Conditioned Food Preference of Unfamiliar Food in Female Rats

ALLEGHENY COLLEGE

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By Julia Lenahan

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor in Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Rodney Clark

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Abstract

In past food aversion studies, with rodent models, a novel food, indicated as a conditioned stimulus (CS) was paired with an unconditioned stimulus (US) like lithium chloride (LiCl). The pairing of a CS and US are utilized to elicit an aversive response. The introduction of the aversive US with the CS, was utilized for rodent models to consume less of the CS in further trials. This research, although pertinent to learning about food aversion, does not look into aversions with familiar foods. Rats exhibit neophobia, the fear of new things, in particular, food. Neophobia is a confounding variable in food aversion research due to the general aversion to novel foods. What happens when a familiar food is paired with an aversive agent like LiCl? Through pairing a familiar food as a CS with an US like LiCl, this experiment investigates if an aversive response to eating familiar food in rodent models can be replicated. Female Sprague-Dawley rats were utilized in this experiment due to lack of using female rats to indicate potential sex differences in past research. Each rat was injected once intraperitoneally (ip) with LiCl, mg/kg was relative to each rodent's body weight. This injection was administered prior to introduction of the novel food in experimentation. Rats were then monitored in trials with a Y-maze and CPP. Rats' intake of the familiar and novel food were recorded as well as entries into each chamber in the CPP and Y-maze. These trials were conducted several times within a week with only one ip injection in the first trial and following trials were monitored for lasting effects of aversion without US. Results indicate there was reduction in food intake and less entries in the familiar food arms of the Y-maze due to illness from LiCl paired with familiar food.

Introduction

Have you ever eaten something and gotten sick from it? It becomes so repulsive that you've never wanted to eat it again? Taste aversion is the simple explanation on why you feel as if you cannot physically eat that food again. Taste aversions arise when an organism eats something and becomes ill from the food. The organism then learns to not eat this food through conditioned taste aversion (CTA). CTA exhibits classical conditioning where two stimuli are paired together. This occurs by pairing these two stimuli in a response where one stimulus corresponds to the other overtime. CTAs are evident in most animals and demonstrate associative learning of palatable rewards and aversive tastes. While humans do form CTAs, the mechanisms underlying these types of associative memory formations are commonly studied in rats. To study this in rodent models, taste aversion is commonly studied through utilizing a familiar food and a novel food. Rats avoid novel foods, and will exhibit neophobia, preferring familiar food over unfamiliar food, (Eckel and Ossenkopp, 1993). Neophobia occurs so that rats avoid the possibility of aversive effects like illness onset usually from harmful, toxic or deadly substances. Neophobia and CTA in nature are used as adaptive tools to avoid aversive effects of substances, (Lin et al., 2014). CTA can be manipulated through several methods, the most common method being injections of LiCl. LiCl creates an aversion to the novel food since it elicits sickness in rats (Tenk et al., 2005). In a typical CTA study, the taste of a novel food is associated with the LiCl injection through classical conditioning. The LiCl injection follows the consumption of the novel food leading to a conditioned aversive response and rats will subsequently avoid the novel food and the familiar food, (Kurz & Levitsky, 1983). Therefore, neophobia is effective in eliciting food preference in rats.

Traditional CTA has a multitude of ways they can be performed. Researchers Shumake et. al looked into LiCl, copper sulfate, cyclophosphamide and red squill for use in CTA, (Shumake et al., 1982). The study looked into the dosage, and route of administration that would be the most effective in creating CTA. Within this study, researchers found that LiCl was the most effective drug for CTA. Low dosages of the drugs, roughly 36 mg/kg, had no aversive effects, a moderate dosage, near 105 mg/kg, lasted 3-5 days, and high doses of around 368 mg/kg, lasted 28 days. Although minimal effective dose has sex differences for female and male rats. Minimal effective dose for inducing aversion for males was lower, (Dacanay et al., 1984). Looking to dose and restrictive food and water has been replicated in newer studies, (Twining et al., 2016). Researchers Twining et al. looked into the dose and restrictive state of morphine, cocaine and LiCl. Researchers found the (suppressive) effects of both morphine and cocaine were greatly reduced when evaluated in either food or water restricted rats. This demonstrates that even amongst morphine and cocaine, LiCl was the most resistant to water and food restriction, (Twining et al., 2016). When studying routes of administration, intraperitoneal injections (ip) were the slowest to create effects of the extinction of CTA, gavage and free ingestion did not produce as strong of an aversion. Ip injections are also found to be easier to manipulate for researchers, resulting in quicker injections and result in low impact of stress on laboratory rats, (Shoyaib, 2020). This study is beneficial for research since it indicates what drugs should be used for aversion, what dosage is crucial for aversion and what administration produces the strongest aversion. Lithium chloride produces aversive effects to rats that help foster conditioned place aversion. In rats, LiCl reduces overall food intake and effects of illness that lead to aversion, (Tenk et al., 2005). LiCl targets food aversion which is crucial to studying conditioned place aversion in rats. In conditioned place aversions (CPA), rats are injected with

LiCl or injected with saline as a control and monitored in a chamber. Rats are injected with LiCl thirty minutes pretreatment and tested on chamber preference between two sides, (Gore et al., 2015). In saline groups, the same measure was done to render similar conditions. After injections, the rats are allowed to choose between the chambers for a designated time. Researchers Tenk et al. found in their experiment that LiCl rats spent significantly less time in the chamber paired with environmental cues that are associated with LiCl, (Tenk et al., 2005). LiCl creates CPP or conditioned place aversion with environmental cues. With the establishment of LiCl reducing food intake and exhibiting CPA, LiCl is a great drug of administration to create CPA.

Y-maze

Y-maze tests are used to look into the neurobehavioral effects of LiCl. LiCl is known to produce gastrointestinal malaise in rats (Cloutier et al., 2018). Previous research indicates that differences in lithium pharmacokinetics have been reported in rats that mimic chronic poisoning, (Hanak et al., 2017). With this in mind, researchers Hanak et al. looked into the neurobehavioral effects LiCl's gastrointestinal malaise may have on rats. Researchers used a Y-maze to investigate lithium-related effects on locomotor activity, anxiety-like behavior, spatial recognition memory and anhedonia in the rat, (Hanak et al., 2017). Y-mazes are typically used to study spatial memory as well as locomotor activity in rats (Conrad et al., 2003). Researchers found that LiCl alters rat behavior and induces hypolocomotion. LiCl effects on rat behavior and hypolocomotion were more prolonged and prominent in rats with rats who are repeatedly exposed to LiCl, (Hanak et al., 2017). Researchers concluded that LiCl's duration of exposure was effective in rat behavior and hypolocomotion. This is important to consider in research since

LiCl can have long-term effects on behavior as well as hypolocomotion as stated. These results indicate additional mechanisms in which LiCl may produce aversive effects long-term, (Yamamoto et al., 1994). In addition to this, these mechanisms may explain a general difference in behavior in rats treated with LiCl.

Sex differences

LiCl also has a significant difference in effects when it comes to overall sex differences. Typical disgust responses in rats like gaping, forelimb flails, chin rubs and paw treads were recorded after an injection of LiCl. Gaping, especially, has been noted as a reliable indicator of anticipatory nausea in rats, (Parker, 2014; Limebeer et al., 2006). When LiCL is paired with a novel cue, CTA occurs in rodent models. In female rats, these disgust responses, especially conditioned gaping, were exhibited at a higher rate than males, (Cloutier et al., 2018). Researchers Cloutier et al. found that there was a link between the toxin dose-related response with sex differences. The potential reasoning for this difference in female and male rats is due to diestrus and proestrus days of a female rats cycle. Researchers found that there were no differences during the estrous cycle in disgust responsiveness but rather in the proestrus cycle, (Cloutier et al., 2018). Female rats exhibited more conditioned gaping responses and increased conditioned disgust responses in trials in their proestrus cycle. Researchers hypothesized that increased levels of estradiol in the proestrus cycle likely enhanced toxin-induced nausea. Estradiol, in previous studies, has been noted to produce ill-ducing properties, (Yuan & Chambers, 1999; Chambers & Hintiryan, 2009). Similar research has suggested that a rise in estradiol at proestrus suggests a negative control in food intake, (Sfikakis et al., 1978; Clarke &

Ossenkopp, 1998). With the effects of disgust responses indicated as more prominent in female rats than male rats it is important to note sex differences that might occur in CTA.

Rats exhibit sex differences in Y-maze pairing and overall short term memory, specifically spatial memory. The Y-maze can assess short term memory through spontaneous alternation. Spontaneous alternation can be assessed by allowing rats to explore all three arms of the maze. This process of exploring all three arms is driven by the curiosity of rats to explore unvisited areas, (Kraeuter et al., 2018). In this experiment, rats were tested in the Y-maze on their spatial memory. Rats were recorded on their total number of arm entries in the maze and spatial memory, (Conrad et al., 2003). Chronic stress did not affect levels of locomotion as measured by arm entries. The Y-maze performance of female rats improved over male rats indicated by higher levels of arm entries, (Conrad et al., 2003). Overall this study showed that sex differences occur in Y-maze performance, with female rats demonstrating an ability to recover quickly from deficits in Y-maze performance, (Frankfurt & Luin, 2015). Sex differences in Y-maze performance, specifically on spatial memory, is important to note with place preference. If female rats are better at spatial memory, females may recognize place preference and achieve more entries in the Y-maze arm that females prefer over the other context.

The pairing of LiCl and CTA also elicits sex differences. In CTA studies, sex differences arise in learning and extinction of cues. Male rats exhibit less resistance to extinction than female rats, (Dalla & Shors, 2009). The less resistance to extinction is important in CTA due to pairing of LiCl and a novel cue. When studying the effects of LiCl on food consumption, a researcher needs to look into the extinction of CTA overtime to see if there are any profound effects of LiCl on CTA. In order to look into food consumption and disgust responses on rats, overtime, a researcher would want to look into the role extinction plays in both of these factors. The

difference in conditioned response is theorized to occur due to organizational effects of gonadal hormones, testosterone and estradiol, specifically during development, (Scimonelli et al., 1999). Modulation of activational effects during the adolescent stage and adulthood play an important role as well in the difference in conditioned response, (McCarthy & Konkle, 2005). Estradiol is a gonadal hormone that is prominent in biological female rats. Estradiol is believed to play a role in the learned response of CTA and spatial memory, (Williams & Meck, 1991). In ovariectomized rats that were given estradiol, extinction of the aversive association were expedited, (Dalla & Shors, 2009). This research demonstrates that sex differences are important to look for in research and is very pertinent in CTA studies with LiCl specifically. The overall learning of CTA in rats also differs due to sex differences. Female rats in CPP needed less of the drug of administration in trials and overall needed less trials to create an aversive association, (Dalla & Shors, 2009). This is very important to consider in research due to the sex differences in learning. Sex differences are exhibited in many species including humans and should be looked into to demonstrate a change in what may occur in learning and drug administration.

Food Utilized in Food Preference

Rats exhibit food preferences in CPP. In CPP paradigms, rats prefer to approach environmental cues that are associated with the consumption of high fat foods (HF) like Cheetos which consist of 10 grams of fat and high sugar foods (HS) such as Froot Loops which consist of 24 grams of sugar, (Privitera et al., 2013). In the CPP paradigm, HF or HS food serves as an unconditioned stimulus (US) that is consumed in a three-way chamber apparatus. Additional rat chow serves as an unconditioned stimulus (UCR). After conditioning rats in the CPP apparatus, rats were then tested for place preference. Researchers Privitera et al. found that rats preferred

the HF or HS foods (US) in these trials. These results are important for CPP research since it demonstrates that HF or HS foods will be preferred by rats even when introduced as a novel food.

LiCl effects on brain regions

LiCl has a long-lasting effect on CTA and the gustatory insular cortex. As previously mentioned, LiCl produces a gastrointestinal malaise in rats, (Cloutier et al., 2018). LiCl, a drug that produces a state of illness, profoundly reduces food consumption and changes basic properties of sensory cortical responses to taste in general, (Stone et al., 2022). CTA, LiCl is paired with an unconditioned stimulus of either food (typically novel food) or flavor (typically saccharin). The association of LiCl and saccharine was looked into in eighty-six rats in the study. Each rat was injected intraperitoneally with LiCl and implanted with electrodes to record neurons for significant associations. After the LiCl and saccharine pairing, all eighty-six rats had developed aversive behaviors to the saccharine thirty minutes later, (Yasoshima & Yamamoto, 1998). The aversive behaviors exhibited were gaping and chin rubbing. After noticing the aversive behaviors all rats were exhibiting, the rat's electrodes were analyzed for significant interactions with aversion and specific brain regions. One hundred and twenty-two unitary interactions were recorded and 20 of these neurons showed a significant enhancement of excitability in the gustatory insular cortex, (Yasoshima & Yamamoto, 1998). This excitable response was in association of the LiCl pairing with the taste aversion. Fourteen of these neurons were significant with short-term excitability and the latter six exhibited long-term excitability, (Yasoshima & Yamamoto, 1998). The interaction between the gustatory insular cortex, taste aversion and LiCl is important to note considering the significance taste aversion can have on

rats in the short-term as well as the long-term. LiCl can produce long-term aversive effects to the stimulus it is paired with.

Significance of Present Study

CTA seeks to help rats with aversive foods through using discrete cues to guide rats what not to eat. My study aims to combat this neophobia and test female rats on food aversion due to sex differences, notably estradiol (Williams & Meck, 1991). Having previous research on male rats and my research on female rats, limit sex differences as another consideration in research. Male rats could be utilized later to replicate this study but there is ample data with using male rats and aversion techniques. In this study, unlike the other studies mentioned, rats will be injected with LiCl or given saline as a control prior to the introduction of novel food, (Tenk et al., 2005). In this process, the rat will develop a preference against the familiar food and the rats will develop a preference to eat the novel food. This is crucial to look into for research purposes due to the fact that rats develop neophobia to novel foods. This research attempts to alter neophobia through producing aversive effects of a known food through ingestion (LiCl). Attenuating aversion conditioning utilized in previous research expands the knowledge on what is known about aversive memory.

Research Question and Hypothesis

For my senior project, my research question is: to what extent can rodent models be conditioned to ingest novel food? Through the manipulation of LiCl administration and food deprivation in Sprague-Dawley rats, food preference as well as place preference was assessed in behavioral apparti. The two behavioral apparti that were conducted this research are the Y-maze

and CPP. To examine food preference and place preference, rats will have free access to both novel and familiar food. Rats will be administered with their treatment group prior to the first trial and monitored throughout trials for the effectiveness of treatment overtime. It was hypothesized that duration after administration would play a role on food intake and number of entries into each behavioral apparti. Rats will be more likely to approach and eat the novel food due to the conditioning of aversive stimuli with the familiar food. The rats exposed to LiCl will prefer novel food, and the saline (control) will prefer familiar food.

Methods

Design

The study was designed as a 2 x 3 Repeated Measures (analysis of variance) ANOVA (LiCl: trials 1-3 set as a time course of administration, and saline: trials 1-3). This was tested as between-subjects design. Each animal was assigned to either the LiCl or the saline conditions (denoted as the control group). The dependent variables that were assessed over time were food intake and number of entries into each behavioral apparatus (either arms or chamber). The behavioral apparti utilized were Y-maze and CPP.

Subjects

Eighteen female Sprague-Dawley rats were utilized in this experiment. The rats were about six months old and their weights ranged from 220-300 grams. Female rats were utilized in this study due to lack of research devoted solely to female rats in CTA studies and previous research studies as a whole. Previous studies have been conducted with only male rats. The underrepresentation of female rats in research dimisses sex differences as a potential barrier to findings, and sexual differentiation of female rats from male rats.

Animals were bred and provided by Allegheny College Carnegie Hall Animal Facility. Prior to experimentation, rats were housed in pairs in plastic tub-style cages with pine shaving bedding and access to standard lab chow and tap water *ad libitum*. During experimentation, rats were housed in cohorts of 6 in hanging wire cages to provide adequate space for each rat. Rats were housed in these hanging wire cages a week prior to experimentation for acclimation. Rats were maintained on a 15:9 hour light:dark cycle and were provided with food and water. Rats had a 23 hour food deprivation prior to trial day of experimentation to entice motivation to eat during trials. After experimentation, rats were granted access to food *ad libitum* until the next

trial. Water remained *ad libitum* throughout experimentation. Rats were checked on daily to ensure health, and cages were cleaned once a week while trays were cleaned three times a week. In addition to this, rats were handled and weighed twice a week prior to experimentation, and daily throughout experimentation. This animal experiment was approved by the Allegheny College Animal Research Committee Protocol and all animal procedures followed The Guide for the Car and Use of Laboratory Animals, 8th Edition.

Materials & Apparti

LiCl was injected intraperitoneally, at a volume of 30 mg/kg (Gaztanaga et al., 2015) and the same volume of saline (1 ml/kg) was injected as a control. Standard laboratory food, noted as familiar food, and Froot Loops, the novel food, serve as the foods to conduct the CTA. To assess CTA, a Y-maze and CPP were used. The Y-maze is a behavioral apparatus that has two arms and a starting base (27.94 x 11.43 x 30.48 cm) (L x W x H). The CPP, another apparatus for CTA. The CPP is a three- chamber apparatus serving two chambers designated for either familiar food or novel food. The middle chamber is denoted as the neutral chamber in order to analyze a two-choice paradigm in rats (95.25 x 34.29 x 30.48 in) (L x W x H).

Procedure

Prior to experimentation, rats were weighed and handled twice a week. During this week prior to experimentation, rats had access to food and water *ad libitum*. After this week, animals were randomly assigned into either treatment group: LiCl or saline. These separate cohorts each consisted of 9 animals that were then utilized for experimentation. All groups were subjected to a 23 hour food deprivation prior to experimentation for each trial. After each trial was conducted,

animals received food *ad libitum* until the next trial where food was restricted again at a 23 hour timeframe. Three separate trials were conducted within a week's worth of time for each experimental group. Throughout experimentation, water was *ad libitum* in the home cage. Thirty minutes pretreatment, the rats were injected with LiCl or saline to ensure the effects of the administered drug. Rats were then monitored post-injection to evaluate signs of illness/malaise. Rats were then run on both Y-maze and CPP to evaluate the presence of a CTA and whether eliciting an aversion to familiar food will create a preference for novel food (approaching as well as consuming the food). These behavioral apparti were then counterbalanced to eliminate confounding variables. One arm of the Y-maze was baited with the familiar food and the other with the novel food (the same process occurs for the CPP testing). Rats will be placed at the base of the Y-maze in the starting zone (otherwise called the neutral chamber in CPP). Rats underwent three trials within this week to record the differences of drug administration throughout the week and development of food preference. To record food preference, the number of entries to each arm/chamber were recorded as well as food intake during trials. Overall behaviors of rats in each behavioral apparti were monitored in the Y-maze and CPP for signs of distress.

After experimentation, rats were then euthanized after experimentation through CO_2 administration.

Conditioned Place Preference (CPP)

CPP is utilized in studies to indicate preference or preferred placement for rats. CPP was conducted with a three-chamber apparatus which aligns with previous research. The middle chamber, indicated as chamber two, was a neutral chamber designated for the rats to have a space in-between the two-choice paradigm. Chambers one and three were designated with either novel

food or familiar food. These chambers are either associated with a drug like LiCl, utilized in this study, or associated with saline serving as the control. LiCl is utilized in food preference and food aversions studies due to the aversive nature of this drug in producing a gastrointestinal malaise in rat models. One side of the chamber will be associated with LiCl to produce a food aversion and unlike other studies, LiCl was paired with the familiar food in an attempt to find an aversion with familiar food rather than a novel food that has been studied previously.

Prior to each trial, a 23 hour food deprivation was implemented to entice motivation to ingest food in trials. After each trial was conducted, rats were given food *ad libitum* for the next 23 hours until food deprivation was put in place again for the next trial. Rats were injected within the first trial with either saline or LiCl in introduction to CPP trials. Rats were then allotted ten minutes to interact with all chambers where entry and food consumption were recorded. Three trials were recorded within a period of a week to monitor the effectiveness of LiCl over time and if food preference would develop overtime. Trials were conducted every other day due to 23-hour food deprivation between trials.

Y-maze

Y-maze is typically utilized in studies to indicate spatial memory for rats. This was implicated in the study to look into the effects of place preference further. Y-maze has two arms and a starting designation. The starting base of the Y-maze was a neutral chamber so the rats have a space in-between the two-choice paradigm. Arms one and two have either novel food or familiar food. These chambers are either associated with a drug like LiCl, utilized in this study, or associated with saline serving as the control. LiCl is utilized in food preference and food aversions studies due to the aversive nature of this drug in producing a gastrointestinal malaise in

rat models. One side of the arm will be associated with LiCl to produce a food aversion and unlike other studies, LiCl was paired with the familiar food in an attempt to find an aversion with familiar food rather than a novel food that has been studied previously.

Prior to each trial, a 23 hour food deprivation was implemented to entice motivation to ingest food in trials. After each trial was conducted, rats were given food *ad libitum* for the next 23 hours until food deprivation was put in place again for the next trial. Rats were injected within the first trial with either saline or LiCl in introduction to Y-maze trials. Rats were then allotted ten minutes to interact with the arms and starting base where entry and food consumption were recorded. Three trials were recorded within a period of a week to monitor the effectiveness of LiCl over time and if food preference would develop overtime. Trials were conducted every other day due to 23-hour food deprivation between trials.

Euthanasia Protocol: CO_2 Administration

Rats were then euthanized after experimentation through CO_2 administration. Rats were placed in plastic tub-style housing containers divided into experimental and control, 9:9 respectively, for termination. This division of grouping was to eliminate overcrowding in plastic tub-style housing containers. A top fitted for CO_2 administration was placed on top of the housing container. A hose attached to the CO_2 cylinder was placed into the housing container and the CO_2 gas was administered into the container at full capacity for 2 minutes. Two minutes of CO_2 administration was set as the standard in order to ensure termination. After CO_2 administration, the CO_2 gas was turned off and rats were assessed 30 minutes after. The 30 minute waiting period was enacted to detect for later movement and additional signs that may

point to the completion of euthanasia. The corpses of the rats were contained in the animal colony freezer.

Statistical Analyses

All statistical analyses were conducted within JASP Software. 2 x 3 Repeated Measures ANOVAs were conducted to test if treatment (LiCl or saline) or time course of administration (trials 1-3). The three effects that were analyzed were: a main effect of either treatment and trials, an interaction between treatment (tx) and trials. Tukey's post hoc analyses (following significant main effects) or one-way ANOVAs (following significant interactions) were performed where appropriate. All statistical significance conducted were set at $p < 0.05$.

The first independent variables are set as the treatment groups: saline or LiCl set as a between-subjects design. The secondary independent variables include each trial the rats were utilized in as separate time points of time after drug administration.

The dependent variables are the number of entries spent in chambers of the CPP or arms of the Y-maze which are calculated by the difference of these scores (familiar food minus novel food). Food intake was also recorded as a dependent variable in the same fashion with food intake recorded with a difference score of familiar food and novel food. The significance level was set to < 0.05, and post-hoc comparisons were performed where appropriate.

Results

All ANOVAs calculated for this experiment were calculated by subtracting the score of the familiar food from the unfamiliar food. These scores were used for the familiar food to unfamiliar food entries as well as the food ingested during trials.

Y-maze Entry Repeated Measures ANOVA

A 2 x 3 Repeated Measures ANOVA – with treatment as the between subject factor, and trials as the within subjects factor – was performed for arm entries in the Y-maze. The results of this statistical analysis indicated a significant interaction between trials and treatment (LiCl vs saline) [$F(2, 32)=7.937$; $p=0.002$]. A Tukey post hoc analysis revealed there was a main effect with LiCl in trial 3 ($M= -1.444$, $SEM= 1.509$) opposed to saline in trial 3 ($M=2.889$, $SEM= 2.619$, $p=0.002$) (Figure 1).

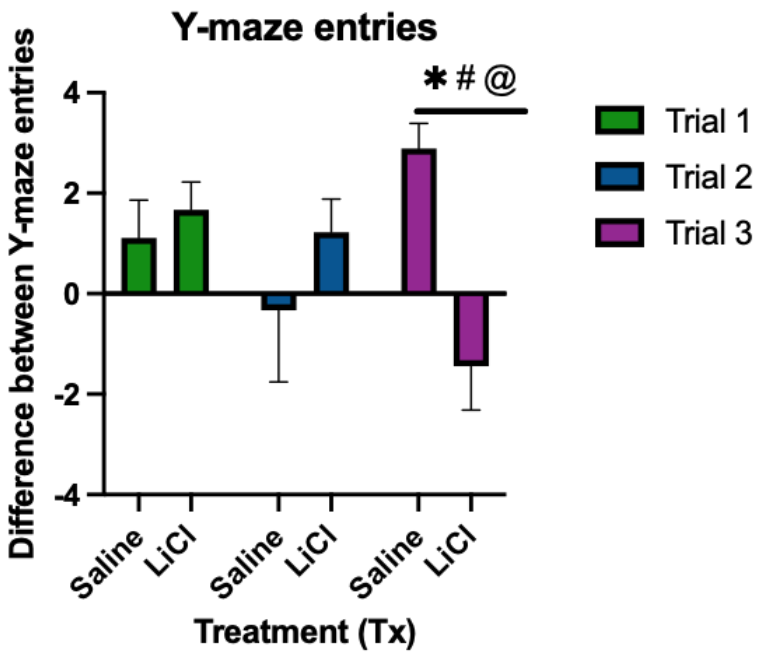

Figure 1. This bar graph displays the average number of entries in the Y-maze which was accounted for by the frequency of entries in each arm (green=trial 1, blue=trial 2, and purple=trial 3). Each error bar is constructed using one standard error from the mean. * = $p < 0.05$, LiCl vs Saline treatment within trials.; # = $p < 0.05$, treatment (tx) x trial interaction; @ = $p < 0.05$, main effect of LiCl, different from trials 1 and 2.

Y-maze Food Consumption Repeated Measures ANOVA

A 2 x 3 Repeated Measures ANOVA – with treatment as the between subject factor, and trials as the within subjects factor – was performed for the difference in food consumption in the Y-maze. The results for this 2 x 3 Repeated Measures ANOVA indicated a significant interaction between trials 2 and 3 and treatment (LiCl vs saline) [$F(2,32)=4.453$; $p= 0.020$]. To examine this interaction, a Tukey post hoc test was conducted and there was no main effect detected (Figure 2).

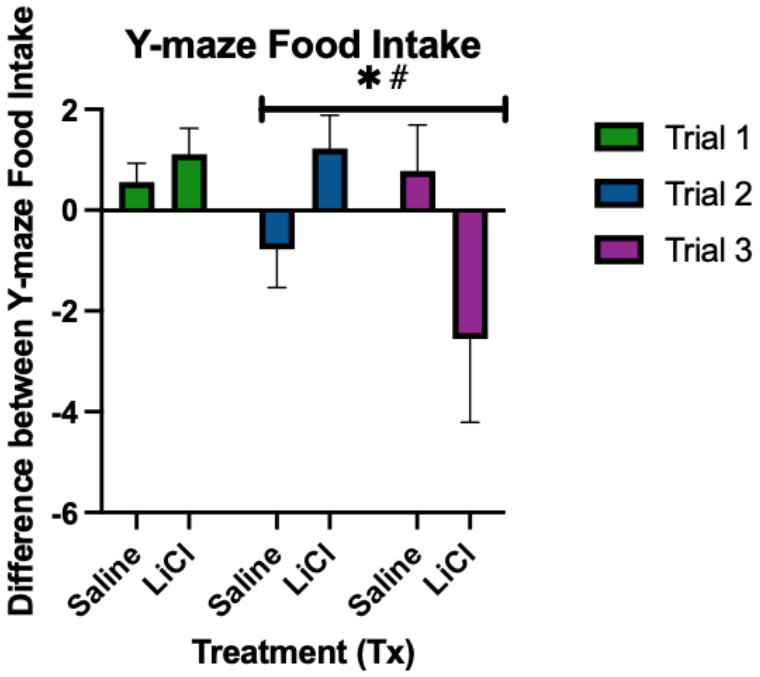

Figure 2. This bar graph displays the average number of food consumed in the Y-maze which was accounted for by the frequency of food consumed in each arm (green=trial 1, blue=trial 2, and purple=trial 3). Each error bar is constructed using one standard error from the mean. * = $p < 0.05$, LiCl vs Saline treatment within trials; # = $p < 0.05$, treatment (tx) x trial interaction.

CPP Entry Repeated Measures ANOVA

A 2 x 3 Repeated Measures ANOVA – with treatment as the between subject factor, and trials as the within subjects factor – was performed for room entries in the CPP apparatus. The results of a 2 x 3 Repeated Measures ANOVA trials x treatment indicated no significant interactions or main effects [$F(2,30)=1.291$; $p=0.290$]. (Figure 3).

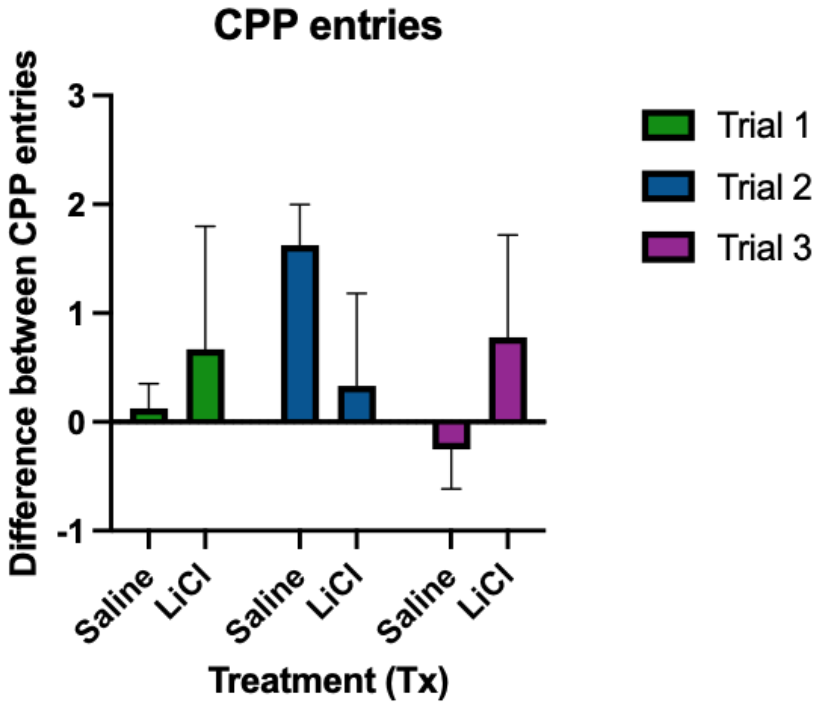

Figure 3. This bar graph displays the average number of entries in the CPP which was accounted for by the frequency of entries in each chamber (green=trial 1, blue=trial 2, and purple=trial 3). Each error bar is constructed using one standard error from the mean.

CPP Food Consumption Repeated Measures ANOVA

The results of a 2 x 3 Repeated Measures – with treatment as the between subject factor, and trials as the within subjects factor – was performed for food consumption in the CPP apparatus ANOVA trials x treatment indicated no significant interactions or main effects [$F(2,32)=0.141$;$p=0.869$] (Figure 4).

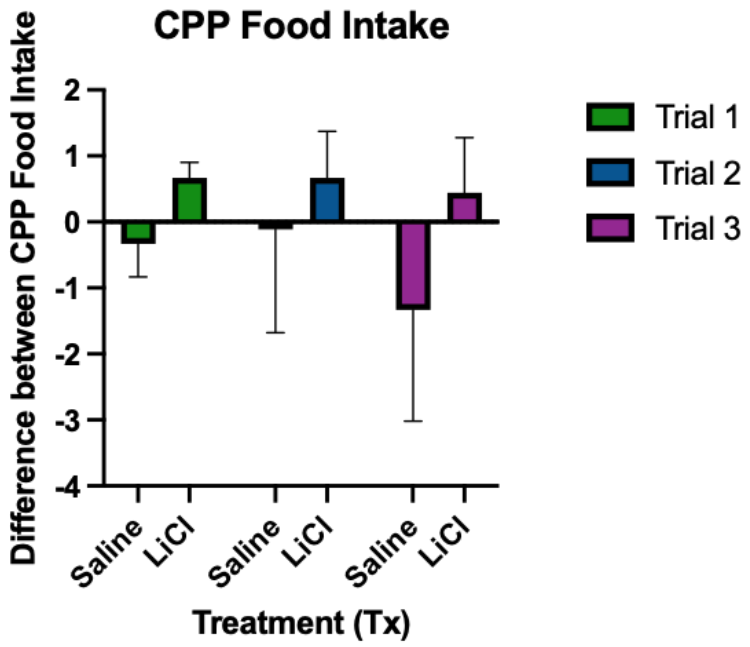

Figure 4. This bar graph displays the average number of food consumed in the CPP which was accounted for by the frequency of food consumed in each chamber (green=trial 1, blue=trial 2, and purple=trial 3). Each error bar is constructed using one standard error from the mean.

Discussion

The purpose of this study was to look at conditioned unfamiliar food preference in female rats. It was hypothesized that aftering injecting rats with LiCl, rats will prefer unfamiliar food, or novel food, over their familiar food. Overall, it appears that, when significant, ingesting novel food was preferred by rats over their familiar food when injected with LiCl. Results of this experiment supported this experiment's hypothesis but contrasts with research presented previously.

Traditional food preference was observed with rats exhibiting gastrointestinal malaise after ingesting novel food, (Sclafani, 1994). In this experiment, as noted, the effects of gastrointestinal malaise with familiar food was studied in part of learning associations with aversive food. LiCl was utilized due to its effects with gastrointestinal malaise, (Cloutier et al., 2018). This study also looked into LiCl effects overtime with pairing with novel food. Previous research has indicated that LiCl can have long-term effects on food aversions, (Yasoshima & Yamamoto, 1998). Due to this, rats were monitored after the single-dose of LiCl throughout the study to look into long term effects. The study supports this hypothesis that LiCl has long-term effects in rats with significance in rats' eating habits and place preference nearing the end of their trials.

In addition to this, conditioned food aversion with familiar food was studied in this experiment to look into the effects of neophobia in rats. Rats are typically reluctant to eat novel food (Neath et al., 2010). With this in mind, the study aims to reduce the effects of neophobia with the administration of gastrointestinal malaise with LiCl to produce conditioned food aversion with familiar food. The study supports this hypothesis with preference to novel food and a reduction of familiar food intake and overall place preference.

Limitations

Due to a small sample size of 18 rats, this sample size is typically insufficient for research and implementation to other studies. Smaller sample size also indicates that it might be insufficient for statistical measurements. Sample size directly affects effect size. When the effect size is smaller, there is not a significant interaction between variables. Even with this in mind, smaller sample sizes produce a larger effect size than studies that have a larger sample size. Effect size, however, tends to be more variable in small sample sizes than large sample sizes. This in return questions whether a smaller sample size in this study was sufficient.

Data collection demonstrated further limitations in this study alongside equipment utilized in this study. The weighing of the food was not adequately rendered in this experiment due to the lack of precision in exact measurements (grams) when weighing food. Further manipulation of place preference and food preference should be implicated in future studies. Rats should be injected in one side of the chamber and the arm if the arm in the Y-maze can be manipulated as such. Prior to injection, rats should be familiarized with the novel food to monitor initial thoughts.

Timing of this experiment should also be noted as a potential limitation. Longer term effects of LiCl administration should be monitored as well as further research with rats ingesting novel food. Multiple cohorts of rats should be investigated in this research due to research analyzing conditioned unfamiliar food preference rather than conditioned familiar food preference. This research may also not be applicable to other studies due to this factor of alternating the type of food experimented on.

Conclusions and Future research

In conclusion, this present study found that rats preferred novel food in both entries as in place preference and food consumption in Y-maze trials. CPP trials were not significant for entries or food consumption. There were, however, some limitations in regards to manipulation of place and food preference to consider.

In order for this study to be expanded on or repeated it is important to consider further manipulation of chamber and arm specific administration for place as well as food preference. Future research should look into implementing male rats to account for sex differences, as well as multiple cohorts or rather a longer timeline conducted. Within this longer timeline, introduction of novel food should be considered as well as a longer assessment of LiCl administration overtime. Further trials should be conducted to have more data to analyze and compare for significance.

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