Generally Physiological

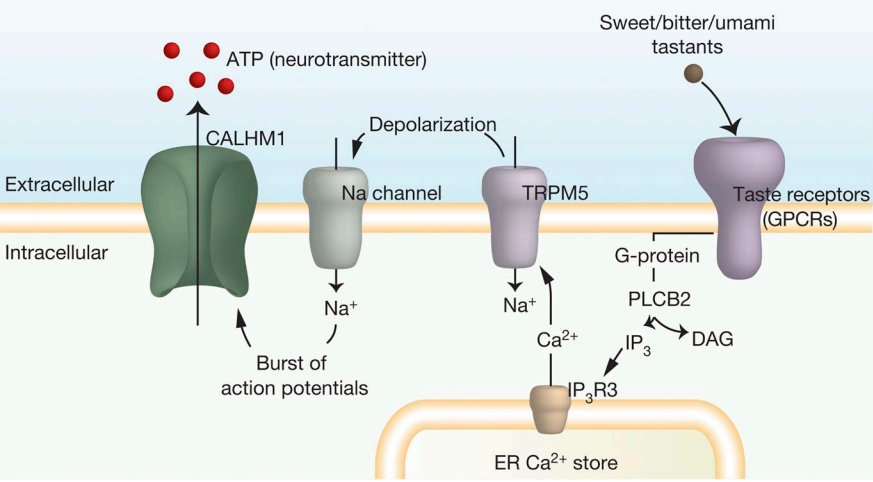
Tasting the bitter and the sweet, honeybee memories, and visualizing calcium throughout entire astrocytes

This month's installment of *Generally Physiological* concerns identification of a channel crucial for the perception of sweet, bitter, and umami tastes; a role for caffeine in honeybee associative olfactory memory; and methods for imaging calcium throughout entire astrocytes.

A taste for CALHM1

Sweet, bitter, and umami (the savory taste elicited by glutamate) substances bind to G protein–coupled taste receptors on type II taste bud cells, activating a signaling pathway in which Ca^{2+} release leads to the Ca^{2+}-dependent activation of TRPM5 channels and thereby membrane depolarization. This elicits action potentials, triggering the nonvesicular release of ATP and thereby the activation of nearby afferent gustatory neurons. Taruno et al. (2013) explored the mechanism underlying ATP release and identified the voltage-gated nonselective ion channel calcium homeostasis modulator 1 (CALHM1) as a critical component of this process. A combination of coexpression studies, RT-PCR analysis, and analyses of mice lacking type II cells revealed that mRNA encoding CALHM1 was present in sweet, bitter, and umami type II cells, but not other taste bud cells. Moreover, whole-cell recordings of single isolated taste bud cells from wild-type and *Cahlm1*$^{-/-}$ mice indicated that CALHM1 mediated a slowly activating nonselective outward current in type II cells. Although mice lacking CALHM1 had morphologically normal taste buds, they showed little if any preference for sweet or umami tastes or avoidance of bitter tastes. Consistent with these behavioral data, gustatory nerve recordings revealed markedly reduced responses to stimulation with sweet, bitter, or umami compounds in *Calhm1*$^{-/-}$ mice, but not to sour compounds or salt (NaCl). CALHM1 can be activated by decreasing extracellular calcium concentration (as well as by membrane depolarization), and its heterologous expression in various cell types promoted the release of ATP in response to depolarization or exposure to low extracellular calcium concentration. Although basal $[Ca^{2+}]_i$, taste-evoked $[Ca^{2+}]_i$, TRPM5 expression, and voltage-gated Na^+ currents were comparable in wild-type and *Cahlm1*$^{-/-}$ type II cells, as was taste bud ATP content, CALHM1 knockout led to the loss of taste-evoked ATP release. The authors thus propose that CALHM1 mediates the release of ATP from type II cells, in a mechanism crucial to the perception of sweet, bitter, and umami tastes.

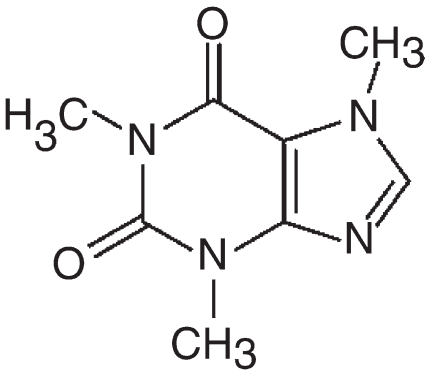
Tasting the bitter and the sweet, a role for caffeine in honeybee associative olfactory memory, and methods for imaging calcium throughout entire astrocytes



Model of taste signaling in type II taste receptor cells, showing ATP release through CALHM1. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. Taruno et al., 495:223–226, copyright 2013.)

Rewarding memories of caffeine?

Bitter substances, like caffeine, contribute to plant defenses by repelling herbivores; indeed, caffeine is toxic in high doses. It thus seems surprising that caffeine is found in the nectar of some plants, where it might be expected to repel pollinating insects. Wright et al. (2013) determined that low concentrations of caffeine (comparable to that in the nectar of *Citrus* and *Coffea* species) enhanced long-term olfactory memory in honeybees: bees were three times more likely to remember a scent associated with a sucrose reward when caffeine was added to the sucrose solution. Bath application of caffeine depolarized

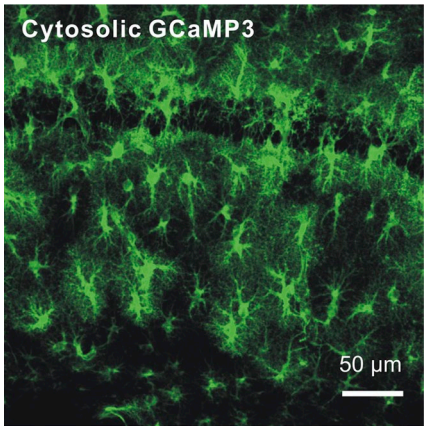


Structure of caffeine.

Kenyon cells in mushroom bodies (a region implicated in the formation of insect olfactory associative memory; see Chittka and Peng, 2013) of intact honeybee brains, an effect blocked by a nicotinic acetylcholine receptor antagonist, and enhanced the response to exogenous acetylcholine. Consistent with caffeine's ability to act as an adenosine receptor antagonist, the adenosine receptor antagonist DPCPX mimicked caffeine's effects on Kenyon cells, suggesting that antagonism of adenosine could be pertinent to caffeine's effects on Kenyon cell excitability (and presumably honeybee olfactory memory). Noting that the concentrations of caffeine in nectar were below those that were aversive, the authors proposed that, by enhancing honeybee olfactory associative memory, caffeine increases foraging efficiency (thereby promoting more effective pollination) independent of its role in defense.

Monitoring localized calcium signals throughout astrocytes

Astrocytes, once relegated to the role of neural "glue," are now known to play crucial roles in brain physiology, which may include their responding to and modulating synaptic activity and regulating local blood flow. Such interactions can be monitored by using fluorescent indicators to visualize calcium signals in astrocytes. However, astrocytic calcium signals have mainly been investigated in somata and large processes (rather than in fine processes adjacent to synapses or endfeet-abutting blood vessels). Moreover, most studies of such neuroglial interactions have used neuron-glia co-cultures, or slices from immature animals (preparations in which neuroglial signaling may be distinct from that in the mature brain). In this issue, Shigetomi et al. provide detailed methods for using genetically encoded calcium indicators (GECIs) introduced through in vivo microinjection of adeno-associated virus (AAV) vectors to visualize calcium signals throughout entire astrocytes in acute hippocampal slices from adult mice. Using cytosolic and membrane-tethered GECIs (cyto-GCaMP3 and Lck-GCaMP3, respectively), Shigetomi et al. (2013) observed numerous spontaneous localized calcium signals (calcium microdomains) throughout entire astrocyte territories, including the branchlets and endfeet. Experiments in which signals in astrocyte endfeet, branches, and somata were simultaneously monitored with cyto-GCaMP3 indicated that that calcium signals in endfeet were independent of those in somata and occurred more frequently. The methods they describe promise to advance our understanding of astrocyte physiology and of their interactions with neurons and with the microvasculature of the brain.



Cytosolic GCaMP3 (cyto-GCaMP3) expressed in astrocytes in the stratum radiatum of adult mice. (From Shigetomi et al., 2013.)

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