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Expectation and expectoration: Information manipulation alters spitting volume, a common proxy for salivary flow

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HIGHLIGHTS

- We manipulated context of a food to see the effect on saliva.
- Tea was presented with labels "tea" and "rabbit hair extract".
- Participants spat out more sample for "rabbit hair extract" than for "tea".
- Reason may be physiological (salivary flow).
- More likely reason is greater motivation to spit effectively for disgusting item.

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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 stimulus is presented to participants may alter salivation via top down cognitive effects and/or behavioral changes (i.e., spitting efficiency). We presented participants with one 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 s of oral exposure in a crossover design with the identical stimulus presented in both contexts; saliva was also collected for 5 min after stimulation while chewing a piece of wax. Participants also completed validated personality instruments to measure food involvement, sensation seeking, sensitivity to reward, and sensitivity to punishment. Our data suggest 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 higher in sensation seeking spat out more sample during the 'food' condition compared to individuals with lower sensation seeking scores, but this difference was absent in the 'non-food' condition. While consistent with a top down cognitive effect on salivary flow, we believe a greater motivation to spit out the 'non-food' stimulus is a more likely explanation. In either case, it is clear the context in which a stimulus is presented alters how much sample/saliva is expectorated, suggesting context needs to be carefully considered in future work on salivary flow.

1. Introduction

Saliva has become an increasingly useful research material across multiple fields. With over 3000 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 with 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, work on saliva predating the proteomic era showed a great deal of

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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 for cognitive and contextual factors to influence salivary composition, salivary flow, and expectoration volume 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 mechanically (by chewing); alternatively, intensely sour products such as lemon juice, acetic acid, and citric acid have all been used to stimulate saliva for collection. However, such 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 [4]. Other studies indicate salivary flow may decrease when an individual is disgusted, potentially activating a stress response [8,9].

Given apparent contextual differences on 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). For expediency and simplicity, many studies collect whole mouth saliva by asking individuals to spit into a container over some fixed period of time, using this as a measure of salivary flow. However, carefully considered, the rate that is obtained (expectoration volume over time) actually confounds spitting efficacy with true salivary flow. That is, this approach will inevitably lead to incomplete collection (of saliva and the stimulus) as some will adhere to oral surfaces or be swallowed. Despite this potential limitation, this method is still widely used in studies of food and saliva (e.g., [10–22]).

Thus, while expectorated volume of saliva over time is clearly an imprecise measure of flow vis-à-vis direct collection from salivary ducts, understanding how context may influence expectoration volume is relevant for interpretation of prior data. Moreover, expectoration based methods are required when seeking to understand how food and saliva interact, as an individual cannot effectively chew a food when devices required for collection from individual ducts are present in the mouth. That is, expectoration based methods, despite potential limitations, are necessitated when the research question of interest pertains to naturalistic interactions with food. The long-term goal of the first author's research program is to understand how interactions between saliva and food influence perception, rather than salivary reflexes or composition per se. Accordingly, the specific goal of the present study was to investigate differences in salivation in response to a food and a non-food. This, in turn, mandated use of an expectoration based method to measure "flow", as placing collection devices in the mouth would inherently make the experience less foodlike, and defeat the contextual manipulation.

To ensure any observed differences in salivary flow (really expectoration volume over time) 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 expectoration volumes (nominally salivary flow rates) during the 5 min 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/expectoration volume, 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 stimulus 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 that the main purpose of this experiment was to test the cognitive influence of expectation (i.e., 'food' versus a 'non food') on salivary response/expectoration volume, 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). Critical to successful deception, 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 studying dry mouth syndrome was entirely consistent with other recent 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;