Measurement of Physiological Changes due to Anticipation of Sour Sensation

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Abstract:

Physiological response to sour stimuli is initiated by the activation of taste receptors in taste-bud cells. When the sour stimuli has been applied repeatedly, memory and experience may lead to anticipatory responses in the human body. These physiological responses are usually attributed to electrical signaling in the brain and muscle, which can be measured non-invasively with an electroencephalograph (EEG) and electromyograph (EMG), while heart rate can be detected with a pulse oximeter. Our findings demonstrate that overall, anticipatory heart rate, EEG and EMG tracings did not differ significantly from baseline values. Upon stimulation with a Warhead candy, however, heart rate and EEG values did change significantly from baseline. Male and female participants also differed in how they responded to sour stimuli. Males were less likely to move their scalene muscle throughout the experiment than females, while female heart rates were less likely to be affected.

Introduction:

Taste sensation can be divided into five basic categories: sweet, bitter, sour, salty, and umami. The ability to detect each taste stimuli relies on the activation of taste receptors in taste-bud cells. Mechanisms involved in this response include membrane receptors, guanine-nucleotide-binding proteins, second messengers, and ion channels (Ugawa et al., 1998). The process of recognizing sour tastes is initiated by protons acting at receptor proteins or channels (Lindemann et al., 2001). Anticipatory responses, also known as feed forward control, could arise if taste recognition is a repeated event. This phenomenon is often linked to learned responses to known stimuli. For example, heart rate can increase prior to engaging in rigorous physical exercise in anticipation of movement. The prediction of an event is based on memory and experience, which contribute to an unexplained predictive anticipatory effect (Mossbridge et al., 2012).

All information, as well as memory and experience, is transmitted through electrical signaling in the body and there are methods for recording that electrical activity. Hummel et al. utilized gustatory event related potentials (ERP) and detected that participants experienced a stronger response to a stronger sour stimuli as compared to a weaker one. However, they detected no significant difference for intensity ratings when comparing sexes nor when stimuli were presented to the left or right side (Hummel et al., 2009). Since the ERP was able to detect these responses to sour stimuli, other basic tests measuring electrical activity should be able to detect similar changes as well.

The electroencephalography (EEG) and electromyography (EMG) measure brain activity and muscle movement, respectively. An EEG is capable of recording complex patterns of neurological activity, and has been used effectively to monitor alertness, awareness of sensation, and cognitive engagement (Teplan, 2002). As all physiological responses involve brain activity, the EEG has the ability to detect whether or not an unpleasant sour taste will trigger electrical signal changes. The EMG is a noninvasive method used clinically to measure muscle movement, which is known to be a part of the human body's "fight or flight" response. Any anticipatory or stress reactions in the muscle will register on the EMG.

The sour taste of a Warhead candy is known to make individuals react by tensing their jaw and muscles around it. Individuals tend to take a moment to brace themselves for the intense reaction they are about to experience. A similar anxious feeling occurs when individuals anticipate giving a speech or getting on a roller coaster. The sympathetic nervous system is activated during stress or anxiety, it often affects the sinoatrial node and increases its rate of firing (Reisner et al., 2007). Interpretation of heart rate data will reveal any deviations from normal resting heart function.

We hypothesize that there would be a discernable difference across all parameters between baseline and anticipatory tracings as well as between baseline and stimulated tracings. The heart rate and the mean amplitude and frequency of the EEG and EMG should go up as a person anticipates a sour taste and even more so when the sour taste is experienced.

Materials and Methods:

Eight males and nine females enrolled in Fundamentals of Human Physiology 435 at the University of Wisconsin Madison participated in this study. We gathered data using electroencephalography (EEG), electromyography (EMG), and heart rate. Data collection was made possible by BIOPAC recording and programming equipment. A combined EEG tracing was used; individual wave data (alpha, beta, gamma, theta) did not prove useful during preliminary testing. The baseline EMG wave was used as well as the integrated EMG tracing. During the experiment, participants were placed in two environments. One was a quiet and dark environment, the other being a dimly lit hallway that was prone to bystanders walking by occasionally, as well as random opening and closing of doors. They were asked to sit in a relaxed position with both feet on the floor, hands on their lap, and eyes closed. Electrodes recording EEG measurements were placed at specific points on the participants' heads. The shield was placed on the mastoid region while the additional two electrodes were placed on each temple. Electrodes recording EMG measurements were placed on the scalene muscles of each participant's dominant side. Finally, a pulse oximeter was attached to the index finger of the participant's dominant hand. Data was collected continuously throughout the duration of the experiment, with a researcher noting the exact time when different phases took place. Each phase could then be identified when analyzing the data.

The first phase of data collection included the initiation of EEG, EMG, and heart rate measurements. Baseline levels were gathered with varying durations for each participant. This was done in order to allow each participant to completely calm down before we began further data collection. A 16 second interval that clearly depicted this calmness was present in all baseline levels collected. Thus, this was the time interval chosen to be analyzed. The period when the proctor informed participants that they would soon ingest a sour Warhead candy is the second phase. This was to ensure that data collected during the second phase is ignored during analysis since response to verbal stimuli would render measurements inaccurate. The third phase of data collection was the anticipatory phase and durations varied depending on the participant. This phase continued until a maximum heart rate was observed, and then participants were told to consume the Warhead candy. The amount of time required for the proctor to provide this instruction was the fourth phase, and would be ignored during data analysis. The fifth phase of data collection recorded the subsequent physiological changes caused upon consumption of the Warhead candy. This phase continued until a maximum heart rate was observed and further data collection was stopped once the heart rate stabilized.

Resting EEG, EMG, and heart rate tracings will be the negative control, as subjects should not experience physiological changes at the beginning of the experiment. The EEG, EMG, and heart rate measurements recorded upon consumption of the Warhead candy will be the positive control. Any deviations from measurements at rest will demonstrate potential physiological changes throughout the testing process.

For EEG and EMG data analysis, the minimum and maximum peak values were recorded from each testing phase (baseline, anticipatory, and stimulated) because these indicate that there was a large amount of neuronal activity taking place at that instant. The absolute values of the minimum and maximum values were compared and the larger of the two was used for further analysis. This method was used because the directionality of depolarization in neurons determines the sign of the EEG and EMG tracings. We wanted the maximum neuronal activity, which is represented by the absolute amplitude of the waves. The percent change in the maximum amplitude was calculated between the baseline versus anticipatory phases and baseline versus stimulated phases for EEG and EMG, while the percent change between those phases were also calculated for the maximum heart rate of each phase. A two-tailed t test was used to analyze the max amplitude peaks between the baseline versus anticipatory phase and the baseline versus stimulated phase for EEG and EMG. The t test was also used to analyze the percent change from baseline to anticipatory and the percent change from baseline to stimulated. The two-tailed t test was used to account for the directionality of the percent changes, and is a more stringent test for significance. The time on the EEG and EMG graphs used for the different phases accounted for excess movement such as movement when the participants had to hold out their hands for the candy, as well as the few seconds where they were told to eat the candy. A one-tailed t test was used to analyze the heart rate data, since directionality was not an issue.

Results:

EEG data: Summary data are shown in **Table 1** and **Figure 1** . The baseline tracing obtained from participant 13 was already at threshold (500 μV); therefore, they were considered an outlier and all EEG tracings (baseline, anticipatory, and stimulated) were omitted from analysis. There was no significant difference between baseline and anticipatory maximum amplitudes (P=0.341). However, a significant increase in activity was observed between baseline and stimulated (P=0.017). The percent change between baseline and anticipatory versus percent change between baseline and stimulated showed no significance (P=0.077). Additional data was analyzed taking gender into account. There were no significant differences between baseline and anticipatory nor baseline and stimulated among males (P=0.803, P=0.118, respectively). There were also no significant differences observed among females within the same measures (P=0.350, 0.267, respectively). There was a significant increase observed in the percent change between baseline and anticipatory versus the percent change between baseline and stimulated among males (P=0.009). However, there was no significant difference observed in the same measures among females (P=0.784).

EMG data: Summary data are shown in **Table 2** and **Figure 2**. There were no significant differences between baseline and anticipatory, baseline and stimulated, nor percent change between baseline and anticipatory versus the percent change between baseline and stimulated (P=0.548, P=0.211, P=0.359, respectively). There were no significant differences observed in the same measures among males (P=0.813, P=0.813, P=0.783, respectively). There were also no significant differences observed in the same measures among females (P=0.083, P=0.159, P=0.213, respectively).

Heart Rate data: Summary data are shown in **Table 3** and **Figure 3**. There were no significant differences observed between baseline and anticipatory (P=0.085). However, a significant increase in heart rate was observed between baseline and stimulated, as well as the percent change between baseline and anticipatory versus the percent change between baseline and stimulated (P=0.001, P=9.892E-7, respectively). There were no significant differences between baseline and anticipatory among both males and females (P=0.078, P=0.259, respectively). However, there were significant increases observed between baseline and stimulated among both males and females (P=0.006, 0.035, respectively). There were also significant increases observed in the percent change between baseline and anticipatory versus the percent change between baseline and stimulated among both males and females (P=0.007, P=3.677E-5, respectively).

Discussion:

A lack of significance between the overall baseline and anticipatory EEG tracings was observed, indicating that there was not a significant increase in brain activity during this time. The idea of anticipation resulting from mental recollection of a past experience with a sour stimuli could not be supported because more brain activity in the form of increased EEG amplitude was not observed. An increase in frequency of the EEG data could also denote increased brain activity; however, frequency could not be measured with the equipment available. There was also no significant difference observed by the EMG at this time, indicating that there were no significant muscular movements made from swallowing anticipatory salivation, tensing, or preparation for the sour sensation they were about to endure. Finally, there was no significant difference in heart rate detected within this time period, further indicating that participants remained calm when they were anticipating the sour sensation.

When observing the overall baseline and stimulated measurements, a significant increase in EEG was visible. As the positive control, this shows that participants did demonstrate increased brain activity when enduring the sensations caused by a sour stimulus. This is an understandable result because the participant is experiencing the sour sensation, rather than attempting to remember that sensation from the past. However, there was no significant difference observed in the EMG data, which fails to indicate a discernible amount of muscle contraction from tensing and salivation due to the sour sensation. Heart rate data shows a significant increase, signifying that an increase in heart rate can be expected when participants consume a sour stimulus. The EEG and heart rate measurements show more significant results than EMG data because their physiological responses most likely occur faster. Furthermore, EMG measurements may be occurring in response to the changes occurring within the brain. Another possible explanation for the lack of change in EMG measurements as compared to those obtained from the EEG and pulse oximeter is that one may mute their physical reactions to a sour stimulus whereas they will typically always display a psychological response.

Overall, data pertaining to the percent change between baseline and anticipatory versus the percent change between baseline and stimulated showed no significant changes in both the EEG and EMG. This informs us that the effect of the sour stimulus did not have a significant impact on the EEG and EMG over time. However, there was a very significant increase in the percent change between baseline and anticipatory versus the percent change between baseline and stimulated in heart rate measurements. From this, we believe that the effect of the sour stimulus had a great impact on participants' heart rates over time.

When taking gender into account, there were no significant differences detected between baseline and anticipatory nor between baseline and stimulated EEG results among males. A lack of increased brain activity over both of these spans indicates that the eight males had no significant reaction to both the anticipation and actual consumption of the sour stimulus. However, there was a significant increase present in the percent change between baseline and anticipatory versus the percent change between baseline and stimulated among males. One possible explanation is that there are certain perceivable gender role differences within our society. While males tend to hold in their emotions, women are less inhibited and are more capable of openly expressing their feelings (Brody, 1985). This restraint in emotional expression exhibited in males could contribute to the significant increase in percent change within the aforementioned domains among men, but not women. The increase in percent change is observed since men are more likely to be cognitively thinking about holding in their displeasure upon consumption.

This would lead to an increased level of EEG activity when exposed to the stressful sour sensation. While the maximum amplitudes detected did not indicate a change in EEG activity between the different phases of testing, the percent changes led us to believe that there was an overall increase in male brain activity over time. The high level of variance in the raw EEG data could have contributed to the non-significant p-values calculated; however, because percent change is a relative value to itself, it is worth noting that there was a significant difference between how anticipation and consumption changed the EEG values for males. EMG results show no significant muscle contraction at any time among men. This could be because the male participants were more calm when experiencing the sour stimulus or because their ability to sense a sour stimulus is not very prevalent. Stimulated heart rates did increase significantly among males compared to baseline levels, supporting the fact that it acted as our positive control. This further supported the idea that an increase in heart rate can be observed upon consumption of a sour stimulus. Anticipatory heart rates were not significantly larger than baseline levels; therefore, men did not demonstrate an increase in cardiac output due to the anticipation of the sour stimulus. Over time a significant increase in the percent change between baseline and anticipatory versus the percent change between baseline and stimulated was observed. This could be due to the greater change in heart rate exhibited when actually consuming the sour stimulus as compared to the heart rate measured during anticipation.

There were no significant differences observed between any measurements taken with the EEG in females. This could be due to females maintaining a relatively more relaxed state throughout the testing process. Similarly, EMG results in females indicate that there were no significant changes between any measurements taken. However, females did show a higher degree of change than males when looking at baseline and anticipatory levels. This signifies that females are more likely to display muscle contraction when anticipating the sensation of a sour stimulus as compared to males. There was also a greater degree of difference detected for females than males when measuring the percent change between baseline and anticipatory versus the percent change between baseline and stimulated. Heart rate changes observed in females were similar to that of males. However, the comparison of baseline and stimulated heart rates indicated a greater significance in males than in females. This could indicate that males are more likely to demonstrate an increase in heart rate due to the consumption of a sour stimulus.

There was a large variation between people who participated in this study, which could be seen in the large range of baseline EEG, EMG, and heart rate, as well as their responses to the stimulus. A large factor in our results was the two locations that were used during this experiment. The first location was s foyer of a building that had random people walking past every now and then. The second location was a dark, isolated room. The difference in locations was beyond our control, and provides a source of error. As seen in the EEG data, our two-tailed t test analysis of the percent change between baseline and anticipatory phase and the percent change between baseline and after eating the candy resulted in a p-value of 0.07677. This is approaching significance, especially after removing an outlier from our data. Any further research should be done with more people and try to conduct all the experiments in one location, removing any variance caused by the data from the first location.

Another interesting source of error was that some people either did not know what a Warhead was or they underestimated the sour taste of a Warhead. This could have caused a large change in brain activity and muscle movement after stimulation, but not during the anticipatory phase. It is likely that if the participants were aware of the degree of sourness of a Warhead that anticipation effects would be greater, as seen in some people who were familiar with Warheads. Another observation is the fact that some participants are more sensitive to tastes, known as supertasters, compared to other participants who are not sensitive to taste, known as nontasters. These factors all cause a large variation in the data recorded.

Some participants did not follow the directions given by the proctor, which caused significant changes in EEG and EMG waves. A few were talking when they were being measured for the baseline tracings, which causes both the EEG and EMG to spike since there was brain activity and muscle movement. One person ingested the Warhead before they were supposed to, not giving enough time for anticipatory changes to take place. Some people had been chewing gum prior to participating, and depending on what kind of gum they had been chewing, their sensitivity to sour tastes could have been dulled or changed in other ways, affecting the data.

The last sources of error that affected the data were due to the BIOPAC equipment used during this experiment. There were 3 electrodes placed for EEG and 3 electrodes placed on one muscle for EMG, 1 of each being the ground. This leads to less accurate data being measured by the one BIOPAC machine for an entire physiological representation. Additionally, the threshold of measurement for EEG (Min value of -500 μV and Max value of 499.98474 μV) was reached multiple times by a few participants. This limits what the true values of these EEG values could have been, as all that is known is they were at least to the threshold. Finally, in the analysis of the data, the data points selected for analysis were not completely uniform due to the nature of how the different phases were determined. Since the phases were determined by when the maximum heart rate was thought to be attained for each phase, different participants varied in response times to change their heart rate. The variation in response times in the heart rate led to a different selection of points in time, which could distort the data.

In order to improve upon the results presented, a consistent non-stimulating environment for testing would be required, especially in order to normalize the EEG data which was the most affected by the environment. Less variation in EEG data could lead to significant findings in the changes from baseline to anticipation, being that true individual baseline data would be much easier to identify in the waveform of the raw data and we would not have to rely on merely identifying the most uniform 16 seconds of the baseline time period, as was the method in this study due to the aforementioned error in the EEG data.

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Tables

Table 1. EEG P-Values

	Baseline/Anticipation	Baseline/Stimulated	%Change
Male	0.802754027	0.118271716	0.009342244
Female	0.349765192	0.267068643	0.783678954
Overall	0.340951748	0.017345942	0.076779892

P-values from 2-tail T-test of EEG max amplitude from the overall and when analyzed by gender for baseline v. anticipation, baseline v. stimulated, and % change from baseline to anticipation.

Table 2. EMG P-Values

	Baseline/Anticipation	Baseline/Stimulated	%Change
Male	0.813312922	0.985915376	0.782630949
Female	0.083240542	0.159405619	0.212871825
Overall	0.547732863	0.210567079	0.358757829

P-values from 2-tail T-test of EMG max amplitude from the overall and when analyzed by gender for baseline v. anticipation, baseline v. stimulated, and % change from baseline to anticipation.

Table 3. HR P-Values

	Baseline/Anticipation	Baseline/Stimulated	%Change
Male	0.078174412	0.006335571	0.007385461
Female	0.258806533	0.035077647	0.0000367737
Overall	0.085130254	0.001318675	0.000000989182

P-values from 1-tail T-test of HR max amplitude from the overall and when analyzed by gender for baseline v. anticipation, baseline v. stimulated, and % change from baseline to anticipation.

Figures and Legends

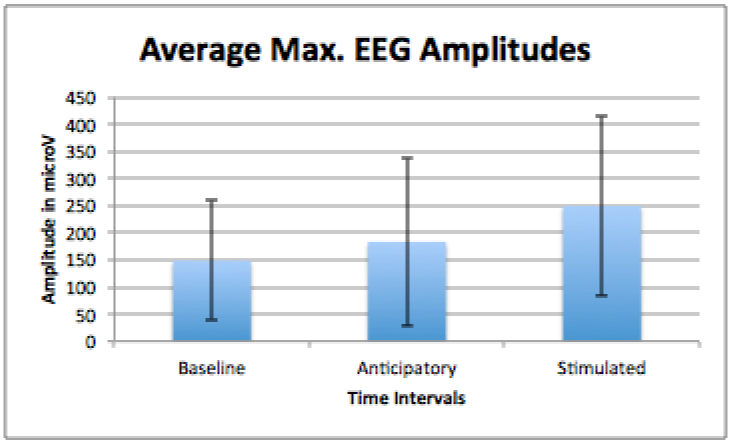

Fig. 1: Graph of the average maximum observed EEG values in microvolts. Data was divided into three time intervals: baseline, anticipatory, and stimulated. Error bars represent standard deviation.

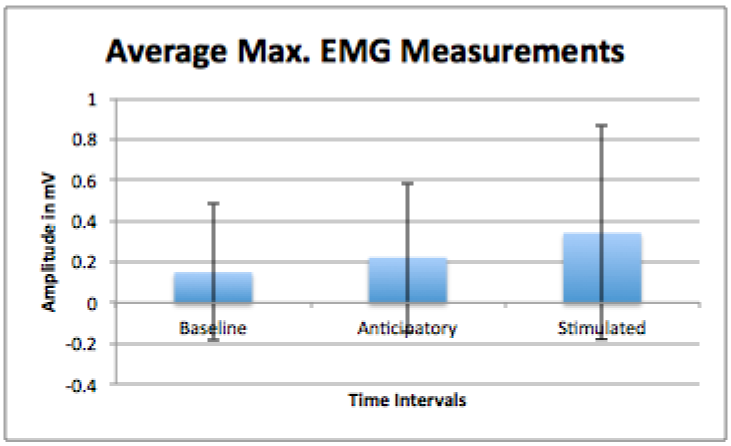

Fig. 2: Graph of the average maximum observed EMG values in millivolts. Data was divided into three time intervals: baseline, anticipatory, and stimulated. Error bars represent standard deviation.

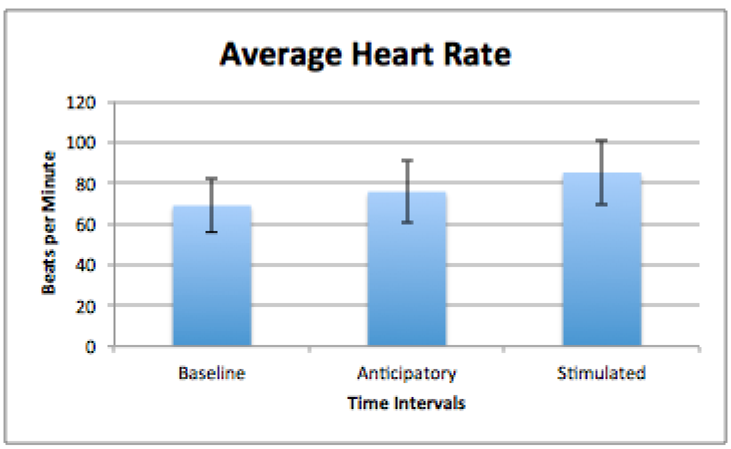

Fig. 3: Graph of the average maximum observed heat rate values in beats per minute. Data was divided into three time intervals: baseline, anticipatory, and stimulated. Error bars represent standard deviation.

Appendix

A pilot study was conducted with three additional, randomly chosen participants to determine if there were any significant changes in anticipatory tracings due to false memories of sour Warhead candies. If participants had not consumed a Warhead since childhood, they may not have had an accurate representation of the candy's sour sensation. This could diminish their anticipatory response due to an inaccurate representation of previously experienced sensations. Results from the pilot study indicated that there was no heightened anticipatory response due to recent exposure to the sour stimulus. Our data indicated that none of the p-values comparing baseline and anticipatory tracings were significant for any of the parameters tested. Furthermore, we hypothesize that consuming a Warhead candy an hour preceding data collection actually desensitized participants to the sour stimulus. Our previous data indicated a significant increase in EEG activity after consumption, but this significance was not present in the results of this follow-up study. Similarly, heart rate values did not indicate a significant change upon receiving the stimulus in the follow-up study. While it is possible that the lack of significance in the follow-up study is due to our low sample size (n=3), the more likely cause of this difference is that our participants experienced a desensitization to the sour stimuli. Participants emphasized that consumption of the Warhead candy for a second time was not as intense as the first, thus supporting our hypothesis. Overall, we conclude that pre-exposure to the sour stimulus will not lead to a significant increase in the anticipatory response; it may have even diminished the anticipatory and stimulated responses.