genomic resource for the research community, but also identified many major players and candidates to uncover the secrets of a fascinating and unique plant family. Clearly, the molecular understanding of their biology offers a guide to 'domesticate' these exciting plants for a variety of applications including, but not restricted to, the development of CO_2-neutral or even CO_2-sequestering biotechnological processes. In this way, these tiny yet fastest growing flowering plants may show great potential and could be one piece of the puzzle to solve some of humanity's most pressing challenges.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Ware, A., Jones, D.H., Flis, P., Chrysanthou, E., Smith, K.E., Kümpers, B.M.C., Yant, L., Atkinson, J.A., Wells, D.M., Bhosale, R., *et al*. (2023). Loss of ancestral function in duckweed roots is accompanied by progressive anatomical reduction and a re-distribution of nutrient transporters. Curr. Biol. *33*, 1795–1802.e4.

2. Liang, Y., Yu, X.H., Anaokar, S., Shi, H., Dahl, W.B., Cai, Y., Luo, G., Chai, J., Cai, Y., Mollá-Moralez, A., *et al*. (2023). Engineering triacylglycerol accumulation in duckweed (Lemna japonica). Plant Biotechnol. J. *21*, 317–330.

3. Ziegler, P., Sree, K.S., and Appenroth, K.J. (2016). Duckweeds for water remediation and toxicity testing. Toxicol. Environ. Chem. *98*, 1127–1154.

4. Michael, T.P., Ernst, E., Hartwick, N., Chu, P., Bryant, D., Gilbert, S., Ortleb, S., Baggs, E.L., Sree, K.S., Appenroth, K.J., *et al*. (2021). Genome and time-of-day transcriptome of Wolffia australiana link morphological minimization with gene loss and less growth control. Genome Res. *31*, 225–238.

5. Wang, W., Haberer, G., Gundlach, H., Gläßer, C., Nussbaumer, T., Luo, M.C., Lomsadye, A., Borodovskz, M., Kerstetter, R.A., Shanklin, J., *et al*. (2014). The Spirodela polyrhiza genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. Nat. Commun. *5*, 3311.

6. Ernst, E., Abramson, B., Acosta, K., Hoang, P.T.N., Mateo-Eizalde, C., Schubert, V., Pasaribu, B., Albert, P.S., Hartwick, N., Colt, K., *et al*. (2025). Duckweed genomes and epigenomes underlie triploid hybridization and clonal reproduction. Curr. Biol. *35*, 1828–1847.e9.

7. Ibanez, V.N., and Quadrana, L. (2023). Shaping inheritance: how distinct reproductive strategies influence DNA methylation memory in plants. Curr. Opin. Genet. Dev. *78*, 102018.

Associative learning: A mechanism for conditioned taste aversion

Matthew E. Carter
Department of Biology, Williams College, Williamstown, MA 01267, USA
Correspondence: mc10@williams.edu
https://doi.org/10.1016/j.cub.2024.12.046

Animals can associate a novel flavor with subsequent sickness and malaise, even when these adverse effects occur minutes to hours later. A new study reveals a mechanism for how this association occurs despite the delay in time.

To survive, animals must distinguish between beneficial foods that provide calories and nutrients and toxic foods that cause harmful effects such as indigestion and sickness[1]. Through experience, animals learn to associate specific flavors with their positive or negative outcomes, thereby improving the ability to make advantageous food choices in the future. Many of these associations occur immediately during consumption — this berry is sweet, this seed is bitter, this plant has painful thorns, this pepper is spicy, and so on. Some associations, however, develop over longer timeframes: for example, conditioned taste aversion (CTA) is a form of associative learning in which an animal learns to avoid a novel flavor after associating it with a delayed aversive outcome, such as illness or gastrointestinal distress[2–4]. Unlike many forms of learning that require close temporal proximity between stimuli, CTA uniquely bridges the delay between food consumption and the subsequent onset of sickness to form a strong and long-lasting aversive memory[2–6].

The phenomenon of CTA presents a fascinating scientific problem — how do animals associate the adverse symptoms of illness with ingestion of a novel flavor that occurred minutes to hours beforehand? In a new study, Zimmerman *et al.*[7] used sophisticated methods in mice to track brain-wide activity patterns over time, uncovering a mechanism that can explain the temporal gap in CTA.

In their work, Zimmerman *et al.*[7] capitalized on the observation that CTA is significantly stronger for novel, unfamiliar flavors compared to familiar ones[3]. A single pairing of a novel flavor with sickness is sufficient to induce a robust aversion. In their study, the authors induced sickness in mice by administering lithium chloride (LiCl), a compound that causes transient gastrointestinal malaise[6]. Consistent with previous findings[5,6,8], pairing a novel, palatable flavor — sweetened grape Kool-Aid — with LiCl administration 60 minutes later resulted in a strong and stable aversion to the flavor (Figure 1).

Where in the brain does the delayed association between a novel flavor and gastrointestinal malaise occur?

Zimmerman *et al.*[7] hypothesized that neurons involved in CTA would be active during three key phases: the initial exposure to the novel flavor; the subsequent experience of malaise; and the re-exposure to the flavor during which avoidance behavior is expressed. To identify these neurons, the authors conducted a brain-wide analysis of expression of Fos, a protein that tends to be upregulated when neurons are active and therefore serves as an indirect marker of neuronal activity[9]. Notably, neurons in the central nucleus of the amygdala (CEA) were active during all three phases (Figure 1). This finding suggested that CEA neurons integrate flavor and malaise signals, serving a crucial role in the storage and retrieval of the aversive memory.

How do individual neurons within the CEA respond during the different stages of CTA formation? Although Fos mapping provides valuable insight into brain-wide neuronal activation patterns, it captures activity only at a single timepoint in each mouse. To overcome this limitation, Zimmerman *et al.*[7] employed state-of-the-art Neuropixel probes[10], enabling stable, long-term recording of hundreds of individual neurons throughout the course of CTA. During initial consumption, about one-third of recorded CEA neurons were activated by the novel flavor, compared to ~10% activated by water consumption and ~17% by familiar flavors (Figure 1). These neurons are presumably activated by upstream circuits mediating sensory perception of the flavor. Immediately after consumption, activity in the novel flavor-encoding neurons decreased, but was subsequently reactivated following LiCl administration while neurons encoding water or familiar flavors remained inactive. Consistently, these same neurons were reactivated upon re-exposure to the flavor two days later, coinciding with the expression of avoidance behavior. These recordings demonstrate that a specific subset of CEA neurons initially responds to a novel flavor, integrates the aversive malaise signal, and forms a stable memory engram that can be reactivated upon subsequent re-exposure to the same flavor.

How do the CEA neurons that respond to a novel flavor receive signals indicating

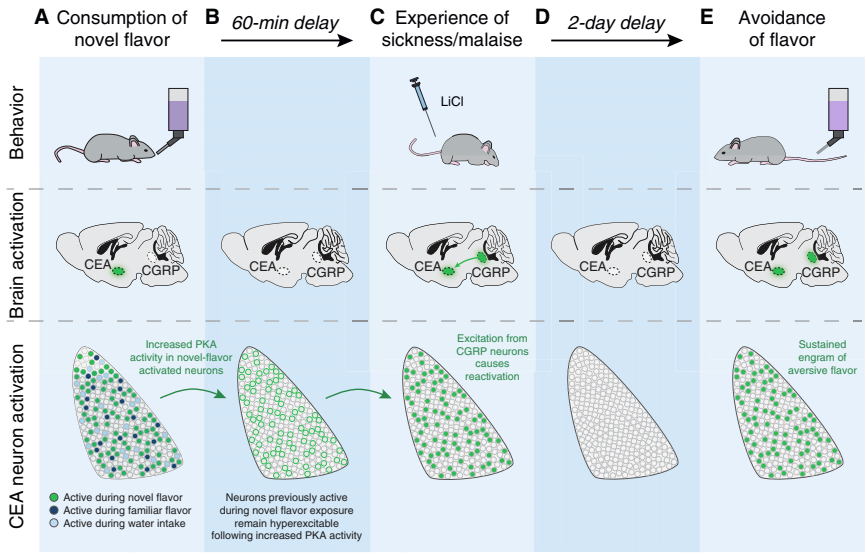

Figure 1. Development of a conditioned taste aversion.
Top row depicts behavioral expression of a CTA across multiple stages, as described in Zimmerman *et al.*[7]. Middle row depicts corresponding activity within the CEA and parabrachial CGRP neurons. Bottom row depicts corresponding activity of individual neurons within the CEA. (A) CTA begins with the consumption of a novel flavor, activating approximately one-third of CEA neurons. (B) During the time between flavor exposure and sickness, activity in CEA neurons decreases. However, a transient increase in PKA activity renders previously activated novel flavor-encoded neurons hyperexcitable. (C) During sickness/malaise, parabrachial CGRP neurons stimulate hyperexcitable CEA neurons, reactivating those originally responsive to the novel flavor. (D) During the next two days, relevant CEA and CGRP neurons remain inactive. (E) Upon re-exposure to the flavor, animals exhibit a strong aversion. CEA neurons encoding the novel flavor now represent a stable memory engram of the aversive experience. Previous studies have shown that CGRP neurons are also reactivated during this avoidance behavior[13].

subsequent sickness? Previous research has shown that information about visceral malaise is conveyed by a population of neurons in the parabrachial nucleus that produce calcitonin gene-related peptide (CGRP)[11]. These CGRP neurons are strongly activated by LiCl and other toxic compounds, and their activity is necessary and sufficient for CTA formation[11–13]. Furthermore, CGRP neurons send robust projections to the CEA[11].

To test whether CGRP neurons activate novel flavor-encoding CEA neurons during malaise, Zimmerman *et al.*[7] exposed animals to a novel flavor and, instead of administering LiCl, stimulated either parabrachial CGRP neurons or their axonal projections within the CEA. Remarkably, this stimulation fully replicated the effects of LiCl, reactivating the same CEA neurons associated with the novel flavor. In separate experiments, ablating CGRP neurons reduced the reactivation of CEA neurons during LiCl administration, demonstrating their essential role in linking malaise signals to flavor-encoding CEA neurons.

The implication is that CGRP neurons in the parabrachial nucleus transmit malaise signals to the CEA, reactivating novel flavor representations and bridging the temporal gap between flavor exposure and delayed sickness (Figure 1). This reactivation likely stabilizes the novel flavor representations into a long-term memory. Notably, the CEA neurons most strongly activated by CGRP stimulation were the same neurons exhibiting the highest activity during memory retrieval two days later. In contrast, novel flavor representations not reactivated by CGRP neuron excitation rapidly degraded, effectively marking those flavors as familiar and safe.

These observations raise another intriguing question: how do CGRP neurons selectively reactivate CEA neurons encoding a novel flavor, but not those encoding familiar flavors? Although CGRP neurons likely form synapses throughout the CEA and have the

capacity to activate a broad population of neurons, only a subset of CEA neurons — those encoding the novel flavor — are subsequently reactivated during malaise. One possibility is that the CEA neurons activated by a novel flavor become selectively primed for potential reactivation by CGRP neurons. Zimmerman *et al.*[7] hypothesized that this priming occurs via a biochemical signaling pathway involving protein kinase A (PKA) and cAMP response element binding protein (CREB), both of which are essential for CTA[14–16]. In this pathway, PKA activation phosphorylates and activates CREB, a transcription factor that regulates gene transcription, ultimately increasing neuronal excitability. Supporting this idea, the authors found that PKA activity increases in CEA neurons in response to a novel flavor, but not to familiar flavors or water. This activation of PKA and CREB could temporarily render novel flavor-encoding CEA neurons hyperexcitable, enabling their selective reactivation by CGRP neurons during sickness, even minutes to hours later. This mechanism reconciles the time delay between flavor exposure and malaise, stabilizing the flavor memory engram for long-term reactivation during future encounters with the flavor.

These initial findings on the PKA/CREB pathway open the door to several future experimental questions. For example, does inhibiting PKA activity or CREB phosphorylation reduce reactivation of CEA neurons during malaise or CGRP stimulation? Does the duration of hyperexcitability in CEA neurons correlate with the time window of CTA formation? What drives the increase in PKA activity in CEA neurons responding to a novel flavor? Investigating how biochemical signaling within CEA neurons aligns with their physiological activity will be essential for a comprehensive understanding of the mechanisms underlying CTA.

Taken together, the results of Zimmerman *et al.*[7] provide a compelling mechanism for how temporal delays between food consumption and adverse outcomes integrate to form a CTA. Future research could explore other forms of associative learning in food intake behaviors involving temporal delays, particularly those linked to beneficial outcomes. For example, gut-derived signals provide the brain with information about the nutritional content of foods hours after ingestion[17–19]. Similarly, delayed water absorption into the bloodstream allows animals to associate foods with higher water content and adjust food preferences to meet hydration needs[20]. Are these beneficial temporal delays processed in the CEA or other brain regions? How are delayed signals for calories, nutrients, and water uniquely encoded to support long-term learning? Addressing these questions will be an exciting direction for future studies, and the framework established by Zimmerman *et al.*[7] provides a robust foundation for exploring these mechanisms.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Rozin, P., and Vollmecke, T.A. (1986). Food likes and dislikes. Annu. Rev. Nutr. *6*, 433–456. https://doi.org/10.1146/annurev.nu.06.070186.002245.

2. Welzl, H., D'Adamo, P., and Lipp, H.P. (2001). Conditioned taste aversion as a learning and memory paradigm. Behav. Brain Res. *125*, 205–213. https://doi.org/10.1016/S0166-4328(01)00302-3.

3. Revusky, S.H., and Bedarf, E.W. (1967). Association of illness with prior ingestion of novel foods. Science *155*, 219–220. https://doi.org/10.1126/science.155.3759.219.

4. Reilly, S., and Bornovalova, M.A. (2005). Conditioned taste aversion and amygdala lesions in the rat: a critical review. Neurosci. Biobehav. Rev. *29*, 1067–1088. https://doi.org/10.1016/j.neubiorev.2005.03.025.

5. Nachman, M. (1970). Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. J. Comp. Physiol. Psychol. *73*, 22–30. https://doi.org/10.1037/h0029807.

6. Ingram, D.K. (1982). Lithium chloride-induced taste aversion in C57BL/6J and DBA/2J mice. J. Gen. Psychol. *106*, 233–249.

7. Zimmerman, C.A., Bolkan, S.S., Pan-Vazquez, A., Wu, B., Keppler, E.F., Meares-Garcia, J.B., Guthman, E.M., Fetcho, R.N., McMannon, B., Lee, J., *et al*. (2025). A neural mechanism for learning from delayed postingestive feedback. Nature, https://doi.org/10.1038/s41586-025-08828-z.

8. Rowland, N.E., Nasrallah, N.A., and Robertson, K.L. (2004). LiCl-induced flavor avoidance compared between rats and mice using a nondeprivation protocol. Am. J. Physiol. Reg. I *286*, R260–R268. https://doi.org/10.1152/Ajpregu.00312.2003.

9. Dragunow, M., and Faull, R. (1989). The use of c-fos as a metabolic marker in neuronal pathway tracing. J. Neurosci. Methods *29*, 261–265. https://doi.org/10.1016/0165-0270(89)90150-7.

10. Steinmetz, N.A., Aydin, C., Lebedeva, A., Okun, M., Pachitariu, M., Bauza, M., Beau, M., Bhagat, J., Bohm, C., Broux, M., *et al*. (2021). Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings. Science *372*, eabf4588. https://doi.org/10.1126/science.abf4588.

11. Carter, M.E., Soden, M.E., Zweifel, L.S., and Palmiter, R.D. (2013). Genetic identification of a neural circuit that suppresses appetite. Nature *503*, 111–114. https://doi.org/10.1038/nature12596.

12. Carter, M.E., Han, S., and Palmiter, R.D. (2015). Parabrachial calcitonin gene-related peptide neurons mediate conditioned taste aversion. J. Neurosci. *35*, 4582–4586. https://doi.org/10.1523/JNEUROSCI.3729-14.2015.

13. Chen, J.Y., Campos, C.A., Jarvie, B.C., and Palmiter, R.D. (2018). Parabrachial CGRP neurons establish and sustain aversive taste memories. Neuron *100*, 891–899.e5. https://doi.org/10.1016/j.neuron.2018.09.032.

14. Lamprecht, R., Hazvi, S., and Dudai, Y. (1997). cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. J. Neurosci. *17*, 8443–8450. https://doi.org/10.1523/JNEUROSCI.17-21-08443.1997.

15. Josselyn, S.A., Kida, S., and Silva, A.J. (2004). Inducible repression of CREB function disrupts amygdala-dependent memory. Neurobiol. Learn. Mem. *82*, 159–163. https://doi.org/10.1016/j.nlm.2004.05.008.

16. Hashikawa, K., Naka, M., Nakayama, D., Matsumoto, N., Neve, R., and Matsuki, N. (2013). Blockade of stimulus convergence in amygdala neurons disrupts taste associative learning. J. Neurosci. *33*, 4958–4963. https://doi.org/10.1523/JNEUROSCI.5462-12.2013.

17. Han, W., Tellez, L.A., Perkins, M.H., Perez, I.O., Qu, T., Ferreira, J., Ferreira, T.L., Quinn, D., Liu, Z.W., Gao, X.B., *et al*. (2018). A neural circuit for gut-induced reward. Cell *175*, 887–888. https://doi.org/10.1016/j.cell.2018.10.018.

18. Buchanan, K.L., Rupprecht, L.E., Kaelberer, M.M., Sahasrabudhe, A., Klein, M.E., Villalobos, J.A., Liu, W.W., Yang, A., Gelman, J., Park, S., *et al*. (2022). The preference for sugar over sweetener depends on a gut sensor cell. Nat. Neurosci. *25*, 191–200. https://doi.org/10.1038/s41593-021-00982-7.

19. Tan, H.E., Sisti, A.C., Jin, H., Vignovich, M., Villavicencio, M., Tsang, K.S., Goffer, Y., and Zuker, C.S. (2020). The gut-brain axis mediates sugar preference. Nature *580*, 511–516. https://doi.org/10.1038/s41586-020-2199-7.

20. Grove, J.C.R., Gray, L.A., La Santa Medina, N., Sivakumar, N., Ahn, J.S., Corpuz, T.V., Berke, J.D., Kreitzer, A.C., and Knight, Z.A. (2022). Dopamine subsystems that track internal states. Nature *608*, 374–380. https://doi.org/10.1038/s41586-022-04954-0.