

**Differences in Taste Sensitivity to
Linoleic Acid between Male and Female Rats**

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Abstract

Obesity is an increasingly serious health problem in the United States, as it is responsible for serious health problems and death. Obesity is linked to ingestion of dietary fats, therefore the identification of a component of dietary fats that may increase ingestion by orosensory stimulation represents a critically important step towards controlling fat intake. Fat is composed of a combination of several different free fatty acids, which have been shown to be detectable by rats using conditioned taste aversion methodology. Linoleic acid appears to be more detectable than oleic acid, both of which have been shown to increase the licking to sweet solutions, presumably by increasing the perceived sweetness of the solutions.

The goal of our research is to discover if varied levels of linoleic acid increases the palatability of sucrose, a desirable tastant, in a dose-dependant manner and whether there is a difference in sensitivity for male and female rats. Neither male nor females showed increased ingestion as the levels of linoleic acid increased, although both males and females did show an increase in licks to increasing concentrations of sucrose, as was expected. No differences between sexes were found for linoleic acid, but a sex difference was found for sucrose concentrations. Microstructure analysis showed that there are increases in the number of bursts with increasing concentrations of linoleic acid, and that the number of licks are regulated by the palatability of the solution rather than satiety. Although the experiment did not reveal a sex difference for taste sensitivity to linoleic acid paired with sucrose, it did show that females have an increased sensitivity to linoleic acid alone, which can be explored through future research.

Introduction

Globally, obesity has become an increasing health problem. The World Health Organization estimates that nearly 400 million adults are obese. This chronic disorder has serious health consequences, including cardiovascular disease, diabetes, heart disease, hypertension, osteoarthritis, and some cancers (World Health Organization, 2000). Increased consumption of fat-rich foods and decreased amounts of sufficient exercise have attributed to the increased prevalence of obesity (Drewnowski, 1997). Likely causes for the elevated intake of fat may be an increase in the palatability of fatty foods, a decreased satiation in response to fats, or a combination of these two factors (Greenberg and Smith, 1996). Current research using the rat model examines the palatability of fatty foods.

The rodent model has provided evidence for the detection and preference of dietary fat using corn oil as a stimulus (Greenberg and Smith, 1996). When rats were presented with mineral oil and corn oil, they showed a preference for corn oil, which is attributed to taste detection because all other sensory properties (smell, texture, and appearance) were the same for each stimuli (Greenberg and Smith, 1996). Smith et al. (2000) found that a conditioned taste aversion (CTA) to a sucrose-corn oil mixture generalized to linoleic acid. Linoleic acid (52%) and oleic acid (31%) make up a large portion of the free fatty acids (FFAs) in corn oil (Gunstone, 1999). Linoleic acid may be chemical in corn oil which can be detected through orosensory cues by the rat. The perception of FFA taste has been attributed to the role of lingual lipase in the production of FFAs from lipids. Lingual lipase can produce approximately 53 mM of oleic acid from triacylglyceride triolein after only 1 s of exposure to the lipids (Kawai and Fushiki, 2003). Fatty acid transporters (FAT) proteins bind to the FFAs, allowing interaction with the taste receptor cells (TRCs) (Fukuwatari et al. 1997). Work by Gilbertson and associates (1997)

showed evidence that free fatty acids inhibit the delayed rectifying K⁺ (DRK) channels in rat taste receptor cells. This activity could represent the taste cue for dietary fat. (Took out cite)

Previously, conditioned taste aversions were utilized to examine the detection of free fatty acids in rats. McCormack and associates (2006) found that rats avoided both linoleic and oleic acids at concentrations greater than or equal to 66 μ M and failed to avoid both 44 μ M linoleic and oleic acid using 2-bottle preference tests. Therefore the behavioral detection threshold for linoleic and oleic acid is in the micromolar concentration range that is only slightly higher than the concentrations used in the Gilbertson et al. (1997) study.

Previous work conducted in our laboratory has demonstrated that the ability to detect linoleic acid is eliminated when the afferent neural pathway, the chorda tympani nerve (CT-deleted N), is compromised prior to the CTA using the 2-bottle preference test paradigm. The rats that received chorda tympani nerve transection (CTX) showed no conditioned avoidance to 88 μ M linoleic acid, whereas those with an intact CTN continued to avoid the LA. Other studies using short duration stimuli have shown that rats could detect and avoid concentrations of linoleic acid between 5 and 20 μ M and oleic acid between 20 and 50 μ M. Through CTA and CTX manipulations, the detection thresholds and neural pathway for FFAs, linoleic and oleic acids, are becoming established.

Pittman and associates (2006a) found that with the addition of 88 μ M linoleic acid or oleic acid to sucrose and glucose there was increased licking behavior across concentrations of those solutions. When 88 μ M LA or OA was added to NaCl, citric acid, and quinine, the licking response was decreased. These results suggest that with innately appetitive taste stimuli, glucose and sucrose, the addition of FFAs enhance those positive effects, while the opposite effect occurs for aversive tastes such as, bitter, salt, and sour. When LA and OA were mixed in concentrations,

proportional to those found in corn oil, there was a similar effect on licking behavior as with the addition of either FFA individually. (Moved from beginning of this paragraph) Similarly in human studies, it has been suggested that fat causes heightened intensity ratings of other tastes. Kanarek, Ryu, and Przypek (1995) suggested that the interaction between fat and salt content might increase the hedonic value and intensity ratings by women. With increased fat content, saltiness ratings also increased. Salbe et al. (2004) found that with increasing fat content in cream solutions there was an increased creaminess rating by human participants.

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Other research conducted by Pittman and associates (2006b) examined the differences between male and female rats in the detection of free fatty acids following a conditioned taste aversion. Both male and female rats conditioned with linoleic acid showed an avoidance at 20, 50, 75, and 100 μM LA, but the amount of avoidance differed. There was a significant difference between males and females at the 50 μM concentration of LA. In order to explore the sensitivity difference more completely, the methodology was modified to more natural feeding environment, no conditioned taste aversion was administered and the rats were water replete in the current study.

The current experiment examines how linoleic acid can influence the consumption of sucrose, comparing between males and females. It is hypothesized that with addition of LA to sucrose concentrations, lick rates would increase as a function of linoleic acid. Differential sensitivity to linoleic acid between the sexes was also examined.

Methods

Subjects

Subjects were twelve male and twelve female Sprague-Dawley rats (greater than 90 days old, obtained from Charles River Laboratories, Wilmington, MA) housed individually in transparent plastic cages in a temperature-controlled colony room on a 12-12 h light-dark cycle with lights on at 7:00 h. Rats had free access to Harland Tekland 8604 rodent chow. Rats were given deionized, distilled water ad libitum during all testing days, and were restricted of water during the two days of training. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Wofford College.

Apparatus

Testing was conducted in the MS-160 gustatory apparatus (DiLog Instruments, Tallahassee, FL). The “Davis Rig” allows the controlled presentation of 16 chemical stimuli and records the licking behavior of the rat at 1-ms resolution. Taste stimuli were presented for controlled durations, wait periods, and interstimulus intervals using the sliding stimulus rack and the controlled lever, opening and closing the access port. The Davis Rig is located in an acoustic isolation chamber with fans that maintain a constant flow of air, in order to minimize olfactory cues. The chamber is lighted with an 8-watt, and rat behavior is monitored using a real-time internet camera.

Phase 1

Two days prior to testing day, the rats were trained in the Davis M-s 160 gustatory apparatus, a device that measures licking patterns to various taste stimuli. During testing, the rats were given access to four bottles of taste stimuli in fifteen second presentations. There were

thirty-two testing trials comprised of eight blocks of stimuli of four stimulus groups. As demonstrated in Table 1, both male and female rats were grouped in a counter-balanced design of stimulus presentations.

Table 1. Counter-balance design of stimulus presentations.

	Day 1	Day 2	Day 3	Day 4
Sucrose	Group 1	Group 2	Group 3	Group 4
Sucrose + 200 uM LA	Group 2	Group 3	Group 4	Group 1
Sucrose + 400 uM LA	Group 3	Group 4	Group 1	Group 2
Sucrose + 800 uM LA	Group 4	Group 1	Group 2	Group 3

Analysis

The number of licks (consumption) and latency until the first lick was recorded for each trial. The dose dependent effects of adding linoleic acid at various sucrose concentrations during short fifteen second trials were calculated for the male and female Sprague-Dawley rats using a lick ratio that normalized taste stimuli responses to each animal's average daily response to water trials, such that a lick ratio of 1.0 is identical to water licking and zero is no response. A mixed factorial ANOVA was used to examine the between-subject effects of sex and with-in subject effects of sucrose concentrations and linoleic acid concentrations.

Phase 2

Twelve male Sprague-Dawley rats were repleted of water prior to testing. The rats were tested across two days in the Davis M-s 160 gustatory apparatus. On each day of testing, the rats were given access to the sixteen taste stimuli in one 90 second presentation.

Microanalysis

Microanalysis of the results was conducted in order to examine the microstructure of licking behavior. The technique follows that of Baird, St. John, and Nguyen (2005). During test trials, licking occurs in bursts, defined as two or more consecutive licks with no interlick interval (ILI) equal to or exceeding 1 s. Burst sizes and durations are calculated based on number of licks per burst and the time for each burst. The pause ratio is the number of pauses divided by the number of ILIs in the meal (number of licks in meal -1).

Results

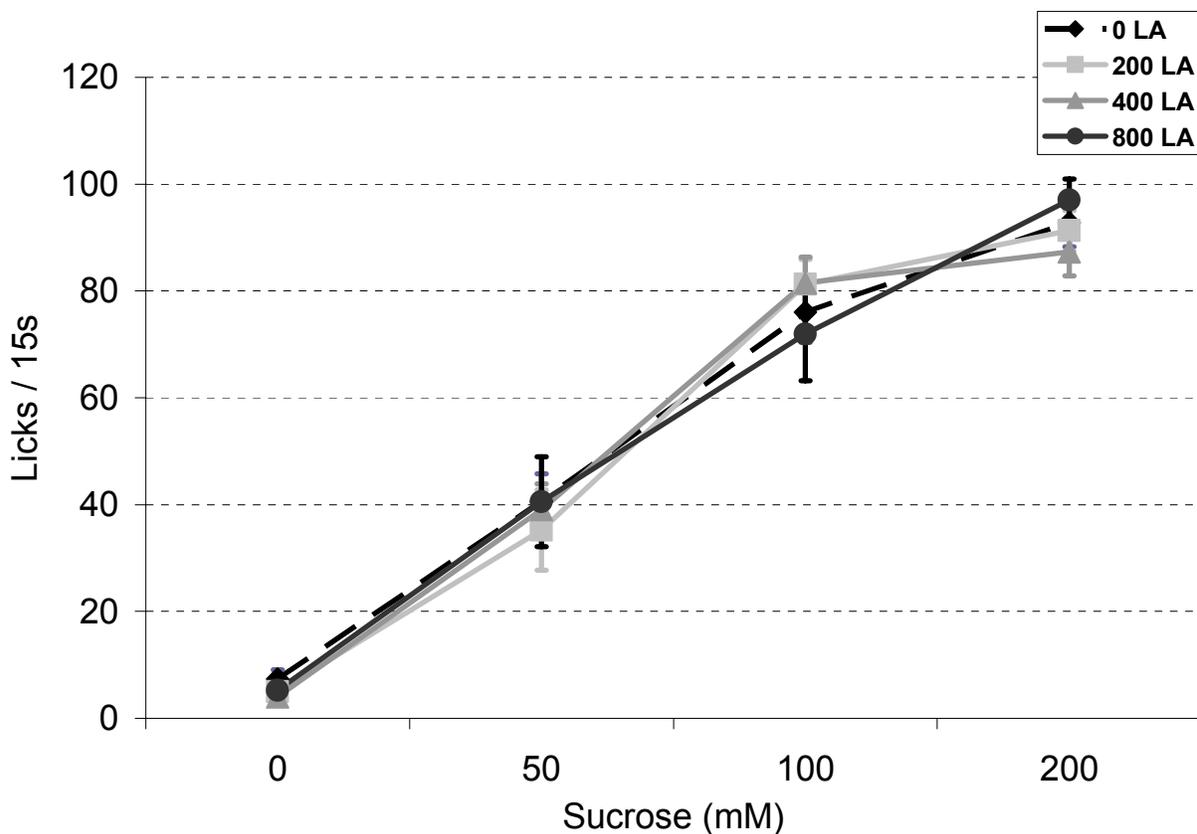


Figure 1. Number of licks in males

As seen in Figure 1, the licking responses increase linearly in males with an increase in sucrose concentrations. No change in number of licks based on increasing LA concentration was observed. In solutions containing 100 mM sucrose, licks differed the most between LA

concentrations, and the most licks occurred at 200 and 400 LA concentrations. At 200 mM sucrose, 800 μ M LA produced the highest amount of licks.

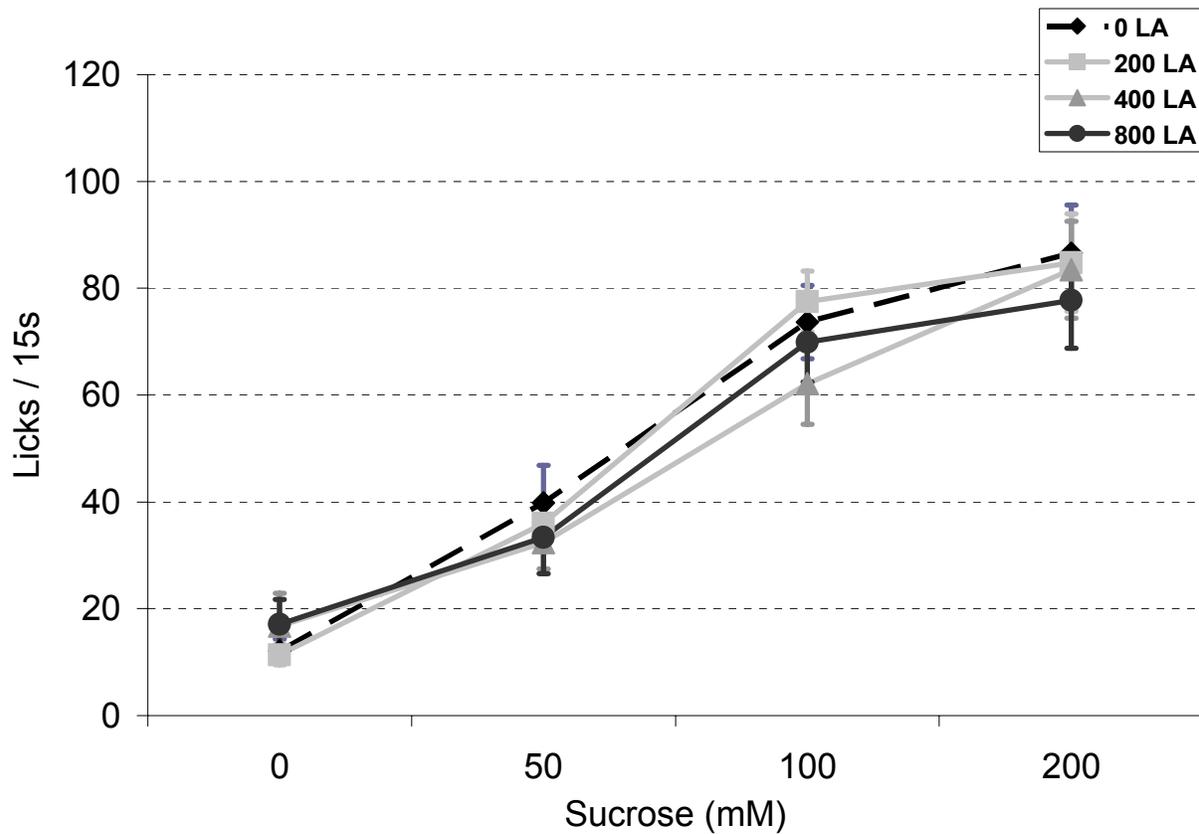


Figure 2. Number of licks in female rats

Figure 2 shows that the number of licks increases as a function of sucrose concentration among female rats. The largest variation in number of licks across LA concentrations occurs in response to solutions containing 100 mM sucrose. However, LA does not significantly change the number of licks to a solution, when compared to the number of licks to a LA-free solution.

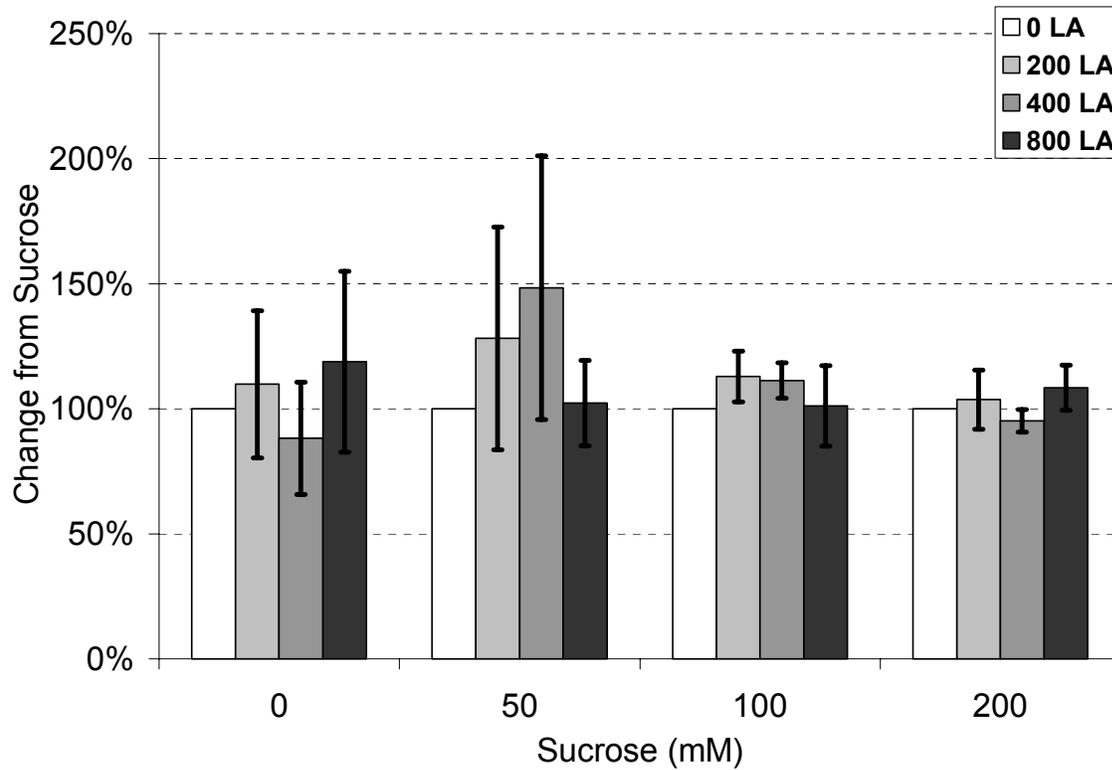


Figure 3. Percent change in licks from sucrose in males rats

In Figure 3, the lick response to solutions containing LA were normalized to the sucrose-only responses producing a percent change measurement. It was found that licks increased with an increase in sucrose concentrations for male rats. The 400 μ g LA concentration produced the maximum licking rate for males at the 50 mM sucrose concentration. LA concentration did not alter the licking rates at 0, 100, or 200 mM sucrose solutions.

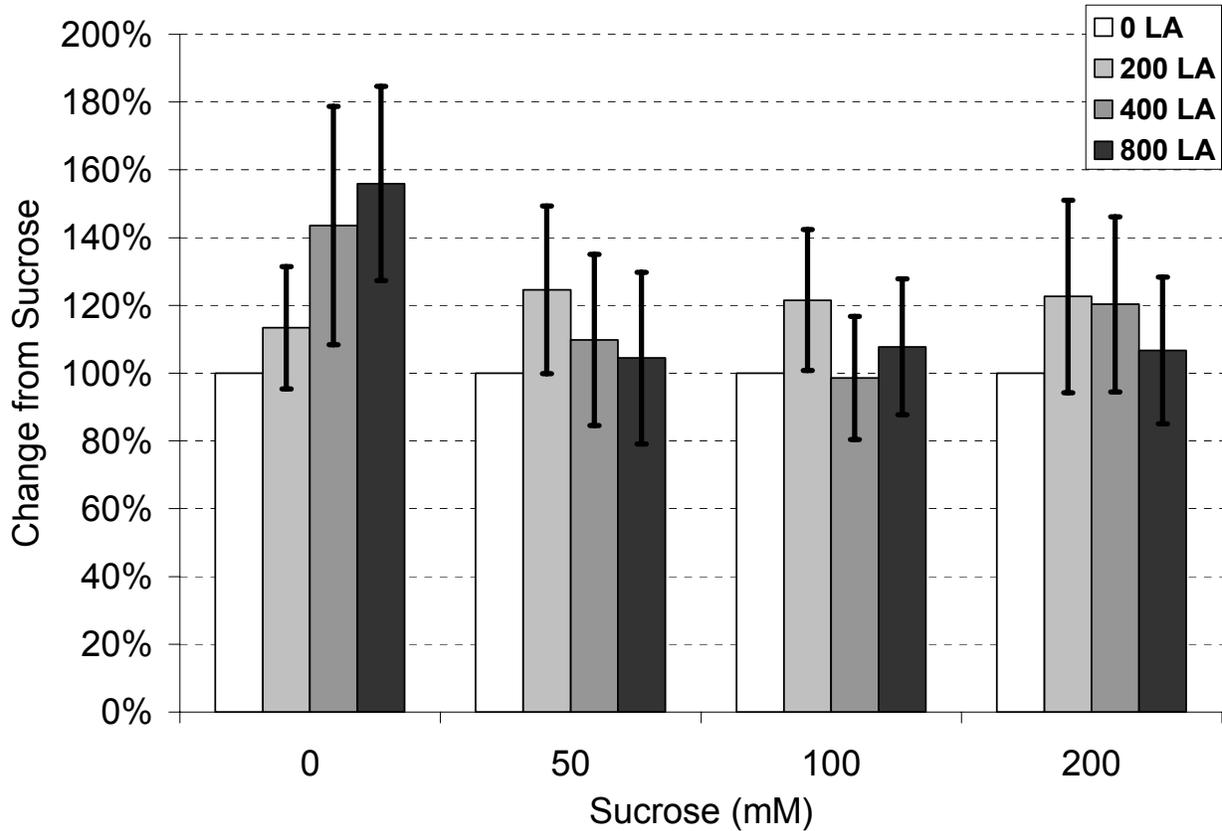


Figure 4. Percent change in licks from sucrose in female rats

As can be seen in Figure 4, female rats increase licking in response to increased LA when no sucrose is present in the solution. When sucrose is introduced, licking does not increase as LA concentration increases. However, there is an overall increase in licking for most solutions containing LA as a whole, when compared to solutions only containing sucrose, although these results were not shown to be statistically significant.

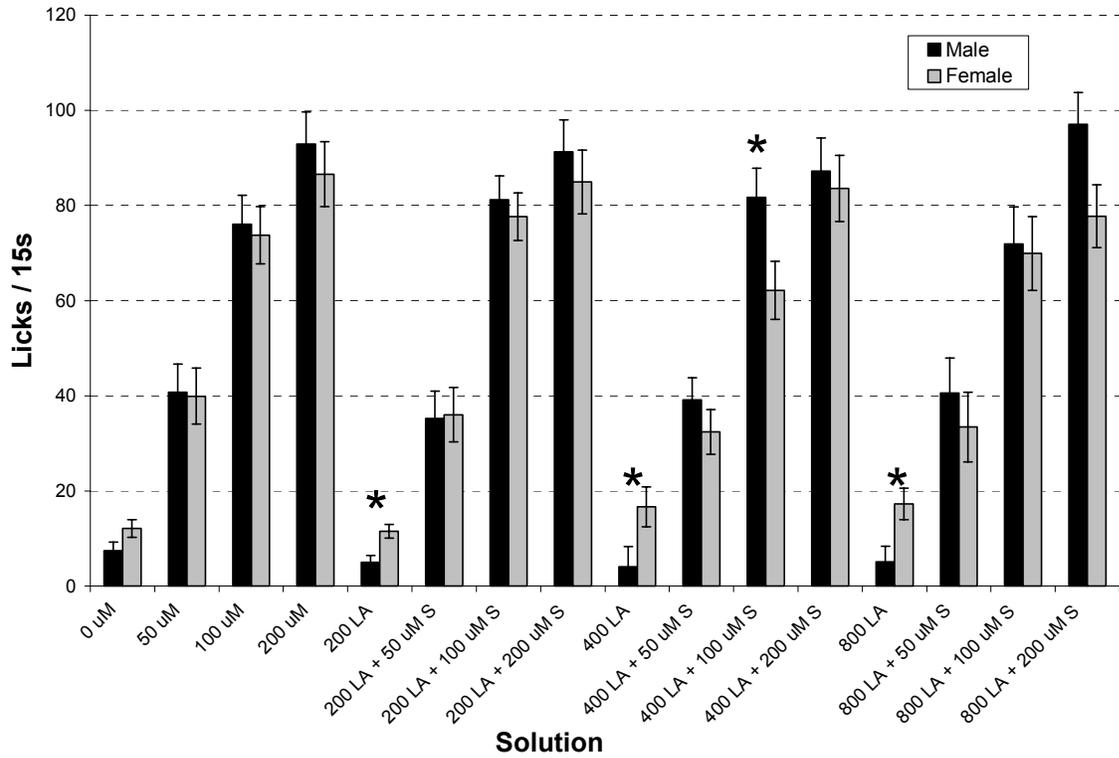


Figure 5. Licks per solution in males and females.

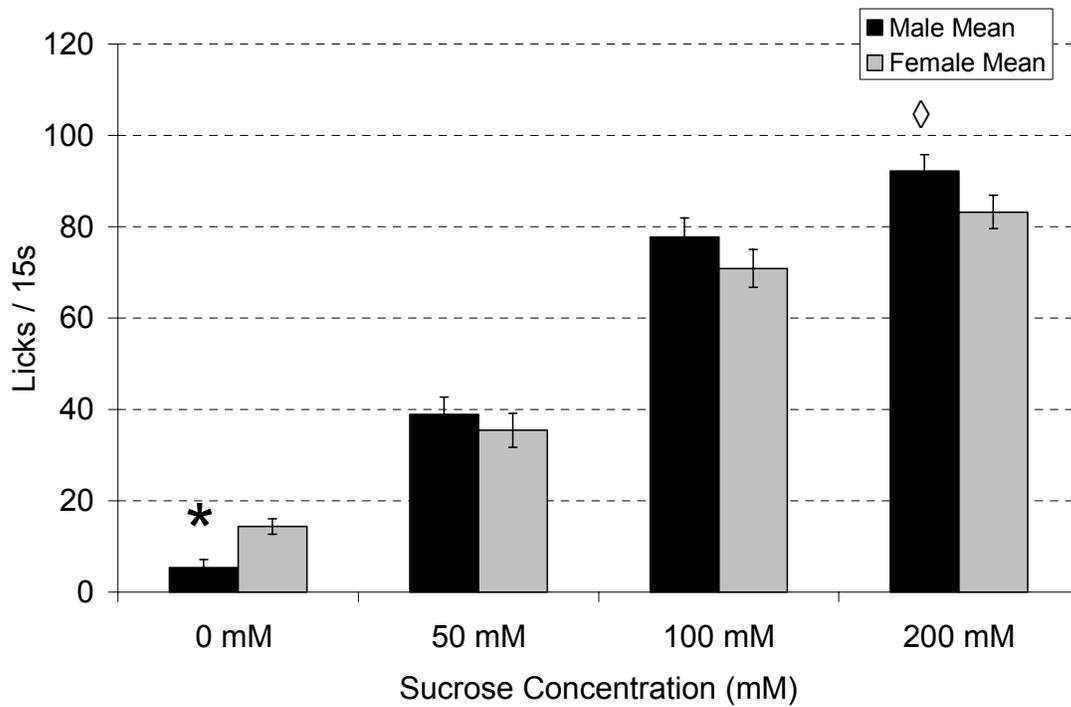


Figure 6. Licks per concentration of sucrose in males and females

A mixed factorial ANOVA was conducted in order to identify any significant main effects or interactions of the independent variables, sucrose concentration, linoleic acid (LA) concentration, and sex, on the dependent variable, licks per 15 s. A between-group statistical analysis of the lick ratio data was collapsed across all four days for sex and a within-group statistical analysis of lick ratio data was collapsed across all four days for sucrose concentrations and LA. There was no main effect of sex, but an interaction was observed between sex and the sucrose solution ($p < 0.05$), as seen in Figure 6. The three-way interaction between sex, sucrose, and LA was not significant, but a paired sample t-test conducted on each of the sixteen stimuli revealed significant effects of the following stimuli: 200 mM sucrose ($p < 0.01$), 400 mM sucrose ($p < 0.05$), 100 μ M LA + 400 mM sucrose ($p < 0.05$), and 800 mM sucrose ($p < 0.05$). The results are displayed in Figure 5.

Microstructure analysis

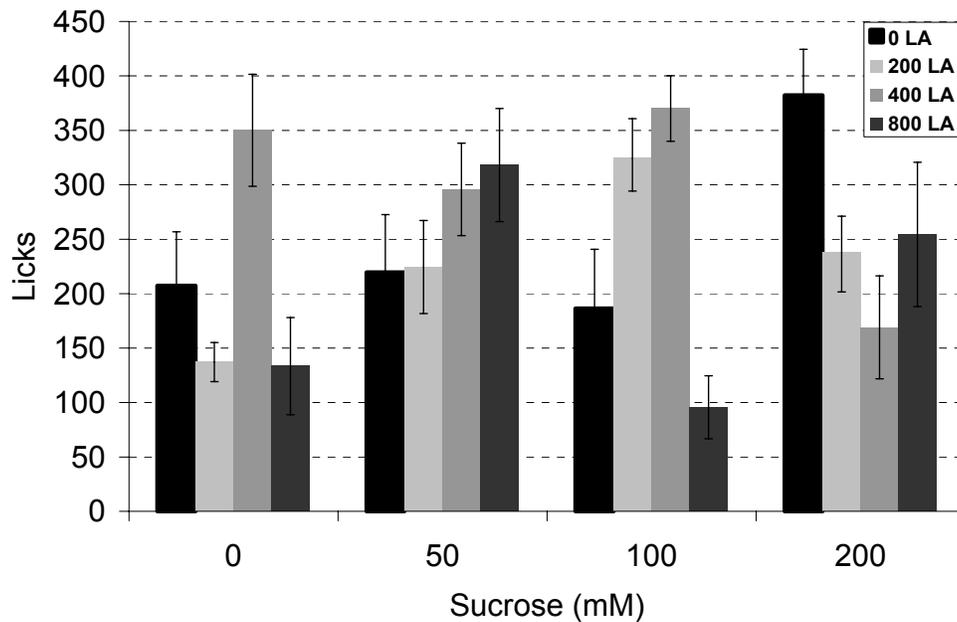


Figure 7. Total number of licks

The total lick counts for the 90s trials are shown in Figure 7. For the 0 mM sucrose concentration, the rats showed increase in licking to 400 μM concentration of linoleic acid. Differences in licking behavior for 50 mM sucrose showed increases in total licks with increasing LA concentrations. LA concentrations of 200 and 400 μM continued to increase for 100 mM sucrose, but decreased for 0 and 800 μM concentrations. There is a significant increase in the 200 mM sucrose with no linoleic acid compared to the other sucrose concentrations.

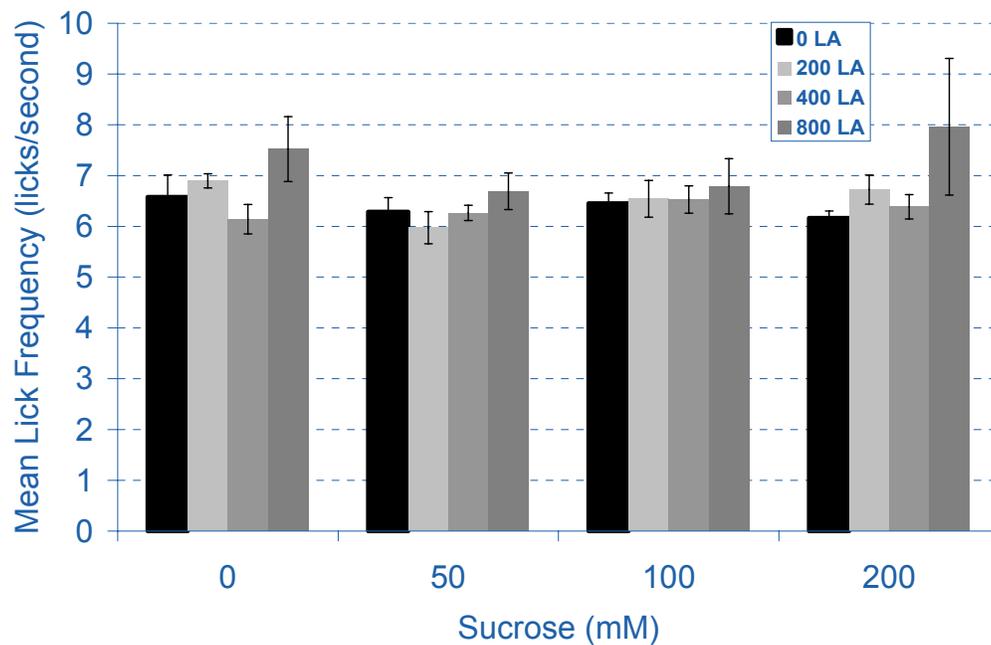


Figure 8. Mean lick frequency for all solutions.

The frequency of licks is shown in Figure 8. The lick frequencies are consistently around 6 licks per second. There were no significant differences due to the different concentrations of sucrose and linoleic acid.

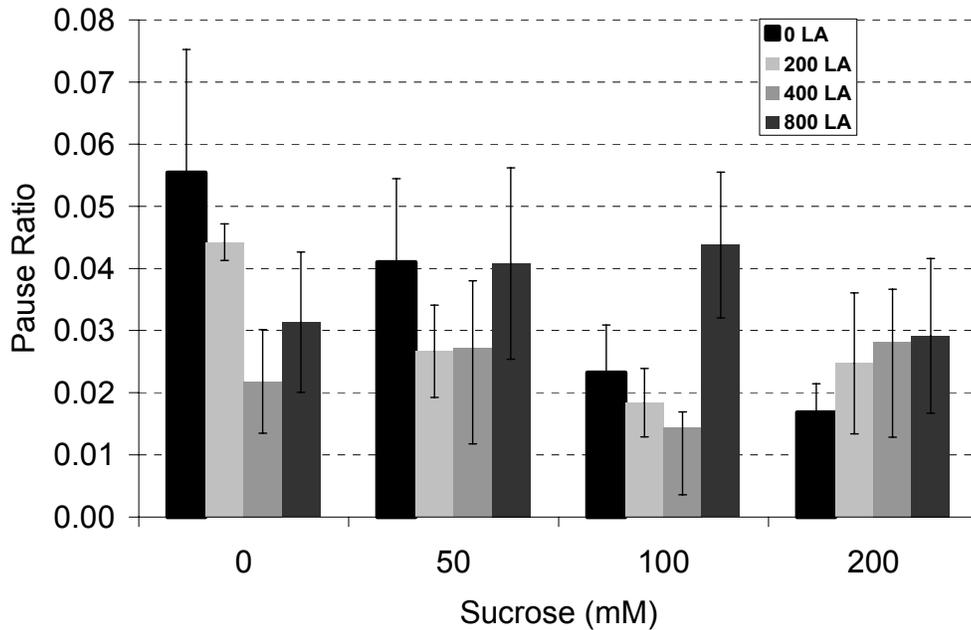


Figure 9. The pause ratios across the trials.

The pause ratio for each concentration is shown in Figure 9. Higher ratios were found for 0 and 200 μM LA and 0 mM sucrose. Ratios were smaller for 200 and 400 μM LA compared to 0 and 800 μM . For the 200 mM concentrations of sucrose, the pause ratios increased as a function of linoleic acid concentration.

Licking behavior may be most affected by lick frequency and pause ratio, which make them the most important factors of the microanalysis. The number of licks varies across the concentrations, without any visible trends. This licking behavior may be affected by lick frequency or by the amount of time spend licking to the solutions. Rats showed consistent licking across all solutions at about 6 licks per second, which is the maximal rate at which they normally lick. The pause ratio did vary by concentration, with smaller ratios for concentrations which the rats licked more (Figure 7).

Discussion

The lick results for the males produced maximal licking for 200 mM sucrose and 800 μ M linoleic acid, as expected. However, licks were minimal compared to other LA concentrations at 100 mM sucrose. Because licks did not greatly differ for all concentrations of LA at each concentration of sucrose, it can not be concluded that there is an increase based on LA concentration for male rats.

The smaller concentrations of LA at 50 mM sucrose produced effects as expected in males, where licking increases relative to LA concentration, but at 800 μ M LA, licking decreased. This could be due to a taste produced by 800 μ M LA with 50 mM of sucrose that was too sweet for palatability. Overall, there was no significant effect of LA on the percent change in licking from baseline. In most cases, LA increased licking, but not in the dose-dependent pattern that was expected. Although LA is known to enhance taste of sucrose based on concentration (Pittman et al, 2006), no such effect was seen in results for male rats.

Sucrose increases the number of times female rats lick to a solution. This is expected because of the pleasant nature of sucrose as a tastant for rats. Linoleic acid did not have an effect on intake at any concentration. Neither a detection threshold for LA nor a preference for sweet solutions when paired with LA was demonstrated in female rats.

Female rats do not show a significant increase in licking to solutions containing varying concentrations of LA. Detection could be occurring, but the LA produced no effect on consumption, even at high concentration levels. Rats are inclined to lick more as the solutions become sweeter, and a ceiling effect could be produced, causing the LA to have little to no effect on number of licks.

The results demonstrated in Figure 6 may be due to the females consuming more water than the males. The observed results in Figure 5 may also be due to the female rats consuming more water and also LA than their male counterparts. The female rats may be more sensitive to the linoleic acid which may have made the water more palatable. The absence of an increase in response to the sucrose solution may be that sucrose was not the best solution in which to test the rats' taste sensitivity.

The results of the microanalysis provide information about licking behavior in a more detailed fashion. The lick total results show that with increasing concentrations of sucrose alone, lick number also increase. This is expected because rats like the taste of sweet solutions. With the addition of linoleic, lick counts varied. Increasing concentrations of linoleic acid in the 50 mM sucrose solutions showed a stepwise trend. This gives some evidence that LA can affect the licking behavior to sucrose; however, the results were not significantly different. For the 100 mM sucrose, increasing concentrations of linoleic acid increased licking to 200 and 400 μ M LA solutions. The licks for the 800 LA at 100 mM of sucrose was decreased, suggesting that the 800 μ M LA made the solution too sweet. Rats tend to show an inverse U-shaped trend for sucrose preference, such that concentrations at the extremes are preferred less than those in between.

The number of bursts for each solution was between 3 and 5 for all solutions. The decrease in the number of bursts for 0 mM sucrose- 800 mM LA may be due to the rats showing an aversion to linoleic acid at a much higher concentration than is probable in natural feeding. The concentrations being used in this experiment are much higher than those found during typical lingual lipase breakdown within the oral cavity (Kawai and Fushiki, 2003). The number of bursts increased for the middle concentrations of linoleic acid, 200 and 400 μ M, with 100 mM

sucrose. This is expected because the concentrations of each solution are within the range preferred by the rats. The number of bursts would increase based on increased licking, Figure 7. The differences between the burst numbers at the 100 mM sucrose concentration are significantly different. No other significant differences can be seen for the number of bursts.

The pause ratio data shows that the number of pauses compared to the number of ILIs change as a function the number of licks. High ratios show that there fewer licks and many pauses, while low ratios show more pauses and many licks (Baird, St. John, & Nguyen, 2005). Higher concentrations of sucrose alone have a smaller ratio, which is related to the increased licking to that solution (Figure 7). The increased ratio for 800 μ M LA – 100 mM sucrose compares to the decrease in licking (Figure 7) and number of bursts (Figure 8).

The microstructure analysis shows that licking behavior to linoleic acid and sucrose is regulated not by satiety, but by the palatability of the solution. If satiety signals are regulating ingestion, then there would be an observable difference in lick frequency, or speed, to various solutions. However, because the rats lick at their maximum speed of 6 licks per second across all solutions, it can be concluded that the rats are not slowing down or speeding up as a result of hunger or fullness. However, the pause ratios during each trial vary for each solution, showing that rats pause during licking depending on the palatability of the solution. This data shows that rats change ingestive behaviors in reaction to the taste of a solution, as opposed to satietal influences.

Linoleic acid did not significantly affect consumption patterns of sucrose. There was not interaction between consumption and sex. Based on these findings, further research is necessary to determine the effects of linoleic acid on the different taste stimuli. Also, research may be conducted to investigate how consumption patterns differ based on sex. Future research using

smaller concentrations of linoleic acid, which are closer to the determined detection thresholds found in previous studies (McCormack et al. 2006). Shortening of the stimulus presentation time and performing the experiments during the normal sleep-wake cycle may produce results closer to natural feeding behavior patterns.

References

- Baird JP, St. John S, Nguyen EA. (2005) Temporal and qualitative dynamics of conditioned taste aversion processing: combined generalization testing and licking microstructure analysis. *Behav Neuro* 119(4): 983-1003.
- Curtis KS, Davis LM, Johnson AL, Therrien KL, Contreas RJ. (2004). Sex differences in behavioral taste responses to and ingestion of sucrose and NaCl solutions by rats. *Physiol Behav* 80: 657-664.
- Curtis KS, Stratford JM, Contreras RJ (2005) Estrogen increases the taste threshold for sucrose in rats. *Physiol Behav* 86: 281-286.
- Drewnowski A. (1997). Why do we like fat? *J Am Diet Assoc* 97(7): S58-2.
- Eckel LA, Moore SR (2004) Diet-induced hyperphagia in the rat is influenced by sex and exercise. *Am J Physiol Regul Integr Comp Physiol* 287: 1080-1085.
- Fukuwatari T, Kawada T, Tsuruta M, Hiraoka T, Iwanga T, Sugimoto E, Fushiki T. (1997). Expression of the putative membrane fatty acid transporter (FAT) in taste buds of the circumvallate papillae in rats. *FEBS Lett* 414: 461-464.
- Greenberg D, Smith GP. (1996). The controls of fat intake. *Psychosom Med* 58: 559-569.
- Gunstone FD. (1999) *Fatty acid and lipid chemistry*. Gaithersburg, MD: Aspen Publishers, Inc.
- Kanarek RB, Ryu M, Przypek J. (1995) Preferences for foods with varying levels of salt and fat differ as a function of dietary restraint and exercise but not menstrual cycle. *Physiol Behav* 57(5): 821-826.
- McCormack DN, Clyburn VL, Pittman DW. (2006). Detection of free fatty acids following a conditioned taste aversion in rats. *Physiol Behav* 87: 582-594.

Moles A, Cooper SJ. (1993) Opioid Modulation of Sucrose Intake in CD-1 Mice: Effects of Gender and Housing Conditions. *Physiol Behav* 58(4): 791-796.

Pittman DW, Labban CE, Anderson AA, O'Conner HE. (2006a). Linoleic and oleic acids alter the licking responses to sweet, salt, sour, and bitter tastants in rats. *Chem Senses* 31(9):835-43.

Pittman DW, Corbin CH, Crawley ME, Smith KR. (2006b). Personal communication. Unpublished results.

Smith JC, Fisher EM, Maleszewski V, McClain B. (2000). Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat. *Physiol Behav* 69: 135-46.

World Health Organization. (2000). Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 894:i-xii, 1-253.