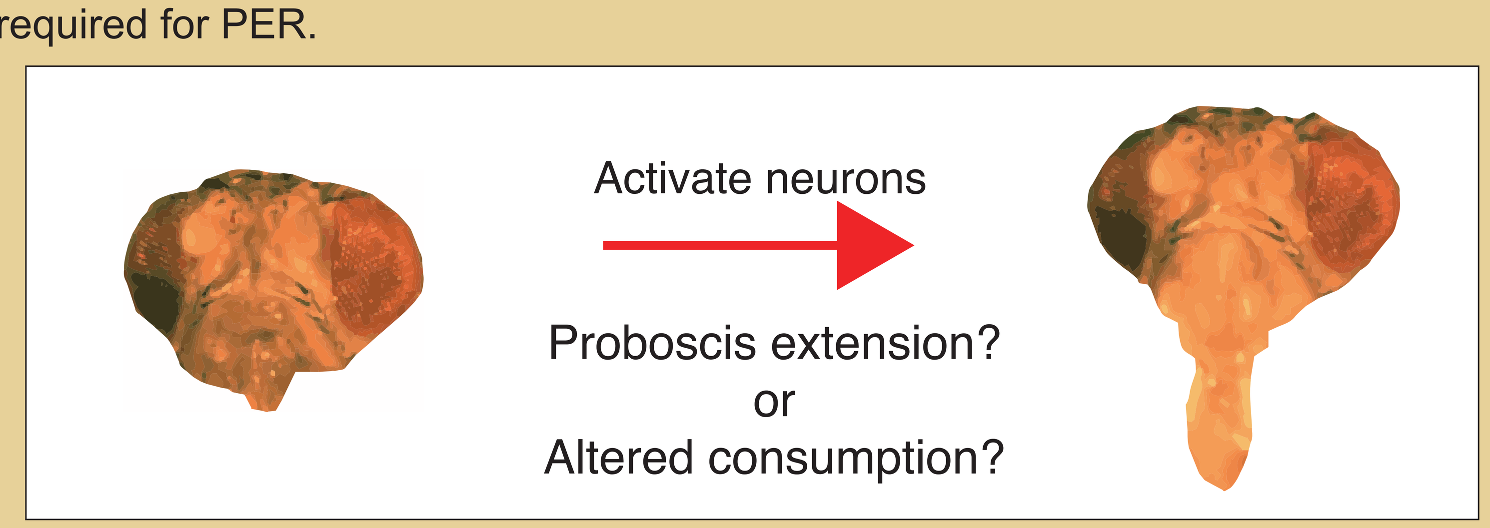
Introduction

Efficient neural control of feeding is essential for an organism's survival. Different taste modalities, such as sweet, bitter and salt, promote or inhibit feeding. However, how these taste modalities are integrated is poorly understood. The fruit fly, *Drosophila melanogaster*, is a powerful model organism to study feeding and taste processing because of its relatively simple neural circuitry and ease in quantifying behavior as proboscis extension. Previous research on *D. melanogaster* has identified distinct taste modalities that inform response to stimuli. I am interested in how these taste modalities (sugar, water, bitter, and salt) are integrated to influence feeding decisions. This will lead to a better understanding of the neural circuitry of the *D. melanogaster* brain and how taste information guides feeding decisions.

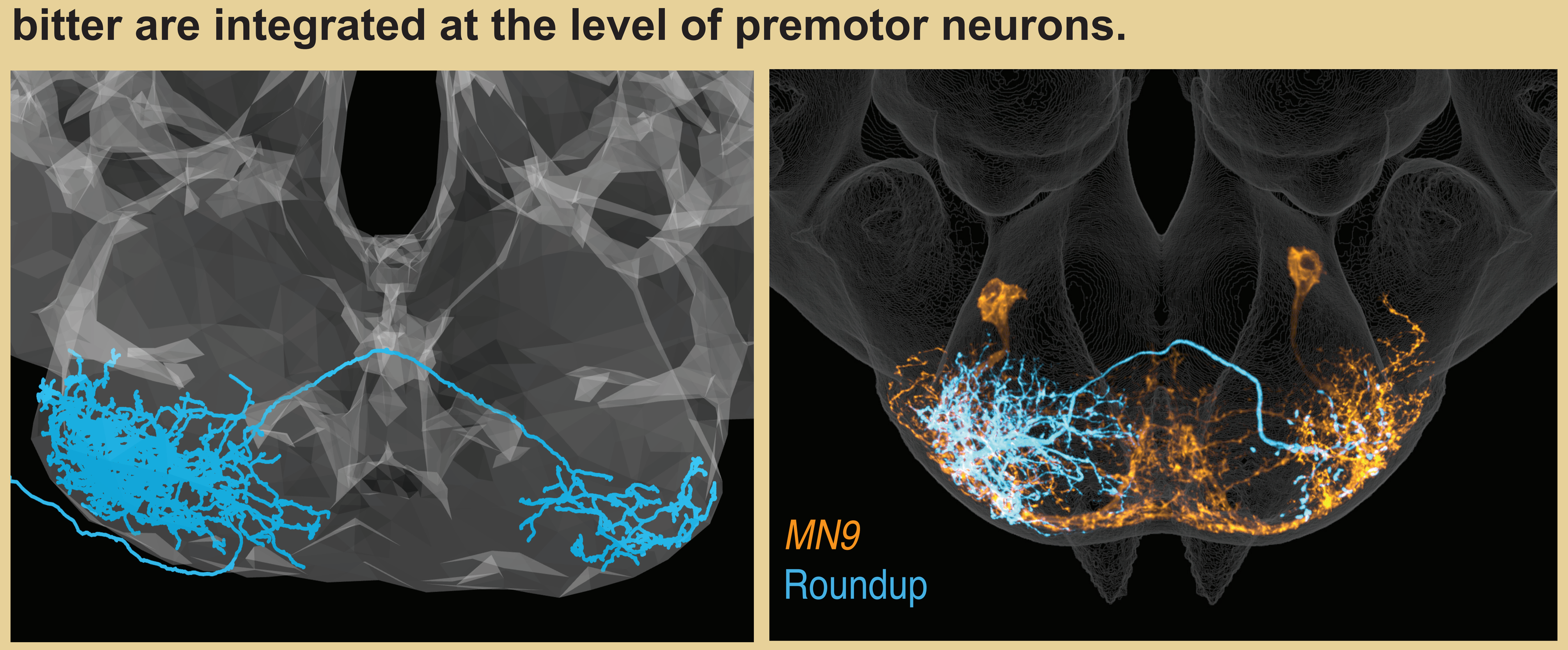
Methods

The region of the *D. melanogaster* brain called the subesophageal zone (SEZ) is known to be involved in sensory-driven action including feeding. The full adult fly brain (FAFB) electron microscopy volume (Zheng et al., 2018) was used to manually reconstruct neurons. 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). The neurons labeled by the split Gal4 lines were optogenetically activated and screened to identify which ones were required for proboscis extension response (PER). Second-order neurons were identified using GFP reconstitution across synaptic partners (GRASP). The second-order neurons were also optogenetically activated and screened via the corresponding split Gal4 lines to see which ones were required for PER.



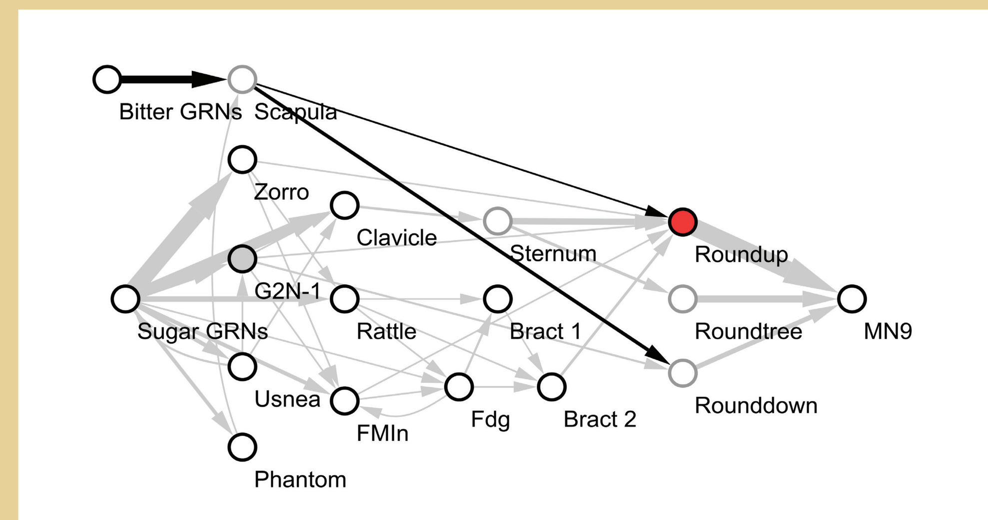
Results

Figure 1: Electron microscopy reconstruction identifies that sugar and bitter are integrated at the level of premotor neurons.



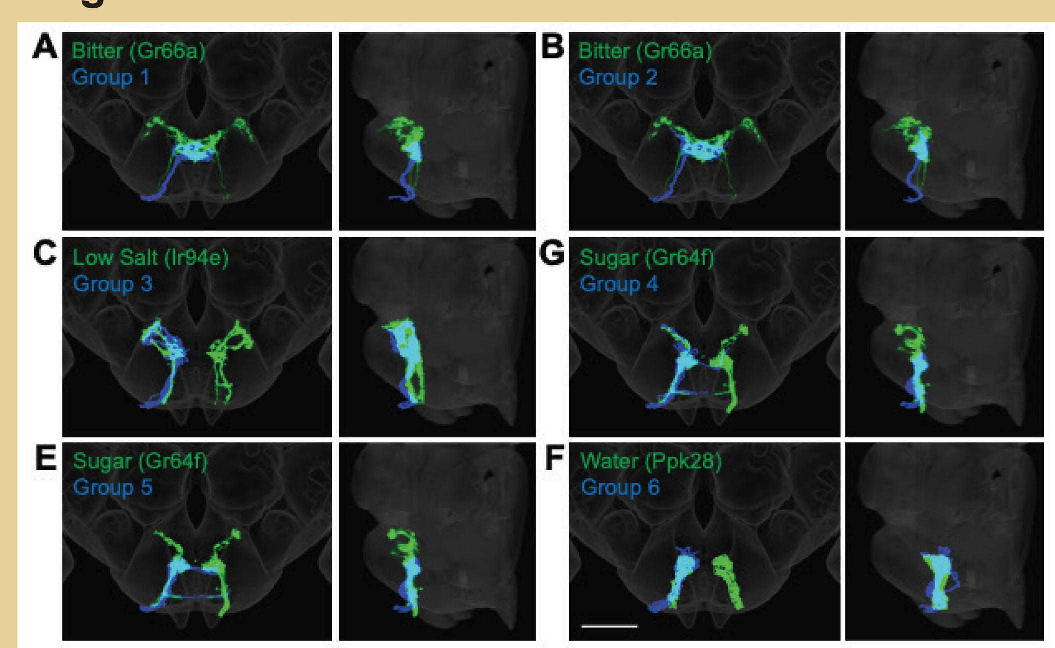
(A) Reconstruction of the premotor neuron Roundup using the annotation platform, CATMAID. Roundup is a neuron sufficient for PER and is responsive to sugar. My electron microscopy reconstruction revealed Roundup is presynaptic to a motor neuron that extends the proboscis, MN9, and postsynaptic to three second-order sugar neurons. We also found Roundup as directly presynaptic to a second-order bitter neuron, Scapula.

(B) Light microscopy image of Roundup and MN9.



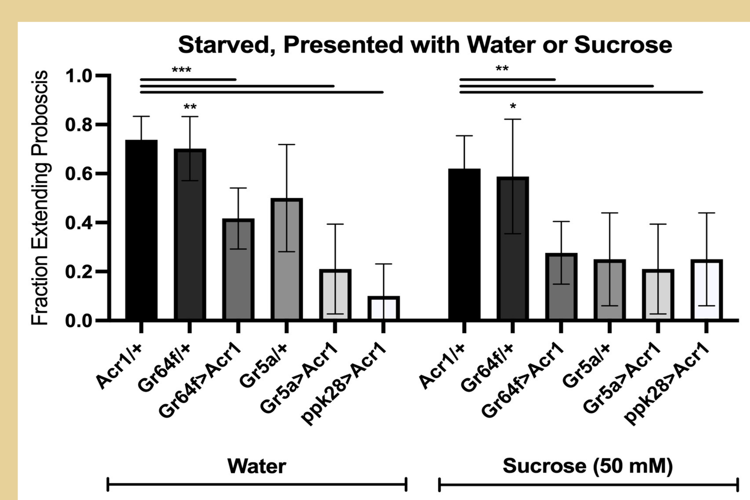
(C) An overall connectivity of the sugar sensorimotor circuit (Shiu, Sterne et al., 2022), showing the site of integration between sugar and bitter taste information.

Figure 2: Visualizing the anatomy of different GRNs shows overlay of sugar and water taste modalities



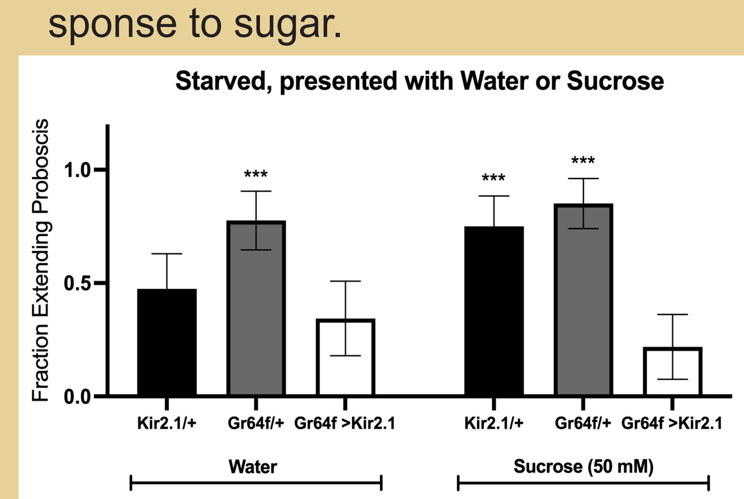
NBLAST comparisons yielded best matches of EM groups and GRNs of different taste classes. A-F (Engert et. al., 2022).

Figure 3: Silencing sugar-sensing neurons inhibits consumption of water

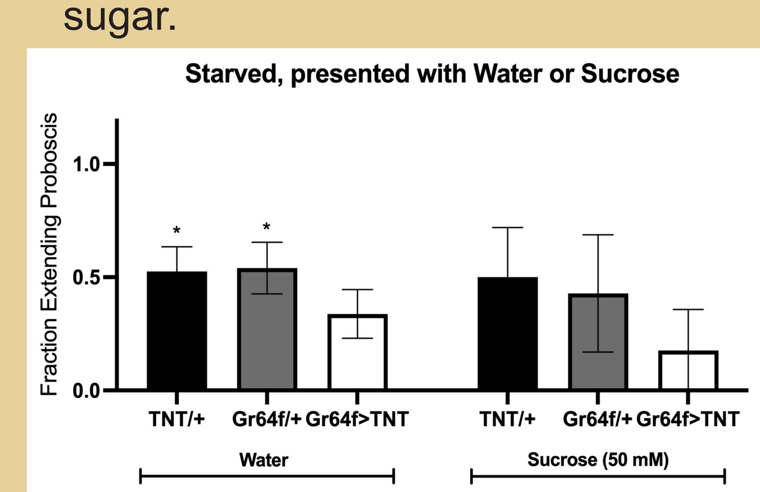


(A) Inactivating the sugar-sensing neuron Gr64f with GtACR1 shows a decreased response to water and sugar. Inactivating the sugar-sensing neuron Gr5a shows a decreased response to water, but not sugar. Inactivating the water-sensing neuron ppk28 shows a decreased response to water, but not sugar.

(B) Inactivating the sugar-sensing neuron Gr64f with the inward rectifying potassium channel Kir2.1 shows decreased response to sugar.



(C) Inactivating the sugar-sensing neuron Gr64f with tetanus toxin (TNT) also causes a decreased response to sugar.



(D) To test that the response to water is based on sensing of low osmolarity, we measured response to 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, demonstrating that proboscis extension to water is not based on mechanosensation but based on sensing the low osmolarity content of stimuli.

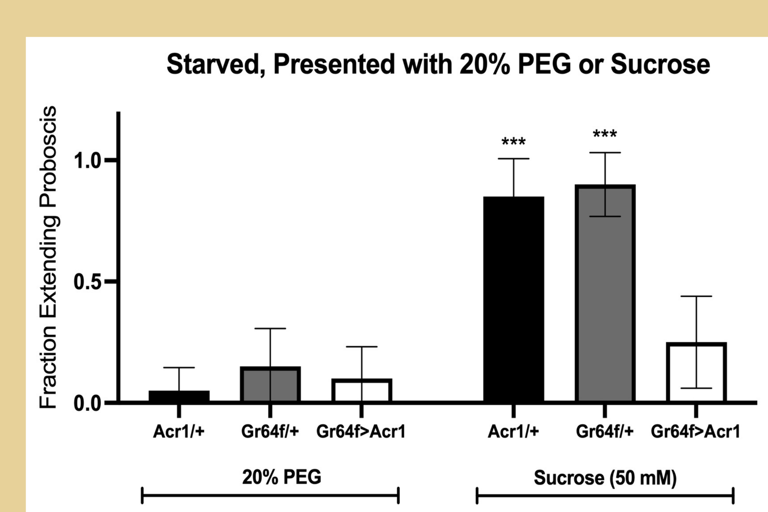
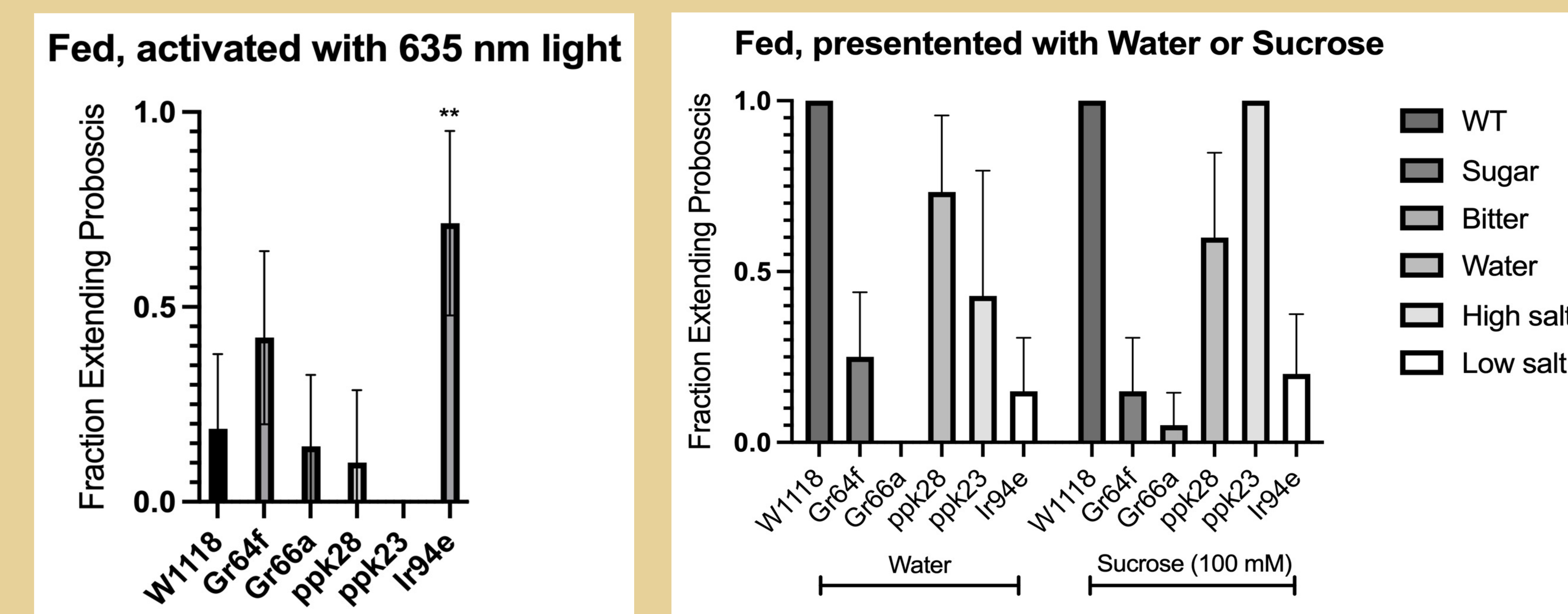


Figure 4: Activation of bitter and salt sensing neurons inhibits proboscis extension

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. These results suggest that sugar and low salt-sensing neurons cause proboscis extension while bitter and putative low-salt sensing neurons also inhibit proboscis extension, suggesting a complex code for aversion and attraction.



Conclusions

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. Additionally, silencing sugar inhibits consumption of water, suggesting an interaction between these two taste modalities. Activation of the putative "low-salt" Ir94e neurons both cause proboscis extension and inhibit proboscis extension to sugar and water, suggesting a complex integration of aversive and appetitive taste modalities.

Future directions

- Analyze the downstream connectivity of taste GRNs
- Repeat taste modalities experiment with a larger number of flies

Acknowledgements

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