The sweet truth: Initial and post-ingestive effects of sugar and protein on taste preferences in rats

Katrina Prantzalos

Follow this and additional works at: https://commons.emich.edu/honors

Part of the Psychology Commons

Recommended Citation
https://commons.emich.edu/honors/633

This Open Access Senior Honors Thesis is brought to you for free and open access by the Honors College at DigitalCommons@EMU. It has been accepted for inclusion in Senior Honors Theses by an authorized administrator of DigitalCommons@EMU. For more information, please contact lib-ir@emich.edu.
The sweet truth: Initial and post-ingestive effects of sugar and protein on taste preferences in rats

Abstract
The purpose of this study is to investigate the food preferences of rats when given food with either a high protein or a high sugar content. We hoped to compare initial preferences and long-term exposure to determine the role of taste, and then post-ingestive effects, to examine if taste preferences are formed on the basis of taste alone or caused by the body’s absorption of nutrients. To achieve this goal, we studied rats’ food choices when presented with high protein yogurt or yogurt with a high sugar content. For half the rats two dishes were placed into the home container (simultaneous condition), for the other half there was only one container (sequential condition). In the simultaneous condition rats were presented with a choice between sweet yogurt and protein yogurt (n = 4). In the sequential condition they were presented with either the sweet or protein yogurt on one day, followed by the opposite on the next day, alternating every day for the duration of the trial. The initial condition was counterbalanced (n = 5 for each initial condition). It was hypothesized that rats would show an initial preference for sugar, which would shift to a preference for protein over the two-week trial period. The results from this experiment show that our initial hypothesis was not supported. Rats simultaneously offered sugar and protein generally preferred sugar, both initially and habitually. However, rats offered only one of the mixtures have an initial preference for protein that rapidly switches over to a preference for sugar. Regardless of testing condition, this preference generally becomes stronger over a two-week testing period. Not only does the interest in sugar increase over time under all conditions, the interest for protein decreases significantly for those in the simultaneous condition. To better substantiate these results, this study should be repeated with a sample size of at least 10 rats per testing condition. A longer timeframe to show changes in consumption would also be advised.

Degree Type
Open Access Senior Honors Thesis

Department
Psychology

First Advisor
Silvia von Kluge

Second Advisor
Joseph Breza

Third Advisor
Carol Freedman-Doan

Keywords
flavor, consumption, diet, sweet
THE SWEET TRUTH: INITIAL AND POST-INGESTIVE EFFECTS OF SUGAR AND PROTEIN ON TASTE PREFERENCES IN RATS

By

Katrina Prantzalos

A Senior Thesis Submitted to the

Eastern Michigan University

Honors College

in Partial Fulfillment of the Requirements for Graduation

with Honors in Psychology

Approved at Ypsilanti, Michigan, on this date 08/01/2018

Silvia von Kluge
Supervising Instructor (Print Name and have signed)

Joseph Bress
Honors Advisor (Print Name and have signed)

Mary K. Ramsey
Department Head (Print Name and have signed)

Carol Freedman-Dean
Honors Director (Print Name and have signed)
Abstract

The purpose of this study is to investigate the food preferences of rats when given food with either a high protein or a high sugar content. We hoped to compare initial preferences and long-term exposure to determine the role of taste, and then post-ingestive effects, to examine if taste preferences are formed on the basis of taste alone or caused by the body’s absorption of nutrients. To achieve this goal, we studied rats’ food choices when presented with high protein yogurt or yogurt with a high sugar content. For half the rats two dishes were placed into the home container (simultaneous condition), for the other half there was only one container (sequential condition). In the simultaneous condition rats were presented with a choice between sweet yogurt and protein yogurt (n = 4). In the sequential condition they were presented with either the sweet or protein yogurt on one day, followed by the opposite on the next day, alternating every day for the duration of the trial. The initial condition was counterbalanced (n = 5 for each initial condition). It was hypothesized that rats would show an initial preference for sugar, which would shift to a preference for protein over the two-week trial period.

The results from this experiment show that our initial hypothesis was not supported. Rats simultaneously offered sugar and protein generally preferred sugar, both initially and habitually. However, rats offered only one of the mixtures have an initial preference for protein that rapidly switches over to a preference for sugar. Regardless of testing condition, this preference generally becomes stronger over a two-week testing period. Not only does the interest in sugar increase over time under all conditions, the interest for protein decreases significantly for those in the simultaneous condition. To better substantiate these results, this study should be repeated with a sample size of at least 10 rats per testing condition. A longer timeframe to show changes in consumption would also be advised.
"Given the obesity epidemic that has recently emerged in developed societies, the specific study of factors that determine food ingestion in humans has become particularly urgent."

*(Bellisle, 2009, p. 539)*

**Introduction**

Identifying the mechanisms of food-related decision making can lead to a better understanding of eating preferences and how those preferences manifest in eating disorders. In particular, it would be helpful to therapies centered on overeating. In this experiment we discuss whether post-ingestive consequences, the food-related stimulation after swallowing, have an effect on taste preference, which will tell us whether it is the taste buds or the post-ingestive effects influence taste preferences most strongly. This could lead to practices in food regulation, which promote eating healthy rather than pleasant foods and to answer questions regarding the development of good eating habits in children particularly picky eaters. For example, the addition of a single food to a daily diet can affect energy balance: the addition of one half of a Hass avocado at lunch leads to greater feelings of satiety in overweight adults for three to five hours and reduced between-meal snacking *(Wien, Haddad, Oda, & Sabaté, 2013)*. This experiment also set a precedent in determining the biological mechanisms of taste preferences. For example, if post-ingestive effects have an effect on eating preferences, we know that the taste buds are not the only influence on taste preferences. This, in turn, could lead to experiments to determine where taste preferences originate; from the brain, the tongue, the gut, and/or anywhere in between. Finally, this study answers questions as to whether presentation of stimuli has an effect on consumption. This could help to understand picky eaters. Thus there is a direct application to children, especially those with disorders such as autism.

It is well known in the conditioned taste aversion literature that taste preferences are not robust or easily conditioned occurrences such as conditioned taste aversions, rather they are
sensitive to other factors than taste, and take time to develop (Drewnowski, 1997; Mehiel & Bolles, 1984; von Kluge, 2018). Many factors are thought to play a role in taste preferences, such as flavor, the perception of stimuli in the mouth from consumption (Small, 2012). Hunger and satiety are also said to play a role in the reception of flavor and the acquisition of taste preferences. Furthermore, it has been suggested that post-ingestive effects of a stimulus, or the food-related stimulation after swallowing, may play a role in food related decision making (Raynor & Epstein, 2000). More strongly, it is argued that differences in the rate of habituation—the change in response, usually a diminished response, to multiple presentations of a stimulus over time—for differing types of stimuli may suggest that consumption has post-ingestive effects that interact with sensory stimuli and may influence habituation and intake (Epstein, Temple, Roemmich, & Bouton, 2009). It is still unknown, however, how all of these factors come together to form a taste preference, or the extent to which each factor may play a role.

Many experiments have been done in hopes of understanding this complex question. In the psychobiological experiments of Le Magnen in 1992, laboratory rats were studied to determine the physiological factors that influence food intake with respect to meal frequency and size. Le Magnen’s work contributed to the idea that behaviors related to food intake were intended to insure the fulfilment of energy and nutrient-dependent needs (Bellisle, 2009). Furthermore, it has been suggested that a beneficial consequence following consumption, such as satiety, leads to a more pleasant interpretation of the sensory characteristics of that food. As a result of these interactions with food, every individual builds a hierarchy of food preferences (Bellisle, 2009).

This has been examined in multiple different nutrient conditioning experiments across several animals, given multiple food options infused with nutrient solutions. In two experiments,
Villalba and Provenza showed that lambs developed food preferences related to the post-ingestive feedback from the consumption of certain amounts of sodium propionate or sodium acetate (1996), as well as with starch (1997). In 1999, Arsenos and Kyriazakis completed similar testing in sheep to show the effects of casein on conditioned taste preferences. Food preferences were developed in the presence of amounts of casein while higher amounts of casein actually led to taste aversions. These researchers showed that food preferences for non-nutritious foods were tied to the amount of volatile fatty acids, starch, and casein, suggesting that the post-ingestive effect of energy plays a role in food-related decision making.

If this post-ingestive consequence – feeling a specific beneficial consequence, like satiety – was the only determinant in food preferences, then we would expect to see what can be called the medicine effect, where we come to love the things that make us feel better. This, however, is not always the case, as other factors tend to influence how we interpret our reactions to food stimuli. For example, aversive post-ingestive conditioning reduces the positive perceptions of Umami taste in sheep (Favreau, Baumont, Ferreira, Dumont, & Ginane, 2010). In further support of this point, it has been found that sucrose added to a preferable solution is more well received by those that are hungry than by those that are sated (Mobini, Chambers, & Yeomans, 2007). This suggests that a flavor preference could be effected by satiety, implying that a post-ingestive response of feeling full may change how you perceive the flavor of a food-related stimulus.

Alternatively, it has been suggested that post-ingestive effects do not alter the sensory factors related to satiation, aside from gastric distention (Raynor & Epstein, 2000). This implies post-ingestive effects are independent of sensory perception of food related stimuli. Furthermore, it was determined that, even though goats use post-ingestive consequences to discover food properties, associate these post ingestive consequences with food flavors, and are capable of
discriminating post-ingestive effects of different foods when tested in sequential conditions, the goats have trouble discerning the different effects when foods are offered simultaneously. These preferences remain unchanged regardless of perception of post-ingestive effects (Duncan et al., 2007). It can thus be concluded that presenting foods sequentially allows for better study of post-ingestive effects, but it is likely that post-ingestive effects played no role in the development of food preferences in this experiment.

Unfortunately, the role of post-ingestive effects in the development of food-related preferences is still unresolved. To better understand the concept, we looked at post-ingestive effects and food related preferences in rats. The objective of our work was to better understand the psychophysiological mechanisms involved in taste preferences. Our specific scientific goal was to determine whether taste preferences are formed on the basis of taste alone or whether post-ingestive responses play a role in the creation of food related preferences. Within our own work, we have defined post-ingestive responses, post-ingestive consequences, and post-ingestive effects to all refer to the positive and negative effects that nutrients have on the body after absorption.

We studied rats' food choices when presented with high protein yogurt or yogurt with a high sugar content. Some of the rats were presented with a simultaneous choice between sweet yogurt and protein yogurt. Other rats were presented sequentially with either the sweet or protein yogurt on one day followed by the other option on the next day. We intended to demonstrate and document rats' preference for sweet when given a choice between food with a high protein or high sugar content and determine whether these preferences changed over time. We also hoped to analyze any differences in initial and later preferences in rats given sweet yogurt or high
THE SWEET TRUTH

protein yogurt daily over a two-week period. Presenting the stimuli in this way could reveal influences of post-ingestive effects on taste preference.

We expected consumption of both substances to increase as the trials continue, as rats became more familiar with, and less fearful of, the newly introduced substances. If, however, preferences play a role in the amount of food consumed, one stimulus will increase more than the other. We hypothesized that rats would show an initial preference for sugar, which would shift to a preference for protein over the two-week trial period. This would imply that post-ingestive effects play a larger role in food preferences than the initial flavor perception of the stimulus, although the conditioning to appreciate the post-ingestive effects (i.e.; long term satiety and/or better nutritional value) would take time to develop.

Methodology

Experimental procedures were approved by the Institutional Care and Usage Committee at Eastern Michigan University (protocol number: 2018-086). All trials took place in the home cages of individually housed rats to maximize the comfort of the animals. Rats were supplied with water ad libitum throughout the study.

For every trial, each rat was weighed and then food deprived for sixteen hours before participation in a session. Through the duration of the trial, the average weight for a rat before food deprivation fell between 486.4g and 500.9g. At the end of the sixteen-hour food deprivation period, the rat was weighed again. Through the duration of the trial, the average weight for a rat following food deprivation fell between 462.3g and 483.1g. Food restriction did not result in greater than or equal to 20% of starting body weight. For one third of the rats, two dishes were placed into the home container (simultaneous condition). The remaining two thirds were presented with only one container (sequential condition). In the simultaneous condition rats were
presented with a choice between sweet yogurt and protein yogurt \((n = 4)\). In the sequential condition they were presented with either the sweet or protein yogurt on one day followed by the alternative on the next day, rotating every day for the duration of the trial. The initial condition was counterbalanced \((n = 5\) for each initial condition). It should be noted that for each trial 217g Oikos plain Greek nonfat yogurt was mixed with either 28g Domino pure cane granulated sugar for the sweet yogurt or 27.68g unflavored BODYTECH whey protein isolate for the protein yogurt. An initial amount of 17.36g to 36.36g of one of the mixtures was placed into each dish. The amount was controlled so that if two dishes were left in the same cage, the amount given in each dish was a similar amount. The dishes were left in the cages for 30 minutes, after which the amount of yogurt consumed, in grams, was recorded. The rats were then returned to their regular diet until the next deprivation period, 7 hours later. The duration of the entire experiment was 16 days, after which the rats were returned to their typical feeding schedule.

**Results**

Due to a limited sample size \((n = 14)\), as well as large variation in results – shown in the boxplots of the simultaneous and sequential conditions for both sugar and protein trials as seen in Figure 1 – nonparametric tests were conducted. By using the medians for comparisons, as opposed to the means, we avoided the issues caused by outliers and the larger spread of the data that are generally seen in mean-dependent analysis methods. Furthermore, we chose to use proportions for all analysis due to the large range (17.36g - 36.36g) in the initial amount of yogurt given at random.

We began the analysis by looking at the boxplots of the average proportion of protein/sugar consumption, from start to finish, of all 14 rats sorted by their test condition: simultaneous, sequential offered protein first, and sequential offered sugar first. Figure 1 shows
the five number summaries for protein testing. In the sequential condition, regardless of the stimulus presented first, the median proportion of protein yogurt consumed was almost 18 times that of the median proportion of protein yogurt consumed in the simultaneous condition. The median consumption for the simultaneous condition was only 4% of the amount of yogurt given, while the median consumption for those presented protein first in the sequential condition was 65% of the amount of yogurt given and the median consumption for those presented sugar first in the sequential condition was 71% of the amount of yogurt given.

Figure 1: The average proportion of protein consumed sorted by testing conditions is 18 times higher for those in the sequential condition than for those in the simultaneous condition. The median consumption for the simultaneous condition was only 4% of the amount of yogurt given, while the median consumption for those in the protein-first sequential condition was 65% of the amount of yogurt given and the median consumption for those in the sugar-first sequential condition was 71% of the amount of yogurt given.

Figure 2 shows the five number summaries for sugar testing. Regardless of condition, the rats ate a larger proportion of the sugar yogurt, with the lowest proportion of sugar yogurt
consumption across all testing being 33% of the amount of yogurt given. There was essentially no difference between the simultaneous and sequential conditions in the median proportion of sugar consumed; the median consumption for the simultaneous condition was 88% of the amount of yogurt given; the median consumption for those in the protein-first sequential condition was 87.5% of the amount of yogurt given; the median consumption for those in the sugar-first sequential condition was 88% of the amount of yogurt given.

![Average Proportion of Sugar Consumed sorted by Testing Condition](image)

*Figure 2: The average proportion of protein consumed sorted by testing conditions is no different for the sequential condition than for those in the simultaneous condition. The median consumption for the simultaneous condition was 88% of the amount of yogurt given. The median consumption for those presented protein first in the sequential condition was 87.5% of the amount of yogurt given. The median consumption for those presented sugar first in the sequential condition was 88% of the amount of yogurt given.*

In order to test the significance of these differences, a nonparametric analysis of variance (Kruskal-Wallis test) was performed. It can be seen that there is no significant difference between the simultaneous condition and either of the sequential conditions for the proportions of
sugar consumed \((p = 0.5689)\). Alternatively, there is a significant difference across at least one of the conditions for the median proportion of protein consumed for rats \((p < 0.05)\). A pairwise comparison using the Wilcoxon rank sum test with Bonferroni adjustment was conducted post hoc to determine differences between groups. Results show that protein consumption is much lower for the simultaneous condition than for the sequential sugar-first \((p < 0.05)\) and sequential protein first \((p < 0.05)\) conditions. There is no significant difference in the proportion of protein consumed between the protein-first or sugar-first sequential conditions \((p = 0.63)\).

Unfortunately, this analysis cannot take into consideration that preferences may change over time. We decided to review the differences between the first and last trial of each condition to test exactly this, as shown in Figures 3 and 4. Figure 3 shows the median differences in the proportions of protein consumed on first and last trials for the simultaneous and sequential conditions. The median absolute deviations are also reported. In the simultaneous condition, the median proportion of protein yogurt consumed in the first and last trials differed by \(-10\% \pm 6\%\) of the respective initial amounts of yogurt given. This means that the median proportion of protein consumed in the initial trial was slightly larger than the median proportion of protein consumed in the final trial. Similarly, in the protein-first sequential group, the median proportion of protein consumed on first trial and the last trial differed by \(-5\% \pm 15\%\) of the initial amounts of yogurt given. Alternatively, in the sugar-first sequential group, the median final proportion of protein yogurt consumed was slightly larger than the median initial proportion consumed in the first trial, differing by \(8\% \pm 10\%\) of the initial amounts of yogurt given.
Figure 3: The median differences in the proportions of protein consumed on first and last trials for the simultaneous and sequential conditions.

Figure 4 shows the median differences in the proportions of sugar consumed on the first and last trials for the simultaneous and sequential conditions. As in Figure 3, the median absolute deviations are also reported. In the simultaneous condition, the median proportion of sugar yogurt consumed in the first and last trials differed by -34% ± 27% of the respective initial amounts of yogurt given. This means that the median proportion of sugar consumed in the initial trial was much smaller than the median proportion of sugar consumed in the final trial. Similarly, in the protein-first sequential group, the median proportion of sugar consumed on first trial and the last trial differed by 20% ± 4% of the initial amounts of yogurt given. Alternatively, in the sugar-first sequential group, the median final proportion of sugar yogurt consumed was slightly larger than the median initial proportion consumed in the first trial, differing by 31% ± 6% of the initial amounts of yogurt given.
With these figures suggesting an upward trend in the consumption of sugar for all conditions, an upward trend in the consumption of protein for the sugar-first sequential condition, and a downward trend in the consumption of protein for the simultaneous condition and protein-first sequential condition, we decided to conduct graphical analyses to better understand the change of protein and sugar preferences over time.
Figure 5: The consumption of protein increases over time for both sequential conditions, but decreases over time for the simultaneous condition, as predicted by linear regression analysis.

Figure 5 shows the change in the median proportion of protein consumption over time. Linear regression analysis shows a no significant change in protein consumption for the protein-first sequential conditions ($p = 0.496$) or the sugar-first sequential condition ($p = 0.287$) but there was a significant decrease for protein consumption in the sequential condition ($p < 0.05$). It should be noted that linear trend-lines may not be the best method of analysis for the protein consumption of the protein-first sequential conditions ($R^2 = 0.08$) or the sugar-first sequential condition ($R^2 = 0.19$). It is a slightly better method for the protein consumption in the simultaneous condition ($R^2 = 0.32$). The given data suggests a possible polynomial trend, or even some oscillating behavior trends, however we drew conclusions from this linear measure, rather than fitting a polynomial trend-line with a higher $R^2$ value to avoid fitting a model too specific to
represent the general population. In ideal conditions, a larger sample size and a longer time frame would resolve this issue.

**Change in Median Proportion of Sugar Consumption Over Time**

![Diagram showing the change in median proportion of sugar consumption over time.]

Figure 6: The consumption of sugar increases over time for all conditions, as predicted by linear regression analysis.

A similar issue was found with the change in median proportion of sugar consumption over time, as shown in Figure 6. Given that rats tended to completely finish all of the sugar-yogurt mixture in later trials, the data generally plateaus early in the experiment. Because of this, a logarithmic trend-line, rather than linear, was fit to the data. Analysis shows a logarithmic increase in sugar consumption for the protein-first sequential conditions (residual standard error = 0.08579, Achieved convergence tolerance = $1.644 \times 10^{-8}$), the sugar-first sequential condition (residual standard error = 0.05026, Achieved convergence tolerance = $4.115 \times 10^{-8}$).
and for protein consumption in the sequential condition (residual standard error = 0.03898, Achieved convergence tolerance = 1.014 x 10^{-7}).

Finally, we compared sugar and protein consumption directly across each of the testing conditions. This is graphically represented in Figure 7 for the simultaneous condition, Figure 8 for the protein-first condition, and Figure 9 for the sugar-first condition. It should be noted that these graphs represent time-series data, rather than a line of best fit, as seen in above graphs.

**Proportion of Stimulus Consumed under the Simultaneous Condition**

![Graph showing proportion of stimulus consumed under the simultaneous condition](image)

*Figure 7: The simultaneous condition consistently showed preferential consumption of sugar over protein.*

The simultaneous condition consistently showed preference for sugar. Initial sugar consumption was 50% of the amount of sugar-yogurt mixture given while initial protein consumption was only 20% of the amount of protein-yogurt mixture given. The difference between the proportions of sugar or protein consumed becomes greater over time, with sugar
consistently being consumed in greater proportions. By the end of the experiment, rats were eating almost 100% of the amount of sugar-yogurt mixture given while protein consumption was less than 10% of the amount of protein-yogurt mixture given.

![Proportion of Stimulus Consumed under the Sequential Protein First Condition](image)

*Figure 8: Rats exposed to protein-first consume a higher proportion of protein on their first exposure, but sugar rapidly becomes the preferred solution.*

The rats in the sequential protein-first condition showed an initial preference for protein during their first exposure, but upon their first exposure to sugar, this preference switched to sugar immediately and remained consistent for the duration of the experiment. It is important to note that towards the end of the experiment, the rats began eating more of the protein yogurt, almost matching the consumption of sugar yogurt.
The rats in the sequential sugar-first condition also showed an initial preference for protein in their first exposure. Upon the second exposure to each stimulus this preference switches to the sugar yogurt mixture. Again, it is important to note that in the sequential protein-first condition, towards the end of the experiment the rats began eating more of the protein yogurt, almost exactly matching the consumption of sugar yogurt by the final trial.

Discussion

The results from this experiment show that our initial hypothesis was not supported. Rats repeatedly offered a choice between sugar and protein will always consume a significantly larger amount of sugar than of protein. When, however, rats are offered the mixtures sequentially over
different days, they have an initial preference for protein that switches to a preference for sugar. The two sequential conditions were statistically indistinguishable, regardless of the mixture consumed.

These results could be indicative of several things, including but not limited to the ideas that sugar has positive post-ingestive effects and that the taste preference for sweet outweighs any possible negative post-ingestive effects that sugar might have. It has been shown that sugar is easier to digest and absorb than the starches in lab chow (Sclafani, 2001). It could also be argued that the rats disliked the taste of the protein used for study. However, this is unlikely, considering the small median differences, with large deviations, between the final and initial proportions of protein consumption.

Furthermore, towards the end of the experiment, in both the sequential sugar-first and protein-first conditions, the rats began eating more of the protein yogurt, approximately matching the consumption of sugar yogurt by the final trial. As this result was not seen in the simultaneous group, it is likely that the flavor of the protein yogurt was not as enjoyable to the rats as the sugar, though some unknown factor made the protein yogurt worth consuming. We hypothesize that the post-ingestive responses did in fact play some role in the consumption larger amounts of protein when protein was the only option, or smaller amounts of protein when there was another option available. The argument could be made that rats were fully sated with the consumption of sugar and thus did not consume the protein yogurt in the simultaneous group. However, this is unlikely given the small amounts of yogurt given, and the varied amount – between 17g and 32g – of yogurt given.

One unexpected result, discontinuous with current literature, is that the consumption of protein decreased when paired simultaneously with sugar. This is inconsistent with research
showing that learned flavor preferences are based on caloric outcome and are independent of initial hedonic value (Mehiel & Bolles, 1984; Mehiel & Bolles 1988). If flavor preferences were strictly based on caloric outcome, independent of hedonic value, then the consumption of both yogurts would have been approximately equal from beginning to end, as the yogurt mixtures have been matched for caloric intake. The most reasonable hypothesis for this outcome is that sensory stimulation influences satiation, and that the effect is not confounded by post-ingestive consequences (Raynor & Epstein, 2000).

Unfortunately, one animal died on the last day of the trials, so its data was not able to be collected. Another died shortly after all trials were completed. While the causes of death were unknown, neither death is suspected to have been caused by anything relating to this experiment. It should be noted, however, that some of the results may be confounded by whatever may have caused their deaths, especially considering the small sample size. Since nonparametric methods were used to counter any outliers, and no connections can be made to the cause of death at this point, all data from these animals was still included in statistical analysis.

In order to better understand the effect of post-ingestive consequences on taste preferences, future studies should consider a longer time frame with a larger sample size, and/or a range of different sweet/sugar concentrations.

**Conclusion**

It was hypothesized that rats would show an initial preference for sugar, which would then shift to a preference for protein over the two-week trial period. The results from this experiment show that our initial hypothesis was not supported. Rats simultaneously offered sugar and protein generally preferred sugar, both initially and over time. However, rats offered only one of the mixtures at a time had an initial preference for protein that rapidly switched to a
preference for sugar. Regardless of testing condition, this preference generally became stronger over the two-week testing period. Not only did the preference for sugar increase over time under all conditions, the preference for protein decreased significantly for those in the simultaneous condition. This is inconsistent with current research showing that learned flavor preferences are based on caloric outcome and are independent of initial hedonic value. To better substantiate these results, this study should be repeated with a sample size of at least 10 rats per testing condition. A longer timeframe to show changes in consumption would also be advised.

**Future Directions**

This study should be repeated with a sample size of at least 10 rats per testing condition (as required by a balanced one-way analysis of variance power calculation). Along with a larger sample size, a different method for predictive analysis may be necessary to show the change in consumption of protein or sugar over time. A longer timeframe to show changes in consumption would also be helpful.

Since the weights of the rats were recorded before each food deprivation, before each trial following the food deprivation, and immediately after the trial before being given regular food, more data analysis could be done to determine if weight was affected throughout this experiment. This could help to determine if satiety played a role in the development of food preferences. For example, a lower weight could indicate more satiety and less food consumption following a trial, which would conflict with other post-ingestive effects.

**References**


Von Kluge, 2018 Personal Communication


Acknowledgements

Thank you Dr. von Kluge for your constant support and encouragement. This project could have never happened without you. I would also like to thank all of the workers in the vivarium who keep the rats living happy and healthy lives.