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Conditioning of human salivary flow using a visual cue for sour candy

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ABSTRACT

Objective: Although the "mouthwatering" to sight, smell, or thought of food is commonly accepted in food and nutrition research, the concept of mouthwatering and human salivary flow conditioning is not well accepted in salivary research. The objective of this study was to revisit whether human salivary flow could be classically conditioned to a previously neutral stimulus.

Design: Sour candy or a non-food control in opaque containers were presented to healthy participants (n = 8). Simple images were consistently paired with container contents. Participants viewed the images for 15 s, then opened the containers and ate (candy) or did not eat (non-food control) the contents. This was repeated 14 times (7 of each stimulus). Order was semi-randomized to ensure one candy and one non-food were presented as the first two and last two stimuli. Saliva was collected with cotton dental rolls during these presentations (first two and last two) after viewing the image for 15 s, but before opening the container.

Results: Participants were successfully conditioned to increase salivary flow in response to the image that predicted candy, as demonstrated by greater weight of saliva in response to 1) the candy-paired image than the non-food-paired image, and 2) the candy-paired image at the end of the first visit compared with the beginning (when the image had no meaning). However, the effect was attenuated during the second visit.

Conclusions: We demonstrate classical conditioning of human salivary flow is achievable, but the effect may not persist to a second visit.

1. Introduction

Despite common use in lay-language, the phenomenon of "mouthwatering" in anticipation of food is contested in the scientific literature. Many in salivary research have argued that mouthwatering is not a sustainable event, at best being a very brief expression of saliva from the submandibular glands, or perhaps just an increase in human awareness of saliva that is already present in the mouth (Carpenter, 2013; Kerr, 1961). Food and nutrition research, however, maintains that mouthwatering is an inherent part of the cephalic phase response: the collection of early physiological events that prepare the oro-gastrointestinal tract for incoming food (Mattes, 2000). Thus, while salivary research contains minimal investigation of mouthwatering in recent years, food and nutrition research continues to use anticipatory or trained saliva to monitor associated responses to food, including hunger (Wooley & Wooley, 1973), desire to eat (Jansen, Stegerman, Roefs, Nederkoorn, & Havermans, 2010; Nederkoorn, Smulders, & Jansen, 2000), dietary restraint (Brunstrom, Yates, & Witcomb, 2004; Ferriday & Brunstrom, 2010; Nederkoorn & Jansen, 2002), and hedonic appeal (Proserpio et al., 2017; Ramaekers et al., 2013; Rogers & Hill, 1989). Reviews on the subject specific to this field can be consulted for the breadth of information available (Keesman, Aarts, Vermeent, Häfner, & Papies, 2016; Mattes, 2000; Wooley & Wooley, 1981).

This disconnect between the fields has become a particular challenge for our laboratory, which focuses on the intersections of psychology of eating, flavor sensation, and salivary biochemistry. As a consequence, we are revisiting the concept of mouthwatering in anticipation to food. In particular, we are focusing on whether salivary flow can be classically conditioned in humans. In classical conditioning, a previously neutral stimulus (e.g. a bell, the conditioned stimulus) is repeatedly associated with an unconditioned stimulus (e.g. eating food) to produce the response (e.g. salivary flow) (Pavlov, 1910). Over time, the previously neutral stimulus will cause the response to occur even in the absence of the unconditioned stimulus. If humans do indeed mouthwater in anticipation of food, then theoretically this process is trained through learning how sight or smell predicts the in-mouth sensations of food. This process is a naturally occurring classical conditioning process—the brain learns that the other sensory cues of a food predict the saliva-stimulating sensations that will occur in the mouth.

The question of whether or not humans can be classically conditioned to salivate has been asked before, with mixed results. Some data indicate conditioning is not possible in humans (Brown & Katz, 1967;

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Brown, 1970; Kerr, 1961; Lashley, 1916), while others show that type of stimulus, time periods between exposures, method and source of saliva collection, and other factors can vastly change the success or failure of a salivary conditioning experiment in humans (Blumberger & Glatzel, 1968; Holland & Matthews, 1970; Ilangakoon & Carpenter, 2011; White, 1978). Type of stimulus is particularly relevant to consider when comparing the literature, as both food-related, (images of food, actual food, observing others eat, etc.; used to represent previously conditioned stimuli) and non-food-related stimuli (buzzers, lights, etc.; used to study the acquisition of conditioning) have been used (Blumberger & Glatzel, 1968; Brothers & Warden, 1950; Holland & Matthews, 1970). Even when conditioning has been documented, the conditioned response can be weaker than the unconditioned response (Blumberger & Glatzel, 1968; Brothers & Warden, 1950).

Consequently, we are revisiting the concept of salivary conditioning in humans. While the concept is not particularly novel, the prevalence of two opposing views justify (indeed, they require) new data to determine whether or not this phenomenon occurs consistently in humans. We hypothesized that if we used a particularly strong salivary stimulus (sour taste), maintained an adequate time period between stimulations, and collected whole mouth saliva rather than isolating a single gland (as the equipment for collecting isolated saliva makes the experience less like normal eating), we would be able to achieve and document conditioning of salivary flow in humans. Notably, our experiment is not designed to test whether salivary glands are actively creating more saliva, but only to measure the amount of saliva that is actually expressed into the oral cavity, as that is the functional end point of interest in ingestive behavior research.

2. Materials & methods

Participants between the ages of 18 and 45 were recruited from Purdue University's campus and surrounding area. Participants that had a history of taste or smell disorders; issues with too much or too little saliva; food allergies; tongue, lip, or cheek piercings; color blindness; or smoked within the past 30 days were excluded. Participants were asked whether or not they liked sour candy and how often they consumed sour candy. Written informed consent was obtained prior to beginning the study, and participants were compensated for their time. All recruiting and testing procedures were approved by the Purdue Institutional Review Board for Human Subjects Research. For all experiments, participants were instructed to drink a 500-mL bottle of water (Ice Mountain Spring Water, Nestle Waters NA) at least 1 h prior to their appointments and to refrain from eating or drinking anything else during the hour prior to testing time. Participants were told that they would receive a series of 14 opaque cups with either two pieces of candy (sour variety, red, strawberry flavored Skittles®, Wrigley) or two pieces of a non-food control (referred to as "paper" hereafter, shown in Fig. 1). The "paper" was actually steel hexnuts, size 10–32, wrapped in light blue adhesive paper; these were used to aid in controlling for the sound and feel of the candies rattling in the cup when it was picked up. On the lids of each opaque container was taped one of two possible simple images (diamond or star, shown in Fig. 1). The images were consistently paired with either candy or paper for each participant. Participants were not explicitly told at the beginning of the experiment which image would be paired with which type of stimulus, but they were told that the image and contents pairing would be consistent. Cups were placed upside down on trays in front of the participant so they could not see the images before it was time to taste each sample. All participants completed two visits at least two days apart. Initial statistical power analysis indicated that 10 participants would be sufficient to detect an effect of conditioning on salivary flow; however, the study was stopped after 8 participants because every participant in the study showed the same pattern for the first visit, and additional testing of two more participants would not have changed the outcome. Further, analysis of the data collected indicated within-subject correlations for salivary flow were much higher than anticipated (0.93 observed, 0.75 used in power calculations).

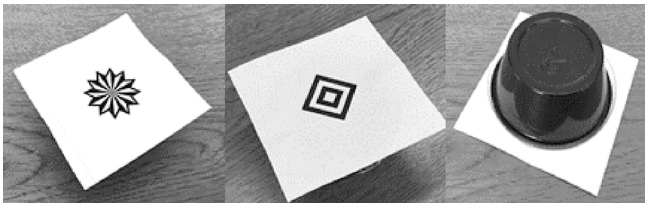

Fig. 1. Images on lids and appearance of cups as seen by participants.

An overview of the conditioning protocol is shown in Fig. 2. A total of 14 sample presentations was conducted for each participant. Half the cups contained candy and the other half paper. Sample order was semi-randomized, ensuring that samples 1 & 2 and 13 & 14 each included one candy and one paper sample. For each sample presentation, participants were instructed to swallow all saliva in his/her mouth, pick up the cup, look at the image on the lid and think about eating the contents for 15 s (timed by researcher). Participants were instructed and reminded not to swallow during the 15 s. For presentations when saliva was collected, the participant next placed two pre-weighed cotton dental rolls in the mouth and rolled them around to collect saliva (approximately 5 s). Participants had not seen the contents of the cup at this point, only the image on the lid. After removing the cotton dental rolls, participants removed the lid of the cup. If the cup contained candy, the participant ate the candy. The participant then rinsed with water, and a three-minute wait was imposed before repeating the process. The overall procedure is shown in Fig. 3.

Preliminary tests indicated that collecting saliva after every sample presentation led to mouth pain, likely because we had removed all the saliva that would buffer against the change in pH caused by the citric acid-coated candies. Because of this, we originally restricted saliva collection to samples 1 & 2, 7 & 8, and 13 & 14 (participants 1–3). Participants still noted some mouth discomfort, so we only collected saliva for samples 1, 2, 13, & 14 for participants 4-8. All data is available in the supplemental data. Participants were not told that saliva would only be collected at specific time points. Instead, they were told that we would collect saliva after some, but not all, samples.

All cotton dental rolls for saliva collection were weighed prior to use, and then again upon removal from the mouth. The initial weight of the rolls was subtracted from the final to calculate the mass of saliva generated. Saliva collection equipment (such as the Lashley cup, commonly used in salivary research) was intentionally avoided, as these methods present an artificial environment that may disrupt the natural eating experience. While simply spitting is commonly used to measure salivary "flow" in the nutrition and food science fields (Dsamou et al., 2012; Murugesh et al., 2015; Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012; Silletti, Bult, & Stieger, 2012), we avoided this method as spitting could be altered by the subject willingness or motivation to expectorate (Running & Hayes, 2016).

Paired *t*-tests were used to compare saliva generated while viewing:

1) Candy image compared with paper image, visit 1, first viewing (samples 1 & 2). These points were not expected to be different, as the images meant nothing at the beginning of the test.
2) Candy image compared with paper image, last time in visit 1, first time in visit 2, and last time in visit 2. At all of these time points, we expected the candy image to stimulate more saliva than the paper image. Respectively, the comparisons at these time points confirm whether or not conditioning was successful (last viewing visit 1); was maintained across days (first time, visit 2); and was maintained/reinforced through the end of the last visit (last time, visit 2).
3) First time compared with last time visit 1 and visit 2, for candy images. In visit 1, the last time was expected to generate more saliva than the first, if conditioning was successful. The test at visit 2 was