Taste Experience Enhances Cortical Response Reliability during Latent Enhancement of Taste Aversion Learning

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Abstract

Learning is not as simple as the association of paired stimuli in a vacuum. For example, benign experience with a taste stimulus weakens future conditioned taste aversions (CTA) to that taste—a phenomenon known as latent inhibition—and enhances later CTA to a novel taste (latent enhancement [LE]; Flores et al., 2016; Flores et al., 2018). Our recent investigations on how benign taste experience impacts cortical responses revealed an increase in the discriminability/salience of Gustatory Cortical (GC) responses to a new taste following experience offering a clue into potential underlying mechanisms for LE on CTA (Flores et al., 2022). Here, we predict that the previously reported increase in response discriminability following taste experience is associated with a reduction of variability that has been shown to promote learning. Our results support this prediction and reveal enhanced trial-to-trial consistency of single-neuron sucrose responses and coherent activity across ensemble neurons before CTA learning. Connecting this result to learning, we further show that the distinction between pre- and post-CTA sucrose responses are indeed greater in rats with prior benign taste experience. Overall, these results suggest that following benign experience, taste coding in GC becomes more reliable (at both the single-neuron and ensemble levels) providing a potential mechanism which may contribute to the stronger CTA acquisition seen in LE of learning.

Keywords: experience, conditioned taste aversion, gustatory cortex, taste palatability, Long Evans rodents

Significance Statement

Animals and humans readily learn the consequences of consuming a specific taste and react by changing their behaviors. We have shown that even seemingly inconsequential and benign taste experiences – which are arguably more common - can enhance taste behavior and learning. The work presented here is the first to evaluate how benign experience alters learning-related cortical processing dynamics using *in-vivo* electrophysiology in freely behaving rats. We report that benign taste experience alters cortical plasticity which underlies the enhancement of learning. This unravels a new area of chemosensory research and may shed light on how daily taste experiences impact the neural dynamics of future taste consumption and learning.

Introduction

Behavioral modifications in response to experience play an essential role in animal survival and decision making. To understand these modifications, researchers typically focus on associative learning paradigms in which experiences are linked to specific consequences. For example, in conditioned taste aversion (CTA), an animal learns to avoid a particular taste (the conditioned stimulus) that was previously paired with malaise (unconditioned stimulus; Garcia et al., 1955).

While often ignored, benign taste experiences (those not linked to negative outcomes) also change behavior and impact associative learning. For example, benign experience with a taste weakens later CTA acquisition towards that same taste (a phenomenon known as latent inhibition; Lubow and Moore, 1959; Lubow, 1973; De la Casa and Lubow, 1995). More recently, we have described a complementary effect wherein experience with salty and/or sour tastes leads to an enhanced aversion towards novel sucrose; we term this phenomenon latent enhancement (LE) of CTA (Flores et al., 2016; Flores et al., 2018). Relative to latent inhibition (Naor and Dudai, 1996; Miranda et al., 2003; Reilly and Schachtman, 2009; Yiannakas and Rosenblum, 2017) what we know about the neural mechanisms underlying LE is still minimal.

Gustatory cortex (GC) is an important node in the central circuit for taste learning and behavior (Yamamoto et al., 1995; Berman and Dudai, 2001; Uematsu et al., 2015; Levitan et al., 2016; Li et al., 2016; Wiaderkiewicz and Reilly, 2023), and recent studies reveal it to be an important part of the neural underpinning of LE as well. Specifically, Flores et al. (2018) reported elevated levels of learning-related c-FOS expression within GC of LE rats and showed that optogenetic inhibition of GC during benign taste experience attenuates LE. These results

support the suggestion that GC integrates benign taste experiences which then boosts future learning.

Of course c-FOS, a gross measure of activity, cannot reveal how benign taste experience affects real-time aspects of taste processing—that is, the way that taste responses evolve through coherent firing epochs related to the detection, identity, and palatability of tastes which have been shown to be behavior- and learning-related (Katz et al., 2001, 2002; Jones et al., 2007). *In-vivo* electrophysiological investigations, however, reveal that these processing dynamics change with benign taste experience, with GC responses towards experienced (familiar) tastes becoming more distinct from each other *via* potentiation of activity related to palatability (Flores et al., 2022; but also see Staszko et al., 2022). Flores et al. (2022) went on to demonstrate that benign experience increases the discriminability of GC responses to a novel taste. This last finding suggests but stops short of testing whether experience-related GC plasticity underlies the LE of aversion learning. Here, we test the specific hypothesis that taste experience alters GC dynamics and plasticity in a way that increases the associability of a novel taste by measuring *in-vivo* taste responses pre-and post-CTA in rats exposed to either tastes (taste-experienced) or water alone (water-experienced).

Our results show that benign taste experience increases the reliability of GC responses towards novel sucrose pre-CTA; trial-to-trial variability in pre-CTA response dynamics, quantified in terms of Fano factor (Churchland et al., 2010b; Yoshida and Katz, 2011), was greatly reduced following taste experience. This reduction of response variability was also observed in terms of increased coherence of state-to-state ensemble transitions pre-CTA. We further revealed that benign taste experience enhances the impact of CTA learning on the GC taste response by increasing the number of responsive neurons as well as the distinctness between pre- and post-CTA sucrose responses. These findings support the hypothesis that

reliable taste responses lead to the enhancement of aversion learning by increasing the associability of a novel stimulus (see Discussion).

Methods

Subjects

Adult female Long Evans rats (N = 8, 250-315g at time of surgery) from Charles River Laboratories served as subjects for these experiments. The same subjects were involved in a previous study on benign taste experience (Flores et al., 2022). Here we focus on learning that occurred after benign taste experience with salty and sour tastes compared to animals who experienced water alone. These rats had surgical implantations of both intra-oral cannula (for taste/water delivery) and multi-channel electrode (for recordings of GC spiking activity) before entering the current study as described in Flores et al. (2022). Animals had *ad libitum* access to food and water prior to all experiments. All procedures were conducted in accordance with the guidelines established by the Brandeis University Institutional Animal Care and Use Committee**.**

Taste Stimuli

Taste solutions consisted of 0.1 M sodium chloride (NaCl; salty taste), 0.2 M citric acid (sour taste), 0.2 M sucrose (sweet taste used for CTA learning/testing), and distilled water (water). We chose these concentrations to remain consistent with our previous investigations of LE (Flores et al., 2016; Flores et al., 2018; Flores et al., 2022).

Acquisition of In-Vivo Electrophysiological Data

Electrophysiology recordings were recorded from each animal (see details below) within a customized Faraday cage (8.5 x 9.5 x 11.5 in), located in an isolated room adjacent to the

animal holding space. The Faraday cage was equipped with one desktop computer (PC) and one Raspberry Pi computer (Model 3B). The Pi computer controlled the taste stimulus presentation by manipulating the opening time and duration of solenoid valves for each taste delivery. The PC collected all electrophysiological recordings from the headstages attached to implanted electrodes (Intan Technologies – RHD2132, see implantation details below). These headstages were capable of sampling every electrode at 30 kHz and digitizing the signal before saving it to the PC. Subsequently, the collected recordings were sorted and analyzed offline using a set of Python analysis codes developed according to the current experimental design (for more details, refer to Mukherjee, 2017).

Only neurons meeting certain criteria were saved in hierarchical data format (HDF) for subsequent data analysis to ensure that only stable and isolated neurons were used. The criteria set the signal-to-noise ratio to higher than 3:1 and required spiking activity to exhibit a clear refractory period of 1 millisecond. We then compared spike times and removed neurons that exhibited significant overlap (at least 50% overlap). This cautious approach minimized the possibility of including the same neurons detected by more than one electrode channel.

Evaluating single-neuron stability across days. To determine if waveforms recorded from a single electrode represented the same neuron across 24 hours (from pre- to post-CTA sessions), we performed a previously described waveform-based held-unit analysis (of conditioned taste aversion; Grossman et al., 2008; Moran and Katz, 2014). A principal component analysis was used to map the shape of each waveform in 2D such that each waveform formed a “cluster”. Clusters that overlapped over the two sessions indicate a high potential of similarity (suggesting that they are the same neuron). Separation of each cluster was calculated using a non-parametric clustering statistic (J_3) for the first and last third time periods of activity for each waveform during each of the sessions (2) being compared.

Waveforms were considered "held" across sessions if the J_3 value between sessions was less than 90% of the entire J_3 distribution.).

Experimental Design and Statistical Analysis

The experimental protocol is summarized in **Figure 1**. On each day, experiments were conducted between 12pm and 2pm in a Plexiglass experimental chamber separate from the rats' home cages. During the experimentation, rats were water deprived for 19 hours/day to ensure task engagement.

Figure 1. Experience sessions and electrophysiology paradigm. A. Representation of Long Evans rodent with intra oral cannula and electrode implantation. **B.** Schematic of the experimental timeline: implantation surgery, 7 days of recovery, 1 day of acclimation (Acc), 3 days of experience with water (W) or tastes (T: water, sodium chloride and citric acid), one day of sucrose consumption (pre-CTA) followed by an injection of lithium chloride (LiCl), and one day of sucrose consumption following CTA learning (post-CTA).

Taste Experience Session. Extensive details regarding taste experience sessions can be found in Flores et al. (2022). In brief, after acclimation to IOC delivery via water presentation (Acc in **Figure 1B**), rats were given trials of either water (control group herein referred to as the water-experienced group) or a taste battery (water, sodium chloride, and citric acid: herein referred to as the taste-experienced group) for three consecutive days (Days 1 – 3, **Figure 1B**). Each session included 120 deliveries of water or the taste battery (40 deliveries of each water, sodium chloride, and citric acid); each delivery involved 40ul bolus of fluid, separated by 15-s inter-trial intervals (Flores et al., 2022).

CTA Conditioning Session. Twenty-four hours after the 3rd taste experience session, rats were given novel sucrose (pre-CTA, **Figure 1B**). Rats were first presented with 5-min access to

a bottle of sucrose in the home cage (maximum consumption was 15ml), after which they were moved to the experimental chamber to receive 120 trials of sucrose via IOC (delivery schedule identical to experience sessions); the bottle consumption ensured the development of CTA, while the IOC deliveries were best for simultaneous *in-vivo* recording of GC activity. Thereafter, each rat received a subcutaneous injection of lithium chloride (LiCl, 0.3 M, 0.5% of bodyweight) to induce CTA learning before being returned to their home cages.

CTA Testing Session. On the fifth day (post-CTA, **Figure 1B**), the strength of CTA was bottle tested: rats were again given access to 15 ml of sucrose for 5 minutes in the home cage. Immediately following behavioral testing animals were placed in the experimental chamber to receive 120 trials of sucrose via IOC while again recording GC taste responses. SPSS was used to perform independent sample *t*-tests comparing sucrose consumption pre- and post-CTA.

Analysis of Electrophysiology Data

Differential recordings of GC spiking activity were acquired during IOC deliveries of sucrose pre- and post-CTA. Single neuron data was subjected to 3 steps of previously described analyses (pre-processing, processing and post-processing) designed to characterize neural responses to stimuli (see Mukherjee, 2017 for in-depth details).

Taste responsivity. A neuron was deemed taste-responsive if its baseline and stimulus firing rates differed significantly. Specifically, paired *t*-tests were conducted to determine whether the evoked firing rate (2 seconds post-delivery) significantly differs from its baseline firing rate (2 seconds prior taste delivery). Alpha level was set at $p < 0.05$ unless specified otherwise (to be conservative when Type I error probability is high).

The impact of CTA on GC activity varies across the first 1.5 sec of a taste response (Grossman et al., 2008; Moran and Katz, 2014). Thus, when evaluating the impact of CTA on GC taste responsivity, we divided taste response into time bins, and compared each bin to baseline activity separately. For one such analysis, the response within 200-700ms (early) post stimulus delivery was isolated to represent the "identity epoch," and the 700-1200ms (late) period was isolated to represent the "palatability epoch" (Katz et al., 2001; Grossman et al., 2008). Data from the first 200ms of the taste response (also called detection epoch) were not used for this analysis because the activity during this particular time bin is known to reflect only somatosensory stimulation (i.e., fluid hitting the tongue) and hardly influenced by CTA learning (Katz et al., 2001; Grossman et al., 2008; Moran and Katz, 2014).

Single neuron trial-to-trial response reliability during CTA conditioning. For single-neuron variability, we used Fano factor (FF) to quantify the variance in taste responses across sucrose trials. As depicted in the following equation, FF is the ratio of the variance and the mean of the firing rates for 120 sucrose trials during CTA conditioning.

$$Fano\ Factor = \frac{Variance_T}{Mean_T}$$

Where *Variance* and *Mean* represent the variance and the mean of firing rates across 120 sucrose trials (*T*) during the CTA conditioning session.

Ensemble (neuron-to-neuron) response reliability during CTA conditioning. While many statistical algorithms have been applied to detect state transitions in neural spiking data (e.g., hidden Markov Modeling, Jones et al., 2007), here we used Bayesian Changepoint Analysis (BCA) to determine the presence of sudden, coherent firing-rate transitions in ensemble taste

responses, which have been shown to exist in taste responses (Katz et al., 2001; Jones et al., 2007; Sadacca et al., 2012) and to be relevant to both naïve behavior (Li et al., 2016; Sadacca et al., 2016) and CTA learning (Grossman et al., 2008; Moran and Katz, 2014; Arieli et al., 2022). Unlike many other methods that provide a single point estimate through maximum likelihood estimation, we chose BCA because, as demonstrated below, it offers a distribution of parameter estimates. This distribution effectively captures how certain the model is about the parameter estimates (in our case, transition times) – the wider the distribution, the more uncertain the model is about the estimates which is crucial for accurately characterizing the quality of state transitions.

To implement BCA, taste-evoked spike trains were collapsed into consecutive 50ms bins, and a Poisson likelihood estimate was used to model the binned spike counts. These models consisted of two sets of latent variables: (1) the emission variables (i.e., firing rates) and (2) the changepoint position variables (i.e., in which time bin changepoints occur); equations and the dependencies between these variables are detailed in **Figure 2**. A modularized pipeline was used to fit and analyze the models across datasets (https://github.com/abuzarmahmood/pytau/tree/development/pytau), enabling efficient comparisons between rats with and without prior taste experience.

Held-single-neuron sucrose responses pre- and post-CTA. To test whether experience impacts the way that single unit GC taste-responses change pre- and post-CTA learning, we measured the Euclidean distance (ED) between each single neuron's taste response pre-CTA and the average firing rate response (first 2 seconds of the taste response) across all neurons (templates) for each taste delivery trial of sucrose post-CTA. Measuring Euclidean Distance helps determine the similarity between two same-length arrays (herein referred to as [post-CTA array] and [pre-CTA array]); for our purposes this measurement helps us determine how CTA

learning impacts responses towards sucrose in single neurons. The larger the distance, the greater the difference between neural responses towards sucrose pre- and post-aversion learning. Here, the array lengths are the number of single neurons held across the 24-hour period (taste experience: N =11 and water experience: N=10. SPSS was used to perform one-way ANOVAs comparing Euclidean distances between sucrose responses pre- and post-CTA.

Results

We have previously shown that benign taste experiences enhance the discriminability of GC responses to both familiar and novel tastes (Flores et al., 2022). While this earlier work focused on the processing of taste stimuli prior to any attempt at associative learning, here we perform a set of investigations into how benign taste experience impacts GC taste responses pre- and post-CTA; CTA being a robust and reliable form of associative learning (**Figure 1**). Our results suggest that response discriminability is associated with an increase in pre-CTA trial-to-trial response consistency. We link this stability to an increase in neuron-to-neuron coherence as ensembles transition from one state to the next. In addition, we also found that CTA reliably impacts sucrose responses in GC (via comparison between pre- and post-CTA responses) but only in the taste-experienced rats. Together our results suggest that benign taste experience alters cortical plasticity in a way that supports the manifestation of the LE phenomenon.

Latent Enhancement of CTA Learning Following Benign Taste Experience

Rats underwent a taste or water experience paradigm identical to previous work illustrating the LE phenomenon (Figure 1, Flores et al., 2016; Flores et al., 2018; Flores et al., 2022) —three days of experience with either water, sodium chloride and citric acid, or water alone. Following the last of these 3 days, all rats underwent aversion training, in which novel sucrose (pre-CTA, **Figure 1**) was paired with the emetic LiCl. We have previously replicated the

original LE phenomenon in multiple experiments (Flores et al., 2016; Flores et al., 2018) using sample sizes of N = 15-20 rats each time showing that benign taste experience enhances learning towards a novel taste when compared to taste-naïve rats. Here, we collected behavior from a smaller subset of rats (N = 7) because our primary research questions rely not on behavior, but on having enough neurons (here, N = 261) for which only a small subset of animals is sufficient.

Figure 3 shows the sucrose consumption during pre- and post-CTA sessions (24 hours apart). Confirming that both groups consumed similar amount of sucrose CS during conditioning, an Independent Samples *t*-test with a 95% confidence interval for the mean difference showed that there was no significant difference in pre-CTA sucrose consumption between water and taste-experienced rats ($p = 0.538$, **Figure 3A**; because Levene's Test revealed a failure of homogeneity of variances, the Welch-Satterthwaite method was used to adjust the degrees of freedom).

While both groups drank similar quantities of sucrose pre-CTA, taste-experienced animals consumed only 14% (0.18 $\pm$ 0.02 mL) of what water-experienced consumed on CTA testing day (1.24g $\pm$ 0.53 mL). An Independent Samples *t*-test showed this difference to be marginally significant (t (4) = -2.676, $p = 0.055$, **Figure 3B**), but given the small sample size, this difference is a testament to the strength of the LE of CTA following benign taste experience.

Taste Experience Improves the Reliability of GC's response to the Conditioned Stimulus.

While our previous results (Flores et al., 2022) demonstrated that benign taste experiences increase the discriminability/salience of ensemble and single-unit Gustatory Cortical (GC) responses to both familiar and new tastes, they fell short of explaining *how* taste experience can enhance learning toward a novel taste. We hypothesized that the increase in discriminability may be tied to an increased reliability of pre-CTA sucrose responses (evident

through higher signal-to-noise ratio (i.e., reduction of noise)). This hypothesis, if it is true, would (not incidentally) also explain how taste experience leads to LE of CTA learning because the reliability of the CS can critically impact the quality of associative learning (Jennings and Bonardi, 2017).

Given this relationship, we hypothesize that, following taste experience, GC's coding of sucrose becomes more reliable and thus more distinct and associable (to the lithium chloride unconditioned stimulus). To test this hypothesis, we specifically examined two distinct ways in which experience might enhance taste response reliability: 1) we asked whether the notorious trial-to-trial variability that is well known to characterize responses to identical stimuli (Shadlen and Newsome, 1994, 1995, 1998) might be reduced by benign taste experience; and 2) taking our cue from more recent work regarding the behavioral relevance of coherent ensemble activity (Moran and Katz, 2014; Mukherjee et al., 2019),we asked whether neuron-to-neuron variability in response dynamics might also be reduced by benign taste experience.

To address the former question, we used Fano factor (FF) to calculate the variability of GC responses across 120 sucrose trials pre-CTA (see methods). The higher the Fano factor, the higher the variability in the response; higher variability should translate into less distinctiveness and reduced associability. Because GC taste response is dynamic (Katz et al., 2001; Jones et al., 2007; Sadacca et al., 2016), and only the palatability epoch has been causally linked to CTA learning (Grossman et al., 2008; Moran and Katz, 2014), we predicted a higher FF in the water-experienced group than in the taste-experienced group when the response transits into the palatability epoch. To test this prediction, the between-group comparison on trial-by-trial variability was analyzed across different time bins of the taste response to capture specific changes by epoch. **Figure 4A** shows that the data are in accord with our prediction. A mixed 2-way ANOVA revealed a significant main effect of experience (*F*

(1, 129) = 6.43, p = 0.012) and Time bin (F (5, 645) = 1.96, p = 0.082), as well as a significant interaction of Group and Time (F (5, 645) = 3.35, p = 0.005). Post-hoc comparisons further revealed that the water-experienced group has significantly larger FFs than the taste-experienced group at baseline (-400-0 ms; $p < 0.05$); this difference then collapses at the beginning of the responses (a result consistent with prior meta-analysis of a range of preparations; Churchland et al., 2010a), only to return during the transition to the late palatability epoch (800 – 2000 ms; $p < 0.05$). The higher variability in the water-experienced group cannot be attributed to lower firing rates, since subsequent analysis on average firing rates failed to identify statistically significant effects of Experience ($F < 1$) or Time by Experience interaction (F (5, 645) = 1.15, p = 0.33; **Figure 4B**).

These results indicate that taste experience specifically reduces the response variability during the palatability epoch—the epoch known to be critically impacted during CTA learning (Grossman et al., 2008; Moran and Katz, 2014; Arieli et al., 2022). Consistent with behavioral work showing enhanced learning with low conditioned stimulus variation (e.g., Jennings and Bonardi, 2017), the increase in across-trial reliability we see here may well make it easier for the rats to learn CTA. Another implication of this result is that, if each neuron in the ensemble is coding more reliably, the within-response transitions of ensemble code itself might be more coherent (see Jones et al., 2007; Sadacca et al., 2016). In the case of GC taste response dynamics (involving state transitions between detection, identity, and palatability epochs), the reliable ensemble coding ensures coherent transitions between epochs.

To test this prediction, we examined whether taste experience impacts GC epoch dynamics, which in turn would alter the coding of a novel CS taste prior to learning. In this ensemble analysis, we target the transitions between identity and palatability (due to this transition's known influence on behavior) to investigate the impact of taste experience on pre-

CTA response dynamics that can benefit later learning (Grossman et al., 2008; Moran and Katz, 2014; Arieli et al., 2022). A state transition has been shown to reflect coherent ensembles of neurons changing their firing rates nearly simultaneously: the more reliable the activity of the neurons in the ensemble, the more coherent the transition. If taste experience makes ensemble response more reliable, we expect enhanced coherence during transitions; an impact that would result in better taste coding setting the foundation for enhanced learning (see Discussion).

To determine whether taste experience impacts transition coherence and ensemble reliability, we ran a Bayesian changepoint analysis (BCA) with a 2-state model on the response period between 200ms to 1500ms; a period with highest likelihood of GC activity transitioning from the identity epoch into the palatability epoch (**Figure 5A**; also see Jones et al., 2007; Sadacca et al., 2016). Rather than a highest likelihood estimate, the BCA model generates a distribution of parameter estimates (of transition time in this case). The variance of these distributions (highest density interval, HDI) reflects how certain the model is about the estimates, allowing us to use HDI as the proxy for the coherence of state transitions. That is, a more reliable coherent transition is expected to result in a narrower distribution (smaller HDI) because the model is more certain about the estimation.

Figure 5B shows the results of this analysis, in which the mean HDIs were plotted for both water-experienced and taste-experienced groups: the rats with taste experience have significantly smaller HDIs than those with only water experience (t (718) = 6.55, p =0.000). We therefore moved on to investigating the two (non-mutually exclusive) mechanisms by which taste experience might improve state transitions (Lin et al., 2021): 1) following benign taste experience, the neurons in GC fire in a more synchronized pattern in response to taste stimuli so that each neuron changes its firing rate closer to the time of the state transitions; or 2)

instead of impacting when neurons respond, taste experience may impact how neurons respond across transitions—the magnitude of the firing rate changes.

To determine whether taste experience improves synchronization of firing across ensemble neurons (i.e., possible mechanism #1), we ran separate changepoint models on each neuron's spiking activity to detect the time of each state transitions per neuron. We then calculated the difference between each neuron's actual transition time and the estimated time of ensemble transition. As shown in **Figure 5C**, we found significantly smaller latencies between estimates from single neurons and ensembles for taste-experienced rats than water-experienced rats (t (67) = 2.41, p = 0.019). This indicates that taste experience causes GC neurons to progress through taste response dynamics in a more synchronized manner. When we compared the firing rates in the 200ms before and after the transitions (i.e., possible mechanism #2), we observed no significant differences between rats with and without taste experience (**Figure 5D**; t (67) = -1.32, p =0.19). Therefore, we conclude that taste experience enhances synchronization of ensemble firing rate changes without changing the magnitude of those changes, which in turn sharpens transitions from one state to the next. These clean transitions may underlie the overall LE of CTA learning in taste-experienced rats.

Taste Experience Enhances Cortical Plasticity during CTA Learning

The results above suggest that benign taste experience decreases the variability of taste responses and state transitions for novel sucrose pre-CTA both between trials and between neurons, which we hypothesize, leads to enhanced associability of that taste during future CTA conditioning (see Discussion). To directly test this prediction, we compared pre-CTA sucrose responsivity to post-CTA responsivity in GC, beginning with the percentage of taste responsive neurons responsive to the sucrose conditioned stimulus.

Previous work investigating the impact of CTA learning on taste processing in the absence of taste experience has reported a significant drop in the percentage of GC neurons responsive to sucrose pre-CTA compared to post-CTA within the late, palatability-coding epoch (Grossman et al., 2008). We replicated this result in our water-experienced rats (**Figure 6**, left panel), although the effect failed to reach significance, likely due to the weak CTA training protocol used here (we purposefully use a mild CTA paradigm to limit floor effects in CTA testing consumption which can make it impossible to detect an LE effect). Conversely, in the taste-experienced group, we found that the percentage of GC neurons responding to sucrose increased following aversion learning: chi-squared analysis revealed that CTA significantly increased the number of taste responsive neurons in the early ($X^2 = 3.93$, $p < .047$) but not in the late epoch ($X^2 < 1$); this impact was not apparent for the water-experienced group ($X^2 < 1$). These results demonstrate that taste experience can modulate the impact of CTA on GC taste processing by increasing GC responsivity during the early epoch of the taste response. Inspired by the reliability analysis (Figures 4 and 5), this increase in responsive neurons, in fact, may result from the fact that GC neurons become more plastic and associable following taste experience which enhances the reliability of CS coding.

The increase in overall responsivity is consistent with the hypothesis that that following benign taste experience, CTA-related plasticity is enhanced in GC neurons due to the reduced variability in taste coding. To directly confirm this hypothesis, we examined the firing rate of single GC neurons held across the 24 hours between pre- and post-CTA sessions. Below, we report findings culled from the modest set of records that exceeded our stringent criteria for 24-hour isolation of single action potentials (water experience: N=10 and taste experience group: N =11. **Figure 7** shows examples of 2 such neurons. Note that CTA induced changes in both responses (the differences between the teal and magenta PSTHs), but the changes were larger in magnitude and longer lasting in GC responses following taste experience.

To test the general accuracy of this observation, we tracked response changes across learning in the entire sample of held neurons, comparing the Euclidean distances (ED) between single-trial post-CTA sucrose responses and the average pre-CTA sucrose responses (the "template") within both taste- and water-experienced groups (see Methods). An independent samples *t*-test performed on these data reveals that post-CTA responses are more distinct (greater ED) from pre-CTA responses for neurons from rats with taste experience than for neurons from water-experienced rats (t (238) = 7.25, $p < 0.001$; **Figure 8A**)—that is, learning changed responses more in LE rats than in control rats.

When more closely evaluating the impact of CTA on GC sucrose responses across individual trials, we found that water-experienced rats showed an unstable impact of CTA learning during post-CTA response. **Figure 8B** shows the PSTHs of two representative neurons (one from each group) from the first 40 (top panels) and last 40 trials (bottom panels) of post-CTA sucrose delivery plotted against all 120 trials pre-CTA for that same neuron. In the water-experienced neuron, the changes in responses toward pre- and post-CTA sucrose were less distinct across the first 40 trials but more distinct during the last 40 trials (**Figure 8B**, left panels). Such within-session differences were not obvious for the neuron from the rat with taste experience (**Figure 8B**, right panels), in which the change in firing rate towards pre- and post-CTA sucrose is consistently distinct across trial blocks.

This between-group difference is quantified for the entire sample of held neurons across all trials in **Figure 8C**, which shows EDs for every trial; the distance from post-CTA and pre-CTA responses can be observed to gradually increase across trials for rats in the water experience group; the correlation between trial number and ED is significantly non-zero ($r = 0.255$, $p = 0.005$). This across-trial difference, however, was not observed in the taste experience group (r

(120) = -0.067, p = 0.469); note, in fact, that EDs for the water-experienced rats only approach the stable EDs for LE rats by the end of the session. In other words, benign taste experience stabilizes future post-CTA responses such that pre-post differences are stably high across the whole post-CTA recording session following taste experience (**Figure 8C**).

Taken together, this pattern of results indicates that following taste experience, CTA learning produces a more stable and larger impact on GC taste response than those in the taste naïve condition. Since the reliability of a conditioned stimulus (like sucrose) can critically influence associative learning (like CTA), we hypothesized that the LE of CTA learning may result from more reliable coding of sucrose. As demonstrated across our results, taste coding is indeed more stable following taste experience within both single-neuron and ensemble activity in GC.

Discussion

This research reveals potential underlying mechanisms for the latent enhancement (LE) of aversion learning that follows benign experience with tastes other than the (novel) conditioned stimulus (Flores et al., 2016; Flores et al., 2018). Inspired by our earlier finding that benign taste experience increases the discriminability of gustatory cortical (GC) ensemble and single-unit responses (Flores et al., 2022), we hypothesized that taste experience could reshape the way that GC processes a taste stimulus by decreasing response variability—an impact that would enhance the associability of a stimulus during aversion learning (and therefore, explain LE). To test this hypothesis, we investigated how taste experience alters the GC taste response to a novel taste (i.e., sucrose) both before (pre) and after (post) CTA learning.

The previous report demonstrated that benign experience impacts the processing of both familiar and novel tastes (Flores et al., 2022); novel sucrose responses became more discriminable and distinct from the response towards water. The current Fano Factor analysis reveals that this enhancement in discriminability is associated with (and likely driven by) an increase in the reliability of taste coding. Specifically, trial-to-trial sucrose responses in GC pre-CTA are less variable in the taste-experienced group than in the taste-naïve group. These results imply that taste experience enhances the reliability of the GC response, increasing signal to noise ratios and in turn laying a foundation for stronger future learning.

It has been well established that reduced variability (both in terms of stimuli and neural responses) and learning go hand-in-hand (see Raviv et al., 2022 for a review). For instance, exposure to multiple tastes together at the time of aversion conditioning, an overshadowing procedure that structurally enhances variability of neural activity (by driving multiple distinct taste responses), leads to reduced aversion learning towards any of those tastes (Pavlov, 1927;

McLaren and Mackintosh, 2002; Flores et al., 2016). When considering the natural response variability towards multiple trials of the same stimulus, it's important to consider the possibility that even this variation might make learning sub-optimal, as the system tries to decide precisely what response pattern should be associated with illness (Shadlen and Newsome, 1994). Thus, the fact that water-experienced rats showed much higher variability within CS-evoked firing rates across trials than those that had taste experience before conditioning can explain LE, in that when the coding of the taste conditioned stimulus is "less stable," the acquisition of learning is slowed and/or weakened.

The increase in response reliability across trials may not be the only mechanism underlying LE of CTA. As noted previously, GC taste dynamics progress through distinct epochs, encompassing detection, identity, and palatability (Katz et al., 2001; Jones et al., 2007; Sadacca et al., 2016). The current work demonstrates that taste experience also impacts the across-neuron coherence of single-trial transitions into the epoch previously shown to be critical for CTA learning (Moran and Katz, 2014), increasing the across-ensemble coherence of this firing-rate change. Given that the cohesion of an ensemble's activity can be linked to an enhanced probability of plasticity (Li, 2018), it is reasonable to suggest that taste experience boosts learning towards the conditioned stimulus (sucrose) by improving the quality (i.e., coherence) of epoch transitions in GC.

Of course, the fact that LE changes learning implies that the impact of taste experience should also be evident when looking at pre-post changes in GC responses. Most of our results in this regard are consistent with those reported in earlier studies (Grossman et al., 2008; Moran and Katz, 2014; Arieli et al., 2022), but we do report two inconsistencies. Most notably, in contrast to Grossman et al. (2008), who described a learning-related reduction in the percentage of taste-responsive neurons, our taste-experience group exhibited increases in the

numbers of GC neurons responding to sucrose post-CTA. The exact cause of this shift in the effect of CTA is not immediately clear, but the difference must be attributable to the influence of taste experience on how GC neurons react to and process novel taste stimuli during conditioning.

In addition to increasing the number of sucrose-responsive neurons, taste experience also enhances the magnitude of post-CTA sucrose responses (compared to pre-CTA responses). These changes align with previously reported CTA-induced GC response changes in taste-naïve rats (Arieli et al., 2022). We would expect that water-experienced animals would show a similar, albeit less pronounced, change in sucrose GC responses after CTA learning, but in fact our analysis revealed only a minimal impact. One potential explanation for this discrepancy, besides the use of different sweet tastes, is the strength of aversion learning and the timing of our *in-vivo* recordings: in order to mitigate potential floor effects on test-day consumption that might obscure the LE phenomenon, we used a LiCl concentration that was half the strength of what is typically used in most CTA studies; this weaker aversion, coupled with the fact that rats were exposed to sucrose before *in-vivo* recording on test day, may have influenced the GC taste-evoked responses captured during our recordings, limiting the observable learning-related changes in GC responses.

The finding that benign taste experience can influence how the brain processes a novel taste stimulus implies that such experience is not actually inconsequential. The impact of benign experience on sensory processing has also been reported in olfaction (e.g., Mandairon et al., 2006) and sensory development (Schiff et al., 2023), but remains understudied despite its omnipresence. Our current *in-vivo* electrophysiological study sheds first light on some potential neural mechanisms that might underlie these experiential and neural dynamics. Future research involving neuronal interventions (optogenetic or chemogenetic inhibition) will be required to

further our understanding of the intricate neural circuitry and mechanisms responsible for the impact of “benign” experience on learning. This, in turn, may offer insights into how everyday experiences impact the neural dynamics of future sensory processing, consumption disorders, and learning and memory formation.

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Figures

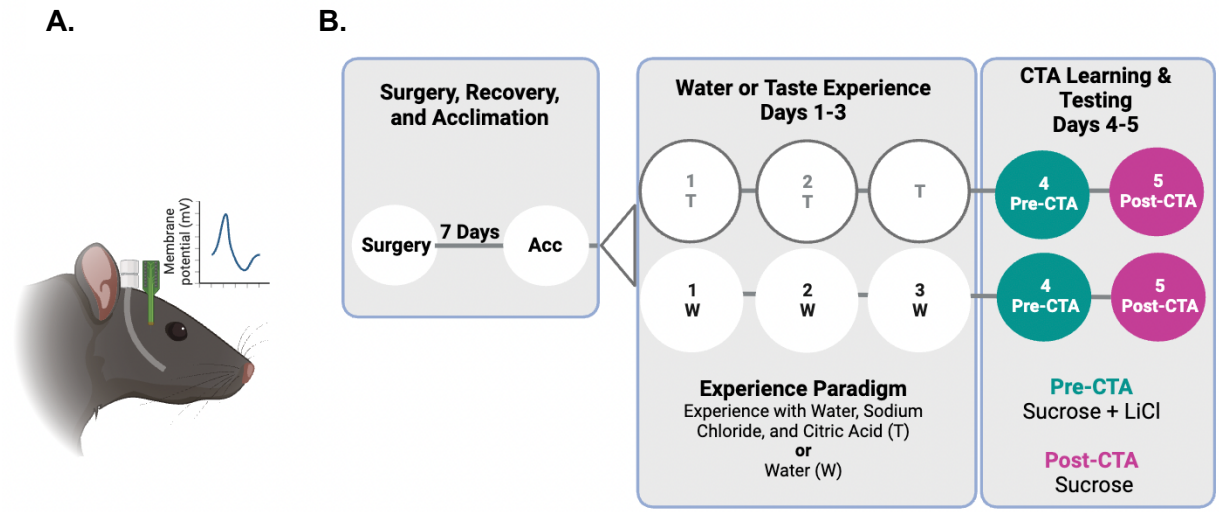

Figure 1. Experience sessions and electrophysiology paradigm. A. Representation of Long Evans rodent with intra oral cannula and electrode implantation. **B.** Schematic of the experimental timeline: implantation surgery, 7 days of recovery, 1 day of acclimation (Acc), 3 days of experience with water (W) or tastes (T: water, sodium chloride and citric acid), one day of sucrose consumption (pre-CTA) followed by an injection of lithium chloride (LiCl), and one day of sucrose consumption following CTA learning (post-CTA). Figure Created with BioRender.com.

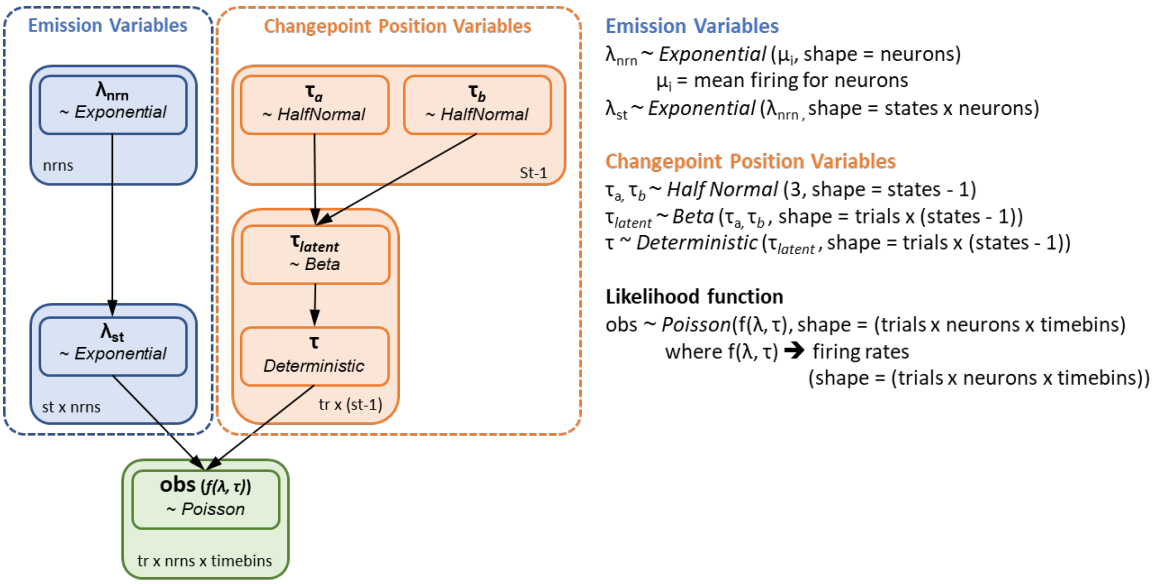

Figure 2. Bayesian changepoint model. Dependencies between the random variables for constructing the changepoint model are shown within a schematic: (number of neurons – *nrns*), (number of states – *st)* and (number of trials – *tr)*.

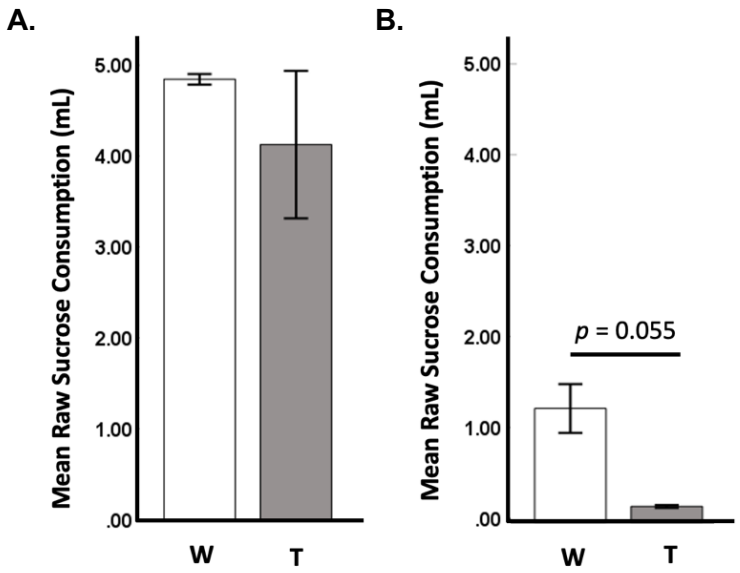

Figure 3. Taste Experience Induces a Latent Enhancement of Aversion Learning Towards a Novel Taste. A. Bar graph demonstrating raw sucrose bottle consumption pre-CTA between water-experienced (W) and taste-experienced groups (T). Sucrose consumption between groups did not significantly differ. **B.** Bar graph demonstrating raw sucrose bottle consumption post-CTA which marginally differed between water-experienced (W) and taste-experienced groups (T) replicating the LE phenomenon.

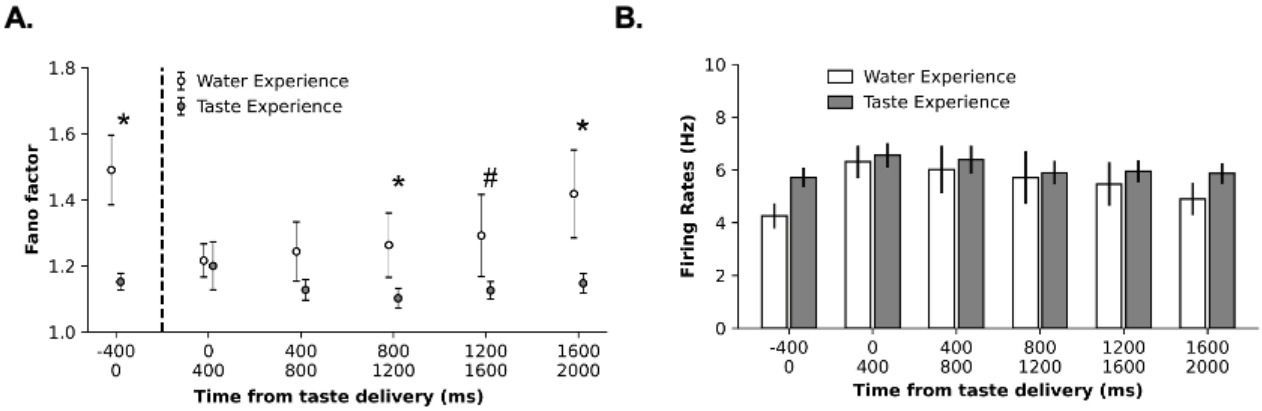

Figure 4. Taste experience improves the reliability of sucrose responses before learning. **A.** Mean Fano factors across each 400ms time bins between groups with either water experience or taste experience. **B.** Mean firing rates across each 400ms time bins between groups with either water experience or taste experience. * $p < 0.05$; # $p = 0.06$. Error bars represent standard error of the mean.

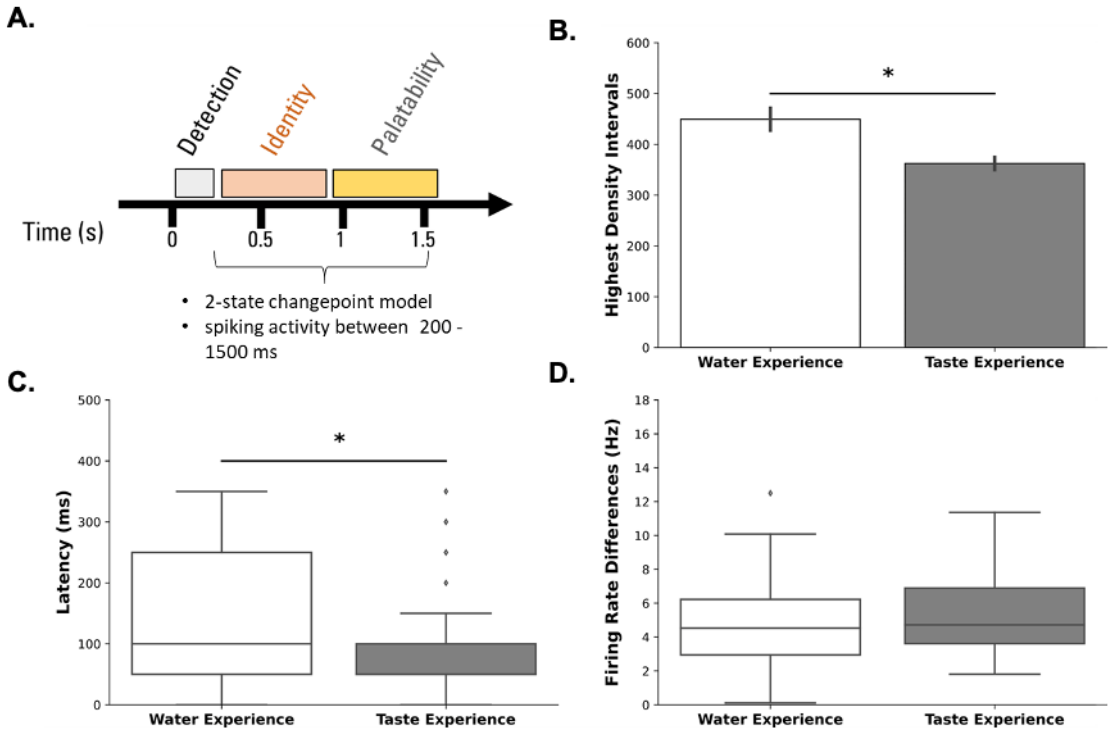

Figure 5. Taste experience sharpens GC state transitions into the palatability state. A. Schematic of GC response dynamic depicting that a 2-state changepoint model was used to fit spiking activity between the identity and palatability states. **B.** Mean highest density intervals (i.e., the uncertainty of the model estimates of the transition time) across Water and Taste Experience Groups. **C.** Mean latency between single neuron transition time and ensemble transition time. **D.** Mean firing-rate changes across transition times across groups. * $p < 0.05$ indicating the significant between-group differences. Error bars represent standard error of the mean.

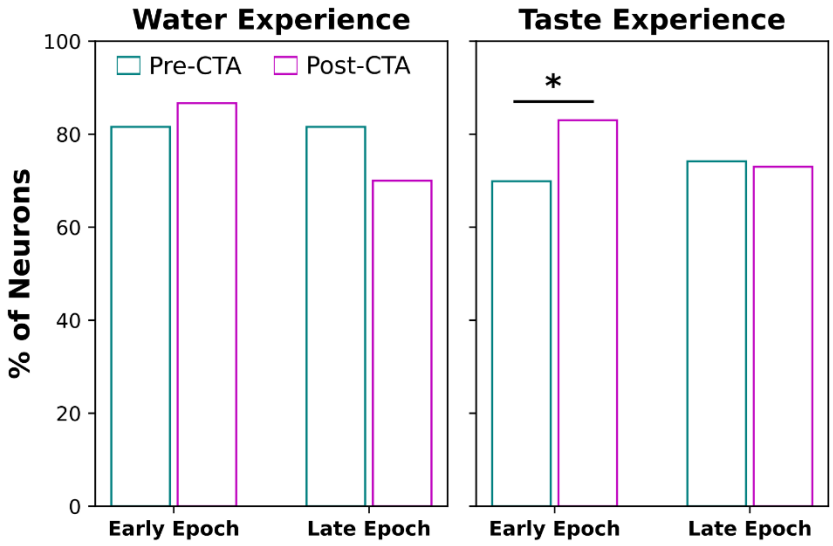

Figure 6. Taste experience increases the cortical responsivity towards sucrose post aversion learning. The bars represent the percentage of GC neurons showing significant taste responses toward sucrose during early (200-700ms) and late (700-1200ms) epochs. The bar colors indicate whether the neurons were recorded from pre-CTA (teal) or post-CTA (magenta) sessions. Our analysis reveals that CTA acquisition increased the percentage of neurons responding to sucrose in the taste-experienced group but not in the water-experienced group. * $p < .05$

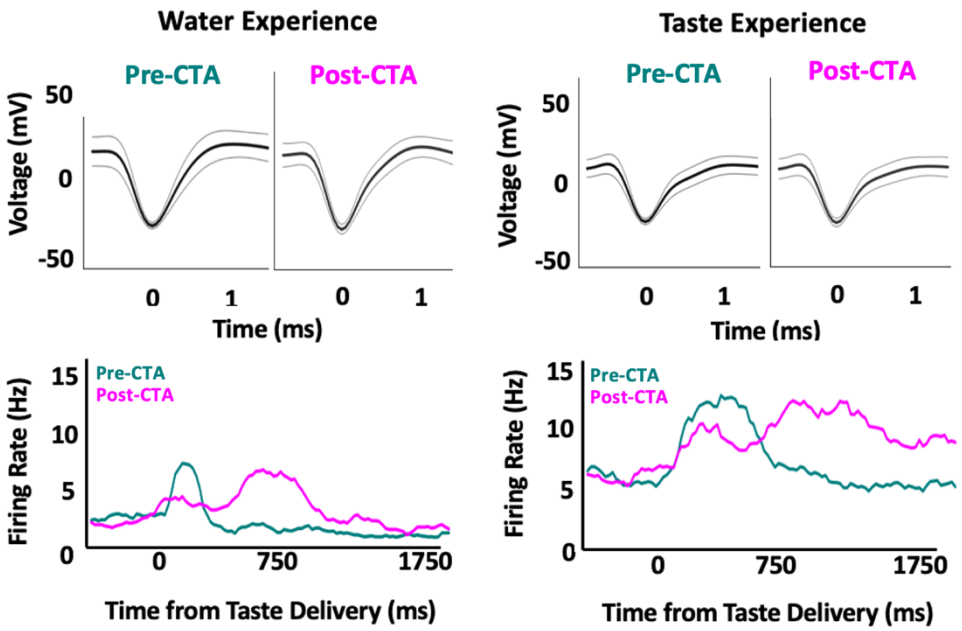

Figure 7. Representative taste-responsive single neurons held across pre- and post-CTA. Waveforms (top panels) and peristimulus histograms (PSTHs; bottom panels) of a single neuron from a water-experienced rat (left) and taste-experienced rat (right) across pre-CTA (teal) and post-CTA (magenta).

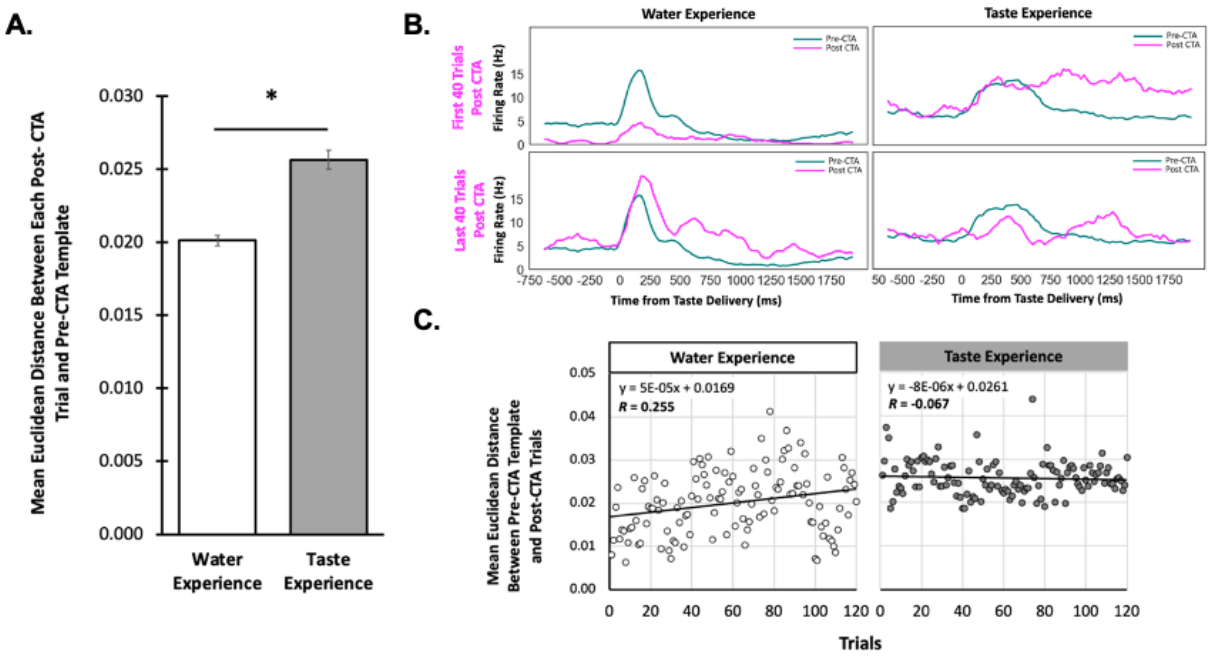

Figure 8. Taste experience stabilizes single-neuron responses. A. Bar Graph demonstrating changes in single-neuron responses to sucrose pre- and post-CTA in both water and taste experience groups. Bars signify mean Euclidean distance between post-CTA sucrose responses and a single pre-CTA template per group. Changes are between water and taste experiences. **B.** Peri-stimulus time histograms of 2 representative neurons (left: Water Experience; right: Taste Experience). Each panel compares the change in firing rate of all 120 pre-CTA trials (teal) to the first 40 (top row) and last 40 (bottom row) sucrose trials post-CTA (magenta). The change between pre- and post-CTA sucrose during the first 40 trials within the water experience group is minimal and increases in later trials. The change between pre- and post-CTA sucrose within the taste experience group is consistent across both early and late trials. **C**. Scatterplots with lines of best fit demonstrating the Euclidean distance between each post-CTA trial and the pre-CTA template across both experience groups. There was a statistically significant positive correlation between the distances and post CTA trials in the water experience group alone. Error bars represent standard error of the mean. * $p < .05$.