THE LONG-TERM EFFECT OF DEVELOPMENTAL EXPOSURE TO CANNABINOIDS DURING LACTATION ON PSYCHOPATHOLOGY

By

Michael Curry

A thesis submitted to the faculty of Radford University
in partial fulfillment of the requirements for the degree of
Master of Arts in the Department of Psychology

Thesis Advisor: Dr. Pamela Jackson

May 2013

Pamela Jackson — 4/26/13

Dr. Pamela Jackson
Thesis Advisor — Date

Jeffrey A. Willner — 4/26/13

Dr. Jeffrey Willner
Committee Member — Date

Jeff Aspel — 4/26/13

Dr. Jeffery Aspelmeier
Committee Member — Date

Abstract

Cannabinoids are the most commonly abused illicit drug during pregnancy (Moreno Trigo, Escuerdo, DeFonseca, & Navarro, 2003). Chronic developmental exposure to exogenous cannabinoids has been shown to alter learning and memory, and emotional regulation (O'Shea, Singh, McGregor, & Mallet, 2004; Newsom & Kelly, 2007). Further research by O'Shea, McGregor, and Mallet (2006) found that repeated perinatal cannabinoid exposure from postnatal day (PD) 4 – PD 25 impaired object recognition and reduced social interaction in adulthood. However, few studies have looked at lactational exposure to cannabinoids and its effects on emotional regulation in offspring. Twenty-nine dams were divided into a cannabinoid-exposed group, a food-yoked control group, or a free-fed control group. The cannabinoid exposed group received daily injections (s.c., 0.35 mg/kg) of CP 55, 940 from the second day after giving birth for 18 days (from PD 2 to PD 19 for the pups), while the control groups received comparable vehicle injections. A food-yoked control group was included because Rubino et al. (2008) found that during cannabinoid injection periods food intake, and body weight decreased, therefore it was necessary to control possible effects of malnutrition associated with decreased food intake. The food-yoked control group was allotted the same amount of food the cannabinoid exposed dam ate on that same PD day. Weight data was collected throughout the study. Behavioral testing of the offspring began on PD 77 after a 58 day washout period. Fifty-eight males were used, two from each litter, to assess emotional regulatory behaviors in adulthood. The behavioral tasks included the elevated plus-maze (EPM), the open-field emergence task, and a sucrose preference test. One male rat ran the EPM while the other ran the emergence task, and both ran the sucrose preference test. The data suggested that the drug-exposed animals and the yoked-control dams lost weight more quickly compared to the control group. In addition, there was a

similar interaction between pup weight and drug group (the food-yoked and experimental pups gained weight at a slower pace compared to the control group). No significant results were found on the EPM, however there was a trend in that the yoked animals had a decreased amount of boli in the open arms compared to the control animals, suggesting decreased anxiety in the yoked animals. Open-field activity measures indicated the yoked animals had increased activity compared to controls. The yoked group was also less anxious than the control group as indicated by spending more time in the center zone per entry. However, all three groups spent the same amount of time in the hide box per entry. The data from the sucrose preference test reflected only slight evidence that lactational exposure to the cannabinoid affected anxiety (neophobia) but not depression (anhedonia); all animals preferred sucrose over plain water initially and across days. The yoked and control animals drank a similar amount of sucrose suggesting there was no indication of neohphobia or anhedonia in the yoked animals. The results suggested that early nutrition deficits may be a serious confound in the attribution of cognitive deficits or emotional changes to marijuana-type drug exposure during early development.

Keywords: developmental cannabinoid exposure, malnutrition, emotional regulation

Michael Curry, M.A.
Department of Psychology, 2013
Radford University

Acknowledgements

A special thanks to my thesis advisor Dr. Pamela Jackson, without her knowledge and insight this project would not have been possible. I would also like to thank my team of graduate and undergraduate researchers, Stephen Casazza, Christopher Hartless, Rina Schick, Heather Boyle, Kody Draper, Lauren English, and Kim Crawford who devoted much of their time toward helping with the completion of this research.

Table of Contents

List of Tables and Figures

The Long-term Effect of Developmental Exposure to CP 55, 940 During Lactation on Emotional Regulation

Archaeological findings indicate that *Cannabis sativia* was used from as early as 8000 BCE as hemp cords and the first medical uses of cannabis were recorded in China around 2700 BCE (Childers & Breivogel, 1998). In 1964 the active ingredient in marijuana (THC) was discovered but, anandamide, one of the endogenous ligands in the endocannabinoid system, was not discovered until 1992 (Childers & Breivogel, 1998). Although there has been a long history of cannabis use, only recently have there been major discoveries about the psychoactive compounds and the brain systems cannabis affects. The endocannabinoid system is comprised of five compounds (the endocannabinoids) that have affinity for the cannabinoid receptors (CBrs) consisting of anandamide, 2-arachidonoylglycerol (2-AG), noladin ether, virodhamine, and N-arachidonoyldopamine (Svizenka, Dubovy, & Sulcova, 2008). Also included in the system are two G-coupled protein receptors (GCPRs) in the central nervous system (CNS) and peripheral tissues around the body as well as enzymes that facilitate the production and degradation of the cannabinoid compounds.

The endocannabinoids have been shown to be involved in nociception, cognition, learning and memory, emotional regulation, reproduction, and immune functions (Fernandez-Ruiz, Berrendero, Hernandez, Romero, & Ramos, 1999; Svizenka et al., 2008; Trezza et al., 2012). Recent evidence demonstrated that CB_1 and CB_2 receptors are found in peripheral tissues and in the CNS. It had previously been thought that CB_1 receptors were exclusively in the CNS, while CB_2 receptors were only in peripheral tissues (Svizenka et al., 2008). Although both receptors exist throughout the body and CNS, CB_1 density is more prevalent in the CNS with receptor sites in the telencephalon, brainstem, spinal cord, and diencephalon. In contrast, CB_2

receptors have higher densities within the immune system, especially in the tonsils and spleen (Svizenka, et al., 2008). In the CNS, the CB_2 receptor sites are similar to the CB_1 sites which are located throughout the brain (Svizenka et al., 2008). Further research by Fride (2008) elaborated that the endocannabinoid CB receptor system plays a critical role in early embryo development, including cell differentiation (contributes to making each neuron a specific type of neuron) and cell migration (which is the process of making cells migrate and form correct brain structures in the correct part of the brain). Furthermore, the cannabinoid system helps the development of other neurotransmitter systems and axonal formation (Fride, 2008; Breedlove, Watson, & Rosenweig, 2011).

Cannabinoid drug abuse has been a consistent problem among pregnant women (Lombard, Hedge, Nagarkatti, & Nagarkatti, 2011; Fried & Smith, 2001). Two human longitudinal studies followed cohorts of children whose mothers consumed marijuana (*Cannabis sativa*) during pregnancy. Data from these studies indicated that when the mother smoked marijuana during pregnancy the deficits in executive function in the offspring could be seen into early adulthood (Fried & Smith, 2001; Day et al., 1992). Drug exposure during and after pregnancy can have adverse behavioral and biological effects on a newborn (as cited in Moreno, Trigo, Escured, de Fonseca, & Navarro, 2003). Delta-9-Tetrahydrocannabinol (THC) is highly lipid soluble and can pass through the blood brain barrier, the placental barrier, and into all parts of the body, leading to defects and abnormalities in the neonatal and developing infant. Furthermore, marijuana and other drugs pose a threat to the developing brain because the blood-brain barrier is incomplete and vulnerable, especially to drug exposure (Moreno et al., 2003).

Although human studies demonstrate the damaging effects of prenatal marijuana exposure, few studies follow children after the age of three (Trezza et al., 2012). Furthermore,

because human data is correlational in nature, extraneous variables such as environmental influences, genetics, and dosage of marijuana cannot be excluded as factors that influence behavior later in life. To counteract the deficiencies in human research, psychologists are turning towards animal models for well-controlled studies that allow researchers to control for extraneous variables and examine possible negative effects of cannabinoid exposure. Research on rodents demonstrates that exposure to exogenous cannabinoid agonists has been shown to have adverse consequences on later behavior (Schneider & Koch, 2003). Agonists such as THC; CP 55, 940; and WIN 55212-2 have been shown to alter emotional and cognitive performance when subjects were exposed during the prenatal period, puberty, and adolescence (Schneider & Koch, 2003; Rubino et al., 2008; Moreno et al., 2003). Schneider and Koch (2003) demonstrated that chronic pubertal treatment of WIN 55212-2 to rats decreased object recognition when the rats were tested later in adulthood. Trezza et al. (2008) demonstrated that prenatal cannabinoid exposure to rats increased anxiety responses in 12 day old pups on ultrasonic vocalization, a highly reliable test for anxiety, furthermore adult rats that were prenatally exposed to THC displayed decreased social interaction.

The dopaminergic, serotonergic, and endocannabinoid systems have all been shown to be involved in emotional regulation (Julien, Advokat, & Comaty, 2011; Trezza et al., 2012). Dopamine has been shown to be involved in the reward system (in this context for drug exposure and addiction) and schizophrenia, while serotonin has been shown to be involved with anxiety and depressive symptoms (Julien et al., 2011). The endocannabinoid, dopamine, and serotonin systems all have intrinsic properties involving emotional regulation (Fride 2008; Julien et al., 2011). Additionally, the endocannabinoid system helps the development and organization of both the dopamine and serotonin systems (Fride, 2008). Prenatal exposure to CP 55,940 should

impair emotional regulation not only because the endocannabinoid system has intrinsic properties for emotional regulation, but also because it alters the development of the dopaminergic and serotonergic systems, which also contribute to emotional regulation (Fride, 2008, Julien et al., 2011; Trezza et al., 2012). Although the opioid peptides are not involved in emotional regulation, prenatal exposure to a cannabinoid agonist has been shown to increase the likelihood of becoming addicted to opiates later in life (Vela et al., 1998, Julien et al., 2011; Rubio et al., 1998).

Perinatal exposure to cannabinoids increases vulnerability to opiate drug addiction later in adulthood (Vela et al., 1998). Opioid receptors were shown to be altered by exposure to THC from gestation day (GD) 5 through postnatal day (PD) 24 (Vela et al., 1998). Rubio et al. (1998) perinatally exposed rats to THC from GD 5 to PD 24 and tested the adult rats on an opioid preference test. Rats exposed to THC perinatally increased self-administration of morphine in adulthood (Rubio et al., 1998). Vela et al. (1998) also looked at how maternal exposure to THC facilitated *mu* opioid receptor binding and self-administration behavior of morphine. There was an increase in *mu* opioid receptor density in THC exposed animals, which led to increased drug-reinforcement behavior on a morphine self-administration task (Vela et al., 1998). Corchero et al. (1998) examined the development of the opioid system after rats had been perinatally exposed to THC during GD 5 – PD 24. Alterations in the opioid system led to an increase in self-administration of opiates and affected the perception of pain. Perinatal exposure to THC decreased the expression of the proenkephalin gene in the caudate-putamen in 70 day old rats, which resulted in marked susceptibility to opiate addiction later in life (Corchero et al., 1998). Vela et al. (1998) demonstrated that perinatal exposure to THC increased self-administration of

morphine in adulthood in rats. However, females were more vulnerable to the reinforcing effects than the males, showing an even higher preference for morphine in adulthood (Vela et al., 1998).

Perinatal exposure has negative consequences on the dopaminergic system as well. DiNeri et al. (2011) showed that maternal exposure (GD 5 – PD 2) to THC decreased D_2 receptor messenger RNA expression in the nucleus accumbens which adversely affected a major reward center in the brain. DiNeri et al. (2011) suggested that this decrease in D_2 receptor messengers can lead to an increase in the likelihood of addiction later in life. Moreno et al. (2003) exposed rats to THC from GD 5 to PD 24 and observed how it altered adult behavior and D_2 sensitivity. An increase in D_2 autoreceptors was found in the dorsal striatum, a major dopaminergic system in the brain. Garcia, de Miguel, Ramos, and Fernandez-Ruiz (1996) looked at perinatal exposure to THC (GD 5 – PD 24) and how it affected responses to amphetamine, a major stimulant. Prenatal exposure to THC was shown to increase drug abuse potential with amphetamine and morphine (Garcia et al., 1996; Moreno et al., 2003). Molina-Holgado, Amaro, Gonzalez, Alvarez, and Leret (1996) found that prenatal exposure to THC altered the 5-hydroxytryptamine or serotonin system (5-HT) in pups. Dams were exposed to THC daily at 5 mg/kg of body weight from GD 5 to the day after birth (plus or minus one day). After the exposure pups were sacrificed on PD 1 and four areas of the brain were examined for alterations in the 5-HT system. Maternal THC exposure decreased levels of 5-HT in the diencephalon for all THC exposed animals, with males having a greater decrease levels of 5-HT compared to females. Molina-Holgado et al. (1996) also demonstrated the negative consequences of maternal exposure to 5 mg/kg THC from GD 5 – PD 1 in the adult rat. After dams were exposed, pups were raised until PD 70 and sacrificed. Brain slices revealed that both male and female rats had decreased levels of serotonin

in the raphe nuclei, septum, hypothalamus, and rostral neostriatum after prenatal exposure to THC.

Although consistent findings show the negative consequences of prenatal exposure, there is a lack in consistency of time of exposure, type of drug used, and dosage of drug. A recent review by Schneider (2009) reported that prenatal treatment periods vary greatly along with how much and what cannabinoid agonist was used. However, even with the varying exposure times and drugs used, effects were found to impair locomotor behavior, emotional regulation, cognitive performance, sensitivity towards drug use, food intake, and weight gain (Schneider, 2009; Rubino et al., 2008). Out of the 21 studies reviewed by Schneider (2009) that looked at perinatal exposure, none looked at pure lactation exposure. For this reason the current study examines the contribution of postnatal cannabinoid exposure during lactation without the prenatal component.

It has been noted that weight gain and food intake decrease during the injection period for cannabinoids. Rubino et al. (2008) demonstrated that when animals are exposed to THC during adolescence it reduced weight gain and decreased food intake. Further research by O'Shea and Mallet (2005) also revealed that THC exposure from PD 4-14 showed a decrease in body weight during the injection period. Because cannabinoid exposure slows down weight gain and decreases food intake it is necessary to control for early deficits in nutrition. Previous research has shown that calorie restriction during lactation produced lasting physiological and behavioral changes in offspring, specifically, that a 25% calorie restriction during lactation significantly lowered cortisol, ACTH, and adrenalin in adulthood (Levay et al., 2010; Levay et al., 2008). Levay et al. (2008) demonstrated that a calorie restriction of 25% for 21 days during the lactation time period significantly reduced body weight gain until PD 42. Furthermore it was

demonstrated that the lactation calorie restricted group had increased anxiety as measured by decreased center zone entries in the open field.

It is important to identify the unique contribution of lactational exposure on rat pup development and emotional regulation in adulthood and the possible role of malnutrition. This is particularly important in rat models because rats are born extremely immature with an age equivalency to 150 days in human gestation. The problem is exacerbated because the rats' brain growth spurt, a time when the brain undergoes rapid changes including myelination and establishment of neuronal connections, occurs during the first 3-4 weeks of life (Schneider, 2009). Because not much is known about how lactational exposure to a cannabinoid agonist will affect emotional behavior, it is necessary to look at lactation exposure to other drugs to demonstrate the damaging consequences. Research conducted by Daly, Hughes, and Woodward (2012) showed that lactational exposure to methadone can have adverse effects on anxiety related behaviors. Female rats that gave birth were exposed to 2.86 mg/kg of methadone daily throughout the lactation period. Pups that were exposed during lactation were tested in the emergence test in an open field on PD 30. They found that these animals had slower emergence speed, which is an indication of anxiety. Furthermore, lactational exposed pups showed increased anxiety in the open field as a function of increased defecation and decreased center-cell entries (Daly et al., 2012). Many studies have found effects of alcohol exposure during lactation. For instance, Bond (1980) demonstrated the adverse effects of exposure to alcohol during lactation. After the pups were exposed to alcohol via lactation an avoidance shock test was conducted at PD 75. Animals exposed to alcohol during lactation showed an impaired performance on the shock-avoidance task.

The present study investigated how exposure to CP 55, 940 during lactation affects development. Furthermore, the study attempts to demonstrate that early exposure to an exogenous cannabinoid will have adverse effects on emotional development. Additionally, it is expected that malnutrition during lactation will alter emotional development. However, impairments associated with malnutrition will not be as severe as drug exposure because the drug exposure is expected to result in malnutrition as well. Tasks used to measure emotional regulation, specifically anxiety and depression, are the sucrose preference task, the elevated plus maze, and the emergence test on an open field. Drug exposed animals are expected to demonstrate signs of neophobia and anhedonia in the sucrose preference task. In addition, drug-exposed animals will show increased anxiety in the emergence task and the elevated plus maze task.

Method

Subjects

All subjects were bred in an animal laboratory at Radford University, which is located in southwestern Virginia. Subjects were offspring of second generation Charles River Laboratory rats. Multiple female rats were paired with one male rat in group cages (74 cm L x 57 cm W x 25 cm D). Once a dam was determined to be pregnant, she was single housed in a standard cage (44 cm L x 22 cm H x 20.5 cm W). After birth, the litter was culled to 8 pups (6 males and 2 females) on PD 1. This procedure was based on body-weight where the smallest pups were sacrificed. Pups were left with the dams until weaning on PD 22.

Mothers were semi-randomly assigned to the drug group, the yoked group or the vehicle group on PD 1 based on pairing body weight. Ideally three sisters were paired with one male and after the dams were pregnant each was assigned to one of the three conditions. This was done in order to minimize genetic variability within a cohort. It was also equally important to maximize genetic variability between cohorts; therefore three other female sisters were paired with a different male. If possible 4-6 females were paired with one male and this was done because if one of the sisters did not get pregnant there was a substitute dam. Also, if a male proved infertile he was removed and another male was put with the females.

At birth, if the dams were in the drug condition, they started receiving a daily dose of the cannabinoid on PD 2 until PD 19. Control and yoked dams were injected with a vehicle solution from PD 2 to PD 19. On PD 22 mothers were removed from the litter. On PD 30, the pups were sexed and housed by gender. Males were housed 3 to a cage. Testing on PD 77 allowed for a 58 day washout period which ensured that the animals were not under direct influence of the drug. If a litter did not have more than 7 pups, it was not used in our study. Rubio et al. (1998) used 2-

3 animals per litter for behavioral testing. In the current study two to three males were selected from each litter and each animal only ran two tasks. A total of 29 dams were used in the study. Dams were weighed through the injection period from PD 2 - PD 19. Subjects were weighed every 7 days from PD 35 through behavioral testing. Subjects were housed socially in standard cages (2-3 to a cage). Just before the sucrose preference test was conducted, subjects were single housed in a stainless-steel hanging cage (25 cm L x 18 cm H x 18 cm W). Subjects were in a temperature ($22^{\circ C} \pm 22^{\circ C}$) and humidity controlled room with a 12:12 light/dark cycle. Food and water was given ad libitum and all data collection was done in the light portion of the cycle. This project was IACUC approved and all animals were treated in accordance with the NIH *Guide for the care and Use of Laboratory Animals*.

Drug Condition

One-third of the dams received a subcutaneous (s.c.) injection of the synthetic cannabinoid agonist, CP 55, 940, and two-thirds received a vehicle injection. The groups were injected from PD 2 through PD 19. The drug,3.5 mg of CP 55,940 ((-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol), Tocris Bioscience, Ellisville, MO), was dissolved in 0.5 mL of ethanol, and combined with 75.0 µL Tween 80 (polyoxyethylene sorbitan monoleate, Sigma-Aldrich, Inc.). The ethanol was completely evaporated using a stream of compressed oxygen. The resulting mixture was combined with 4.96 mL of physiological saline to produce a solution of 5.0 mL (O'Shea et al., 2004). The final preparation was administered by s.c. injection at a dose of 0.35 mg/kg body weight, staying consistent throughout injections. Vehicle solution was physiological saline, and tween 80 and was also administered based on body weight.

Design

Food consumption was monitored across the three groups during the lactation period. The drug and control groups had free access to food throughout the injection period. A second control group's food was yoked with the drug group to control for decreased food intake associated with drug exposure. Food consumption was monitored every day starting from PD 2 to PD 20. For the control and drug groups, food was increased as needed to ensure that the animal had free access. If the dam was in the yoked control condition, her food allotment was fixed on what a matched drug mother ate over that same day of injection.

The behavioral tasks selected have been shown to be reliable assessments of anxiety and or depression in rats. Two male animals were picked from the litter to run two tasks each on the following tasks: sucrose preference task, elevated plus maze, and an emergence test. Half the subjects ran the elevated plus maze, the other half ran the emergence task and all animals ran the sucrose preference test. All apparati were cleaned between subjects with a vinegar water solution, 1 part vinegar, 10 parts water, to remove scents from previous animals in the apparati. Measurements for the elevated plus maze and open-field emergence task were conducted in rooms containing overhead lights and white noise to mask extraneous sounds. In addition, both tasks were videotaped. The animals were single housed for the duration of sucrose preference task. White noise was not administered in the sucrose preference task.

Elevated Plus Maze (EPM)

The elevated plus maze task measured anxiety. Behavioral testing started on PD 77. Anxiety was measured by the number of open arm entries and how much time was spent in open arms. More open arm entries and more time spent in open arms were an indication of decreased anxiety. Boli has also been shown to be a reliable measure of anxiety as well. The more the animals defecated the higher their level of anxious. Trezza et al. (2008) found that animals

exposed to 5mg/kg of THC from GD 15- PD 9 had increased anxiety as indicated by not entering open arms as much as the control animals.

Apparatus. The plus maze is raised 50 cm above the ground and consists of 4 arms. Each arm is 61.0 cm long and 11.5 wide. The center platform measures 12.5 cm long by 12.5 cm wide. The four arms extend from the center platform to create a plus sign. Two opposite sides were enclosed by walls that were 40 cm high. The other two arms were not enclosed and only had a 2.4 cm safety lip surrounding the open arms. A video camera was mounted directly above the plus-maze connected to a TV monitor and each session was recorded on a VCR, for coding purposes. The maze was conducted in standard room lighting. White noise was provided in the background to eliminate peripheral noise.

Procedure. Subjects were brought into the running room on a cart in a cage and were allowed to remain in their cage for 5 minutes before testing began. Rats were placed in the center of the plus-maze. The experimenter exited the room and proceeded to monitor the rat's behavior from an adjoining room. The session lasted for 10 minutes and behavior was coded at a later time by experimenters who were blind to the group conditions. Entry into an arm was defined by when the front half on the body crossed the beginning of the arm. Increased enclosed arm entries, amount of time spent in enclosed arms, and increased boli (feces) were indicators of higher levels of anxiety. Lower levels of anxiety were indicated by the opposite: increased time or entries into the open arms and decreased boli.

Open-Field Emergence Test

The emergence test is a standard measure of anxiety in rodent models. It measured exploratory behavior and avoidance of open cells in the open field. Animals were placed in a hide box with a door that faces the open field. Anxiety was measured by time spent in the hide

box and in the center zone compared to the periphery. More time spent in the periphery and less time in the center per entry were indicators of increased anxiety. Anxiety was further measured by hide box time per entry. Navarro, Rubio, and Rodriguez- de Fonseca (1994) injected female rats with THC from GD 5 – PD 24. Their rats were tested on PD 70 and THC exposed rats were found to have increased emergence latency times, another sign of anxiety.

Apparatus. The apparatus is a wooden rectangular box (103x103x46) painted white. The floor was divided into a 4x4 pattern making 16 distinct cells. One of the cells (25.5x25.5x15) is covered with the hide box. The open field was illuminated with a 25 watt light bulb with a camera fixed above the box to record data. The animals were tracked using AnyMaze software. White noised was used to eliminate excessive background noise and a vinegar solution (1 part vinegar 10 parts water) was used to eliminate olfactory cues between trials.

Procedure. Experimental subjects were brought to the running room on a cart in a holding tub and remained nearby for at least 5 minutes prior to the start of testing. This task consisted of two 10-minute trials, to measure emergence latency, hide box time per entry, activity, and time spent in the center zone per entry. Emergence latency measured how long it takes initially for the animal to come out of the hide box, where hide box time per entry was how much time the animal spent in the hide box every visit. Activity was measured by total distance traveled during each trial. Emergence into the open field was defined as half of the body being out of the hide box. Longer emergence latency, increased hide box time per entry, and decreased mean center zone time per visit are measures of increased anxiety. A camera recorded each trial and the behavior was coded by blind observers to determine each of the behaviors at a later time if the AnyMaze software failed to track the rat. White noise was played to eliminate background noise and a vinegar wash was used after each trial to wipe down the box. At the beginning of

each trial, the rat was placed in the hide box for 1 minute and then the door was removed, which allowed the animal the opportunity to explore the open field.

Sucrose Preference Task

The sucrose preference task is a rat model used to measure both symptoms of anxiety and depression. The first part of the sucrose preference task was a test for neophobia. This was conducted during the first hour of the sucrose preference task. Anxiety was demonstrated if the animals were afraid of the novel stimulus and did not consume the sucrose solution. Anhedonia is a depression symptom demonstrated by the lack of interest or inability to experience pleasure. This was measured by the percent of sucrose consumption by the rats across three days. Rubino et al. (2008) injected animals with THC during adolescence and tested these animals in adulthood on the sucrose preference test. Control animals drank significantly more 2 % sucrose water on day two and day three compared to animals that were injected with THC, indicating anhedonia.

Apparatus. The sucrose preference task was conducted using single hanging-cages and sucrose solution and plain water. Animals were single-housed 24 hours prior to the test to acclimate to the housing conditions. Plain water and the 2 % sucrose solution were kept refrigerated. Two plastic tubes (13.5 cm L x 2.5 cm W) were filled with approximately 30.0 mL of plain water and sucrose solution and were used to measure neophobic behavior during the first hour. Larger glass bottles (~150 mL) were filled with greater portions of the two solutions and sealed with rubber stoppers and were given to the animals over the next 72 hours to capture a measure of anhedonia. Measurements were taken every 24 hrs.

Procedure. The sucrose preference task is a 4-day procedure with 5 measurements, two of these measurements were for neophobia and 3 were for anhedonia. Each measurement was

used to compare the amount of sucrose solution consumed compared to plain water. Neophobia was measured by recording the amount of both solutions consumed over 1 hr. Measures were taken at half hour intervals. Two plastic tubes were filled with approximately 30 mL of solution in each bottle. Pre-measurements were taken in weight (g) and mL. The mL was assessed at 30 min and at 1 hour the mL and weight was taken at the end of the neophobic measurement. Decreased sucrose consumption compared to plain water is indicative of neophobic behavior.

Once the neophobic measures were completed, two larger bottles replaced the smaller one to start measurements on anhedonia. The larger bottles were left on for continuous access for 3 days and weighed 3 times 24 hrs apart. The rats received approximately 40g of standard food chow daily. If the solutions in either bottle ran out, the correct solution was refilled, weighed, and placed back on the cage. When the bottles were taken off they were replaced on the opposite side of the previous day. A decrease in percent sucrose consumed over the 3 days compared to the control animals is indicative of anhedonia.

Results

Statistical Plan

The effects of malnutrition, vehicle and CP 55,940 cannabinoid treatment were assessed using repeated measures and univariate Analysis of Variance (ANOVAs) for many of the analyses. Where appropriate, post-hoc analyses were conducted using least significant differences (LSD). Independent and/or paired samples *t*-tests were also used to analyze simple effects. The current project used focused testing to interpret and analyze much of the data because the effects of drug exposure versus malnutrition were expected to have different effects on behavior. Focused testing is an alternative way to analyze results; it allows the researcher to answer specific research questions that might be missed when using omnibus tests (Rosentahal, Rosnow, & Rubin, 2000). Specifically, the current study's hypothesis was focused on the differences between the drug group and the control group, and the yoked group versus the control group. This was done because malnutrition has been associated with drug exposure and it is important to tease apart the effects associated with drug exposure and the effects associated with malnutrition. Focused testing was used because omnibus ANOVAs do not appropriately address the hypothesis and are not focused enough for the current research (Rosentahal et al., 2000). PASW Statistics 18 and Microsoft Excel were used to analyze the data and to produce graphs.

Health Checks

Body weight has been used to assess health and development in rats. A series of weight checks were conducted throughout the study to ensure that any group differences could be attributed to the proper manipulation preceding behavioral data collection. The first weight check compared the weight of the dams when they were initially paired with a male. An ANOVA

indicated that there were no significant differences between the drug, yoked, and control groups when they were first paired with a male. The means and *F* ratios are provided in Table 1. The second weight check was on the day after mothers gave birth on PD 1. There were no significant differences in the mothers' weights on the day after they gave birth either. The three groups of mothers did not differ before the manipulation began.

Other indicators of initial health were how many pups were born and how many died or were consumed. The ANOVA revealed no difference in how many pups were born between the drug, yoked, and control litters. Attrition during the injection period was measured. Litters that had to be dropped from the study due to the reduction in number of pups were included in this ANOVA. There were no significant differences in attrition between the drug, yoked, and control groups. See Table 1 for a description of the data. Based on these analyses it is clear that the dams and litters were not different before drug treatment began.

Table 1

Health checks before group assignment. Standard deviations are presented below the means.

	Drug (N = 11)	Yoked (N = 9)	Control (N = 9)	*F*	df	η^2
Mean weight of dam on day of first pairing	384.18 (32.08)	386.22 (75.20)	390.56 (49.36)	.04	(2,26)	<.01
Mean weight of dam on day following birth	432.00 (36.86)	426.75 (83.08)	425.71 (77.28)	.03	(2,23)	<.01
Average number of pups born	12.15 (3.19)	11.7 (2.87)	12.33 (3.06)	.33	(2,26)	.03
Pup attrition	.27 (.49)	.11 (.33)	.44 (.53)	1.22	(2,26)	.09

Food Intake During the Injection Period

Food intake was measured from PD 2through PD 20. A 3 x 19 repeated measures ANOVA was run comparing all groups. Unlike the other analyses, it was necessary to identify whether food consumption differed from the drug and yoked group, as well as the drug and control group, across individual days. A significant interaction revealed that all the groups ate the same amount at the beginning of the injection period and at the end, but drug exposure decreased food consumption after the injections started, $F(36, 342) = 1.83$, $p = .004$, $\eta^2 = .16$ (see Figure 1). Additionally, there was a main effect of group where the control animals consumed more food than the drug and yoked animals regardless of day, $F(2, 19) = 9.69$, $p = .001$, $\eta^2 = .51$. A main effect of day revealed that all animals increased the amount of food consumed, $F(18, 342) = 48.31$, $p < .001$, $\eta^2 = .72$. This is due to the need for additional fuel to feed growing pups. Least significant differences (LSD) post hoc analyses revealed that the control group ate significantly more food than the drug group, $p = .001$, and the yoked group, $p = .001$. There was not a significant difference in the amount of food eaten between the drug and yoked groups. See Figure 1 for a representation of the data. It was also important to measure how much food was consumed on average by each group because it determined how much less food the drug and yoked group ate compared to the control group. On average the drug group consumed 35.17, the yoked group consumed 34.26, and the control group consumed 49.54 grams of food. The drug group ate 71.00% of what the control group ate and the yoked group ate 69.57% of what the control group ate, which corresponds to a 30% reduction in food consumed.

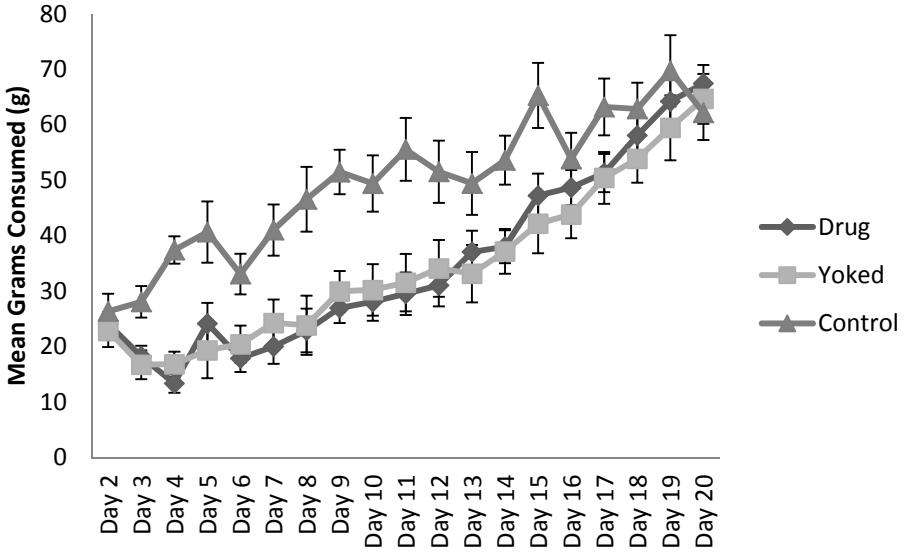

Figure 1- Displays mean grams of food consumed across postnatal days, with SEMs. Control animals ate more food across days compared to drug and yoked animals. All groups increased food consumption across days. The rate of food consumption increased faster for the drug and yoked groups in the third week.

Body Weight

Mother's weight during the injection period. Dams were weighed each day during the 18-day injection period. In order to see how weight loss translated to the pups, it was necessary to run the repeated measures on the same day that the pups were weighed. Therefore, a 2 x 4 repeated measures ANOVA (drug group versus the control group) was conducted to see the effect of drug exposure on weight gain on postnatal days 2, 8, 14, and 20. Drug exposed dams lost weight at a faster rate than the control dams, as revealed by the significant interaction, $F(3, 54) = 10.99$, $p < .001$, $\eta^2 = .38$. There was a significant effect of day, meaning that all dams lost weight across days, $F(3, 54) = 33.12$, $p < .001$, $\eta^2 = 65$. There was no significant effect of drug group indicating that no one group weighed heavier regardless of day, $F(1, 18) = 1.91$, $p = .184$, $\eta^2 = 10$. In order to assess what days the groups differed, *t*-tests were run for each PD day. On PD 2 drug exposed dams did not differ from control dams $t(18) = .267$, $p = .793$, $d = .13$. The *t*-test revealed that on PD 8 drug exposed dams approached weighing significantly less than controls $t(18) = -1.87$, $p = .077$, $d = -.88$. Drug exposed dams weighed significantly less on PD 14 compared to control dams, $t(18) = -2.19$, $p = .042$, $d = -1.03$. On PD 20 a trend was also observed that the drug animals were approaching weighing less than the control animals, $t(18) = -1.74$, $p = .099$, $d = -.82$.

A similar 2 x 4 repeated measures ANOVA was also run comparing the yoked dams to the control dams' body weight during the injection period. The interaction revealed yoked dams lost weight at a faster rate across days compared to the control dams, $F(3, 48) = 8.18$, $p < .001$, $\eta^2 = .34$. Furthermore, a significant effect of day indicates that all dams, regardless of group, were losing weight across days, $F(3, 48) = 25.11$, $p < .001$, $\eta^2 = .61$. There was no main effect of group, averaged across day the groups did not differ in weight, $F(1, 16) = 1.24$, $p = .281$, $\eta^2 =$

.07. To see where the groups differed *t*–tests were run starting on PD 2 and every 6 days after. There were no significant differences at all: on PD 2 ($t(16) = -.02$, $p = .987$, $d = -.01$), PD 8 ($t(16) = -1.50$, $p = .153$, $d = -.75$), PD 14 ($t(16) = -1.64$, $p = .120$, $d = -.82$), or PD 20 ($t(16) = -1.35$, $p = .197$, $d = -.68$) This indicated that the overall interaction of group by day was significant but it cannot be pinned to any one day during the injection period. Data is displayed in Figure 2.

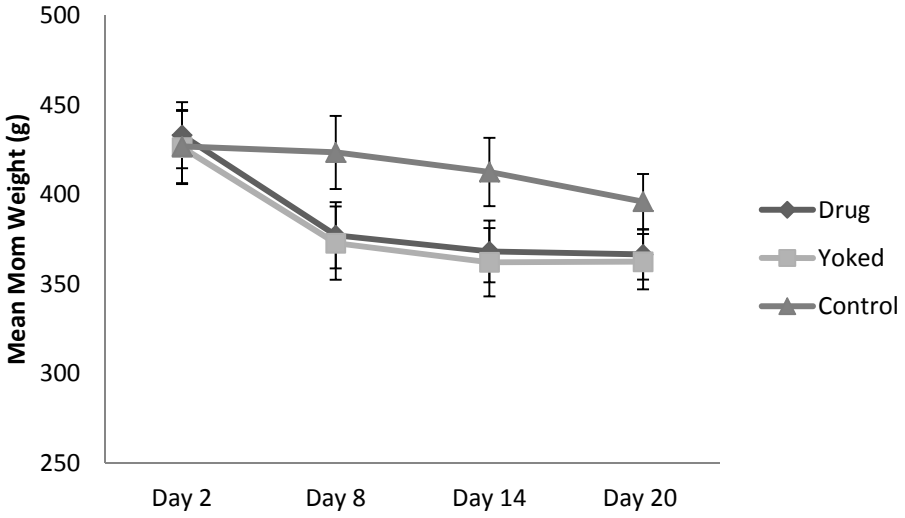

Figure 2- The average mom weight on PD 2, PD 8, PD 14, and PD 20. Weights are represented in means with SEMs. The interaction reveals that both drug and yoked animals lost weight faster across days than the control group. A main effect of day indicated that all animals lost weight across days regardless of drug condition. Individual analyses for each day revealed that the drug group differed from the control group on PD 14 only.

Pup weights during the injection period. Pups were weighed once every six days during the injection period to assess the growth and health of the pups. A 2 x 4 repeated measures ANOVA for the drug group versus the control group was conducted to see the effect of drug exposure on weight gain on postnatal days 2, 8, 14, and 20. A significant interaction revealed that control animals gained weight quicker across days compared to drug exposed pups, $F(3, 54) = 15.62$, $p < .001$, $\eta^2 = .47$.The means are graphed in Figure 3. A main effect of day indicated that all animals gained weight during the injection period, $F(3, 54) = 794.07$, $p < .001$, $\eta^2 = .98$. Unlike the mothers, the control pups weighed more than the drug exposed pups overall during the injection period, $F(1, 18) = 17.43$, $p = .001$, $\eta^2 = .49$. In order to determine if the groups differed on individual days, independent samples *t*-test were run. On PD 2 the weights did not significantly differ, meaning the drug and control animals weighed the same at the beginning of the injection period ($t(18) = -.38$, $p = .706$, $d = -.18$). Control animals weighed heavier on PD 8 compared to drug exposed animals ($t(18) = -3.66$, $p = .002$, $d = -.86$), and on PD 14 ($t(18) = -4.45$, $p < .001$, $d = -1.05$) and on the final day of the injection period ($t(18) = -3.97$, $p = .001$, $d = -1.87$).

The same repeated measures ANOVA was run comparing the yoked pups to the control pups. Overall, the yoked pups, gained weight less quickly over the injection period. This was demonstrated by a significant interaction, $F(3, 45) = 18.20$, $p < .001$, $\eta^2 = .55$. A main effect of day indicates that all animals gained weight over the injection period, $F(3, 45) = 938.50$, $p < .001$, $\eta^2 = .98$. Also, yoked pups weighed less over the injection period indicated by a main effect of group, $F(1, 15) = 16.36$, $p < .001$, $\eta^2 = .52$. Independent samples *t*-test were run at each day to see at what days the groups differed from each other. On PD 2 the weights did not significantly differ from each other indicating that the pups did not weigh differently at the start of the

injection period ($t(15) = -.108$, $p = .915$, $d = -.06$). Control animals weighed more at PD 8 compared to yoked animals ($t(16) = -3.01$, $p = .008$, $d = -1.51$) and on PD 14 ($t(16) = -4.3$, $p = .001$, $d = -2.17$) and PD 20 ($t(16) = -4.42$, $p < .001$, $d = -2.21$). See Figure 3 for a representation of the data.

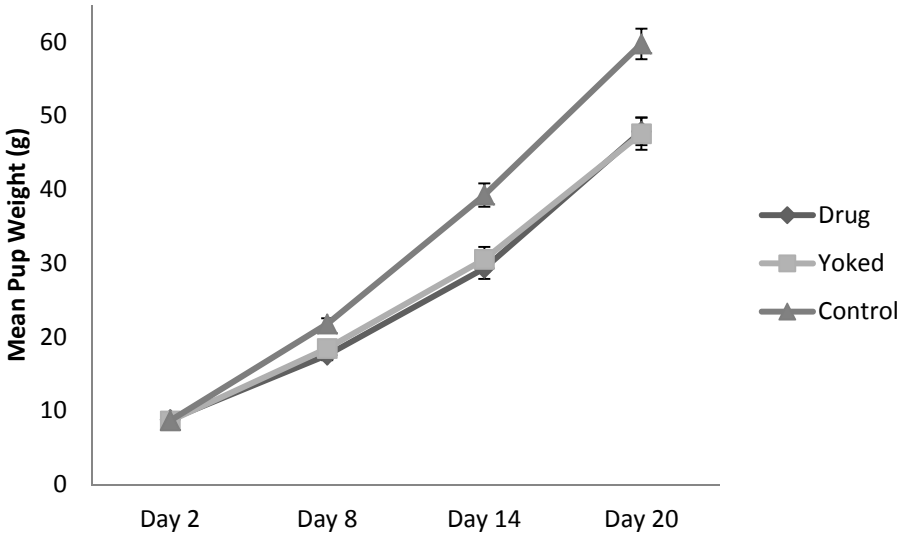

Figure 3- The average weight of the litter of pups on each postnatal day weighed during the injection period. Weights are represented in means with SEMs. The interaction reveals that drug and yoked animals gained weight slower across days than the control group. All animals gained weight across days regardless of drug condition. Control animals weighed more than the drug and yoked groups on all days except PD 2, the first day of injections.

Pup washout weights. Pups were weighed every 7 days starting on PD 35 and ending on PD 56. This was done to measure health development after drug and food deprivation. A 2 x 4 repeated measures ANOVA was used to measure the effect of drug exposure on weight gain. There was no significant interaction between the drug and control groups ($F(3, 45) = 2.03$, $p = .123$, $\eta^2 = .12$) . However, there was a significant effect of day, meaning all animals gained weight during the washout period, $F(3, 45) = 872.57$, $p < .001$, $\eta^2 = .98$. Control animals weighed more than the drug animals as indicated by a main effect of group, $F(1, 15) = 5.03$, $p = .04$, $\eta^2 = .25$.

The same repeated measures ANOVA was used to compare the yoked and control animals. The comparison between the yoked and control animals had the same pattern. There was no significant interaction ($F(3,42) = 2.10$, $p = .114$, $\eta^2 = .13$), however, there was a significant effect of day meaning all animals gained weight regardless of group, $F(3, 42) = 845.88$, $p < .001$, $\eta^2 = .98$. A main effect of group revealed that the control animals on average were heavier than the yoked animals, $F(1, 14) = 7.69$, $p = .015$, $\eta^2 = .34$. The means are displayed in Figure 4.

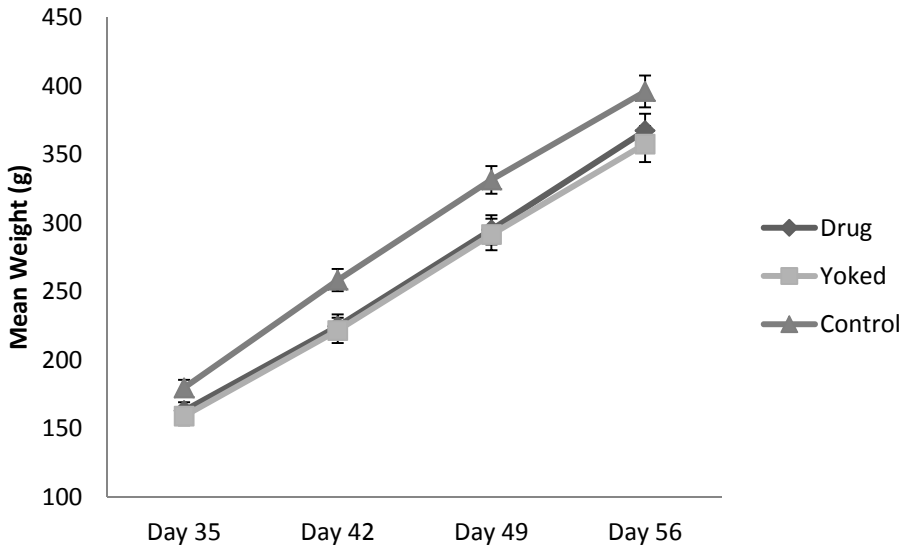

Figure 4- The average weight of individual pups on postnatal days 35, 42, 49 and 56. Weights are represented in means with SEMs. All animals gained weight across days regardless of drug condition. Averaging across days, the control animals weighed more than both yoked and drug groups.

Elevated Plus Maze

The EPM is a well-established measure of anxiety in rats. This was assessed by looking at mean percentage of time spent in open arms, mean percentage of entries into open arms, and mean number of boli produced. More time and entries into open arms was an indication of decreased anxiety and increased boli was an indication of increased anxiety. Independent samples *t*- tests were used to analyze the results. Overall the drug and control group did not significantly differ on any of the measures taken on the EPM. There was no significant difference in mean percentage of time spent in open arms, $t(16) = .920$, $p = .371$, $d = .46$ (Figure 5), mean percentage of entries in open arms, $t(16) = -.188$, $p = .853$, $d = -.09$ (Figure 6), and number of boli produced in the open arms $t(16) = -.394$, $p = .699$, $d = .20$ or enclosed arms $t(16) = -.981$, $p = .341$, $d = -.49$ (Figure 7). This indicated that the drug and control animals displayed equivalent levels of anxiety on the EPM.

An independent samples *t*-test were used to compare the yoked and control groups. The analysis revealed a similar pattern. There was no significant difference in mean percentage of time spent in open arms, $t(14) = .73$, $p = .480$, $d = .39$ (Figure 5), or mean percentage of entries in open arms, $t(14) = .09$, $p = .931$, $d = .05$ (Figure 6). However, there was a trend in amount of boli produced in the open arms which indicated the yoked animals were less anxious than the controls, $t(14) = -1.77$, $p = .098$, $d = -.95$, although this effect was not quite significant and was not seen in boli produced on the enclosed arms, $t(14) = -.518$, $p = .612$, $d = .28$. Means for the EPM are displayed in Figures 5, 6, and 7. Figure 5 represents mean percent of time spent in open arms. Figure 6 is mean percentage of entries into open arms and Figure 7 is the mean amount of boli produced.

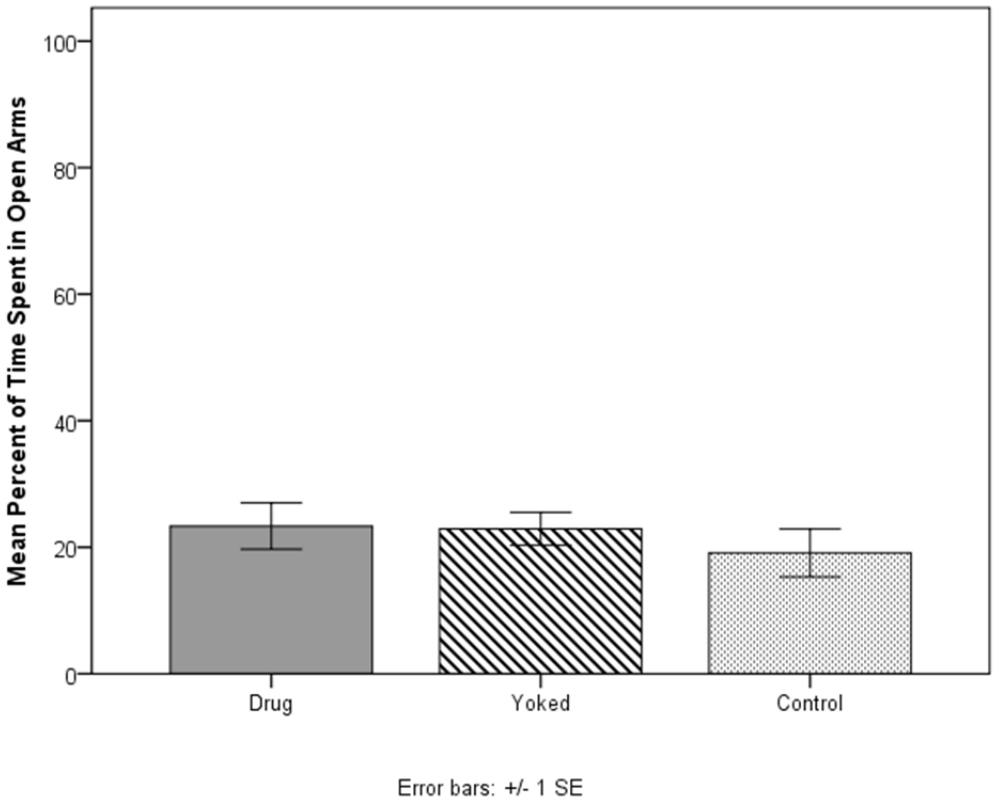

Figure 5 - The mean percentage of time spent in open arms compared to enclosed arms. It is represented in mean percentage with SEMs. No significant differences were found.

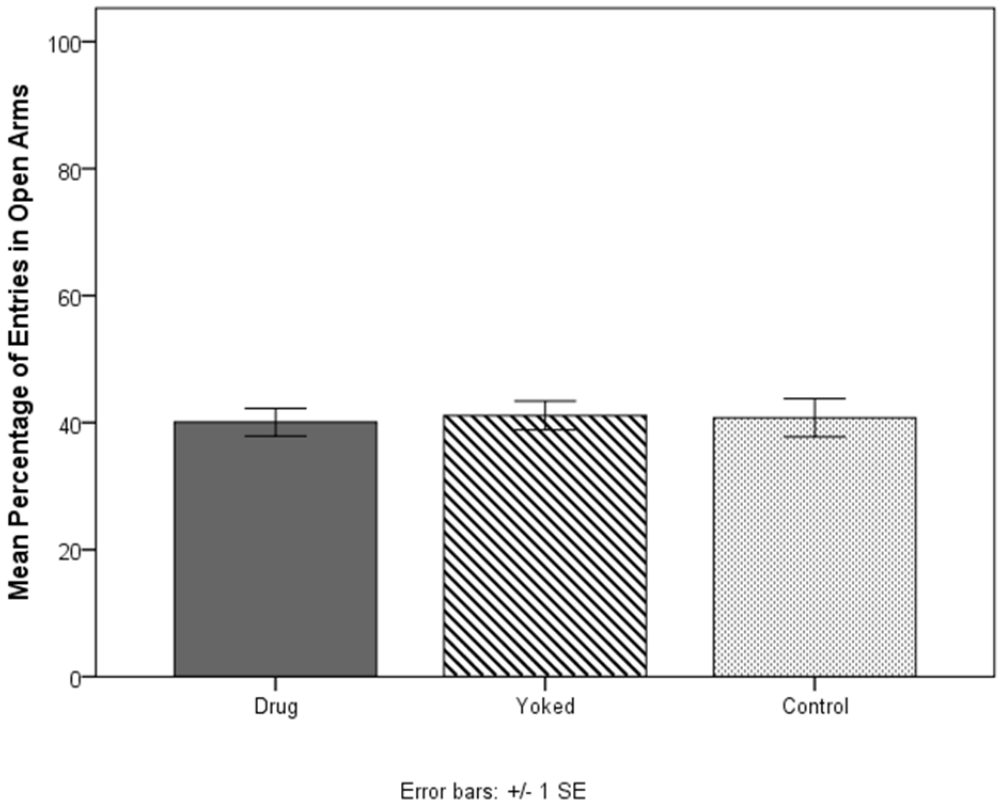

Figure 6- The mean percent of entries into open arms out of total entries into open and enclosed arms. It is represented in mean percentage of entries with SEMs. No significant effect was found as a function of group.

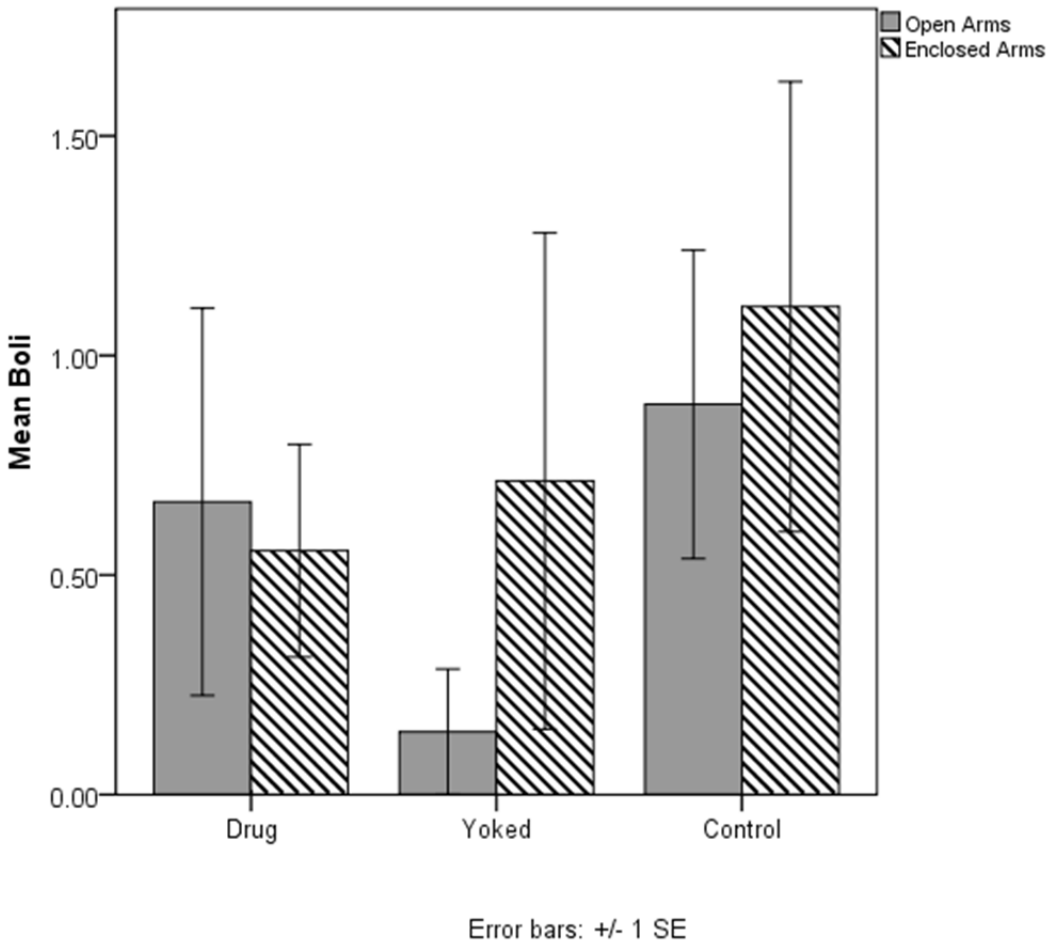

Figure 7- Represents the mean number of boli on the open versus the enclosed arms on the EPM. The mean boli is presented with SEMs. Both drug and control groups produced similar amounts of boli. Although not significant, there was a trend suggesting that the yoked animals were less anxious than the control animals as measured by boli produced in the open arms.

Open-Field Emergence

The emergence task is a well-known task that assesses anxiety and activity measures in an open field. Latency to emerge from the hide box, avoidance of the center cells, and increased boli production are all indications of increased anxiety, as well as spending more time in the hide box. Distance traveled in meters measured activity/hyperactivity in rats. Two repeated measures ANOVA were used to analyze the data on the two trials of this task. One ANOVA measured drug versus control and the other measured yoked versus control.

Activity. The activity data was measured by using AnyMaze software. A 2 x 2 repeated measures ANOVA comparing the drug group to the control group across trials revealed that there was no interaction, $F(1, 13) = .01$, $p = .938$, $\eta^2 < .01$, main effect of day, $F(1, 13) = 3.42$, $p = .087$, $\eta^2 = .21$, or main effect of group , $F(1, 13) = .01$, $p = .944$, $\eta^2 < .01$. This indicated that they traveled a similar amount of distance on trial 1 and trial 2. The means are graphed in Figure 8. The same repeated measures ANOVA was run comparing the yoked and control animals. There was no interaction $F(1, 14) = .21$, $p = .657$, $\eta^2 = .01$ and there was no main effect of day $F(1, 14) = 2.48$, $p = .138$, $\eta^2 = .15$. However, the yoked animals had an increased amount of activity, $F(1, 14) = 5.12$, $p = .04$, $\eta^2 = .27$. See Figure 8 for a depiction.

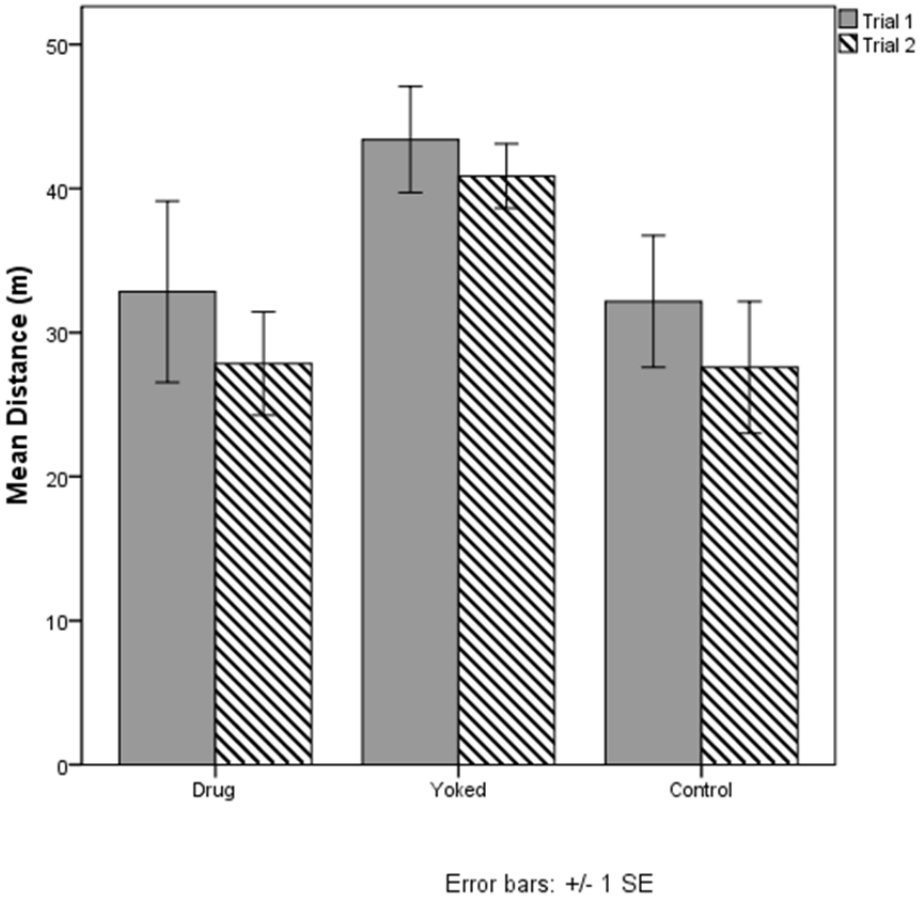

Figure 8- Mean distance traveled in the open field where all bars are displaying SEMs. The drug group did not significantly differ in meters traveled compared to the control group. The yoked group, regardless of day, traveled significantly further in the open field compared to the control group.

Anxiety. Emergence to first exit from the hide box was one measure of anxiety. The longer it took to emerge was a sign of increased anxiety. A full emergence was when half the rat was out of the hide box. A 2x2 ANOVA comparing the drug and the control group across trials revealed that there was no interaction, $F(1, 13) = .01$, $p = .918$, $\eta^2 < .01$, no main effect of group, $F(1, 13) = 1.19$, $p = .294$, $\eta^2 = .08$, nor an effect of day, $F(1, 13) = .03$, $p = .866$, $\eta^2 < .01$. The same pattern of results was seen when comparing the yoked and control group across trials. There was no interaction $F(1, 14) = .03$, $p = .859$, $\eta^2 < .01$, no main effect of group, $F(1, 14) = .412$, $p = .531$, $\eta^2 = .03$, nor an effect of day, $F(1, 14) = .06$, $p = .804$, $\eta^2 = .01$. All groups regardless of trial had similar latency to first emergence times. Means are displayed in Figure 9.

Hide box time per entry was also used to measure anxiety. An increase in time spent and entries into the hide box was an indication of increased anxiety. Because the yoked animals were more active than the controls, the average amount of time spent per entry was calculated for each animal on both trials. The 2 x 2 repeated measures ANOVA for drug versus control group across trials revealed there was no interaction, $F(1, 13) = .06$, $p = .809$, $\eta^2 = .01$, no main effect of group, $F(1,13) = .43$, $p = .522$, $\eta^2 = .03$, nor a main effect of day, $F(1, 13) = .21$, $p = .652$, $\eta^2 = .02$. The drug group and control group spent an equal amount of time in the hide box per entry. This suggested a similar amount of anxiety on the emergence task. The 2 x 2 repeated measures ANOVA for yoked versus control groups revealed the same pattern of results, the yoked animals displayed a similar level of anxiety as the control animals. There was no interaction, $F(1, 14) = .38$, $p = .548$, $\eta^2 = .03$, no main effect of group, $F(1,14) = 2.26$, $p = .155$, $\eta^2 = .14$, nor a main effect of day, $F(1, 14) = .03$, $p = .874$, $\eta^2 < .01$ .See Figure 10 for a representation of the data.

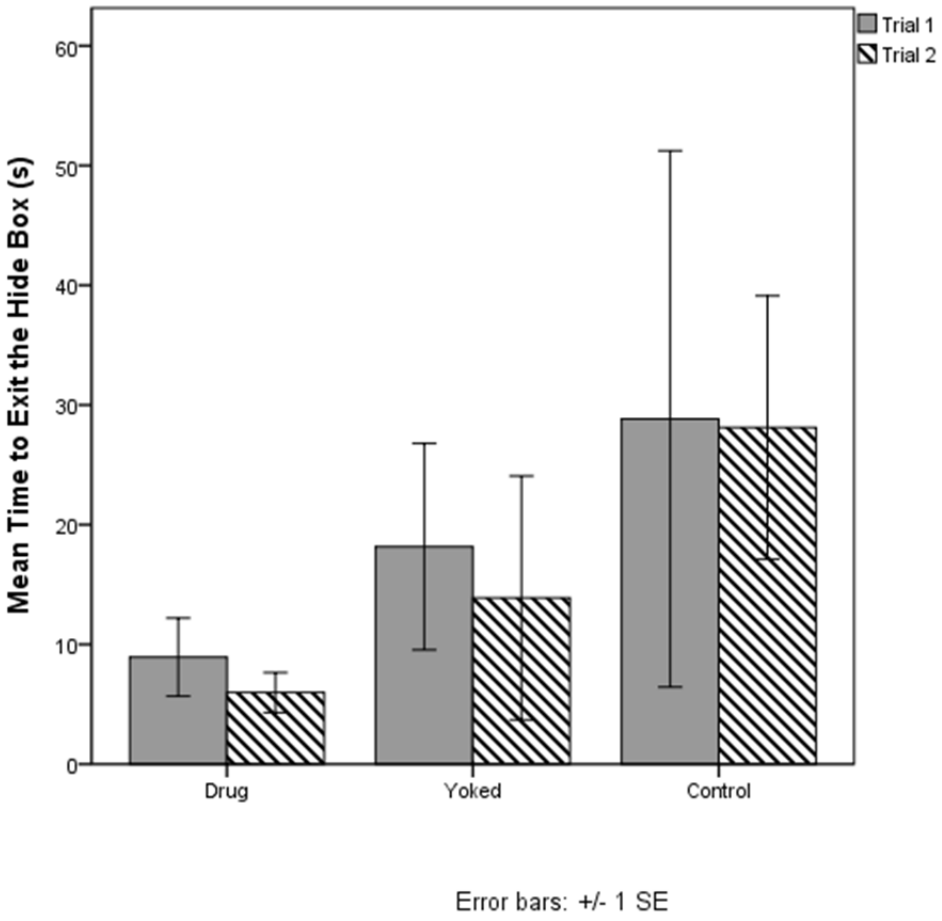

Figure 9 – Mean time to exit the hide box on trial 1 and trial 2 of the open-field emergence task with SEM displayed. Regardless of group or trial, all animals had a similar latency to emerge time.

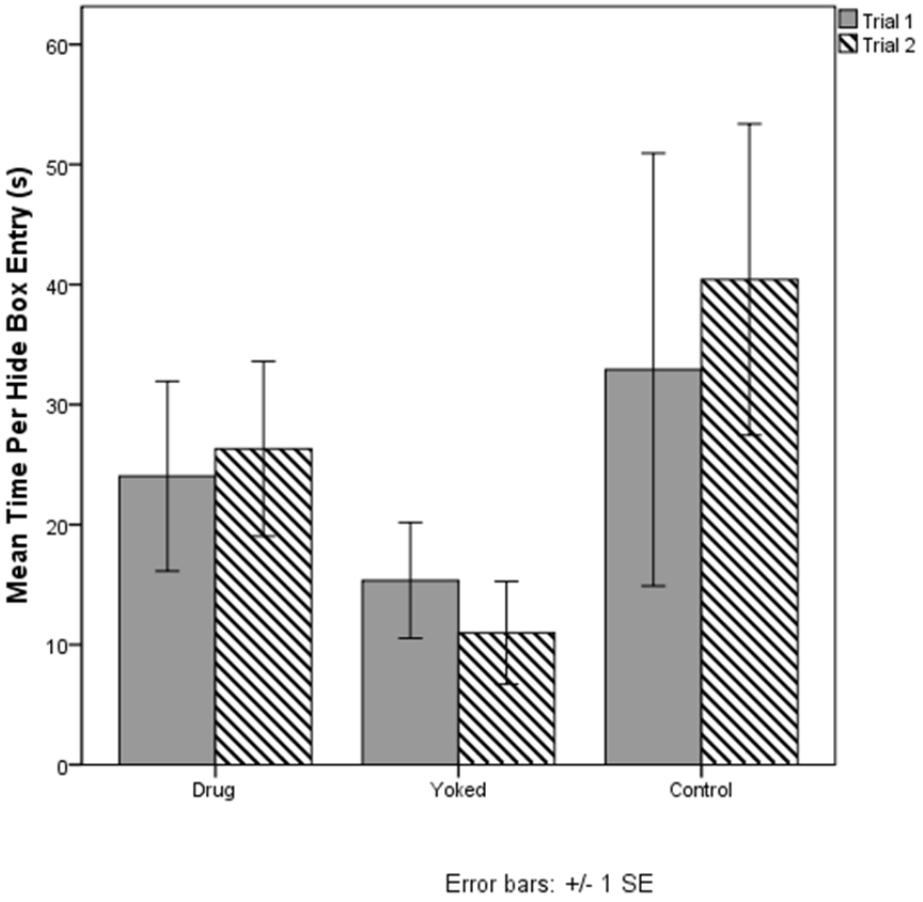

Figure 10- Mean time per hide box entry on trial 1 and trial 2 on the open-field emergence task with SEMs displayed. All animals, regardless of condition or trial, spent a comparable time in the hide box per entry.

Defecation has also been shown to be a reliable measure to assess anxiety in rats; increased boli production is an indication of increased anxiety. The 2 x 2 repeated measures ANOVA for drug versus control groups across trials found no interaction, $F(1, 13) = .13$, $p = .725$, $\eta^2 = .01$, no group effect, $F(1, 13) = .02$, $p = .891$, $\eta^2 < .00$, nor an effect of day, $F(1, 13) = .01$, $p = .907$, $\eta^2 < .00$. The drug group and control group produced similar amounts of boli, suggesting a similar amount of anxiety on the emergence task. Yoked versus control group revealed the same pattern, there was no interaction, $F(1, 13) = .08$, $p = .782$, $\eta^2 = .01$, no group effect, $F(1, 13) = .14$, $p = .713$, $\eta^2 = .01$, nor an effect of day, $F(1, 13) = .72$, $p = .41$, $\eta^2 = .05$. The yoked animals did not differ from the controls animals in terms of stress levels within the open field. Means are displayed in Figure 11.

More time spent in the center zone of the open field compared to the periphery is an indication of decreased anxiety in rats. However, because the yoked group had increased activity in the open field, mean time per entry into the center zone was used to measure anxiety in order to control for the increased activity. The 2 x 2 repeated measures ANOVA was used to analyze the results for drug and control groups across trials. There were no significant differences between the drug group and the control group. There was no significant interaction, $F(1, 13) = .43$, $p = .523$, $\eta^2 = .03$, no main effect of group, $F(1, 13) = .24$, $p = .633$, $\eta^2 = .02$, and there was no main effect of day, $F(1, 13) = 3.19$, $p = .098$, $\eta^2 = .20$ All animals avoided the center cells, suggesting a similar level of anxiety. The same repeated measures ANOVA was run comparing the yoked and control animals. There was no significant interaction, $F(1, 14) = .40$, $p = .539$, $\eta^2 = .03$, nor a main effect of day, $F(1, 14) = .18$, $p = .674$, $\eta^2 = .01$. However, regardless of trial, the yoked animals were less anxious compared to the control animals, $F(1, 14) = 4.08$, $p = .046$, $\eta^2 =$

.27. The yoked animals spent more time per entry in the center cells. The means are displayed in Figure 12.

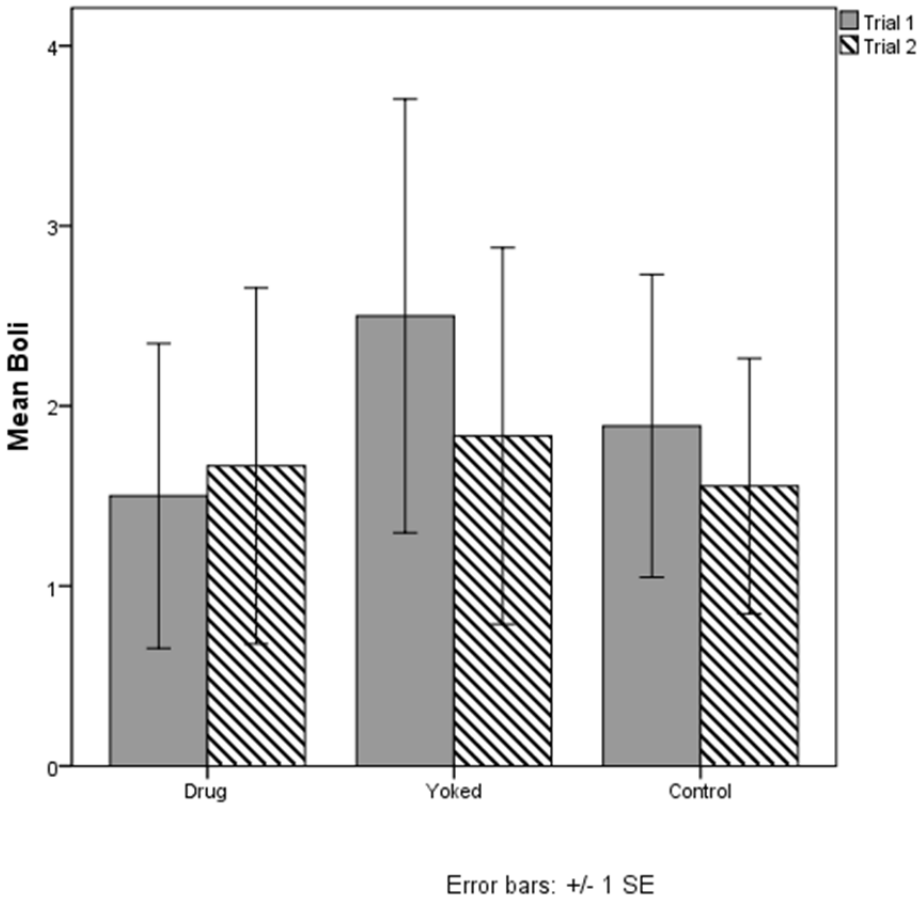

Figure 11- Mean boli production during the emergence task on trial 1 and trial 2 with all bars displaying SEMs. Both drug and control groups produced similar amounts of boli on the open field. The same pattern was seen when comparing the yoked and control groups.

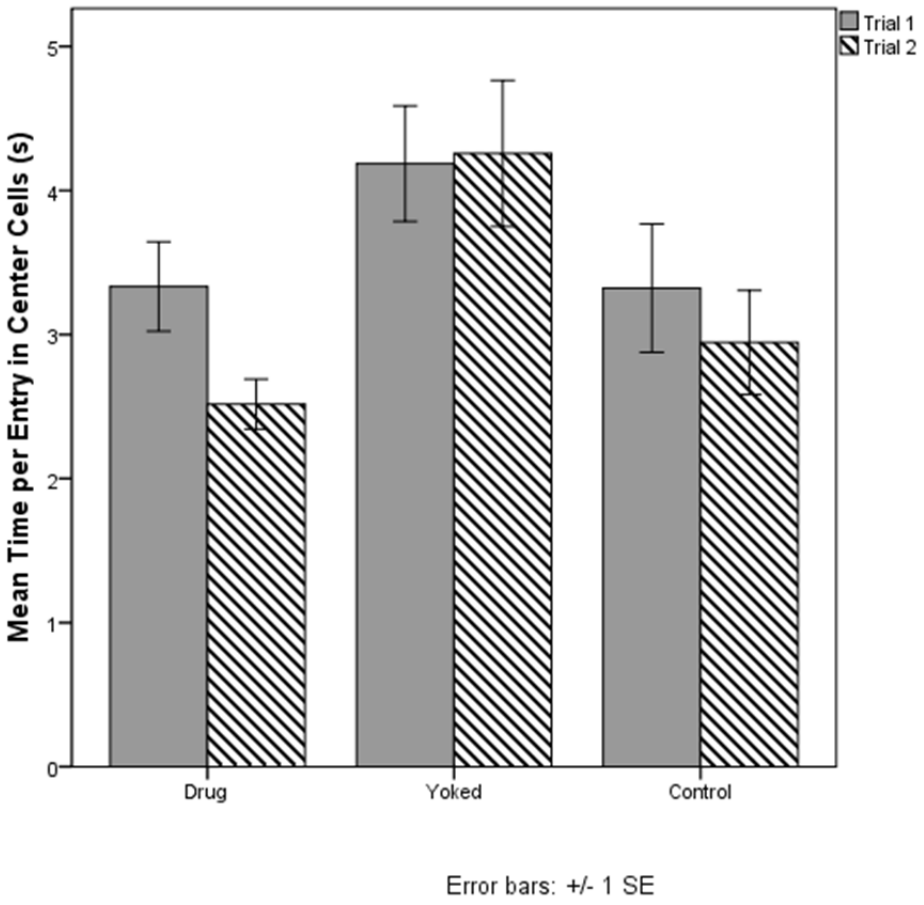

Figure 12- Mean time spent in the center cells each time the rat entered on trial 1 and trial 2 of the open field emergence task. SEMs are displayed on the bars. The drug and control animals displayed the same amount of anxiety as a function of time spent in the center cell per entry. The yoked animals on average spent more time in the center per entry compared to the control animals, an indication of decreased anxiety.

Sucrose Preference Task

Neophobia. Anxiety in the sucrose preference task was measured by the percent of sucrose drank during the first hour of the task. Measurements were taken in mL at 30 minutes and in mL and g at 1 hour. Decreased sucrose consumption was a suggestion of fear to a new stimulus (neophobia). Results were analyzed using independent samples *t*-tests. Overall there was a trend that the drug animals were slightly more neophobic than the control animals at the 30 min mark, $t(32) = -1.92$, $p = .063$, $d = -.68$. Means are displayed in Figure 13. This trend did not continue into the measures taken at 60 min in grams, $t(32) = -1.24$, $p = .224$, $d = -.44$. These data suggest slightly more anxiety initially in the drug group compared to the controls. Means are displayed in Figure 14. The same statistical plan was used to compare the yoked and control animals. There was no significant difference at 30 min ($t(26) = -.17$, $p = 869$, $d = .07$) or 1 hr ($t(26) = .74$, $p = .463$, $d = .29$). This suggests both the yoked and control groups had a similar low level of anxiety towards the novel substance.

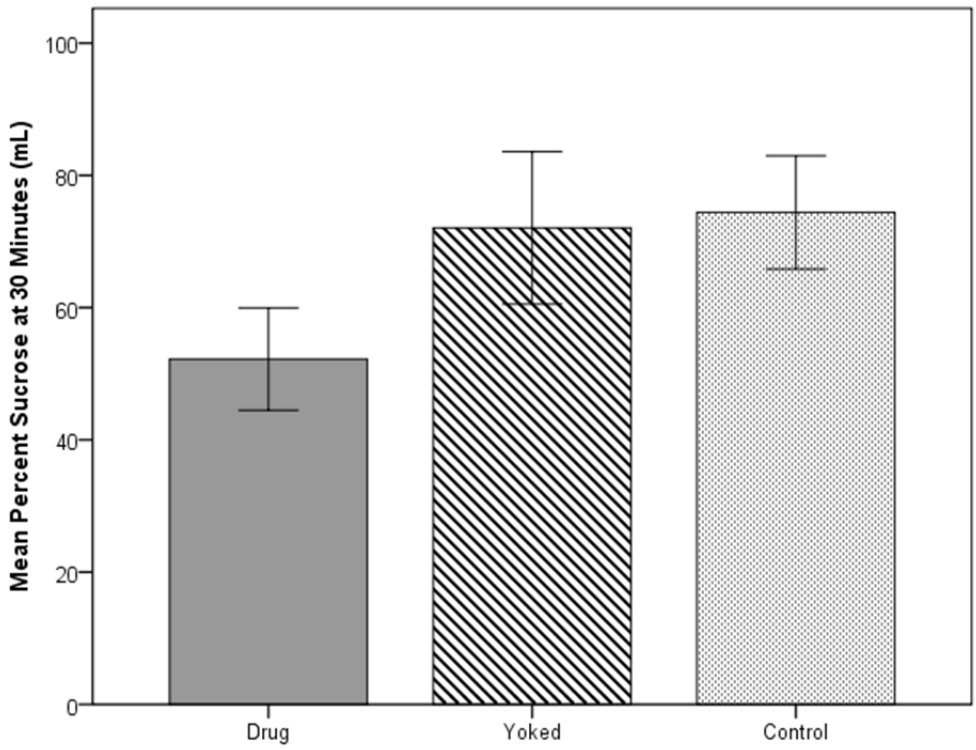

Figure 13- Mean percent of sucrose consumed at 30 min, with SEMs also represented. There is a trend suggesting that the drug group was more anxious compared to the control group. No difference was observed between the yoked and control groups.

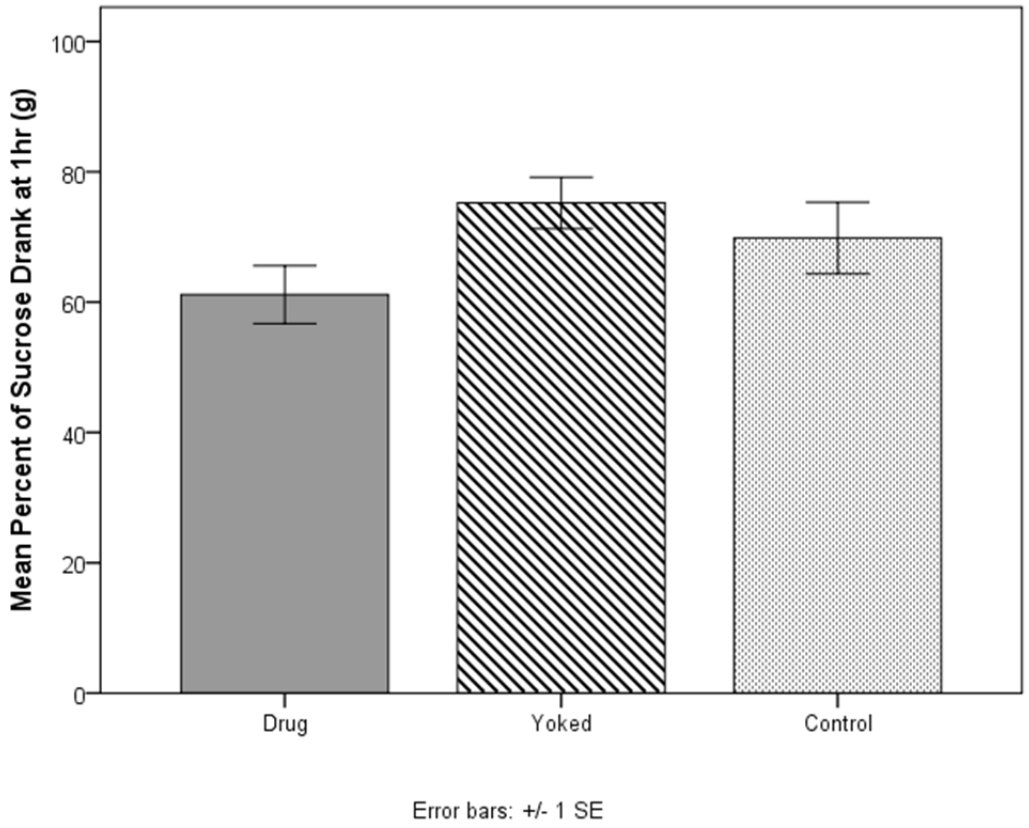

Figure 14- Mean percent of sucrose drank at 1 hour using a weight measure (grams) with SEMs also represented. There was no difference when comparing the drug group to the control group. The was also no difference when comparing the yoked and control groups.

Anhedonia. Depressive symptoms in the sucrose preference task was measured by the percent of sucrose drank across 3 days. A 2 x 3 repeated measures ANOVA was used to compare the drug group to the control group. There was no significant interaction, $F(2, 64) = .10$, $p = .902$, $\eta^2 < .01$, no main effect of group, $F(1, 32) = .81$, $p = .376$, $\eta^2 = .03$, and there was no main effect of day, $F(2, 64) = 1.50$, $p = .231$, $\eta^2 = .05$; both groups drank an equal percentage of sucrose water across days. Means are displayed in Figure 14. The same repeated measures ANOVA was used to analyze the yoked group compared to the control group. The same pattern of results was found: there was no interaction, $F(2, 52) = .22$, $p = .801$, $\eta^2 = .01$, no main effect of group, $F(1, 26) = 2.22$, $p = .148$, $\eta^2 = .08$, and there was no main effect of day, $F(2, 52) = .55$, $p = .587$, $\eta^2 = .02$ Both the yoked and control groups consumed a similar percentage of sucrose water across days. See Figure 15 for a display of the results. None of the animals showed signs of depressive symptoms.

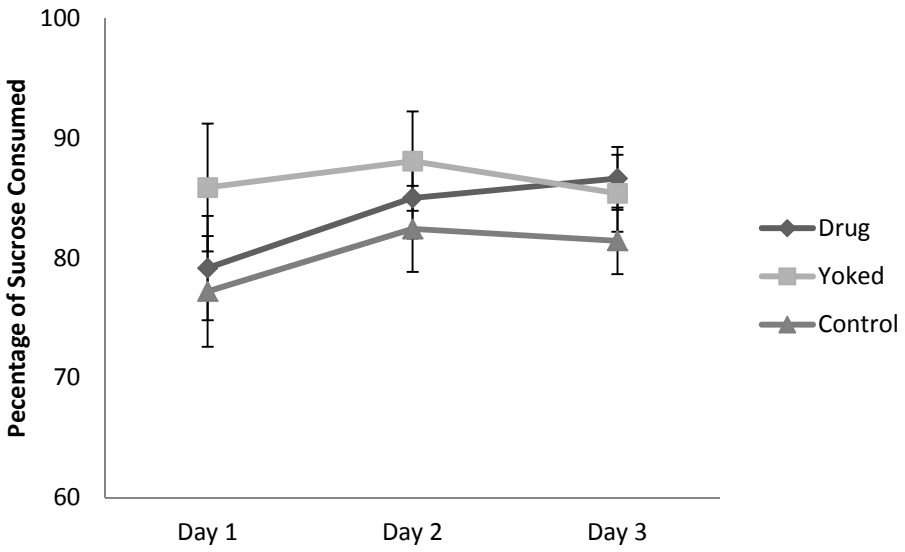

Figure 15- Percentage of sucrose consumed compared to plain water with SEMs displayed. No groups differed in the amount of sucrose consumed across days.

Discussion

It was hypothesized that lactation exposure to cannabinoids would alter physical development as a function of decreased food intake during the injection period, and increase anxiety and depressive symptoms compared to the control group. It was also hypothesized that malnutrition during lactation would hinder weight development as a function of decreased food intake and increase anxiety and depressive symptoms. However, impairments from malnutrition alone were not expected to be as severe as the impairments observed in the drug exposed group. The results partially support the original hypothesis in that drug and yoked animals consumed less food than the control group. Additionally, the original hypothesis was supported in that drug and yoked groups gained weight at a slower rate during the injection period and beyond compared to control animals. The hypothesis was not supported by the alterations in anxiety and depressive symptoms when comparing the drug and control groups. Overall there were no significant differences in anxiety or depressive symptoms as a function of postnatal cannabinoid exposure. Although there was a trend in the neophobia measure on the sucrose preference task, indicating an increase in anxiety, the effect was not quite significant. The drug and control group displayed similar amounts of anxiety and depressive symptoms on all behavioral tasks. However, there was evidence to support altered emotionality in the yoked group compared to the control group. The yoked group displayed increased locomotor activity and decreased anxiety in the open-field emergence task suggesting that malnutrition alone affects behavior.

Food intake was monitored from PD 2 to PD 20 and injections occurred on PD 2 through PD 19. It was expected that drug mothers would consume less food than the control mothers throughout the injection period, and the yoked mothers were only fed the amount that the drug mothers consumed. As expected, the control group consumed more food than drug and yoked

groups, and all dams consumed more food across days. The significant interaction effect revealed that the drug mothers caught up to the control mothers in food consumption by the end of the injection period, which suggests that tolerance to the appetite suppressant effects of the drug developed after approximately two weeks.

Rubino et al. (2008) found similar results that cannabinoid exposed animals decreased food intake during the injection period in adolescent rats. This was seen only during the injection period and body weights rebounded when the injections stopped. Further evidence by McGregor, Issakidis and Prior (1996) found that acute exposure to CP 55, 940 also decreased food intake in adult male rats. The current study is in line with previous research that exposure to cannabinoids decreases food intake in animals. Furthermore, Levay et al. (2010) reported that food intake increased across the lactation period. They found that food intake increased incrementally throughout the gestation and lactation time periods in free fed mothers. Food consumption must keep pace with the developing pup because as they grow they need more food. It is evident that the current results are in line with previous research in terms of food intake in the control animals. The yoked animals followed the same pattern as the drug animals because they were fed the same amount the drug animals had eaten, and there was no drug exposure that slowed down their eating, therefore they consumed all the food given to them. O'Shea et al. (2004) mentioned that animals exposed to cannabinoids can develop a tolerance which could affect the amount of food consumed later in the injection period although this was not reported in their study.

It was expected that all mothers would lose weight during the injection period (as they recovered from the pregnancy and breast fed the pups) while all pups would gain weight regardless of condition. Additionally, it was expected that the control dams would lose weight at

a slower rate than drug and yoked dams. Similarly, it was expected that the control pups would gain weight faster than the drug and yoked pups across days. As expected all mothers lost weight and all pups gained weight. Also, drug and yoked mothers lost weight at a quicker rate during the injection period than the control animals. A similar pattern was seen in the pups where the drug and yoked animals gained weight at a slower rate than the control pups. All pup weights were equivalent at the start of the injection period but the drug group started to gain weight less quickly by PD 8. Weight gain effects in pups were seen into adolescence. Drug animals were significantly lighter than control animals until PD 49 and yoked animals were lighter than control animals until PD 56.

Similar results were found by O'Shea and Mallet (2005) in that exposure to a moderate dose of THC from PD 4 to 14 reduced weight gain throughout the injection period. They observed this effect through PD 52 to 53. Rubino et al. (2008) reported that adolescents injected with THC from PD 35 to 45 had slower weight gain as well. Further evidence by McGregor, Issakidis, and Prior (1996) found that chronic CP 55, 940 exposure reduced body weight in adult male rats. It is evident that cannabinoid exposure slows weight gain during developmental exposure and that the effect lasts well beyond the exposure period. The current study validates previous findings because similar patterns in weight loss of the dams and weight gain in the pups were observed. It is evident from these findings that a yoked group must be included in order to control for malnutrition and alterations to weight gain. Although, weight development was impaired throughout the injection and washout period, this effect dissipated before behavioral testing began on PD 77.

Levay et al. (2008) also found reduced weight gain in the mothers and slower weight gain in pups when looking at calorie restriction. They discovered that a 25% calorie restriction during

lactation reduced the mothers' weight quicker and the pups gained weight slower during the restriction time period. Furthermore, Levay et al. (2008) found that this weight impairment was seen until PD 42, suggesting that developmental exposure to calorie restriction in the dams can have long lasting changes in the pups. Additional research by Levay et al. (2010) found the same pattern that a mild lactation calorie restriction of 25 % produces weight change in pups throughout exposure time period. Levay et al. (2010) also found that lactational calorie restricted offspring had decreased serum corticosterone, adrenalin, and ACTH between 12-14 weeks of age. In the current study the yoked group had a 30.43% calorie restriction and the drug group had a calorie restriction respectively.

Data collected on the elevated plus maze indicated drug exposure did not alter emotionality compared to control animals. Specifically, the drug and control groups did not differ on percent of time spent on open arms, both groups preferred to spend time in the enclosed arms. Furthermore, both groups preferred to enter the enclosed arms more compared to open arms. Finally, the drug group and the control group defecated the same amount on the EPM. The drug group and control group had similar levels of anxiety on the EPM. In comparing the yoked group to the control group a similar pattern of results emerged. Both groups preferred the enclosed arms as a function of time and entries. However, there was a slight indication that the yoked animals were less anxious as a function of boli. Although not quite statistically significant, the yoked animals produced less boli on the open arms compared to the control animals.

Previous research on early developmental exposure to cannabinoids is inconsistent when it comes to the EPM. Trezza et al. (2008) found that after they exposed animals to 5 mg/kg of THC from GD 15 to PD 9 there was an increase in anxiety on the EPM in adulthood. THC exposed animals had a decreased percentage of time spent on open arms and decreased

percentage of entries to open arms. However, contrasting research by Rubio et al. (1995) found animals exposed to 5 mg/kg of THC from GD 5 to PD 24 showed a decrease in anxiety in adulthood. Animals that were exposed to THC had increased percent open arm entries and spent more time in the open arms (Rubio et al., 1995). Further differences between these two studies can be seen in the duration of the trial. Trezza et al. only ran animals on the EPM for 5 min while Rubio et al. ran each animal for 15 min. Additionally, Rubio et al. specifically states that each animal habituated for 5 min before the trial whereas Trezza et al. specified no habituation time. Because the current study ran the EPM for 10 min it is possible that it was long enough to wash out any initial anxiety effects as seen by Trezza et al. (2008) and short enough to miss the decreased anxiety with a longer trial described by Rubio et al. (1995).

Similar inconsistencies exist in the literature on calorie restriction during early developmental time periods. Levay et al. (2008) exposed mothers to 25% calorie restriction during the lactation time period. Offspring were then run on the EPM in adulthood. The calorie restricted dams did not produce more anxious offspring as a function of open arm entries or time spent in open arms. Conversely, Periea-da-Silva, Cabral-Filho, & de-Oliveria, (2009) found that early malnutrition decreased anxiety in the EPM. Mothers' in the malnutrition group received food chow with 10% less protein than the control groups. In adulthood, offspring were run on the EPM and malnourished offspring showed decreased anxiety as a function of increased entries and time spent in open arms. Further research by Fraga et al. (2011) demonstrated that pups malnourished during lactation showed increased anxiety on the EPM. Dams were exposed to bromocriptine, which made the dams lactate less for the last three days of nursing. Offspring were then run on the EPM in adulthood and it was demonstrated that the malnourished pups had increased anxiety by entering and spending less time in open arms. There are inconsistencies

with type of malnutrition where Levay et al. simply restricted calorie intake, Pereira-da-Silva et al. reduced the amount of protein each animal was allotted, and Fraga et al. only restricted nutrition for the last three days of lactation via a drug. The current research found some indication that the yoked animals were less anxious, however due to the lack of consistency in the literature, and the very small effect found here, the results are ambiguous at best.

The emergence task did not show any indication of changes in anxiety or activity in the drug animals compared to the control animals. Specifically, in the amount of boli, mean time spent in the hide box per entry, and mean time spent in the center zone per entry. Drug and control animals displayed an equal amount of anxiety and activity throughout the emergence task. This did not hold true for when comparing the yoked and control groups. The yoked group had an increase in activity and a decrease in anxiety. The increased in distance traveled suggests that the yoked animals had increased locomotor activity. Overall, the yoked group showed decreased anxiety by showing an increased time spent in center cells per entry. This measure was used because the number of entries into the center cells was influenced by the increase in activity of the yoked animals, therefore it was necessary to get an average time spent in the center zone per entry. There was no difference between the yoked and control group when measuring the amount of boli, but there was a not quite significant decrease in mean time per entry into the hide box for the yoked group compared to the control group. Taken together with the almost significant decrease in boli in the EPM, there might be some evidence to suggest a mild decrease in anxiety in the yoked animals compared to the controls.

The literature also demonstrates that differences exist when looking at locomotor activity in cannabinoid exposed animals. For instance, Rubio et al. (1995) found that only female rats that received THC during GD 5 – PD 24 had increased locomotor activity in adulthood and the

male rats showed no difference compared to controls. Additionally, Navarro et al. (1994) found that activity was increased in cannabinoid offspring after being exposed from GD 5 – PD 24. This effect was seen in preweanling male and female rats and only in adult females at PD 70. This suggests that the effects are no longer evident in adult males as Rubio et al. found. The current study looked at males in the open field task at PD 77, and our results agreed with Rubio et al. and Navarro et al. in terms of adult male activity levels after infant cannabinoid exposure. With respect to the emergence task itself our results are consistent with previous research. O'Shea et al. (2004) found that when rats were perinatally exposed to CP 55,940 they showed no increase in anxiety on the emergence task compared to controls. A similar light-dark emergence task was run by Navarro et al. (1994). This emergence task assessed latency to exit the dark side of an open field to the lit side of the open field. This is similar to the current study because it assessed how quickly rats emerge from a dark 'safe' box to a lit open field. Results from Navarro et al. (1994) indicated that males exposed to THC during GD 5- PD24 had a similar latency to emerge to the open field as control animals, which is similar to what the current study found.

The yoked animals showed more activity and decreased anxiety on the emergence task. Previous findings indicated offspring of calorie restricted dams during lactation were more anxious as measured by entering the center of the open field less (Levay et al., 2008). However, Levay et al. (2008) also found that mothers in the lactation calorie restriction group had better pup retrieval and better nesting conditions, suggesting increased maternal care. A follow up study by Levay et al. (2010) found that lactational calorie restricted mothers' offspring had significantly lower levels of cortisol. Increased maternal care has been shown to increase activity in pups and decrease anxiety (Caldji et al., 1998; Urarte et al., 2007). Caldji et al. (1998) found that adult offspring of dams who had increased maternal care had increased exploration in the

open field test and Urarte et al. (2007) found increased maternal care decreased anxiety as measured by shorter latencies to enter the center zone. It is possible that in the current study the yoked dams had increased maternal care which then lead to alterations in activity and anxiety measures in adulthood. Both drug and yoked groups were calorie restricted but only the yoked group had any alterations on behavior. Because of the drug's immediate effects, the drug moms probably didn't compensate by paying more attention to their pups. However, these results need to be further investigated by looking at maternal care and cortisol levels in yoked dams. It is also possible that the increase in activity was a function of decreased anxiety in the yoked animals. If the yoked animals were less anxious it is entirely possible that the decreased anxiety lead to an increase in activity. Furthermore, because all subjects were ran during the light cycle, when rats are less active, it is also possible that malnutrition in the yoked group affected their circadian rhythm. Although this is possible, it is very consistent in the literature that all animals were run during the light portion of their day/night cycle, making it unlikely.

It was hypothesized that drug exposure and malnutrition would increase neophobia on the sucrose preference task. Measurements were taken at 30 min and 1 hr. There was a suggestion that drug exposure increased anxiety compared to control animals at 30 min., where drug animals drank slightly less sucrose at 30 min compared to control, although the effect was not significant. The effect had dissipated by one hour. Additionally, yoked animals did not display any neophobia on the sucrose preference task. Anhedonia was measured over the next 3 days. Both drug and yoked groups did not show any signs of anhedonia compared to the control group, all groups preferred to drink the sucrose and in approximately equal amounts across days.

All animals having an increased preference for sucrose has been widely established. Because all animals prefer sucrose it is important to see how other studies conducted the sucrose

preference task in order to determine why the drug animals did not exhibit neophobia/ anhedonia. Mourlon et al. (2010) found that maternal deprivation (dam separated from the pups) does not decrease sucrose consumption in rats. Early maternal deprivation took place during PD 1 to PD 14 where mothers were removed from the cages for 3 hrs. Additionally, Mourlon et al. (2010) only allotted the animals to drink the sucrose for 60 min each day, where the current study allowed for 24 hr access to the sucrose solution. Their offspring were tested on a 1% sucrose solution preference task and it revealed that all animals preferred the 1% sucrose solution. Additionally, Ueji and Yamamoto (2012) found that 10 week old rats preferred a 2% sucrose solution over a 30% sucrose solution. This suggests that a 2% solution might be more preferable to rats. If a 2% solution is more preferable to rats it is possible that it washed out any possible effects that could be seen in the current sucrose preference task. Methodological differences could explain the reason why all animals preferred sucrose. Ueji and Yamamoto only allowed for a two day exposure to sucrose and they paired a 2% solution with a 30% solution of sucrose. Further research needs to be done to determine the exact solution concentration to accurately assess anxiety and depression that is associated with this task. Rubino et al. also used a 2% sucrose solution and found anhedonia in animals treated with cannabinoids during adolescents. However, in their analysis they only looked at day 2 and 3 for decreased sucrose consumption. It is possible that malnutrition and cannabinoid exposure during infancy do not cause depressive symptoms in adult rats.

The results of the current study partially support the original hypotheses. Decreased food intake associated with drug exposure has been shown to decrease weight during the injection period and into the washout period of drug exposure. These effects are also seen within the yoked group where weight loss is associated with decreased food consumption that

has long lasting effects into the washout period. Food intake, not drug exposure itself, is causing the decrease in body weight. Exposure to cannabinoids during lactation did not show any lasting behavioral changes in the pups when they were adults. Although it is tempting to question whether or not the drug was ever transferred to the pup, it has been demonstrated that cannabinoids are present in breast milk in rats (Jakuboivc, Hattori, & McGeer, 1977). It could be that the exposure time period was not sensitive enough to negatively alter emotional regulation in offspring. However, malnutrition alone increased activity and decreased anxiety. This suggests that early nutritional insults have long lasting emotional changes into adulthood. Additionally, because of the large effect sizes seen the in current project, it is possible that a low sample size masked effects that actually existed. Typically previous research would have about 10 – 12 animals per group where the current project had 9 – 11 in each group. Increasing the sample size would allow the current project to have sufficient power and see any effects that actually exists. In conclusion, the current study is an indication that closer attention needs to be drawn to the effects of malnutrition during early stages of development when examining cannabinoid drug exposure. Early nutrition deficits may confound the attribution of emotional alterations associated with early cannabinoid drug exposure. The current study demonstrates that including a food restricted group that corresponds with the food decrease associated with drug exposure is necessary because food restriction alone causes emotional alterations.

References

Bond, N. W. (1980). Postnatal alcohol exposure in the rat: Its effects on avoidance conditioning, Hebb-Williams maze performance, maternal behavior, and pup development. *Physiological Psychology, 8, 4,* 437-443.

Breedlove, S. M., Watson, N. V., & Rosenzweig, M. R. (2011*). Biological Psychology: A I ntroduction to Behavioral, Cognitive, and Clinical Neuroscience.* Sunderland, Massachusetts: Sinauer Associates.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Neurobiology, 95,* 5335-5340.

Childers, S. R., & Breivogel, C. S. (1998). Cannabis and endogenous cannabinoid systems. *Drug and Alcohol Dependence, 51,* 173-187.

Corchero, J., Garcia-Gil, L., Manzanares, J., Fernandez-Ruiz, J. J., Fuentes, J. A., & Ramos, J. A. (1998). Prenatal tetrahydrocannabinol exposure reduces proenkephalin gene expression in the caudate-putamen of adult female rats. *Life Sciences, 63*(10), 843-850. doi:10.1016/S0024-3205(98)00341-5.

Day, N., Cornelius, M., Goldschmidt, L., Richardson, G., Robles, N., & Taylor, P. (1992). The effects of prenatal tobacco and marijuana use on offspring growth from birth through 3 years of age. *Neurotoxicology and Tetralogy 14,* 407-414.

Daly, F. M., Hughes, R. N., & Woodward, L. J. (2012). Subsequent anxiety-related behavior in rats exposed to low-dose methadone during gestation, lactation, or both periods consecutively. *Pharmacology, Biochemistry and Behavior, 102,* 381-389.

DiNieri, J., Wang, X., Szutorisz, H., Spano, S., Kaur, J., Casaccia, P., Dow-Edwards, D.,& Hurd, Y. (2011). Maternal cannabis use alter ventral striatal dopamine D_2 gene regulation in the offspring. *Biological Psychiatry, 70*(8), 763-769.

Fernandez-Ruiz, J., Berrendero, F., Hernandez, M. & Ramos, J. (2000). The endogenous cannabinoid systems and brain development. *Trends in Neuroscience, 23*(1), 14-20.

Fernandez-Ruiz, J. J., Berrendero, F., Hernandez, M. L., Romero, J., & Ramos, J. A. (1999). Role of endocannabinoids in brain development. *Life Science, 65,* 725-736.

Field, P. A. (1976). Short and long-term effects of pre-natal cannabis inhalation upon rat offspring. *Psychopharmacology, 50(3),* 285-291.

Fraga, M. C., Moura, E. G., Silva, J. O., Bonomo, I. T., Filgueiras, C. C., Abreu-Villaca, Y., Passos, M. C. F., Lisboa, P. C., & Manhaes, A. C. (2011). Maternal prolactin inhibition at the end of lactation affects learning/memory and anxiety-like behaviors but not novelty-seeking in adult rat progeny. *Pharmacology, Biochemistry and Behavior, 100,* 165-173. doi:10.1016.j.pbb.2011.07.007.

Fried, P. A., & Smith, A. M. (2001). A literature review of the consequences of prenatal marihuana exposure: An emerging theme of a deficiency in apects of executive function. *Neurotoxicology and Teratology, 23*(1), 1-11.

Fride, E. (2008). Multiple roles for the endocannabinoid system during the earliest stages of life: Pre- and postnatal development. *Journal of Neuroendocrinology, 20,* 75-81.

Garcia, L., de Miguel, R., Ramos, J., & Fernandez-Ruiz, J., (1996). Prenatal tetrahydrocannabinol exposure in rats modifies the responsiveness of midbrain dopaminergic neurons in adulthood to a variety of challenges with dopaminergic drugs. *Drug and Alcohol Dependence, 42*(3), 155-166.

Jakubovic, A., Hattori,T., & McGeer P. L. (1977). Radioactivity in suckled rats after giving 14C-tetrahydrocannbinol to the mother. *European Journal of Pharmacology, 22*, 221-223.

Julien, R. M., Advokat, C. D., & Comaty, J. E. (2010). *A Primer of Drug Action.* New York, New York: Worth Publishers.

Levay, E. A., Paolini, A. G., Govic, A., Haze, A., Penman, J., & Kent, S. (2008). Anxiety-like behavior in adult rats perinatally exposed to maternal calorie restriction. *Behavioural Brain Research, 191,* 164-172. doi:10.1016/j.bbr.2008.03.021

Levay, E. A., Paolini, A. G., Govic, A., Haze, A., Penman, J., & Kent, S. (2010). HPA and sympathoadrenal activity of adult rats perinatally exposed to maternal mild calorie restriction. *Behavioural Brain Research, 208,* 202-208. doi:10.1016/j.bbr.2009.11.033

Lombard, C., Hegde, V. L., Nagarkatti, M., & Nagarkatti, P. S. (2011). Perinatal exposure to delta 9- tetrahydrocannabinol triggers profound defects in T cell differentiation and function in fetal and postnatal stages of life, including decreased responsiveness to HIV antigens. *The Journal of Pharmacology and Experimental Therapeutics*, *339*(2), 607-617.

McGregor, I. S., Issakidis, C. N., & Prior, G. (1996). Aversive effects of synthetic cannabinoid CP 55, 940 in rats. *Pharmacology Biochemistry and Behavior, 53,* 657- 664. doi:0091-3057(95)02066-7

Molina-Holgado, F., Alvarez, F. J., Gonzalez, I., Antonio, M. T., & Leret, M. L. (1997). Maternal exposure to delta 9- tetrahydrocannabinol (delta 9 – THC) alters indolamine levels and turnover in adult male and female rat brain regions. *Brain Research Bulletin, 43,* 173-178.

Molina-Holgado, F., Amaro, A., Gonzalez, M. I., Alvarez, F. J., & Leret, M. L. (1996). Effect of maternal delta 9- tetrahydrocannabinol on developing serotonergic system. *European Journal of Pharmacology, 316,* 39-42.

Moreno, M., Trigo, J. M., Escuredo, L., De Fonseca, F., & Navarro, M. (2003). Prenatal exposure to tetrahydrocannabinol increases presynaptic dopamine D2 receptor sensitivity: a behavioral study in rats. *Pharmacology Biochemistry and Behavior, 75*(3), 565-575.

Mourlon, V., Baudin, A., Blanc, O., Lauber, A., Giros, B., Naudon, L., & Dauge, V. (2010). Maternal deprivation induces depressive-like behaviours only in female rats. *Behavioural Brain Research, 213,* 278-287. doi: 10.1016/j.bbr.2010.05.017

Navarro, M., Rubio, P., & Rodriguez de Fonesca, F. (1994). Sex-dimorphic psychomotor activation after perinatal exposure to delta-9-tetrahydrocannabinol: An ontogenic study in Wistar rats. *Psychopharmacology, 116*(4), 414-422.

Newsom, R. J., & Kelly, S. J. (2007). Prenatal delta-9-tetrahydrocannabinol exposure disrupts social and open field behavior in adult male rats. *Neurotoxicology and Teratology, 30*(3), 213-219.

O'Shea, M., & Mallet, P. E. (2005). Impaired learning in adulthood following neonatal delta-nine-THC exposure. *Behavioral Pharmacology, 16,* 455-461. doi: 10.1097/00008877-200509000-00019.

O'Shea, M., McGregor, I. S., & Mallet, P. E. (2006). Repeated cannabinoid exposure during perinatal, adolescent or early adults ages produces similar long-lasting deficits in object recognition and reduced social interaction in rats. *Journal of Psychopharmacology, 20*(5), 611-621.

O'Shea, M., Singh, M. E., McGregor, I. S., & Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *Journal of Psychopharmacology, 18,* 502–508.

Pereira-da-Silva, M. S., Cabral-Filho, J. e., & de-Oliveria, L. M. (2009). Effect of early malnutrition and environmental stimulation in the performance of rats in the elevated plus maze. *Behavioral Brain Research, 205,* 286-289. doi:10.1016/j.bbr.2009.05.031

Rosenthal, R., Rosnow, R. L., & Rubin, D. B. (2000). *Contrasts and effect sizes in behavioral research: a correlational approach*. Cambridge: Cambridge University Press.

Rubio, P., De Fonseca, F. R., Martin-Calderon, J. L., Del Arco, I., Bartolome, S., Villanua, M. A., & Navarro, M. (1998). Maternal exposure to low doses of tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. *Pharmacology Biochemistry and Behavior, 61*(3), 229-238. doi:10.1016/S0091-3057(98)00099-9.

Rubino, T., Vigano, D.,Realini, N., Guidali, C., Braida, D., Capurro, V., Castigiloni, C., Cherubino, F., Romualdi, P., Candeletti, S., Sala, M., & Parolaro, D. (2008). Chronic delta-9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: Behavioral and biochemical correlates. *Neuropsychopharmacology, 33*(11), 2760-2771.

Schneider, M., & Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ration task in adult rats. *Neuropsychopharmacology, 28(10),* 1760-1769. doi: 10.1038/sj.npp.1300225.

Schneider, M. (2009). Cannabis use in pregnancy and early life and its consequences: animal models. *European Archives Psychiatry Clinical Neuroscience, 259,* 383-393.

Svizenska, I., Dubovy, P., & Sulcova, A. (2008). Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures- A short review. *Pharmacology, Biochemistry, and Behavior, 90,* 501-511.

Trezza, V., Campolongo, P., Cassano, T., Macheda, T., Dipasquale, P., Carratu, M. Gaetani, S., & Cuomo, V. (2008). Effects of prenatal exposure to tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats. *Psychoparmacology, 198*(4), 529-537.

Trezza, V., Campolongo, P., Manduca, A., Morena, M., Palmery, M., Vanderschuren, L. J. M. J., & Cuomo, V. (2012). Altering endocannabinoid neurotransmission at critical developmental ages: impact on rodent emotionality and cognitive performance. *Frontiers in Behavioral Neuroscience, 6,* 1-12. doi:10.3389/fnbeh.2012.00002

Ueji, K., & Yamamoto, T. (2012). Flavor learning in weanling rats and its retention. *Physiology & Behavior, 106,* 417- 422. doi: 10.1016/j.physbeh.2012.021

Uriarte, N., Breigerion, M. K., Benetti, F., Rosa, X. F., & Lucion, A. B. (2007). Effects of maternal care on the development, emotionality, and reproductive functions in male and female rats. *Wiley InterSciences,* 452-462. doi:10.1002/dev20241.

Vela, G., Martin, S., Garcia-Gil, L., Crespo, J., Ruiz-Gayo, M., Fernandez-Ruiz, J., Garcia-Lecumberri, C., Peoaprat, D., Fuentes, J, Ramos, J, & Ambrosio, E. (1998). Maternal exposure to tetrahydrocannabinol facilitates morphine self-administration behavior and changes regional binding to central mu opioid receptors in adult offspring female rats. *Brain Research, 807,* 101-109.