Cell

Acid Tongues Cause Sour Thoughts

Michael J. Krashes[1,2] and Alexander T. Chesler[3,*]
[1]Diabetes, Endocrinology, and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Bethesda, MD 20892, USA
[2]National Institute on Drug Abuse (NIDA), NIH, Baltimore, MD 21224, USA
[3]National Center for Complementary and Integrative Health (NCCIH), NIH, Bethesda MD 20892, USA
*Correspondence: alexander.chesler@nih.gov
https://doi.org/10.1016/j.cell.2019.09.013

Animals use their sense of taste to evaluate the quality and safety of food before ingestion. In this issue of *Cell*, Zhang and colleagues provide a comprehensive exploration into the elusive mechanisms underlying sour detection.

As you walk into the café for a much-needed coffee, your sensory systems work in concert to tell you about your surroundings. As you take your first sip, activation of taste receptors signals just the right combination of bitterness and sweetness that you have learned precedes being awake and happy. However, taste is also a last line of defense before ingestion. If the milk in your coffee has spoiled, the unexpected sourness would impel you to spit it out. It is this latter sensation that Zhang et al. in this issue of *Cell* dissect down to its molecular and cellular components, from "tongue to brain" (Zhang et al., 2019).

Over the past few decades, studies from Charles Zuker, Nicholas Ryba, and colleagues have made great strides in uncovering the molecular and neural substrates for taste sensation. A compendium of work has laid out how the selective expression of specific receptors endows individual taste receptor cells (TRCs) with tuning limited to one of the five taste modalities: sweet, bitter, salt, unami, and sour (Yarmolinsky et al., 2009). For example, ectopic expression of bitter receptors in sweet TRCs caused mice to become attracted to things they normally find disgusting and even lethal (Mueller et al., 2005). These types of switches in sensory and behavioral functions also occur when connections between the tongue and neurons in the geniculate ganglion that relay taste information to the brainstem are rewired (Lee et al., 2017). Together with other experiments, a persuasive argument has been constructed that each type of taste is represented in the brain via distinct pathways. Now, work from Zhang et al. provides a treasure trove of information regarding the selective pathways for sour sensation.

Similar to other tastes, a dedicated class of TRCs responds solely to acids. Sour TRCs express the molecule PKD2L1, and genetic ablation of these cells causes profound deficits in physiological and behavioral responses to sour but not to other tastes (Huang et al., 2006). Interestingly, recordings from these PKD2L1-positive TRCs by Emily Liman's group uncovered an unusual proton-conductance that they proposed was generated by the elusive sour receptor (Chang et al., 2010). In a major breakthrough, the Liman group used the biophysical signature of this proton conductance as the basis of an expression cloning screen to identify Otop1 as the founding member of an evolutionarily conserved family of proton channels. This work showed that Otop1 is both sufficient to confer a proton conductance in non-native cells and necessary for this type of acid-evoked response in TRCs (Tu et al., 2018).

But, is Otop1 the sour taste receptor? In their current study, Zhang et al. provide compelling data that the answer is a resounding yes. Strikingly, they now show that responses to a range of acids in both taste nerve recordings and geniculate ganglia imaging are completely absent from Otop1-knockout mice. Similar loss of function experiments were also just reported by the Liman group (Teng et al., 2019). Equally fascinating, in a "lemon to lemonade" experiment, Zhang et al. drive expression of Otop1 in sweet TRCs using a genetic replacement strategy and show these mice now have "sweet and sour" responding taste neurons. These data also mean that the Otop1 proton conductance is sufficient to drive signaling in very different types of TRCs. It is tempting to speculate that now that sour also activates sweet TRCs, these mice will find acids much more appetitive, like drinking lemonade.

The geniculate ganglion has a "one taste per neuron" organization with very few polymodal cells (Barretto et al., 2015). Zhang et al. find a limited number of transcriptionally distinct cell types within the geniculate and propose a distinct cluster for each taste modality. Indeed, *in vivo* functional imaging supports this idea, as geniculate neurons marked using a PENK-cre mouse selectively respond to sour in an Otop1-dependent fashion, whereas those marked by spondin1-cre respond only to sweet. The single-cell data also give us a glimpse of intriguing molecular differences between the clusters that likely have functional meaning. For example, PENK is not just a useful marker but also encodes proenkephalin—well-known neuropeptide ligands for opioid receptors, a class of GPCRs expressed throughout the brainstem that potently modulate neural activity.

Following this logical thread into the brain, Zhang et al. looked for dedicated sour neurons in the nucleus of the solitary tract (NTS), the primary target of taste neurons in the brainstem. Using an *in silico* approach by combing through the Allen Brain Atlas, they

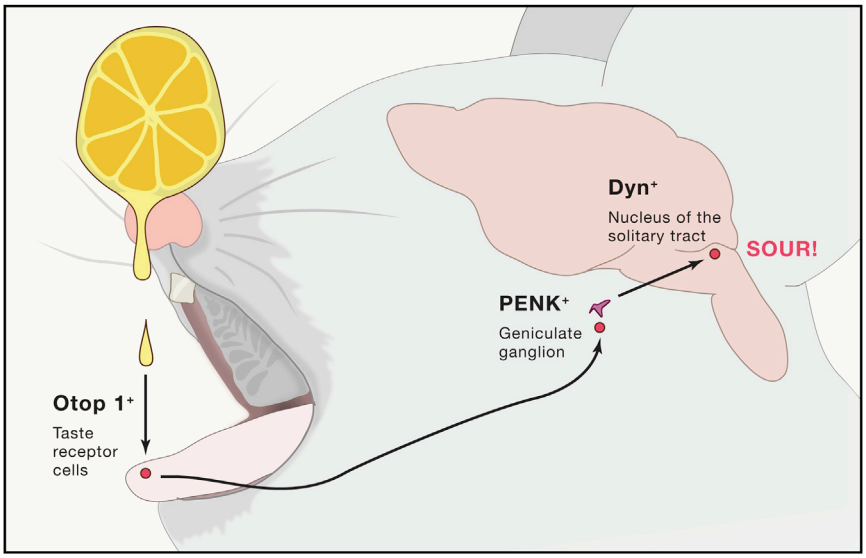

Figure 1. A Dedicated Pathway for Sour Taste

searched for genes with interesting expression patterns within the NTS. One gene that caught their eye was dynorphin, since it was expressed at the site where taste inputs arrive at the NTS. Recordings from this population of neurons using fiber photometry revealed robust increases in calcium responses to only sour tastes. Although dynorphin+ NTS neurons are not responsive to salt, bitter, sweet, or umami via the tongue, it is tempting to speculate they may respond to other types of stimuli from other chemosensory systems, such as vagal or trigeminal. A more thorough look at what other inputs have been labeled outside the geniculate from their rabies-tracing experiments will begin to provide clues.

If dynorphin-cre-positive NTS neurons are part of a dedicated pathway for sour (Figure 1), then their activation should evoke "sour" behaviors. In a cleverly designed behavioral task, the authors trained mice to report bitter versus sour by going left or right after sampling a drinking spout spiked randomly with these tastes. They then asked which way a trained mouse goes when they optically stimulate the dynorphin-cre NTS neurons while giving them water. As predicted, this type of optogenetic stimulation results in mice picking the sour side. But are these mice really experiencing sour taste, or are they choosing the side that's more sour-like or less bitter? Do these animals exhibit grimacing, retching, or noticeable aversion to this artificial encoding of sour? Evaluating the downstream activity in the brain evoked by optical stimulation of dynorphin-cre NTS neurons with those evoked by sour tastes will be an exciting path forward.

Interestingly, recent work from Yuki Oka's lab found that direct light activation of sour TRCs marked by the expression of the gene PKD2L1 promoted vigorous licking, contrary to what one might expect if these cells signal sour aversion (Zocchi et al., 2017). Instead, they propose that the sour TRCs are activated by the washing out of saliva by water, generating a signal that thirsty mice should drink more. At first pass, these results seem directly contrary to those reported here, as Zhang et al. show that optogenetic activation of sour-responsive NTS neurons abruptly stops licking. If these brainstem neurons are part of a labeled line for sour taste, then how can these two results be reconciled?

When protons hit the mouth, taste is not the only sensory system paying attention. Trigeminal afferents densely innervate the oral cavity, and many of them express the non-selective cation channel Trpv1, which is gated by heat, capsaicin, and protons. Activation of Trpv1-expressing trigeminal afferents results in a burning, stinging sensation, and at least some of the aversiveness of sour is believed to be mediated through these neurons (Liman et al., 2014). Since common experience tells us that it is very unlikely that nociceptors are activated by drinking water, perhaps co-activation of multiple sensory systems is required for sour aversion rather than attraction? Indeed, Zhang et al. show that deletion of Otop1 has minimal effects on sour aversion, but when coupled with ablation of Trpv1-positive afferents, there is an absence of avoidance to a physiologically relevant range of acid concentrations. Perhaps then, the role of dynorphin NTS neurons is to integrate responses of sour TRCs with those from Trpv1 trigeminal afferents? If so, these neurons might play a specific role in aversion, whereas other NTS cell types might respond to water and drive drinking. Alternatively, the dynorphin NTS neurons may be heterogenous, some responding to water, some to stronger acid; inquiries that can be answered employing imaging strategies that permit individual cellular resolution.

Here, Zuker and colleagues have synthesized a wealth of data and approaches to produce a comprehensive model for how sour taste reaches the brain. This work lays the foundation for future studies that will undoubtably find critical details about taste representation and how the combined activity of sensory systems produces experiences, emotions, and memories. In short, while the line may be labeled, that doesn't mean it is any less fascinating.

REFERENCES

Barretto, R.P., Gillis-Smith, S., Chandrashekar, J., Yarmolinsky, D.A., Schnitzer, M.J., Ryba, N.J., and Zuker, C.S. (2015). The neural representation of taste quality at the periphery. Nature *517*, 373–376.

Chang, R.B., Waters, H., and Liman, E.R. (2010). A proton current drives action potentials in genetically identified sour taste cells. Proc. Natl. Acad. Sci. USA *107*, 22320–22325.

Huang, A.L., Chen, X., Hoon, M.A., Chandrashekar, J., Guo, W., Tränkner, D., Ryba, N.J., and Zuker, C.S. (2006). The cells and logic for mammalian sour taste detection. Nature *442*, 934–938.

Lee, H., Macpherson, L.J., Parada, C.A., Zuker, C.S., and Ryba, N.J.P. (2017). Rewiring the taste system. Nature *548*, 330–333.

Liman, E.R., Zhang, Y.V., and Montell, C. (2014). Peripheral coding of taste. Neuron *81*, 984–1000.

Mueller, K.L., Hoon, M.A., Erlenbach, I., Chandrashekar, J., Zuker, C.S., and Ryba, N.J. (2005). The receptors and coding logic for bitter taste. Nature *434*, 225–229.

Teng, B., Wilson, C.E., Tu, Y.-H., Joshi, N.R., Kinnamon, S.C., and Liman, E.R. (2019). Cellular and Neural Responses to Sour Stimuli Require the Proton Channel Otop1. Curr. Biol. Published online September 19, 2019. https://doi.org/10.1016/j.cub.2019.08.077.

Tu, Y.H., Cooper, A.J., Teng, B., Chang, R.B., Artiga, D.J., Turner, H.N., Mulhall, E.M., Ye, W., Smith, A.D., and Liman, E.R. (2018). An evolutionarily conserved gene family encodes proton-selective ion channels. Science *359*, 1047–1050.

Yarmolinsky, D.A., Zuker, C.S., and Ryba, N.J. (2009). Common sense about taste: from mammals to insects. Cell *139*, 234–244.

Zhang, J., Jin, H., Zhang, W., Ding, C., O'Keeffe, S., Ye, M., and Zuker, C.S. (2019). Sour Sensing from the Tongue to the Brain. Cell *179*, this issue, 392–402.

Zocchi, D., Wennemuth, G., and Oka, Y. (2017). The cellular mechanism for water detection in the mammalian taste system. Nat. Neurosci. *20*, 927–933.

Sleep and the Balance between Memory and Forgetting

Hong-Viet V. Ngo[1,2] and Jan Born[2,3,*]
[1]School of Psychology, University of Birmingham, Birmingham B15 2TT, UK
[2]Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen 72076, Germany
[3]Centre for Integrative Neuroscience, University of Tübingen, Tübingen 72076, Germany
*Correspondence: jan.born@uni-tuebingen.de
https://doi.org/10.1016/j.cell.2019.09.007

Slow oscillations and delta waves are neuronal activity rhythms that hallmark sleep, but until now their respective functional roles have been impossible to tease apart. Utilizing a closed-loop optogenetic approach in rats, Kim et al. (2019) dissociated the functions of these two canonical rhythms, showing they support the consolidation and forgetting of memories, respectively.

Whereas the processing of specific information in the brain occurs through the dynamic firing patterns of distributed neuronal ensembles, the brain's state (such as sleep and wakefulness) is instead determined by concurrent field potential oscillations (Buzsáki and Draguhn, 2004). These oscillations, originating predominantly from synchronized membrane depolarization (termed "up states") and hyperpolarization (down states) in neuronal networks, essentially modulate information processing and synaptic memory formation by providing time windows of coherently facilitated and disfacilitated spiking activity in these networks (Bergmann and Born, 2018). Slow oscillations (SOs) and delta waves are similar field potential rhythms in the low 0.5–4 Hz frequency band that are a hallmark of sleep and specifically the state of slow-wave sleep (SWS). SOs are thought to be somewhat slower in frequency and higher in amplitude than delta waves, but a precise differentiation of the two putatively distinct oscillatory phenomena cannot be made based on the field potential signal itself. In fact, it was so far unclear whether the two oscillations serve specific functions at all, leading many to disregard this issue in studies. In an elegant study using the timed disturbance of spiking activity in rats in this issue of *Cell*, Kim et al. (2019) succeeded in dissociating the functions of SO and delta waves for memory processing, revealing that SOs support the preservation of a memory, whereas delta waves favor its forgetting.

In their study, the authors first trained rats on a brain machine interface (BMI) task where they gradually learned (by reward) to control the activity of one or two target neurons in the motor cortex. After training, the rats were allowed to sleep for about 1 h, and then performance on the BMI task was reassessed. During the period of sleep after training, they recorded the re-activations of those neuron ensembles that encoded the task at training. Such ensemble reactivations occur mainly during the slow rhythms of SWS and are thought to mediate the well-known memory effect of sleep. Importantly, in their experiments the authors simultaneously disturbed motor cortex firing activity during the sleep after training, using an optogenetic stimulation via an inhibitory opsin, which prompted an immediate reduction in neuronal firing rates in the targeted motor area. In order to precisely time this suppressive stimulation, it was applied in a "closed-loop controlled" manner, which means that the optogenetic stimulation was applied whenever the on-line analysis of the recording identified an up state (depolarizing phase of the oscillation) of either an SO or a delta wave. Thus, firing activity was reduced only during SO up states or delta wave up states. Compared with no stimulation or random stimulation