Adolescent Cannabinoid Exposure and Novelty Seeking: Effects on Memory in Adult Female Long-Evans Rats

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Abstract

Past research has shown that adolescent exposure to synthetic cannabinoids in rats can produce lasting effects on recognition memory (Schneider, Schömig, & Leweke, 2008). Persistent deficits in spatial memory in adult rats after they were chronically exposed to the synthetic cannabinoid WIN55, 212-2 during adolescence have also been shown (Abush & Akirav, 2012). Yet, Mateos et al. (2011) found that spatial memory deficits were only negatively affected in female rats, and not males. Due to the variability of results in the cannabinoid literature the current study incorporated a second variable of individual novelty seeking behaviors. For novelty-seeking, high responders (HR) are more exploratory, and appear less anxious while low responders (LR) are less exploratory and appear more anxious. Cannabinoid studies have also shown that administration can negatively affect food intake (Miller & Drew, 1974). Due to the negative physiological and cognitive effects of malnutrition, it is important to account for the lack of food intake and understand its effects on memory in adulthood. Additionally, cannabinoid research also lacks information on the influences of the estrous cycle on memory. Frye, Duffy, and Walf (2007) showed that rats differ in behavior across the estrous cycle, with those in the proestrus and estrus phase outperforming female rats in diestrus phase on a spatial memory task; because it is suggested that estrous cycle influences memory it is important to include it as a factor in the current study. The current study investigated how adolescent exposure to the synthetic cannabinoid CP 55,940 and novelty seeking effect memory in adult female Long-Evans rats. The study utilized a yoked-control group to control for the reduction in food intake of drug animals. All subjects were monitored for the estrous phase before the start the behavioral tasks. Results indicated that drug, and yoked animals ate significantly less food than control animals during injections. Drug and yoked animals also

weighed less than control animals during the injection period. Data from the body-weight analyses suggest that phenotype also affects food intake and weight gain. Neither drug group nor phenotype effected anxiety or activity levels. Drug animals demonstrated the best spatial memory and object recognition memory was significantly affected by group, estrous cycle, and object phenotype. Overall, this study showed that cannabinoid exposure significantly decreased food intake in adolescent rats. There was no evidence that demonstrated that animals retained differences in activity as adults (did not retain their distance phenotype). In addition, the current study provided evidence that estrous cycle and phenotype influence spatial and recognition memory after cannabinoid exposure. Future cannabinoid studies should monitor food intake in order to prevent weight loss in drug animals and continue to investigate the impact that natural differences and hormones may have on behavior, and memory in animals.

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Chapter 1: Introduction

Since 1996, twenty-three states and the District of Columbia have permitted the use of medical marijuana for an assortment of medical conditions. Moreover, residents of Alaska, Colorado, Oregon, and Washington state have additionally voted to pass legislation that would legalize the sale and distribution of recreational marijuana among those citizens that are 21 years of age or older. The National Survey on Drug Use and Health (NSDUH; SAMHSA, 2014) observed that adolescent perceptions of risk associated with monthly marijuana use have steadily declined since 2007, decreasing from 34.4 percent to 24.2 percent in 2013 (p.73). Consistent with the decline in perceived risk of marijuana, the prevalence of use among youths has increased from 6.7 to 7.1 percent between the years 2007 and 2013 (SAMHSA). Among the adolescents who reported use in 2013, 14.2 percent were 16-17 years old, and 5.8 percent were 14-15 years old (SAMHSA, p.73).

Overall, the National Survey on Drug Use and Health revealed that 5.7 million persons aged 12 or older reported using marijuana on a daily basis and 2.4 million persons initiated use for the first time in 2013 (56.6 percent were under the age of 18). Among those who had initiated use before age 21, the average age of initiation was 16.2 years of age. Likewise, the NSDUH (SAMHSA) reported that age of first use was associated with marijuana dependence and abuse, revealing that 11.5% of people who reported first use at age 14 or younger were dependent or abused marijuana as adults. This is compared to 2.6 percent of adults whose age of first use was age 18 or older and were dependent and abused marijuana. Due to the rise in adolescent marijuana use, and declining perception of risk, the current study investigated the effects of chronic adolescent marijuana use in adulthood.

Chapter 2: Overview of the Literature

Although childhood is characterized by a plethora of developing systems, adolescence can be defined as the developmental period wherein the body and the brain grow into adulthood. During adolescence, the brain experiences a growth in connectivity, and cognitive abilities mature, such as the ability to plan, self-regulate, and use working memory (Bossong & Niesink, 2010). As a consequence of increased neural growth, these dramatic changes make adolescents particularly susceptible to the use of exogenous substances like marijuana (Bossong & Niesink, 2010).

Although research in the endocannabinoid system is still in its infancy, studies have demonstrated the important role the endocannabinoid system has throughout the peripheral and central nervous system (Pacher & Mechoulam, 2011). Two cannabinoid (CB) receptors have been identified and are spread throughout the nervous system: CB_1 and CB_2 (Bossong & Niesink, 2010). The CB_1 receptor is more prevalent in the central nervous system whereas the CB_2 receptor is most prevalent in the peripheral nervous system (Bossong & Niesink, 2010; Pacher & Mechoulam, 2011). CB_1 receptors are located in high levels in brain areas such as the cerebellum, basal ganglia, hippocampus, and cortex (Bossong & Niesink, 2010). The CB_2 receptor is found throughout the peripheral nervous system, such as the heart, kidney, lung and liver, and moderates inflammatory/immune responses to neurodegenerative, pain, organ, and cancer conditions (Pacher & Mechoulam, 2011).

Although marijuana influences the peripheral nervous system, of more importance to the current project are its effects on the central nervous system (CNS). Alongside classification of CB receptors, several endogenous CB ligands have been identified: anandamide and 2-arachidonoylglycerol (2-AG; Bossong & Niesink, 2010). Unlike most other neurotransmitters,

the endocannabinoids are released from the post-synaptic neuron and bind at the CB receptors on the pre-synaptic neuron. Endocannabinoids regulate and maintain glutamate homeostasis throughout the brain (Bossong & Niesink, 2010). This regulation of glutamate prevents excitotoxicity induced by the influx of Ca^{2+}. As a result, endogenous CB ligands act as messengers that relay messages to the presynaptic neuron to cease Ca^{2+} action at NMDA receptors (Bossong & Niesink; Pacher & Mechoulam). As a result, the endocannabinoid system has important neuroprotective functions due to the prevention of cellular apoptosis from excessive Ca^{2+} (Bossong & Niesink, 2010). Campbell and Gowran (2007) support the neuroprotective role of the endocannabinoid system stating that in cases of neuronal damage, increases in endogenous cannabinoids occur and cells that lack the CB_1 receptor are more susceptible to injury. Such occurrences suggest that an early augmentation in endocannabinoid levels may protect against the actions of harmful substances (Campbell & Gowran, 2007).

Exogenous Cannabinoids

Chronic use of Δ9-tetrahydrocannabinol (THC; the main psychoactive ingredient in marijuana) and synthetic cannabinoids produce alterations to the endocannabinoid system through two different mechanisms: down-regulation, and desensitization of CB receptors (Bossong & Niesink, 2010). Burston et al. (2010) had consistent findings after administering chronic doses of THC to adolescent and adult Long-Evans rats. Burston and colleagues discovered that desensitization of the CB_1 receptor occurred throughout the brains of both adult and adolescent rats. More specifically, Burston et al. (2010) found that desensitization was greater in female adolescent rats in the hippocampus, ventral midbrain, and prefrontal cortex. Burtson et al. also revealed that there was a significant reduction in CB_1 density in the prefrontal cortex, striatum, hypothalamus, and ventral midbrain after chronic exposure to THC. Likewise,

Rubino et al. (2008) found that repeated exposure to a high dose of THC in adolescence produced a significant decrease in CB_1 receptor binding in adolescent female rats across many brain areas: prefrontal cortex, caudate putamen, hypothalamus, hippocampus, amygdala, thalamus, periaqueductal gray, substania nigra, ventral tegmental area (VTA), and cerebellum. Despite the reduction in binding levels during adolescence, Rubino et al. only found down regulation and desensitization of CB_1 receptors in the nucleus accumbens (NAc), and amygdala in female adult rats (with down-regulation only in the VTA). Pistis et al. (2004) further found that both adult and adolescent rats administered an acute dose of WIN-55212.2 exhibited less stimulation in the VTA and NAc when exposed to the same dose of WIN two weeks after the last injection. Ellgren et al. (2008) also discovered that after intermittent exposure to a low dose of THC there was a significant increase in CB_1 density in the NAc in vehicle-treated animals, and that after one dose of THC there was an increase in CB_1 receptor density in the same area. Receptor density levels have been seen in rodent models to induce tolerance and often results in the need for more stimulation to acquire the same effects, thus supporting the idea of CB tolerance in rats (Rubino et al., 2008; Burston et al., 2010). Overall, there is some evidence that suggests that adolescent exposure to cannabinoids may lead to changes in behavior via alterations in CB receptors in various structures throughout the central nervous system.

Memory. In addition to changes in CB receptors, some studies have suggested that cannabinoids induce negative effects on cognitive functions such as spatial memory, and object recognition. Verrico, Gu, Peterson, Sampson, and Lewis (2014) studied the effects of repeated THC exposure on memory in adolescent rhesus monkeys (*Macaca mulatta*). Verrico et al. exposed the monkeys to THC for 6 months and testing their spatial/object memory 23 hours after administration throughout the 6-month period. Results revealed that chronic exposure did not

produce impairments in object recognition throughout the 6 months, but did impair spatial memory. These results further demonstrate the deficiencies that follow repeated cannabinoid exposure and reveals the propensity that cannabinoids have to impair working memory functioning. Wise, Thorpe, and Lichtman (2009) also demonstrated spatial memory deficits in a radial arm maze task in rats that received intrahippocampal injections of THC and CP 55,940 (a CB agonist). Results showed that drug animals exhibited significantly more errors in the radial arm task than vehicle-treated rats. Further results revealed that when administered rimonabant (a CB antagonist) the memory impairing effect of CP 55,940 and THC was blocked; however, when rimonabant was administered dorsal to the hippocampus it failed to block those memory disruptive effects.

Likewise, several rodent studies have demonstrated that chronic administration of cannabinoids during adolescence disrupts memory for object recognition in adulthood. For example, O'Shea, McGregor, and Mallet (2006) discovered that rats administered CP 55,940 exhibited a significant impairment in object recognition memory. Specifically, results showed that rats administered CP 55,940 demonstrated a significantly lower preference for the novel object than vehicle-treated counterparts indicating impaired recognition memory. Mateos et al. (2011) found that adolescent rats that were exposed to chronic doses of CP 55,940 exhibited deficits in memory as adults. Results revealed that female drug animals had significant impairment in spatial memory as demonstrated by a significant reduction in exploration of a displaced object. However, unlike previously mentioned studies, Mateos et al. (2011) did not show the same deficit in recognition memory in females but did see recognition deficits in males.

Additional research has suggested that the effects of cannabinoids on object recognition memory are dependent on the amount of time between last injection and the time of behavioral

data collection (Abush & Akirav, 2012). In a study investigating chronic adolescent exposure to WIN55,212-2 (Postnatal Day 45 to Postnatal Day 60), Abush and Akirav revealed that rats that had a 10-day delay between last injection and behavior collection had significant impairments in recognition memory compared to rats that were tested after 30 and 75 days post-injection period. However, impairments in spatial memory in an object-placement task persisted even after 75 days post-injection (Abush & Akirav, 2012). Results from Abush and Akirav are not consistent with the previously mentioned research that found persistent deficits in recognition memory. Results were consistent with Mateos et al. findings that chronic use impairs spatial memory. Overall, there is a lack of consistent findings on the long-term effects of cannabinoid exposure on spatial and recognition memory and further research is required to understand the potential confounding variables.

Development. Studies involving adolescent rats have revealed a dichotomy between adolescent behavioral responses and adult responses. As mentioned previously, the neural growth during adolescence has been shown to be sensitive to the administration of exogenous cannabinoids such as marijuana and synthetic CB agonists (Bossong & Niesink, 2010). This alteration results in lasting effects in the adult brain, and as a consequence, on behavior (Quinn et al., 2008). As such, it is important to understand how adult and adolescent exposures differ from each other. For example, in a study conducted on adolescent wistar rats, Quinn et al. (2008) found that adolescent rats administered repeated-doses of THC had impaired recognition memory when tested 12 days post-injection. However, adult rats with chronic THC exposure exhibited no impairments on recognition memory compared to controls. Overall, the adult rats were not affected to the same degree as the adolescent rats (Quinn et al., 2008). Additional studies also demonstrate that adolescent exposure to cannabinoids is more deleterious than adult

exposure. Researchers found that chronic exposure to WIN 55,212-2 produced lasting disturbances on object recognition memory in adolescent-treated rats, but not in adult-treated rats (Schneider, Schömig, & Leweke, 2008). While both adult and adolescent rats exhibited impairments in object exploration after acute exposure, only adolescent rats demonstrated a long-term effect after chronic exposure to Win55,212-2 (Schneider, Schömig, & Leweke, 2008). Overall, chronic exposure to exogenous cannabinoids affects adolescent rats more significantly than adult exposed rats. Additionally, as mentioned in Chapter 1, the rate of use in adolescent humans has increased over time and because of that it is increasingly important to understand the potential long-term effects that cannabinoids may have after adolescent use.

Food intake. Many animal studies have suggested that synthetic cannabinoids have a negative effect on food intake and body weight when administered to rodents (Miller & Drew, 1974; Mateos et al., 2011; Rubino et al., 2008; Biscaia et al., 2003). Mateos et al. and Rubino et al. demonstrated that chronic exposure to cannabinoids decreased food intake and inhibited weight gain. Biscaia et al. also found that drug exposure significantly reduced food intake in rats and significantly reduced the body weight. Rubino et al. (2008) and Mateos et al. (2011) were consistent with Biscaia et al. and revealed a significant decrease in food intake in drug animals and in body weight, however food intake was shown to increase after cessation of exposure during adolescence and body weight recovery was exhibited before entrance into adulthood. Research has found that nutrition in early life is an important aspect that is related to the growth and formation of the central nervous system of any organism (Laus, Vales, Costa, & Almeida, 2011). Fukuda, Francolin-Silva, and Almeida (2002) demonstrated that rats that were malnourished experienced greater difficulty during a spatial-navigation task. These results suggest that malnutrition may potentially affect learning and impair the consolidation of spatial

memory (Fukuda, Francolin-Silva, & Almeida, 2002). As a result, it is important for new cannabinoid research to address the potential confounds that reduced caloric intake during adolescence can have on rat behavior, cognition, and affect; it is fundamental for future cannabinoid studies to separate the drug effects from the negative effects of malnutrition during administration.

Sex Differences

While much research has been devoted to adolescence, the majority of cannabinoid research has been conducted on male rats, and less on female rats. Studies have demonstrated that males and females differ in a number of behavioral and physiological ways in response to cannabinoid administration (Craft, 2005). In a review article, Craft, Marusich, and Wiley (2013) discuss differences across males and females stating that rodent cannabinoid studies on cognition, spatial learning, and memory showed that females had greater deficits than male rats. In a study involving adolescent exposure to cannabinoids in male and female rats, Rubino et al. (2008) discovered that female rats exhibited more immobility in the forced swim task, suggestive of depression. Rubino et al. further revealed that female rats had a significant reduction in the function of the CB_1 receptor after chronic exposure to THC in areas like the amygdala, and ventral-tegmental area. These results suggest an alteration in the emotional systems of female rats, which result in depressive and anxious behaviors following cannabinoid administration. Differences in memory were also seen in Mateos et al. (2011), as discussed previously, wherein females had spatial memory deficits, and males had recognition memory deficits. Because research has suggested that cannabinoid exposure can differentially affect the sexes, future research is needed in order to understand how cannabinoid exposure effects memory in female rats.

Estrous Cycle. Although current research in cannabinoids has begun to include females, most studies have yet to account for the estrous phase (hormone cycle). Studies that have researched the interaction between estrous cycle and cannabinoid modulation after exposure have reported findings consistent with the past research on sex differences. Females are consistently more sensitive than males to the effects of THC during adolescence, regarding emotional behavior (Riebe, Hill, Lee, Hilard, & Gorzalka, 2010). It has been hypothesized by others that observed sex differences could be dependent on the levels of hormones in circulation. In support, Riebe et al. (2010) reported that CB_1 density and binding was influenced by estradiol administration and ovarian intactness. More specifically, Riebe et al. found that in females, cannabinoid receptor site density in the hypothalamus and amygdala were significantly increased by estradiol administration in intact-female rats and female rats that were ovariectomized and received estradiol. Further, Riebe et al. (2010) reported that overall, cannabinoid receptor expression was regulated by the hormone estradiol and varied by brain-region, demonstrating that the presence of hormones may mediate the physiological effects of CB agonists.

Other studies in female estrous cycle have focused on the natural hormone fluctuations that occur throughout the estrous cycle instead of on exogenous hormone administration. In a female rat, vaginal opening typically occurs between postnatal days 32 and 36; after vaginal opening a rat will begin cycling through the phases of the estrous cycle which typically lasts between four to five days (Goldman, Murr, & Cooper, 2007; Spear, 2000). Hormone levels vary across the estrous cycle, with estrogen at the highest levels at the beginning of the proestrus phase and progesterone at the end; both begin to decrease throughout the estrus phase. Levels of estrogen and progesterone are lowest in the diestrus 1 phase and begin to rise in the diestrus 2 phase (Goldman et al., 2007; Warren & Juraska, 1997).

Past research has demonstrated that females throughout varying phases of the estrous cycle differ in behavior and affect (Marcondes, Miguel, Melo & Spadari-Bratfisch, 2001). For example, Marcondes et al. (2001) discovered that female rats in the proestrus phase spent more time in the open arms of the EPM than females in the diestrus phase, demonstrating an anxiety-relieving effect for rats in proestrus and an anxiety-provoking effect for rats in diestrus. The anxiogenic effect was eliminated after administering estradiol to the diestrus female rats, suggesting that the elevated levels of estrogen in proestrus attenuates the negative effects of stress in the brain (Marcondes et al., 2001). Marcondes et al. also revealed that estradiol levels in proestrus rats were 2.5 times higher than rats in any of the three other phases (diestrus 1, diestrus 2, and estrus). Thus, it is important to evaluate these two extremes in order to understand whether estrogen has a role in how cannabinoids differentially affect female rats across various phases of the estrous cycle.

Warren and Juraska (1997) further demonstrate the role of estrogen in behavior in a study that investigated how spatial learning differed across the proestrus and estrus phases of the estrous cycle. Utilizing the Morris Water Maze, Warren and Juraska examined female rats in the proestrus phase compared to female rats in the estrus phase when faced with a task that required the use of spatial recognition and learning. The researchers found that performance in the water maze differed significantly across phase and that rats in the estrus phase outperformed those rats in the proestrus phase. These results are contrary to research conducted by Berry, McMahan, and Gallagher (1997) that found no difference in performance in the Morris water maze between rats in either the proestrus or the estrus phases. Frye, Duffy, and Walf (2007) also found that when female rats were tested on spatial memory in an object placement task animals in proestrus and estrus outperformed those in diestrus phase. Frye et al. demonstrated that when animals were

administered progesterone or estradiol immediately after the first trial, it significantly improved their spatial memory compared to control animals. Conrad et al. (2004) had congruent findings when comparing rats in the proestrus phase versus the estrus phase on a spatial Y-maze task. Results from the Y-maze task showed that rats in proestrus and estrus performed similarly and exhibited good spatial memory regardless of phase (Conrad et al. did not include diestrus phase as a group). As such, it is important for new research to investigate whether sex differences in spatial learning and recognition are due to female estrous cycle or to the effects of cannabis and synthetic cannabinoids.

Individual Differences: Rodent Models of Novelty-Seeking

Recent animal research has begun to emphasize individual characteristics across rats, distinguishing certain characteristics and biological correlates. One individual difference of particular importance is novelty seeking in rats. Past research has shown that rats differ in their reactivity to forced-exposure to novelty. More specifically, research has been able to separate two classifications of characteristics based on the locomotor activity a rat demonstrates in a novel open-field apparatus: high responders versus low responders. The novelty-seeking phenotype describes high-responder rats as being more active and mobile in both vertical and horizontal activity making high-responder rats appear to be less anxious than their counterparts (Pawlak, Ho, & Schwarting, 2008). Likewise, low-responder rats are characterized by remarkably lower locomotor activity scores and an apparent increase in anxious behaviors (Pawlak et al., 2008).

Although high-responder rats appear to be less anxious behaviorally, studies have also revealed that high-responders tend to have a significant increase in prolonged secretion of corticosterone when compared to their counterparts (Stead et al., 2006). As an example, Clinton,

Watson, and Akil (2014) found that selectively bred high-responder rats had a significant increase in corticosterone and adrenocorticotrophic hormone after exposure to a novel task compared to low-responder rats. Research has also revealed that high and low-responder rats also significantly differ in working memory. High-responders were found to have a significant decrease in discrimination of a novel and familiar object as compared to low-responder rats (Antoniou et al., 2008). Furthermore, research has revealed that CB agonists also affect neurotransmitter levels in a phenotype-dependent manner. Acute administration of a low dose of THC was found to increase glutamate levels in both high-responder and low-responder rats in both the prefrontal cortex and caudate putamen (Galanopoulos et al., 2011). However, acute administration of a high-dose of THC was found to increase glutamate levels in high-responder rats in the hippocampus. Galanopoulos et al. (2011) also discovered that when administered a low dose of THC, high-responder rats displayed heightened ambulatory distance when compared to low responder rats given the same dose of THC. Due to a lack of research in individual differences in cannabinoid research, it is important to understand how basic phenotypic differences between rats may influence studies measuring behavioral data.

Proposed Study

Due to the decline in perception of risk associated with marijuana and the increase in adolescent use, it is becoming increasingly important to understand the long-term effects of chronic adolescent marijuana use on cognition and brain functioning in adulthood. Furthermore, because attention has mostly focused on male rats it is important to expand cannabinoid research to the female gender and understand the influences the estrous cycle has on drug exposure, behavior, locomotion and cognition. In order to isolate the effects of adolescent cannabinoid exposure it is necessary to control and limit confounding factors such as sex and individual

differences. Future research must focus on the estrous cycle, and understand novelty-seeking phenotypes as a potential variable in the effect of chronic cannabinoid exposure in female rats.

The current study investigated how chronic adolescent cannabinoid exposure and novelty seeking interact and affect recognition and spatial memory in adult female Long-Evans rats. Spatial memory was assessed utilizing the object placement task in an open-field arena, and recognition memory was assessed utilizing the similar object placement task. Due to the evidence that cannabinoid administration induces malnutrition in rats, the current study introduced a yoked-food control group that received the same amount of food that drug animals consumed. Estrous samples were collected via swab to understand the interaction between cannabinoids, phenotype and hormone levels on behavior. Estrous phase was also split into two groups based off previous research indicating that animals in the proestrus and estrus phase were similar in memory performance and have elevated levels of hormones (Frye et al., 2007); proestrus and estrus phases versus diestrus I and II phases. Additionally, each rat was screened for phenotype before the injection period.

For the current study, it was expected that the drug animals would have a significant reduction in food intake and body weight during the injection period; yoked-control animals were also predicted to show reduced body weight due to the manipulation of food restriction. It was predicted that drug animals would exhibit greater anxiety and poorer spatial and recognition memory than control animals; it was also predicted that yoked-animals would show a deficit due to the malnutrition at a vulnerable age. It was hypothesized that high-responder rats would have greater activity and spend more time in the anxiety-provoking center-zone (CZ) of an open-field apparatus when compared to low-responder animals. In terms of memory, it was hypothesized that rats in proestrus/estrus phase would exhibit better recognition and spatial memory than rats

in the other phases. The present study contributes to cannabinoid research by investigating interactions between cannabinoids, novelty-seeking, and estrous cycling on adult cognition in rats. The present study also illustrates the importance of food monitoring in cannabinoid research to control for the lack of proper nutrition in the drug-group.

Chapter 3: Method

Subjects

Fifty-six female naïve Long-Evans rats were bred in the behavioral and cognitive neuroscience animal lab at Radford University. All animals were bred from animals originating at Charles River Laboratories. One male was paired with one female and housed in a standard plastic cage during breeding (44 cm L x 22 cm H x 20.5 cm W). After pup birth on postnatal day zero (PND 0), the litter was culled to 12 pups (six males and six females if possible) between PND 1 and PND 2. Pups remained with the dam until weaning on PND 22, when they were sexed and housed by gender.

Phenotype screening was completed for all females within a litter on PND 28 and 29. Three female rats from each litter were semi-randomly assigned to either the drug (n=18), yoked-food control (n=18) or control (n=18) groups on PND 34 based on phenotype and bodyweight.

Rats were given free access to food and water except during the injection period (PND 35 to 48) when the yoked-food manipulation animals were only given the amount of food that the drug animals consumed the previous day. Animals were housed individually in stainless steel hanging cages (25cm x 20cm x 18cm) during the injection period. After the injection period, animals were returned to group housing and placed on free feed. They were weighed every five days during the drug washout period (PND 49 to PND 77). Behavioral testing began on PND 77.

Subjects were housed in a humidity and temperature controlled room with a 12-hour light and dark cycle. This study was IACUC approved, and all procedures were consistent with the NIH Guide for the Care and Use of Laboratory Animals.

Drug Manipulation

The synthetic cannabinoid agonist CP 55,940 ((-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol) was injected intraperitoneally at a dose of 0.35 mg/kg from PND 35 through PND 48 (CP 55,940; R&D Systems, Minneapolis, MN). For mixing the drug solution, 3.5 mg of CP 55,940 was thoroughly mixed with 75.0 µm of Tween 80 (polyoxyethylene sorbitan monoleate), and 0.35 mL of ethanol. After thorough mixing, the ethanol was evaporated using compressed oxygen; the remainder of the solution was mixed with 9.925 mL of saline (O'Shea, Singh, McGregor, & Mallet, 2004). Drug animals were then administered 0.35 mg/kg of the mixed drug. The control and yoked-control groups received 0.35 mg/kg of a vehicle injection of saline mixed the same way as described, without CP 55,940. Throughout the injection period, food intake was monitored and weighed daily for all animals.

Estrous Cycle Determination

The lavage technique was used to determine estrous phase, as described in Warren and Juraska (1997). Estrous phase was classified by the cells that were present on the slide. Proestrus contains large, round, nucleated cells. Estrus contains cells that are mostly cornified. Diestrus 1 has mostly leukocytes and cornified cells, and diestrus 2 has a majority of leukocytes and nucleated epithelial cells (Warren & Juraska, 1997; Goldman et al., 2007; Stackman, Blasberg, Langan, & Clark, 1997). For this study, estrous phase was separated into two groups for analyses: Group 1 (proestrus and estrus phase—highest estrogen, and progesterone hormone levels) and Group 2 (diestrus I and II—lowest hormone levels).

Phenotype Screening

Novelty-seeking phenotype testing was conducted in a small room created by two gray curtains and two white walls with posters. Locomotor activity was measured in dimly lit

conditions in a large wooden, white-painted open-field arena (103 cm X 103 cm square X 44.5 cm tall walls). The object used in phenotyping on Day 2 was a small multi-colored globe (9.4 cm tall). Phenotype screening was conducted on PND 28 and 29. Animals were classified into two phenotypes: distance phenotype and object phenotype based on their horizontal activity and exploration of the object (Philpot & Wecker, 2008; Antoniou et al., 2008). For Distance phenotype: high-responders (HR) had increased horizontal activity (distance traveled) and low responders (LR) had decreased activity. For object phenotype, HR animals had increased exploration of the object on Day 2, and LR had decreased exploration. For both phenotype, animals were classified using a median wherein all animals above the median were HR, and all animals below were LR—this includes all of the females from every litter in the study. Classification of phenotype was not constant for distance and object phenotype; some animals were HR for distance phenotype, but LR for object phenotype and vice versa.

On each phenotype test day, all female animals from a litter were removed from their home cage, weighed and placed in a plastic holding cage with shredded paper bedding and allowed to habituate to the test room for 10 minutes before data collection. White noise was created by a Sleep Easy machine. On PND 28, the rats were placed into the apparatus for 15 minutes and allowed to explore the empty arena. After the trial, the animals were placed back into a separate holding cage and the apparatus was cleaned using a vinegar solution (10%). When all female animals had finished the trial, the animals were placed back into their home cage. The second trial was conducted on PND 29, and was set-up similarly to the first trial except a novel object was placed in the center of the apparatus. After the 10-minute habituation to the test room, the animals were placed in the apparatus and allowed to explore for 5 minutes; at the end of the trial the animal was placed in a separate holding tub, and the apparatus was cleaned using 10%

vinegar. Data collection utilized the AnyMaze software to record vertical and horizontal activity and exploration of the object. Adolescent novelty-phenotype screening procedures were modeled after Philpot and Wecker (2008), Galanopuolos et al. (2011), Antoniou et al., (2008) and Aydin, Oztan, and Isgor (2012).

Object Placement as an Adult

Behavioral testing for object placement began on PND 77 and continued through PND 81. Habituation to the apparatus consisted of four trials. Each trial was on a consecutive day and lasted 10 minutes. Data from the first habituation trial were used for anxiety and activity measures in the open-field. On PND 81, rats were exposed to two trials (sample and test phase) that each lasted five minutes and were separated by a ten-minute inter-trial interval. In the sample phase, two objects were placed in separate corners in the arena, and in the test phase, one object was moved to a different location. Each contact with the objects was recorded. A contact was defined as the animal being 0.64 to 1.27 centimeters away from an object (if the subject sniffed at, or looked at the object within a distance close enough to touch with their whiskers). Turning away from the object was not considered an exploration or contact (Mateos et al., 2011). Good spatial memory was defined as a significant increase in time spent with the moved object during the test trial. Object layout was counterbalanced across groups; animals were always put in the center of the field facing the wall opposite the objects.

Apparatus. The open-field arena was located in a small room that contained one table, one door, two cameras hanging from the ceiling, and two posters on different walls. The open-field arena was made of wood and painted white (61cm x 61cm x 36 cm). Objects utilized in the object placement task were two clear, multi-colored, plastic cylinders (12.7 cm tall). Each object was attached to the floor of the open-field via Velcro. Each session was recorded via video

camera, and saved on a DVR. AnyMaze tracking software was utilized to track the movement of the rats within the open-field and in relation to the objects.

Procedure. Before the first habituation trial (PND 77), and the test day (PND 81), a vaginal lavage was taken to record estrous phase of the subject. The subject was then weighed and placed in a plastic holding cage with littermates and allowed to habituate to the test room for 10 minutes. Before and after each trial, the arena and objects were cleaned using a vinegar solution (10%). During habituation from PND 77 to 80, subjects were placed in the apparatus and allowed ten minutes to explore. At the end of each habituation, trial animals were returned to their home cage in the colony room. On test day, PND 81, subjects were placed in the open field and were given five minutes to explore the objects. At the end of the sample phase, subjects were placed in a holding cage for ten minutes with their littermates. For the test phase, animals were then placed back into the center of the open-field and were given another five minutes to explore the displaced and familiar object. Exploration of the objects was monitored by researchers in an adjacent room via DVR and AnyMaze. Procedures for the object placement task were modeled after Mateos et al. (2011), Abush, and Akirav (2012).

Object Recognition as an Adult

Behavioral testing for the object recognition task began four days after the object placement test day on PND 85 and continued through PND 87. Habituation occurred on two consecutive days, with each trial lasting five minutes. On PND 87, two separate five-minute trials were conducted (sample and test phase). The first trial utilized two identical objects that were placed in different locations within the apparatus. During the second trial, one object was exchanged with a novel object. Object contact and exploration was defined the same as for the

object placement task. Good recognition memory was indicated when there was a significant increase in exploration of the novel object during the test phase.

Apparatus. The object recognition task was conducted in the same room and apparatus as the object placement task. The objects for the object recognition task were different from the object placement task and were an orange and black, plastic shampoo bottle (familiar objects; 19.05 cm tall) and a white-painted wooden object (novel object; 11.4 cm tall). Each session was taped using a video recorder, DVR, and captured by the AnyMaze software.

Procedure. Test day procedures were consistent with the object placement task. On each of the two habituation trials, the subject was placed in the apparatus and allowed five minutes to explore. On PND 87, a vaginal lavage was taken before starting the object recognition trials. The animals were then given 10 minutes to habituate to the test room. Afterwards they were given 5 minutes to explore the two identical shampoo bottles for the first trial. After the sample phase, rats were returned to a holding cage with their littermates for 30 minutes before completing the test phase. After 30 minutes, one shampoo bottle was removed and replaced with the novel wooden object. The rat was then placed in the apparatus and given five minutes to explore the novel and familiar object. Exploration of the objects in both trials was recorded by researchers and AnyMaze from an adjacent room. Procedures for the object recognition task were modeled after Mateos et al. (2011), Abush and Akirav (2012), and O'Shea et al., (2006).

Chapter 4: Results

Because the yoked-control animals were not a true control group, and were exposed to food manipulation, all analyses were conducted separately to compare the drug versus control animals and the yoked versus the control animals. Estrous cycle was divided into two groups for analysis: proestrus and estrus phase versus diestrus I and II phase. Furthermore, food intake and body weight analyses were conducted for both the distance phenotype and the object phenotype, which are described below. All behavioral tasks were analyzed using the estrous cycle, drug group, and phenotype as independent variables (2 x 2 x 2 ANOVA). Only significant results were reported; All ANOVA results are reported in the Appendix.

Novelty Seeking Phenotype Classification

For the current study, two separate novelty-seeking phenotype measures were used. The distance and the object phenotype measures were analyzed using a 2 (group) by 2 (phenotype) analysis of variance (ANOVA). The first median split was for distance traveled (meters) on Day 1 of phenotype screening (distance phenotype; PND 28). All females (within every litter) with a distance greater than 39.031 meters were classified as high responders (HR) and animals below were low responders (LR). High responders were significantly more active than LR rats on day 1 for all three drug groups, $F(1, 54)= 78.03$, $p<.001$. Figure 1 shows future distance phenotype split across groups for the animals that were semi-randomly chosen to be used in the study. A median split for mean time spent (s) with the object on Day 2 was also utilized for analyses (Object Phenotype; PND 29). All animals that spent greater than 11.6 seconds with the object were classified as HR and animals below were classified as LR. Overall the HR rats chosen to be used in the study spent significantly more time with the object than LR rats on day 2, $F(1, 54)= 51.07$, $p<.001$. Figure 2 shows the object phenotype split for study animals in each drug group.

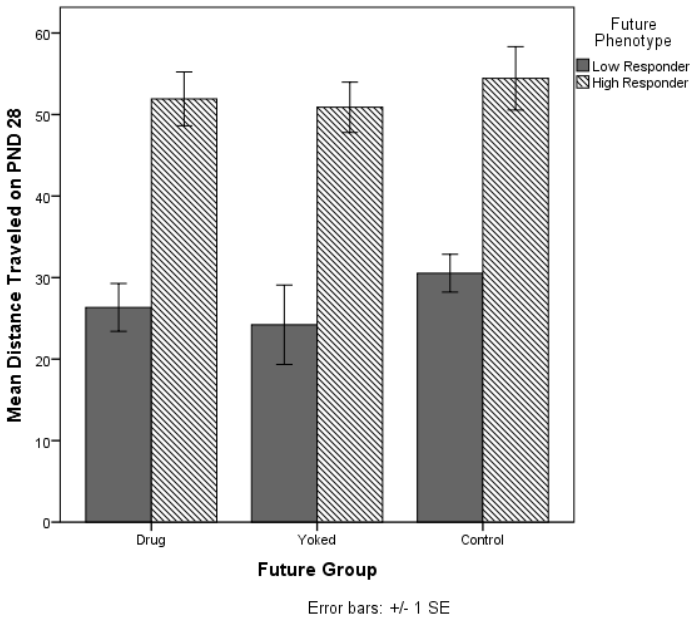

Figure 1. Mean distance traveled (meters) for low responder and high responder animals in the distance phenotype behavioral task.

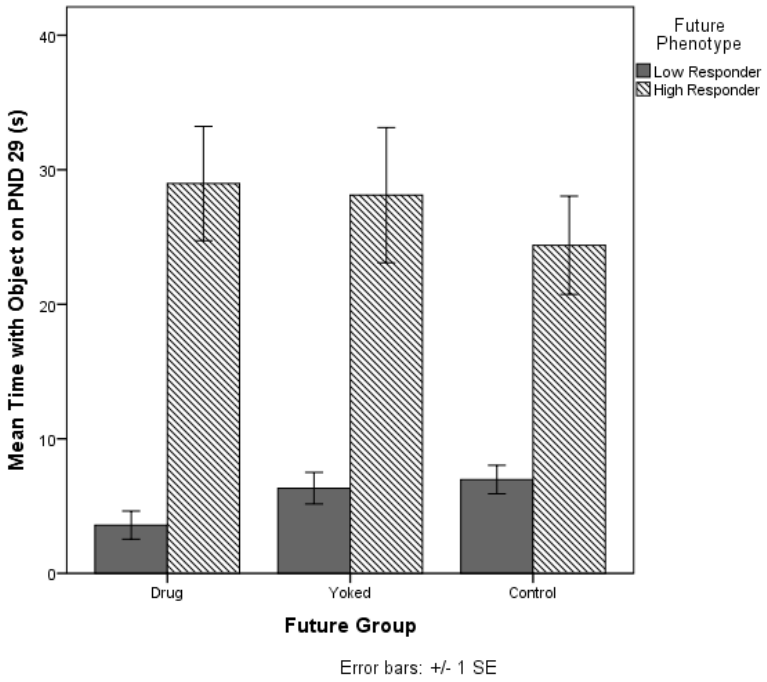

Figure 2. Mean time spent with the object (in seconds) on Day 2 for low responder and high responder animals on the object phenotype behavioral task.

Food Intake

All ANOVA statistics can be found in the Appendix. Results are separated by phenotype, with distance phenotype analyses being reported first for drug versus control and then yoked versus control, followed by the same analyses for object phenotype.

PND 35. Two 2 x 2 between-subject ANOVAs were conducted for drug group and distance phenotype to analyze food intake on PND 35. The first analysis compared drug versus control animals by distance phenotype. There was no main effect of group or distance phenotype. There were no interaction effects. The second analysis was conducted for yoked vs. control animals and distance phenotype. There was no main effect of group or distance phenotype. There were no interaction effects. These analyses show that all groups of animals consumed similar quantities of food on PND 35 (start of injections).

The same two 2 x 2 between-subjects analyses of variance (ANOVA) were conducted for drug group and object phenotype to analyze food intake on the first day of injections (PND 35). There were no significant main effects or interactions for either the drug versus control nor yoked versus control animals. These results also confirm that all animals consumed equivalent amounts of food at the beginning of the injection period regardless of phenotype.

Distance Phenotype. Two 2 (drug group) x 2 (distance phenotype) x 14 (days) repeated measures ANOVAs were conducted on food intake between PND 36 and PND 49.

Drug versus Control. There was a significant main effect for day, $F(13, 442) = 29.22$, $p<.001$, partial $\eta^2= .462$. As can be seen in Figures 3 and 4, these results show that food consumption increased significantly during the injection period for all animals regardless of group or distance phenotype. The main effect for group was significant, $F(1, 34)= 46.20$, $p<.001$, partial $\eta^2= .576$, indicating that food intake in drug animals ($M= 19.17$, $SD= 1.57$) was

significantly lower when compared to controls (*M*= 23.33, *SD*= 2.09). There were no significant interaction effects between drug and phenotype for food intake. Distance phenotype did not affect food intake during injections. Overall, drug animals consumed less food than controls suggesting that drug exposure caused reduced nutrition in rats.

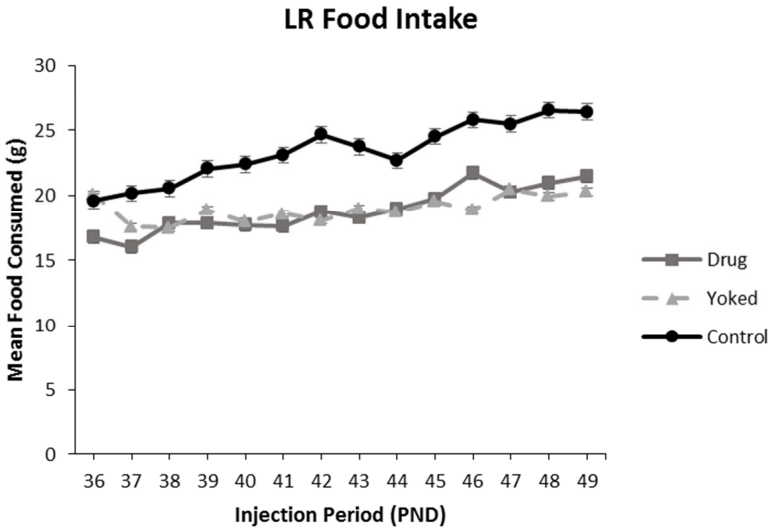

Figure 3. Mean amount (g) of food intake for LR animals across drug group during the injection period (PND 36 to 49)

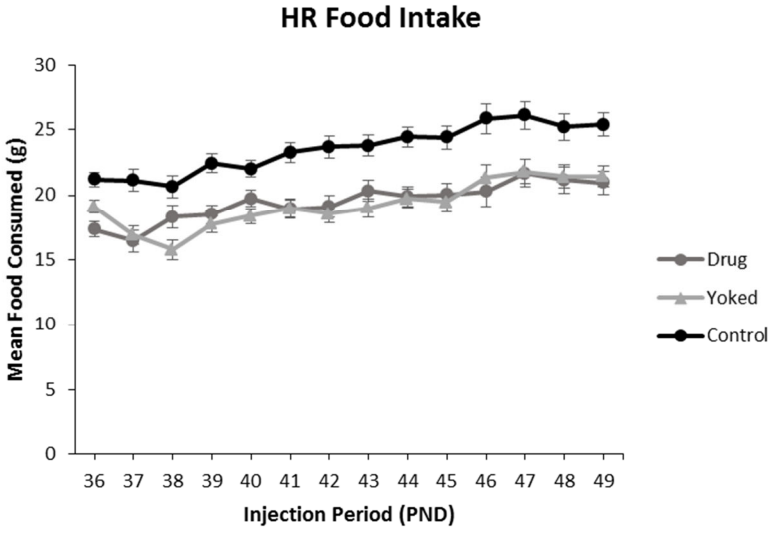

Figure 4. Mean amount (g) of food intake for HR animals across drug group during the injection period (PND 36 to 49)

Yoked versus Control. Analyses for yoked versus control animals showed a significant main effect for day, $F(13, 442) = 20.27$, $p<.001$, partial $\eta^2= .374$. The main effect for group was also significant, $F(1, 34)= 43.32$, $p<.001$, partial $\eta^2= .560$. As shown in Figure 3 and 4, food consumption increased across the injection period for all animals, but food intake in yoked animals ($M= 19.16$, $SD= 1.58$) was significantly lower when compared to controls ($M= 23.33$, $SD= 2.09$) demonstrating a diminished consumption of food and nutrients. There was also a significant interaction between group by day, $F(13, 442)= 10.09$, $p= .003$, partial $\eta^2= .229$, indicating that the yoked animals ate less food over the injection period than control animals.

Because the group by day interaction was significant, a one-way ANOVA was conducted to measure food intake for each day of the injection period (PND 36 to 49) which shows that the yoked and control groups were not eating significantly different amounts of food on PND 36, $F(1, 36)= 3.72$, $p= .062$, partial $\eta^2= .094$. On PND 37, however, there was a significant main effect for group showing that control animals ($M= 20.65$, $SD= 2.02$) were consuming significantly more food than yoked animals ($M= 17.11$, $SD= 2.36$), $F(1, 36)= 24.68$, $p< .001$, partial $\eta^2= .407$. The main effect for group continued for the rest of the injection period, PND 38 to PND 49. Overall, by PND 37 yoked animals were consuming significantly less food than control animals. This was expected due to the intentional food manipulation placed on the yoked-group.

Object Phenotype. The second group of analyses was conducted utilizing the object phenotype for a 2 (group) by 2 (object phenotype) by 14 (day) repeated measures ANOVA to analyze food intake over the injection period (PND 36 to PND 49).

Drug versus Control. There was a significant main effect for day, $F(13, 442) = 30.51$, $p<.001$, partial $\eta^2= .473$. The main effect for group was also significant, $F(1, 34)= 51.51$, $p<.001$, partial $\eta^2= .602$. As shown in Figure 5 and 6, drug animals ($M= 19.18$, $SD= 1.57$) ate significantly less food than control animals ($M= 23.31$, $SD= 2.09$). There were no interaction effects, nor any effect of object phenotype. These results indicate that food consumption increased across the injection period for all animals. Overall, the data also show that drug animals had significantly reduced food intake when exposed to CP 55,940.

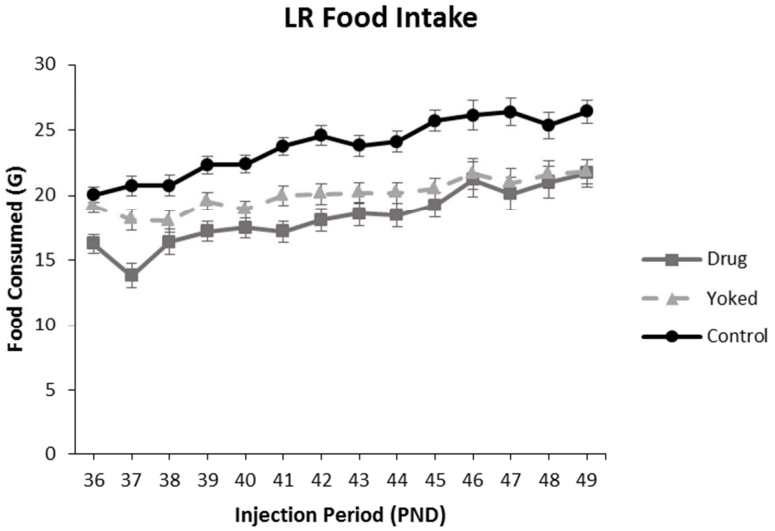

Figure 5. Mean amount (grams) of food intake for LR animals across drug group during the injection period (PND 36 to 49)

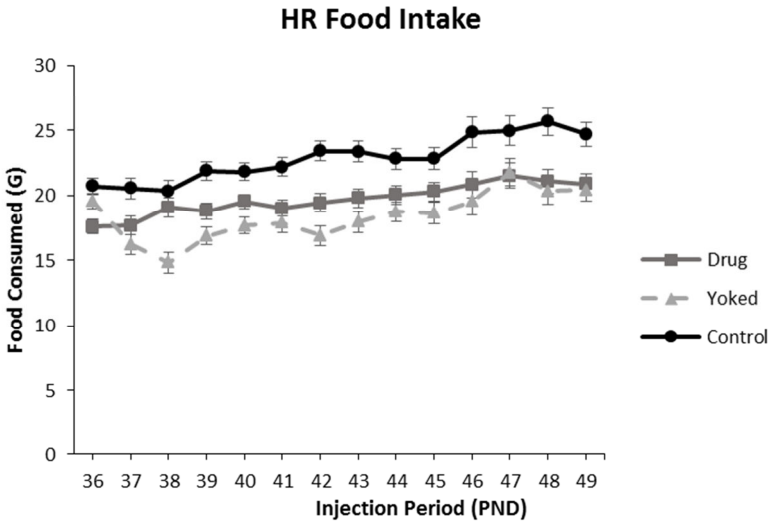

Figure 6. Mean amount (grams) of food intake for HR animals across drug group during the injection period (PND 36 to 49)

Yoked versus Control. There was a significant main effect for day, $F(13, 442)= 24.32$, $p<.001$, partial $\eta^2= .417$, showing that food intake increased for all animals during the injection period. There was a significant interaction between day and group, $F(13, 442)= 2.41$, $p= .004$, partial $\eta^2= .066$, showing that yoked animals gained less weight over the injection period than control animals. There was also a significant main effect for group, $F(1, 34)= 50.71$, $p<.001$, partial $\eta^2= .599$. Overall, yoked animals ($M= 19.16$, $SD= 1.58$) consumed significantly less food than control animals ($M= 23.31$, $SD= 2.09$), as expected because the yoked animals had an imposed food manipulation. There was also a main effect for object phenotype, $F(1, 34)= 5.06$, $p= .031$, partial $\eta^2= .130$. HR animals ate less ($M= 20.49$, $SD= 2.78$) on average than LR animals ($M= 22.01$, $SD= 2.68$) during the injection period. There were no significant interaction effects with phenotype. Overall, the yoked animals ate less than the control animals and the HR animals ate significantly less on average than the LR animals.

Because the day by group interaction was significant, a one-way ANOVA for food intake for each day of the injection period was conducted for the yoked versus control animals. The yoked and control groups were not eating significantly different amounts of food on PND 36, $F(1, 36)= 3.72$, $p= .062$, partial $\eta^2= .094$. On PND 37, there was a significant main effect for group showing that control animals ($M= 20.65$, $SD= 2.02$) were consuming significantly more food than yoked animals ($M= 17.11$, $SD= 2.36$), $F(1, 36)= 24.68$, $p< .001$, partial $\eta^2= .407$. The main effect for group continued to be significant for each day following PND 37 (PND 38 to 49). Overall, this shows that on PND 37 the yoked animals were starting to eat significantly less food than control animals.

Conclusions. Overall, drug animals ate significantly less than control animals during the injection period. Yoked animals also ate significantly less than controls and ate less throughout

the injection period, but this was expected due to the food manipulation imposed on yoked animals. For the object phenotype, HR animals ate significantly less than LR animals (yoked and control animals only) —this was not seen in the distance phenotype animals. However, drug and yoked animals were not matched based on phenotype, meaning that an HR drug rat may have been paired with an LR yoked animal within each phenotype. This may have caused the observed reduction in food intake in the HR group compared to the LR group, and potentially affected the results in food intake.

Body Weight: Injection Period

All ANOVA statistics can be found in the Appendix. Results are separated by phenotype, with distance phenotype analyses being reported first for drug versus control and then yoked versus control, followed by the same analyses for object phenotype.

PND 35. Two 2 x 2 between-subject ANOVAs were conducted for drug group (drug vs. control and then yoked vs. control) and distance phenotype to analyze body weight (g) on PND 35. There were no main effects of group or distance phenotype. There were no interaction effects between drug group and phenotype. These analyses show that all groups of animals weighed similarly on PND 35 and did not vary as a function of group or distance phenotype before injections or food deprivation began.

Two 2 x 2 between-subject ANOVAs were conducted for drug group (drug vs. control and then yoked vs. control) and object phenotype to analyze body weight (g) on PND 35. There were no main effects of group or distance phenotype. There were no interaction effects between drug group and phenotype. These analyses show that all animals weighed similarly on PND 35 and did not vary as a function of group or object phenotype before injections or food deprivation began.

Distance Phenotype. When analyzing body weight for the injection period two 2 (drug group) x 2 (distance phenotype) x 14 (day) repeated measures ANOVAs were conducted for drug versus control and yoked versus control.

Drug versus control. There was a significant main effect for day, $F(13, 416)= 915.73$, $p<.001$, partial $\eta^2= .966$. These results show that body weight increased significantly during the injection period for all animals regardless of group or phenotype. There was a significant interaction between day and group indicating that control animals gained weight at a higher rate

than the drug animals during the injection period, $F(13, 416)= 29.62$, $p < .001$, partial $\eta^2= .481$. There was also a significant 3-way interaction effect among day, group, and phenotype, $F(13, 416)= 2.34$, $p= .005$, partial $\eta^2= .068$; see Figure 7 and 8. The main effect for group was also significant, $F(1, 32)= 12.81$, $p= .001$, partial $\eta^2= .286$. Specifically, body weight (g) in drug animals ($M= 157.34$, $SD= 12.84$) was significantly lower when compared to controls ($M= 174.82$, $SD= 15.76$). There was no significant main effect for phenotype. Overall, drug animals gained less weight than controls suggesting that drug exposure reduced body weight during the injection period.

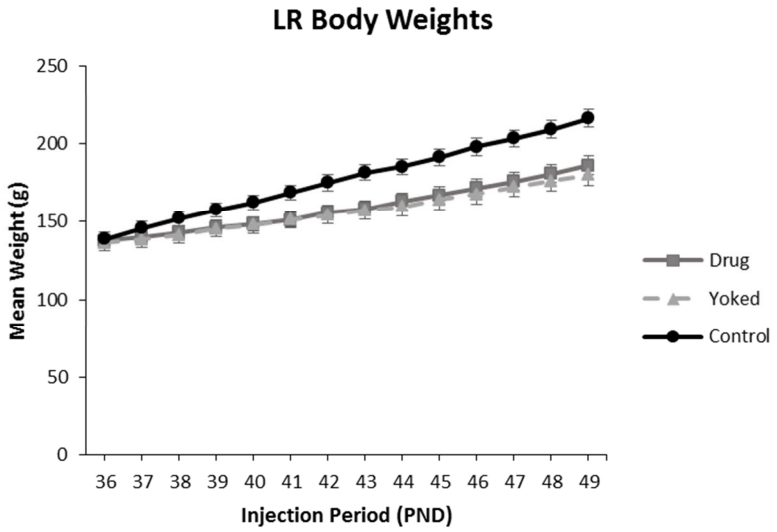

Figure 7. Mean body weight (g) for LR animals during the injection period (PND 36 to 49)

Figure 8. Mean body weight (g) for HR animals during the injection period (PND 36 to 49)

In order to interpret the significant 3-way interactions, tests of the simple interactions were conducted. Two 2 (group) x 14 (day) repeated measures ANOVA was conducted to measure bodyweight for the drug versus control LR animals, and then the HR animals. Results showed a significant interaction for group by day for LR animals, $F(13, 182)= 29.99$, $p< .001$, partial $\eta^2= .682$, and HR animals, $F(13, 234)= 7.36$, $p< .001$, partial $\eta^2= .290$. For LR and HR animals, the control group gained more weight over the injection period than drug animals from PND 36 to 49 but the effect size was larger for the LR groups. A one-way ANOVA analyzing bodyweight for drug versus control was conducted for each day of the injection period for only the LR animals. Analyses for PND 36 to 40 were not significant indicating that the drug and control animals did not weigh significantly different. On PND 41, there was a significant main effect for group showing that the LR drug animals ($M= 151.28$, $SD= 13.42$) weighed significantly less than LR control animals ($M= 169.05$, $SD= 16.16$), $F(1, 14)= 5.73$, $p= .031$, partial $\eta^2= .290$; the significant main effect continued for the rest of the injection period (PND 42 to 49). A one-way ANOVA analyzing bodyweight for drug versus control animals was also conducted for each day of the injection period for only the HR animals. The HR drug animals were not significantly different than control HR rats on PND 36, $F(1, 18)= 1.845$, $p= .191$, partial $\eta^2= .093$. On PND 37, there was a significant main effect for group indicating that that the HR drug animals ($M= 133.82$, $SD= 10.51$) weighed significantly less than the HR control animals ($M= 144.72$, $SD= 12.45$), $F(1, 18)= 4.47$, $p= .049$, partial $\eta^2= .199$; this significant main effect for group continued for the rest of the injection period. Overall, the LR drug and control animals weighed significantly different by PND 41 and the HR drug and control animals weighed significantly different by PND 37.

Yoked versus Control. Analyses for yoked versus control animals showed a significant main effect for day, $F(13, 416)= 808.83$, $p<.001$, partial $\eta^2= .962$. There was a significant interaction effect between group and day, $F(13, 416)= 34.25$, $p<.001$, partial $\eta^2= .517$, showing that the yoked animals gained less weight than control animals. There was also a significant 3-way interaction between group, phenotype, and day, $F(13, 416)= 3.46$, $p<.001$, partial $\eta^2= .097$. The main effect for group was also significant, $F(1, 32)= 11.16$, $p= .002$, partial $\eta^2= .259$, showing that yoked ($M= 159.82$, $SD= 11.34$) animals weighed significantly less than control ($M= 174.82$, $SD= 15.76$) animals. These results indicate that body weight increased across the injection period for all animals. Overall, LR yoked animals appeared to be more negatively affected when compared to LR control animals and HR yoked animals. See Figures 7 and 8.

To better interpret the significant 3-way interactions, tests of simple interactions were conducted. Two 2 (group) by 14 (day) repeated measures ANOVA analyzing body weight were conducted for yoked versus control HR animals, and then again for LR animals. The results showed a significant interaction for group by day for LR animals, $F(13, 156)= 40.04$, $p< .001$, partial $\eta^2= .769$, and HR animals, $F(13, 260)= 9.57$, $p< .001$, partial $\eta^2= .324$. For LR and HR animals, the control group gained more weight over the injection period than drug animals but the effect size was larger for the LR groups. A test of simple main effects was also conducted utilizing a one-way ANOVA to measure body weight on each day of the injection period (PND 36 to 49) for yoked versus control LR animals. The main effect for group on PND 36 to 41 was not significant demonstrating that the yoked and control animals did not weigh significantly different on those days. On PND 42, there was a significant main effect for group showing that the LR yoked animals ($M= 154.37$, $SD= 15.00$) weighed significantly less than LR control animals ($M= 175.23$, $SD= 17.34$), $F(1, 12)= 5.54$, $p= .036$, partial $\eta^2= .316$; the significant main

effect for group continued for the rest of the injection period. The same test of simple main effects for the HR yoked and control animals showed no significant difference for PND 36 to 40. Results did show a significant main effect on PND 41, revealing that HR yoked animals (*M*= 153.83, *SD*= 9.80) weighed significantly less than HR control animals (*M*= 164.80, *SD*= 14.21), $F(1, 20)= 4.57$, $p= .045$, partial $\eta^2= .186$; this significant main effect for group continued for the rest of the injection period. Overall, LR yoked animals gained less weight throughout the injection period than LR control animals, and weighed significantly less than controls by PND 42 whereas the HR yoked animals weighed significantly less than HR controls by PND 41. See Figures 7 and 8.

Object Phenotype. The second group of analyses was conducted utilizing the object phenotype for a 2 (group) by 2 (object phenotype) by 14 (day) repeated measures ANOVA to analyze body weight (g) during the injection period.

Drug versus Control. There was a significant main effect for day, $F(13, 416) = 830.79$, $p<.001$, partial $\eta^2= .963$. There was a significant interaction effect for group and day indicating that drug animals gained less weight during the injection period than control animals, $F(13, 416)= 26.43$, $p<.001$, partial $\eta^2= .452$. To interpret the group by day interaction, a one-way ANOVA for drug versus control was used to analyze body weight on each PND of the injection period. The results showed that the drug and control groups were not significantly different on PND 36, $F(1, 36)= 1.05$, $p= .313$, partial $\eta^2= .028$, or PND 37, $F(1, 36)= 3.97$, $p= .054$, partial $\eta^2= .099$. There was a significant simple main effect on PND 38, $F(1, 36)= 5.22$, $p= .028$, partial $\eta^2= .127$, indicating that the drug animals (*M*= 139.75, *SD*= 11.95) weighed significantly less than the control group (*M*= 149.29, *SD*= 13.76) by PND 38 of the injection period. This simple main effect for group remained significant for the rest of the injection period. The overall main

effect for group was also significant, F (1, 32)= 14.67, p=.001, partial η^2= .314. On average drug animals (M= 157.34, SD= 12.84) weighed significantly less than control animals (M= 174.82, SD= 15.76). There were no further interaction effects with phenotype. Overall, the data shows that drug animals gained less weight throughout the injection period and weighed less, on average, than controls suggesting that CB exposure during adolescence disrupts normal weight gain patterns in rats probably due to the reduction in food intake. See Figures 9 and 10.

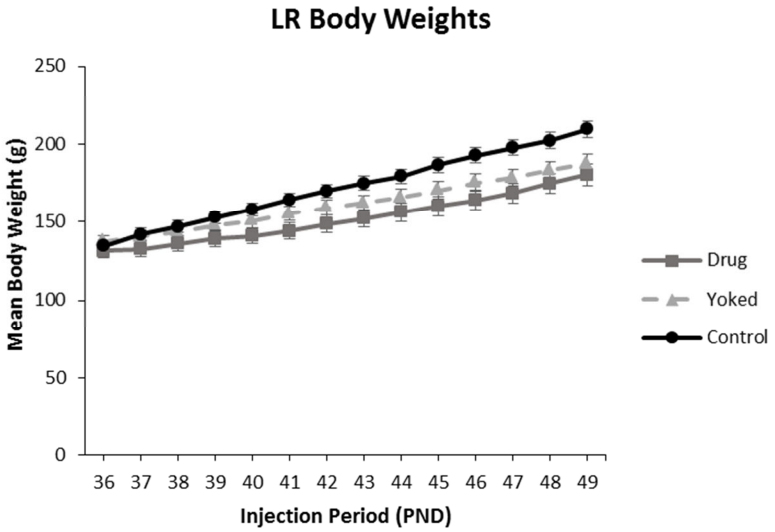

Figure 9. Mean body weight (g) for LR animals during the injection period (PND 36 to 49)

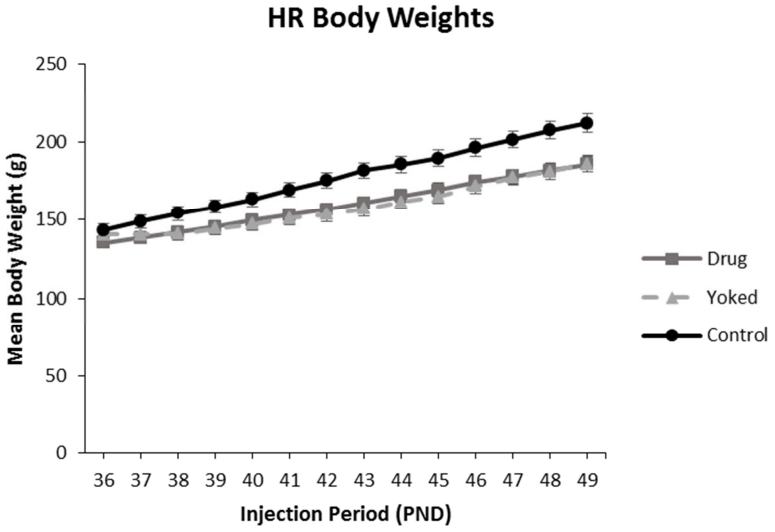

Figure 10. Mean body weight (g) for HR animals during the injection period (PND 36 to 49)

Yoked versus Control. There was a significant main effect for day, $F(13, 416)= 825.40$, $p<.001$, partial $\eta^2= .963$, showing that body weight increased for all animals during the injection period, as shown in Figures 9 and 10. There was a significant interaction between day and group, $F(13, 416)= 29.26$, $p<.001$, partial $\eta^2= .478$, showing that control animals had a greater increase in weight than yoked animals over the injection period. There was an overall main effect for group, $F(1, 32)= 10.44$, $p= .003$, partial $\eta^2= .246$, showing that yoked animals ($M= 159.82$, $SD= 11.34$) weighed significantly less than control animals ($M= 174.82$, $SD= 15.76$) during the injection period. There was no main effect for phenotype and no interaction effect between phenotype and drug group. Overall, the data shows that yoked animals had less weight gain throughout the injection period compared to the control animals. Results indicate that the food deprivation experienced by the yoked group lead to a significant reduction in weight gain during adolescence.

A one-way ANOVA was conducted to compare body weight for yoked versus control on each day of the injection period (PND 36 to 49) in order to interpret the significant day by group interaction. There was no main effect for group from PND 36 to 38 indicating that the yoked group was not significantly different from the control group. There was a significant main effect for group on PND 39, $F(1, 36)= 4.27$, $p= .046$, partial $\eta^2= .106$, showing that the yoked and control groups weighed significantly different; this significant main effect of group remained significant for the rest of the injection period (PND 40 to 49). Overall, the yoked group gained less weight during the injection period, and was significantly different from the control group from PND 39 through the end of the injection period.

Conclusions. Overall, all groups of animals started the injection period weighing the same but diverged quickly as the injection period progressed. For distance phenotype, LR drug

animals weighed significantly less than controls by PND 41, however HR drug animals weighed significantly less than HR controls by PND 37, this may suggest that HR animals may have been more affected by injections. The distance LR yoked rats weighed significantly less than controls by PND 42, and HR yoked animals weighed significantly less than controls by PND 41. However, there were no interactions between drug group and object phenotype when analyzing body weight. This may indicate that distance phenotype had a greater effect on weight gain than object phenotype when exposed to chronic injections. The different results between object and distance phenotype may also reflect activity influences—the distance HR drug rats may have weighed significantly less than controls at an earlier time due to a potential increase in activity in the cage.

Body Weight: Post-Injection Period

All ANOVA statistics can be found in the Appendix. Results are separated by phenotype, with distance phenotype analyses being reported first for drug versus control and then yoked versus control, followed by the same analyses for object phenotype.

Distance Phenotype. A 2 (drug group) x 2 (distance phenotype) x 5 (day) repeated measures ANOVA was conducted to measure the body weight (g) of animals during the post-injection period; animals were weighed every five days (PND 50 to 70).

Drug versus Control. There was a significant main effect for day indicating that all rats gained weight throughout the post-injection period, $F(4, 128)= 1009.43$, $p<.001$, partial $\eta^2= .969$. There was a significant day by group interaction, $F(4, 128)= 11.52$, $p<.001$, partial $\eta^2= .265$, showing that drug animals gained weight faster than controls. There was also a significant day by phenotype interaction, $F(4, 128)= 7.24$, $p<.001$, partial $\eta^2= .184$, showing that the LR groups gained weight faster than HR animals. There was also a main effect of group, $F(1, 32)= 2.90$, $p=$

.035, partial η^2= .132, showing that on average drug animals (*M*= 237.78, *SD*= 19.14) weighed less than control animals (*M*= 254.82, *SD*= 27.22). In sum, all groups of animals gained weight throughout the post-injection period, but the drug group and LR animals gained weight faster than the other animals; on average, however, drug animals did weigh less than control animals regardless of weight gain throughout the period. See Figure 11 and 12.

A one-way ANOVA was conducted separately on each PND that the animals were weighed during the washout period (50, 55, 60, 65, 70) for drug versus control animals. There was a significant main effect for group on PND 50, $F(1, 36)= 18.43$, $p< .001$, partial $\eta^2= .339$, and PND 55, $F(1, 36)= 7.68$, $p= .009$, partial $\eta^2= .176$, demonstrating that the drug and control groups were still significantly different in terms of bodyweight. Results revealed that on PND 60 there was no longer a main effect for group on body weight indicating that the drug animals (*M*= 242.54, *SD*= 20.23) were not significantly different from the control animals (*M*= 258.31, *SD*= 30.28), $F(1, 36)= 3.02$, $p= .091$, partial $\eta^2= .077$. Analyses on PND 65 and 70 also show that the groups did not significantly differ in body weight. Due to the significant day by phenotype interaction, the same analyses were conducted for phenotype on each day of the washout period (PND 50 to 70; drug and control animals only). There was no significant main effect of phenotype for bodyweight from PND 50 to 65. There was a significant main effect of phenotype on PND 70, ($F(1, 34)= 4.43$, $p= .043$, partial $\eta^2= .115$), showing that LR animals (*M*= 294.09, *SD*= 28.73) weighed more than HR animals (*M*= 275.07, *SD*= 25.46). Overall, drug animals gained more weight during the post-injection period than control animals, and on PND 60, were no longer significantly different from the control animals. LR animals also gained weight faster than HR animals, and weighed significantly more than HR animals on PND 70 of the post-injection period.

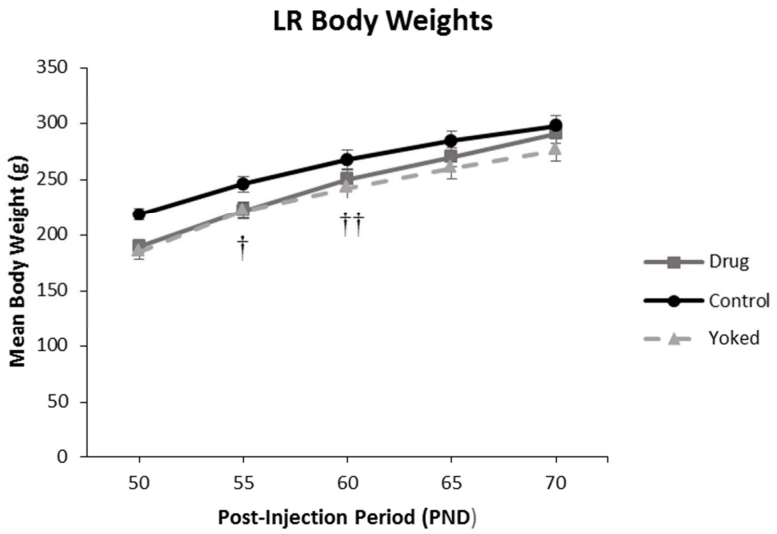

Figure 11. Mean body weight (g) for LR animals in the distance phenotype for the Post-injection period (PND 50 to 70). The † denotes that there was no longer a significant difference for yoked group, and †† denotes no difference for drug group

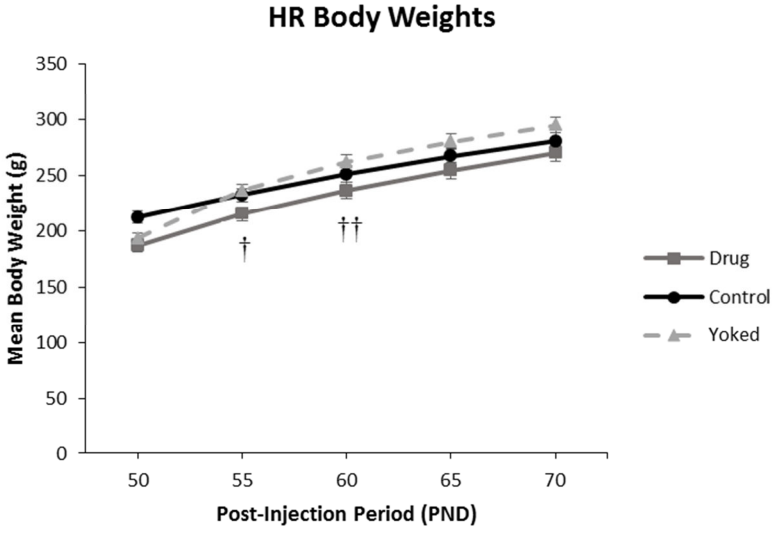

Figure 12. Mean body weight (g) for HR animals in the distance phenotype for the Post-injection period (PND 50 to 70). The † denotes that there was no longer a significant difference for yoked group, and †† denotes no difference for drug group

Yoked versus Control. The 3-way interaction between group, phenotype, and day was significant, $F(4, 136)= 4.72$, $p=.001$, partial $\eta^2= .122$. In order to test the simple interactions, a 2 (group) by 5 (day) repeated measures ANOVA was conducted to measure body weight for the HR and then again for the LR animals. The results revealed a significant interaction between group and day for HR animals, $F(4, 80)= 26.05$, $p< .001$, partial $\eta^2= .566$, but not for LR animals, $F(4, 48)= 2.17$, $p= .086$, partial $\eta^2= .153$. A one-way ANOVA for bodyweight on each day of the washout period (PND 50, 55, 60, 65, 70) for the yoked versus control HR animals was then conducted. There was a significant simple main effect for PND 50, showing that the HR yoked group weighed significantly less than the HR control group, $F(1, 20)= 7.03$, $p= .015$, partial $\eta^2= .260$. There was no longer a significant simple main effect on PND 55, $F(1, 20)= .269$, $p= .610$, partial $\eta^2= .013$, showing that the groups were no longer significantly different; for PND 60 to 70, there was also no simple main effect for group. However, as shown in Figures 11 and 12, the HR yoked animals did surpass the HR control animals in weight although this effect was not statistically significant. Additionally, a second 2 (phenotype) by 5 (day) repeated measures ANOVA for bodyweight during the post injection period was conducted for the yoked animals. The results showed a trend for LR yoked animals to gain less weight throughout the post-injection period than HR yoked animals, $F(4, 64)= 2.32$, $p= .066$, partial $\eta^2= .127$.

Results from the 2 (group) by 2 (phenotype) by 5 (day) repeated measures ANOVA for bodyweight also revealed a significant interaction between group and day, $F(4, 136)= 21.09$, $p< .001$, partial $\eta^2= .383$, indicating that the yoked animals gained more weight throughout the washout period than the controls. A one-way ANOVA for drug group was conducted separately on each PND the animals were weighed during the washout period (50, 55, 60, 65, 70). Results revealed that the yoked animals no longer weighed significantly different than control animals on

PND 55, $F(1, 36)= .416$, $p= .523$, partial $\eta^2= .011$. There was an overall main effect for day, $F(4, 136)= 1046.61$, $p<.001$, partial $\eta^2= .969$, indicating that all animals gained weight over the post-injection period. Overall, the HR yoked animals gained more weight than LR yoked animals and weighed more than the HR control animals at the end of the post-injection period; there were significant group interactions for LR animals.

Conclusions. Overall, the drug and yoked animals gained weight faster than control animals during the post-injection period (see Figures 11 and 12). In drug and control animals, the LR rats gained more weight throughout the washout period than HR animals, and by PND 70 the LR animals weighed significantly more than the HR animals. Drug animals no longer had significantly lower body weights than the control animals on PND 60, and yoked animals were no longer significantly different than controls on PND 55—these lack of group differences remained non-consistent throughout the rest of the post-injection period. Additionally, the HR yoked animals gained more weight than both HR control and LR yoked animals.

Object Phenotype. A 2 (drug group) x 2 (object phenotype) x 5 (day) repeated measures ANOVA was conducted to measure body weight (g) for animals during the post-injection period; animals were weighed every five days (PND 50 to 70).

Drug versus Control. There was a significant main effect for day indicating that all rats gained weight throughout the post-injection period, $F(4, 128)= 807.31$, $p<.001$, partial $\eta^2= .962$. There was a significant day by group interaction, $F(4, 128)= 9.61$, $p<.001$, partial $\eta^2= .231$, indicating that the drug animals gained more weight than the control animals across the washout period. There was also a main effect of group, $F(1, 32)= 4.44$, $p= .043$, partial $\eta^2= .122$, showing that on average drug animals ($M= 237.78$, $SD= 19.14$) weighed less than control animals ($M=$

254.82, *SD*= 27.22). There were no other significant main effects or interactions with phenotype. Overall, drug animals gained weight faster than control animals during the post-injection period.

A one-way ANOVA was conducted separately on each PND the animals were weighed during the washout period (50, 55, 60, 65, 70) for drug versus control in order to better understand the significant day by group interaction. There was a significant main effect for group on PND 50, $F(1, 36)= 18.43$, $p< .001$, partial $\eta^2= .339$, and 55, $F(1, 36)= 7.68$, $p= .009$, partial $\eta^2= .176$. Results revealed that on PND 60 there was no longer a main effect for group on body weight. This indicates that the drug animals (*M*= 241.16, *SD*= 20.57) no longer weighed significantly different than the control animals (*M*= 256.02, *SD*= 31.08) by PND 60, $F(1, 36)= 3.02$, $p= .091$, partial $\eta^2= .077$. The main effect remained inconsistent for PND 65 and 70 as well. See Figure 13 and 14.

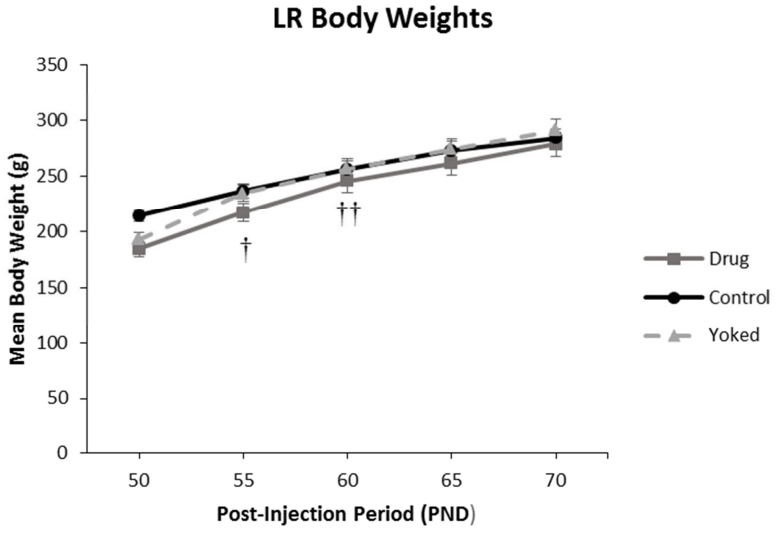

Figure 13. Mean body weight (g) for LR animals in the object phenotype for the post-injection period in the drug and control groups. The † denotes that there was no longer a significant difference for yoked group, and †† denotes no difference for drug group.

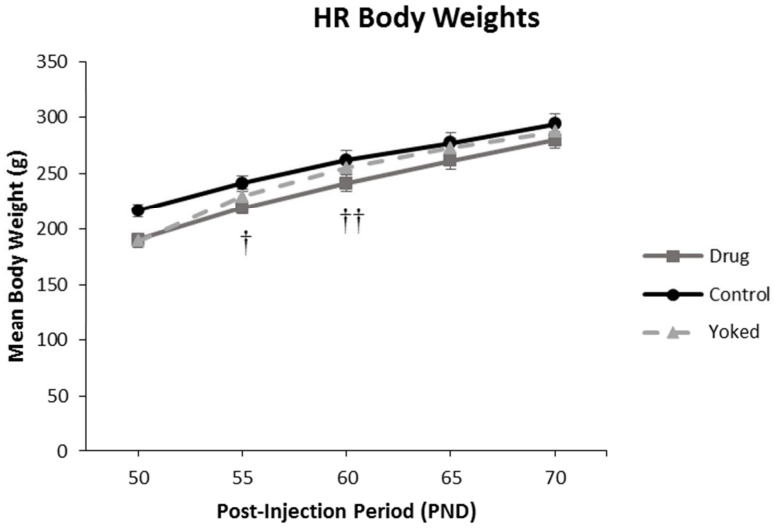

Figure 14. Mean body weight (g) for HR drug and HR control animals in the object phenotype for the Post-injection period. The † denotes that there was no longer a significant difference for yoked group, and †† denotes no difference for drug group

Yoked versus Control. There was a main effect for day, $F(4, 128)= 946.01$, $p<.001$, partial $\eta^2= .967$, indicating that all animals gained weight over the post-injection period. There was a significant interaction between group and day, $F(4, 128)= 21.09$, $p< .001$, partial $\eta^2= .397$, indicating that the yoked animals gained more weight. There were no other significant main effects or interactions for group or phenotype. Overall, the results show that all groups gained weight over the washout period, but the yoked animals gained weight faster than the control animals during the washout period.

A one-way ANOVA was conducted separately on each PND the animals were weighed during the washout period (PND 50 to 70) for yoked versus control groups. There was a significant main effect for group on PND 50, $F(1, 36)= 16.37$, $p< .001$, partial $\eta^2= .313$, showing that yoked animals weighed less than control animals. Results revealed that on PND 55 there was no longer a main effect for group on body weight indicating that the yoked animals ($M= 231.68$, $SD= 17.64$) were no longer significantly different from control ($M= 236.18$, $SD= 24.73$) animals, $F(1, 36)= .416$, $p= .523$, partial $\eta^2= .011$. The main effect for group remained non-significant for PND 60, 65, and 70. See Figure 13 and 14.

Conclusions. Overall, the drug and yoked animals gained weight faster than control animals during the post-injection period. Drug animals were no longer significantly different from the control animals on PND 60, and yoked animals no longer weighed significantly less than controls by PND 55 showing that the significant body weight difference did not persist during the post-injection period after injections. Object phenotype did not significantly affect body weight gain during the post-injection period whereas distance phenotype did interact with drug group on body weight gain. For drug and control animals, the LR animals weighed more

than HR animals; in addition, HR yoked animals had a trend to weigh more than LR animals, but this effect did not persist for the entire washout period.

Open Field Task: Activity

Activity analyses for the open field task were conducted using several 2 (group) x 2 (distance phenotype) x 2 (estrous) between-subjects ANOVAs to measure differences in the distance traveled (m) on the first day of habituation for object placement. Distance phenotype was used for analyses because it best reflects the measures being analyzed for the open-field activity data. Results reported will be as follows: drug vs. control then yoked vs. control. All ANOVA statistics can be found in the Appendix.

Drug versus Control. There were no main effects for group when comparing drug versus control animals. There was no main effect for phenotype, or estrous group and no interaction effects between drug group, phenotype, and estrous group. Overall, activity levels were equivalent across all groups. In addition, the previous significant difference between HR and LR animals on distance traveled disappeared with the adult animals showing no variability in activity level as a function of their distance phenotype as adults. See Figure 15.

Yoked versus Control. There were no significant main effects or interaction effects for phenotype, estrous group, or drug group in this analysis as well, demonstrating that all animals had similar activity levels regardless of drug group, phenotype, or estrous phase. HR and LR groups were again no longer significantly different for distance traveled when tested as adults, potentially showing an effect of aging. See Figure 15.

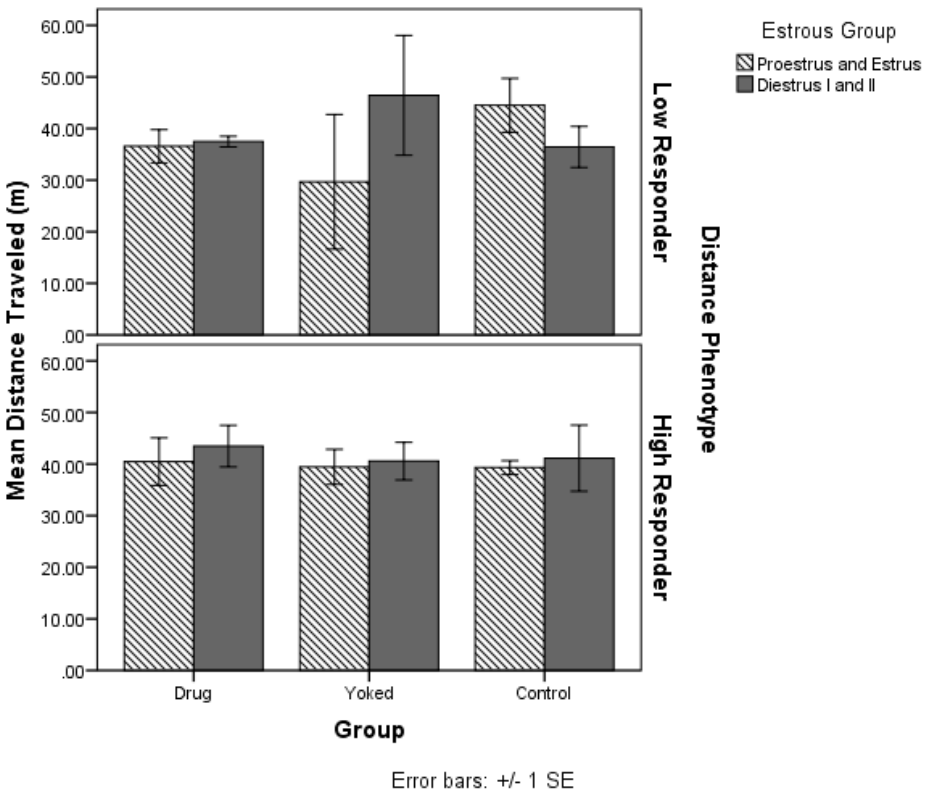

Figure 15. The mean distance traveled (m) during the first day of habituation to the new open field for each group as a function of the distance phenotype

Open Field Task: Anxiety

Analyses for the open field task were conducted using several 2 (group) x 2 (distance phenotype) x 2 (estrous phase) between-subjects ANOVAs to measure differences in the mean percent of time spent in the center zone (CZ) during the first habituation trial on the new open field. Results are reported as follows: drug vs. control and yoked vs. control. All ANOVA statistics can be found in the Appendix.

Drug versus Control. There were no main effects for group when comparing drug versus control animals. There were no main effects or interaction effects between drug group, phenotype, or estrous phase. Overall, neither estrous phase, drug exposure nor phenotype influenced anxiety as demonstrated by similar amounts of time spent in the CZ across animals. See Figure 16.

Yoked versus Control. There were no main effects for group, phenotype, or estrous. There were no interaction effects between phenotype, estrous, and group. Overall, anxiety was not affected by food deprivation during the injection period (yoked group), phenotype, or estrous phase and was equivalent across all animals. See Figure 16.

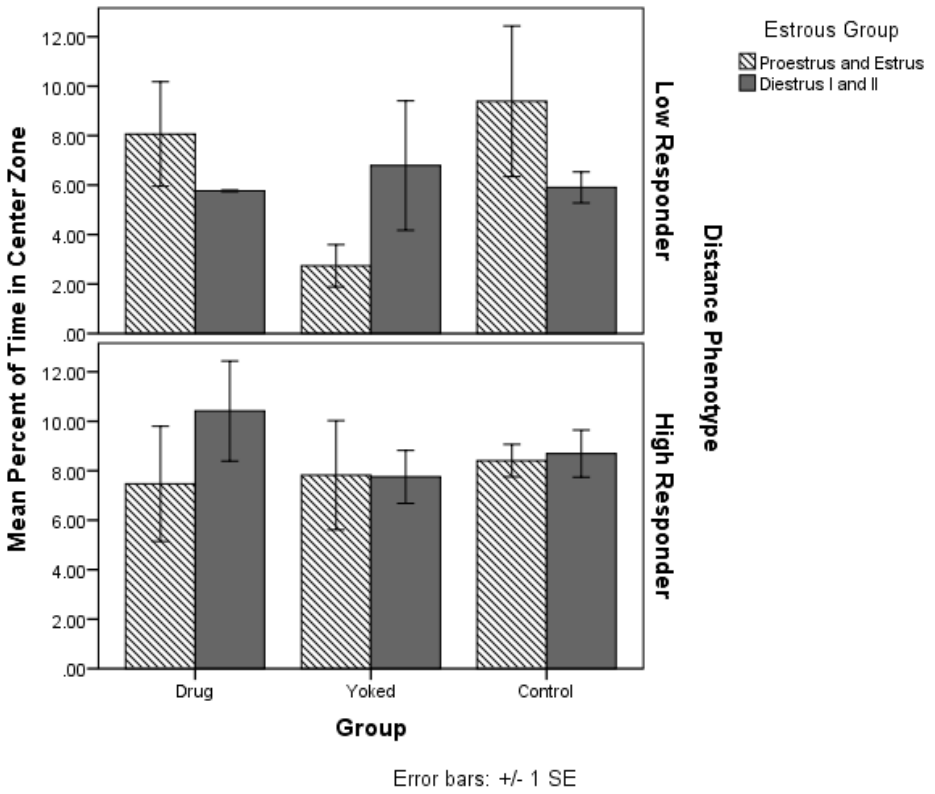

Figure 16. The mean percent of time spent in the center zone (CZ) for habituation day one of the open field task for group. Striped versus solid bars represent estrous group, and rows are separated by phenotype

Object Placement

Analyses for object placement utilized a 2 (group) by 2 (object phenotype) by 2 (estrous) by 2 (object) mixed-model repeated measures ANOVA to analyze the time spent with object A and object B during the test trial (Trial 2). Object A was the familiar object that remained in a constant location, and object B was moved to a different location during the test trial. Good spatial memory was defined by an increase in time spent with object B when compared to object A for Trial 2. Separate analyses were conducted to compare the drug and control animals and then the yoked and control animals. Object phenotype was used for the following analyses because of the similarity in measures and tasks used for object phenotype split and the current data. All ANOVA analyses can be found in the Appendix.

Drug versus Control. There was a significant main effect for the object, demonstrating that all groups of rats spent more time with object B (M= 21.40, SD= 16.28) than A (M= 14.31, SD= 14.66), $F(1, 29)= 12.94$, $p= .001$, partial $\eta^2= .309$. There was a near significant interaction between group and object, $F(1, 31)= 3.37$, $p= .071$, partial $\eta^2= .108$, showing that the drug animals (M= 25.51, SD= 15.31) spent more time with the moved object than controls (M= 17.51, SD= 16.59). There was also a significant interaction effect between group and phenotype, $F(1, 29)= 4.62$, $p= .040$, partial $\eta^2= .137$, showing that LR drug animals spent the most time with object B when compared to object A during Trial 2 (see Figure 17). To test simple effects, a 2 (group) by 2 (object) repeated measures ANOVA was conducted for LR animals to analyze time spent with object A versus B on Trial 2. The results showed a significant object by group interaction, $F(1, 15)= 4.89$, $p= .043$, partial $\eta^2= .246$, revealing that the LR drug animals had a significant increase in exploration of the moved object when compared to the familiar object. There was also a significant simple main effect of group, $F(1, 15)= 6.93$, $p=.019$, partial $\eta^2= .316$

indicating that the LR drug animals (*M*= 32.18, *SD*= 13.72) spent more time with the objects in general than LR control animals (*M*= 11.95, *SD*= 13.83) during Trial 2, $F(1, 15)= 8.87$, $p= .009$, partial $\eta^2= .371$. The same repeated measures ANOVA was conducted for HR drug and control animals and showed that there were no significant simple interactions between group and object, $F(1, 18)= .594$, $p= .451$, partial $\eta^2= .032$. There was a significant simple main effect for the object factor indicating that all HR animals had a significant increase in exploration of the moved object (*M*= 22.36, *SD*= 16.16) compared to the familiar object (*M*= 15.21, *SD*= 16.76) during Trial 2, $F(1, 18)= 6.04$, $p= .024$, partial $\eta^2= .251$. There were no overall main effects for estrous or interactions with phenotype or drug group. Overall, drug exposure in the LR animals produced the best spatial memory compared to LR control animals as indicated by a significant increase of exploration of the moved object compared to object A; there were significant differences between group and phenotype in the HR animals demonstrating that, overall, all HR animals spent more time with the moved object on Trial 2 regardless of group.

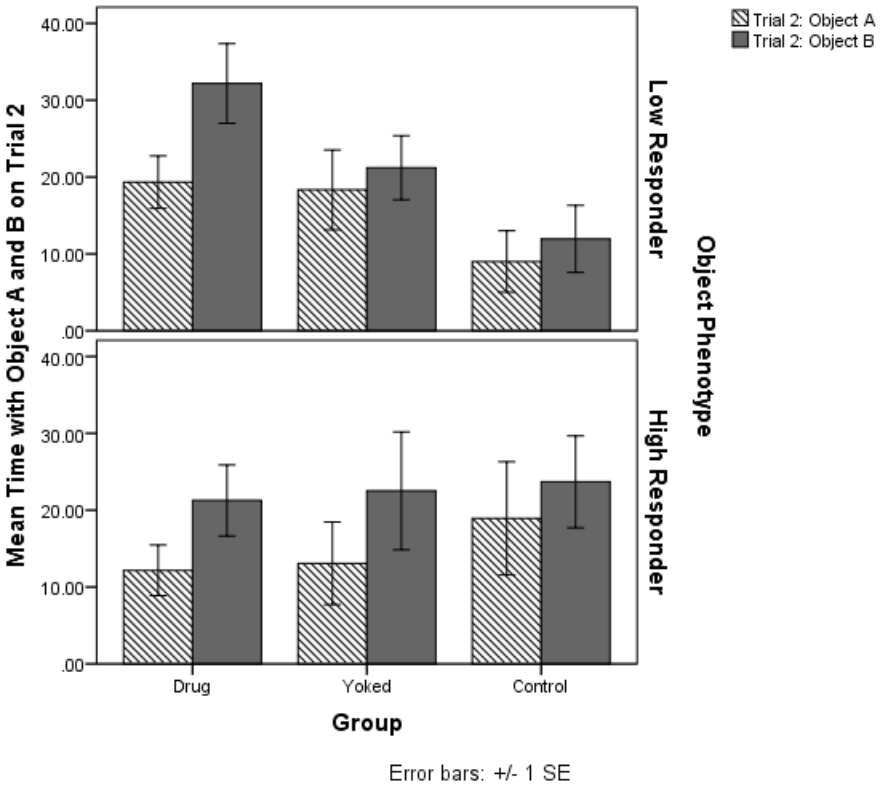

Figure 17. Mean time spent (s) with Object A and Object B during Trial 2 of the Object Placement Task for object phenotype and drug group

Yoked versus Control. Due to lack of yoked animals in the proestrus/estrus phase, the estrous cycle was not included as a variable in the yoked vs. control analyses which were conducted using a 2 (group) x 2 (phenotype) x 2 (object) repeated-measures ANOVA. There was a significant main effect for object, $F(1, 29)= 8.98$, $p= .005$, partial $\eta^2= .214$, demonstrating that all animals spent more time with object B ($M= 19.63$, $SD= 17.19$) than A ($M= 14.68$, $SD= 16.68$). There were no other significant main effects or interaction effects between phenotype and group. Overall, all animals noticed that the object had moved in Trial 2 regardless of group or phenotype. However, as seen in Figure 17, neither the yoked or control groups had a significant increase in exploration of the moved object over the familiar object on Trial 2.

Object Recognition

Analyses for object recognition were conducted using a 2 (group) by 2 (object phenotype) by 2 (estrous) by 2 (object) repeated measures ANOVA to analyze the time spent with object A and object B during Trial 2. Good recognition memory was defined by an increase in time spent with the novel object (B) when compared to the familiar object (A) on trial 2. Separate analyses were conducted to compare the drug and control animals and then the yoked and control animals. Object phenotype was used for the following analyses because of the similarity in measures and tasks used for object phenotype split and the current data. All ANOVA statistics can be found in the Appendix.

Drug versus Control. There was a significant main effect for object, $F(1, 27)= 22.09$, $p< .001$, partial $\eta^2= .450$, demonstrating that all animals spent more time with the novel object B ($M= 35.03$, $SD= 25.31$) than A ($M= 15.55$, $SD= 14.06$). There were no other significant main effects of group, phenotype, or estrous. However, as seen in Figure 18, there was a significant 3-way interaction between group, phenotype, and estrous group, $F(1, 27)= 4.37$, $p= .046$, partial

η^2= .139. In order to test this using simple interactions, a 2 (group) by 2 (estrous) x 2 (object) repeated measures ANOVA was conducted for time spent with object A versus B on trial 2 for HR animals and then again for LR animals. Results revealed that there was a significant group by estrous interaction for HR, $F(1, 16)= 5.14$, $p= .038$, partial $\eta^2= .243$, but not for LR animals, $F(1, 11)= .914$, $p= .359$, partial $\eta^2= .077$ (See Figure 18). Specifically, this showed that the HR control animals in diestrus spent more time investigating both objects than HR control animals in the proestrus group; however, this was not observed in the HR drug animals. There was a simple main effect for object in LR animals showing that all LR animals spent more time with the novel object than the familiar object on Trial 2, $F(1, 11)= 9.15$, $p= .012$, partial $\eta^2= .454$. A 2 (group) by 2 (object) repeated measures ANOVA was conducted for HR animals in the proestrus group and compared the time spent with object A versus B. There were no simple interactions between group and object, and no main effect of group, but there was a significant simple main effect for object, $F(1, 8)= 7.47$, $p= .026$, partial $\eta^2= .483$, demonstrating that all HR animals in the proestrus group spent more time with the novel object over the familiar object (see Figure 19). A 2 (group) by 2 (object) repeated measures ANOVA was also conducted for HR animals in the diestrus group and compared the time spent with object A versus B. There were no simple interactions or main effects for group, but there was a simple main effect for object, $F(1, 8)= 8.18$, $p= .021$, partial $\eta^2= .506$, indicating that all animals recognized the novel object on Trial 2. Overall, the results show that all animals had a significant increase in exploration of the novel object when compared to the familiar object on Trial 2.

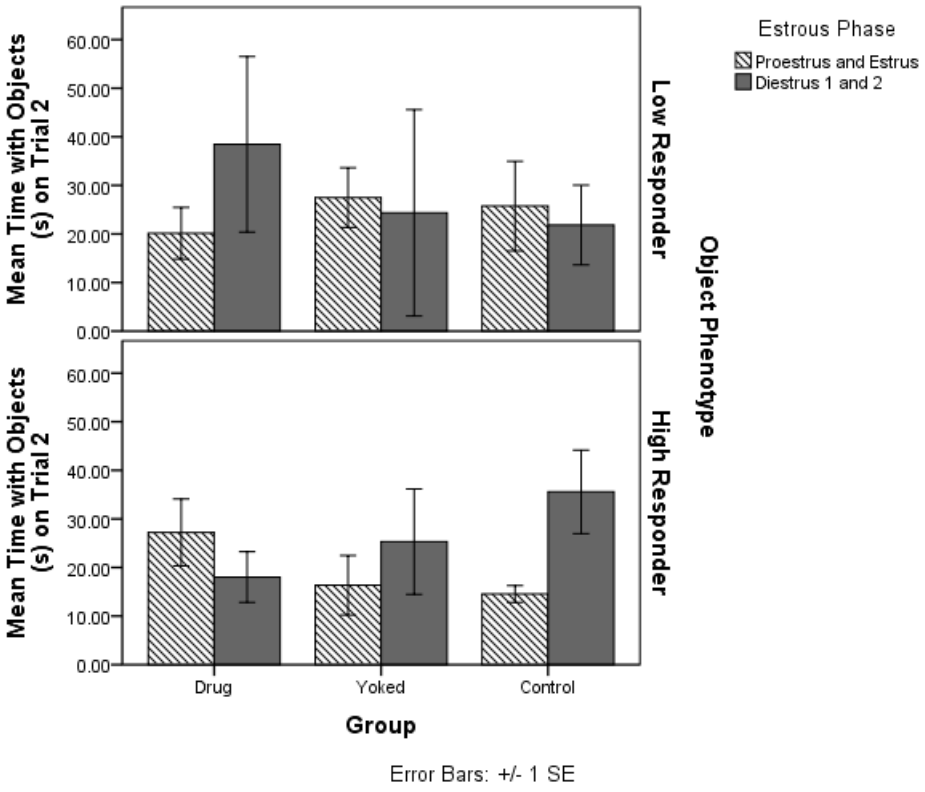

Figure 18. Mean time (s) spent with the objects during Trial 2 of the object recognition task for drug group. Rows are separated by object phenotype, and the bars represent estrous group.

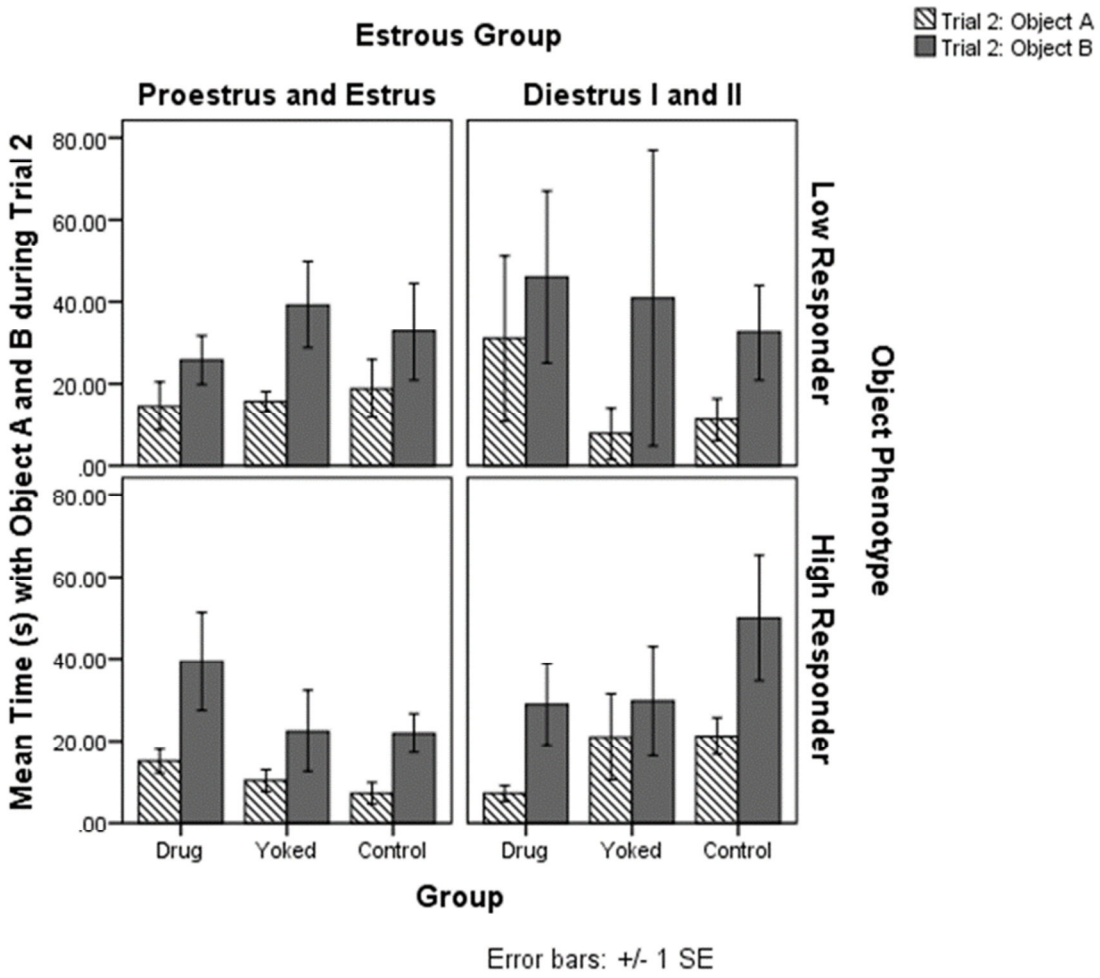

Figure 19. Mean time (s) spent with object A and B during Trial 2 of the object recognition task for drug group. Rows are separated by object phenotype, and columns are separated by estrous group

A 2 (group) by 2(estrous) ANOVA was conducted for object A and then again for object B for the drug versus control animals in order to further investigate the significant group by estrous interaction in the HR animals. The results revealed that there were no significant simple main effects or interactions between group and estrous on time spent with the novel object (object B), $F(1, 16)= 2.59$, $p= .127$, partial $\eta^2= .140$, indicating that none of the estrous groups spent significantly more time with the novel object when compared to other groups (see Figure 19). There was a significant interaction between estrous and drug group for time spent with object A (familiar object) during trial 2, $F(1, 16)= 11.43$, $p= .004$, partial $\eta^2= .417$. A one-way ANOVA was conducted to analyze the time with object A for the HR drug versus control animals in the diestrus group. The results revealed that the HR control animals in the diestrus group spent significantly more time with the familiar object than the HR drug animals in the diestrus group, $F(1, 8)= 8.19$, $p= .021$, partial $\eta^2= .506$ (see Figure 20). A one-way ANOVA was conducted to analyze the time with object A for the HR drug versus control animals in the proestrus group. There was no main effect for group on time with the familiar object for the HR animals in the proestrus group, $F(1, 8)= 3.49$, $p= .099$, partial $\eta^2= .303$, indicating that the drug and control animals in the HR proestrus group were not significantly different. Overall, the HR control animals in the diestrus group explored the familiar object more than the HR control animals in the proestrus group, and HR drug animals in the diestrus group.

A second one-way ANOVA was conducted to measure time with the familiar object for HR control animals in the proestrus group versus the diestrus group. The results showed that HR control animals in diestrus also spent significantly more time with the familiar object than HR control animals in the proestrus/estrus group, $F(1, 7)= 6.27$, $p= .041$, partial $\eta^2= .472$. There was a trend for HR drug animals in the proestrus group to spend more time with the familiar object

than HR drug animals in the diestrus group, $F(1, 9)= 4.57$, $p= .061$, partial $\eta^2= .337$ (see Figure 20).

Overall, all LR and HR animals spent more time with the novel object than the familiar object. However, there was a between-subject's interaction in HR between estrous and group that showed phenotype influenced how the drug exposure and estrous phase influenced time with the objects. Further, none of the groups spent significantly different amounts of time with the novel object, but the HR control animals in the diestrus group spent significantly more time with the familiar object than HR drug animals in the diestrus group, and HR control animals in the proestrus group. These results suggest that none of the groups were significantly different in time spent with the novel object, but do suggest that for control animals, estrous group had a significant impact on time spent with the familiar object—HR control animals in diestrus spent more time with the familiar object than HR control proestrus animals. The results also suggest that the drug exposure may have impacted the effects of the estrous cycle, this is implicated because the HR diestrus animals spent significantly more time with the familiar object than the HR drug animals in the diestrus groups.

Yoked versus Control. There was a significant main effect for the objects, $F(1, 28)= 24.58$, $p< .001$, partial $\eta^2= .467$, demonstrating that all animals spent more time with the novel object ($M= 33.55$, $SD= 26.33$) than the familiar object ($M= 15.21$, $SD= 12.70$; see Figure 19). There were no other significant main effects or interaction effects between estrous, phenotype and group. Overall, all animals noticed the novel object and showed good recognition memory as demonstrated by increased time spent with object B over A during trial 2 of the task regardless of group, phenotype, and estrous phase. However, Figure 19 suggests that the diestrus yoked animals do not show a significant recognition of the novel object.

Conclusions. The results overall demonstrate that drug exposure alone did not produce deficits in object recognition as demonstrated by the lack of a significant main effect of drug group. Specifically, the data showed that in HR control animals, the proestrus group decreased exploration compared to the diestrus phase HR control animals while the LR control and drug animals were similar regardless of estrous. This may explain why there were no significant increases in exploration between the two objects—the estrous group may have interacted with phenotype, and affected recognition memory in the control animals. Drug animals were also influenced differently by estrous when compared to control animals; the HR drug animals in diestrus had significantly less time with the familiar object than HR control animals in the diestrus group. This may indicate that drug exposure may disrupt the normal influences of the estrous cycle on recognition memory. However, because HR drug animals in the diestrus phase had a tendency to have decreased exploration of the familiar object when compared to HR drug animals in the proestrus group, the results still represent that estrous does impact object recognition of familiar objects. Overall the results indicate that drug exposure, estrous phase, and phenotype affect the amount of time spent with the familiar object on trial 2, but not the novel object for drug and control animals.

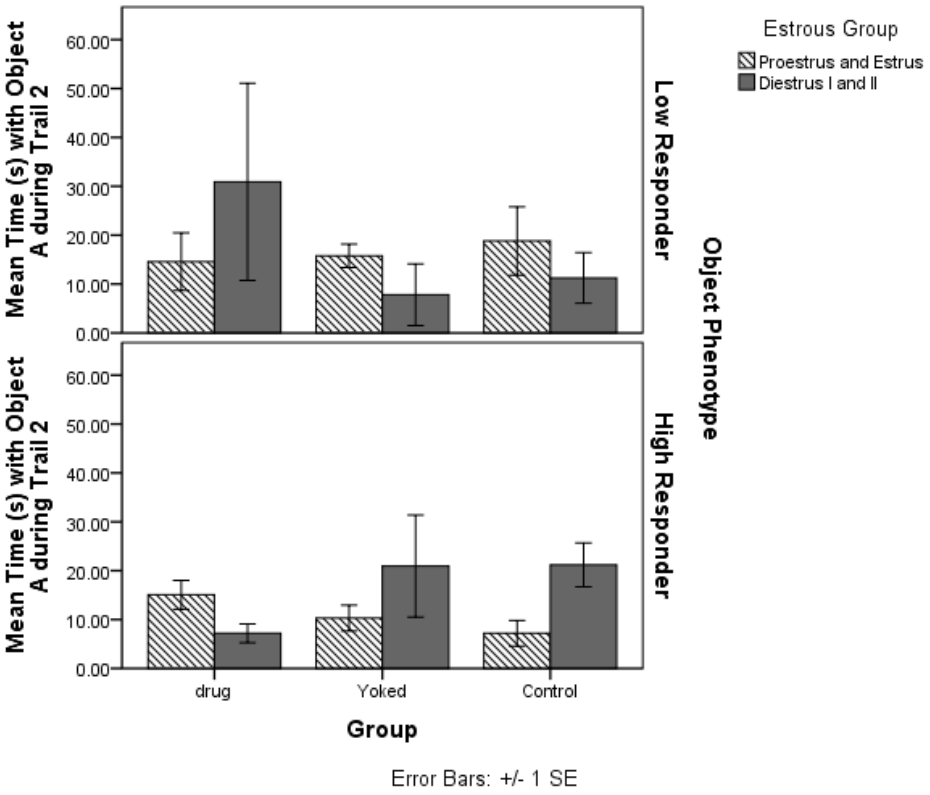

Figure 20. Mean time (s) spent with object A (familiar object) during Trial 2 of the object recognition task for drug group. Rows are separated by object phenotype, and columns are separate by estrous group

Chapter 5: Discussion

The current study investigated the effects that the synthetic cannabinoid CP 55,940 has on food intake, activity, anxiety, spatial location memory and object recognition. Due to the variability in adolescent cannabinoid research, however, the current study also investigated how factors such as individual differences in novelty seeking and hormone cycling influenced those behaviors.

The hypothesis that chronic adolescent drug exposure would negatively affect food intake was supported. Results showed that drug animals weighed and ate less than the normal control animals. This is congruent with past research that has also suggested that cannabinoid exposure has a negative impact on food intake in rats, and that drug animals had less food intake and lower body weight than controls (Biscaia et al., 2003; Mateos et al., 201; Rubino et al., 2008). Food intake in the yoked group was also significantly decreased compared to the control group. This was expected due to the nature of the food manipulation placed on the yoked group. However, results also indicated that in the yoked versus control analyses, the object HR ate significantly less than the object LR. Further body weight analyses for the drug versus control animals also showed that weight gain during the injection period was also affected by phenotype, as demonstrated by a significant interaction between group, distance phenotype and day for the drug versus control animals. This interaction revealed that the LR drug animals weighed significantly less than LR control on PND 41, whereas the HR drug animals weighed significantly less than HR control animals on PND 37—this was only the third day of injections. This interaction was not observed in analyses with the object phenotype groups, and was not present in the yoked versus control analyses (despite the main effect mentioned previously for food intake in the object phenotype). One potential reason for the delay in weight gain in the

distance HR drug animals may be that the HR drug animals were more affected by the new single house environment and repeated injections than the LR drug animals, resulting in the HR drug animals having significantly impaired weight gain earlier in the injection period than the LR drug animals. According to Dellu, Piazza, Moal, and Simon (1996), HR animals have a prolonged excretion of corticosterone (stress hormone) when introduced to novelty. Since all animals were introduced to a novel, single housing environment during injections, HR animals may have experienced elevated stress that may have interacted with the drug exposure and limited the weight gain in the HR drug animals.

Lastly, our results showed that body weight increased after the cessation of injections, this is also consistent with Biscaia et al. (2003) who also found that drug animal body weights were no longer different from controls within 10 to 15 days after the end of the injection period. Despite the non-significant differences in the body weight of animals in the drug group, there was a significant difference for distance phenotype. In the distance phenotype, HR yoked animals gained weight faster than other groups, and surpassed the control animals in body weight (although this increased weight gain did not quite reach significance). This is consistent with Pawlak et al. (2008) who discussed that food-deprived HR rats consumed food at a faster rate than LR animals—an action that would lead to a faster weight gain in the distance HR yoked animals. However, LR animals (drug and control animals only; distance phenotype) gained weight faster than HR animals over the injection period, and weighed significantly more than HR animals on PND 70 of the post-injection period. This is opposite of what would be expected (HR animals should gain more) given that the HR rats have been reported to consume food at a faster rate after deprivation. This difference could potentially involve physiological differences between HR and LR animals, and the effects of stress on feeding/body weight gain. Maniam and

Morris (2012) state that stress can reduce food intake in rats suggesting that the distance HR animals may have less weight gain during washout because of a prolonged stress response to the injection period. This may also potentially reflect a degree of helplessness in HR animals, meaning that the prolonged stress response from injections resulted in decreased food consumption or other actions that may inhibit weight gain. It may also be that the distance HR animals were more active when grouped housed with their litter-mates—an action that would allow them to burn more calories and slow weight gain. This would be consistent with results from Aydin, Frohmader, and Akil (2015) that discovered HR animals exhibited more depressive symptoms than LR animals after a 14-day injection regime—a complete phenotypic switch from the original screening. More research is needed, however, in order to understand the nature of the relationship between drug exposure and phenotype on food intake and weight gain.

The second hypothesis that cannabinoid exposure would negatively affect spatial memory and object recognition memory was not supported. This is due to the results from the current study revealing that the LR drug animals exhibited the best spatial memory when compared to LR control animals (HR animals did not have a significant interaction between group and the objects). However, because drug exposure influenced LR animals more than HR animals, this may suggest that the endocannabinoid system interacts with the HPA axis to influence spatial memory, or to increase interest in novelty—the LR control animals spent less time with the moved object than LR drugs, so the CB exposure may have facilitated either curiosity, or raised awareness of the environment in those animals. The hypothesis that estrous cycle would influence spatial memory was not supported. Results from the current study did not reveal any main effects for estrous group for drug versus control animals, and did not show any interactions with any of the other study variables. This is inconsistent with Frye et al. (2007) who found that

proestrus and estrus rats have better spatial memory than diestrus animals. This inconsistency with past research may be due to the lack of animals in each of the estrous groups—some groups only had a couple animals when estrous was included as an independent variable. As such, additional research that incorporates a larger sample could help expand the literature for estrous cycle and the potential interactions it may have with CB exposure. Overall, LR drug animals demonstrated good spatial memory compared to LR control animals; for HR, drug group did not affect memory indicating that all HR animals spent more time with the moved object on Trial 2 compared to the familiar object.

For object recognition memory, animals were not different in terms of drug group (not including the other variables; no main effects for group). These results are not consistent with other cannabinoid research that suggests CB exposure has either no effect or a negative effect on spatial memory. Studies from O'Shea et al. (2004; 2006) revealed that adolescent drug exposure resulted in poor object recognition memory in adulthood when compared to controls. Likewise, one study suggested that cannabinoid exposure reduced spatial memory in animals but had no effect on recognition memory (Mateos et al., 2011; Abush & Akirav, 2012; Verrico et al., 2014). However, the current study also found that drug group significantly interacted with estrous phase and phenotype on object recognition and partially supported the hypothesis that estrous cycle would influence spatial and recognition memory. Specifically, there was a significant group by estrous interaction for HR but not for LR animals. Additionally, all HR animals in the proestrus phase and all animals in the diestrus phase had a significant increase in exploration of the novel object on Trial 2, demonstrating good recognition memory. However, HR control animals in the proestrus/estrus phase had decreased exploration of the familiar object compared to the diestrus phase HR control animals, suggesting that the HR control animals in the diestrus group may not

have remembered the novel object, or noticed the familiar object on Trial 2. These results are consistent with Walf et al. (2006) who demonstrated that rats in the proestrus phase performed better than rats in the diestrus phase on recognition memory tasks. For HR drug animals, there was a trend for animals in the proestrus group to spend more time with the familiar object than animals in the diestrus. This potential estrous effect in drug animals is opposite from the HR control animals, and may reflect a complex interaction between the endocannabinoid system, HPA axis (for phenotype), and estrogen/progesterone hormones. Differences between estrous group and drug group may reflect the physiological interactions with estrogen and the endocannabinoid system—CB receptor density is highest during diestrus and lowest during estrus (Gorzalka & Dang, 2012). Additionally, estradiol has been shown to influence the mechanism of action of CB agonists and increase their ability to suppress GABA transmission; this may potentially impact memory (Gorzalka & Dang, 2012). Overall, the current study found that estrous cycle, and phenotype influence object recognition memory and suggests that some of the variability in cannabinoid memory studies may be attributed to a lack of accountability for individual differences, and hormone levels. Most importantly, due to the lack of research and literature on novelty-seeking and estrous phase it is important that future studies expand on this study in order to understand how they are related/the nature of the interaction between phenotype and estrous cycle on memory.

The third hypothesis that HR animals would be significantly more active and less anxious than LR rats was not supported. This study showed a lack of significance between phenotype, drug group and estrous cycle on both measures. One potential reason for the lack of significance could be the timing of the phenotype screening. For example, Philpot and Wecker (2008) showed that adolescent rats had greater activity when compared to young adults of the same phenotype;

they also revealed that there was a greater rate of HR animals in adolescent rats than the young adult group. This may mean that animals in the current study had a natural decline in activity due to age, and not drug or estrous exposure. There was no observed effect of drug group, phenotype, or estrous on activity or anxiety. This suggests that drug exposure and food deprivation do not cause anxiety or reduce anxiety. This is inconsistent with O'Shea et al. (2006) who found that chronic cannabinoid exposure increased anxiety when tested in adulthood. The results also suggest that estrous cycle has no impact on activity or anxiety as well. This is not consistent with Marcondes et al. (2001) who found that animals in the proestrus phase were less anxious than diestrus phase animals. One potential reason for this discrepancies with both estrous and cannabinoid literature could be the low number of animals in the groups (Drug x Phenotype x Estrous)—more animals may have resulted in different results. As shown in Figure 16, several groups of animals had a large amount of variability that could be improved with more subjects. Overall, future research in adolescent cannabinoid exposure may benefit from using selectively bred HR and LR animals, and not outbred animals screened at an early age, to ensure that novelty seeking phenotype remains consistent throughout the lifespan and to better understand potential influences on anxiety and drug exposure. Future cannabinoid research may also benefit from screening for phenotype again after the injection period. Additionally, future cannabinoid research should focus on the influential power of estrous cycle in order to understand how the endocannabinoid system and hormone levels may interact with each other to change affect and activity.

There were a couple of limiting factors in the current study. The first limiting factor is the low number of animals in each group when estrous was added as an independent variable. Estrous was not included for analysis in object placement for the yoked versus control group

analysis because there was only one animal in a group—because of this we were unable to investigate the potential influences of estrous on spatial memory. An additional limiting factor is the timing of the phenotype screening. Some research suggests that adolescent rats are naturally more exploratory, and results from this study show that differences in activity did disappear in the same animals when tested as adults. However, object phenotype did influence memory on the object placement and recognition tasks. This may suggest that some of the novelty-seeking behavior observed was due to individual differences in approach anxiety, not novel environment exploration or activity differences.

Overall, the results do not support previous cannabinoid research that shows that chronic adolescent exposure to CB impairs spatial and recognition memory, it also shows the importance of monitoring food intake in drug animals. Cannabinoid studies in the future should continue to investigate the effects of cannabinoid exposure on food intake to isolate the effects of drug exposure from potential malnutrition and start investigating a way to reduce the impact of drug exposure on food intake. Also, estrous phase and phenotype affected recognition memory for the drug versus control animals. Moreover, the current study showed that LR animals were not affected by estrous (or drug) whereas the HR animals maintained the group x estrous interaction demonstrating that HR control animals in diestrus spent the most time with the familiar object, indicating that estrous phase can increase or decrease interest or memory of a familiar object (see Figure 20) . This current study further demonstrates the importance for female rat studies to include estrous as a potential influencing factor. Overall, the current study attempted to investigate the cause of variability in cannabinoid research and demonstrated that estrous cycle and novelty-seeking phenotype can potentially be responsible for producing different results across studies.

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Appendix A: ANOVA Results

ANOVA Summary Tables for all Analyses

Food Intake: Distance Phenotype

Table 1

Mean Food Consumed During Injection Period for Drug vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 29.22$, $p< .001$, partial $\eta^2= .462$
Day x Group	$F(13, 442)= 1.18$, $p= .294$, partial $\eta^2= .033$
Day x Distance Phenotype	$F(13, 442)= 1.07$, $p= .381$, partial $\eta^2= .031$
Day x Group x Phenotype	$F(13, 442)= .839$, $p= .618$, partial $\eta^2= .024$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 2

Mean Food Consumed During Injection Period for Drug vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 46.20$, $p< .001$, partial $\eta^2= .576$
Distance Phenotype	$F(1, 34)= .776$, $p= .384$, partial $\eta^2= .022$
Group x Distance Phenotype	$F(1, 34)= .037$, $p= .849$, partial $\eta^2= .001$

Note. Between subjects ANOVA is represented

Table 3

Mean Food Consumed During Injection Period for Yoked vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 20.27$, $p< .001$, partial $\eta^2= .374$
Day x Group	$F(13, 442)= 2.95$, $< .001$, partial $\eta^2= .080$
Day x Distance Phenotype	$F(13, 442)= .840$, $p= .617$, partial $\eta^2= .024$
Day x Group x Phenotype	$F(13, 442)= 1.26$, $p= .237$, partial $\eta^2= .036$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 4

Mean Food Consumed During Injection Period for Yoked vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 43.32$, $p< .001$, partial $\eta^2= .560$
Distance Phenotype	$F(1, 34)= .348$, $p= .559$, partial $\eta^2= .010$
Group x Distance Phenotype	$F(1, 34)= .005$, $p= .945$, partial $\eta^2= .000$

Note. Between subjects ANOVA is represented

Table 5

Mean Food Consumed for Yoked Versus Control Animals on Each PND of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 36)= 3.72$, $p= .062$, partial $\eta^2= .094$
PND 37	$F(1, 36)= 24.68$, $p< .001$, partial $\eta^2= .407$
PND 38	$F(1, 36)= 20.87$, $p< .001$, partial $\eta^2= .367$
PND 39	$F(1, 36)= 27.96$, $p< .001$, partial $\eta^2= .437$
PND 40	$F(1, 36)= 34.95$, $p< .001$, partial $\eta^2= .493$
PND 41	$F(1, 36)= 32.65$, $p< .001$, partial $\eta^2= .476$
PND 42	$F(1, 36)= 46.67$, $p< .001$, partial $\eta^2= .564$
PND 43	$F(1, 36)= 29.83$, $p< .001$, partial $\eta^2= .453$
PND 44	$F(1, 36)= 25.03$, $p< .001$, partial $\eta^2= .410$
PND 45	$F(1, 36)= 28.04$, $p< .001$, partial $\eta^2= .438$
PND 46	$F(1, 36)= 16.20$, $p< .001$, partial $\eta^2= .310$
PND 47	$F(1, 36)= 15.85$, $p< .001$, partial $\eta^2= .306$
PND 48	$F(1, 36)= 16.64$, $p< .001$, partial $\eta^2= .316$
PND 49	$F(1, 36)= 23.96$, $p< .001$, partial $\eta^2= .400$

Note. Significance indicates there was a significant Main effect for group on that PND

Food Intake: Object Phenotype

Table 6

Mean Food Consumed During Injection Period for Drug vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 30.51$, $p< .001$, partial $\eta^2= .473$
Day x Group	$F(13, 442)= 1.01$, $p= .433$, partial $\eta^2= .029$
Day x Object Phenotype	$F(13, 442)= 1.71$, $p= .056$, partial $\eta^2= .048$
Day x Group x Phenotype	$F(13, 442)= 1.02$, $p= .431$, partial $\eta^2= .029$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 7

Mean Food Consumed During Injection Period for Drug vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 51.51$, $p< .001$, partial $\eta^2= .602$
Object Phenotype	$F(1, 34)= .131$, $p= .720$, partial $\eta^2= .004$
Group x Distance Phenotype	$F(1, 34)= 3.45$, $p= .072$, partial $\eta^2= .092$

Note. Between subjects ANOVA is represented

Table 8

Mean Food Consumed During Injection Period for Yoked vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 24.32$, $p< .001$, partial $\eta^2= .417$
Day x Group	$F(13, 442)= 2.41$, $p= .004$, partial $\eta^2= .066$
Day x Object Phenotype	$F(13, 442)= 1.40$, $p= .153$, partial $\eta^2= .040$
Day x Group x Phenotype	$F(13, 442)= 1.06$, $p= .391$, partial $\eta^2= .030$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 9

Mean Food Consumed During Injection Period for Yoked vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 50.71$, $p< .001$, partial $\eta^2= .599$
Object Phenotype	$F(1, 34)= 5.06$, $p= .031$, partial $\eta^2= .130$
Group x Distance Phenotype	$F(1, 34)= .477$, $p= .494$, partial $\eta^2= .014$

Note. Between subjects ANOVA is represented

Bodyweight for Injection Period: Distance Phenotype

Table 10

Mean Body Weight (g) During Injection Period for Drug vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 416)= 915.73$, $p< .001$, partial $\eta^2= .966$
Day x Group	$F(13, 416)= 29.62$, $p< .001$, partial $\eta^2= .481$
Day x Distance Phenotype	$F(13, 416)= .496$, $p= .927$, partial $\eta^2= .015$
Day x Group x Phenotype	$F(13, 416)= 2.34$, $p= .005$, partial $\eta^2= .068$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 11

Mean Body Weight During Injection Period for Drug vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 32)= 12.81$, $p= .001$, partial $\eta^2= .286$
Distance Phenotype	$F(1, 32)= .729$, $p= .400$, partial $\eta^2= .022$
Group x Distance Phenotype	$F(1, 32)= .033$, $p= .857$, partial $\eta^2= .001$

Note. Between subjects ANOVA is represented

Table 12

Mean Bodyweight (g) During the Injection Period for LR Drug versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(13, 182)= 586.79$, $p< .001$, partial $\eta^2= .977$
Day x Group	$F(13, 182)= 29.99$, $p< .001$, partial $\eta^2= .682$
Group	$F(1, 14)= 5.74$, $p= .031$, partial $\eta^2= .291$

Note. Both within-subjects and between subjects effects are depicted

Table 13

Mean Bodyweight (g) During the Injection period for HR Drug versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(13, 234)= 412.88$, $p< .001$, partial $\eta^2= .958$
Day x Group	$F(13, 234)= 7.36$, $p< .001$, partial $\eta^2= .290$
Group	$F(1, 18)= 7.09$, $p= .016$, partial $\eta^2= .283$

Note. Both within- and between subject effects are depicted

Table 14

Mean Body Weight for LR Drug Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 14)= .027$, $p= .872$, partial $\eta^2= .002$
PND 37	$F(1, 14)= .663$, $p= .429$, partial $\eta^2= .045$
PND 38	$F(1, 14)= 1.53$, $p= .236$, partial $\eta^2= .099$
PND 39	$F(1, 14)= 2.65$, $p= .126$, partial $\eta^2= .159$
PND 40	$F(1, 14)= 3.54$, $p= .081$, partial $\eta^2= .202$
PND 41	$F(1, 14)= 5.73$, $p= .031$, partial $\eta^2= .290$
PND 42	$F(1, 14)= 5.75$, $p= .031$, partial $\eta^2= .291$
PND 43	$F(1, 14)= 9.06$, $p= .009$, partial $\eta^2= .393$
PND 44	$F(1, 14)= 7.12$, $p= .018$, partial $\eta^2= .337$
PND 45	$F(1, 14)= 8.32$, $p= .012$, partial $\eta^2= .373$
PND 46	$F(1, 14)= 9.10$, $p= .009$, partial $\eta^2= .394$

PND 47	$F(1, 14)= 10.34$, $p= .006$, partial $\eta^2= .425$
PND 48	$F(1, 14)= 10.47$, $p= .006$, partial $\eta^2= .428$
PND 49	$F(1, 14)= 10.83$, $p= .005$, partial $\eta^2= .436$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 15

Mean Body Weight for HR Drug Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 18)= 1.85$, $p= .191$, partial $\eta^2= .093$
PND 37	$F(1, 18)= 4.47$, $p= .049$, partial $\eta^2= .199$
PND 38	$F(1, 18)= 4.36$, $p= .051$, partial $\eta^2= .195$
PND 39	$F(1, 18)= 5.29$, $p= .034$, partial $\eta^2= .227$
PND 40	$F(1, 18)= 5.66$, $p= .029$, partial $\eta^2= .239$
PND 41	$F(1, 18)= 6.50$, $p= .020$, partial $\eta^2= .265$
PND 42	$F(1, 18)= 8.09$, $p= .011$, partial $\eta^2= .310$
PND 43	$F(1, 18)= 7.47$, $p= .014$, partial $\eta^2= .293$
PND 44	$F(1, 18)= 7.89$, $p= .012$, partial $\eta^2= .305$
PND 45	$F(1, 18)= 7.78$, $p= .012$, partial $\eta^2= .302$
PND 46	$F(1, 18)= 7.88$, $p= .012$, partial $\eta^2= .305$
PND 47	$F(1, 18)= 8.31$, $p= .010$, partial $\eta^2= .316$
PND 48	$F(1, 18)= 8.25$, $p= .010$, partial $\eta^2= .314$
PND 49	$F(1, 18)= 8.66$, $p= .009$, partial $\eta^2= .325$

Note. Significance indicates there was a signficant Main effect for group on that PND

Table 16

Mean Body Weight (g) During Injection Period for Yoked vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 416)= 808.83$, $p< .001$, partial $\eta^2= .962$
Day x Group	$F(13, 416)= 34.25$, $p< .001$, partial $\eta^2= .517$
Day x Distance Phenotype	$F(13, 416)= .697$, $p= .767$, partial $\eta^2= .021$
Day x Group x Phenotype	$F(13, 416)= 3.46$, $p< .001$, partial $\eta^2= .097$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 17

Mean Food Consumed During Injection Period for Yoked vs. Control and Distance Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 32)= 11.16$, $p= .002$, partial $\eta^2= .259$
Distance Phenotype	$F(1, 32)= .003$, $p= .959$, partial $\eta^2= .000$
Group x Distance Phenotype	$F(1, 32)= 1.01$, $p= .321$, partial $\eta^2= .031$

Note. Between subjects ANOVA is represented

Table 18

Mean Bodyweight (g) During the Injection period for LR yoked versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(13, 234)= 565.15$, $p< .001$, partial $\eta^2= .979$
Day x Group	$F(13, 234)= 40.04$, $p< .001$, partial $\eta^2= .769$
Group	$F(1, 12)= 6.31$, $p= .027$, partial $\eta^2= .345$

Note. Both within- and between subject effects are depicted

Table 19

Mean Bodyweight (g) During the Injection period for HR yoked versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(13, 234)= 421.90$, $p< .001$, partial $\eta^2= .955$
Day x Group	$F(13, 234)= 9.57$, $p< .001$, partial $\eta^2= .324$
Group	$F(1, 20)= 4.07$, $p= .057$, partial $\eta^2= .169$

Note. Both within- and between subject effects are depicted

Table 20

Mean Body Weight for LR Yoked Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 12)= .177$, $p= .681$, partial $\eta^2= .015$
PND 37	$F(1, 12)= 1.05$, $p= .326$, partial $\eta^2= .081$
PND 38	$F(1, 12)= 2.05$, $p= .178$, partial $\eta^2= .146$
PND 39	$F(1, 12)= 2.47$, $p= .142$, partial $\eta^2= .170$
PND 40	$F(1, 12)= 3.40$, $p= .090$, partial $\eta^2= .221$
PND 41	$F(1, 12)= 4.41$, $p= .058$, partial $\eta^2= .268$
PND 42	$F(1, 12)= 5.54$, $p= .036$, partial $\eta^2= .316$
PND 43	$F(1, 12)= 7.81$, $p= .016$, partial $\eta^2= .394$
PND 44	$F(1, 12)= 8.27$, $p= .014$, partial $\eta^2= .408$
PND 45	$F(1, 12)= 8.16$, $p= .014$, partial $\eta^2= .405$
PND 46	$F(1, 12)= 10.54$, $p= .007$, partial $\eta^2= .468$
PND 47	$F(1, 12)= 11.85$, $p= .005$, partial $\eta^2= .497$
PND 48	$F(1, 12)= 13.13$, $p= .003$, partial $\eta^2= .523$
PND 49	$F(1, 12)= 14.45$, $p= .003$, partial $\eta^2= .546$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 21

Mean Body Weight for HR Yoked Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 20)= .209$, $p= .653$, partial $\eta^2= .010$
PND 37	$F(1, 20)= .337$, $p= .568$, partial $\eta^2= .017$
PND 38	$F(1, 20)= 1.49$, $p= .236$, partial $\eta^2= .069$
PND 39	$F(1, 20)= 2.21$, $p= .152$, partial $\eta^2= .100$
PND 40	$F(1, 20)= 3.35$, $p= .082$, partial $\eta^2= .144$
PND 41	$F(1, 20)= 4.57$, $p= .045$, partial $\eta^2= .186$
PND 42	$F(1, 20)= 5.86$, $p= .025$, partial $\eta^2= .227$
PND 43	$F(1, 20)= 6.42$, $p= .020$, partial $\eta^2= .243$
PND 44	$F(1, 20)= 5.69$, $p= .027$, partial $\eta^2= .221$

PND 45	$F(1, 20)= 6.51$, $p= .019$, partial $\eta^2= .245$
PND 46	$F(1, 20)= 5.38$, $p= .031$, partial $\eta^2= .212$
PND 47	$F(1, 20)= 6.01$, $p= .024$, partial $\eta^2= .231$
PND 48	$F(1, 20)= 5.84$, $p= .025$, partial $\eta^2= .226$
PND 49	$F(1, 12)= 4.84$, $p= .040$, partial $\eta^2= .195$

Note. Significance indicates there was a significant Main effect for group on that PND

Body Weight for Injection Period: Object Phenotype

Table 22

Mean Body Weight (g) During Injection Period for Drug vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 885.08$, $p< .001$, partial $\eta^2= .963$
Day x Group	$F(13, 442)= 26.33$, $p< .001$, partial $\eta^2= .436$
Day x Object Phenotype	$F(13, 442)= .524$, $p= .910$, partial $\eta^2= .015$
Day x Group x Phenotype	$F(13, 442)= 1.67$, $p=.065$, partial $\eta^2= .047$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 23

Mean Food Consumed During Injection Period for Drug vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 13.38$, $p= .001$, partial $\eta^2= .282$
Object Phenotype	$F(1, 34)= 1.18$, $p= .284$, partial $\eta^2= .034$
Group x Object Phenotype	$F(1, 34)= .385$, $p= .539$, partial $\eta^2= .011$

Note. Between subjects ANOVA is represented

Table 24

Mean Body Weight for Drug Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 36)= 1.05$, $p= .313$, partial $\eta^2= .028$
PND 37	$F(1, 36)= 3.97$, $p= .054$, partial $\eta^2= .099$
PND 38	$F(1, 36)= 5.22$, $p= .028$, partial $\eta^2= .127$
PND 39	$F(1, 36)= 7.36$, $p= .010$, partial $\eta^2= .170$
PND 40	$F(1, 36)= 8.75$, $p= .005$, partial $\eta^2= .195$
PND 41	$F(1, 36)= 11.72$, $p= .002$, partial $\eta^2= .246$
PND 42	$F(1, 36)= 13.61$, $p= .001$, partial $\eta^2= .274$
PND 43	$F(1, 36)= 16.15$, $p< .001$, partial $\eta^2= .310$
PND 44	$F(1, 36)= 14.72$, $p< .001$, partial $\eta^2= .290$
PND 45	$F(1, 36)= 15.75$, $p< .001$, partial $\eta^2= .304$
PND 46	$F(1, 36)= 16.10$, $p< .001$, partial $\eta^2= .309$
PND 47	$F(1, 36)= 17.50$, $p< .001$, partial $\eta^2= .327$
PND 48	$F(1, 36)= 17.34$, $p< .001$, partial $\eta^2= .325$
PND 49	$F(1, 36)= 17.54$, $p< .001$, partial $\eta^2= .328$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 25

Mean Body Weight (g) During Injection Period for Yoked vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Day	$F(13, 442)= 865.66$, $p< .001$, partial $\eta^2= .962$
Day x Group	$F(13, 442)= 29.75$, $p< .001$, partial $\eta^2= .467$
Day x Object Phenotype	$F(13, 442)= 1.99$, $p= .020$, partial $\eta^2= .055$
Day x Group x Phenotype	$F(13, 442)= 1.26$, $p=.236$, partial $\eta^2= .036$

Note. Within-subjects subjects ANOVA is represented for PND 36 to 49

Table 26

Mean Food Consumed During Injection Period for Yoked vs. Control and Object Phenotype

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 8.63$, $p= .006$, partial $\eta^2= .202$
Distance Phenotype	$F(1, 34)= .002$, $p= .964$, partial $\eta^2= .000$
Group x Object Phenotype	$F(1, 34)= .290$, $p= .594$, partial $\eta^2= .008$

Note. Between subjects ANOVA is represented

Table 27

Mean Body Weight for Yoked Versus Control Animals for Each Day of the Injection Period

Main Effect	F-Statement
PND 36	$F(1, 36)= .142$, $p= .709$, partial $\eta^2= .006$
PND 37	$F(1, 36)= .846$, $p= .364$, partial $\eta^2= .023$
PND 38	$F(1, 36)= 3.11$, $p= .086$, partial $\eta^2= .079$
PND 39	$F(1, 36)= 4.27$, $p= .046$, partial $\eta^2= .106$
PND 40	$F(1, 36)= 6.07$, $p= .019$, partial $\eta^2= .144$
PND 41	$F(1, 36)= 7.85$, $p= .008$, partial $\eta^2= .179$
PND 42	$F(1, 36)= 10.78$, $p= .002$, partial $\eta^2= .235$
PND 43	$F(1, 36)= 13.27$, $p= .001$, partial $\eta^2= .269$
PND 44	$F(1, 36)= 12.64$, $p= .001$, partial $\eta^2= .260$
PND 45	$F(1, 36)= 13.69$, $p= .001$, partial $\eta^2= .276$
PND 46	$F(1, 36)= 13.39$, $p= .001$, partial $\eta^2= .271$
PND 47	$F(1, 36)= 14.66$, $p< .001$, partial $\eta^2= .289$
PND 48	$F(1, 36)= 15.29$, $p< .001$, partial $\eta^2= .298$
PND 49	$F(1, 36)= 14.33$, $p= .001$, partial $\eta^2= .285$

Note. Significance indicates there was a significant Main effect for group on that PND

Body Weight for Post-Injection Period: Distance Phenotype

Table 28

Mean Body Weight (g) for Post-Injection Period for Drug vs. Control

Main Effect or Interaction	F-statement
Day	$F(4, 136)= 1016.06$, $p< .001$, partial $\eta^2= .968$
Day x Group	$F(4, 136)= 10.53$, $p< .001$, partial $\eta^2= .237$
Day x Distance Phenotype	$F(4, 136)= 5.67$, $p< .001$, partial $\eta^2= .143$
Day x Group x Phenotype	$F(4, 136)= .467$, $p=.760$, partial $\eta^2= .014$

Note. Within-subjects subjects ANOVA is represented for PND 50 to 70

Table 29

Mean Body Weight (g) for Post-Injection Period for Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 4.31$, $p= .046$, partial $\eta^2= .112$
Distance Phenotype	$F(1, 34)= 1.38$, $p= .248$, partial $\eta^2= .039$
Group x Distance Phenotype	$F(1, 34)= .001$, $p= .978$, partial $\eta^2= .000$

Note. Between subjects ANOVA is represented

Table 30

Mean Body Weight for Drug Versus Control Animals for the Post-injection Period

Main Effect	F-Statement
PND 50	$F(1, 36)= 18.43$, $p< .001$, partial $\eta^2= .339$
PND 55	$F(1, 36)= 7.68$, $p= .009$, partial $\eta^2= .176$
PND 60	$F(1, 36)= 3.02$, $p= .091$, partial $\eta^2= .077$
PND 65	$F(1, 36)= 2.25$, $p= .143$, partial $\eta^2= .059$
PND 70	$F(1, 36)= .827$, $p= .369$, partial $\eta^2= .022$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 31

Mean Body Weight for HR versus LR groups for the Post-injection Period (Includes Animals in the Drug and Control Groups)

Main Effect	F-Statement
PND 50	$F(1, 34)= .450$, $p= .507$, partial $\eta^2= .013$
PND 55	$F(1, 34)= 1.92$, $p= .175$, partial $\eta^2= .053$
PND 60	$F(1, 34)= 3.19$, $p= .083$, partial $\eta^2= .086$
PND 65	$F(1, 34)= 3.34$, $p= .076$, partial $\eta^2= .089$
PND 70	$F(1, 34)= .4.43$, $p= .043$, partial $\eta^2= .115$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 32

Mean Body Weight (g) for Post-Injection Period for Yoked vs. Control

Main Effect or Interaction	F-statement
Day	$F(4, 128)= 979.09$, $p< .001$, partial $\eta^2= .968$
Day x Group	$F(4, 128)= 18.52$, $p< .001$, partial $\eta^2= .367$
Day x Object Phenotype	$F(4, 128)= .023$, $p= .999$, partial $\eta^2= .001$
Day x Group x Phenotype	$F(4, 128)= 4.57$, $p=.002$, partial $\eta^2= .125$

Note. Within-subjects subjects ANOVA is represented for PND 50 to 70

Table 33

Mean Body Weight (g) for Post-Injection Period for Yoked vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 32)= 1.82$, $p= .187$, partial $\eta^2= .054$
Distance Phenotype	$F(1, 32)= .010$, $p= .992$, partial $\eta^2= .000$
Group x Distance Phenotype	$F(1, 32)= 3.86$, $p= .058$, partial $\eta^2= .108$

Note. Between subjects ANOVA is represented

Table 34

Mean Bodyweight (g) During the Post-injection period for LR Yoked versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(4, 48)= 411.14$, $p< .001$, partial $\eta^2= .972$
Day x Group	$F(4, 48)= 2.17$, $p= .086$, partial $\eta^2= .153$
Group	$F(1, 12)= 3.68$, $p= .079$, partial $\eta^2= .235$

Note. Both within- and between subject effects are depicted

Table 35

Mean Bodyweight (g) During the Post-injection period for HR Yoked versus Control Animals

Main Effect or Interaction	F-Statement
Day	$F(4, 80)= 623.44$, $p< .001$, partial $\eta^2= .969$
Day x Group	$F(4, 48)= 26.05$, $p< .001$, partial $\eta^2= .566$
Group	$F(1, 20)= .280$, $p= .602$, partial $\eta^2= .014$

Note. Both within- and between subject effects are depicted

Table 36

Mean Body Weight for HR Yoked Versus Control Animals for Each Day of the Post-injection Period

Main Effect	F-Statement
PND 50	$F(1, 12)= 11.52$, $p= .005$, partial $\eta^2= .490$
PND 55	$F(1, 12)= 3.68$, $p= .079$, partial $\eta^2= .235$
PND 60	$F(1, 12)= 2.61$, $p= .133$, partial $\eta^2= .178$
PND 65	$F(1, 12)= 2.62$, $p= .132$, partial $\eta^2= .179$
PND 70	$F(1, 12)= 2.06$, $p= .177$, partial $\eta^2= .146$

Note. Significance indicates there was a significant Main effect for group on that PND

Table 37

Mean Body Weight for Yoked Versus Control Animals for the Post-injection Period

Main Effect	F-Statement
PND 50	$F(1, 36)= 16.37$, $p< .001$, partial $\eta^2= .313$
PND 55	$F(1, 36)= .416$, $p= .523$, partial $\eta^2= .011$
PND 60	$F(1, 36)= .003$, $p= .953$, partial $\eta^2= .000$
PND 65	$F(1, 36)= .006$, $p= .937$, partial $\eta^2= .000$
PND 70	$F(1, 36)= .087$, $p= .770$, partial $\eta^2= .002$

Note. Significance indicates there was a significant Main effect for group on that PND

Body Weight for Post-Injection Period: Object Phenotype

Table 38

Mean Body Weight (g) for Post-Injection Period for Drug vs. Control

Main Effect or Interaction	F-statement
Day	$F(4, 136)= 860.51$, $p< .001$, partial $\eta^2= .962$
Day x Group	$F(4, 136)= 9.13$, $p< .001$, partial $\eta^2= .212$
Day x Object Phenotype	$F(4, 136)= .637$, $p= .637$, partial $\eta^2= .018$
Day x Group x Phenotype	$F(4, 136)= .632$, $p=.640$, partial $\eta^2= .018$

Note. Within-subjects subjects ANOVA is represented for PND 50 to 70

Table 39

Mean Body Weight (g) for Post-Injection Period for Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 34)= 4.29$, $p= .046$, partial $\eta^2= .112$
Object Phenotype	$F(1, 34)= .107$, $p= .746$, partial $\eta^2= .003$
Group x Object Phenotype	$F(1, 34)= .031$, $p= .861$, partial $\eta^2= .001$

Note. Between subjects ANOVA is represented

Table 40

Mean Body Weight (g) for Post-Injection Period for Yoked vs. Control

Main Effect or Interaction	F-statement
Day	$F(4, 136)= 1035.60$, $p< .001$, partial $\eta^2= .968$
Day x Group	$F(4, 136)= 24.60$, $p< .001$, partial $\eta^2= .420$
Day x Object Phenotype	$F(4, 136)= .287$, $p= .886$, partial $\eta^2= .008$
Day x Group x Phenotype	$F(4, 136)= .502$, $p=.734$, partial $\eta^2= .015$

Note. Within-subjects subjects ANOVA is represented for PND 50 to 70

Table 41

Mean Body Weight (g) for Post-Injection Period for Yoked vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 34)= .409$, $p= .527$, partial $\eta^2= .012$
Object Phenotype	$F(1, 34)= .016$, $p= .900$, partial $\eta^2= .000$
Group x Object Phenotype	$F(1, 34)= .081$, $p= .777$, partial $\eta^2= .002$

Note. Between subjects ANOVA is represented

Open Field Task: Activity

Table 42

Mean Distance Traveled between Yoked vs Control

Main Effect or Interaction	F-statement
Group	$F(1, 30)= .111$, $p= .741$, partial $\eta^2= .004$
Estrous	$F(1, 30)= .547$, $p= .465$, partial $\eta^2= .018$
Distance Phenotype	$F(1, 30)= .047$, $p= .830$, partial $\eta^2= .002$
Group x Estrous	$F(1, 30)= 2.356$, $p= .135$, partial $\eta^2= .073$
Group x Phenotype	$F(1, 30)= .078$, $p= .783$, partial $\eta^2= .003$
Estrous x Phenotype	$F(1, 30)= .133$, $p= .717$, partial $\eta^2= .004$
Group x Estrous x Phenotype	$F(1, 30)= 2.627$, $p= .116$, partial $\eta^2= .081$

Notes. Distance is measured in meters. Distance phenotype is used for this table.

Table 43

Mean Distance Traveled between Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 29)= ..078$, $p= .782$, partial $\eta^2= .003$
Estrous	$F(1, 29)= .037$, $p= .849$, partial $\eta^2= .001$
Distance Phenotype	$F(1, 29)= .595$, $p= .447$, partial $\eta^2= .020$
Group x Estrous	$F(1, 29)= .694$, $p= .412$, partial $\eta^2= .023$
Group x Phenotype	$F(1, 29)= .723$, $p= .402$, partial $\eta^2= .024$
Estrous x Phenotype	$F(1, 29)= .965$, $p= .334$, partial $\eta^2= .032$
Group x Estrous x Phenotype	$F(1, 29)= .405$, $p= .529$, partial $\eta^2= .014$

Notes. Distance is measured in meters. Distance phenotype is used for this table.

Open Field Task: Anxiety

Table 44

Mean Percent of Time in Center Zone for Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 29)= .014$, $p= .905$, partial $\eta^2< .001$
Estrous	$F(1, 29)= .202$, $p= .656$, partial $\eta^2= .007$
Distance Phenotype	$F(1, 29)= 1.065$, $p= .311$, partial $\eta^2= .035$
Group x Estrous	$F(1, 29)= .460$, $p= .503$, partial $\eta^2= .016$
Group x Phenotype	$F(1, 29)= .158$, $p= .694$, partial $\eta^2= .005$
Estrous x Phenotype	$F(1, 29)= 2.520$, $p= .123$, partial $\eta^2= .080$
Group x Estrous x Phenotype	$F(1, 29)= .068$, $p= .797$, partial $\eta^2= .002$

Notes. Distance phenotype is used for this table.

Table 45

Mean Percent of Time in CZ for Yoked vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 30)= 2.456$, $p= .128$, partial $\eta^2= .076$
Estrous	$F(1, 30)= .028$, $p= .868$, partial $\eta^2= .001$
Distance Phenotype	$F(1, 30)= 2.838$, $p= .102$, partial $\eta^2= .086$
Group x Estrous	$F(1, 30)= 2.379$, $p= .133$, partial $\eta^2= .073$
Group x Phenotype	$F(1, 30)= .830$, $p= .370$, partial $\eta^2= .027$
Estrous x Phenotype	$F(1, 30)= .006$, $p= .941$, partial $\eta^2 <.001$
Group x Estrous x Phenotype	$F(1, 30)= 2.867$, $p= .101$, partial $\eta^2= .087$

Notes. Distance phenotype is used for this table.

Object Placement

Table 46

Mean Time in Contact with Object A vs. B between Drug vs. Control

Main Effect or Interaction	F-statement
Object	$F(1, 29)= 12.94$, $p= .001$, partial $\eta^2= .309$
Object x Estrous	$F(1, 29)= .723$, $p= .402$, partial $\eta^2= .024$
Object x Object Phenotype	$F(1, 29)= .003$, $p= .955$, partial $\eta^2= .000$
Object x Group x Estrous	$F(1, 29)= .277$, $p= .603$, partial $\eta^2= .009$
Object x Group x Phenotype	$F(1, 29)= .093$, $p= .762$, partial $\eta^2= .003$
Object x Estrous x Phenotype	$F(1, 29)= .604$, $p= .443$, partial $\eta^2= .020$
Object x Group x Estrous x Phenotype	$F(1, 29)= .857$, $p= .362$, partial $\eta^2 = .029$

Notes. Represents within-subjects ANOVA on the Object Placement task.

Table 47

Mean Time in Contact with Object A vs. B between Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 29)= 1.375$, $p= .250$, partial $\eta^2= .045$
Estrous	$F(1, 29)= .652$, $p= .426$, partial $\eta^2= .022$
Object Phenotype	$F(1, 29)= .064$, $p= .802$, partial $\eta^2= .002$
Group x Phenotype	$F(1, 29)= 4.62$, $p= .040$, partial $\eta^2= .137$
Group x Estrous	$F(1, 29)= .238$, $p= .629$, partial $\eta^2= .008$
Estrous x Phenotype	$F(1, 29)= .752$, $p= .393$, partial $\eta^2= .025$
Group x Estrous x Phenotype	$F(1, 29)= .807$, $p= .376$, partial $\eta^2= .027$

Note. Between subjects ANOVA is represented

Table 48

Mean Time in Contact with Object A versus B for LR Drug versus Control Animals

Main Effect or Interaction	F-Statement
Object	$F(1, 15)= 12.46$, $p= .003$, partial $\eta^2= .454$
Object x Group	$F(1, 15)= 4.89$, $p= .043$, partial $\eta^2= .246$
Group	$F(1, 15)= 6.93$, $p= .019$, partial $\eta^2= .316$

Note. Both within- and between subject effects are depicted

Table 49

Mean Time in Contact with Object A versus B for HR Drug versus Control Animals

Main Effect or Interaction	F-Statement
Object	$F(1, 18)= 6.04$, $p= .024$, partial $\eta^2= .251$
Object x Group	$F(1, 18)= .594$, $p= .451$, partial $\eta^2= .032$
Group	$F(1, 18)= .435$, $p= .518$, partial $\eta^2= .024$

Note. Both within- and between subject effects are depicted

Table 50

Mean Time in Contact with Object A vs. B between Yoked vs. Control

Main Effect or Interaction	F-statement
Object	$F(1, 29)= 5.74$, $p< .023$, partial $\eta^2= .165$
Object x Group	$F(1, 29)= .570$, $p= .456$, partial $\eta^2= .019$
Object x Estrous	$F(1, 29)= .332$, $p= .569$, partial $\eta^2= .011$
Object x Object Phenotype	$F(1, 29)= .516$, $p= .478$, partial $\eta^2= .017$
Object x Group x Estrous	$F(1, 29)= .538$, $p= .469$, partial $\eta^2= .018$
Object x Group x Phenotype	$F(1, 29)= .231$, $p= .634$, partial $\eta^2= .008$
Object x Estrous x Phenotype	$F(1, 29)= .051$, $p= .823$, partial $\eta^2= .002$
Object x Group x Estrous x Phenotype	$F(1, 29)= .134$, $p= .717$, partial $\eta^2 = .005$

Notes. Represents within-subjects ANOVA on the Object Placement task.

Table 51

Mean Time in Contact with Object A vs. B between Yoked vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 29)= .000$, $p= .988$, partial $\eta^2= .000$
Estrous	$F(1, 29)= .506$, $p= .483$, partial $\eta^2= .017$
Object Phenotype	$F(1, 29)= .916$, $p= .346$, partial $\eta^2= .031$
Group x Phenotype	$F(1, 29)= .712$, $p= .406$, partial $\eta^2= .024$
Group x Estrous	$F(1, 29)= .223$, $p= .641$, partial $\eta^2= .008$
Estrous x Phenotype	$F(1, 29)= .631$, $p= .433$, partial $\eta^2= .021$
Group x Estrous x Phenotype	$F(1, 29)= .280$, $p= .601$, partial $\eta^2= .010$

Note. Between subjects ANOVA is represented

Object Recognition

Table 52

Mean Time in Contact with Object A vs. B between Drug vs. Control

Main Effect or Interaction	F-statement
Object	$F(1, 27)= 22.09$, $p< .001$, partial $\eta^2= .451$
Object * Group	$F(1, 27)= .040$, $p= .844$, partial $\eta^2= .001$
Object * Estrous	$F(1, 27)= .497$, $p= .487$, partial $\eta^2= .018$
Object * Phenotype	$F(1, 27)= .775$, $p= .387$, partial $\eta^2= .028$
Object * Group * Estrous	$F(1, 27)= .391$, $p= .537$, partial $\eta^2= .014$
Object * Group * Phenotype	$F(1, 27)= .125$, $p= .727$, partial $\eta^2= .005$
Object *Estrous* Phenotype	$F(1, 27)= .000$, $p= .997$, partial $\eta^2= .000$
Object * Group * Estrous * Phenotype	$F(1, 27)= .127$, $p= .682$, partial $\eta^2= .006$

Note. Within-subject ANOVA depicted

Table 53

Mean Time in Contact with Object A vs. B between Drug vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 27)= .059$, $p= .809$, partial $\eta^2= 002$
Estrous	$F(1, 27)= 1.095$, $p= .305$, partial $\eta^2= .039$
Object Phenotype	$F(1, 27)= .185$, $p= .670$, partial $\eta^2= .007$
Group x Estrous	$F(1, 27)= .102$, $p= .752$, partial $\eta^2= .004$
Estrous x Phenotype	$F(1, 27)= .011$, $p= .919$, partial $\eta^2= .000$
Group x Phenotype	$F(1, 27)= .402$, $p= .531$, partial $\eta^2= .015$
Group x Estrous x Phenotype	$F(1, 27)= 4.37$, $p= .046$, partial $\eta^2= .139$

Notes. Between-subjects ANOVA depicted

Table 54

Mean Time in Contact with Object A versus B for Drug group by Estrous Group LR animals

Main Effect or Interaction	F-Statement
Object	$F(1, 11)= 9.15$, $p= .012$, partial $\eta^2= .454$
Object x Group	$F(1, 11)= .191$, $p= .670$, partial $\eta^2= .017$
Object x Estrous	$F(1, 11)= .308$, $p= .590$, partial $\eta^2= .027$
Object x Estrous x Group	$F(1, 11)= .028$, $p= .870$, partial $\eta^2= .003$
Group	$F(1, 11)= .225$, $p= .645$, partial $\eta^2= .020$
Estrous	$F(1, 11)= .385$, $p= .547$, partial $\eta^2= .034$
Group x Estrous	$F(1, 11)= .914$, $p= .359$, partial $\eta^2= .077$

Note. Both within- and between subject effects are depicted

Table 55

Mean Time in Contact with Object A versus B for Drug group by Estrous Group HR animals

Main Effect or Interaction	F-Statement
Object	$F(1, 16)= 15.48$, $p= .001$, partial $\eta^2= .492$
Object x Group	$F(1, 16)= .012$, $p= .915$, partial $\eta^2= .001$
Object x Estrous	$F(1, 16)= .250$, $p= .624$, partial $\eta^2= .015$
Object x Estrous x Group	$F(1, 16)= .536$, $p= .475$, partial $\eta^2= .032$
Group	$F(1, 16)= .135$, $p= .718$, partial $\eta^2= .008$
Estrous	$F(1, 16)= .788$, $p= .338$, partial $\eta^2= .047$
Group x Estrous	$F(1, 16)= 5.14$, $p= .038$, partial $\eta^2= .243$

Note. Both within- and between subject effects are depicted

Table 56

Mean Time in Contact with Object A versus B for Drug versus control HR animals in the Proestrus Group

Main Effect or Interaction	F-Statement
Object	$F(1, 8)= 7.47$, $p= .026$, partial $\eta^2= .483$
Object x Group	$F(1, 8)= .448$, $p= .522$, partial $\eta^2= .053$
Group	$F(1, 8)= 2.11$, $p= .184$, partial $\eta^2= .209$

Note. Both within- and between subject effects are depicted

Table 57

Mean Time in Contact with Object A versus B for Drug versus control HR animals in the Diestrus Group

Main Effect or Interaction	F-Statement
Object	$F(1, 8)= 8.18$, $p= .021$, partial $\eta^2= .506$
Object x Group	$F(1, 8)= .162$, $p= .698$, partial $\eta^2= .020$
Group	$F(1, 8)= 3.05$, $p= .119$, partial $\eta^2= .276$

Note. Both within- and between subject effects are depicted

Table 58

Mean Time in Contact with Object A for HR animals across Drug group and Estrous Group

Main Effect or Interaction	F-Statement
Group	$F(1, 16)= .897$, $p= .358$, partial $\eta^2= .053$
Estrous	$F(1, 16)= .901$, $p= .357$, partial $\eta^2= .053$
Group x Estrous	$F(1, 16)= 11.43$, $p= .004$, partial $\eta^2= .417$

Note. Between subject effects are depicted

Table 59

Mean Time in Contact with Object B for HR animals across Drug group and Estrous Group

Main Effect or Interaction	F-Statement
Group	$F(1, 16)= .023$, $p= .880$, partial $\eta^2= .001$
Estrous	$F(1, 16)= .537$, $p= .474$, partial $\eta^2= .032$
Group x Estrous	$F(1, 16)= 2.59$, $p= .127$, partial $\eta^2= .140$

Note. Between subject effects are depicted

Table 60

Mean Time in Contact with Object A for HR animals for Drug versus Control

Main Effect or Interaction	F-Statement
Group (Diestrus Group)	$F(1, 8)= 8.20$, $p= .021$, partial $\eta^2= .506$
Group (Proestrus Group)	$F(1, 8)= 3.49$, $p= .099$, partial $\eta^2= .303$

Note. The main effect is for drug group for the HR animals in either the Diestrus or Proestrus Groups

Table 61

Mean Time in Contact with Object A for HR Control animals Across Estrous Group

Main Effect or Interaction	F-Statement
Estrous	$F(1, 7)= 6.27$, $p= .041$, partial $\eta^2= .472$

Note. The main effect is for estrous group for the HR control animals

Table 62

Mean Time in Contact with Object A vs. B between Yoked vs. Control

Main Effect or Interaction	F-statement
Object	$F(1, 28)= 24.58$, $p< .001$, partial $\eta^2= .467$
Object * Group	$F(1, 28)= .001$, $p= .970$, partial $\eta^2= .000$
Object * Estrous	$F(1, 28)= .771$, $p= .388$, partial $\eta^2= .027$
Object * Phenotype	$F(1, 28)= .749$, $p= .394$, partial $\eta^2= .026$
Object * Group * Estrous	$F(1, 28)= .229$, $p= .636$, partial $\eta^2= .008$
Object * Group * Phenotype	$F(1, 28)= 1.968$, $p= .172$, partial $\eta^2= .066$
Object *Estrous* Phenotype	$F(1, 28)= .041$, $p= .842$, partial $\eta^2= .001$
Object * Group * Estrous * Phenotype	$F(1, 28)= .393$, $p= .536$, partial $\eta^2= .014$

Notes. Within-subjects ANOVA depicted

Table 63

Mean Time in Contact with Object A vs. B between Yoked vs. Control

Main Effect or Interaction	F-statement
Group	$F(1, 28)= .026$, $p= .874$, partial $\eta^2= .001$
Estrous	$F(1, 28)= .762$, $p= .390$, partial $\eta^2= .026$
Object Phenotype	$F(1, 28)= .084$, $p= .774$, partial $\eta^2= .003$
Group x Estrous	$F(1, 28)= .184$, $p= .671$, partial $\eta^2= .007$
Estrous x Phenotype	$F(1, 28)= 1.978$, $p= .171$, partial $\eta^2= .066$
Group x Phenotype	$F(1, 28)= .234$, $p= .632$, partial $\eta^2= .008$
Group x Phenotype x Estrous	$F(1, 28)= .237$, $p= .630$, partial $\eta^2= .008$

Notes. Between-subjects ANOVA depicted