The Role of AgRP Neurons in the Formation of a Flavor Preference

Heather Schneps, COL, 2023; Dr. Amber Alhadeff, COL, Neuroscience

Background

Humans and animals are motivated to eat by the combination of flavors and nutrients in foods, but how do we know which foods are nutritive?

- Flavor-nutrient learning is a paradigm used to investigate how mice learn to associate specific flavors with nutritive and caloric intake (Myers, 2018). Infusions of either glucose or fat paired with consumption of a particular flavor can condition a preference for that flavor, versus a flavor paired with infusion of water, when mice are presented with a two-bottle choice (Sclafani, 2011). The nutrient-paired flavor is the conditioned stimulus (CS+), and the water-paired flavor is referred to as CS-.
- Hypothalamic agouti-related protein (AgRP) neurons are essential for feeding behavior. When stimulated, AgRP neurons evoke feeding in rodent models within minutes (Belgardt et al., 2009; Krashes, 2011).
- **Hypothesis:** As nutritive infusions into the gut lead to reductions in AgRP activity (Goldstein et al., 2021), stimulating AgRP neurons will disrupt the learning of flavor-nutrient associations.

Scientific Question: Do AgRP neurons play an important role in developing learned associations between flavors and nutritive intake?

Strategy: We utilized a flavor-nutrient paradigm in combination with optogenetic stimulation of AgRP neurons to understand the effect of AgRP neuron activation on the development of a flavor preference and the gradual disappearance (extinction) of a flavor preference.

Cohorts

- Gastric catheters for directly infusing nutrients into the gut, optic fibers for stimulating AgRP neurons were surgically implanted in all mice

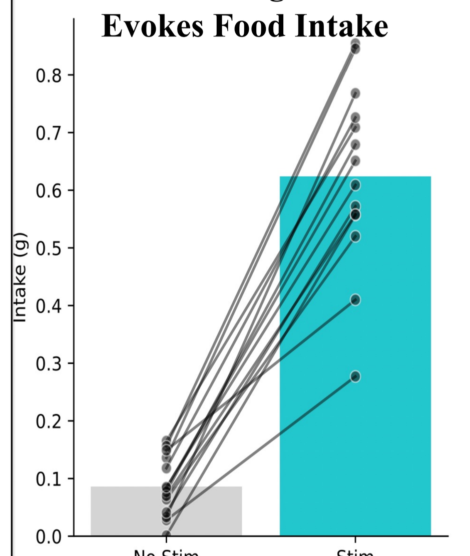
Experimental Group: AgRP-Cre x Ai32 (ChR2-expressing) mice
Control Group: AgRP-Cre x Ai9 (tdTomato) mice

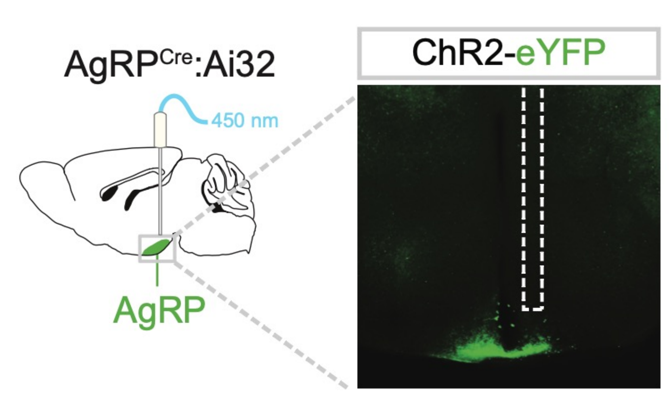
- Expression of ChR2 in the experimental mice, but not the controls, enabled laser stimulation to provoke action potentials in AgRP neurons

Procedure

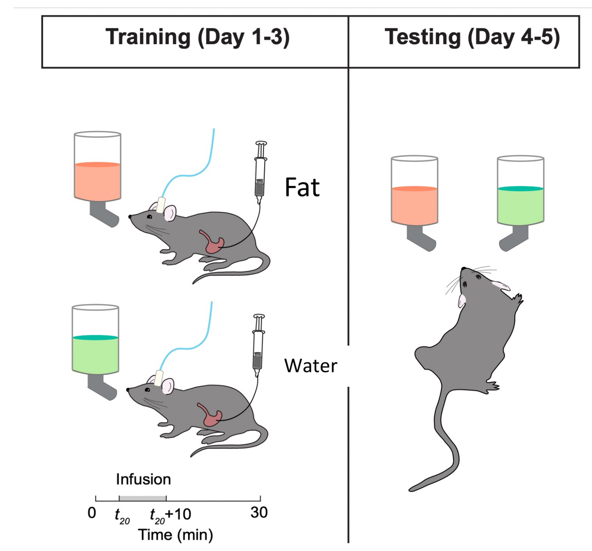
1. 1-bottle habituation in MedAssociates operant boxes (4 days)
2. 1-bottle training: 20 licks of flavored solution (0.05% KoolAid in 0.05% Sodium Saccharin) triggered a 0.6 mL infusion of 16.67% glucose or 6.67% intralipid for CS+ flavors and water for CS- flavors. Solutions were infused over 10 minutes in the ad lib procedure and 5 minutes in the pair-fed procedure (3 days, CS- and CS+ sessions each day)
3. 2-bottle habituation (1 day)
4. 2-bottle testing: measured CS-, CS+ licks (2 or more days)
5. Calculated mean preference index (PI):
   PI = (average # CS+ licks)/(average sum of CS+, CS- licks)

Stimulation of AgRP Neurons Evokes Food Intake

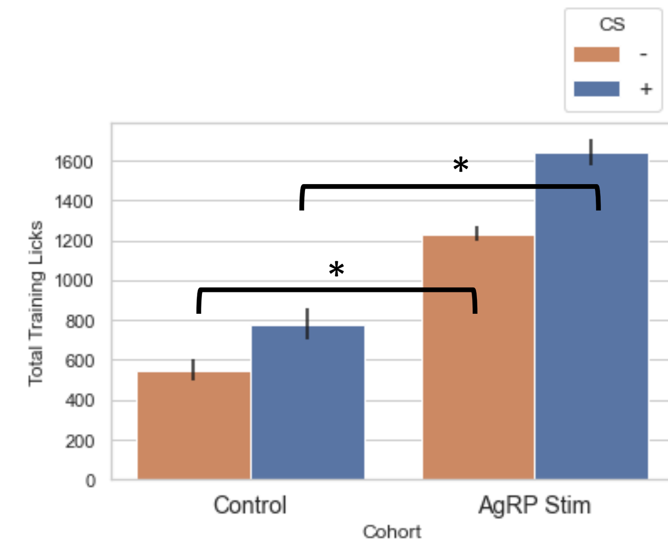


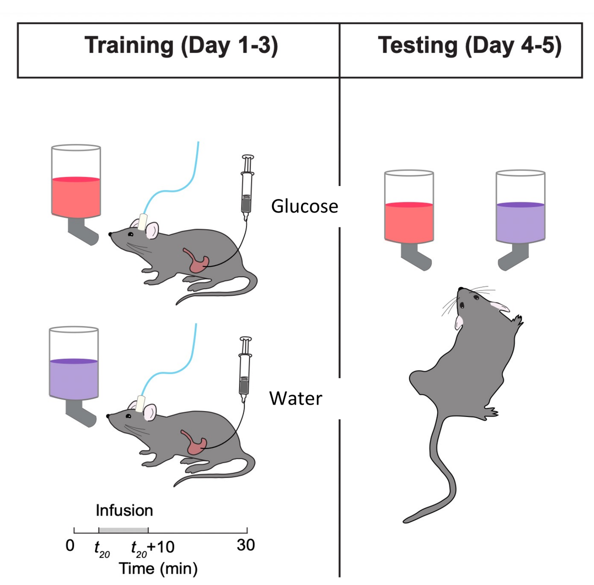


AgRP Neuron Stimulation During Training Increases Flavor Consumption During Both Training and Testing

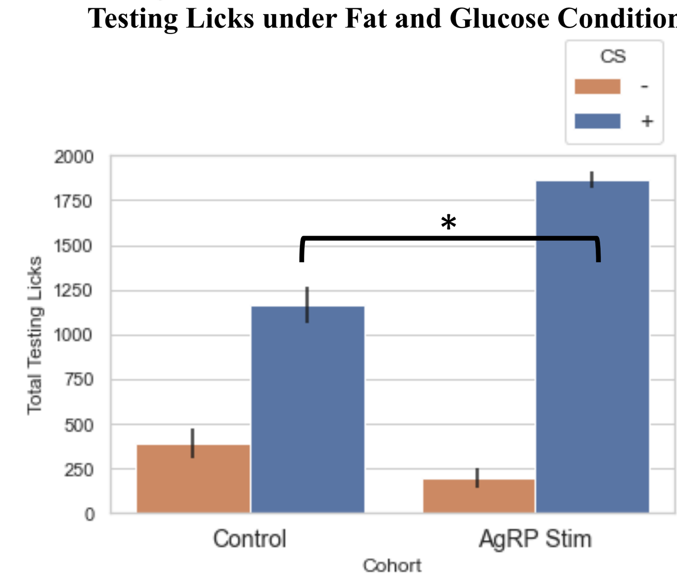


AgRP Neuron Stimulation Increases Training Licks under Fat and Glucose Conditions



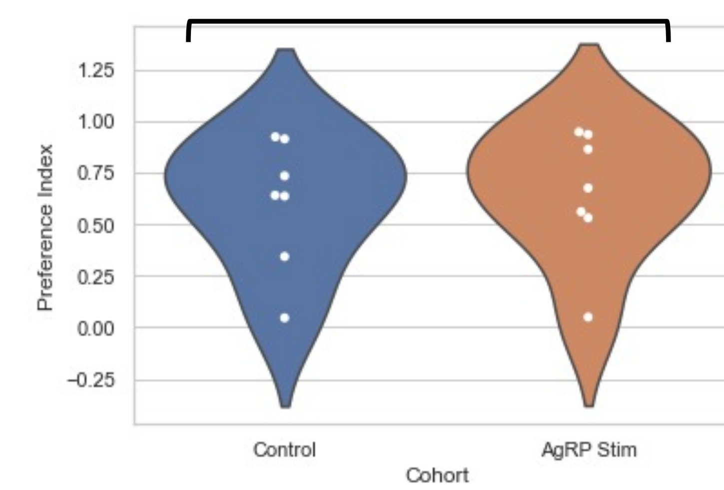


AgRP Neuron Stimulation Increases CS+ Testing Licks under Fat and Glucose Conditions

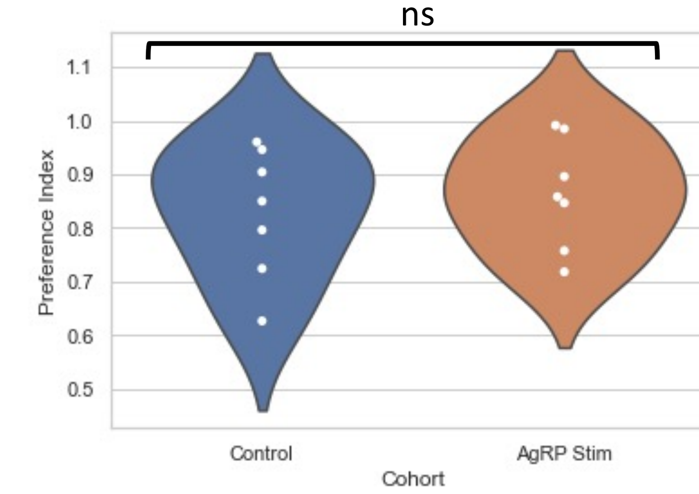


Limiting Training Intake Prevents Stimulation-Induced Potentiation of Flavor-Nutrient Preferences

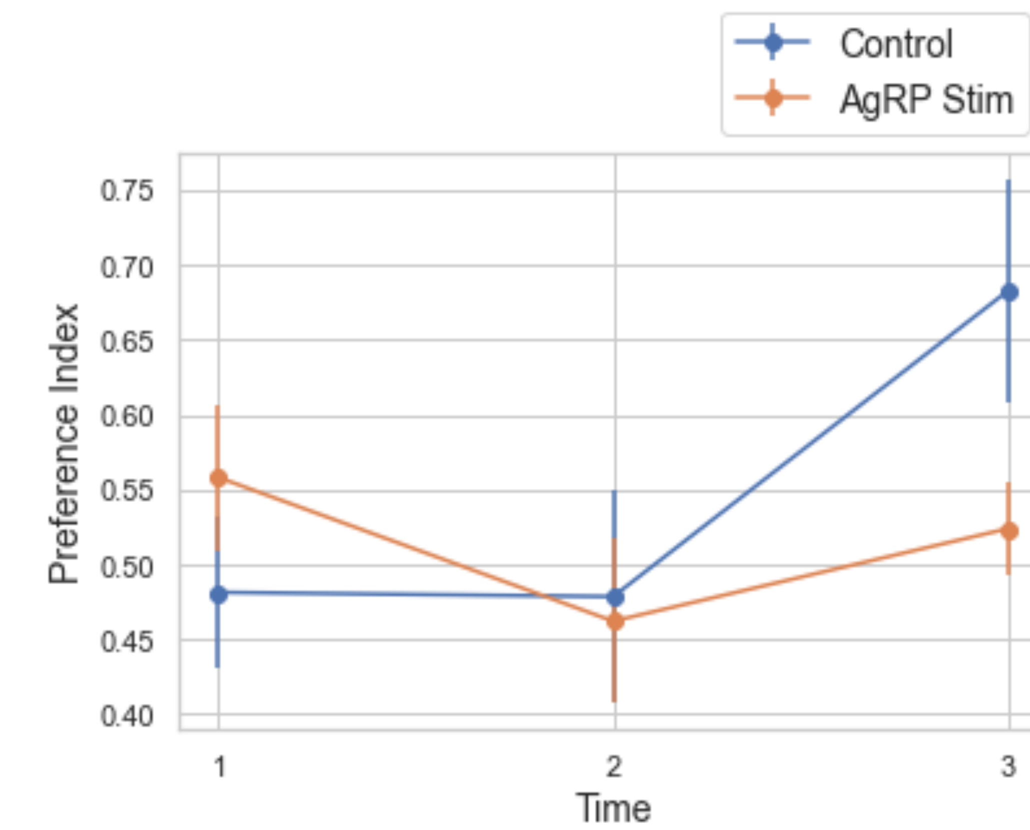
Fat as Unconditioned Stimulus (US)



Glucose as Unconditioned Stimulus (US)



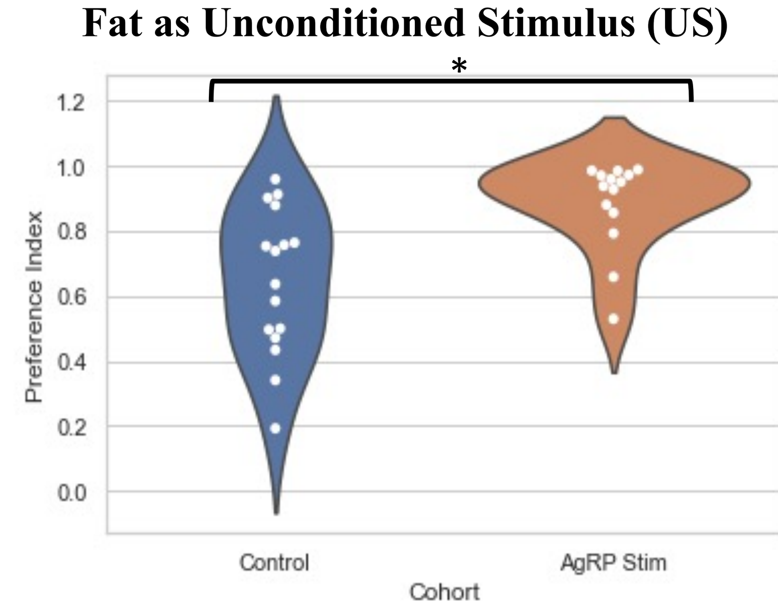
AgRP Neuron Stimulation May Impact Retention of Glucose Flavor-Nutrient Associations



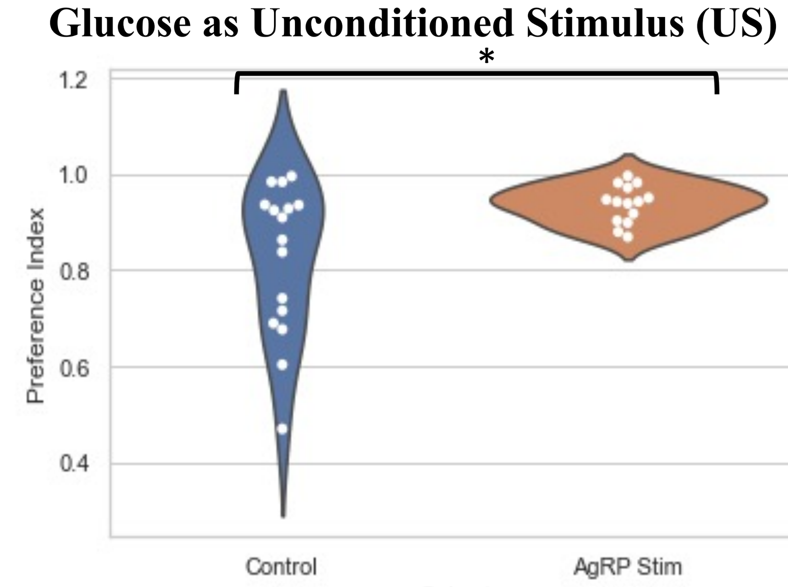
AgRP Neuron Stimulation Potentiates Flavor-Nutrient Preferences for Fat and Glucose

Preference Index (PI) = (# CS+ licks)/(# CS+ licks + # CS- licks)

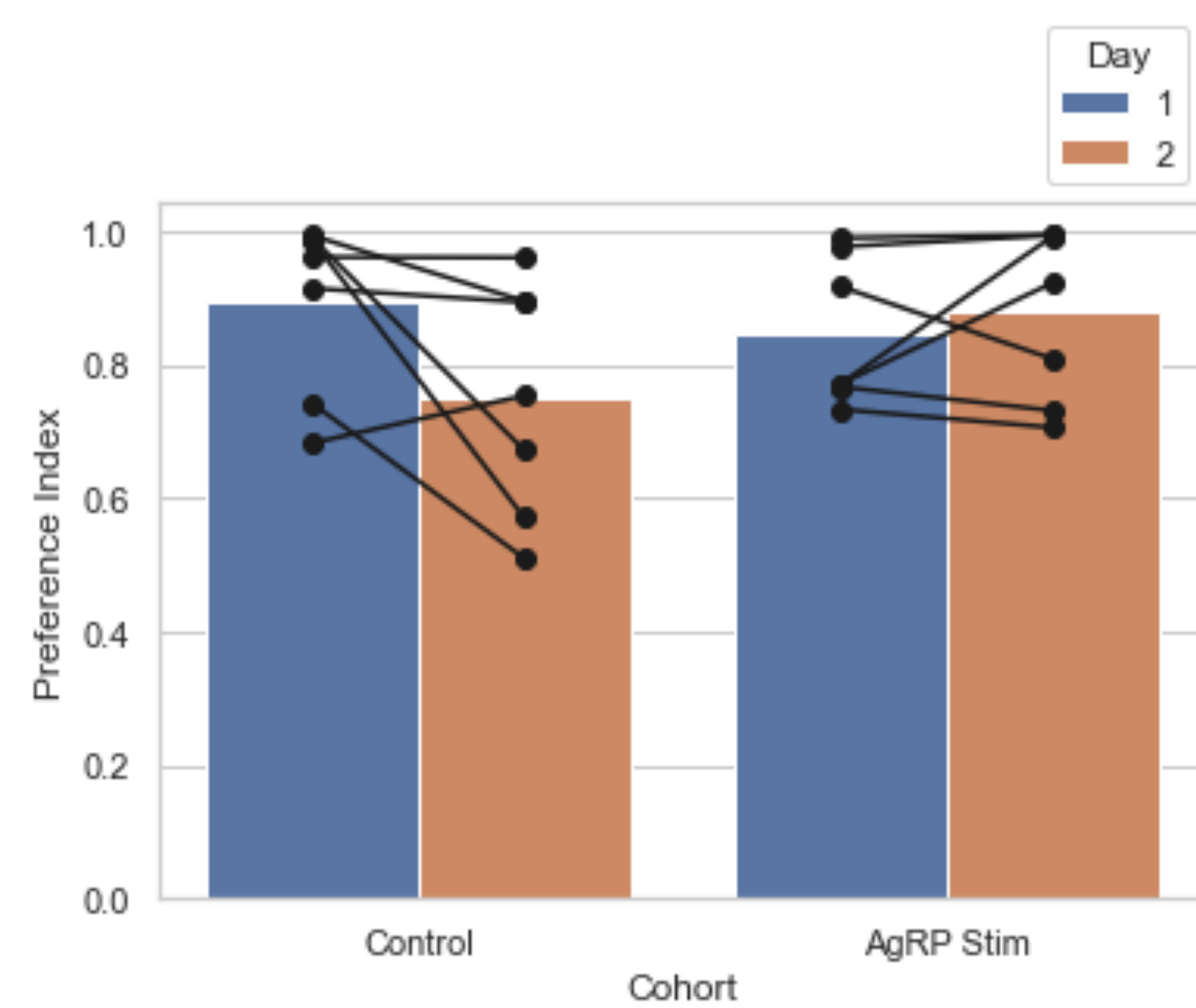
Fat as Unconditioned Stimulus (US)



Glucose as Unconditioned Stimulus (US)



AgRP Neuron Stimulation May Prolong Flavor-Nutrient Preferences for Glucose



Conclusions

- AgRP neuron stimulation during training potentiates a flavor-nutrient preference for fat or glucose in a consumption-based manner, contrary to our original hypothesis
- AgRP neuron stimulation may influence the longevity of flavor-nutrient preferences for glucose
- Further research into how AgRP neurons and other neuron populations contribute to flavor-nutrient learning may allow for the development of new treatments to counteract the obesity epidemic

Acknowledgements

I would like to thank my P.I. Dr. Amber Alhadeff, mentor Nathaniel Nyema, colleague Elizabeth Gold, and high school research assistant Jah'Seek'I Joslyn for their immense contributions to this project.

Funding

This project was funded by the Velay Women's Science Research Fellowship and the CURF Mary L. And Matthew S. Santirocco College Alumni Society Undergraduate Research Grant.

References

Alhadeff, A. L., Su, Z., Hernandez, E., Klima, M. L., Phillips, S. Z., Holland, R. A., Guo, C., Hantman, A. W., De Jonghe, B. C., & Betley, J. N. (2018). A neural circuit for the suppression of pain by a competing need state. *Cell, 173*(1), 140-152.e15. https://doi.org/10.1016/j.cell.2018.02.057

Belgardt, B. F., Okamura, T., & Brüning, J. C. (2009). Hormone and glucose signalling in POMC and AgRP neurons. *The Journal of physiology*, *587*(Pt 22), 5305–5314. https://doi.org/10.1113/jphysiol.2009.179192

Berrios, J., Li, C., Madara, J. C., Garfield, A. S., Steger, J. S., Krashes, M. J., & Lowell, B. B. (2021). Food cue regulation of AGRP hunger neurons guides learning. *Nature (London), 595*(7869), 695-700. https://doi.org/10.1038/s41586-021-03729-3

Betley, J. N., Xu, S., Cao, Z. F. H., Gong, R., Magnus, C. J., Yu, Y., & Sternson, S. M. (2015). Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature*, *521*(7551), 180. https://link.gale.com/apps/doc/A463816530/SCIC?u=upenn_main&sid=summon&xid=0cd9d1a6

Chen, Y., Lin, Y.-C., Zimmerman, C. A., Essner, R. A., & Knight, Z. A. (2016). Hunger neurons drive feeding through a sustained, positive reinforcement signal. *eLife*, *5*. https://link.gale.com/apps/doc/A473116833/AONE?u=upenn_main&sid=summon&xid=4d7eac33

Goldstein, N., McKnight, A. D., Carty, J. R. E., Arnold, M., Betley, J. N., & Alhadeff, A. L. (2021). Hypothalamic detection of macronutrients via multiple gut-brain pathways.*Cell Metabolism, 33*(3), 676-687.e5. https://doi.org/10.1016/j.cmet.2020.12.018

Krashes, M. J. *et al.* Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J. Clin. Invest. 121, 1424-1428 (2011)

Myers, K. P. (2018). The convergence of psychology and neurobiology in flavor-nutrient learning. *Appetite*, *122*, 36–43. doi:10.1016/j.appet.2017.03.048

Sclafani, A. (2011). Flavor conditioning by nutrients in the gut. *Appetite*, *57*, S39–S40. doi:10.1016/j.appet.2011.05.266

Su, Z., Alhadeff, A. L., & Betley, J. N. (2017). Nutritive, post-ingestive signals are the primary regulators of AgRP neuron activity. *Cell Reports (Cambridge), 21*(10), 2724-2736. https://doi.org/10.1016/j.celrep.2017.11.036