Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

The Interactions between Bitter and Sweet Taste Processing in *Drosophila*

Yu-Chieh Chen
Interdepartmental Neuroscience Program, University of California, Riverside, California 92521
Review of French et al.

In *Drosophila*, the detection of sweet and bitter compounds is mediated, for the most part, by distinct gustatory receptors (Grs) expressed in sweet and bitter gustatory receptor neurons (GRNs), respectively. The GRNs are intermixed within hair-like structures called taste sensilla that are distributed in different body parts, including the labelum, pharynx, distal segments of the legs (tarsi), wing margins, and ovipositor.

Engaging in appropriate feeding behaviors in response to different tastants, such as acceptance of appetitive (sweet) foods and rejection of toxic (bitter) foods, are essential for survival of animals. To promote behavioral avoidance, some bitter compounds have been proposed to not only activate bitter GRNs but also inhibit sweet GRNs. Activation profiles of labelar and tarsal sensilla in response to several bitter compounds have been well characterized using single sensillum recordings (Weiss et al., 2011; Ling et al., 2014). In addition, the suppression of sweet taste by bitter compounds has been observed in *Lepidoptera* (Schoonhoven and van Loon, 2002), leech (Li et al., 2001), and *Drosophila* (Meunier et al., 2003; König et al., 2014) with both electrophysiological and behavioral analyses. Moreover, a recent study suggests that an odorant binding protein, OBP49a, expressed in gustatory organs, is required for sweet neuron inhibition by bitter compounds (Jeong et al., 2013). However, the relative contribution of the two bitter sensing pathways (i.e., activation of bitter GRNs and inhibition of sweet GRNs) in controlling feeding behaviors, and whether they are independent of each other, is unclear. The detailed genetic, physiological, and behavioral analyses of these pathways are also lacking. A recent article in *The Journal of Neuroscience* by French et al. (2015) parses out the two pathways by assessing electrophysiological and behavioral responses of flies to sucrose mixed with either strychnine (which activates bitter GRNs and inhibits sweet GRNs) or L-canavanine (which only activates bitter GRNs) (Lee et al., 2012).

To quantify food acceptance behavior, French et al. (2015) presented flies with sugar/bitter mixtures to two main taste organs (labelum and tarsi) and scored indices for both proboscis extension reflex (PER) and proboscis retraction (PR). Flies robustly extended their proboscis upon sugar stimulation, but PER was reduced when aversive compounds were mixed with sugar. Mixing strychnine but not L-canavanine with sucrose reduced PER, suggesting that inhibiting sweet GRNs is a key factor for reducing PER. In contrast, both strychnine and L-canavanine reduced PR, indicating that bitter GRNs are involved in this behavior. Consistent with this idea, genetic ablation of *Gr66a*-expressing bitter GRNs greatly reduced PR but had no effect on PER to sucrose mixed with either strychnine or L-canavanine. In addition to PER/PR assays that have relatively short time scales, the authors used multiple choice capillary feeder (MultiCAFE) and binary choice feeding assays to evaluate food consumption and preference over 2 h (French et al., 2015, their Fig. 4). Similar to the PER/PR results, mixing either strychnine or L-canavanine with sucrose significantly decreased food consumption and induced food rejection. Interestingly, flies without functional bitter GRNs still avoided strychnine-mixed food but not L-canavanine-mixed food in both MultiCAFE and binary choice feeding assays, arguing that inhibition of sweet GRNs by strychnine is sufficient to trigger avoidance in the absence of bitter GRNs.

French et al. (2015) next examined the cellular basis of sugar inhibition by comparing electrophysiological recordings from different types of labelar sensilla upon presenting sucrose alone or sucrose/bitter mixtures. They found that two additional bitter compounds, caffeine and nicotine, did not inhibit responses to sugar (French et al., 2015, their Fig. 5). Notably, since caffeine alone activates bitter GRNs in s- and i-type sensilla (Weiss et al., 2011), one might expect the total spike number evoked by the sucrose/caffeine mixture to be higher than that evoked by sucrose alone, simply by having both sucrose- and caffeine-induced spikes. Unexpectedly, this was not seen in their s6 and i9 sensillum recordings (French et al.,

Received April 21, 2015; revised May 26, 2015; accepted May 29, 2015.
I thank Dr. Anupama Dahanukar for her helpful comments and editorial assistance on the manuscript.
The author declares no competing financial interests.
Correspondence should be addressed to Yu-Chieh Chen, Interdepartmental Neuroscience Program, 900 University Avenue, University of California, Riverside, CA 92521. E-mail: ychen107@ucr.edu.
DOI:10.1523/JNEUROSCI.1552-15.2015
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2015, their Fig. 5*D*,*E*). The simplest explanation is that sugar stimuli suppress bitter GRN activity if presented at high concentrations, but this idea requires further investigation, especially given that such a mechanism could potentially be harmful to animals.

Why do bitter compounds differ in their ability to inhibit sweet GRNs? One intriguing hypothesis is that the ability to inhibit sugar responses correlates with the toxicity of bitter compounds: the more toxic the compound, the more potent its sugar inhibition ability. Alternatively, different bitter compounds might inhibit responses induced by different sugars by selectively targeting distinct sweet Grs. However, no dose-dependent responses to the panel of bitter compounds were reported in this study and it is expected that the ability of bitter compounds to inhibit sugar responses depends on the concentrations of each substance. This hypothesis is supported by a recent study where sweet GRN activity was completely abolished with 5 mM caffeine mixed with 10 mM sucrose (instead of 100 mM sucrose used in French et al., 2015) (Jeong et al., 2013). Future studies that examine dose-dependent responses for a larger panel of bitter compounds, tested in sugar/bitter mixtures with 10 and 100 mM sucrose as well as other sugars, will yield deeper insight into the capacity of bitter compounds to inhibit different sugar responses.

Several possible mechanisms may contribute to the suppression of sweet GRNs by bitter compounds. One possibility is that sweet and bitter GRNs directly interact with each other. For example, in the *Drosophila* olfactory system, nonsynaptic lateral inhibition of an olfactory receptor neuron (ORN) is observed when transiently activating its neighboring ORN (Su et al., 2012). The labelar i9 sensillum may be an excellent place to address whether such ephaptic interaction also occurs in gustatory sensilla, because it houses only one sweet and one bitter GRN. To do this, French et al. (2015) expressed channelrhodopsin 2 (ChR2) in *Gr66a*-expressing bitter GRNs and, within the time course of the sucrose stimulus, presented blue light to transiently activate bitter GRNs. Interestingly, they found no reduction of sustained activity of sweet GRNs during optogenetic activation of bitter GRNs, suggesting the lateral inhibition does not occur in gustatory system (French et al., 2015, their Fig. 8).

Another mechanism by which bitter compounds may suppress sweet GRN activity is through inhibitory GABAergic interneurons. This type of presynaptic modulation mediated by $GABA_B$ receptors has been shown in both olfactory and gustatory systems (Wilson and Laurent, 2005; Olsen and Wilson, 2008; Root et al., 2008; Chu et al., 2014). A recent study by Chu et al. (2014) demonstrated that both sweet (labeled by *Gr64f-LexA*) and bitter (labeled by *Gr66a-LexA*) GRNs in the labelum have synaptic connections with GABAergic interneurons (labeled by *GAD1-Gal4*). The fact that $GABA_B$ receptors are expressed only in sweet but not bitter GRNs allows suppression of sweet GRNs upon bitter GRN activation through presynaptic gain control. Based on this finding, one would expect optogenetic activation of bitter GRNs to inhibit sweet GRNs through GABAergic interneuron circuits. However, the results by French et al. (2015) are inconsistent with this idea, because optogenetic activation of bitter GRNs had no effect on the activity of sweet GRNs (which express Gr64f) in the i9 sensillum. Given that other types of sensilla were not assessed, it is possible that the bitter GRN in the i9 sensillum has no synaptic contact with GABAergic interneurons. Considering that *Gr66a*-labeled bitter GRNs are present in all s- and i-type sensilla, it is conceivable that the GFP-reconstituted signals of *Gr66a-LexA* and *GAD1-Gal4* drivers shown by Chu et al. (2014) were from other types of labelar sensilla but not the i9 sensillum. It is also possible that GABAergic modulation is restricted to axon terminals and affects synaptic transmission rather than spike activity obtained from single sensillum recordings. Future studies using *Gr47b-Gal4* (which is only expressed in bitter GRNs of i8, i9, and i10 sensilla) to perform GFP reconstitution across synaptic partners (GRASP) assays will be more specific to determine whether neurons within the i9 sensillum make synaptic contact with GABAergic interneurons. Moreover, optogenetic activation of GABAergic interneurons during recordings of sugar-evoked activity in sweet GRNs in different labelar sensilla will provide more direct information about the involvement of GABAergic interneurons in suppressing sweet GRN activity.

In conclusion, French et al. (2015) provide insights into the cellular and behavioral contributions of sweet GRN inhibition by bitter compounds in both labelar and tarsal hairs. The dispensability of bitter GRNs for sweet GRN inhibition suggests that bitter compounds may directly modulate sweet GRN activity. With the aid of optogenetics, the authors demonstrated that unlike the olfactory system, GRNs do not have ephaptic interactions, although the involvement of GABAergic interneurons needs to be further evaluated. Their work not only confirms the behavioral role of sweet GRN inhibition but also provides an optogenetic experimental design with which to uncover mechanisms that integrate different taste inputs.

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