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Meifeng (Maia) Yang April 9, 2024

Taste-elicited short-term memory leads to prolonged modulation of feeding behavior in *Drosophila melanogaster*

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Department of Biology

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Abstract

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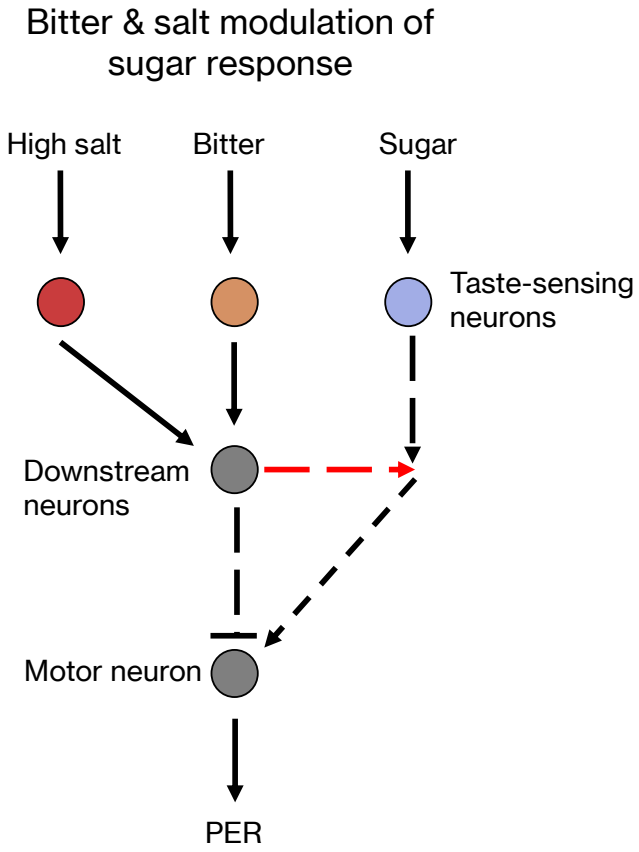

The ability to process and integrate taste inputs and generate appropriate behavior is essential to animal survival. Taste cues can modulate both immediate and future feeding behavior. Taste modulation of future feeding behavior has been well studied through the context of associative learning, where two stimuli are paired, but is less studied through the context of short-term memory, where the two stimuli do not overlap in time. Using *Drosophila melanogaster*, we studied how future feeding decision is modulated by the short-term memory of a previous taste experience, and how this modulation is influenced by internal state, time,

intensity of tastant, and type of tastant. We found that brief exposure to bitter and salt suppressed future feeding responses to sugar. This suppression is stronger when the animal is hungry and when the intensity of tastant is stronger. In addition, any suppression disappears over the course of a few minutes, supporting a short-term memory model. The ability to store memory of external stimuli and process with the context of internal state allows animals to integrate information and generate flexible behavioral responses.

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Introduction

Background

The *Drosophila* taste system

Taste is one of the most important sensory cues that help us navigate the world and survive. Attractive tastes, such as sugar, signal nutrition and calories, while aversive tastes, such as bitter, warn animals of potential toxins. The fruit fly *Drosophila melanogaster* is one of the most commonly used model to study structural, behavioral, and molecular processes, including taste processing, due to the extensive and sophisticated research on *Drosophila*. For a small organism, *Drosophila* has a considerably large number of genes -14,000 compared to human's 21,000 genes (Pandey & Nichols, 2011). Over 65% of human disease-associated genes have homologues in flies, and with the genetic tools available to target and study individual genes and cells in *Drosophila*, we can perform experiments otherwise infeasible on humans (Michele Markstein, 2018). Amongst the myriad phenomena we are trying to understand, knowledge on the regulation and processing of taste can help us understand how we perceive and interact with the world.

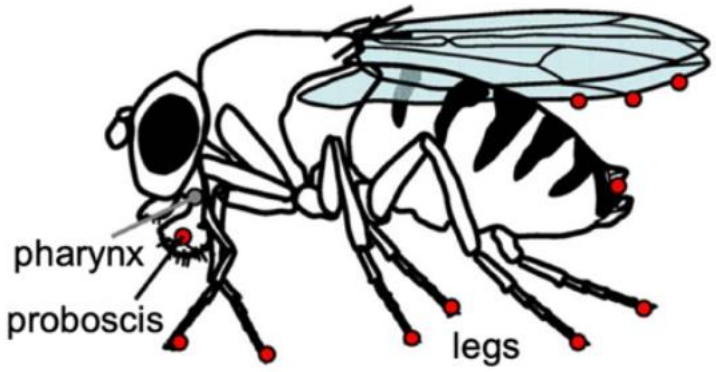


Figure 1. Taste-sensing cells distribution in *Drosophila melanogaster* (Devineni, 2022).

Similar to humans, *Drosophila* detects basic tastes like bitter, sweet, sour, salt, and umami (Thorne et al., 2004). However, they do so via taste receptors across multiple organs, mainly the labellum (tip of the proboscis), pharynx, and legs (Figure 1), which then activate neural pathways in the brain to elicit appetitive or aversive behaviors (Masek & Keene, 2016). To initiate feeding, the fly extends its proboscis to contact the food source (Thoma et al., 2017). This behavior is known as the "proboscis extension response", hereby abbreviated as PER. PER is a convenient proxy for measuring feeding without having to weigh minuscule changes in fly or food weight. Flies are known to be attracted to sugar, repelled by bitter, and attracted to low levels of salt (Jaeger et al., 2018; Scott, 2018). However, there is less knowledge on how appetitive and aversive tastes interact with each other and how fly behaviors are influenced by such interactions.

Behavioral flexibility and modulation in Drosophila

Drosophila behavior is flexibly modulated by many factors. Different tastes activate largely distinct taste-sensing neurons to promote or suppress feeding (Liman et al., 2014). The presence of one taste can cause immediate modulation on the response to another taste (Chu et al., 2014). Internal state (hunger, thirst, etc.) can switch the fly's preference for a food from avoidance to attractance (Devineni et al., 2019). The pairing of an aversive taste with an appetitive taste (e.g. bitter with sweet) can suppress the fly's future PER to sugar via taste-associative learning regulated by the mushroom body, the brain region that regulates experiential learning (Kirkhart & Scott, 2015). However, there is less knowledge on another type of modulation that is not immediate modulation or associative learning: taste-elicited short-term memory modulation of subsequent feeding behavior in *Drosophila*. For instance, if a foraging fly encounters an unpalatable food source, then the fly encounters a palatable source, will the

previous brief experience affect the fly's decision now to feed? A recent study done by Deere and Devineni found that a brief exposure to bitter taste suppressed subsequent feeding responses to sugar, and this modulation disappeared quickly: PER was significantly suppressed 20 seconds after bitter presentation and no longer suppressed 5 minutes after (Deere & Devineni, 2022). Given that the bitter stimulus was no longer present at the time of this suppression, they proposed that this modulation is caused by a short-term memory from the brief experience, showing that taste stimuli not overlapping in time can also modulate feeding responses (Deere & Devineni, 2022). Since the taste receptor neuron activation is not simultaneous with the behavior modulation, it may be mediated by slow-decaying activity in downstream neurons, causing prolonged behavioral modulation. This type of behavioral flexibility enables the fly to integrate taste information as it samples different sources, generating an understanding of local food quality and helping it make feeding decisions (Deere & Devineni, 2022). To better understand behavioral flexibility and taste pathway cross-modulation, though, we still need to investigate how fly internal state, as well as the concentration and type of tastant and the time delay between taste exposures, contribute to feeding behavior modulation.

Hypothesis and Goals

To further understand how one taste experience modulates future response to another taste, this project addressed four major questions: 1) What is the effect of internal state (hunger) on PER modulation? 2) What is the effect of the time delay between bitter stimulation and subsequent sucrose stimulation on the suppression of PER to sucrose? 3) What is the effect of aversive (bitter) tastant concentration on the suppression of PER to sucrose? 4) What is the effect of different types of aversive tastant (i.e. tastants other than bitter) on suppression of PER to

sucrose? These questions will shed light on how the brain integrates non-overlapping experiences and stimuli to generate appropriate behavioral responses, ensuring success when foraging in a natural environment.

Hypothesis for Question 1 (effect of hunger): Previous studies have shown that hunger affects how *Drosophila* responds to tastants. For example, a study by Inagaki et al. found that starvation increases fly sensitivity to sugar but decreases sensitivity to bitter (Inagaki et al., 2014). Another by Deere et al. studying associative learning found that hunger increases learned attraction to sugar and increases learned aversion to bitter (Deere et al., 2022). Since hunger increased experience-dependent modulation from associative learning, it may also increase short-term memory dependent modulation.

Hypothesis for Question 2 (effect of delay time): Since the suppression of PER to sucrose is based on short-term memory from previous bitter experience, we expect any suppression of PER to be transient and disappear a few minutes post-experience. Deere & Devineni had previously shown that the modulatory effect was strong after a 20 second delay and disappears after 5 minutes (Deere & Devineni, 2022), and we hypothesize that modulation decays gradually between those time points.

Hypothesis for Question 3 (effect of tastant intensity): We expect that a higher intensity of aversive taste solution (bitter or salt) will lead to a stronger suppression of subsequent PER to sucrose.

Hypothesis for Question 4 (effect of tastant): Finally, we considered the effect of different tastants on feeding behavior modulation by using salt instead of bitter. It has been

shown that flies are averse to high levels of salt (Jaeger et al., 2018). Thus, we hypothesize that subsequent PER suppression by salt exposure may be weaker than bitter.

Together, testing these hypotheses will promote our understanding of how different taste circuits in the brain modulate one another across time, demonstrating the flexibility of the brain.

Materials and Methods

We approached these questions through two methods, one presenting the flies with real tastants and the other using optogenetics to stimulate specific taste neurons. In both, feeding modulation was assessed by comparing PER responses to sugar before and after tastant exposure.

Fly stocks and maintenance:

Flies were raised at 25° on cornmeal-molasses food. Experiments were performed on mated females, 3-7 days old. For experiments using starved flies, flies were food-deprived with water for 1 day.

Flies used for all optogenetic experiments were maintained in darkness and fed on food with 1mM trans-retinal for 3 days prior to testing. For optogenetic experiments using starved flies, flies were first fed with 1 mM trans-retinal for 3 days, then were food-deprived with a wet Kimwipe with water containing 1 mM trans-retinal for 1 day.

Salt Dose-response PER assays

The purpose of a dose-response PER assay is to test fly aversion to a certain taste. In this case, we want to determine concentrations of salt that cause aversion. PER is measured for sugar alone or sugar with increasing concentrations of salt to reflect salt aversion.

Flies were first anesthetized on ice, then immobilized facing belly-up on a slide with myristic acid wax, with the two anterior pairs of legs glued. This allows for easy stimulation of the proboscis with tastants (Figure 2). Flies were then placed in a humidified, dark chamber for 30-60 minutes to recover from gluing. Flies were water-satiated before testing to ensure their feeding responses did not stem from thirst. Tastants were delivered with small, thin wicks made of Kimwipe by briefly applying the wick to the fly's proboscis. During water satiation, the Kimwipe wick is presented to the fly until the fly no longer displays PER, indicating water satiation. During experiments to test PER with taste solutions like salt, sucrose, or quinine, the tastant-soaked wick briefly stimulates the proboscis for 1 second, delivering a taste to the fly.

Tastants used were water, 100 mM sucrose, 250 mM NaCl+100 mM sucrose, 500 mM NaCl+100 mM sucrose, 1. M NaCl+100 mM sucrose, 3 M NaCl+100 mM sucrose, and 500 mM sucrose. Flies were tested sequentially in batches of 15-22, and the percentage of flies showing PER was recorded, with only full proboscis extensions being counted as "PER". As mentioned above, we used PER as a proxy for feeding response. At the end of each experiment, flies were tested with a positive control of 500mM sucrose, and they were excluded if they did not respond, as this is an indication of bad physical health. For each experiment, at least 4 independent sets of flies were tested, representing a minimum of 60 flies.

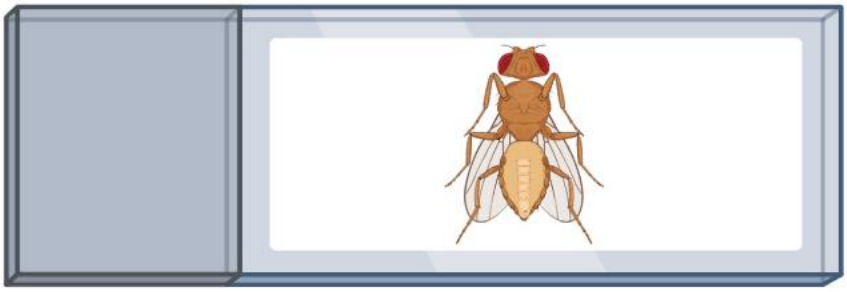


Figure 2. PER experiment set-up. Fly is secured onto a slide with myristic acid facing up, allowing for proboscis stimulation and PER observation (diagram not to scale).

Short-term memory PER assays

Short-term memory modulation PER assays with real tastants differ from dose-response assays in that the former investigates modulation by short-term memory of taste while the latter investigates the instantaneous modulation of feeding response by two concurring tastes. The flies were glued and water satiated in the same way as described above, and the same 500 mM control was used. They were then stimulated with 100mM sucrose to get a baseline PER and subsequently presented with the aversive tastant (bitter or salt) of a pre-determined concentration. They were allowed to rest for a brief, pre-determined amount of time before being stimulated with 100mM sucrose again to measure PER. The percentage of flies showing PER pre-exposure was compared to that post-exposure. The difference between baseline PER and post-exposure PER reflects whether negative short-term memory can indeed influence the flies' feeding behaviors (Figure 3A).

For optogenetic experiments activating bitter-sensing neurons, we used binary systems Gal4/UAS and lexA/lexAop for conditional gene expression to target specific neurons. Gal4 and lexA are transcriptional activators that drive effectors UAS and lexAop, respectively, thus causing the transcription of the target gene downstream of the promoter region (Riabinina &

Potter, 2016). We used driver lines Gr66a-lexA and Gr33a-Gal4 to drive lexAop-Chrimson and UAS-Chrimson, respectively. Both driver lines label bitter-sensing neurons in *Drosophila.* Chrimson is a light-sensitive cation channel that, when stimulated by red light of 620-750 nm, will cause neuron depolarization and activation (Klapoetke et al., 2014).

For optogenetic short-term memory PER assays, flies were glued in individual dishes to ensure that the light stimulates one fly at a time. The optogenetics experiment set-up is very similar to memory modulation PER set-up. Taking the bitter modulation experiment as an example, we used transgenic flies with their bitter-sensing neurons labeled by the Gr33a-Gal4 line, so that they will "taste" bitterness when light-stimulated. The experiment flow is the same: establish baseline PER with sucrose, use light to activate bitter-sensing neurons, wait, present sucrose again, compare the PER values before and after bitter exposure (Figure 3B).

To activate taste neurons with Chrimson, a red (617 nm) LED of varying intensity was turned on for 1 second, and the light was positioned 6 centimeters away from the flies. The light was controlled with an Arduino. We used the Arduino IDE to program three buttons on an Arduino Board connected to a red LED. The program uses pulse width modulation (PWM) to control the voltage delivered to the LED, thus controlling the output light intensity, allowing us to assign different light intensities to each button, mimicking different concentrations of bitter tastant.

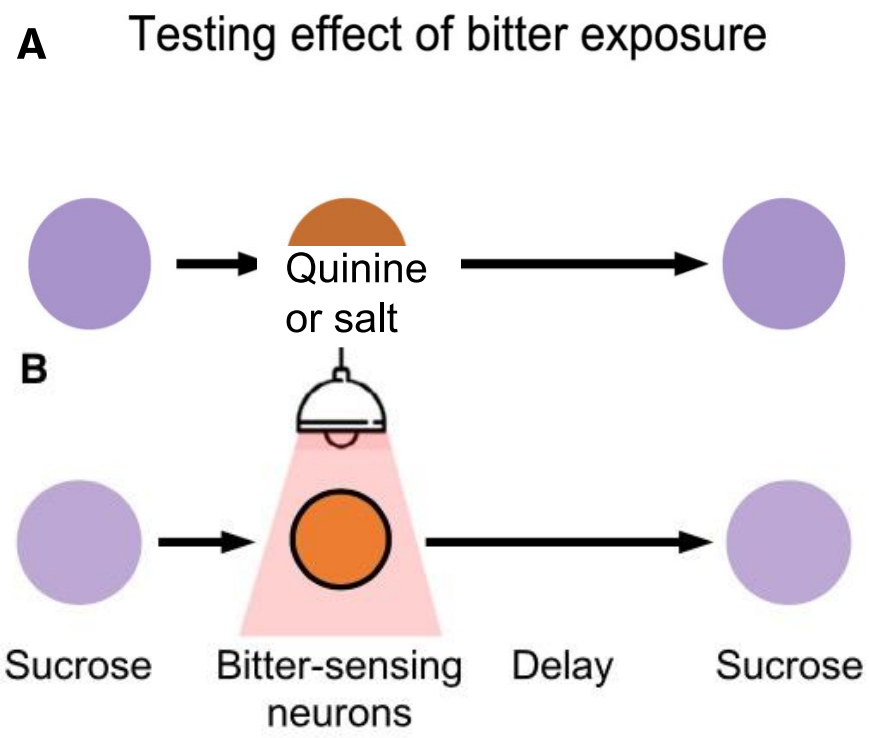


Figure 3. Adapted experiment flow for short-term memory behavior modulation (Deere & Devineni, 2022). A) Using real tastants to generate short-term taste memories. B) Using optogenetics to generate short-term taste memories.

Taste solutions

All taste solutions were prepared with Milli-Q water and remade ~every month to ensure freshness. Stocks were kept in the refrigerator at 4 °C.

Statistical analysis

Statistical analyses were performed using GraphPad Prism, version 9. We used paired t-tests to compare two groups, one-way ANOVAs to compare more than two groups, followed by multiple comparisons tests. To test the significance of each bar compareds to the theoretical mean (0), we used one sample t- and Wilcoxon tests. All graphs represent mean ± SEM.

Resource table

Reagent type (species) or resource	**Designation**	**Source or reference**	**Identifiers**
Genetic reagent, *D. melanogaster*	*Gr33a-Gal4*	Moon et al., 2009	BDSC: 31425
Genetic reagent, *D. melanogaster*	*Gr66a-lexA/CyO*	Thistle et al. (2012)	BDSC: 93024
Genetic reagent, *D. melanogaster*	*UAS-Chrimson-TdT^{VK5}*	Duistermars et al., 2018	N/A
Genetic reagent, *D. melanogaster*	*lexAop-CS-Chrim(attp40)*	D. Kim; Hattori et al. (2017)	BDSC 44277
Genetic reagent, *D. melanogaster*	*Wild-type control 2U (isoCJ1)*	Dubnau et al., 2001	N/A
Chemical compound, drug	Sucrose	Sigma-Aldrich	S9378
Chemical compound, drug	Quinine hydrochloride dihydrate	Sigma-Aldrich	Q1125
Chemical compound, drug	Sodium chloride	Sigma-Aldrich	S9888
Chemical compound, drug	All trans-retinal	Sigma-Aldrich	R2500
Chemical compound, drug	Myristic acid	Sigma-Aldrich	M3128
Software, programming	Arduino IDE 2.3.2	Arduino	https://www.arduino.cc/en/software
Software, algorithm	GraphPad Prism, version 9	GraphPad software	www.graphpad.com/scientific-software/prism

Table 1. Resource table of key materials used. Table includes the reagents, fly strains, and software used in the experiment.

Results

Recapitulation of published results confirms bitter stimulation & optogenetic bitter neuron activation suppresses future PER to sucrose

1) Bitter experience suppresses future PER to sucrose

We began by recapitulating previous results showing that short-term memory created by brief bitter taste exposure modulates future feeding responses in Drosophila (Deere & Devineni, 2022). We expected that bitter exposure would decrease future feeding responses, and we measured modulation through PER. We prepared 2U (wild-type) flies for PER according to the methods described above. We measured the flies' PER to 100 mM sucrose, briefly stimulated the flies with a 10 mM quinine solution, then allowed a delay period of either 20 seconds or 5 minutes before briefly stimulating them with sucrose again to measure their PER to sucrose. To identify behavioral modulation, we compared the post-bitter PER percentage to the pre-bitter PER percentage.

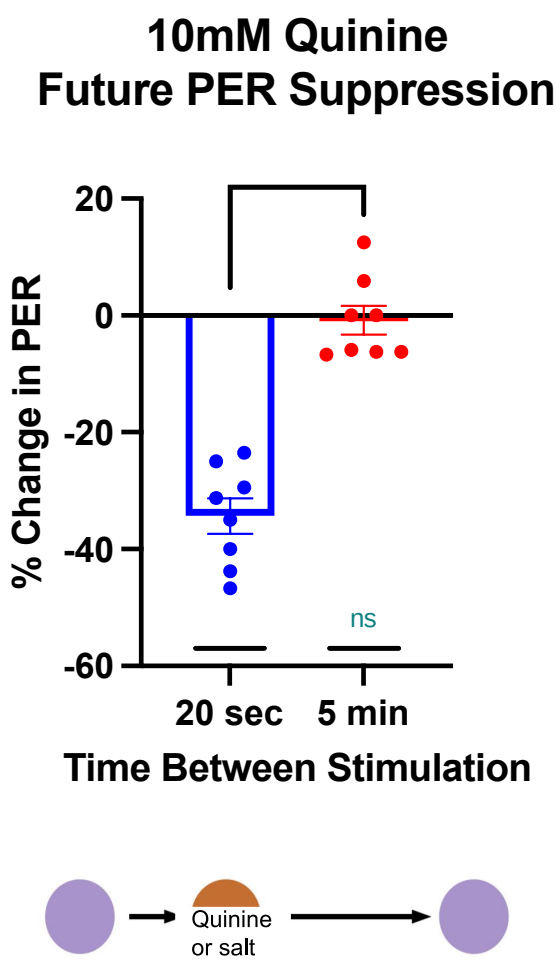


Figure 4. Bitter stimulation suppresses subsequent PER to sucrose. Stimulation with 10 mM quinine causes a significant suppression of PER to sucrose after a 20-second delay (n=8 sets of flies for a total of 132 flies tested). Time conditions were compared using paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test.

We compared the initial, pre-bitter exposure PER to the post-bitter exposure PER after a 20 second delay by subtracting the initial percentage of flies that showed PER to sucrose stimulation from the post-bitter PER to sucrose percentage, meaning that a negative bar represents PER suppression while a positive bar represents PER enhancement. Indeed, the percentage of flies that showed PER to sucrose significantly decreased from 74% to 39%--a 35% reduction (Figure 4). However, the suppression in PER is no longer observed if the delay between bitter exposure and second sucrose stimulation is 5 minutes (Fig. 4). Congruent to

previous results (Deere & Devineni, 2022), this suggests that the behavioral modulation observed is time-dependent and short-term, as it disappears within 5 minutes. With this foundational validation, we could then vary experimental conditions to further study behavioral flexibility within the short-term memory modulation paradigm.

2) Optogenetic activation of bitter-sensing neuron suppresses subsequent PER to sucrose

We next repeated published results of directly activating bitter-sensing neurons to recapitulate the observations of using bitter tastant (Deere & Devineni, 2022). This is to ensure that the prolonged suppression of PER to sucrose after bitter exposure is caused by short-term memory, not by residual bitter tastant on the proboscis.

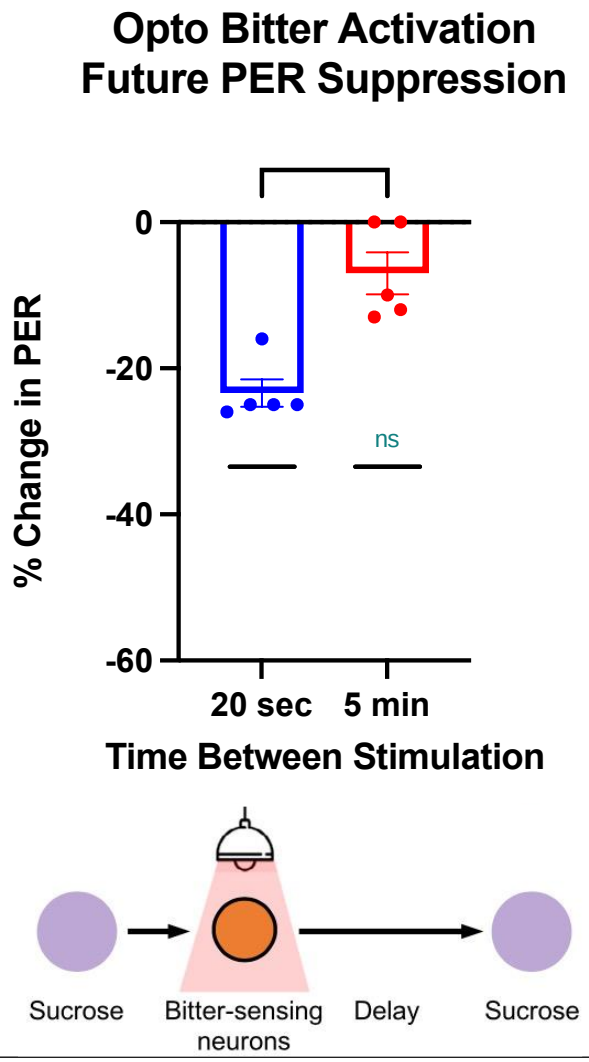


Figure 5. Optogenetic activation of bitter-sensing neurons suppresses subsequent PER to sucrose. The figure shows activation of bitter-sensing neurons marked by Gr66a-lexA. Activation was able to significantly suppress flies' PER to sucrose after a 20-second delay (n=5 sets of flies for a total of 105 flies tested). Time conditions were compared using paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test.

To test whether optogenetic activation of bitter-sensing neurons suppresses the flies' subsequent PER to sucrose, we used 1-day starved Gr66a-lexA/lexAop-Chrimson transgenic flies labelling bitter-sensing neurons. We set up the optogenetics PER assay using methods detailed above. Instead of bitter tastant, we activated the flies' bitter-sensing neurons with the highest light intensity on our LED (255). The pre- and post-bitter stimulation PER values were

compared to see if the brief experience influenced the flies' subsequent feeding decisions. Consistently, the flies responded to the sucrose stimulus 23% less 20 seconds after optogenetic bitter-sensing neuron activation than before optogenetic activation (Figure 5), but their response to sucrose returned comparable to baseline 5 minutes after bitter optogenetic activation. These results are consistent with stimulation with real bitter compounds, confirming that the modulatory effects of bitter compound stimulation are not due to residual bitterness on the fly's proboscis. In addition, these results confirm that we were able to recapitulate previous results (Deere & Devineni, 2022), thus allowing us to repeat these experiments while varying additional conditions.

Bitter short-term memory suppression of subsequent PER to sucrose

1) Hunger state affects optogenetic bitter suppression of subsequent PER to sucrose

We investigated the effect of hunger state on feeding behavior modulation—will satiation decrease the effect of short-term memory PER modulation?

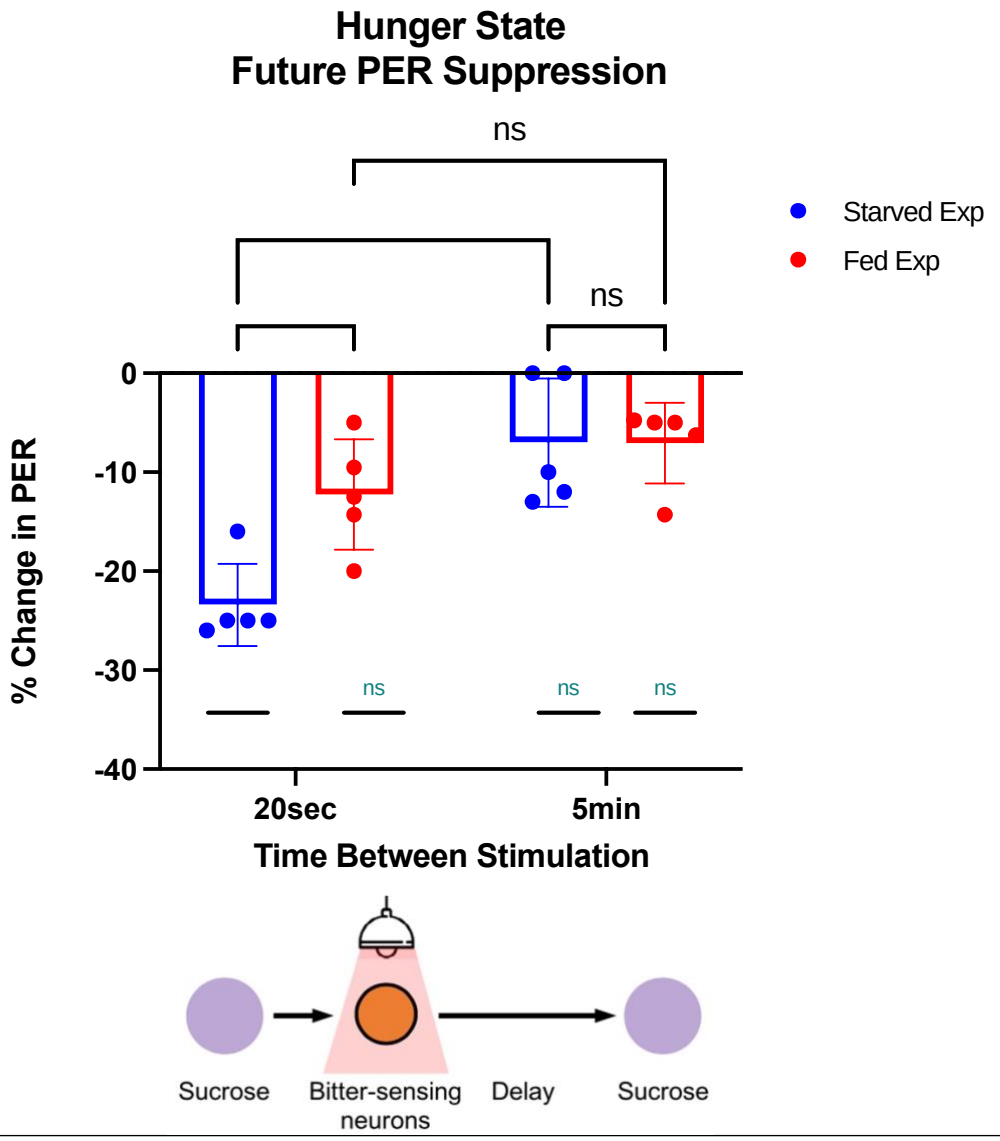


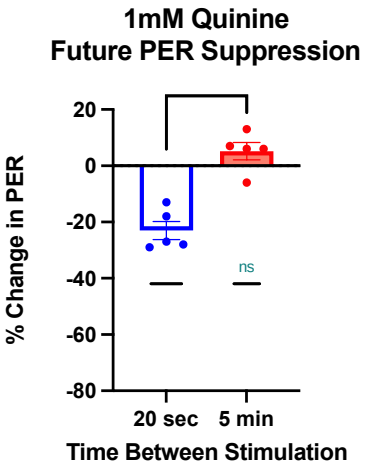
Figure 6. Hunger state influences optogenetic bitter modulation of subsequent PER to sucrose stimulation. Graph shows change in the percentage of flies that displayed PER to sucrose after bitter optogenetic activation vs before bitteer activation by time-delay and starvation state. PER is significantly suppressed 20 seconds after bitter optogenetic activation in the 1-day starved condition (n=5 sets of flies for a total of 105 flies tested). PER was not significantly suppressed in either time delays in the non-starved condition (n=5 sets of flies for a total of 91 flies tested). Time conditions were compared using a one-way ANOVA followed by multiple comparisons; individual conditions were tested using one sample t-test and Wilcoxon test.

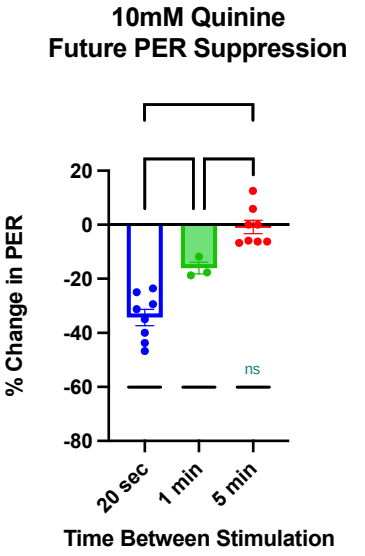
We first investigated the effect of hunger state on short-term memory suppression of subsequent PER to sucrose. Using Gr66a-lexA/lexAop-Chrimson transgenic flies again,

compared results of starved flies with non-starved flies. When flies were not starved, they responded to the sucrose stimulus 12% less 20 seconds after optogenetic bitter-sensing neuron activation than before optogenetic activation (Figure 6). With a 5 minutes delay, the suppression was not significant compared to 0 (Fig. 6). Modulation by optogenetic bitter activation induced short-term memory was significantly less when flies were not starved compared to starved (Figure 6), suggesting that this phenomenon is very much state-dependent. This furthers previous findings on how hunger state influences acute response to taste and learned response to taste (Inagaki et al., 2014; Deere et al., 2022) and demonstrates that behavioral flexibility extends to short-term memory.

2) & 3) Bitter short-term memory suppresses subsequent PER to sucrose in a dose- and time-dependent manner

Since we were able to show subsequent PER suppression with bitter tastant and optogenetic activation of bitter-sensing neurons, we moved on to address the questions laid out earlier. Starting with the first two questions of whether short-term memory-based PER suppression is influenced by the concentration/intensity of the tastant and the length of time post-experience, we varied the concentration of the quinine solution and the time delay. We tested quinine concentrations of 1 mM, 10 mM, and 50 mM and time delays of 20 seconds, 1 minute, and 5 minutes. For each concentration of quinine tested, we tested different time delays to characterize the time course of the memory modulation.





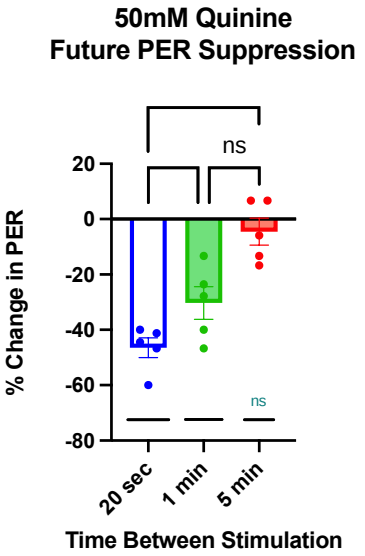


Figure 7. Time- and concentration-dependent suppression of subsequent PER to sucrose by quinine stimulation. Graphs show subsequent PER suppression effects of 1 mM, 10 mM, and 50 mM quinine. Quinine suppresses subsequent PER to sucrose in a concentration- and time-dependent manner. We did not test the 1-minute delay condition for 1 mM quinine due to the relatively small modulatory effect observed with the 20-second delay. For the 1 mM condition, we have n=5 sets of flies for a total of 83 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test. For the 10 mM condition, we have n=8 sets of flies for a total of 132 flies tested. Time conditions were compared using a one-way ANOVA followed by multiple comparisons; individual conditions were tested using one sample t-test and Wilcoxon test. For the 50 mM condition, we have n=5 sets of flies for a total of 80 flies tested. Time conditions were compared using a one-way ANOVA followed by multiple comparisons; individual conditions were tested using one sample t-test and Wilcoxon test.

We observed that PER is strongly suppressed 20 seconds after bitter exposure for all quinine concentrations tested. 1 mM quinine led to a 23% decrease in PER to sucrose post-bitter exposure, 10 mM led to a 34% decrease, and 50 mM led to 46% decrease (Figure 7). For each concentration, the magnitude of PER suppression decreased as the time delay increased. With 10 mM and 50 mM quinine , PER suppression is still significant after 1 minute (suppressed by 16% and 30%, respectively), and only becomes non-significant after 5 minutes (Fig. 7).

Our findings provide further insight into how short-term memory created by a brief taste experience modulates subsequent feeding decisions in *Drosophila*. As the concentration of the aversive tastant increases, it creates a more negative experience, leading to a stronger feeding response suppression. We also characterized the modulation time course and found that modulation decays gradually over 5 minutes, with stronger modulation caused by a more aversive experience decaying slower and weaker modulation decaying faster.

We hypothesized that different light intensities activate neurons to different levels, mimicking the effect of different quinine concentrations. To determine if different light intensities were sufficient to activate bitter-sensing neurons, we activated the bitter-sensing neurons with light intensities of 50, 80, and 255 (out of a maximum of 255; controls LED output) while stimulating the flies with sucrose and measured the percentage of flies displaying PER. If lower light intensities are incapable of sufficiently activating the bitter-sensing neurons and suppressing acute PER to sucrose, then we cannot use them to test short-term memory PER suppression.

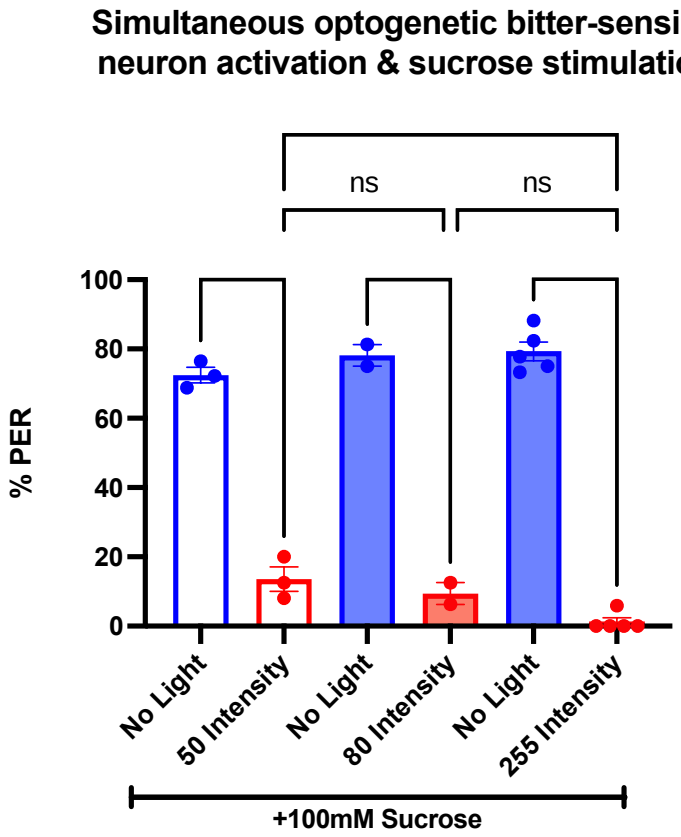
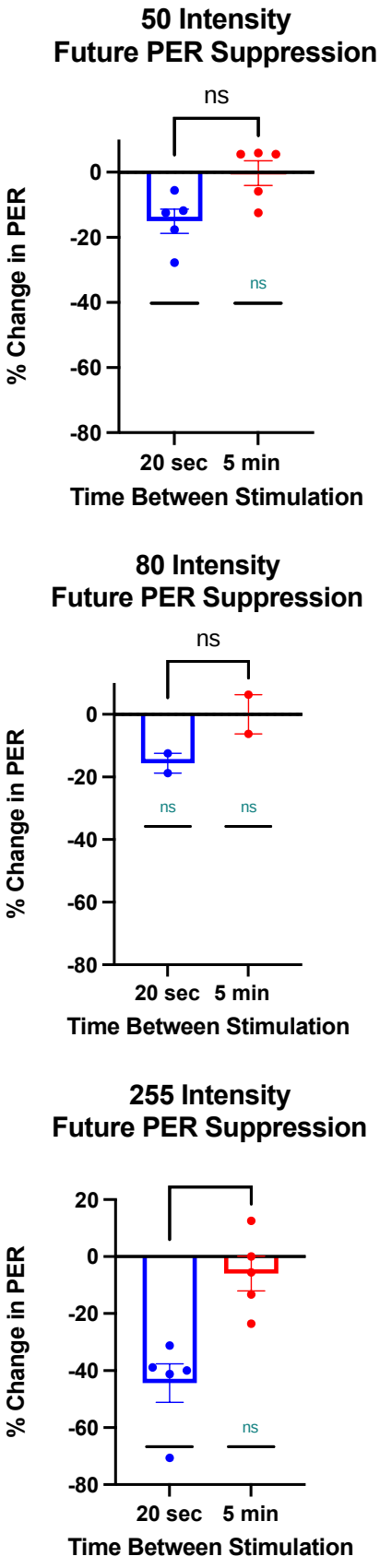


Figure 9. Optogenetically activating bitter-sensing neurons while stimulating with sucrose. Graph shows the percentage of flies that showed PER sucrose stimulation while activating bitter-sensing neurons with different light intensities. PER to sucrose was significantly suppressed at all 3 light intensities (n=12 sets of flies for a total of 202 flies tested). Conditions were compared using a one-way ANOVA followed by multiple comparisons.

When we simultaneously activated bitter-sensing neurons and stimulated the flies' proboscis with sucrose, there was a significant suppression of PER to sucrose for all three light intensities. 14% of flies showed PER with 50-intensity bitter neuron activation, 9% showed PER with 80-intensity bitter activation, and 1% showed PER with 255-intensity bitter activation (Figure 9). This confirms that even lower light intensities lead to sufficient activation of bitter-sensing neurons, allowing us to test the effect of memory modulation with lower intensities.



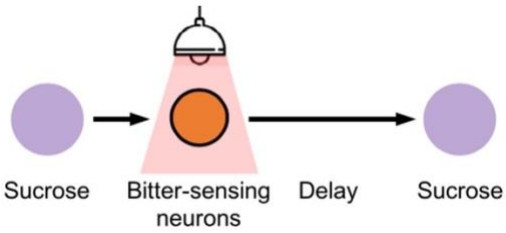


Figure 10. Short-term memory suppression of future PER to sucrose by different light intensities. Graph compares PER suppression by time delay and light intensities. The PER suppression caused by intensities 50 and 80 were not significant compared to baseline (0), while 255 intensity caused a significant suppression in PER to sucrose 20 seconds after bitter-sensing neuron activation. For the 50-intensity condition, we have n=5 sets of flies for a total of 86 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test. For the 80-intensity condition, we have n=2 sets of flies for a total of 32 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test. For the 255-intensity condition, we have n=5 sets of flies for a total of 84 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test.

After confirming that the light intensities we chose could all sufficiently activate bitter-sensing neurons, we moved on to test optogenetic short-term bitter memory modulation of subsequent PER to sucrose. We tested intensities of 50, 80, and 255 and time delays of 20 seconds and 5 minutes. Despite being able to acutely suppress PER to sucrose, the 50 and 80 intensities caused much weaker suppression of subsequent PER to sucrose after a 20 second delay (Figure 10). Only the 255 intensity significantly suppressed PER to sucrose, causing a 44% decrease in PER 20 seconds after bitter activation compared to before (Fig. 10). This is intriguing because lower light intensities were able to acutely suppress PER to sucrose.

Salt short-term memory modulation

Drosophilae are averse to high salt in salt + sugar dose-response PER experiments

With the knowledge that bitter compounds like quinine can create short-term memory that suppresses subsequent PER, we wanted to test whether this effect could be observed with other aversive compounds like salt. Salt is an interesting compound because it can be appetitive or repelling to *Drosophila* depending on its concentration. A study by Jaeger et showed that when *Drosophilae* are raised under normal conditions (not salt deprived), they are attracted to low concentrations of salt but are averse to higher concentrations (Jaeger et al., 2018). Will high salt, then, suppress subsequent PER to sucrose like that caused by bitter compounds? We aimed to 1) confirm which salt concentrations are aversive to our 2U (wild-type) flies, 2) determine if aversive salt tastants can modulate *Drosophilae*'s subsequent feeding decisions, and 3) determine whether this modulation is time- and concentration-dependent.

Jaeger et al. found that salt concentrations of 250 mM, 500 mM, and 1 M in a 100 mM sucrose solution significantly suppressed flies' PER compared to 100mM sucrose alone (Jaeger

et al., 2018). We started with our first aim of confirming the aversiveness of these concentrations with our wild-type flies through dose-response PER experiments. Unlike experiments with bitter, the flies were not starved to ensure that they were not deprived of salt, which has been shown to reduce salt aversion (Jaeger et al., 2018). We prepared flies similar to bitter PER experiments and tested them with 250 mM, 500 mM, 1 M, or 3 M of NaCl mixed with 100 mM sucrose. The percentage of flies showed PER to each concentration of salt solution was compared to sucrose alone to determine salt suppression of PER.

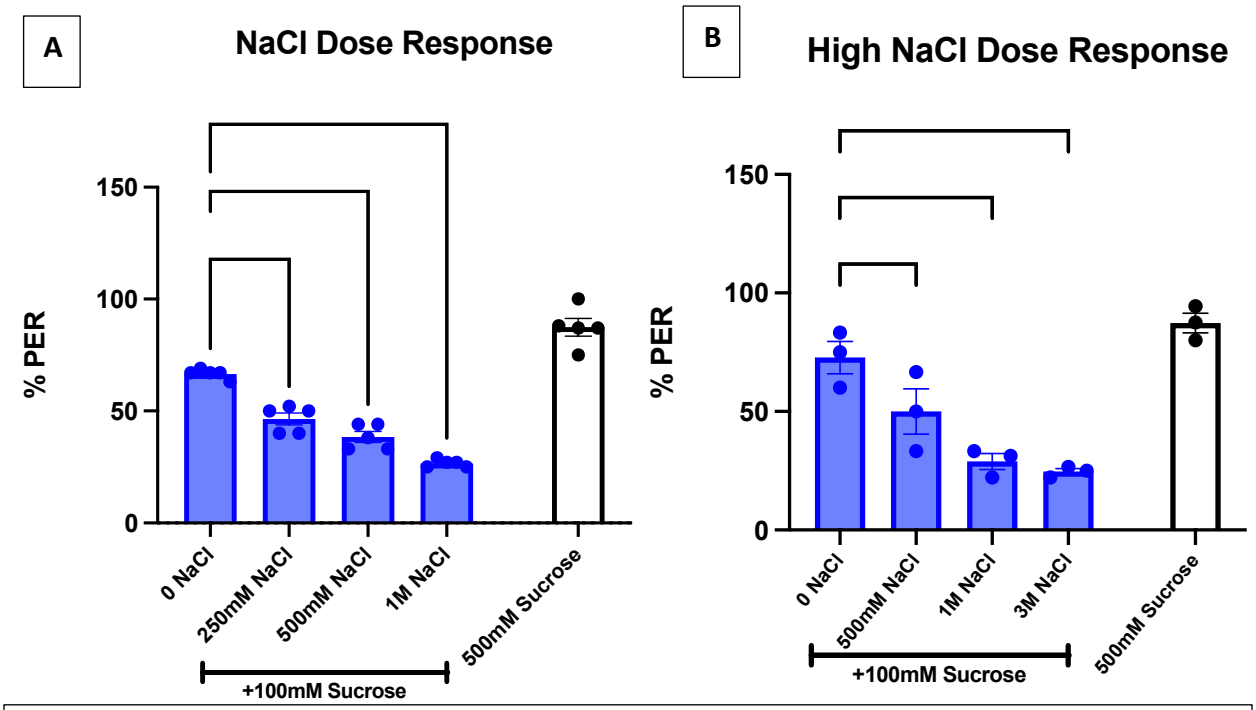


Figure 11. Salt dose-response experiments to determine concentrations of salt that cause aversion. A) Acute PER suppression by salt concentrations of 250 mM, 500 mM, and 1 M. B) Acute PER suppression by salt concentrations of 500 mM, 1 M, and 3 M. 1 M and 3 M of salt significantly suppressed PER in both experiments. For NaCl dose-response, we have n=6 for a total of 107 flies tested. Conditions were compared using a one-way ANOVA followed by multiple comparisons. For high NaCl dose-response, we have n=3 for a total of 49 flies tested. Conditions were compared using a one-way ANOVA followed by multiple comparisons.

All concentrations of salt caused aversion, with salt solutions 250 mM and 500 mM causing less acute suppression in PER (a 20% and 26% decrease compared to PER to sucrose alone, respectively) than salt concentrations of 1 M and 3 M, which strongly suppressed the flies' acute PER by 42% and 43%, respectively, subtracting from PER to sucrose alone (Fig. 11). Based on these results, we determined the salt concentrations to use for PER experiments testing behavior modulation caused by short-term memory, 1 M and 3 M of NaCl, because they elicited strong aversion.

High salt concentrations suppress PER in a dose- and time-dependent manner

We focused on using high salt to recapture the time- and concentration-dependent PER suppression observed with bitter stimulation.

We prepared 2U (wild type) flies for PER and tested them with a 1 M or 3 M salt solution and brief delays of 20 seconds or 5 minutes. We compared the post-salt exposure percentage of flies that responded to sucrose stimulation to the pre-salt exposure percentage.

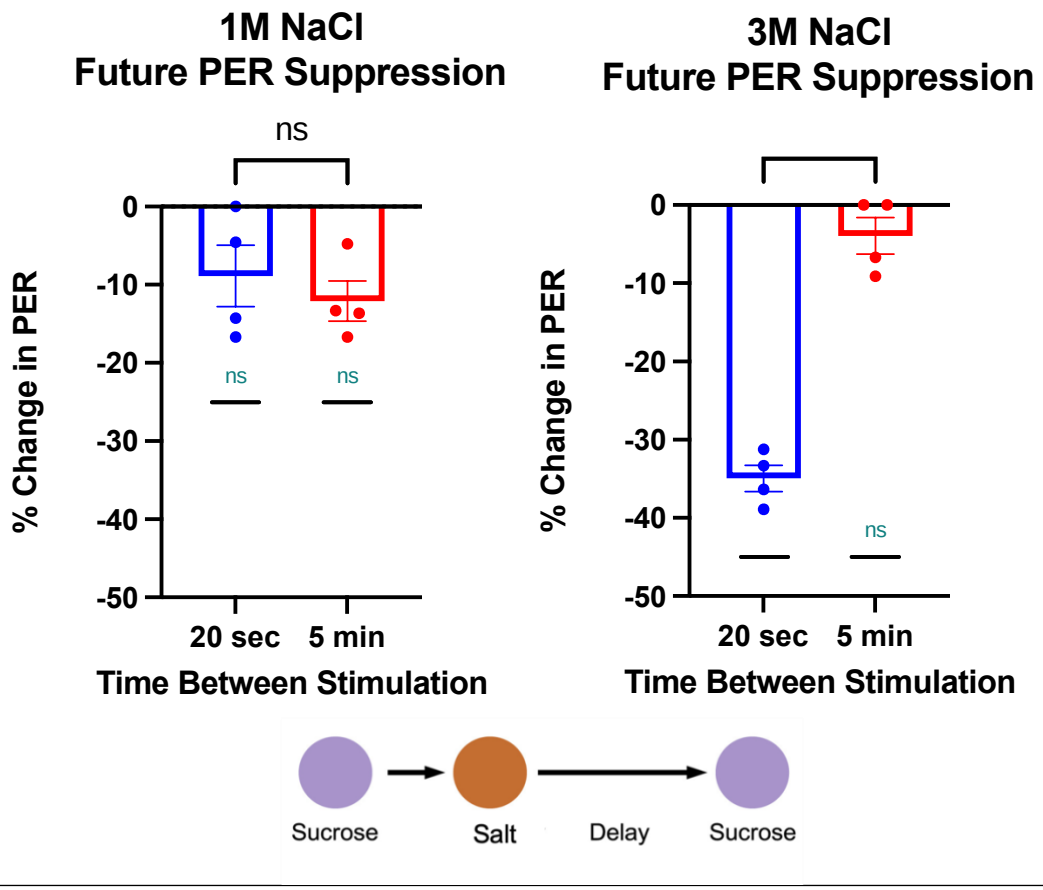


Figure 12. Salt modulation of subsequent PER to sucrose. Graph shows the modulatory effects of 1 M and 3 M of salt after a 20-second or 5-minute delay. Suppression of PER to sucrose 20 seconds after salt stimulation was only significant with 3 M salt, not significant with 1 M salt. For the 1 M NaCl condition, we have n=4 sets of flies for a total of 76 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test. For the 3 M NaCl condition, we have n=4 sets of flies for a total of 71 flies tested. Time conditions were compared using a paired t-test; individual conditions were tested using one sample t-test and Wilcoxon test.

Despite the strong PER suppression observed in acute dose-response experiments with 1 M and 3 M NaCl, the modulatory effect of 1 M NaCl was non-significant. With a 20 second delay between salt stimulation and subsequent sucrose stimulation, the percentage of flies that showed PER to sucrose decreased 9% compared to pre-salt exposure. With a 5-minute delay, the percentage decreased 12%. Neither suppression of response to sucrose stimulation was statistically significant (Figure 12). 3 M NaCl, however, considerably suppressed the flies'

subsequent response to sucrose presentation after a 20-second delay, returning to non-significant after a 5-minute delay (Fig. 12). Our results suggest that salt solution concentrated enough to acutely suppress PER to sucrose may not necessarily elicit short-term memory modulation of subsequent PER behavior.

Discussion

In this study, we built on a newly characterized model of behavioral modulation in the taste system. The model showed that both brief, direct taste exposure and optogenetic simulation of aversive tastes suppresses future PER to sucrose over the time course of <5 minutes. We found that 1) internal hunger state of flies plays a role in short-term memory suppression of future PER: if the fly is not hungry, its feeding behavior will less likely be modulated by short-term memory. 2) time delay affects short-term memory suppression of future PER: short-term memory decays quickly over the time course of 5 minutes. The strength of PER suppression decreases accordingly—the suppression observed after a 20-second delay is stronger than after a 1-minute delay; the same holds true for a 1-minute compared to a 5-minute delay. 3) the concentration of aversive tastant or level of optogenetic activation affects the magnitude and length of modulation of future feeding behavior. The higher the neuronal activity, the more robust and long-lasting the behavioral modulation. 4) aversive tastes other than bitter, namely salt, can also elicit short-term memory that decreases the likelihood of future PER to surcose. Interestingly, although high concentrations of salt (1 M and 3 M) were aversive to flies, only 3M caused short-term memory PER modulation. This may mean that only very high salt concentrations are able to create short term memories that modulate subsequent PER.

In this study, we showed that hunger state, time, concentration of tastant, and type of tastant influence the fly's subsequent feeding decision. This type of flexible short-term memory modulation is adaptive for animals in a natural environment. As it samples different foods over time, the animal could integrate taste information based on its urgency to feed and its recent encounters with nearby food sources to generate an understanding of local food quality and thus make the most appropriate feeding decisions.

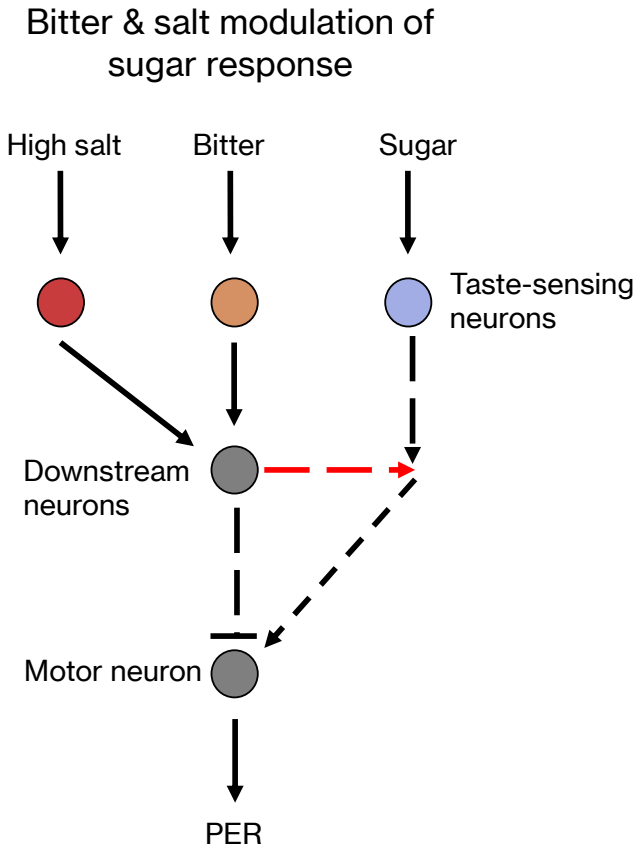


Figure 13. Model for salt and bitter modulation of future PER (adapted from Deere & Devineni, 2022). Dash lines show connections that may be indirect; red dash lines show downstream salt/bitter pathways that cause prolonged suppression of responses to sugar. Yellow highlight show proposed area of cross-modulation.

Our results furthered our understanding of the taste system. Firstly, we found that hunger state influences short-term memory induced behavior modulation: flies that are not hungry are less likely to form short term memory from a brief, negative experience and change their subsequent feeding behaviors accordingly. This is consistent with Deere et al.'s results on hunger-state regulation of associative learning (Deere et al., 2022). Our finding reflects the flexibility of the taste system—when there is no urgency for food, there is no need for the organism to make immediate switches in feeding behavior. Secondly, we found that the magnitude and longevity of short-term memory induced behavioral modulation is positively correlated with the concentration of the aversive stimulus, and the modulation decays gradually over 5 minutes. This suggests that the stronger the taste-sensing neurons are activated, the stronger the post-stimulus downstream neuron activity (Figure 13). Finally, we found that salt can also create short-term memories that modulate subsequent feeding behavior. This again highlights the flexibility of the taste system and of memory modulation. The fly processes information of a compound depending on the concentration of the compound to meet both nutritional and taste needs, and high concentrations of salt was observed to cause prolonged aversion and suppression of PER (Figure 13). These results build on the recently established behavioral modulation paradigm shedding light on some of the nuances underlying short-term memory modulation and laying the groundwork for future work to investigate the cellular mechanisms of the taste circuits.

The next steps of this study will be testing more conditions for salt modulation and optogenetically activating salt-sensing neurons. In addition, although the PER assay allowed us to accurately control the timing and duration of taste-stimulation, it puts the fly in a non-natural condition. We hope to investigate short-term memory modulation in a more naturalistic

environment in the future. Finally, based on our proposed model of modulation (Figure 13), we hope to determine the site and mechanism of taste circuit modulation that causes prolonged PER suppression. Nonetheless, this study provides the foundational information for behavior mechanisms, allowing for future work to identify the underlying neural mechanisms.

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