ASSOCIATIVE LEARNING AND THIAMINE-BASED FLAVOR PREFERENCE

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

ASSOCIATIVE LEARNING AND THIAMINE-BASED FLAVOR PREFERENCE

presented by Rachel Richardson,

a candidate for the degree of Master of Arts,

and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

The current experiment explored the extent to which thiamine deficiency at the time of conditioning is important as well as the importance of thiamine deficiency at the time of testing. This experiment controlled for previous experiments’ flaws. Our experiment controlled for the time of thiamine deprivation, whether it was during the time of conditioning or at the time of test. Based on our results, thiamine deficiency did not influence consumption of a flavored solution that had been paired with thiamine. This was true for thiamine deficiency at the time of conditioning pairings and at the time of testing.
Introduction

Thiamine is a B vitamin found in many parts of our bodies and is used by many tissues. Thiamine is not produced by organisms’ bodies, but rather ingested through different foods. This vitamin is important in the use of carbohydrates, metabolizing glucose, and the function of our heart, muscles and nerves. Thiamine is a molecule that helps in carbohydrate metabolism in two different pathways (Martin, 2014). The absence of thiamine in our diet can lead to many complications, including complications in humans. First, thiamine helps break down carbohydrates, as previously mentioned. Through this process, sugars and other molecules are broken down, which aid in the proper function of biochemical reactions and help to synthesize brain chemicals. Thiamine is necessary for all organs to function correctly, but can be particularly important for the heart and nervous system. Thiamine deficiency can cause heart failure, retention of sodium and water in the blood, as well as increased blood flow through the vessels. In humans, thiamine deficiency is mostly seen as a result of alcohol-related neurological disorder, Wernicke-Korsakoff Syndrome. Some characteristics of Wernicke-Korsakoff Syndrome are dementia, ataxia, confusion, and the disorder can lead to a coma or death.

Other studies have examined the effect of thiamine deficiency has on learning, behavioral changes and memory impairments. For instance, Langlais and Savage (1995) examined thiamine deficiency on spontaneous alteration, exploratory activity, and the rats’ ability to learn and remember. The researchers used a model of Wernicke-Korsakoff Syndrome by using rats that were made thiamine deficient by pyrithiamine. The results showed that the thiamine deficient animals were significantly impaired in learning the
specific tasks, compared to the control animals. However, some of the thiamine deficient animals in the study were unable to learn specific tasks. The researchers also noticed that the thiamine deficient animals that could not learn certain tasks had significant reductions in thickness of their parietal and frontal cortex, as well as neuronal loss, which was thought to be a result of the deficiency. The results of this study show that many behaviors may be affected by thiamine deficiency, and thiamine deficiency impairs learning and memory.

Many studies have been conducted examining the effects of thiamine deficiency in rats. A study by McCandless and Shenker (1968) examined thiamine deficiency and other biomedical assessments in different brain regions. This study shows that when thiamine deficiency is produced by a thiamine deficient diet, neurological symptoms didn’t become apparent until 4.5-5 weeks. “These [symptoms] consisted of ataxia, impaired righting response, opisthotonic posturing, and drowsiness” (McCandless, 2010). These deficits could be reversed within 16-36 hours, if subjects are given an injection of thiamine. This study helped define the range that is critical for neurological issues to start occurring after thiamine depletion begins.

Other studies examining thiamine deficiency and its effects on the brain noticed that there was a decrease in serotonin uptake in thiamine deficient rats. This decrease in serotonin led to hypothermia, and in some studies, seizures occurred. Fournier and Butterworth (1990) evaluated thiamine deficiency in rats soon after birth. In this study, pregnant rats were made thiamine deficient. Their offspring were studied at 13 days after birth. The researchers noticed that these offspring had decreased levels of pyruvate dehydrogenase, alpha ketoglutarate dehydrogenase, and transketolase. They concluded
that, “These changes could certainly have deleterious effects on brain development during critical periods for metabolic activity” (McCandless, 2010). Harper (1942) also studied the effect of thiamine deficiency in rats. These researchers found that glucose absorption in the intestine was greatly decreased in thiamine deficient rats as opposed to non-thiamine deficient rats (McCandless, 2010).

In the 1960’s and 1970’s, many studies examined thiamine deficiency and its role in conditioning with flavored solutions and subsequent taste preference. It is important to examine if a preference for a flavor can be developed through flavor-nutrient pairings. Such a finding will show that associative learning processes are involved in this form of health promotion, and whether nutrients and the experience of nutritional benefits can have affective properties (as shown by a behavioral preference) that support such associations.

Rozin and Kalat (1971), as well as many other investigators, have found that when an organism is lacking in a certain nutrient or vitamin they will seek out that missing nutrient/vitamin. Rozin and Kalat called this phenomenon “specific hungers”. It is possible that organisms use associative processes to learn about these relationships. If the nutrient or the substance containing the nutrient has a distinctive flavor, and consuming that substance produces improved health, then a preference for the substance may occur. If this process occurs, then it can demonstrate that associative processes are involved in the specific hunger behaviors. Although associative learning is implied in the way that these occurrences are described, insufficient work has been conducted in which the eventually preferred substance does not itself contain the needed nutrient. That is, all of the demonstrations of preferences for substances involving a needed nutrient contain
that needed nutrient. The exceptions are described in some detail below. It seems very likely that a preference for a flavor (e.g., cherry) that has been paired with a needed nutrient can be demonstrated, as it is now known that rats come to prefer a flavor that has been paired with other metabolically important stimuli (e.g., flavors paired with calories, Fedorchak & Bolles, 1987). This study seeks to determine if associative learning is involved in preferences for a flavor paired with thiamine when thiamine is a needed nutrient.

Studies examining associations and thiamine deficiency began with experiments by Garcia, Ervin, Yorke, and Koelling (1967). This study gave all rats thiamine depletion, and later the rats were allowed to drink saccharin followed by an injection of thiamine. These rats consumed a large amount of saccharin at test because the saccharin had been exposed at the time of thiamine repletion. A control condition received saccharin during depletion of thiamine instead of receiving saccharin during repletion, and this group consumed less saccharin at test. Specifically, the study gave the saccharin to the control rats a few days into a thiamine deficiency phase, and therefore it is likely that the control rats were already becoming deficient at the time of their saccharin exposure. Of course, flavors associated with recovery should be more preferred than flavors associated with deficiency. Seward and Greathouse (1973) followed up the study done by Garcia et al. by intentionally testing the preference of flavors associated with illness (thiamine deprivation) and also recovery-paired flavors. In their study, experimental rats received exposure to a saccharin solution prior to each thiamine injection at the time that the rats were thiamine depleted. Control rats received saccharin solution two days after the thiamine injection. Their findings showed that the control group’s saccharin consumption
decreased, rather than increased, indicating that the saccharin may have been paired with
the onset of a deprivation period. The control group may have exhibited an aversion to
the saccharin, rather than the experimental group acquiring a preference for the solution.

Zahorik, Maier, and Pies (1974) mention that the results of the Garcia et al. study
were not clear because these rats could have developed a taste preference to the flavor
paired with recovery of thiamine as Garcia et al. suggested, or the group difference may
have occurred because the control group displayed an aversion to the flavor paired with
the deficiency of thiamine, or a combination of both factors.

Zahorik and Maier (1969) attempted to remedy this problem by giving the rats a
choice between consumption of the two flavors at test – a novel (control) flavor as one
alternative and a (experimental) flavor given during the time of thiamine repletion as the
other test flavor. By comparing the conditioned flavor with a “neutral flavor”, a clear
preference might be detected. In this experiment, they found that the rats preferred the
flavor paired with thiamine repletion. However, Rozin and Kalat (1971) argued that the
preference for the flavor paired with recovery was due to it being a familiar flavor (as
mentioned, the other “control” flavor was novel at the time of testing). Neophobia is a
common response to novel substances for rats and could explain their test result.

Zahorik et al. (1974) pointed out that the test flavor should be equally familiar to
both groups at the time of testing to avoid neophobia as a confounding variable, and they
also included a control group that did not receive thiamine deficiency. Animals tested this
way showed a greater preference for the “recovery flavor” and differential neophobia
could not account for this difference.
Zahorik et al. conducted an experiment to specifically examine the role of neophobia using four different groups that were tested using two flavors. The first group was used to replicate previous findings. This group of rats was tested on their preference between the recovery flavor (a flavor paired with injections of thiamine for thiamine-deprived rats) and a novel flavor. The second group was given the choice between the recovery flavor and a flavor that they had been introduced to earlier (to reduce any neophobia). The third group was given a choice between a novel flavor and a familiar flavor (neither of these control flavors were associated with thiamine but were used simply to examine the potential for neophobia). The fourth group was given a choice between the recovery flavor and a familiar flavor (the latter control flavor had never been paired with thiamine). This latter group may qualify as a “conservative control”, since differential neophobia could not explain the result. These results showed a preference for the recovery flavor relative to the familiar flavor or the novel flavor.

Another important issue involves the issue of whether thiamine deficiency at the time of conditioning (flavor-thiamine pairings) and/or at the time of test is necessary to show a preference. Zahorik (1977) states in a book chapter on this topic, “It has been known for many years that thiamine deficient rats will ingest large quantities of foods containing the required nutrient and that these foods continue to be eaten in large amounts even after recovery from deficiency is complete”. Therefore, Zahorik claims that thiamine deprivation state at the time of testing may not matter. In other words, the rats may prefer the flavor at test even when not thiamine deprived at test (as long as it was previously conditioned).
Alternatively, do subjects need to be deprived of a nutrient to form the flavor-nutrient association? Berridge and Schulkin (1989) explored whether depletion at the time of conditioning is essential in nutrient-based taste preference. In this study, rats were given a flavor-salt pairing in Phase 1, when they were not sodium deprived. Berridge and Schulkin manipulated whether the rats were sodium deficient at the time of test or not. After Phase 1, the experimental group was made sodium deficient and then tested on the previously paired flavor. Results showed that the rats that were sodium deprived at test showed a flavor preference for the flavor previously paired with salt. The animals that were not sodium deficient during testing showed no preference. This opposes Zahorik’s emphasis on deprivation at the time of conditioning being of sole importance, rather than deprivation state at the time of test. Berridge and Schulkin showed that associations between the flavor and the sodium could be formed, even when the animals are not deprived during conditioning.

In sum, many earlier studies provided some evidence that flavor-thiamine pairings given during a time of thiamine deficiency produces a preference for the flavor; but these studies have features in their design that make interpretation of the results difficult. The results could be due to differential neophobia, or due to an aversion by the control group instead of a preference in the experimental condition.

Moreover, given Zahorik’s claim, there is some reason to question whether the deprivation state at the time of testing is important. That is, would two groups that are given flavor-thiamine pairings at a time that they are thiamine-deprived both show a comparable preference at test even when one group is thiamine deprived at test and one is not thiamine deprived at test. Garcia et al. state that deprivation at test does matter (see
Seward & Greathouse, 1973), but Zahorik and Seward and Greathouse claim that deprivation state at test does not matter.

There are other shortcomings in previous work, besides those already described. One potential confound in previous studies is that not all groups experienced thiamine deficiency at some point during the experiment. Every rat should be subjected to the somewhat comparable treatment at some point in the study. The present experiment controlled for this potential confound because each rat was subjected to thiamine deficiency at some point during the experiment. This experiment also tested whether nutrient deprivation is important at each phase of the study (conditioning phase or the test phase).

I hypothesized that the rats that are thiamine deprived during conditioning and during the test would learn the saccharin-thiamine association, and would come to prefer the flavor that was paired with thiamine. I also hypothesized that, consistent with Zahorik’s view, rats that are thiamine-deprived at the time of conditioning but not at the time of test will also show a flavor preference. Despite Berridge and Schulkin’s finding, I predicted that rats that receive conditioning while thiamine repleted but are thiamine deprived at test would not show a flavor preference.

Materials and Methods

Animals. Forty-four, adult male, Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN), approximately 200 grams in body weight were used in this study. Animals were individually housed. The animals had access to food and water ad libitum, except where noted below, and were maintained on a 16 hour light/8 hour dark cycle
(lights on at 7 a.m.). Animals were fed either their normal rat chow (Purina Rat Chow, 5008, St. Louis, MO) or a thiamine-free version of rat chow (Harlan Laboratories, Inc.-Teklad Diets, Madison, WI, TD.85027, RX: 1391154), depending on their group membership and the time when they are to experience thiamine deficiency.

**Procedure.** All procedures occurred during the light portion of the light/dark cycle, and were carried out in accordance with federal animal usage guidelines, and were approved by the University of Missouri Animal Care and Use Committee (ACUC Protocol 7988). The animals were randomly divided into four groups. To make sure that all four groups received similar experiences with thiamine deficiency, all rats received two deficiency periods. This period contained the flavor-thiamine conditioning pairings for some groups, whereas this conditioning occurred during a non-deficiency period for other groups.

Experimental Group 1 were conditioned while thiamine deficient and tested when not thiamine deficient (see Figure 1 for the full procedure). This group was fed a normal diet starting on Day 1 and this diet continued until Day 7. Following the normal diet, the rats were put on a thiamine deficient diet from Day 8 until Day 28. This group experienced its conditioning period (on the deficient diet) from Day 29 until Day 45. The rats were then placed back on the normal diet from Day 46 until Day 67. This group will then have one last period on the deficient diet from Day 68 until Day 89, followed by five days on the normal diet. After these 94 days, the rats were tested.

Experimental Group 2 was conditioned when thiamine deficient and tested while thiamine deficient (see Figure 1). This group was fed a normal diet starting on Day 1, and continued on this diet until Day 7. Following the normal diet, the rats were put on a
thiamine deficient diet from Day 8 until Day 28. This group experienced conditioning (on the deficient diet) from Day 29 until Day 45. The rats were then placed back on the normal diet from Day 46 until Day 72. This group then had one last period on the deficient diet from Day 72 until Day 93. After these 93 days, the rats were tested.

Control Group 1 was conditioned while not thiamine deficient and tested while not thiamine deficient (see Figure 1). This group was fed a deficient diet starting on Day 1 and this continued until Day 21. Following the deficient diet period, the rats were put on a normal diet from Day 22 until Day 29. This group also experienced its conditioning period (on the normal diet) from Day 29 until Day 45. The rats were then be placed back on the deficient diet from Day 46 until Day 62. This group then had a period on the normal diet from Day 62 until Day 67. From Day 67 until Day 88, the rats were placed on the deficient diet again, followed by five days of normal diet (days 88-93). After these 93 days, the rats were tested.

Control Group 2 was conditioned while not thiamine deficient and was tested while thiamine deficient (see Figure 1). This group was fed a deficient diet starting on Day 1 and this continued until Day 21. Following the deficient diet period, the rats were put on a normal diet from Day 22 until Day 29. This group also went through its conditioning period (on the normal diet) from Day 29 until Day 45. The rats were then placed back on the deficient diet from Day 46 until Day 62. This group then had a period on the normal diet from Day 62 until Day 72. From Day 72 until Day 93, the rats were placed on the deficient diet again. After these 93 days, the rats were tested.

Each rat was given four conditioning trials and the trials were separated by 3 days (each conditioning trial involved a 4-day “cycle”). During the conditioning trials, a 4%
(v/v) banana solution (Kroger Artificial Banana Flavoring, Cincinnati, Ohio) was used as the flavor. Rats were given this diluted banana flavoring using an infusion procedure. This procedure involved putting 2 ml of banana flavor in a needleless syringe and slowly administering the fluid to the rat orally with this syringe. Before the rats were given the banana-flavored infusions, they were given two “practice” infusions (a day or two prior to the conditioning trial) using water. These infusions were given to allow the rats to acclimate to the infusion procedure. Immediately following the banana infusion on the conditioning trials, the rats were injected (IP) with 0.2 mg/kg of thiamine. The injection volume was 0.8 mL/kg.

The test was conducted by giving the rats 4% banana solution (as was used for conditioning) for a 24-hour period. The rats were allowed to drink this solution ad lib. Solution was placed in bottles on the rats’ home cages during the test period. The banana solution was weighed prior to being placed on the cages and then at specific intervals throughout the 24-hour period. The banana solution was weighed 15 minutes after being put on the home cage, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours. After the 24-hour test period, the banana solution was taken off the cages and replaced with regular water bottles.

Results and Discussion

As mentioned above, I hypothesized that the rats that were thiamine deprived during conditioning and during the test would learn the saccharin-thiamine association and would come to prefer the flavor that was paired with thiamine during the test period. I also hypothesize that, consistent with Zahorik’s view, rats that were thiamine-deprived
at the time of conditioning, but not at the time of test, would also show a flavor preference. Following our hypothesis, that is, Experimental Group 2 would consume more banana flavoring at test than Control Group 1 or Control Group 2. Despite Berridge and Schulkin’s finding, I predicted that rats that received conditioning while thiamine repleted but are thiamine deprived at test would not show a flavor preference. Following our hypothesis, I predicted that Control Group 2 would show no flavor preference. Control Group 1 would also show no preference during test.

As mentioned in the Procedure section, there was a test period where banana flavoring was places on the animals’ home cages. The banana flavoring was weighed at specific times throughout the 24-hour test period. These data were used in the repeated measures analysis of variance. The results were analyzed by using a repeated measures analysis of variance. The repeated measures ANOVA revealed a significant effect of interval, $F(7, 266) = 207.46$, $p < 0.01$, suggesting that cumulative consumption increased over time. There was no main effect of group, $F(3, 38) = .93$, $p > 0.05$, and no significant interaction, $F(21, 266) = 0.78$, $p > 0.05$. We also conducted a Tukey HSD to compare the mean differences of consumption between each group. Group 1 and Group 2’s means differed by 0.01959507. The mean difference between Group 1 and Group 3 was -0.0963873. The difference in means between groups 1 and 4 was 0.10303575. The mean difference between groups 2 and 3 was -0.06923380. Group 2 and Group 4’s mean difference was 0.08344068. Finally, the mean difference for Group 3 and Group 4 was 0.15267447. The following will be a description of consumption of banana solution over the 24-hour period, with measurements (bottle weights) taken at minute 0, 15, 30, 60, 120, 240, 480, 960, and at minute 1440. At minute 15, the mean consumption of banana
flavoring for Group 1 was 1.209. Group 2 was 1.160, Group 3 was .750, and Group 4 was .682. The mean consumptions of banana solution at the 30-minute time period for these four groups (in order) were 1.718, 1.790, 1.100, and .873. The mean consumptions of banana solution at one hour for the groups were 2.100, 2.110, 1.770, and 1.355. Looking at the last time point, at 1440 minutes, the mean consumptions for the groups were 41.2, 38.690, 34.410, and 31.436.

In addition, three other ANOVAs were conducted, collapsing across certain groups. The first was an ANOVA collapsing across the two groups that were deficient during conditioning (Groups 1 and 2) compared to a collapsing of the two groups that were not deficient during conditioning (Groups 3 and 4). This comparison was only conducted at the time point of 960 minutes. This obtained no main effect of thiamine deficiency during conditioning on consumption, F(1,40) = 2.073, p > .158. A second ANOVA compared these collapsed conditions (Groups 1 and 2 versus 3 and 4) at the final time point of 1440 minutes. This analysis produced no main effect of thiamine deficiency during conditioning on consumption, F(1,40) = 2.480, p > .123. Finally, a repeated measures ANOVA conducted on this same comparison of groups but using last two time points – 960 minutes and 1440 minutes was conducted. There was no main effect of thiamine deficiency and there was no interaction conditions and the two time points, F(1,40) = 1.816, p > .185.

The individual body weights of the rats can be seen below in Figure 1A. The mean body weights of the rats can be seen below in Figure 1B.
Figure 1A shows the individual body weights (in grams) of each rat in each group taken in July 2014, when the rats were about 70 days old. The first 11 points represent rats in the group that were thiamine deficient during conditioning and not thiamine deficient during test. The next 10 points represent rats in the group that were thiamine deficient during conditioning and during test. The following 10 points represent rats in the group that were not thiamine deficient during conditioning and not thiamine deficient during test. The last 11 points represent rats in the group that were not thiamine deficient during conditioning and thiamine deficient during test.
Figure 1B shows the mean body weights (in grams) of the rats in each group. At this time, the rats were about 70 days old. The error bars represent the standard error of the mean.
Figure 1C shows the mean body weights of the rats, by group. Note that the animals were about 40 days old when these weighs were recorded. For this first weight, all four groups’ weights were averaged to compare with the averages weights of the Baseline animals. Day 44-49 represents one weight. Groups 1 and 2 were weighed at Day 44 and Groups 3 and 4 were weighed at Day 49.

The cumulative banana consumption collected during the test period can be seen below in Figure 2. The hypothesis that the rats that were thiamine deprived during conditioning and during the test would learn the saccharin-thiamine association and would come to prefer the flavor that was paired with thiamine during the test period was not supported. It was also hypothesized that the group that was not thiamine deprived during conditioning or test would consume less banana flavoring than the other groups. As seen below, that hypothesis was not supported either.
Figure 2 shows the cumulative banana consumption over the 24-hour test period. As shown, there was no significant difference in the groups’ consumption rates. Please note that the abscissa is not to scale. Each time point has double the value of the one directly preceding it. Ex: 240 is double the value of 120.
In Figure 2, it is shown that there is not much difference between group consumption. The group that was thiamine deprived during the time of conditioning and at the time of test was expected to have a much higher consumption rate than the other groups. The group of rats that was not thiamine deficient during the time of conditioning or at the time of test was hypothesized to have a much lower rate of consumption than the other groups. As shown, these two groups’ data points are located in-between the other groups’ data points on the graph in Figure 2.

It is assumed that the amount of thiamine used in everyday foods does not have a distinctive flavor. After examining published studies, it was learned that in humans “…flavor compounds [were] observed when solutions of thiamine…were heated” (Arnold, 390). Stacey and Sullivan (2003) came to a similar conclusion. The researchers investigated whether thiamine could be detected in beer. In this study 100ml samples of beer were presented to participants. These samples were either containing 0mg of thiamine or 10mg of thiamine. They noted that, “participants did not differentiate between beer fortified with thiamine and normal beer”. This study’s findings were also consistent with findings by Stacey (2003, 378).

The present study extended previous studies by attempting to improve on their shortcomings. As previously mentioned, the studies conducted on this topic possessed certain flaws that we hoped to correct. One correction we made was to include a control group for each condition. For example, instead of just using one experimental group receiving thiamine only at the time of conditioning and one control group receiving thiamine at both test and conditioning (as was done in one earlier report, as discussed in the introduction above), we used a group for each specific condition that could be
studied. We also controlled for a potential confound in which all animals were not subjected to the same amount of thiamine deficiency in most of the earlier work, and some groups did not get thiamine deficiency at all. There were also shortcomings involved in the results of some of the earlier studies, and we hoped to avoid these problems.

There are many reasons why I believe the results were not significant and the manipulation was without an effect. One reason could be because certain groups did not get exposed to enough thiamine deficiency to make them sufficiently deficient to differentiate their learning from other groups’ learning. This is because the campus ACUC committee insisted we use a maximum of 24 days of thiamine deprivation in order to avoid serious health problems with the rats. Studies we based our experiment upon used as many as 60 days of thiamine deficiency. These studies were also conducted in the 60’s and 70’s, and they had very different animal care and housing guidelines to abide by. With the implications currently set in place to provide a comfortable environment for our research animals, I believe it would be nearly impossible to conduct a study, similar to those mentioned, using 60 consecutive days of thiamine deficiency. Although this was likely the primary reason the experiment did not generate significant results, it is still reasonable to ask whether conditioning occurred. With the absence of deficiency symptoms and the lack of additional testing to determine the extent of the animals’ true physiological deprivation state (or lack thereof), it is difficult to assume the exact state the animals were in during the experiment. If the animals were only slightly deprived, could they have developed a mild conditioned preference for the flavor associated with
the thiamine, as opposed to the stronger preference we had hoped to find. Given the null result, it is also possible that a different flavor (CS) could have been more effective.

Another reason for the present null result could be that the rats never became deprived of thiamine. We bought our thiamine deficient food diet expecting it to be completely absent of thiamine. It has come to my attention that presumably thiamine-deficient diets that actually contained substantial amounts of thiamine has been a problem to researchers in the past. While attending a poster session at the meeting of the Society for Neuroscience in Washington D.C. (2014), a researcher mentioned he had conducted similar research to the present project and his thiamine deficient diet was ineffective in producing an effect in his experiment. He informed me that he had tested his thiamine deficient food after the experiment’s completion and found that it was not actually thiamine deficient. By the time this conference occurred, it was too late to check the content of our thiamine deficient food. Throughout the experiment, we never noticed any signs of thiamine deficiency. However, because the campus ACUC committee insisted we use a maximum of 24 days of thiamine deprivation in order to avoid serious health problems with the rats (they claimed that a longer period could produce neurological damage as well as problems with righting responses and dramatic weight loss), we were not sufficiently alerted to the possibility that the diet was not exerting any effect. Clearly the issue regarding the role of thiamine deficiency on preferences for flavors that have been paired with thiamine remains somewhat unresolved.

Future work could extend the present study. It would be beneficial to test the thiamine deficient food to verify its validity. It would also be beneficial to test thiamine levels in the animals’ blood at different stages throughout treatments (the beginning,
middle, and end of thiamine deprivation and repletion) to have a baseline and comparison amounts to support the variables manipulated in this research. If future work is done to test these specific hypotheses, I would like to rerun the present study with the recommendations and improvements mentioned. It would also be interesting to investigate sodium in the same way as was done here. This would give us a better idea on how different nutrients play a role in conditioning (e.g., Berridge & Schulkin, 1989). Given the relatively large amount of published work examining thiamine deficiency and associative processes, including work by Zahorik, Seward and Greathouse, and others that were discussed above we are more knowledgeable about these (thiamine-based) effects. There is less certainty about the use of sodium and other adaptive chemicals or nutrients with respect to need and how this need affects associative processes. An experiment conducted that is similar to this one, but studies sodium rather than thiamine, would help us acquire new information on the advantages of sodium in this type of experiment and also give us helpful insight on the difference that nutrients that possess flavor (sodium chloride) and those that do not possess flavor (thiamine) have on conditioning and learning.
REFERENCES


Figure 3

Timeline showing different groups, days of thiamine deficient diet and normal diet, as well as conditioning and test periods.