McNair Scholars Research Journal

Volume 2 | Issue 1 Article 9

2-12-2010

The Sensitivity in Methods of Measuring Conditioned Flavor Aversions and Conditioned Flavor Preferences

Jacinda T. Bunting-Dady
Eastern Michigan University, jbuntin1@emich.edu

THE SENSITIVITY IN METHODS OF MEASURING CONDITIONED FLAVOR AVERSIONS AND CONDITIONED FLAVOR PREFERENCES

Jacinda T. Bunting
Dr. Kenneth Rusiniak, Mentor

ABSTRACT

This project investigated a multiple measurement procedure to assess conditioned flavor aversions (CFA) and conditioned flavor preferences (CFP) in male albino rats. Volume consumed is currently the most common and often the sole method used. Most studies employ group designs, whereas this study used a single-subject design to compare behavior patterns and responses between individual rats. Response measurements include: total licks, lick rate, lick patterns, volume (ml) consumed, volume (ml) per lick. Strong CFA showed consistent decreases in total licks, lick rate, total volume, and volume per lick. CFP was evident, although not consistent, in total licks, lick rate, total volume (ml), and volume (ml) per lick. Volume per lick measurement in CFP revealed that three of the four rats drank more per lick on the posttest flavor day after training. This measure may be a good indicator of CFP. This study provides normative data for evaluating the effects of drugs on neurotransmitters that modulate CFA and CFP.

INTRODUCTION

Our understanding of learning and memory requires two different but converging approaches to analysis, namely behavioral and neurological (Delprato & Rusiniak, 1991; Timberlake, 1993). This integrative approach proposes that a coordinated interaction of multiple brain structures is required for learning and memory to occur and has been described as processes and systems (Wig, Buckner, & Schacter, 2009). These memory-related circuits are also very important for different types of learning and memory to occur (Squire, 1992; White & McDonald, 2002).

Current work in cognitive neuroscience involving humans suggests that learning and memory rely on three neural systems. The first

system consists of the prefrontal cortex and the medial temporal lobe and its associated regions. This system is believed to be involved in the conscious recollection of training, or what is empirically known in behavioral psychology as "explicit memory." *Explicit memory* is involved in visual, verbal, and motor learning tasks. The second system consists of the basal ganglia and neocortex, associated with "implicit memory." *Implicit memory* refers to the situation in which the subject can demonstrate knowledge or a skill using actions but cannot explicitly retrieve information verbally or consciously. A third system includes the amygdala and its associated structures (e.g., the limbic system), which form the neural basis for "emotional memory." *Emotional memory* has characteristics of both implicit and explicit memory. The neural circuits involved in emotional memory are unique in that they involve the amygdala. Learning involved in eating is often studied using an associative learning model that involves emotional conditioning.

Associative learning is the connection or association of stimuli and responses. The neurological basis of associative learning is often researched using animal models. Animal research also plays a key role in our understanding of physiological processes, specifically those affecting neural disorders (Carroll & Overmier, 2001). The rat has served as a useful tool for identifying structures in the brain that are particularly important in associative learning.

Typically, the neural basis of learning and memory has been investigated using a range of techniques, including brain lesions in animals learning various tasks (Pezuk et al., 2008), fos-like immunoreactivity in rats (Bernstein & Koh, 2007), electrical recording from brain areas while an animal performs learning tasks (Shatskikh et al., 2006), recordings from slices of hippocampal tissue, and the injection of drugs that either inhibit or elicit learning and memory in the rat (Davies et al., 2007; Golden, 2007). The study of brain processes in relation to behavior is an approach that entails assessing both neural and behavioral responses to stimuli. Such studies allow us to collect data that could not be collected using human subjects.

As in human memory models, several brain structures in the rat have been identified as being associated with different types of memory functions. Animal research is also believed to be a source of insight into the evolution of the human body and its functions. This use of animal models has been particularly instrumental in shaping our understanding of how the brain handles information involved in associative learning processes. Interestingly, rodents, particularly the rat, have proven to be

an especially important experimental vertebrate to study memory systems. For example, the hippocampus is hypothesized to be involved in several types of learning and memory, including declarative memory (Squire, 1992), spatial memories (O'Keefe & Burgess, 1996; Clement, Blahna, & Nekovarova, 2008), configural learning (Rickard & Grafman, 1998), consolidation (Remondes & Schuman, 2004), relational and conjunction memory (Moses & Ryan, 2006), and taste learning (Stone, Grine, & Katz, 2005).

The hypothalamus structure is believed to play a role in the process of learning and memory involved in eating and drinking. The amygdala stimulates the hippocampus and cerebral cortex, which are both important for memory storage (de Quervain, Roozendaal, & McGaugh, 1998). The former system may be homologous to the prefrontal-medial temporal system in humans, as well as the limbic system.

The rat is often used to study associative learning in eating behaviors using two learning models, *conditioned flavor aversion* (*CFA*) and *conditioned flavor preference* (*CFP*). The study of learning and memory processes involved in conditioned flavor aversion has been especially useful. This process occurs when a subject associates the taste and odor of a certain flavor with symptoms of illness caused by a toxic, spoiled, or poisonous substance (Delprato & Rusiniak, 1991; Garcia, Hankins, & Rusiniak, 1974; Golden, 2007; Green & Garcia, 1971; Lipinski et al., 1995). Conditioned flavor aversion is unique in that it can be acquired often in a single conditioning trial; when this was first discovered, it violated the basic principles of learning and memory known at the time (Garcia & Ervin, 1968; Garcia, Hankins, & Rusiniak, 1974; Green & Garcia 1971; Revusky & Garcia 1970; Rozin & Kalat, 1971).

This discovery indicated that there may be various biological constraints on learning and memory. It was also useful in elaborating brain regions involved in emotional learning. That is, the brain has evolved different neural memory systems that affect different learning and memory functions (Wig, Buckner, & Schacter, 2009). Many brain regions are believed to be involved in CFA learning including the amygdala (Yamamoto, 2008), nucleus accumbens (Ramirez-Lugo, Nunez-Jaramillo, & Bermudez-Rattoni, 2007), and the hippocampus (Stone, Grimes, & Katz, 2005). While the underlying neurological processes involved in CFA learning are still not known, researchers are consistently using different methods of assessment to increase our understanding of CFA.

Volume, weight, and percent consumed are the most common methods used to obtain aversion data in an eating study. The implications

for CFA study have ranged from studies on obesity and drug abuse to its relation to the eating behaviors in humans after chemotherapy treatment.

Conditioned flavor preference, known as the complement of CFA, occurs when an association is developed between a neutral flavor and a positive nutritional after-affect. The role of flavor in nutritional obesity and other addictive behaviors has been one focus of CFP studies (Scalfani, 2001). Many animals, including humans, base their consumatory behavior on the flavor of food (Golden, 2007). Flavor preferences acquired during consumatory behaviors are believed to develop due to orosensory as well as nutritional values (Sclafani, 2001). Another characteristic of preference that has been proposed is wanting in the absence of liking (Robinson and Berridge, 1993). Behaviorally, the cues associated with taking drugs that were investigated by Robinson and Berridge (1993) can be extended into cues related to eating behaviors.

The increase in consumption measured by volume and energy levels has recently been the measurement focus when investigating the effects that adding to the palatability of food has on CFP studies (Sclafani, 2001). Nevertheless, behavioral gestures and a multiple measurement procedure may reveal preferences or avoidances that may not have been evident by measuring only the volume consumed or the energy level of the subject. In addition, small molecule neurotransmitters have recently been investigated for their role in CFA and CFP. Glutamate is one of these neurotransmitters. Most recently, glutamate receptors have been shown to be critical for associative learning (Simonyi et al., 2009).

The glutamate receptor that is sensitive to N-Methyl-D-Aspartate (NMDA) is widely distributed in the central nervous system (Golden, 2007) and has been demonstrated to moderate several different kinds of learning. This NMDA receptor is associated with many of the primary functions of the nervous system and is currently researched in the pharmacological management of seizures, a variety of neurological disorders, pain, central nervous system abnormalities, neurological activity and development (Haberny et al., 2002). Memory is one of these primary functions and there is an increasing interest in the field of behavioral pharmacology for agents that may block this NMDA receptor.

The study of the nutritional values involved in flavor preferences as well as research with a focus on the biological components of flavor aversions has lead to the study of neurotransmitters. This area of research has most recently expanded to the study of their role in preference and avoidance learning and the effects of drugs that inhibit the neurotransmitters functions.

The central nervous system readily penetrates the anticonvulsant compound of MK-801{(+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate)} (Wong et al., 1986). Several researchers have found that MK-801, a non-competitive antagonist, disrupts CFA learning. A MK-801 injection distributed directly into the amygdala has been shown to reduce neophobia and rejection response to a novel flavor, suggesting that glutamate responses in the amygdala may be directly associated with CFA (Tucci, Rada, & Hernandez, 1998).

This project was designed to obtain baseline data in preparation for our future work which will focus on blocking the NMDA receptors and the affects that different dose levels of the NMDA antagonist will have on CFA and CFP learning. More specifically, the role of the NMDA receptors in the associative learning processes of CFA and CFP while under the influence of MK-801 and APV-5. The compound APV (DL-2-amino 5-phosphonovaleric acid) is a competitive NMDA antagonist and will also be used to block the neurotransmitters proposed to be involved in CFA and CFP learning.

We observed orofacial gestures, licks, and volume consumed to investigate whether a multiple measurement procedure may be a more sensitive method of measuring CFA and CFP. In previous studies, behaviors have been observed as identifiers of an aversion reaction, and we adopted some of these criteria for the current study, namely lip smacking, hesitant lick patterns, wiping of the mouth area (which resembles grooming behaviors), as well as grabbing and biting the drinking spout (Delprato & Rusiniak, 1991). Future analysis of the orofacial data collected during the current study will use behavioral criteria similar to Delprato and Rusiniak (1991) and Berridge, Grill, and Norgren (1981) to score behaviors on a temporal basis.

The basic methodology of this experiment follows several published reports. The general behavioral procedures used for the CFA group were similar to those used by Garcia, Hankins, and Rusiniak (1974), Delprato and Rusiniak (1991), and Lipinski, Rusiniak, Hillard, and Davis (1995). The methodology of the preference study follows those used by Rusiniak, Steigerwald, Arsnov, and Spencer (2008). The overall goal of this study is to identify a baseline for several consumption measurement methods used in a typical CFA and CFP study. This baseline data will then be used to determine which method of measurement is most efficient for assessing CFA and CFP learning in a drug study. The assessment of CFA and CFP may require multiple measurement techniques in order to detect any disruptions due to the drugs MK-801 and APV-5. Such techniques were developed during the pilot study.

METHODS

Animals

The experiment was conducted on male albino rats (200g–300g) obtained from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed individually in standard suspended wire mesh cages in a colony maintained on a 12L:12D cycle, with lights on from 0600 to 1800h. Purina Lab Chow and water was available at all times, except as noted.

Materials and Procedures

Apparatus. Plastic boxes (15 x 15 x 17cm) were placed on brass grids. Rats had access to a single metal drinking spout with a ball-bearing tip (ATCO TD-30, Napa, CA) provided through a small hole at one end of the box. The boxes were enclosed in a larger sound-attenuating chamber equipped with white noise (65 dB re 20 $\mu N/m^2$), which masked extraneous sounds (Rusiniak, Garcia, & Hankins, 1976). A video recording device (Cyber-shot® Digital Camera DSC-HX1 Imaging Device: 1/2.4 type [7.63mm] Exmor CMOS Sensor, Megapixel: 9.1MP, Recording Media: 8G Flash Memory and 11MB of Internal Flash memory) was placed in the sound-attenuating chamber to assess orofacial gestures and general agitation measures (Berridge, Grill, & Norgren, 1981).

Procedure. An electronic drinkometer circuit (Grason-Stadler E4600A-1) monitored licks recorded during the 5 minute sessions (Rusiniak, Garcia, & Hankins, 1976). Drinkometer results were printed on strips and later analyzed using a temporal basis contact with the spout. A videotape recording of each rat's behavioral responses during training and test day were analyzed using frame by frame playback (Adobe Soundbooth CS3, Version 1.0). Each rat was scored for orofacial gestures and agitation using the consummatory response criteria similar to Berridge, Grill, and Norgren (1981), Berridge and Grill (1983), and Delprato and Rusiniak (1991). Volume consumption in ml was measured using a single 50 ml calibrated centrifuge tube equipped with a rubber stopper and stainless steel sipper tube.

General behavioral procedures. After several weeks of adjustment to the colony, the experimental procedures began. Each day the animals were weighed, handled, and given access to water and food. Rats were then pre-adapted to drinking in the apparatus. Water was removed from the home cages, and the animals were trained to consume their entire daily fluid consumption during a single 5 min

session in the experimental apparatus (Rusiniak, Garcia, & Hankins, 1976). Animals maintained 85% of their free-feeding weight by post-session feedings, and by daily feedings on the days that sessions were not conducted (Weatherly et al., 2005).

Training. Drinking sessions were reduced gradually from 1h to 5 min; trials then remained at 5 min for the remainder of the pre-adaption period. Drinking sessions occurred at the same time each day maintaining a 23.75 h deprivation period. Water was provided in a single 50 ml calibrated centrifuge tube equipped with a rubber stopper and stainless steel sipper tube. Consumption in ml was measured daily. Lick data and video recordings of the sessions were obtained during training as well. Rats were also familiarized with the tested fluids on one pretest flavor session to reduce the effect of novelty (Green & Garcia, 1971; D. Mitchell, D. Scott, & L. Mitchell, 1977) on one flavor pretest session.

Experimental procedures.

Conditioned flavor aversion group (n = 4). A total of 4 rats were assigned to the CFA group. Two rats (CFA rat #1 and CFA rat #2) were assigned to the pilot study and 2 rats (CFA rat #3 and CFA rat #4) were assigned to the current study. Experimental conditions in the pilot study were identical to the current study with the exception of the temporal distribution of the illness agent.

Approximately 2 days before experimental sessions began all rats received sham intubations to habituate them to the intragastric infusion procedure. During this habituation process, a plastic infant feeding tube (Kendall/Curity, 38 cm, ref# 1155722) was passed down the throat into the stomach and approximately 3 ml of water was infused.

Experimental procedures began for all rats on Day 1. Rats #1 and #3 ($n = 2$) were pre-exposed to cherry flavor (4.25 g powdered cherry Kool-Aid and 0.1% saccharine), rats #2 and #4 ($n = 2$) were pre-exposed to grape flavor (4.25 g powdered grape Kool-Aid and 0.1% saccharine). On days 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, and 18, all rats received water in the apparatus during a standard 5 min session. On days 4 and 7, 2 rats in the current study received an intragastric infusion of the illness agent (0.15 M LiCl at 127 mg/kg) 30 min after receiving the assigned flavor. In the pilot study, 2 rats received the illness agent immediately after the flavor. The dose used for all rats was well below that which animals will self-administer and causes a brief gastric malaise (Rusiniak, Hilliard, & Poschel, 1993).

On day 10, each rat in each flavor group was tested with only

their assigned flavor. On days, 13, 16, and 19 rats were tested again with only their assigned flavor, without the coupling of the illness agent, to obtain extinction data.

Conditioned flavor preference group (n = 4). A total of 4 rats were in the CFP group. Two rats were in the pilot study (CFP rat #1 and CFP rat #2) and 2 rats were in the current study (CFP rat #3 and CFP rat #4). Experimental conditions in the pilot study were identical to the experimental conditions in the current study.

Experimental procedures began for all rats on Day 1. All rats received milk nutrient for a 5 min session in the apparatus. On day 2, rats #1 and #3 (n = 2) were pre-exposed to cherry flavor (4.25 g powdered cherry Kool-Aid and 0.1% saccharine), rats #2 and #4 (n =2) were pre-exposed to grape flavor (4.25 g powdered grape Kool-Aid and 0.1% saccharine). On days 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 18, 19, and 18, all rats received water in the apparatus during a standard session. On days 5, 8, 11, 14, and 17 rats received an assigned flavor mixed with a carbohydrate nutrient (20% polycose). On day 20, rats in both flavor groups of the pilot study and the current study were tested with only the assigned flavor. Results were measured by lick patterns, orofacial gestures, and volume in ml during all training, test, and water days.

RESULTS

Conditioned Flavor Aversion

Flavor pretest and posttest results. Aversion measures in total licks, lick rate, volume (ml), and volume per lick were compared between the pretest flavor day and posttest flavor day (post-treatment flavor alone). Strong conditioned flavor aversion was evident and consistent in several measures of flavor consumption: total licks, lick rate, total volume (ml), volume (ml) per lick indicated on the data strips all decreased (see Table 1 and Figure 1).

Total licks. The number of total licks before and after conditioning on flavor days is shown in Table 1. Total number of licks per each 5 min session was analyzed for each individual subject. All rats decreased in the total number of licks during posttest day session. In the total lick measurement results, rats in the pilot study (CFA rat #1 and CFA rat #2) both stopped licking after the initial lick. Rats in the current study (CFA rat #3 and CFA rat #4) continued to lick (CFA rat #3 = 29 licks, and CFA rat #4 = 15 licks), but they did not drink any measurable volume.

Lick rate. The lick rate before and after conditioning on flavor days and water days is shown on Table 2. The number of licks while in contact with the drinking spout was analyzed for each individual subject, to observe licks per contact second on the spout. Licks per second decreased in most rats, except CFA rat #2, which increased in lick rate during both the posttest flavor day and the posttest water day. CFA rat #2 and CFA rat #3 both increased in lick rate and CFA rat #1 had the same lick rate on pretest flavor day and pretest water day. CFA rat #4 drank at an increased rate on pretest water day in comparison to the pretest flavor day.

Volume (ml). Volume consumption before and after conditioning on flavor days is shown on Table 1. Volume was measured in (ml) before and after each 5 min session on pretest flavor days, and posttest flavor day. Volume consumption decreased for all rats on the posttest flavor day.

Volume (ml)/lick. Volume consumption before and after conditioning on flavor days is shown on Table 1. Consumption in ml/lick ranged from 0.004 to 0.011 ml/lick. None of the rats showed a significant volume of consumption on the posttest day.

Lick pattern. The lick pattern before and after conditioning on flavor days and water days can be reviewed for one rat in Figure 1. Lick pattern displayed a strong aversion had developed. The lick pattern also displayed an increased in drinking behaviors on posttest water day.

CONDITIONED FLAVOR AVERSION (CFA) FLAVOR EXPOSURE DAYS						
RAT #	**LICKS**		**VOLUME**		**VOLUME (ml)/ LICK**	
	Flavor Pretest	Flavor Posttest	Flavor Pretest	Flavor Posttest	Flavor Pretest	Flavor Posttest
1	647	1	6	0	0.009	0
2	398	1	4.5	0	0.011	0
3	227	29	1.5	1	0.007	0
4	58	15	0.25	0	0.004	0

Table 1 Conditioned Flavor Aversion: Number of licks, volume (ml), and volume (ml) per lick on pretest flavor day and posttest flavor day.

LICKS PER CONTACT SECOND						
RAT #	Flavor Pretest	Flavor Posttest	+ or –	Flavor Pretest	Flavor Posttest	+ or –
1	3	0	**(-) 3**	3	3	**0**
2	3	4	**(+) 1**	4	5	**(+) 1**
3	4	3	**(-) 1**	4	5	**(+) 1**
4	3	1	**(-) 2**	4	4	**0**

Table 2. Conditioned Flavor Aversion: Number of licks per contact second on pretest flavor day and posttest flavor day and baseline water pretest and posttest days.

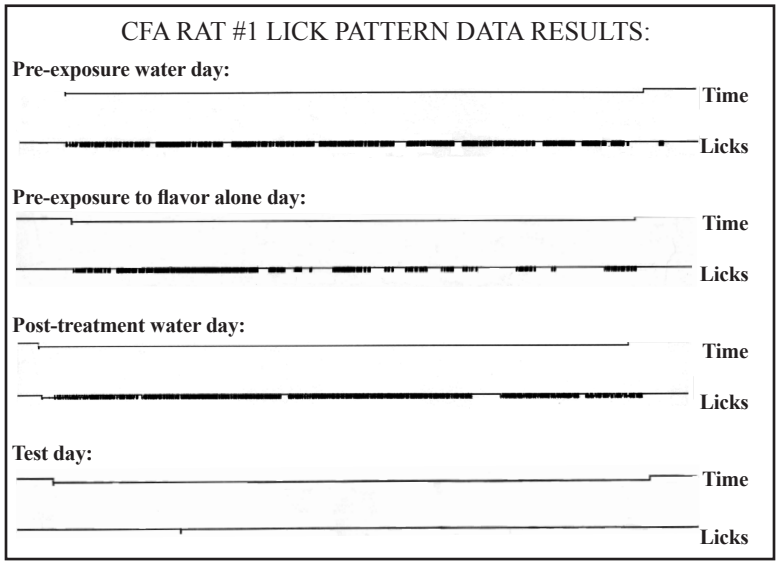

Figure 1. Conditioned Flavor Aversion Group: The top line indicates the start and stop of the five-minute session. The bottom line shows the licking behavior pattern. Black marks indicate contact with the spout.

Baseline water pretest and posttest results.

Consumption before and after conditioning on water days is shown on Table 3. A comparison of total licks, volume (ml), and volume (ml) per lick on pretest water day and posttest water day showed that water consumption before and after treatment remained relatively constant. Notably, water volume consumed increased during the pilot study and decreased in the current study.

CONDITIONED FLAVOR AVERSION (CFA) WATER BASELINE						
RAT #	**LICKS**		**VOLUME**		**VOLUME (ml)/ LICK**	
	Water Pretest	Water Posttest	Water Pretest	Water Posttest	Water Pretest	Water Posttest
1	884	**793**	10.5	**12.5**	0.012	0.012
2	1027	**704**	10	**11**	0.01	**0.009**
3	933	1077	11.5	**8.5**	0.012	0.008
4	843	862	10	**9**	0.012	0.008

Table 3. Conditioned Flavor Aversion: Number of licks, volume (ml), and volume (ml) per lick on pretest water day and posttest water day.

Conditioned Flavor Preference

Flavor pretest and posttest results. Preference measures in total licks, lick rate, volume (ml), and volume per lick were compared between the pretest flavor day and posttest flavor day (post-treatment flavor alone). Conditioned flavor preference was evident, although not consistent, in all measures of flavor consumption: total licks, lick rate, total volume (ml), and volume (ml) per lick. The derived measure of consumption, volume (ml) per lick, revealed an interesting feature of conditioned flavor preference. Animals drank more per lick on the posttest flavor day after training. Three of the four rats increased in the amount consumed per lick (i.e., gulping). Therefore, this measure may be a good indicator of conditioned flavor preference. Moderate conditioned taste preference was evident in the volume (ml) consumed, and in volume (ml) per lick. In the pilot study and the current study, different measures detected CFP. In both studies, volume was the most sensitive followed by volume per licks. Absolute number of licks was the least sensitive of the measures. Licking patterns shown on the lick strips were not a clear indicator of conditioned flavor preference.

Total licks. Total licks before and after conditioning on flavor days is shown in Table 4. Total number of licks for each 5 min session was analyzed for each individual subject. A consistent amount of increase in the consumption on posttest flavor day was not evident in the total licks. Not all rats increased in the number of licks. Those rats that did increase were not consistent when measuring the level of increase that occurred.

Lick rate. The lick rates of the subjects before and after conditioning on flavor days are shown in Table 5. The number of licks while in contact with the drinking spout was analyzed for each individual subject to observed licks per contact second on the spout. Lick rates during test day varied between the pretest and posttest flavor days, and the pretest and posttest water days.

Volume. Volume consumed before and after conditioning on flavor days is shown in Table 4. Volume was measured in (ml) before and after each 5 min session on pretest flavor days, posttest flavor day, pretest water day, and posttest water day. All rats displayed an increase in volume consumption on posttest flavor day. This increase in consumption ranged from 1.0 to 4.5 ml.

Volume (ml)/lick. Volume per lick before and after conditioning is shown on Table 4. Volume (ml) consumption was measured on pretest and posttest flavor days for each rat. These data were analyzed for each individual subject to observe the amount of consumption in (ml) per lick recorded. Consumption in ml/lick on the pretest flavor day ranged from 0.006 to 0.015 ml/lick. The consumption in ml/lick was measured on posttest flavor day. Rat #2 displayed an increased of 0.007 ml/lick and rat #3 displayed a decreased of 0.003 ml/lick.

Lick pattern. The lick pattern before and after conditioning on flavor days and water days is shown in Figure 2. Licking patterns shown on the lick strips were not a clear indicator of conditioned flavor preference.

CONDITIONED FLAVOR PREFERENCE (CFP)								
LICKS			**VOLUME**			**VOLUME (ml)/ LICK**		
Flavor Pretest	Flavor Posttest	+ or –	Flavor Pretest	Flavor Posttest	+ or –	Flavor Pretest	Flavor Posttest	+ or –
771	702	**(–) 69**	6.50	10.50	**(+)4.00**	0.008	0.015	**(+) 0.007**
34	438	**(+) 404**	0.50	5.25	**(+) 4.50**	0.015	0.012	**(–) 0.003**
169	339	**(+) 170**	1.00	3.50	**(+) 2.50**	0.006	0.01	**(+) 0.004**
215	358	**(+) 143**	1.50	2.25	**(+) 1.00**	0.007	0.009	**(+) 0.002**

Table 4. Conditioned Flavor Preference: Number of licks, volume (ml), and volume (ml) per lick on pretest flavor day and posttest flavor day. A (+) indicates an increase and (-) indicates a decrease.

LICKS PER CONTACT SECOND						
Rat	Licks per Contact Second Flavor Pretest	Licks per Contact Second Flavor Posttest	+ or –	Licks per Contact Second Pretest Water	Licks per Contact Second Posttest Water	+ or –
1	4	4	**0**	4	4	**0**
2	4	3	**(-) 1**	5	5	**0**
3	4	3	**(-) 1**	5	5	**0**
4	3	4	**(+) 1**	5	4	**(-) 1**

Table 5. Conditioned Flavor Preference: Number of licks per contact second on pretest flavor day, posttest flavor day, pretest water day, and posttest water day.

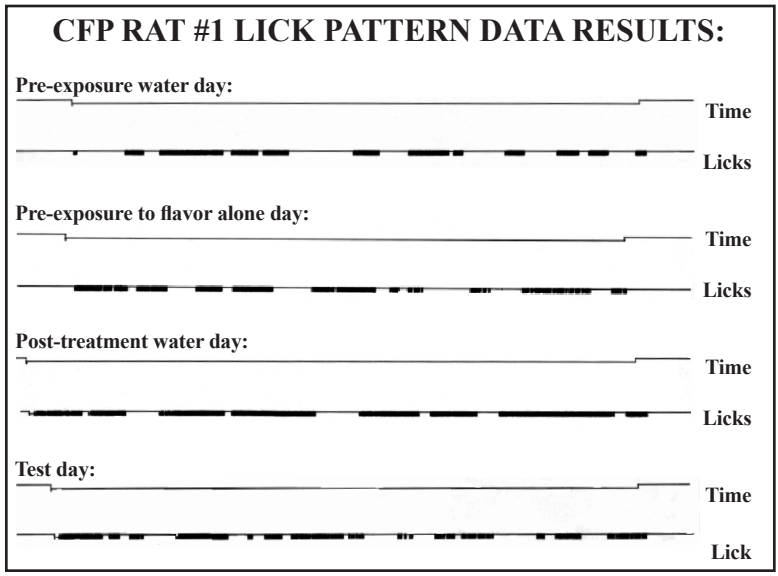

Figure 2. Conditioned Flavor Preference: The top line indicates the start and stop of the five minute session. The bottom line shows the licking behavior pattern. Black marks indicate contact with the spout.

DISCUSSION

Conditioned flavor aversion was evident in all rats. All of the methods of measurement used in this study indicated a flavor aversion had occurred, except for pattern of licks. Changes were evident in total licks, lick rate, total volume (ml), and volume (ml) per lick. Although there was a difference between the rats in the pilot study and the rats

in the current study, the total number of licks on posttest flavor day decreased for all rats. When reviewing individual results between subjects, licks reveal a different pattern in the total licks between the pilot study rats and the current studies rats. The total licks on posttest day suggested that the rats in the current study continued to return to the spout (several times) during the session. The total licks measurement suggested behaviors similar to tasting, not drinking.

The rats in the pilot study took one initial lick and did not return to the spout. Notably, several external factors may have influenced these data. First, the rats in the pilot study received the LiCl immediately after the flavor, whereas the rats in the current study received the LiCl 30 min after the flavor. Second, the drinkometer is unable to distinguish between a lick and any other contact with the spout. The rat may have come into contact with the spout through behaviors other than licking. This alternative contact could then have registered as a lick, giving a false count of licking behaviors. Third, the rats tongue may have come into contact with the spout without depressing the ball in the spout to release the fluid (i.e., tasting).

The lick rate decreased in all but one rat on posttest day. The rat with the increased lick rate on posttest flavor day also increased in lick rate during the water sessions. This increase in the rate of licking may be indicative of the predetermined drinking behaviors of that rat.

The volume decreased to 0 ml for all rats on posttest flavor day. None of the rats in the conditioned flavor aversion group showed a significant volume of consumption on posttest flavor day. Volume consumed did increase during the posttest water day for the rats in the pilot study and decreased in the current study. This may be due to experimental conditions as well as predetermined drinking behaviors.

The volume (ml)/lick measure consistently showed a strong flavor aversion in all rats on posttest day. On pretest day, volume (ml)/lick varied in all rats. This variance in consumption per lick may also be due to predetermined drinking behaviors of the rats. The low volume of intake also influenced the volume (ml)/lick results. The lick pattern strips served as a clear indicator that conditioned flavor aversion had occurred. Pretest flavor day and posttest flavor day, when compared, reveal a decrease in drinking behaviors. The lick pattern shown on the drinkometer strips was a useful method of measurement in detecting conditioned flavor aversion.

Conditioned flavor preference was evident, although not consistent, in all methods of measurement. An increase in the number of licks was not evident in all rats, but lick measures from posttest flavor day and pretest flavor day did show an increase in consumption. Those rats that did

increase in the number of licks did not do so consistently. Lick rates varied for most rats when comparing pretest and posttest flavor day to pretest and posttest water day. Lick rates during posttest were consistent with lick rates during pretest and posttest water day. Volume measures are commonly used to investigate conditioned flavor preference. While all rats increased in the volume consumed, the amount of increase that occurred varied. The increase in the volume consumed ranged from 1.0 to 4.5 ml, while rats that statistically drank the same amount did so at a different rate.

The derived measure of volume (ml)/lick revealed interesting features of drinking behaviors in the rats. On pretest flavor day, the rats varied widely in volume (ml)/lick consumed. This variance in the initial intake of the novel flavor was detected in all of the rats in the CFA group as well, suggesting predetermined drinking behaviors varied among the rats. Posttest flavor day volume (ml)/lick also varied in all rats in the conditioned flavor preference group. This comparison of how much is being consumed per lick revealed that the rats engaged in sipping and gulping behaviors on posttest flavor day. The volume (ml)/lick measure did not reveal a strong preference, although observing these sipping and gulping behaviors more closely in combination with other measurements may serve as a useful indicator of the level of flavor preference that has occurred.

By reviewing only the amount of consumption using only one measure in CFA and CFP studies, we cannot conclude anything about the neurological and behavioral systems that influence these learning models. In both the CFA and CFP group, when analyzing the total number of licks the rat takes in a drinking session, the drinkometer counts any contact with the spout as a lick. Again, other behaviors could have caused the drinkometer to register a lick: grabbing the spout, sniffing the spout, lip smacking, etc. This measure is non-informative of these behaviors. Video analysis of these behaviors may prove more useful in obtaining an accurate count of licks.

The lick rate measurement did not show a consistent decrease for the CFA rat or a consistent increase for the CFP rats. This was not considered an ideal measurement for either of the learning models. While aversion and preference were both detected via measurement of volume, in that both groups altered the volume of their drinking, this measure proved to be most useful when in combination with licks. In the CFP group volume (ml)/lick may offer sensitivity to the detection of a flavor preference.

A more thorough analysis of the way we measure CFA and CFP would expand our understanding of the behaviors associated with aversions and preferences. These learning models are essential motivational states in eating disorders and interestingly, eating behaviors of subjects

recovering from chemotherapy (Bernstein, 1985). Data from the current study will be used as baseline data for our future study involving the neurological affects of NMDA antagonists on conditioned flavor aversion and conditioned flavor preference. The behaviors associated with CFA and CFP while under the influence of an antagonist will be reported using the multiple-measurements method used in the current study. We propose that by using the multiple-measurement methods design used in this study we may better detect the effects of the NMDA antagonists, MK-801 and APV-5, on CFA and CFP.

REFERENCES

Bernstein, I., (1985). Learned food aversions in the progression of cancer and its treatment. *Annals of The New York Academy of Sciences*, *443*, 365–380.

Bernstein, I., Koh, M. (2007). Molecular signaling during taste aversion learning. *Chemical Senses*, *32*, 99–103.

Berridge, K. & Grill, H. (1983). Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. *Behavioral Neuroscience, 97*, 563–573.

Berridge, K., Grill, H., & Norgen. R. (1981). Relation of consummatory responses and preabsorptive insulin release to palatability and learned taste aversions. *Journal of Comparative and Physiological Psychology*, *95* (3), 363–382.

Berridge, K. & Robinson, T. (1993). The neural basis of drug craving: An incentive-sensitization theory of addition. *Brain Research Reviews, 18*, 247–291.

Burgess, N. & O'Keefe, J. (1996). Cognitive graphs, resistive grids, and the hippocampal representation of space. *Journal of General Physiology, 107*, 659–662.

Carroll, M. & Overmier, J.B. (2001). Advancing human welfare through behavioral science. *Animal Research and Human Health*. Washington, DC: American Psychological Association.

Davies, M., Kirov, S., & Andrew, R. (2007). Whole isolated neocortical and hippocampal preparations and their use in imaging studies. *Journal of Neuroscience Methods*, *166* (2), 203–16.

Delprato, D. & Rusiniak, K. (1991). Response patterns in shock avoidance and illness aversion. *In Fear Avoidance and Phobias*, 285–315.

de Quervain, D., Roozendaal, B., & McGaugh, J. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature, 394, 787–790.

Garcia, J., & Ervin, F. (1968). Gustatory-visceral and telereceptor-cutaneous conditioning: Adaptation in internal and external milieus. *Communications in Behavioral Biology, 1*, 389–415.

Garcia, J., Hankins, W., & Rusiniak, K. (1974). Behavioral regulation of the milieu interne in man and rat. *Science*, *185*, 824–831.

Golden, G. (2007). Behavioral and neural characterization of conditioned flavor-taste preferences. *Dissertation submitted to the Department of Biological Science*. Florida State University.

Green, K. & Garcia. J. (1971). Recuperation from illness: Flavor enhancement for rats. *Science*, *173*, 749–751.

Haberny, K., Paule, M., Scallet, A., Sistare, F., Lester, D., Hanig, J., & Slikker, Jr., W. (2002). Ontogeny of the N-Methyl-D-Aspartate NMDA receptor system and susceptibility to neurotoxicity. *Toxicological Sciences, 68*, 9–17.

Klement, G., Blahna, K., & Nekovarova, T. (2008). Novel behavioral tasks for studying spatial cognition in rats. *Physiological Research, 57*, S161–S165.

Lipinski, W., Rusiniak, K., Hillard, M., & Davis. R. (1995). Nerve growth factor facilitates conditioned taste aversion in normal rats. *Brain Research*, *692*, 143–153.

Mitchell, D., Scott, D. W., & Mitchell, L. K. (1977). Attenuated and enhanced neophobia in the taste-aversion delay of reinforcement effect. *Animal Learning and Behavior, 5*, 99–102.

Moses, S. & Ryan, J. (2006). A comparison and evaluation of the predictions of relational and conjunctive accounts of hippocampal function. *Hippocampus, 16*, 43–65.

Palmerino, C.C., Rusiniak, K. W., & Garcia, J. (1980). Flavor-Illness Aversions: The peculiar roles of odor and taste in memory for poison. *Science, 208*, 753–755.

Pezuk, P., Aydin, E., Aksoy, A., & Canbeyli R., (2008). Effects of BNST lesions in female rats on forced swimming and navigational learning. *Brain Research, 1228*, 199–207.

Ramirez-Lugo, L., Nunez-Jaramillo, L., & Bermudez-Rattoni, F. (2007). Taste memory formation: Role of the nucleus accumbens. *Chemical Senses, 32*, 93–97.

Revusky & Garcia J. (1970) Learned associations over long delays. In G. Bower and J. Spence (Eds.), *Psychology of learning and motivation: Advances in research and theory 4.* New York: Academic Press.

Remondes, M., & Schuman, E. (2004). Role for cortical input to hippocampal area CA 1 in the consolidation of long-term memory. *Nature, 431*, 699–703.

Richard, T. & Grafman, J. (1998). Loosing their configural mind: Amnesic patients fail and transverse patterning. *Journal of Cognitive Neuroscience, 10*, 509–516.

Rozin, P., & Kalat, J. (1971). Specific hungers and poison avoidance as adaptive specializations of learning. *Psychological Review, 78*, 459–486.

Rusiniak, K., Hilliard, M. & Poschel, E.J. (1993). Individual differences in lithium chloride sensitivity in rats. *Michigan Academician*, *25*, 145–157.

Rusiniak, Steigerwald, Arsnov, & Spencer. (2008). Flavor preference conditioned by recovery from lithium illness or calories in rats. *Paper presented at the Annual Meeting of the Michigan Academy of Science, Arts, and Letters, Kalamazoo, MI.*

Sclafani, A. (2001). Psychobiology of food preferences. *International Journal of Obesity, 25*, S13–S16.

Shatskikh TN, Raghavendra M, Zhao Q, Cui Z, & Holmes GL. (2006). Electrical induction of spikes in the hippocampus impairs recognition capacity and spatial memory in rats. *Epilepsy & Behavior: E&B*, *9* (4), 549–56.

Simonyi, A., Serfozo, P., Parker, K., Ramsey, A., & Schachtman, T. (2009). Metabotropic glutamate receptor 5 in conditioned taste aversion. *Neurobiology of Learning and Memory, 10*, 1016.

Squire, L.R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review*, *99*, 195–231.

Stone, M., Grimes, B., & Katz, D. (2005). Hippocampal inactivation enhances taste learning. *Learning and Memory, 12*, 579–586.

Timberlake, W. (1993). Behavior systems and reinforcement: An integrative approach. *Journal of Experimental Analysis of Behavior, 60*, 105–128.

Tucci, S., Rada, P., & Hernandez, L. (1998). Role of glutamate in the amygdala and lateral hypothalamus in conditioned taste aversion. *Brain Research, 813*, 44–49.

Weatherly, J., Nurnberger, J., Arthur, E., & King, B. (2005). Duration, response, and location: The influences of upcoming 32% sucrose on rats' licking or lever pressing for 1% liquid sucrose. *Behavioral Processes, 70*, 80–90.

White, N. & McDonald, R. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning & Memory*, *77*, 125–184.

Wig, G., Buckner, R., & Schacter, D. (2009). Repetition priming influences distinct brain systems: Evidence from task-evoked data and resting-state correlations. *Journal of Neurophysiology, 101*, 2632–2648.

Willner, J., Gallagher, M., Graham, P., & Crooks, G. B. (1992). N-Methyl-D-Aspartate Antagonist D-APV selectively disrupts taste-potentiated odor aversions. *Behavioral Neuroscience, 106*, 315–323.

Wong, E., Kemp, J., Priestley, T., Knight, A., Woodruff, G., & Iverson (1986). The anticonvulsant MK-801 is a potent N-Methyl-D-Aspartate antagonist. *Neurobiology, 83*, 7104–7108.

Yamamoto, T., Shimura, T., Sako, N., Yasoshima, Y., & Sakai, N. (1994). Neural substrates for conditioned taste aversion in the rat. *Behavioral Brain Research, 65* (2), 123–137.