The Physiological Effects of Anticipation In Regards to Sour Taste Experience

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Key Words: Anticipation, Electromyography, Feed-Forward, Galvanic Skin Response, Heart Rate, Sour

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Abstract

After consuming sour food, the body's response is to produce saliva in order to neutralize the acidity. The physiological response of increased saliva production in response to sour taste is accomplished by increased muscle contraction and salivary gland activity. Additionally, increased heart rate and sweat production is observed when subjects are administered a sour stimulus. In this experiment, the feedforward response to sour food was tested by feeding participants Warheads Sour Candy. The purpose of this experiment was to determine if the responses between eating a sour candy and the anticipation of eating one were similar. Participants were unknowingly given a Warhead and then their physiological responses (electromyography, galvanic skin response, heart rate) were recorded. They were then presented with a second Warhead to measure their anticipatory (feedforward) response. The results of this study showed statistical significance for heart rate between anticipation and stimulation. There was no significance observed for galvanic skin response (GSR) and electromyography (EMG). Our hypothesis was not fully proven, thus further studies will be needed to more definitively explain the physiological effects of sour food and possible feedforward mechanism involved.

Introduction

For humans there are 5 categories of taste sensation: sour, sweet, umami, bitter and salty, all of which have specialized cells that reside on the taste buds of the tongue. These various tastes are detected and transduced by many different pathways, all of which lead to varying physiological responses. (Iwata et al., 2014). Sour taste causes some of the most interesting physiological responses such as puckering of the facial muscles, increased saliva production and increased heart rate. The human body has evolutionarily developed to respond to sour food by

producing more saliva, which dilutes the acid that causes the sour sensation (Takayanagi et al, 1995). There is also evidence to suggest that muscle contraction in the neck during puckering stimulates saliva production (Lohmander, Kiliardis, and Sjögreen, 2011). Additionally, studies have shown that when a sour food is consumed, both heart rate and sweat production increase (Rousmans, 2000). These studies document typical physiological responses to sour food when consumed, but it remains unknown what occurs when people are *anticipating* the consumption of sour food.

Pavlov discovered that animals can be conditioned to display specific anticipatory behavior towards various foods they have experienced in the past (Preston, Knutson, and Kringelbach, 2014). A more recently discovered and applicable phenomenon called feedforward control may also be of consequence. Feedforward control occurs when one knows that a certain stimuli that they have already experienced in the past is about to occur, and results in the typical physiological responses before the stimulus is even applied (Raff, Strang, and Widmaier, 2015). In terms of anticipatory behavior in regards to taste, it has been shown that when subjects were allowed to look at certain foods associated with a sour taste, salivary flow increased due to anticipatory effects. (Christenson, 1984). Additionally, according to more recent studies, rats and humans display stereotypical expectation and anticipatory responses to certain foods when either trained to do so or when given visual cues relating to various tastes (O'Doherty et al, 2002, Gardner and Fontanini, 2014). While there have been many studies that researched physiological responses to sour food and anticipation of sour food, there has been little research that has combined these two ideas.

Thus, a question is raised: How do the physiological effects that occur during anticipation of consuming a sour food (sweating, heart rate, and muscle activity) compare to those that occur when the food is consumed? We hypothesize that similar physiological effects will occur during consumption of, and in anticipation of consumption of sour food. We believe that once the subject has eaten the sour food once, their body will be familiar with the response necessary to cope with sour foods and will remember this stimulus. Additionally, we believe that by informing the subject that they are going to eat the sour food again, their brain will process this information, remember the previous response to this stimulus, and the physiological changes will begin to occur before the subject eats the sour food.

To test our hypothesis, we will carry out three physiological measurements to determine if the anticipation of eating a "Warhead" (a sour candy) will create the same effects as actually eating one. We will measure muscle activity in the neck (correlated with salivary gland activity), amount of sweat that is produced, and the heart rate of the participant throughout the experiment to record their reactions when consuming a Warhead compared to when they are anticipating consuming one, thus testing our hypothesis.

Materials

In this study, physiological changes due to anticipation of consuming sour foods were tested. These changes were observed by measuring heart rate levels, muscle activity in the neck (which will indirectly measure salivary gland activity) via electromyography (EMG), and galvanic skin response which measures sweat production (GSR), all according to the Biopac Manual. A Pulse Oximeter Model 9843 (Nonin, Medical, Inc.) was used to measure heart rate.

To measure galvanic skin response (GSR), a BSL Electrodermal Activity (EDA) Finger Electrode Xdcr *SS3LA* (BIOPAC Systems Inc.) was used, along with an Isotonic Recording Electrode Gel *101*. Finally, a BSL TP Electrode Lead Adapter *SS1LA* (BIOPAC Systems Inc.) connected to Adhesive EEG disks *ADD204* for 4 mm electrode was used to measure electromyography (EMG) in salivary muscles. The EMG required Electrode Gel *100*, 227 g tube. Both the GSR and EMG were used in conjunction with BIOPAC software (Biopac Systems Inc., Model MP36). Other miscellaneous materials included a food survey, participation consent form, Premium Plus saltine crackers, and Warheads Extreme Sour Hard Candy (Black Cherry flavor).

Methods

Before beginning data collection with any participants, the heart rate monitor, galvanic skin response, and electromyography equipment were tested on an experimenter before and after eating a Warhead. The resting rate of all three measurements was taken before administration of a Warhead. The Warhead was then consumed, causing all three measurements to display substantial deviation from the observed baseline. This information served as a positive control to demonstrate that measurable change is attainable in the physiological tests (heart rate, GSR, EMG). The positive control data can be seen in Figures 1, 2 and Table 1.

Prior to active participation, students in Physiology 435, Lab section 602, were given a food survey to fill out, checking off items they would be willing to consume (Appendix A). This helped determine who would be willing to eat a Warhead without revealing the goal of the study. Students who were not willing to eat sour candy were removed from consideration. Consent

forms were formatted and approved by the UW Madison Physiology Department, and were sent to eligible students via email (Appendix B).

To begin, students were led into a room that was secluded and prevented any outside distractions or stimuli that may have affected the results. Participants were instructed to sit up straight with both feet firmly planted on the floor in front of them while looking straight ahead. Participants were hooked up to all three physiological measurement instruments at the beginning of the experiment which remained attached throughout the duration. The GSR was placed on the index and middle fingers of the participant's dominant hand, the pulse oximeter on the index finger of the non-dominant hand, and three EEG electrodes were placed under the mandible as shown in Figure 3. Participants were also instructed to place their hands on their laps, palm up, to ensure consistent positioning of all subjects during data collection. EMG and GSR measurements were measured continuously via BIOPAC and saved; the measurements were later statistically analyzed. The participant's resting heart rate was taken before any experimentation began. The participant was then knowingly given a saltine cracker to normalize each participant's palate so that everyone consumed the same food and went back to baseline before ingesting the Warhead.

After the participant finished the saltine, they were instructed to close their eyes and were given a Warhead without being told what they were eating. Heart rate was recorded every 5 seconds during the consumption of the Warhead for a total of 35 seconds. Participants were told to not move their jaw at all while eating the Warhead to prevent erroneous muscle activity measurements from being recorded, and were instead told to use their tongue to move the Warhead around in their mouth if the sour sensation became too extreme. Time on the BIOPAC measurements was noted when the Warhead was first ingested. After one minute, participants

spat out the Warhead and were given one minute to return to baseline levels and were offered water if needed. To eliminate any residual sour flavor and to normalize their palate again, participants were given another saltine cracker.

Participants were then told they were going to consume another Warhead candy. Heart rate was recorded immediately after they were told this. A time period of 15 seconds was allowed before handing the subjects the Warhead to consume to allow for 3 heart rate recordings and also ample time for muscle activity and GSR responses to occur. The subjects were then allowed to consume the Warhead with continuous recording of all data, in order to collect extra data in case it was needed later on for analysis. The BIOPAC time was recorded when the participants were told about the candy, and when they finally consumed the candy.

To begin data analysis, a baseline was recorded for each physiological instrument. All were taken from the same time point and before the participant was given the first saltine. To determine stimulation of the Warhead, the time frame from when the participant first puts the Warhead in their mouth until about 30 seconds later is taken into account. Within that time frame, the largest value was taken for heart rate and GSR and the largest amplitude signal is taken for EMG. To determine the anticipation of the Warhead, the time frame from when the participant is told they will eat another Warhead until they are actually given the second Warhead is highlighted. The values are collected the same as they were for stimulation.

Results

EMG. To extract data from the recordings to analyze, the EMG trace with the highest amplitude within the time interval for each treatment was used. A two-tailed t-test was then conducted to analyze two treatments comparative to each other, cross-analyzing all treatments, with the results as follows. Figure 5 summarizes the results pertaining to the main interest for this study, comparing anticipation of ingesting a Warhead and eating a Warhead (also referred to as "stimulation treatment" elsewhere). A difference of 0.3886 mV was found between the two treatments which was insignificant ($p = 0.0917$). In addition to this analysis, the baseline/stimulation was also compared (difference of 2.127 mV, $p = 4.595 \times 10^{-5}$) and baseline/anticipation (1.7388 mV, $p = 6.117 \times 10^{-6}$), both of which resulted in significant differences between the two treatments (Tables 3 and 4).

GSR. To extract GSR data from the recordings to analyze, the maximum value within the time interval was found for each treatment. A two-tailed t-test was then conducted to cross-analyze all treatments. Figure 6 shows that the difference of 0.0839 microsiemens between the anticipation treatment and the Warhead (stimulation) treatment was insignificant ($p = 0.706$). Additionally, there was found to be no significant difference between the baseline and anticipation treatments (0.7157 microsiemens, $p = 0.06961$), but there was a significant difference of 0.7997 microsiemens ($p = 0.00842$) that was found between the baseline and stimulation treatments (Tables 3 and 4).

Heart Rate. To extract data to analyze for heart rate, all heart rate data points within a treatment were averaged to create a mean value for each treatment. A two-tailed t-test was then conducted to cross-analyze all treatments and test for significance. Figure 7 shows that there was

a significant difference for the mean heart rate of the anticipation treatment compared to the stimulation treatment ($p = 0.0369$) with a difference of 3.789 beats/min. For the baseline/stimulation comparison, there was a difference of 15.263 beats/min between the two treatments with a significant associated p-value of 3.406 x 10^-9 (Table 3). As for the baseline/anticipation comparison, there was a difference of 11.474 beats/min with a significant associated p-value of 5.942 x 10^-6 (Table 4).

Discussion

The results of this study do not fully support our hypothesis that there is no difference between the physiological effects of consuming a sour food and anticipating the consumption of a sour food. There was a significant difference ($p < 0.05$) observed between the stimulant and anticipatory response in the heart rate measurements. It appears that the anticipation response was not similar enough to the initial stimulus to confirm our hypothesis. In EMG and GSR measurements, there was no significant difference found ($p > 0.05$) between the anticipatory and stimulus responses (Table 2). This suggests that our hypothesis was correct and the physiological responses for anticipation and stimulation of sour taste are extremely similar.

However, there is statistical significance when comparing anticipation and stimulation values to baseline values. Although this was not a part of our original hypothesis, it is important to note that there were statistically significant physiological differences between the tests and the baseline because this provides the basis for our hypothesis. The values for stimulation and anticipation may not have been close enough to one another, but both their values differed

significantly more from the baseline. This helps supports our hypothesis by showing that stimulation and anticipation responses both greatly differed from the baseline measurements.

Limitations

Some limitations with experimental conditions may have affected the results of this study. The placement of the EMG electrodes may have skewed the data because movements of the jaw might have been recorded when the focus was on puckering of the cheeks or saliva production. During one participant's experiment, the EMG sensors became disconnected and therefore recording stopped, reducing the sample size for EMG. During a few other participants' trials, GSR readings had a tendency to gradually decline throughout the experiment. Even though increased responses were observed when the Warhead was administered, due to the gradual decline these data points were still skewed in comparison to the baseline. There were also confounding variables that could have impacted the study. For example, inconsistency in Warhead flavor could have led to variability in sour flavor and therefore a variability in response. Participants that talked or laughed while eating the Warhead could have caused peaks in the EMG reading not due to puckering. Additionally, having participants close their eyes before eating the first Warhead could have triggered a nervous response not related to the sour candy.

Overall this study had a strong experimental design that stayed consistent throughout the entire study. Some ways that we stayed consistent were by following a strict experimental procedure with each participant and having each instrument managed by the same experimenter throughout the study. Also, based off findings in a study by Christina et al., we tried to eliminate adverse taste effects that could have been caused by various foods each participant might have

ingested up to 30 minutes prior to the study by feeding each participant one saltine cracker before the stimulation trial and the anticipation trial to attempt to cleanse their palate.

Future Directions

In future experiments, there could be beneficial changes made in order to better the study. In response to the skewed EMG data from participant jaw movement, the electrode placement could be moved from the neck to the upper cheek. This would allow pucker response to be measured without the electrodes being activated by unrelated jaw movements. Additionally, more time for the anticipatory response should have been allowed for the participants to build a more defined physiological effect. Our hypothesis was also harder to prove compared to the converse, and as such, it would be suggested for future studies to try to prove the existence of significant differences between groups, and not attempt to prove no such difference occurs.

Conclusion

Studies have shown that when subjects experience anticipation it is usually in the form of responses such as increased salivary flow, heart rate, and sweat production (Christenson, 1984). The purpose of the study was to investigate the feedforward response of anticipating the consumption of sour food. While statistically significant data supporting our hypothesis of no difference between anticipation and stimulation wasn't found, we did find a statistically significant difference between each and the baseline. These findings do support the occurrence of feedforward mechanisms in the human body. Understanding these mechanisms is important when applied clinically to other instances involving anticipation. One such situation would be where a person associates stress with a certain object, and seeing this object causes an

anticipatory response. Both of these situations involve seeing a stimulant and having an innate anticipatory reaction causing distress.

Acknowledgements

We would like to thank Dr. Lokuta, the teaching assistants and peer learning volunteers involved with Physiology 435 for assisting us during the research process, and the University of Wisconsin-Madison for allowing us to use their facilities to carry out our study. Additional thanks to Alan Sayler for assisting with statistical analysis. Lastly, we would like to thank our fellow students that participated in our study.

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Figures & Tables

Figure 1: This figure shows the positive control data for the galvanic skin response. The red arrow is pointing to the baseline measurement (minimal sweating). The green arrow is pointing to the exact moment the Warhead was ingested by the subject. The purple arrow is pointing to the sharp increase in galvanic response, or sweating, in the subjects middle and index finger after consumption of the Warhead.

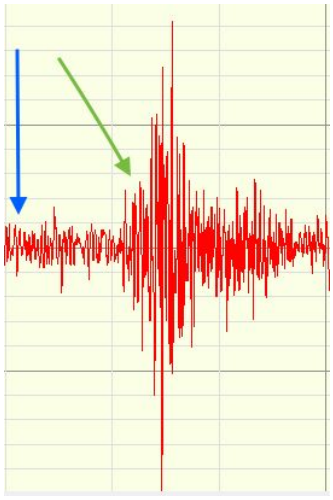

Figure 2: This figure shows the positive control data for the electromyography (EMG). The blue arrow is pointing to before the Warhead was ingested, or the baseline data for salivary muscle responses. The green arrow is pointing to when the Warhead was ingested, showing an immediate response. This response was the puckering of the subject's mouth, causing a reading on the EMG.

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Time Administered	Heart Rate (ppm)
Baseline (No Warhead)	58
Warhead Ingested (0 seconds)	69
5 seconds	66
10 seconds	71
15 seconds	74
20 seconds	72
25 seconds	72
30 seconds	68

Table 1: This table shows the positive control data for heart rate. A baseline heart rate was taken, to compare back to when determining if there was a response to the sour candy or not. The Warhead was then administered to the subject and their heart rate was monitored starting there and for the following 30 seconds.

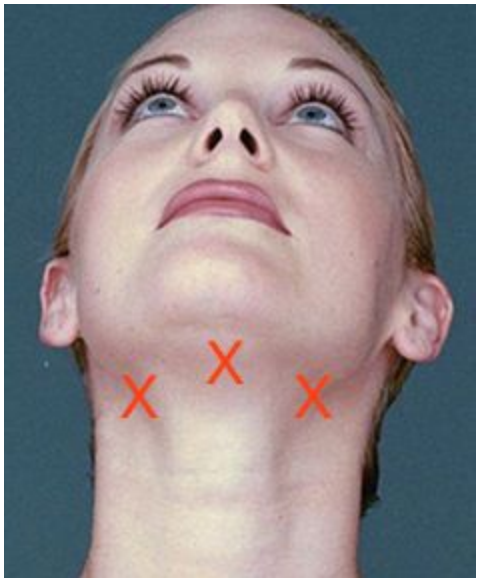

Figure 3: This figure demonstrates the placement of the EEG electrodes on a participant's neck. The red X's mark where one would place each electrode, which are used to measure muscle movement in this area, associated with puckering and salivation.

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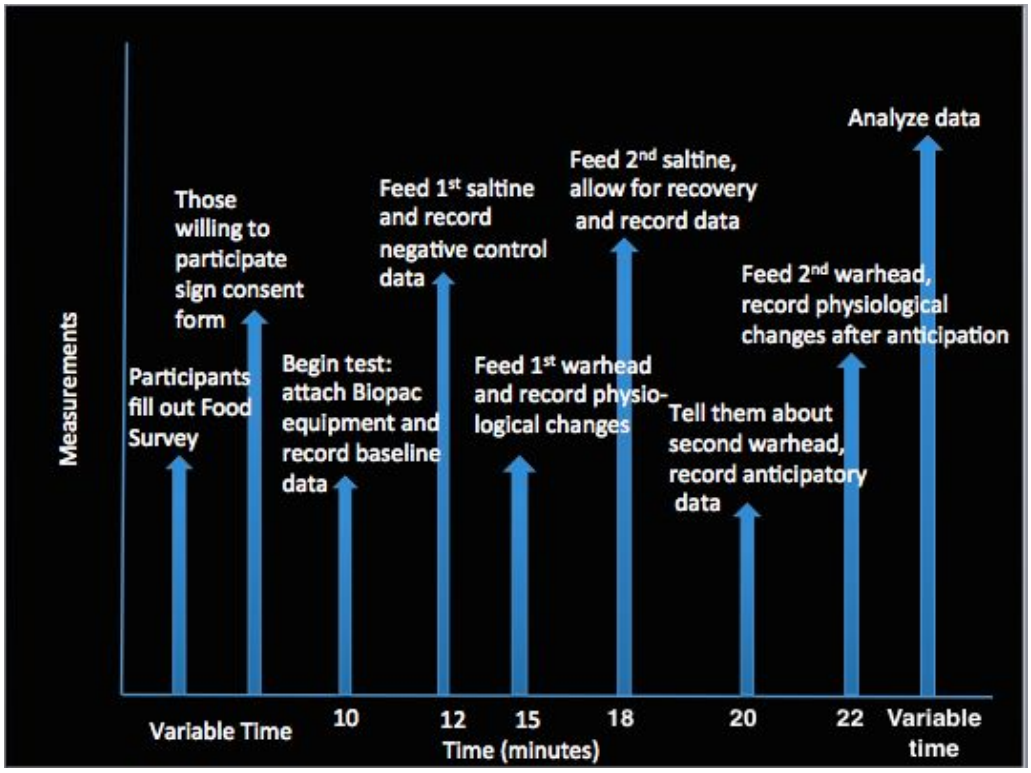

Figure 4: This figure is a flowchart of the timeline of events for our experiment. It clearly lists out each summarized step, including the few that were taken before actual testing began.

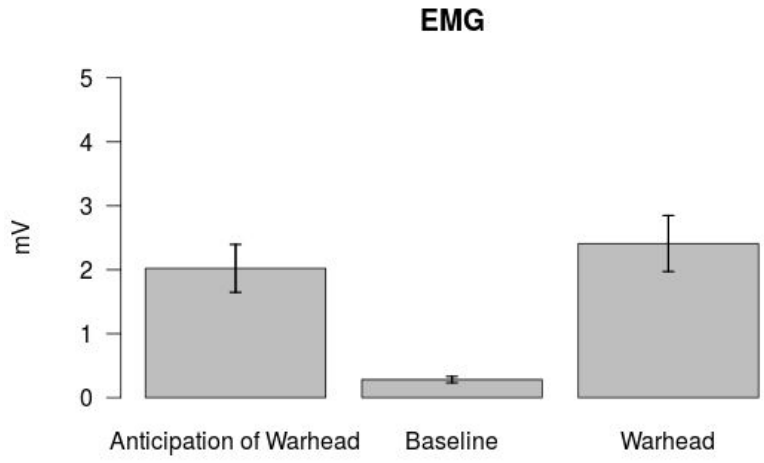

Figure 5: Graph of the average maximum EMG measurements for the anticipation treatment and the Warhead (stimulation) treatment. There was no significant difference between the two conditions ($p = 0.0917$, two-tailed t-test).

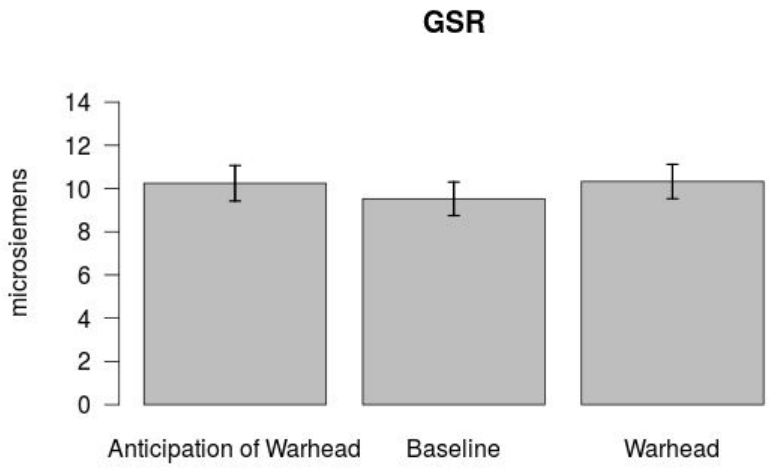

Figure 6: Graph of the average maximum GSR measurements from the anticipation treatment and the Warhead (stimulation) treatment. There was no significant difference between the two treatments ($p = 0.706$, two-tailed t-test).

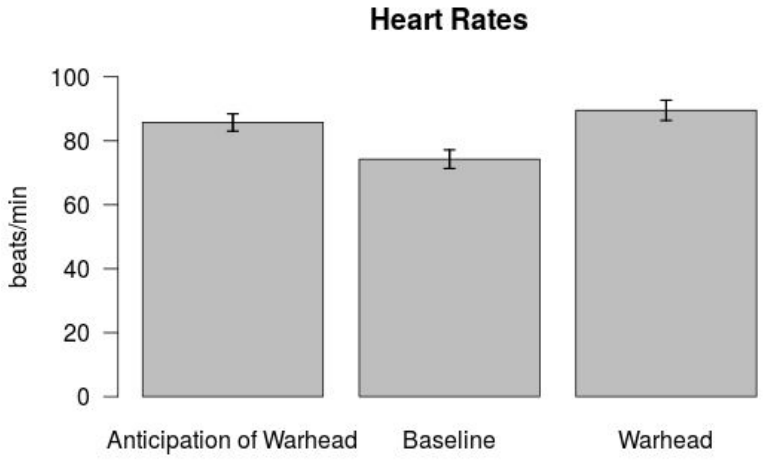

Figure 7: Graph of the average heart rate during the anticipation treatment and the Warhead (stimulation) treatment. There was a significant difference between the two ($p = 0.0369$, two-tailed t-test).

	Anticipation Versus Stimulation Tests		
Measurement	**EMG (mV)**	**GSR (microsiemens)**	**Heart Rate (bpm)**
Change	**0.3886**	**0.0839**	**3.789**
P-Value	**0.0917**	**0.7086**	**0.0369**

Table 2. P-values for all three measurements for the anticipation treatment and the ingestion of a Warhead treatment. The only test measurement in which there was a significant difference between treatments was heart rate.

	Baseline Versus Stimulation Tests		
Measurement	**EMG (mV)**	**GSR (microsiemens)**	**Heart Rate (bpm)**
Change	**2.127**	**0.7997**	**15.263**
P-Value	**4.59497E-05**	**0.008427532**	**3.40615E-09**

Table 3. P-values for all three measurements for the baseline treatment and the ingestion of a Warhead treatment. All three measurements showed a significant difference between treatments with EMG and heart rate resulting in extremely small p-values.

	Baseline Versus Anticipation Tests		
Measurement	**EMG (mV)**	**GSR (microsiemens)**	**Heart Rate (bpm)**
Change	**1.7388**	**0.7157**	**11.4738**
P-Value	**6.11755E-05**	**0.069518441**	**5.9426E-06**

Table 4. P-values for all three measurements for the baseline treatment and the anticipation treatment. Heart rate and EMG resulted in significant differences between the two treatments, while GSR was slightly above the 0.05 benchmark for significance.

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Appendix A

Food Choice Survey

We hope you will be a fit for our new physiological study! Please check below, agree or disagree, which foods you would be willing to consume. Please include a reachable email:

Food Type	Agree	Disagree
Sweet		
Sugary Candy		
Ice Cream		
Pastries (i.e. macaroons, cupcakes, etc.)		
Bitter		
Alcohol		
Grapefruit		
Caffeine (coffee)		
Sour		
Lemons/Limes		
Sour Candy (i.e. Warheads, sour straws, etc.)		
Sour Drinks – Green Apple soda, etc.		
Salty		
Potato Chips		
Bacon (or other cured meat)		
Pizza		
Umami		
Mushrooms		
Fish (salmon, cod, etc.)		
Green Tea		
Textured		
Mushy (i.e. mashed potatoes)		
Crunchy (chips, toasted bread, etc.)		
Slimy/Juicy (i.e. tomatoes, cooked mushrooms, etc.)		
Thank you for completing our survey! We will review the results and get back to you on whether or not you are qualified to participate in our study.		

Appendix B

Consent to Participate in a Research Study
UW-Madison ● Madison, WI

Title of Study: Physiology 435 Lab 602, Group 12

Investigators:

Name: Erin Denton **Email:** edenton@wisc.edu
Name: Luke Fowler **Email:** lmfowler@wisc.edu
Name: Chloe Lang **Email:** cblang2@wisc.edu

Introduction
- You are being asked to be in a research study of physiological responses to consumption of certain foods.
- You were selected as a possible participant because of your answers to a food survey that was previously distributed.
- We ask that you read this form and ask any questions that you may have before agreeing to be in the study.

Purpose of Study
- The purpose of the study is to determine what physiological responses occur in response to eating certain foods.
- Ultimately, this research may be published in an online scientific journal.

Description of the Study Procedures
- If you agree to be in this study, you will be asked to do the following things:
  - Be measured by three different Biopac devices
  - Consume food given to you by experiment conductors while in different conditions.
- You can expect your participation in the experiment to last approximately 20 minutes.

Risks/Discomforts of Being in this Study
- There are no reasonable foreseeable (or expected) risks. There may be unknown risks.

Benefits of Being in the Study
- There are no expected benefits to the participants in this study.

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Confidentiality

- This study is anonymous. We will not be collecting or retaining any information about your identity.
- Refrain from sharing specific study design details, research purpose, or any of your experiences from this experiment, as it is crucial for our future participants to have no prior knowledge of the specifics of this study.

Right to Refuse or Withdraw

- The decision to participate in this study is entirely up to you. You may refuse to take part in the study *at any time* without affecting your relationship with the investigators of this study or the University of Wisconsin-Madison. Your decision will not result in any loss or benefits to which you are otherwise entitled.

Right to Ask Questions and Report Concerns

- You have the right to ask questions about this research study and to have those questions answered by me before, during or after the research. If you have any further questions about the study, at any time feel free to contact one of the investigators using the contact information at the top of the form. If you'd like, a summary of the results of the study will be sent to you.

Consent

- Your signature below indicates that you have decided to volunteer as a research participant for this study, and that you have read and understood the information provided above. You will be given a signed and dated copy of this form to keep, along with any other printed materials deemed necessary by the study investigators.

Subject's Name (print): _____

Subject's Signature: _____ Date: _____

Investigator's Signature: _____ Date: _____