2016

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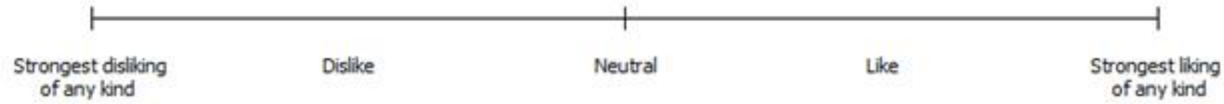

Expectation, expectoration and disgust: information manipulation alters spitting efficiency, a proxy for salivary flow

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Keywords: Stimulated saliva, disgust, expectation, salivary flow

Abstract

Saliva is becoming an increasingly useful research material across multiple fields of inquiry, including biomedical, dental, psychological, nutritional, and food choice research. However, both the flow rate and protein composition of stimulated saliva differ as a function of the collection method. We hypothesized that the context in which a salivary stimulus is presented to participants may alter salivation via behavioral (i.e., spitting efficiency) or top down cognitive effects. We presented participants with a constant stimulus (commercially available green tea) in two distinct contexts, once where the tea was described as a food item ("tea") and once where it was described as a disgusting non-food item ("rabbit hair extract"). Saliva and the expectorated stimulus were collected following 15 seconds of oral exposure in a crossover design with the identical stimulus presented under both contextual conditions; saliva was also collected for 5 minutes after stimulation while chewing a piece of wax. Participants also completed several validated personality instruments to measure food involvement, sensation seeking, sensitivity to reward, and sensitivity to punishment. Our data indicate participants spat out more sample when told they received the 'non-food' stimulus compared to the 'food' stimulus, particularly when they were given the non-food stimulus first. Further, individuals who were more sensation seeking spat out more sample during the 'food' condition compared to individuals with lower sensation seeking scores, but this difference was not observed during the 'non-food' condition. We interpret these data as showing either a greater motivation to spit for the 'non-food' stimulus, or a top down cognitive effect on salivary flow: that is, the context in which a salivary stimulus is presented alters how much sample/saliva is expectorated, and this effect may interact with personality factors.

1. Introduction

Saliva has become an increasingly useful research material across multiple fields. With over 3,000 proteins in saliva, many with functions that remain unclear, saliva may hold great potential for diagnostic use above and beyond its fundamental physical roles in the mouth [1]. Unstimulated saliva (also called resting saliva) differs from stimulated saliva, and stimulated saliva differs depending on the type of stimulation [2, 3], an observation that dates back to Pavlov. Based on his work in dogs, Pavlov proposed 'alimentary' (food stimulated) saliva was thicker, more mucous-like while 'defensive' (acid stimulated) saliva was thinner and more watery [4].

Saliva is reflexively secreted due to taste, mechanical, and to some degree olfactory stimulation generating afferent signals to the brain; these signals are then modified by other input before efferent signals stimulate salivary glands via autonomic nerves [2, 5]. While these direct, sensory stimulations of the salivary reflex dominate salivary flow rates, previous work on saliva predating the proteomic era showed a great deal of variation in salivary responses depending on cognitive factors, such as mood, personality [3], conditioning [6], instructions/feedback [3, 7], and flavor expectation [3]. Given growing interest in saliva as diagnostic tool via –omic approaches, the potential of cognitive factors to influence salivary composition and flow needs to be reconsidered.

Many studies that collect saliva use some oral stimulus to help generate saliva. For example, parafilm (wax) has been widely used to collect saliva stimulated by chewing; alternatively, intensely sour products such as lemon juice, acetic acid, and citric acid have all been used to collect saliva stimulated by acidity. However, these studies have rarely considered whether the participant receiving the stimulus considered the item to be ‘food’ or not. The origins of classic Pavlovian conditioning tells us that dogs will salivate when they expect to receive food, but that this saliva is different from saliva in response to an acid stimulus [4]. Other studies indicate salivary flow may decrease when an individual is disgusted, potentially activating a stress response [8, 9].

Given such contextual differences in salivation, we hypothesized the context in which a stimulus is presented may alter key salivary parameters, especially when salivary “flow” is measured via expectoration (rather than direct collection from the salivary ducts). Notably, while the expectoration method certainly has some inherent flaws in that it will inevitably lead to incomplete collection of all saliva and stimulus (some will adhere to oral surfaces), this collection method remains widely used in studies of food and saliva (e.g., [10-22]).

Thus, while expectorated saliva volume is clearly an imprecise measure of flow vis-à-vis direct collection, understanding how context influences collection volume is relevant for interpretation of prior data. Moreover, expectoration methods are necessitated when seeking to understand how food and saliva interact, as an individual cannot effectively chew when the mouth contains devices required for collection from individual ducts. That is, the expectoration method, despite potential limitations, is necessitated when the research question of interest pertains to naturalistic interactions with food. The specific goal of this study was to investigate differences in salivation in response to a food and a non-food. This aim mandated use of an expectoration based measure of “flow,” as inserting collection devices in the mouth would inherently make the experience less like eating “food”, and defeat the our contextual cognitive manipulation.

To ensure any observed differences in salivation were not due to differences in the stimulus itself, the exact same item (a commercially available tea) was presented to participants twice: once with the stated context that it was food ("*tea*") included as a control in the experiment, and once with the stated context that it was not food ("*rabbit hair extract*"). To justify and rationalize ingestion/tasting of rabbit hair extract, we told participants a cover story that a) animals such as rabbits in increase salivation when licking themselves (true), b) we believe rabbit hair may naturally contain a substance which stimulates saliva during licking and grooming (false), and c) we were studying this extract as potential treatment for dry mouth syndrome (false), a deception which was approved by the local institutional review board. Participants thus expected to receive an item that would stimulate salivation, but was presented with as strong of a non-food, disgust-evoking context as we thought would be potentially plausible. We expected one of two outcomes: 1) participants would salivate (or more precisely, expectorate) more during "*rabbit hair*" stimulation because the item would be viewed as a threat and would need to be purged from the mouth, and also simply because we told them they would salivate more with "*rabbit hair*" compared to "*tea*"; or 2) participants would salivate/expectorate less during "*rabbit hair*" stimulation because they were disgusted and the product was unappetizing. Other outcome measures included salivary flow rates during the 5 minutes after stimulation, protein content after stimulation, and sensory ratings of the stimuli.

2. Materials and Methods

To test whether participants' expectations of a stimulus ('food' versus 'not food') would change their salivary response, the concept of "rabbit hair extract" was invented by the first author. To our knowledge, no actual “rabbit hair extract” product exists. This item was chosen as rabbit hair is not a typical food product, but could conceivably be produced industrially from food grade sources, as rabbit meat is commercially available in North America. We specifically selected this item to induce disgust, under the premise that hair of an animal violates typical assumptions of what is considered edible in the United States. Disgust is a particularly strong motivator for food rejection [25]. Further, the plausibility of “rabbit hair extract” as a means to stimulate salivary production was propagated through the fact that animals do in fact increase salivation when grooming themselves [26], so the participants were told that compounds in the rabbit hair extract would promote saliva production. We chose to tell participants that the item would stimulate saliva, rather than decrease it, because in most studies attempting to collect saliva, this is how the stimulant would be described (i.e., as a aid to collection). Collectively, this allowed us to present a stimulus with a label that would ideally invoke disgust and be perceived as ‘non-food’ while still presumably being plausible as food grade salivary stimulus.

Given the main purpose of this experiment was to test the cognitive influence of expectation on salivary response (i.e., ‘food’ versus a ‘non food’ stimulus), participants were recruited to participate in a study on the stimulation of saliva. Recruiting documents advertised that subjects would be tasting tea and “rabbit hair extract”, a product supposedly designed as a natural supplement to stimulate salivary production for the treatment of dry mouth syndrome. In reality, participants tasted the exact same tea twice, once labeled as “*tea*” and once labeled as “*rabbit hair extract*.”

Eligible participants (n=56; 11 men) were recruited from a database maintained by the Sensory Evaluation Center at Penn State. This database consists of large number of age diverse individuals (1500+) who have previously expressed an interest in participating in studies in our facility; it is not a typical psychology study pool comprised by undergraduates, and this is the first deception study we have ever recruited from this population (additional details below). Our facility also works on drug delivery systems (e.g., [27-29]) and sensory biology (e.g., [30, 31]), so the idea that we might be working on dry mouth syndrome was not incongruent with other recent study recruitment efforts. Here, eligibility criteria included: between 18 and 45 years of age, no known defects in taste or smell, no food allergies, no tongue/lip/cheek piercings, no smoking within the past 30 days, not suffering from dry mouth, no history of choking or difficulty swallowing, and willing to taste the samples and provide saliva. All participants were told the test would involve tasting tea and rabbit hair extract. During the experiment, several participants failed to comply with instructions during the saliva collection phases. These participants were excluded from the saliva flow rate analysis (completers n=40; 7 men). Further, some participants failed the internal controls for using the general Labeled Magnitude Scale correctly for intensity ratings, and so were excluded from any analysis on the sensory data (completers n=51; 10 men). All participants signed written, informed consent documents (which did *not* contain the true purpose of the study) at the beginning of the experiment. At the conclusion of the experiment, participants signed separate debriefing forms containing details on the deception; these forms indicated they understood the deception and elected to remain in the study. That is, per standard ethical guidelines, participants were offered the opportunity to withdraw consent and have their data destroyed once they learned of the deception and the real goals of the study; none chose to do so. The entire protocol, including the use of deception during initial consent, was approved by the Penn State Institutional Review Board (protocol number: STUDY00002951).

Two samples were prepared per participant. Each participant received 15 grams of one sample labeled "*tea*" and another sample labeled "*rabbit hair extract*" in counterbalanced order. Samples were served cold, approximately 4°C. Both samples were actually the same commercially available unsweetened tea product (Soft Floral Rose Green Tea by Teas' Tea, purchased locally). To select this tea, both authors and other members of our research team tasted over 20 commercially available ready to drink tea based beverages – this specific tea was chosen as it had an easily noticeable but not grossly unpleasant off flavor that our team collectively felt made it plausible when labeled both as a food ("*tea*") as a non-food ("*rabbit hair extract*") item.

All surveys and sensory data were collected using CompuSense Cloud software (Guelph, ONT). Upon arriving for the experiment, participants were given an 8oz bottle of water to consume immediately. For the next 20 minutes, participants completed the consent process, our standard orientation warm-up procedure for a general labeled magnitude scale (gLMS, [32], supplemental data) and a personality survey (either Arnett's Inventory of Sensation Seeking (AISS, [33]) or the English language version of the Sensitivity to Reward/Sensitivity to Punishment Questionnaire (SPSRQ, [34-36]). Next, participants sat in individual sensory booths, where they were given the first sample in a 50mL centrifuge tube that had been pre-weighed. Participants were instructed, as a group, to take the entire sample into his or her mouth and swish it around for 15 seconds. An experimenter counted down the 15 seconds aloud. Participants then expectorated the entire sample (and any accumulated saliva) back into the centrifuge tube. These samples (saliva + expectorated stimulus) were immediately weighed, aliquoted and frozen on dry ice. Next, participants received a 2x2 inch piece of parafilm. Participants were instructed to chew on the piece of parafilm and expectorate all saliva for the next 5 minutes. Saliva was expectorated into a second pre-weighed 50mL centrifuge tube that was held in a cup of regular ice. During the chewing period, participants rated sensory aspects of the sample. Hedonic ratings were collected on a bipolar, horizontal visual analog scale, with the ends of the scale being labeled 'strongest disliking of any kind' (left side) and 'strongest liking of any kind' (right side); the midpoint of the scale was labeled 'neutral', as shown in Supplemental Figure 1. This scale has been described previously (e.g., [37, 38]. Ratings of perceived intensity were collected using a horizontal gLMS with marks to indicate no sensation (0), barely detectable (1.4), weak (6), moderate (17), strong (35), very strong (51), and strongest imaginable sensation of any kind (100). Separate scales were used to collect intensity ratings for: overall flavor, odor, sweetness, sourness, saltiness, and bitterness. A text box was also provided for open-ended comments on the stimulus. After the 5 minutes elapsed, saliva (following chewing on parafilm) was also immediately weighed, aliquoted, and frozen on dry ice. After the first sample, participants drank another 8 oz bottle of water. Another 20-minute wait was imposed, during which participants filled out the other personality survey (either the AISS or SPSRQ, whichever they had not yet completed, with the order of presentation of personality measures counterbalanced across participants). Participants then tasted the second sample using the same procedure described above. After both samples had been tasted, panelists completed a final survey containing the Food Involvement Scale (FIS, [39]), medication usage, tea consumption, as well as an open comment box on whether the rabbit hair 'worked' to stimulate saliva; this was included as a way to gauge whether a participant had figured out that the "*rabbit hair extract*" was a ruse.

For all participants, the experiment was conducted in the afternoon between the hours of 1300 and 1600. All tests for each participant were conducted the same session, which lasted about 90 minutes.

Salivary flow rates (or, as later described, expectoration efficiency) were calculated by difference, using the before and after weights of the pre-weighed 50mL centrifuge tubes and subtracting the weight of the tea (15g) for the "*tea"* and "*rabbit hair"* stimulated samples. Protein content of the chewing saliva was measured using the BCA assay (Thermo Scientific Pierce, Rockford, IL). The two saliva samples collected in the "*tea"* and "*rabbit hair"* conditions were not measured for protein content, as each contained expectorated tea along with saliva, and the precise ratio of tea to saliva would require development of a new technique to measure the proportion of the constituents. That is, although all participants were given the same amount of tea, the presence of some negative values in our data indicated not all participants expectorated completely, and residual amounts of tea and saliva remaining in the mouth were unknown and not necessarily equivalent across individuals.

For readability and clarity throughout the remainder of the manuscript, when the sample was presented in a 'food' context (e.g., labeled as "*tea*") it will be referred to as "Tea," while when it was presented in a 'non-food' context (e.g., labeled as "*rabbit hair extract*") it will be referred to as "Rabbit Hair." Also, saliva collected during the stimulation with these two samples (the 15 seconds of swishing) will be referred to as sample stimulated saliva, while saliva collected during the phase of chewing parafilm (5 min) will be referred to as chewing stimulated saliva.

Data were analyzed using repeated measures ANOVA in SAS 9.4 (Cary, NC) for salivary flow rates, protein content, and sensory data comparing the Tea and Rabbit Hair conditions. For chewing stimulated salivary flow, residual analysis indicated violation of normality assumptions, so data for flow rates were $\log_{10}$ transformed. For sensory intensity data, data were square root transformed to meet normality assumptions (a $\log_{10}$ transform was also attempted, but the data still violated model assumptions). Affective (hedonic) ratings were analyzed by Spearman rho correlations and by Wilcoxon Sign Rank tests (for difference between rating for Tea and for Rabbit Hair) because of 'lumping' in the data (many ratings just barely above or below neutral). These data were also analyzed for interaction effects with order by conducting separate analyze on those who received Rabbit Hair first compared to those who received Tea first. Correlations between sensory data and personality questionnaires and medication use were analyzed using Spearman's rho in SAS 9.4. Medication use was coded as binomial data for use of: any medication, allergy medicines, and thyroid or antidepressant medicines. After observing the effects for Tea/Rabbit Hair stimulated saliva and the numerous negative values in these conditions, data were reanalyzed for participants with: only negative flow rates (indicating incomplete spitting, N=13, 4 who tasted Rabbit Hair first), one negative and one positive flow rate (N=9, 5 who tasted Rabbit Hair first), and only positive flow rates (N=18, 12 who tasted Rabbit Hair first). In the final group of only positive flow rates, model assumptions about the normality of the residuals were violated, so the data were $\log_{10}$-transformed to correct for this.

3. Results

3.1 Sensory data

There was evidence of an interaction with order for hedonic ratings of the samples. Those who received Tea first then Rabbit Hair gave higher ratings to Tea (median difference of Tea – Rabbit Hair = 0.7; p=0.02). While this value is significantly differs from zero, the median difference is very small, less than a full point on a 100 point line scale. No difference in hedonic ratings was apparent for participants who received Rabbit Hair followed by Tea (median difference Tea – Rabbit Hair = -0.2, p=0.79; see Figure 1). Ratings for bitterness were negatively correlated with ratings for liking in both samples (Table 1). There also was a trend for an interaction with order in bitterness ratings, with those who received Rabbit Hair first perceiving the Rabbit Hair as slightly less bitter than the Tea (p=0.06, p=0.87 for Tea first group).

3.2 Salivary parameters

For the stimulated salivary flow (or expectoration) during the 15 seconds of oral exposure, we observed significant main effects for condition (Tea vs. Rabbit Hair: F(1,38)= 7.80; p=0.008), order of tasting (Rabbit Hair first versus Tea first: F(1,38)= 4.88; p=0.03), as well as an interaction between condition and order (F(1,38)= 7.80; p=0.008). Figures 2 and 3 summarize these effects. For the stimulated saliva, there was evidence that participants salivated more both when they received Rabbit Hair first compared to Tea first (between subject), and when told they were tasting Rabbit Hair compared to Tea (within subject). However, in light of the interaction effect, the significant main effect appears to be driven almost entirely by the group who received Tea first: those who received Rabbit Hair first showed little difference in stimulated saliva between conditions, instead maintaining high salivation across both. One outlier was present in the Rabbit Hair first group (noted with # in Figure 3); however, while removing this individual led to slight increase in Rabbit Hair versus Tea stimulated saliva for the Rabbit Hair first group as well, the overall effects did not change with removal. No differences were observed for chewing salivary flow rates, or for chewing saliva protein content. Notably, individual salivary flow rates were highly correlated within each subject for both chewing and sample stimulated saliva (Table 1).

Considering the large number of negative values in the Tea/Rabbit Hair stimulated salivary "flow" rate, the data were reanalyzed by whether participants had negative values for both Tea and Rabbit (incomplete expectoration in both conditions), negative values for only one condition, or positive values for both conditions. Results from this analysis indicate that the effects on "flow rate" were driven by the individuals with negative values in both conditions (N=13). A summary of the findings for this post-hoc separation of groups is shown in Table 2.

3.3 Individual factors: Sensation Seeking, Sensitivity to Punishment, Sensitivity to Reward, Food Involvement, and Medications

Individuals who were more sensitive to punishment (from the SPSRQ) gave lower hedonic ratings in both conditions (Tea: rho=-0.37, p=0.008; Rabbit Hair: rho=-0.34, p=0.01); they also reported more bitterness in both conditions (Tea: rho=0.47, p=0.0006; Rabbit Hair: rho=0.41, p=0.003). Those who were more Sensation Seeking salivated more than those who were less sensation seeking, but only for the Tea condition (Tea: rho=0.36, p=0.02; Rabbit Hair: rho=0.19, p=0.24). In direct contrast to two separate studies from our facility [37, 40], here we unexpectedly found those who were more sensation seeking were also more sensitive to punishment (rho=0.41, p=0.003) and less sensitive to reward (-0.40, p=0.003). However, the current study's sample may not be entirely representative of the wider population, as the individuals who participated in this test had to be willing to consume "*rabbit hair extract*"; some personality factors may incline an individual to participate in such an intentionally disgusting protocol in return for monetary reward. Thus, it is possible the discrepancy with prior data vis-à-vis personality may be random variation or a selection effect arising from willingness to participate, so caution is warranted in interpreting these particular results. No other significant correlations were observed for sensitivity to reward or punishment (SPSRQ), sensation seeking (AISS), food involvement (FIS), or medication use with sensory or salivary measures.

4. Discussion

The main findings of this study were that a) participants salivated or expectorated more when told they were tasting "*rabbit hair extract*" compared to "*tea*," especially when they tasted tea first or if they had low expectorated weights of sample + saliva, and b) participants who were more sensitive to punishment gave lower affective ratings and higher bitterness ratings both "*tea*" and "*rabbit hair extract*." Also, participants with higher sensation seeking scores salivated or expectorated more for "*tea*" than those who were less sensation seeking, but this difference was not observed for "*rabbit hair extract*." Another outcome, which we interpret cautiously due to the small effect, is that those who received "*tea*" first gave slightly higher liking ratings to the "*tea*" compared to "*rabbit hair extract*," while this difference was not evident in those who received "*rabbit hair extract*" first. Implications of these findings are discussed in detail below.

Given evidence for significant differences in salivary "flow" that occurred when participants were told the sample was Rabbit Hair compared to Tea, we can think of three distinct explanations for this observation. First, being told the stimulus was rabbit hair extract may have successfully elicited disgust and thus triggered a reflex that stimulated additional saliva production to flush the disgusting stimulus from the oral cavity. Another possible explanation is that participants salivated more because we told them the Rabbit Hair may function as a stimulant for saliva production. It may be that participants salivated more simply because they expected to salivate more (open-ended comments collected at the end of the study indicated that most participants believed they salivated more during the Rabbit Hair exposure compared to Tea). Finally, a third explanation for the observed effect is that participants were disgusted by the rabbit hair extract and thus spat more forcefully and/or efficiently, thereby leaving less sample/saliva in the mouth. Given that the overall effect was driven predominantly by the group with negative values for "flow" in both the Tea and Rabbit Hair conditions, we believe this last explanation is the most plausible. All possibilities are discussed below.

The first possibility – that exposure to a disgusting or non-food stimulus may actually stimulate salivation – is congruent with prior literature on salivary responses. This explanation would indicate a top-down effect of cognition on salivation. Individuals who received the Rabbit Hair second displayed the most robust effect, while those who received the Rabbit Hair first may have simply retained the increased salivation into the second session (perhaps through being reminded of the first sample). Disgust reactions include potential for contamination – that is, that one disgusting object may contaminate another, originally non-disgusting object – and such contamination cannot be washed away [41, 42]. Consequently, in the environment of the test with the continual reminders of "*rabbit hair extract*" the first experience with the sample may have contaminated the latter experience, thereby maintaining the higher salivation for the Rabbit Hair first group.

The second explanation for the higher salivation during stimulation with Rabbit Hair – that participants salivated more simply because we told them they might – would also indicate a top-down effect of cognition on salivation. This would essentially indicate a placebo effect, and the potential for this effect was increased by the intentionally un-blinded nature of our experiment. Presumably, the order effect could be explained because the participants who received Rabbit Hair first believed that the stimulus was making them salivate, and so continued to salivate more even during the second stimulation. However, no differences were observed during the chewing phases. Notably, previous work showed participants could decrease, but not increase, parotid salivary secretion rate upon demand with practice and immediate feedback from the experimenter [7]. Others have been able to increase acceleration of salivary flow with monetary reward [43] or with mouth movements or swallowing [44].

The final possibility – that participants were simply more motivated to spit more completely under the Rabbit Hair condition – is likely the dominant contributor for the observed effects. Individuals who spat the least (showing negative values for both Tea and Rabbit Hair stimulated salivary "flow") drove the effect for a difference in the expectorated weights between the two cognitive conditions. Thus, the effect on "flow" observed here may be more accurately interpreted as an effect on expectoration efficiency. The inadequate expectorators (those who spat out less than the 15g of original sample, indicating both sample and saliva were retained in the mouth) may have been more motivated to spit out the entire sample when it was labeled as Rabbit Hair, leading to greater spitting efficiency. However, it needs to be noted that this factor (spitting efficiency) is inherently confounded with salivary flow rate, as those with low flow rates will have less saliva to spit out, increasing the chance they will have a negative value for the difference between expectorated sample and original sample weight.

Regardless, the effect observed here appears to be predominantly due to behavioral differences in spitting due to the stimulus context. While other measures for salivary flow (such as cannulation of the ducts or Lashley cups) would certainly more precisely measure salivary flow rather than expectoration, these devices are not practical for studies attempting to measure how saliva interacts with food in a normal eating situation. The current study aimed to test a “food” versus “non food” context specifically, and such devices would have inevitably shifted the perception of the experiment toward the “non food” context. Nonetheless, the findings of different expectoration weights due to context are a particularly important design consideration for future work on how food interacts with saliva. Previously, many studies [10-22]have used expectoration to measure salivary flow in studies involving real food or taste stimuli. While many studies using expectoration to measure salivary flow rate use smaller stimulus volumes, this is widely variable (from a few drops to 15mL [10-22]); ad libutim sip or bite sizes are closer to 15mL in volume [21, 23] and spoonful sizes are around 7-9 grams (close to 7-9 mL, but dependent on density) [24], so the quantity used in the current study (15g, approximately 15mL) is more in line with actual eating behavior. Again, as the purpose of this experiment was to investigate how the context of the stimulus changed the salivary parameters, keeping the environment as similar to regular eating as possible was critical. Giving extremely small volumes would make even the “food” sample less similar to normal eating, inherently confounding our results. From the outcomes shown in this experiment, using expectoration as a proxy for salivary flow rate is not advisable if there could be a cognitive or expected difference between the samples.

Despite the main effect likely being driven by inefficient expectoration, it is important to note that numerous studies, many several decades old, reported differences in salivation due to top-down cognitive effects when using dental rolls or isolated parotid collection methods rather than expectoration [3]. Early work also found differences in salivation due to: sexual arousal [45]; sustained attention [46]; simple suggestion (i.e., water described as sour caused more salivation, and citric acid described as water caused less [47]); threat, fear, and aggression [48]; and depression [49]. For an older but thorough review of parotid responses with numerous examples of top-down effects, the interested reader is referred to [3]. All of these reports buttress the interpretation that cognitive factors and/or emotional state could also alter salivary flow rates, despite the fact that our observed differences appear to be primarily from expectoration efficiency.

Notably, ratings from the current study indicate affective responses did not markedly differ between the two samples, so if the "*rabbit hair extract*" did actually inspire a sense of disgust, asking participants to rate liking/disliking on a generalized hedonic scale did not successfully capture this feeling. However, to invoke a sense of disgust, negative hedonic values are not necessary. Work in the disgust literature has consistently demonstrated that objects with acceptable sensory attributes can still be disgusting, or can be contaminated by other disgusting objects [41, 42, 50]. For example, veal or horse or dog could simultaneously be highly liked in blind tasting when based solely on the sensory experience, but still be highly disgusting for an individual participant given animal welfare or cultural reasons. Further, we should reemphasize that all participants here had already indicated they were willing to taste "*rabbit hair extract*"; participants who would have been very strongly disgusted by such a product would not have signed up (i.e., a potential selection bias). Nonetheless, open ended comments suggest participants certainly expected the "*rabbit hair extract*" to be disgusting. Their comments included: "not as bad as I expected," "intimidating," "resembled tea, but much less enjoyable," "surprised that it didn't smell or taste funny," and "[I was] nervous about the rabbit."

As differences in expectoration were observed due to cognitive factors, personality factors would seem to have strong potential to influence these differences. In previous work, disgust, but not simply negative affect, has been shown to up-regulate the oral immune response, though this was induced only through images and no differences in salivary flow rate (unstimulated, passive drool) were observed [51]. This lack of change in flow rate may be because disgust would be greater for an item actually entering the mouth, rather than simply viewing the disgusting item [42]. Here, while it appears that greater sensation seeking individuals may have salivated more during Tea stimulation compared to those who were lower in sensation seeking, this effect was not particularly strong (see Table 1). However, the presence of such an effect for Tea but not Rabbit Hair is intriguing; it may indicate that the perception of the item as a disgusting and/or non-food stimulus moderated the effect of sensation seeking on expectoration/salivation. Potentially, spitting could be viewed as socially risky behavior (at least in western culture), so this may interact with personality to influence spitting efficiency. That said, motivation to remove a disgusting/non-food item from the mouth may override any such effect during the Rabbit Hair exposure. Accordingly, additional work would be needed to confirm these interactions, and to elucidate why they occur.

That sensitivity to punishment correlated with lower liking and higher bitterness ratings for the stimuli is intriguing, but not entirely unexpected. Bitterness is often seen as a negative attribute in foods (with the caveat that preference for bitter foods can be learned with positive post-ingestive rewards – e.g., coffee and caffeine or beer and ethanol/bitter hops). However, separating affective responses and sensory ratings has been a perpetual challenge in the sensory community, as participants often group these aspects together or substitute one for the other [52]. Sweetness is classically seen as being inherently rewarding [53, 54], and bitterness is considered to be innately aversive. It may not be totally unreasonable to extend this slightly to conclude bitterness is inherently "punishing" (prior caveats about learned associations not withstanding). Indeed, while sweetness is accepted, bitterness is rejected even in utero and infancy [55-58]. This concept – e.g., the relationship between disliking, aversion and punishment – warrants additional nuanced investigation. Such work should also be conducted with specific attention paid to recruitment methods, as fundamental differences in personality are likely present in individuals who willing to sign up for a test using "*rabbit hair extract*" as compared to those who chose not to respond to our screener.

Limitations in this work are certainly apparent in the method of saliva collection. Expectoration weights are highly variable, as some liquid will naturally be retained on the oral surfaces, yet also inherently confounded with the salivary flow rate of the individuals (people with lots of saliva will spit out more saliva). To our knowledge, data are not available on what physiological parameters might influence retention of tasted stimuli when participants are instructed to expectorate, though data indicate that thicker liquids are more retained by surfaces in swallowing {Bülow, 2003, Videoradiographic analysis of how carbonated thin liquids and thickened liquids affect the physiology of swallowing in subjects with aspiration on thin liquids}. The tea used in the current study was of similar consistency to water, however. While the 15g bolus used in the current study allows for a large variation by participants to accidently swallow some of the sample, the lowest value for differences in sampled verses expectoration weights was around -1.2 g, indicating a loss of less than 1 mL. Other studies, using less sample, may have less opportunity for swallowing/inefficient expectoration, but would also be less relevant to normal sip sizes, which are in the 15g or 15mL range [21, 23]. Again, in order to keep this experiment as similar to normal eating as possible, only changing the label of the "not food" stimulus rather than the entire environment, some limitations were inevitable.

5. Conclusions

Present data demonstrate that the context in which a salivary stimulus is delivered to participants can alter spitting efficiency (a commonly used proxy for salivary flow rate), with a non-food/disgusting context resulting in greater spitting efficiency than the exact same item presented as a food. Further, personality factors appear to influence both spitting efficiency and the sensory ratings for stimuli, though more work is needed to understand these effects. While additional work is also needed to understand the mechanism behind the increased amount of saliva collected in the non-food condition, present data indicate that at a purely pragmatic level, both the context in which a stimulus is presented and the personalities of participants should be carefully considered in studies using saliva as a research material, especially for studies that measuring flow rates or expectoration over brief periods.

Acknowledgements

The authors thank Jennifer Meengs MS RD and the Sensory Evaluation Center staff for their assistance in collecting these data, and our participants for taking part.

Conflict of Interest Disclosure

CAR has no conflicts of interests to declare. JEH has received speaking or consulting fees from corporate clients in the food industry. Additionally, the Sensory Evaluation Center at Penn State conducts routine consumer tests for industrial clients in the food, packaging and packaged goods industries to facilitate experiential learning for undergraduate and graduate students. None of these organizations have had any role in study conception, design or interpretation, or the decision to publish these data.

Funding

This work was supported by the Pennsylvania State University as well as United States Department of Agriculture Hatch Project [PEN04565] funds. The authors also receive partial salary support from a National Institutes of Health grant to JEH for unrelated work.

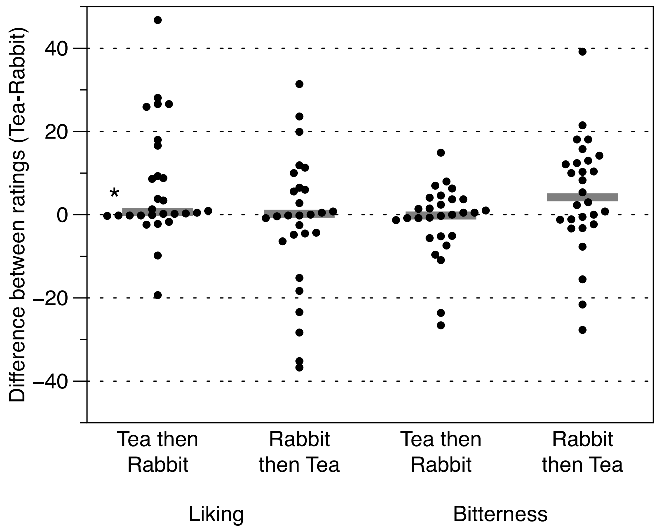


Figure 1: Change in liking and bitterness ratings by order of tasting. Horizontal lines indicate median values. For liking, the star indicates the median value for the change in liking was significantly different from zero (p=0.02; Wilcoxon Sign Rank test) for the Tea then Rabbit group. A trend may also be present for slightly higher bitterness ratings in Tea among those who received Rabbit then Tea (p=0.06; Wilcoxon Sign Rank test).

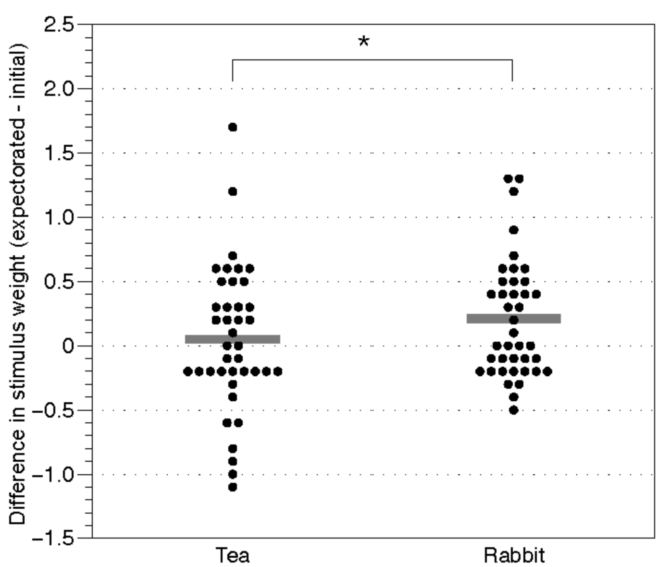


Figure 2: Salivary flow/spitting efficiency during stimulation with tea over 15 seconds, when presented in tubes labeled either “Tea” or “Rabbit Hair.” Tubes contained 15 g of sample, and were accurately weighted before and after presentation. Flow rate was determined by mass differences, so negative values presumably indicate incomplete expectoration of sample. Horizontal lines indicate the group mean, which was significantly higher (p=0.008) in the Rabbit Hair condition.

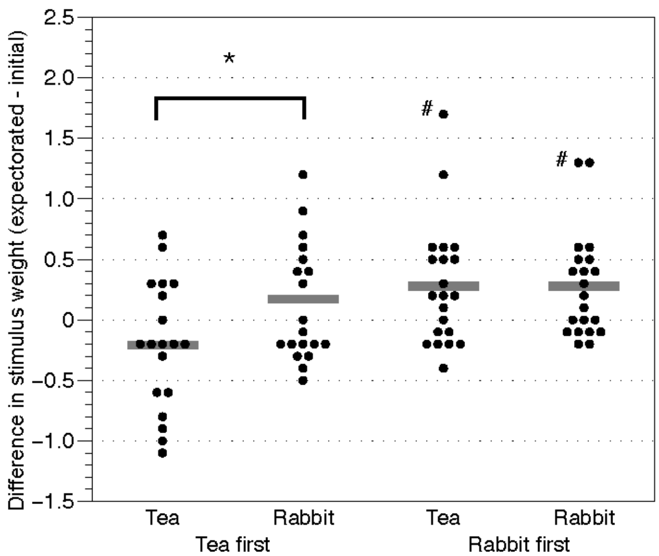


Figure 3: Same data as Figure 2 (salivary flow/spitting efficiency during stimulation) but stratified by order of tasting. Horizontal lines indicate group means. The main effect of condition (Tea versus Rabbit Hair) was functionally driven by those in the Tea first condition, where flow was significantly higher (* p=0.006) in the Rabbit Hair condition; the means did not differ by condition in the Rabbit first group. The # marks an outlier, but removing this individual did not change overall data patterns.

Table 1: Spearman correlations

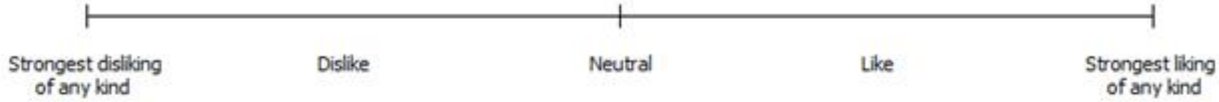
		Rho	p-value
Salivation (N=40, non-compliant participants removed)			
Tea: Stimulated Saliva	Rabbit: Stimulated Saliva	0.71	<.0001
Rabbit: Chewing Saliva	Tea: Chewing Saliva	0.90	<.0001
Tea: Stimulated Saliva	Sensation Seeking	0.36	0.02
Rabbit: Stimulated Saliva	*Sensation Seeking*	*0.19*	*0.24*
Sensory and personality (N=51, those who did not use scale reliably removed)			
Tea: Liking	Rabbit: Liking	0.57	<.0001
Tea: Bitterness	Rabbit: Bitterness	0.53	<.0001
Rabbit: Bitterness	Rabbit: Liking	-0.45	0.0008
Tea: Bitterness	Tea: Liking	-0.37	0.007
Tea: Bitterness	Sens. to Punishment	0.47	0.0006
Tea: Liking	Sens. to Punishment	-0.37	0.008
Rabbit: Bitterness	Sens. to Punishment	0.41	0.003
Rabbit: Liking	Sens. to Punishment	-0.34	0.01
Tea: Bitterness	Tea Consumption	-0.38	0.007
Rabbit: Bitterness	*Tea Consumption*	*-0.27*	*0.06*
Tea: Liking	Tea Consumption	0.29	0.04
Rabbit: Liking	*Tea Consumption*	*0.26*	*0.06*
Sensation Seeking	Sens. to Reward	-0.40	0.003
Sensation Seeking	Sens. to Punishment	0.41	0.003

Table 2: Analysis of Tea/Rabbit Hair stimulated saliva "flow" by expectoration efficiency

	F	p-value
Both negative values: N=13 (4 with Rabbit Hair first)		
Main effect: Order*	F(1,11)=4.51	0.057
Main effect: "Tea" vs. "Rabbit Hair"	**F(1,11)=6.22**	**0.030**
Interaction	F(1,11)=1.80	0.21
One positive and one negative value: N=9 (5 with Rabbit Hair first)		
Main effect: Order*	F(1,7)=0.00	0.95
Main effect: "Tea" vs. "Rabbit Hair"	F(1,7)=1.27	0.30
Interaction	F(1,7)=1.92	0.21
Both positive values: N=18 (12 with Rabbit Hair first)		
Main effect: Order*	F(1,16)=0.06	0.81
Main effect: "Tea" vs. "Rabbit Hair"	F(1,16)=2.28	0.15
Interaction	F(1,16)=2.79	0.11

*Order effect had very low power in these decreased sample groups

Supplemental figure:



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