Physiology & Behavior 274 (2024) 114430

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Salivary proteins rescue within-session suppression and conditioned avoidance in response to an intragastric quinine infusion

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ARTICLE INFO

Keywords:
Saliva
Bitter
Post-oral

ABSTRACT

A subset of salivary proteins (SPs) upregulates in response to a quinine-containing diet. The presence of these SPs then results in decreased bitter taste responding and taste nerve signaling. Bitter taste receptors in the oral cavity are also found in the stomach and intestines and contribute to behaviors that are influenced by post-oral signaling. It has been previously demonstrated that after several pairings of post-orally infused bitter stimuli and a neutral flavor, animals learn to avoid the flavor that was paired with gastric bitter, this is referred to as conditioned avoidance. Furthermore, animals will decrease licking of a neutral solution within a test session, when licking is paired with an intragastric bitter infusion; this has been described as within-session suppression. We used these paradigms to test the role of SPs in behaviors influenced by post-oral signaling. In both paradigms, the animal is given a test solution directly into the stomach (with or without quinine, and with or without SPs), and the infusions are self-administered by licking to a neutral solution (Kool-Aid). Quinine successfully conditioned a flavor avoidance, but, in a separate trial, we were unable to detect conditioning in the presence of SPs from donor animals. Likewise, quinine was able to suppress licking within the conditioned suppression paradigm, but the effect of the bitter was blocked in the presence of saliva containing SPs. Together, these data suggest that behaviors driven by post-oral signaling can be altered by SPs.

1. Introduction

Taste information plays an important role in diet acceptance. Taste conveys nutritional and safety information about the foods that we consume. For example, sweet[1] taste is thought to signal highly caloric food and increases motivation to consume the foods. In contrast, bitter taste is often used as a warning system for toxins. Plants produce plant secondary compounds (PSCs) to protect themselves from herbivory. These compounds are often reported as bitter and bind to bitter T2R taste receptors. PSCs are often not dangerous to mammalian consumers and in fact, are found in healthy vegetables. The bitter taste of vegetables contributes to high variability in the acceptance of healthy foods across age. Broadly speaking, humans and animals tend to avoid bitter compounds and when consuming bitter foods will decrease food intake compared to more palatable foods. The decrease in food intake has been proposed to be due to both the hedonic evaluation of the food [5,8] and the activation of post-oral bitter receptors which can delay gastric emptying [9] and are related to the release of satiety hormones [4,10,11].

We have previously demonstrated that exposure to a bitter diet upregulates salivary protein (SP) expression and these proteins can, in turn, increase acceptance of the bitter stimulus [13–15,24]. After induction of these diet-related SPs, rats show changes in feeding behaviors associated with both orosensory (rate of feeding) and post-oral feedback (meal size). Furthermore, saliva collected from donor rats with upregulated SPs alters taste responses to bitter stimuli in naïve rats [13].

Briefly, the presence of SPs decreases negative/aversive oromotor responses (e.g., gapes) to quinine in a taste reactivity paradigm. These data suggest that SPs alter bitter acceptance by altering the sensitivity to the bitterness, or perceived concentration of quinine. Here, we plan to use our donor saliva technique to investigate the role of SPs on post-oral feedback.

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[1] Note that references to "bitter," "sweet" etc. are used to describe compounds that activate receptor populations associated with these taste qualities in humans. We can never be sure that the perceptual experience of a rodent is identical to the human experience described by these adjectives.

https://doi.org/10.1016/j.physbeh.2023.114430
Received 2 August 2023; Received in revised form 4 October 2023; Accepted 6 December 2023
Available online 7 December 2023
0031-9384/Published by Elsevier Inc.

Expression of bitter taste receptors has been previously identified in the gastrointestinal tract [2,25]. Stimulation of bitter T2R receptors in the gut through intragastric infusions can mediate behaviors such as conditioned taste/flavor aversions and within-session suppression [9]. Intragastric infusions of a bitter stimulus such as denatonium benzoate can decrease within-session licking to a salt solution [19] and a palatable maltodextrin + saccharin solution [9] within a trial. Rats can also condition a flavor avoidance in a 2-bottle preference test to a Kool-Aid flavor that was repeatedly paired with an intragastric infusion of 10 mM denatonium (but not 2.5 mM). To experimentally test whether SPs can alter post-oral feedback as they do in taste reactivity, we asked if we could block conditioned avoidance to a quinine-paired Kool-Aid flavor or within-session suppression driven by an intragastric infusion of quinine by delivering donor saliva in the infusate.

2. General methods

2.1. Subjects

Male Long Evans rats were between 400 and 550 g at study onset. The colony room was maintained at 20 ± 2 °C with a 12:12 h light/dark cycle. All training and testing were conducted during the lights-on phase. Rats were single-housed and given access to water and standard rodent chow ad lib (Envigo 2018) except when noted otherwise. Animals were cared for, and all testing was conducted, in compliance with the University at Buffalo Institutional Animal Care and Use Committee.

2.2. Surgeries

Using sterile technique, indwelling intragastric silastic catheters (0.76 mm I.D x 1.65 mm O.D.; Dow Inc.,) were surgically implanted under isoflurane anesthesia (2–3 %). Briefly, silastic catheters were fixed to the fundic region of the stomach with a purse-string suture. A piece of polypropylene mesh (Bard Devices Inc.) was slipped over the silastic tubing and pressed against the stomach to form an additional anchor. The catheter was routed through the intraperitoneal cavity and out a midline incision in the muscle wall, it then passed through the subcutaneous space to the shoulder blades. The tubing was then connected to a backport (Plastics One) that was secured by suture between the shoulder blades. Backports were sealed when not in use by commercially available dust caps. Rats were given two weeks post-op recovery before training and testing, and catheters were flushed daily with 2 mL of saline to maintain patency.

2.3. Donor saliva collections

Donor saliva was collected from rats that are separate from the experimental rats used in the trials. We use injections of isoproterenol to approximate the SP profile after bitter diet exposure. Isoproterenol increases the relative expression of a subset of SPs and the total concentration of SPs. The pattern of SP expression after the drug treatment overlaps with the pattern we see after quinine dietary exposure [12,13]. We have demonstrated that this pattern of expression, when diluted to naturally occurring concentrations, is capable of altering taste-driven behaviors including diet acceptance [16] and taste reactivity measurements [13]. This method of collecting donor saliva is used over taking donor saliva from diet induced animals because drug treatment results in much higher volumes (3mls vs 80ul) and higher protein concentrations (~10 mg/mL compared to ~3 mg/mL) than dietary treatment. After protein measurement to determine the concentration we dilute the donor saliva to a concentration that is biologically relevant with artificial saliva (0.015 M NaCl, 0.022 M KCl, 0.003 M $CaCl^2$, 0.0006 M $MgCl^2$) [17]. This further increases the volume we can deliver. High volumes are essential when running these types of infusion studies.

Donor saliva collection is described in detail in [13]. Briefly, rats were injected with pilocarpine (Sigma-Aldrich; 2 mg/mL) and isoproterenol (Sigma-Aldrich; 0.2 mg/mL) subcutaneously while under isoflurane anesthesia (2–3 %). Using a 1000 uL pipette, saliva was aspirated from around the teeth and under the tongue. SP concentration was measured using the Pierce Protein BCA assay (ThermoFisher Scientific). All donor saliva collected for these studies was combined into a single homogenous sample and was determined to be 12.2 mg/mL. Artificial saliva was used to dilute this concentration to 3 or 5 mg/mL as noted in the methods for each study. Once the saliva was diluted, we call this whole saliva.

2.4. Apparatus

Rats were trained and tested in an electronic esophagus which has been described in detail in [6]. Briefly, the electronic esophagus consists of a stainless-steel cage (24 × 29 × 33 cm) with a lickometer attached at the front of the cage and a swivel arm that holds infusion tubing. The infusion tubing was threaded into the cage through the top access slot and connected to the rat during testing. The infusion pumps are operated by the lickometer; when the rat licks from the sipper tube, the custom software (LIB1FT5 QuickBasic Infusion Program, as described in [6]) records the number of licks to the stimulus and delivers a 3-s infusion at a rate of 1.3 mL/min for every 20 licks to the stimulus. The number of licks at each min was recorded by the computer and saved for subsequent analysis.

2.5. Electronic esophagus training

After post-op recovery, all rats were trained in the electronic esophagus for at least 15 min a day across four days. On day one, rats were placed in the chamber for 15 min and given a single bottle of water to acclimate to the chamber itself. On day 2, rats were placed in the chamber, given a bottle of water and the backports were attached to the swivel to acclimate the animal to the tether, but no infusions were administered. On days three and four, rats were placed in the chamber, given a single water bottle, attached to the tether, and given an infusion of water when they licked the water bottle. Rats were required to lick at least 500 licks within that 15 min session each day to move forward in the training and were rerun ~30 mins after the last session if licks failed to reach 500. All rats were water deprived during training and testing in both experiments.

2.6. Conditioned suppression

Rats ($n = 8$) were used to test the role of SPs in modifying post-oral feedback during a conditioned avoidance paradigm. To replicate conditioned avoidance of a novel flavor with a gastric infusion of quinine (positive control), we first trained the animals to associate quinine and water intragastric infusions with distinct Kool-Aid flavors. After the electronic esophagus training period (described above), rats began six days of conditioning training. Rats were chronically water deprived (no supplemental water given) and given 15 min access to 0.05 % cherry or grape Kool-Aid mixed with 0.1 M NaCl. Rats were infused with 2 mM quinine hydrochloride dihydrate (Sigma-Aldrich) or water, and infusions were yoked to the animals licking as described above. Each gastric stimulus (quinine or water) was paired with the assigned Kool-Aid three times in an alternating pattern for a total of six conditioning sessions (e.g., water/cherry, quinine/grape, water/cherry, quinine/grape, etc.). All pairings and exposures were counterbalanced such that half of the animals received one Kool-Aid flavor (e.g., grape) with quinine and the other flavor (e.g., cherry) with water. The reverse was true for the other half of the rats. At the end of the conditioning, rats were given a "practice" 2-bottle preference test in the home cage and were presented with two novel Kool-Aid flavors (e.g., Sharkleberry Fin and Blue Raspberry Lemonade) with 0.1 M NaCl over the course of two days. This data was not analyzed; this was intended only to encourage the rats

to explore each bottle and learn the solutions are different. After the practice 2-bottle preference test, animals were then given the conditioned solutions for two days in 30-min 2-bottle preference tests in the home cage. Bottles were weighed before and after the test to measure total intake in grams. Bottle sides switched on day 2 to avoid a side preference. Food and enrichment were removed from the cage during this time and returned ~30 min after the end of the test.

To determine if SPs could alter the post-oral effects of quinine in the conditioned suppression test, we replicated the previous conditioning but presented rats with 0.05 % orange and lemon-lime Kool-Aid flavors and paired them with artificial saliva alone (no quinine) or whole saliva at 5 mg/mL with quinine (2 mM). We used artificial saliva alone in the control condition instead of water to control for the ionic composition of saliva. Again, conditioning lasted six days with daily 15-min sessions and all pairings were counterbalanced between rats. Rats were then given a practice 2-bottle preference test followed by the real 2-bottle preference test with the conditioned solutions as described above.

A strength of our design was that we were able to confirm the concentration of quinine was effective before using whole saliva in the infusions. This is important because donor saliva is difficult to collect. However, we were unable to account for the possibility of an order effect. To address this, we included an additional control condition. To control for the effects of time and experience in the paradigm, we ran the first conditioning paradigm again with the same Kool-Aid flavors to confirm that the animals were still able to demonstrate the association.

Total intake measures for the 2-bottle preference tests were compared by paired samples *t*-test for each conditioning session using Systat 13. Outlier tests were performed on the preference scores in all three conditioning trials using GraphPad Grubbs' Outlier calculator.

2.7. Within-session suppression

A new group of rats ($n = 7$) was cannulated for the within-session suppression paradigm. Training for rats ($n = 7$) in the within-session paradigm was identical to that of the conditioned suppression paradigm. Kool-Aid flavors were randomly paired with infusates (Table 1) for each animal, and the order of infusion presentations was randomized for each animal. Animals were infused with water, 2 mM quinine hydrochloride dihydrate, whole saliva at 5 mg/mL, 2 mM quinine + whole saliva at 3 mg/mL, 2 mM quinine + whole saliva at 5 mg/mL, and 2 mM quinine + bovine serum albumin (BSA) at 5 mg/mL. In the conditioning trial (above), we compared behavior between artificial and whole saliva because we wanted to control for the ionic profile but were attempting to reduce the amount of donor saliva necessary for the study as it is difficult to collect. Here, we included whole saliva as a negative control group to address the role of the proteins alone. Kool-Aid flavors included cherry, grape, orange, lemon-lime, black cherry, and watermelon. These studies were run in two testing cohorts. Infusates (Table 1) and Kool-Aid flavors (cherry, grape, orange, lemon-lime, black cherry, and watermelon) did not differ between our testing cohorts, however, we added different taste stimuli to the Kool-Aid to drive licking behavior. Our first cohort was offered Kool-Aid containing saccharin ($n = 3$) as in [9]. The second cohort was given Kool-Aid containing salt ($n = 4$) as in [19]. We switched to salt in the second cohort because we found in other work in the lab that it increased licking behavior over saccharin (data not shown) and one of our goals was to maximize intake to be able to see suppression. We saw no differences in these two cohorts however, and therefore combined them into a single group. Animals were chronically water restricted and no supplemental water was given, they consumed all their fluid in the test conditions. Sessions were 15 min each day for 6 consecutive days.

Table 1
List of solutions infused and the hypotheses tested.

Infusates	Hypothesis tested
Water (control)	Baseline licking
2 mM quinine	Positive control; To demonstrate lick suppression
5 mg/mL SPs	Negative control; Test the effect of SPs alone
2 mM quinine + 3 mg/mL SPs	Test the effect of SP on licking suppression
2 mM quinine + 5 mg/mL SPs	Test the effect of SP on licking suppression
2 mM quinine + 5 mg/mL bovine serum albumin (BSA)	Test the effect of protein on licking suppression

Licks to each infusion were compared by within-subjects ANOVA with time and infusion as repeated measures factors. Average cumulative licks at the 5-minute and 15-minute time points were compared between all infusion sessions by within-subjects ANOVA with infusion as a repeated measures factor. ANOVAs and post-hoc *t*-tests were conducted using Systat 13.

3. Results

3.1. Conditioned avoidance: the presence of salivary proteins rescues a conditioned avoidance of a quinine-paired Kool-Aid flavor

Here we asked whether SPs could disrupt a conditioned avoidance of a Kool-Aid flavor paired with an infusion of 2 mM quinine in a 2-bottle preference test across three conditioning sessions. No animals were determined to be outliers (two-standard deviations from the mean) for any of the trials. In the first conditioning session, rats were conditioned to pair 2 mM quinine and water with grape and cherry Kool-Aid flavors. Surprisingly, rats consumed more of the quinine-paired solution during conditioning compared to the water-paired solution ($t(7) = -2.405$, $p = 0.047$). This appears to be due to a large intake in some of the animals on the first day of conditioning (Fig. 1A). During the preference trials, rats drank more of the water-paired Kool-Aid flavor and drank less of the quinine-paired Kool-Aid flavor ($t(7) = -5.350$, $p = 0.001$, Fig. 1B) which confirms rats were conditioned to avoid the quinine-paired Kool-Aid flavor.

In the second round, rats were conditioned to pair artificial saliva and 2 mM quinine + whole saliva at 5 mg/mL with orange and lemon-lime Kool-Aid flavors. Rats consumed similar amounts to the quinine + whole saliva and artificial saliva-paired solutions during conditioning ($t(7) = -1.601$, $p = 0.153$, Fig. 2A). Rats did not show any conditioned preference or avoidance of the quinine + whole saliva paired Kool-Aid flavor (CS+) compared to the artificial saliva-paired Kool-Aid flavor (CS-; $t(7) = -1.702$, $p = 0.132$, Fig. 2B) which supports the idea that SPs block conditioned avoidance of a quinine-paired Kool-Aid flavor.

We then retested 2 mM quinine and water to confirm that time did not reduce the ability of the animal to demonstrate a conditioned avoidance of quinine-paired Kool-Aid. Rats consumed similar amounts to the quinine- and water-paired solutions during conditioning ($t(7) = 0.863$, $p = 0.417$; Fig. 3A) but rats drank less of the quinine-paired Kool-Aid flavor (CS+) compared to the water-paired Kool-Aid flavor in the 2-bottle test (CS-; $t(7) = -2.343$, $p = 0.052$, Fig. 3B). Therefore, rats continue to show sensitivity to the quinine infusions despite having extensive experience with quinine.

3.2. Within-Session suppression: salivary proteins block the post-oral bitter-induced within-session intake

It has been previously shown that the gastric infusion of denatonium elicits a decrease in behavioral responses to a neutral Kool-Aid flavor [9] and NaCl [19]. Here, we test whether infusing SPs with quinine into the gut can block the within-session decreased licking response induced by a quinine gut infusion.

The infusate and time within session both affected licking behavior (infusion: $F(5,25) = 4.268$, $p = 0.006$, time: $F(14,70) = 25.922$, $p < 0.001$, infusion x time interaction: $F(70,350) = 2.743$, $p < 0.001$;

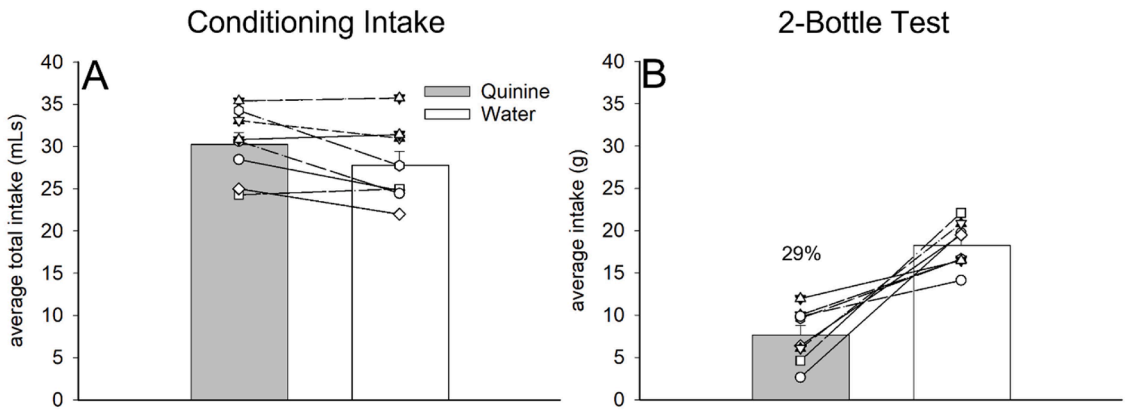

Fig. 1. Kool-Aid intake during conditioning and 2-bottle test for quinine and water infusates. Data are average total intake (mLs) for the 3-pairings during the conditioning phase (A) and average intake (g) for the 2-bottle test after conditioning (B). A: There was a difference in intake between each pairing (grape/cherry Kool-Aid + quinine/water) during the conditioning phase that could be attributed to higher intake during the first two days of conditioning ($p = 0.047$). B: Rats consumed significantly less of the quinine-paired Kool-Aid flavor compared to the water-paired flavor ($p = 0.001$). The number above the quinine bar is the average preference score for the quinine-paired solution.

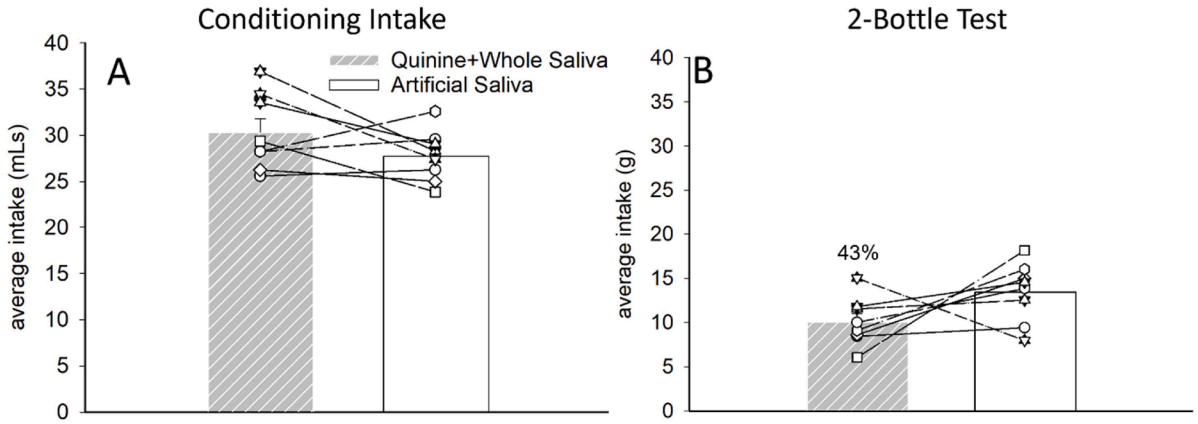

Fig. 2. Kool-Aid intake during conditioning and 2-bottle test for quinine + whole saliva and artificial saliva infusates. Data are average total intake (mLs) for the 3-pairings during the conditioning phase (A) and average intake (g) for the 2-bottle test after conditioning (B). A: Consumption of each Kool-Aid flavor (orange/lemon-lime) was similar in the conditioning phase ($p = 0.153$). B: Rats consumed similar amounts of each Kool-Aid flavor in the 2-bottle test ($p = 0.132$). The number above the quinine bar is the average preference score for the quinine-paired solution.

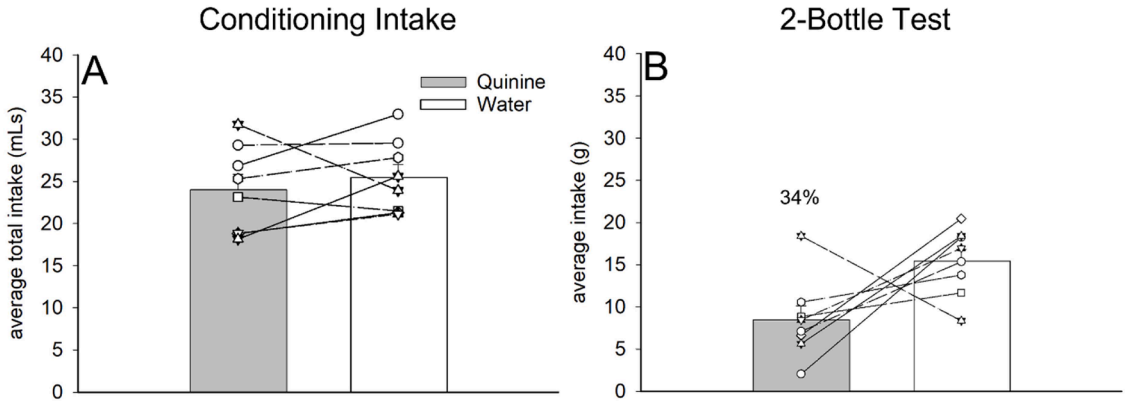

Fig. 3. Kool-Aid intake during conditioning and 2-bottle test for quinine and water infusates. Data are average total intake (mLs) for the 3-pairings during the conditioning phase (A) and average intake (g) for the 2-bottle test after conditioning (B). A: Consumption of each Kool-Aid flavor (grape/cherry Kool-Aid) was similar during the conditioning phase ($p = 0.417$). B: There was a trend in consumption such that rats consumed less of the quinine-paired Kool-Aid flavor compared to the water-paired flavor ($p = 0.052$). The number above the quinine bar is the average preference score for the quinine-paired solution.

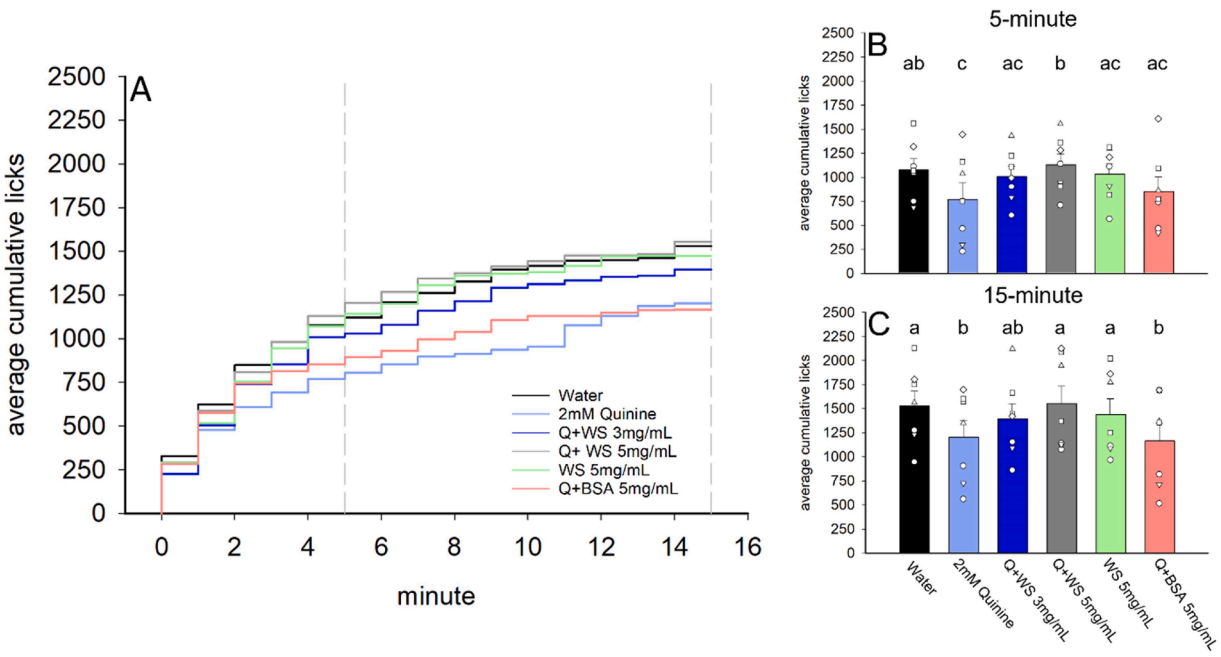

Fig. 4. Within-session licking for infusates throughout the 15-minute session in a single representative animal (A), and average cumulative licks ± SEM at the 5-min (B) and 15-min (C) timepoints. A: Graph shows average cumulative licks across time in a single representative animal. Each line represents different infusates and gray dashed lines represent the 5-min and 15-min timepoints. B and C: Graphs show average cumulative licks with each bar representing a different infusate at the 5-min (B) and 15-min (C) timepoints. Infusions include water, 2 mM quinine, quinine + whole saliva at 3 mg/mL (in figure: "Q+WS 3 mg/mL"), quinine + whole saliva at 5 mg/mL (in figure: "Q+WS 5 mg/mL"), whole saliva at 5 mg/mL (in figure: "WS 5 mg/mL"), and quinine + BSA at 5 mg/mL (in figure: "Q+BSA 5 mg/mL").

Fig. 4A). We focused our analysis on 5- (early in the test; Fig. 4B) and 15-min (total intake in the test; Fig. 4C) time points. Generally, the effects we saw in the total intake were also present at 5 min into the trial, although in some cases were less pronounced, suggesting that the effect of the infusate has a rapid effect on licking behavior. Rats licked less when infused with quinine compared to water (5 min: $t(6) = 2.955$, $p = 0.025$; 15 min: $t(6) = 7.250$, $p < 0.001$). This suppression is partially rescued when quinine is infused with whole saliva at 3 mg/mL. Licking when infused with quinine + whole saliva at 3 mg/mL was intermediate to water-infused licking and quinine alone, but different from neither (Q: 5-min: $t(6) = 0.341$, $p = 0.745$; 15-min: $t(6) = 0.947$, $p = 0.380$), water: 5-min: $t(6) = -0.907$, $p = 0.399$; 15-min: $t(6) = -1.987$, $p = 0.094$). Animals infused with quinine + whole saliva at 5 mg/mL licked significantly more than when they were infused with quinine alone (5-min: $t(6) = -2.414$, $p = 0.052$; 15-min: $t(6) = -3.332$, $p = 0.016$) and no different from licking that is paired with a water infusion (5-min: $t(6) = -0.369$, $p = 0.724$; 15-min: $t(6) = -0.162$, $p = 0.876$). Rats showed licking patterns that did not differ when infused with whole saliva or water (5 min: $t(6) = 0.331$, $p = 0.752$; 15 min: $t(6) = 0.398$, $p = 0.704$). Rats continued to show suppression in licking behavior when infused with quinine + BSA at 5 mg/mL, similar to an infusion of quinine alone (5 min: $t(6) = -0.826$, $p = 0.441$; 15 min: $t(6) = 0.019$, $p = 0.985$).

3.3. General discussion

We have previously shown that exposure to quinine or tannic acid-containing diets upregulated a subset of SPs, and this is correlated with increased intake, rate of feeding, brief-access licking, detection threshold, and decreased taste-nerve responding to quinine [13–15,24]. We have also described a relationship between the upregulation of SPs and an increase in meal size suggesting that SPs may also be contributing to changes in post-oral feedback. We have also demonstrated a causal relationship between SPs and decreased bitter taste perception using a taste reactivity paradigm. Briefly, rats show decreased aversive oro-motor movements when orally infused with a quinine + whole saliva cocktail compared to an infusion of quinine alone. In light of these data and literature suggesting that some SPs can survive the highly acidic environment in the gut [1,23], we hypothesized that SPs may also act to alter responding of post-oral receptors. In the present study, we explored the effect of SPs on behaviors driven by post-oral feedback independent of bitter diet experience by using intragastric silastic catheters that allowed us to infuse solutions into the gut while bypassing the oral cavity. The benefit of this paradigm is that rats can self-administer the gastric infusion with their own licking behaviors and can increase or decrease their infusion by increasing/decreasing their licking to the neutral solutions, in this case, random Kool-Aid flavors with 0.1 M NaCl (or 0.2 % saccharin). Intragastric infusions of prototypical bitter stimuli such as denatonium have been shown to elicit an avoidance response to the lick stimuli [9,19] while intragastric infusions of preferred stimuli such as glucose result in increased intake of the lick stimulus [18,21,22].

To determine if SPs altered post-oral feedback, we first established a conditioned flavor avoidance to an infusion of quinine. We adopted the protocol established by the Sclafani lab [21]. An intragastric infusion of quinine elicited a conditioned avoidance of the paired Kool-Aid flavor suggesting activation of bitter T2R receptors decreased intake. The increased intake of the quinine-paired Kool-Aid flavor during the conditioning was surprising as many others have shown altered intake of the CS+ Kool-Aid flavor during the conditioning phase [21]. We attribute this to an increased intake on the first and second days of conditioning that may have been elevated by our water deprivation paradigm. Intake of quinine-paired Kool-Aid flavor decreased throughout the rest of the conditioning sessions. We extended our water deprivation training in experiment 2 and then replicated suppression within session.

When infused with whole saliva, we saw no differences in intake during the conditioning phase and we were unable to detect a conditioned avoidance to the quinine + whole saliva-paired Kool-Aid flavor. This is consistent with findings from our previous work using the taste reactivity paradigm where animals decreased aversive response to an oral infusion of quinine when SPs were present. There are, however, limitations to this study, first, there is variation in the animal's responses to the quinine-paired Kool-Aid after the saliva conditioning trial, with preference scores ranging (0.24–0.65) and representing quinine

avoidance (preference score < 0.045, $n = 3$), indifference (preference score: 0.045–0.55, $n = 4$) and quinine preference (preference score > 0.55, $n = 1$). This variation suggests that animals may vary in their sensitivity to bitter driven conditioning. Second, to control for time and experience, we retested the quinine and water pairings to confirm animals could still display a conditioned avoidance after SP exposure. We re-used the original Kool-Aid flavors in this second test assuming we would see differences in licking during the conditioning phase, which would suggest that they remembered the old association, but we did not see differences during the conditioning phase. After re-conditioning they displayed the avoidance behavior in the 2-bottle test which supports the idea that the animals could relearn the avoidance. We cannot, however, entirely rule out that the observed outcome in this second round could be attributed to a learned response due to prior experience. With evidence suggesting a role for SPs altering post-oral feedback, but in face of these limitations, we decided to try a second paradigm: within-session suppression.

Animals can modify their licking behavior within ~2 to 6 min of an intragastric infusion to compensate for the post-oral experience [9,19]. In our paradigm, rats decreased their licking behavior within the first 5 min of the 15-min session when infused with quinine compared to a water baseline infusion demonstrating activation of bitter T2R receptors in the gut. It has been shown that denatonium in the gastrointestinal tract stimulates the release of cholecystokinin [4,11], the same process could be mediating the decrease in intake when quinine is infused. Rats did not suppress licking behavior when infused with quinine + whole saliva, which supports our hypothesis that SPs alter within-session suppression of an intragastric quinine infusion. There is a concentration-dependent effect of SPs such that a higher concentration of SPs (5 mg/mL) results in complete rescued suppression while SPs at 3 mg/mL show a partial rescue. The 5 mg/mL saliva with quinine seemed to elevate intake at the 5-min time point over that of whole saliva alone. This effect was transient, and there was no difference at 15-minutes. While these data suggest the effect of the SPs is very early in the trial, we do not know why this treatment would be different from the whole saliva alone. Overall, the rescue effect is consistent with our previous work looking at SPs on bitter taste where decreased protein concentration results in increased aversive movements to an oral infusion of quinine in a concentration-dependent manner [13]. Whole saliva alone at 5 mg/mL did not alter licking behavior and the response was similar to water infusion suggesting that the presence of the SPs does not increase licking. The proteins also appear to be specific: an infusion of BSA did not recover the quinine-induced suppression. These data suggest that the effect of SPs on consumption is specific to reducing "bitterness" and this effect is specific to SPs themselves not protein in general.

3.4. Conclusions and future work

Saliva is mixed into every bite of food a consumer eats during bolus formation. These experiments suggest that SPs alter how that food is interacting with the gut in ways that modify behaviors within a meal. Along with our previous taste reactivity data, these data demonstrate that SPs impact on consumption and modify feeding behaviors specifically to bitter compounds by decreasing the salience of quinine along the alimentary tract.

Our lab has begun identifying sex differences in protein expression patterns and timing of SP upregulation after bitter diet onset. While we work to better define the role of hormones in the expression of SPs, we have focused this work on males only. We will be addressing similar hypotheses with females in the future. Future work will also focus on protein identification and the mechanism SPs use to alter taste and post-oral feedback. There are multiple hypotheses that have been proposed to describe the way SPs are interacting and modifying taste stimuli. It is possible that SPs bind to the stimulus and prevent it from binding to the receptor. There is evidence that proteins form complexes with stimuli [3,7] and that these complexes survive the gut [1,23]. Another hypothesis is that the essential SPs are enzymes that break down the stimulus into a new product that does not activate a bitter receptor [20]. Lastly, SPs could bind to the bitter receptor itself and modulate the response to the stimulus. Understanding the mechanism by which SPs interact with post-oral bitter receptors will increase our ability to alter receptor activation in ways that could modulate meal size.

Funding

This research was supported by the National Science Foundation (NSF), United States of America IOS1942291 and NIH R01 DC016869. In part, supported by the National Institute of Health, National Institute of General Medical Science Awards (R25 GM095459 and T32 GM144920) to University at Buffalo (V.A-G. Trainee).

Author contributions

Experiments were designed by A-MT, LEM and VA-G, Data were collected by VA-G and LEM, Data were analyzed by VA-G, LEM and A-MT, Manuscript was prepared by A-MT, VA-G and LEM.

Declaration of Competing Interest

The authors declare no competing financial interests.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank Anthony Sclafani for donating the equipment to the lab and for his training and advice on the paradigm, and Samantha Brooker, Kimberly F. James, Kamila Nixon, and Miranda Berkebile for their technical assistance.

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