EFFECT OF AN ADOLESCENT WESTERN DIET ON THE BRAIN, CANNABINOID SENSITIVITY, AND IMPULSIVE EATING BEHAVIOR

by

RAWAD KHALIL BASMA

(Under the Direction of Emily Noble)

ABSTRACT

Adolescence is a critical period for neurodevelopment, where environmental factors have long-term effects on the brain. Increased consumption of a Western Diet (WD) has raised concern regarding long-term cognitive consequences. This study investigated how adolescent WD consumption influences the adult brain and behavior, particularly impulsive action for sucrose. We further investigated how an adolescent WD impacts sensitivity to cannabinoids and cannabinoid responses. Male rats were fed WD or chow in their adolescence, after which all rats switched to a chow diet so that we could exclusively measure lasting effects of adolescent WD consumption. WD-fed male rats exhibited increased caloric intake and sustained body weight gain while on the diet, but there was no long-term effect of an adolescent WD on impulsive behavior during adulthood. This contrasts with our prior studies in female rats, where WD-fed rats increased impulsive responding, suggesting sex dependent effects of WD on impulsive responding for sucrose. Cannabinoids, which increase impulsive responding for sucrose in

females, had no effect on impulsive responding for food in males. However, preliminary findings suggest that rats fed a WD during adolescence demonstrate exacerbated hyperphagic responses to the cannabinoid receptor agonist CP55940. These findings emphasize the need for further research on sex differences in dietary effects on neurodevelopmental outcomes.

Index Words: Adolescence, Critical Period, Cannabinoid, Western Diet, Impulsivity, Adult Behavior

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DEDICATION

This thesis is dedicated to everyone who played a role in my life over the last five years. To my parents Khalil and Lama, who have supported me in every sense of the word, thank you for all of your help along the way. To my sister Rand, who is the main influence for who I am today, thank you for being you. To all of my friends both domestic and abroad, thank you for all the good times and memories to come. To my pets, Mia and Meru, thank you for being great emotional support animals and cuddling me when I need it most. To anyone reading this, thanks for lending your eyes.

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INTRODUCTION AND LITERATURE REVIEW

Introduction

The stage of life from birth through adolescence is a critical period of brain development, which is when brain plasticity is at its peak and the brain is susceptible to the greatest impact from environmental factors such as diet[1]. While much of the brain has transitioned to a more fixed state by the end of childhood, adolescence is a still a critical period for regions of the brain such as the prefrontal cortex and hippocampus, and these regions play an important role in modulating impulse control[2-5]. Understanding the impact of dietary factors on the brain is becoming of increased importance due to the rise of consumption of the Western Diet (WD), which has been extensively shown to negatively impact the brain and neurocognitive functions in both humans and in animal models[6, 7]. A WD is defined as containing a high percentage of saturated fat and refined sugars making the food highly palatable[8]. In the U.S., despite its energy density, consuming a WD is highly affordable and easily accessible, thus, with the increasing cost of living and groceries, poor dietary outcomes around increased consumption of the WD is expected to increase in future years [9]. Thus, the need for investigating the long-term impact of an adolescent WD consumption on brain function is high, as adolescents are some of the highest consumers of saturated fat and added sugars, with 85% of males and 78% of females exceed the recommended 10% total caloric intake from saturated fats, as well as 61% of males and 57% of females exceeding the recommended intake for added sugars. The stage of life

from birth through adolescence is a critical period of brain development, which is when brain plasticity is at its peak and the brain is susceptible to the greatest impact from environmental factors such as diet[1]. While much of the brain has transitioned to a more fixed state by the end of childhood, adolescence is a still a critical period for regions of the brain such as the prefrontal cortex and hippocampus, and these regions play an important role in modulating impulse control[2-5]. Understanding the impact of dietary factors on the brain is becoming of increased importance due to the rise of consumption of the Western Diet (WD), which has been extensively shown to negatively impact the brain and neurocognitive functions in both humans and in animal models[6, 7]. A WD is defined as containing a high percentage of saturated fat and refined sugars making the food highly palatable[8]. In the U.S., despite its energy density, consuming a WD is highly affordable and easily accessible, thus, with the increasing cost of living and groceries, poor dietary outcomes around increased consumption of the WD is expected to increase in future years [9]. Thus, the need for investigating the long-term impact of an adolescent WD consumption on brain function is high, as adolescents are some of the highest consumers of saturated fat and added sugars, with 85% of males and 78% of females exceed the recommended 10% total caloric intake from saturated fats, as well as 61% of males and 57% of females exceeding the recommended intake for added sugars[10]. As of 2018, reports highlight that children aged 2-19 consume 67% of their calories from ultra processed food containing high proportions of saturated fats and added sugars [11].

Along with a high prevalence of consumption of the WD, Americans are increasingly using more recreational drugs, such as cannabis[12]. Cannabis acts on cannabinoid receptors, membrane bound G-protein coupled receptors that cannabinoids bind to that mediate the effects of the cannabinoid system[13]. The cannabinoid system modulates various physiological

functions including energy homeostasis, hyperphagia, pain management, anxiety, and appetite regulation[13-16]. States are revaluating their long-standing precedent of cannabis as an illegal drug, with 18 states and Washington DC fully legalizing cannabis, with an additional 18 states medically legalizing it as well[17]. Because of this recent legality shift, many users, both adult and adolescent, are being led to think cannabis consumption is of little to no consequence[12]. Data suggests that over the last two decades marijuana consumption has doubled with at least 19% of Americans regularly use cannabis, making it the most widely consumed psychoactive drug[18]. Cannabinoid use may not be of little or no consequence, for example, in humans use of cannabinoids has been suggested to exacerbate symptoms of trait impulsivity, and to reduce activation of brain regions such as the prefrontal cortex [19] . Therefore, understanding the effects of cannabis, and particularly the consequence on behaviors such as impulsivity, is of critical importance.

Western Diet consumption during adolescence: impact on brain development

Consumption of a WD at an early age does not only increase risk for obesity, heart disease, and many other preventable chronic health conditions, it also alters brain development and can lead to lasting effects on behavior during adulthood [20, 21].

It is well-known that diets high in saturated fats and added sugars impair cognitive function. Greenwood and Winocur (1990) demonstrated that rats fed a high saturated fat diet exhibited deficits in learning and memory tasks[22]. These findings were further supported by the same authors in 1999 when they concluded that learning deficits in rats induced by a high saturated fat diet were independent of obesity and were linked to impaired functioning of the

hippocampus—a region of the brain which plays a vital role in meal size, meal onset, and food impulsivity[23]. Further research focused on the restricting the consumption of a WD to adolescence have suggested that the effects of these dietary factors on the hippocampus may be long lasting when the diets are consumed during this critical period of development. Nyaradi et al. (2014) showed that WD consumption during adolescence disrupts learning, memory, and executive function leading to non-reversible long-term deficits in cognitive functioning[6]. Evidence from rodent models shows that dietary factors that make up a WD can have an impact on the brain and hippocampal function that are not easily reversible. Boitard et al. (2012) found that adolescent rats fed a WD had lasting impairments in learning outcomes when compared with adults on the same diet[24]. Evidence suggests that diets high in sugar consumed during adolescence negatively affect hippocampus dependent learning and memory function [25-28]. Further research investigating the neurological effects of consumption of a high fat diet (HFD) during adolescence found that HFD fed rats had significantly more impaired hippocampal dependent memory [29-31]. The synthesis of high sugar consumption and HFD produced similar findings in other studies. It was concluded that consuming both HFD with added sugars resulted in loss of hippocampal memory function in various memory assessments [32-34] Further research conducted by Labouesse et al. (2017) uncovered a mechanism for the neurological stress induced by the WD involving synaptic modulator reelin (RELN) as a critical mediator of this vulnerability[35]. The researches propose this is because peri adolescent WD consumption selectively downregulates prefrontal RELN+ cells and that augmenting the medial prefrontal cortex (mPFC) RELN levels using transgenic methods or prefrontal pharmacology prevents the WD-induced prefrontal cognitive deficits[35]. The study goes on to further identify N-methyl-D-aspartate-dependent long-term depression (NMDA-LTD) at prefrontal excitatory synapses as a

synaptic signature because perinatal WD consumption abolishes NMDA-LTD, a function that is restored by RELN overexpression[35]. This study provides novel mechanistic insight into the vulnerability of the adolescent mPFC towards nutritional stress, such as WD's. A study conducted by Kanoski et al. (2007) found that unrestricted access to a high fat diet (HFD) impaired discrimination reversal learning and reduced brain derived neutrophic factor (BDNF) in the prefrontal cortex and ventral hippocampus[36]. Rats who were given the HFD diet responded more than controls to the previously rewarded cue at the outset of discrimination reversal[36]. Because of this mounting evidence of WD adjacent feeds effect on the brain, one of the aims of this thesis is to determine the impact of a WD consumption during adolescence on neural function as measured by impulsivity testing.

Hargrave et al. (2016) proposed a "vicious cycle" model, where WD induced cognitive impairments disrupt the ability to properly regulate eating behaviors, which leads to further overconsumption of unhealthy foods[37]. This positive feedback loop could explain why individuals who habitually consume WD struggle with dietary self-regulation, however the vicious cycle hypothesis has not been fully investigated. There is some scientific support, as the hippocampus, which is negatively impacted by a WD, has been shown also to modulate eating behaviors. For example, lesions to the hippocampus make animals not able to discriminate whether they are hungry or full, confirming the hippocampus role in feeding behaviors[34]. Another study was conducted investigating the potential mechanisms behind hippocampal dependent eating behaviors. This study found that ventral hippocampus (vHPC) ghrelin signaling produced behvaior that indicated a perceived state of hunger, irrespective of satiety signals in the body including cholecystokinin, exendin-4 (a glucagon-like peptide-1 receptor agonist), amylin, and mechanical distension of the stomach[38]. Imaging of the brain revealed this behavior

occurred due to a connection that exists between vHPC neurons to the lateral hypothalamic area orexin (hypocretin)-producing neurons that then project to the laterodorsal tegmental nucleus in the hindbrain[38]. While these studies show that the WD negatively impacts hippocampal dependent spatial memory function, episodic memory, and eating behaviors, the impact of a WD on other types of behaviors, such as hippocampal dependent eating behaviors remains unexplored. One goal of this research was to fill this critical knowledge gap.

Impulsive Behavior

Impulsivity is defined as acting rapidly without full consideration of the consequences. Impulsive behavior plays a role in various physiological processes such as substance abuse, compulsive overeating, and obesity. Studies in humans have shown that diet score and impulsive behavior are negatively correlated[39], and even resulted in self-senses of heightened urgency among human subjects[40]. Despite the documentation of these associations between diet and impulsive behavior, it is unknown how WD consumption as an adolescent will affect behavior into adulthood, which is a knowledge gap we seek to address.

Several brain regions are critically involved in regulating impulse control, and notably the hippocampus is one of them. Noble et al. (2019) identified a neural pathway linking the hypothalamus to the hippocampus, melanin concentrating hormone producing neurons, that when selectively activated increased impulsive responding for food, indicating that the hippocampus is important for food impulsive behavior[25]. Specifically, melanin concentrating hormone increased impulsive responding in the Differential Low Rates of Responding (DRL) task, and preference for immediate rewards in the delay discounting task[25]. Mechanistic

insight was uncovered by Hsu et al (2018) when they revealed a novel neural pathway which highlights the role the hippocampus and prefrontal cortex play in higher order feeding behavior. Monosynaptic glutamatergic ventral hippocampal field CA1 (vCA1) to mPFC connectivity inhibits food-motivated behaviors through vCA1 glucagon-like peptide-1 receptor (GLP-1R)[2]. Through this pathway, researchers discovered that communication to mPFC NMDA receptors inhibits impulsive response for palatable food[2]. Given that the hippocampus and prefrontal cortex are in a critical period of development during adolescence, and that a WD consumption during adolescence has been shown to impact these brain regions[2, 25], we hypothesized that a WD consumed during adolescence would increase hippocampal dependent impulsive eating behavior.

While the hippocampus is important for impulse control likely through impacting inhibitory control, other brain regions may impact impulsivity through affecting reward signaling. Jacques et al. (2019) reviewed evidence showing that excessive sugar consumption alters the mesocorticolimbic dopamine system, which is central to reward processing and impulsivity[41]. The overconsumption of sugar leads to dopaminergic dysregulation, reinforcing impulsive eating behaviors similar to that of someone suffering from a substance abuse disorder[42]. Increases in dopamine signaling as the result of increases sugar consumption delays the release of acetylcholine during feeding, which then postpones satiety signaling leading to overconsumption of food[43].

The Relationship Between the Cannabinoid System and the Western Diet

Emerging research suggests that the WD impacts the endocannabinoid system (ECS), a crucial regulator of appetite, metabolism, and various neurophysiological processes[44, 45]. The ECS comprises endogenous cannabinoids (AEA and 2-AG), cannabinoid receptors (CB1 and CB2), and enzymes involved in cannabinoid biosynthesis and degradation[16]. Studies indicate that chronic consumption of a WD enhances endocannabinoid signaling, particularly at peripheral CB1 receptors within the intestine, which heavily associate with hyperphagia and obesity[44, 46]. Argueta et al. (2017) found that mice fed a WD exhibited increased levels of the endocannabinoids 2-arachidonoyl-sn-glycerol (2-AG) and anandamide (AEA) in both the upper small intestine and plasma [34]. These increases in AEA and 2-AG correlated with increased gene expression of *fatty acid amide hydrolase and monoacylglycerol lipase*, which are AEA and 2-AG respective degradation enzymes, within the jejunum and plasma [34]. Furthermore, the increases of AEA and 2-AG among WD-fed rats corresponded with decreased expression of cannabinoid *receptor 1 and cannabinoid receptor 2,* the cannabinoid receptor 1 and 2 coding gene, compared to standard chow-fed rats [34]. Pharmacological inhibition of peripheral CB1 receptors normalized feeding behaviors, suggesting that enhanced cannabinoid signaling in the periphery contributes to overeating [34].

The dysregulation of cannabinoid receptors and metabolic enzymes in animals on a WD suggest that the WD has a direct impact on the ECS, however whether a WD has a lasting impact on the ECS when consumed during adolescence remains unknown. In addition to overeating, Avalos et al. (2020) showed that activation of CB1 receptors in the intestinal epithelium are the main driver for preferences toward WD, suggesting the presence of further interactions between the WD and the cannabinoid system[46]. In this study, Avalos et al. showed that ECS signaling

is increased in the gut in mice after consumption of palatable fatty foods, which in turn increases consumption of fatty foods [36]. The authors then conducted a systemic blockade of CB1 receptors via CB1 receptor antagonist AM251 which reduced WD preference in mice [36]. Similarly, genetically modified mice lacking CB1 receptors in the intestinal epithelium displayed reduced preferences for WD, supporting the notion that the ECS is driving the consumption of palatable, high fat/high sugar diets via intestinal endocannabinoid signaling. For this reason, it is likely that edible cannabinoids may have an impact on the regulation of food palatable food consumption, which is a topic of investigation of this thesis. Beyond metabolic implications, the WD also potentially alters cannabinoid sensitivity in the brain, particularly in the hippocampus, as WD consumption has been shown to alter drug sensitivity in this brain region[47]. Rosenberger et al. (2016) explored the interaction between WD and midazolam which is a type of benzodiazepine, a class of depressant drugs used to combat spasms and anxiety, revealing that rats fed a WD had exacerbated hippocampal dependent spatial memory deficits following midazolam treatment [37]. These results suggest that metabolic disruptions induced by the WD could heighten the brain's sensitivity to pharmacological agents that act on the central nervous system, such as cannabinoids, however this avenue is yet to be further explored.

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Chapter 2

ADOLESCENT EXPOSURE TO THE WESTERN DIET EFFECTS ON ADULT BEHAVIOR AND THE RELATIONSHIP WITH THE CANNABINOID SYSTEM

1 Introduction

The period from birth through adolescence is a critical for brain development, as this is when brain plasticity is at its peak and the brain is most influenced by environmental factors like diet[1]. While much of the brain becomes more fixed by the end of childhood, regions like the prefrontal cortex and hippocampus remain sensitive during adolescence and play a key role in many feeding behaviors[34, 35]. TheWestern Diet (WD), which is rich in saturated fats and refined sugars, has been shown to negatively affect neurocognitive function in both humans and animals, especially learning and memory function that relies on regions such as the hippocampus and prefrontal cortex[21, 23]. Adolescents exceed the recommended intake of saturated fats and added sugars [10]and this along with the fact that their brains are still developing makes them potentially at risk for long-term neurocognitive dysfunction. Indeed, prior studies have shown that a WD consumption during adolesence has long term consequences for hippocampal dependent spatial and episodic memory function [28, 32, 33, 48]. However, less well known is the impact of an adolescent WD consumption on hippocampus- and PFC-dependent eating behaviors, such as food impulsivity. Therefore we set out to determine whether adolescent WD consumption impacts food impulsive action during adulthood. We tested animals after they had been off of the WD such that we could assess the isolated effects of the adolescent WD on brain development.

When planning how to test the Western Diet's (WD) effects on adult behavior, it was crucial that we did not bias our results by having the test subjects exposed to the diet longer than necessary. This is because other studies have already shown what happens when WD is consumed throughout the lifespan, and if the diet is consumed past the critical period, we could skew the results. In rodents, the literature has related human ages to rodent ages to give researchers a better frame of reference of how to time their studies. The literature found that adulthood begins postnatal day (PND) 63 and in male rats' puberty occurs between PND 42-49[49]. We decided we would give rats a WD as early as possible on PND 26 and follow through with this diet until adulthood at PND 63.

As an additional aim of these studies, we investigated whether an adolescent WD consumption impacts cannabinoid-induced feeding behavior. There is an increasing use of recreational drugs like cannabis in the U.S.[12]. Cannabis acts on the cannabinoid system, which regulates key physiological functions such as appetite and energy balance[13, 16]. The interactions between the WD effects on the brain and the the role the ECS plays is less understood. This thesis seeks to bridge this gap in knowledge. Basic mechanisms underlying how cannabinoids modulate behavior and metabolic processes in past studies usually were conducted using intraperitoneal injections of cannabinoid receptor ligands as the route of administration. The main benefit of injectable cannabinoid administration is that the dose in the blood can be controlled. However, cannabinoid drugs are not typically injected whether it be recreational or clinical settings; instead, cannabinoids are smoked or taken orally. Therefore, our lab has developed a novel model of edible cannabinoid consumption where CP55940, a THC-like cannabinoid, is dissolved in coconut oil and incorporated into a gelatin-based edible with

enhanced palatability[50]. The precise dose for each subject is then portioned according to the weight of the animal.

2. **Methods**

2.1 Animals

Twenty-four male Sprague Dawley rats (Inotiv, Indianapolis, IN, USA) arrived on post-natal day (PND) 24 and were individually housed in shoebox cages on a 12:12 reverse light/dark cycle in a temperature-controlled vivarium (22°C). Body weight, food intake, and fluid intake measurements were recorded three times a week on Monday, Wednesday, and Friday. Once behavioral testing began, food intake was recorded five times a week on every testing day. All procedures were approved by the Institute of Animal Care and Use Committee at the University of Georgia (Athens, GA, USA) (protocol number A2022 06-035-A12).

2.2 Experimental Groups / Diet intervention

Rats were divided into two experimental groups that were matched for initial body weights on PND 26 two days after arrival: Standard chow diet (LabDiet 5053, Picolab Rodent Diet 2013.1% kcal fat, n = 12) with two bottles of water or a Western Diet (WD) (Research Diets D12451, 45% kcal from fat; WD; n = 12) (Best Flavors, BF 8414, Orange, CA) with the choice of water or a 11% high fructose corn syrup solution (HFCS). The treatment group was kept on the WD until PND 63. After PND 63 both groups were fed standard chow diet and water until conclusion of study.

2.3 Differential Low Rates of Responding Task

DRL task takes place in an operant chamber designed to test impulsive action in rodents. The operant chamber functions via two retractable levers and one food receptacle. Rats are trained to associate one of the levers with earning a sucrose reinforcer (45 mg sucrose pellets, F0023, Bio-Serv, Flemington, NJ).Once rats associate the active lever with reward, rats must learn to withhold pressing on the active lever for a number of seconds that is progressively increased as training through the task ensues (0, 5, 10, or 20 seconds). The first week of training entails a fixed ratio 1 schedule (DRL0), where a lever press results in an immediate pellet delivery to the food receptacle. Automatic dispensary of a pellet occurs if a rat does not press the lever for 600 seconds (AutoShaping). A week after DRL0, a five second delay is installed (DRL5). So, rats must wait for 5 seconds after each lever press before pressing again or the reinforced lever will not deliver a sucrose pellet. Failure to wait for the required time results in a reset of the timer and no reinforcer earned. During the subsequent two weeks a ten second period must occur between presses for a sucrose reinforcer to be earned (DRL10). The last two weeks of training requires a twenty-second delay between lever presses to earn a reinforcer (DRL20). Training sessions occur over 45 minutes (one training session per day). During each session, a light above the active lever flashes for presses that result in a reward. In the final week of training, a tone accompanies active presses that result in a reward. Rats are officially trained in the task when they earn a stable efficiency score over multiple days. After training through DRL0-DRL10 is complete, DRL20 training occurs for three weeks before final testing of the task occurs for one day. Cannabinoid receptor agonist testing occurred for one day and followed a between subject's design. Animals were tested using a counterbalanced design on the DRL20 task. An efficiency score is calculated by dividing the number of pellets earned by the number of lever presses on

the reinforced (active) lever. The number of active and inactive presses, as well as the number of pellets earned were measured, and efficiency scores were calculated. All training and testing occurred during the dark phase.

2.4 Cannabinoid administration via gelatin-based edibles

Cannabinoids are lipophilic, so the presence of a lipophilic solvent, in this case coconut oil, is the vital component to successful drug delivery. Edibles were made in the laboratory via a 1:1:1 ratio of coconut oil and lecithin; Jello and potassium sorbate; and set gelatin. The nonselective dual cannabinoid receptor agonist CP55940 (Item No. 13608; Cayman Chemical, Ann Arbor, MI, USA) was first dissolved in 14 grams of coconut oil (Simple Truth Organic) and ¾ tsp lecithin (Earthfare, Athens, GA, USA) at the concentration of 0.18 mg/mL. In a separate container, 11.3 grams Jello and ¼ tsp potassium sorbate were added to 18 mL boiling deionized water after removal from the heat. The use of Jello allowed for multiple flavors to be employed and enhanced palatability, while potassium sorbate was used as a mold inhibitor. In a third vessel, 5 grams of gelatin is combined with 10 mL of water and allowed to set. Next, equal parts Jello and coconut oil mixtures are combined on a stir plate with constant stirring while a third equal part of set gelatin is added. The mixture is constantly stirred on a hot plate at 35-45°C. After five minutes of constant stirring, the homogenous mixture is portioned into two-milliliter molds using a syringe and allowed to set overnight at 4°C. Vehicle edibles are made in the exact same manner, absent of drug. This recipe makes a total of 12 2-mL edibles.

Edibles were delivered to subjects at 0.12 mg/kg during the DRL testing day and first food intake testing day by portioning the edible according to the weight of the animal. This does was chosen based on [50]. The two-milliliter molded edible contains 0.06 mg/mL (0.12 mg/2mL). Vehicle edibles are portioned and administered based on 2 mL/kg to match the size of

the drug edible. Once administered, edibles were voluntarily consumed by the rodents in two minutes or less. Bedding was meticulously scoured for any unconsumed edible, and rodents with leftover edible were excluded from analyses (n=0).

2.5 Chow intake following ingestion of edible cannabinoid receptor agonist

Three days prior to the first ingestion of a CB1 agonist containing edible, rats were habituated to the vehicle edible by giving them one-half of a whole 2 mL edible to consume. Food intake experiments followed a within subject's design. On the DRL CB1 agonist testing day, chow was removed 2 hours before the start of the dark cycle. Thirty minutes prior to the start of the dark cycle, rats received either CP55940-containg edible at 0.12 mg/kg or vehicle edible. At the beginning of the dark cycle, standard chow was returned to their cages, and total food intake data were collected on the hour for the first 4 hours after food was returned at the onset of the dark cycle, as well as 24 hours after food was returned.

2.6 Statistical Analyses

Data were analyzed by GraphPad Prism (Version 10.4.0). Body weight and calorie intake data were analyzed via a two-way repeated measures ANOVA with Bonferroni's multiple comparison test for post hoc analyses. DRL data were analyzed via a Student's two-tailed unpaired t-test.

3 Results

3.1 Body weight and food intake

Body weight and food intake were measured 3 times a week (MWF) 1 hour before the dark cycle began. Once DRL task started, food intake data was recorded 5 days per week. There was a time x diet interaction for body weight gain ($F_{(40, 880)} = 9.648$; P<.0001) with a main effect of diet ($F_{(1, 22)} = 10.48$; P=.0038) and time ($F_{(1.86, 40.94)} = 4642$; P<.0001). Post hoc analyses revealed that body weights were significantly heavier in the WD-fed animals on measurements 13-17, which occurred during the last measurement days while animals were still on the WD (Fig 1A). There was a significant time x diet interaction for calorie intake ($F_{(52, 1144)} = 2.008$; P<.0001) with main effect of time ($F_{(1.159, 25.5)} = 6799$; P<.0001) but no significant main effect of diet ($F_{(1, 22)} = 4.166$; P=.0534) (Fig 1B).

3.2 Western Diet Calorie Distribution

The WD was made up of a high fat, high sugar food pellets (45%kcal from fat) and an 11% high fructose corn syrup solution. Rats consumed roughly 75% of their kcals from the food pellets and ~25% of their kcals from the 11% high fructose corn syrup solution. There was a time x calorie source interaction ($F_{(3, 66)} = 4.517$; P= .006) with main effect of time ($F_{(1.839, 40.45)} = 48.59$ P<.0001) and calorie source ($F_{(1, 22)} = 181.6$; P<.0001). Post hoc analyses revealed that average kcal intake for the HFD was the same during weeks 2-4, but significantly lower during the first week of introducing the diet when the animals were the smallest (P<.001). Consumption of the sugar sweetened beverage was increased in weeks 2-4 compared with week 1 and was significantly elevated in week 4 compared with week 3 (P<.05).

3.3 Differential Low Rates of Responding (DRL) Training

Rats were trained in the DRL task in the operant chambers (Fig 3A). During training, rats were fasted when they were placed into the operant chambers. Two hours before the start of the dark cycle, access to food was removed, and food was returned after completion of the task. Rats completed the training in three cohorts, counterbalanced by diet such that there were equal numbers of each diet group in each cohort, with the last cohort finishing the training three hours into the dark cycle. Across the 14-day training period of DRL 20, there was a main effect of training day on on active lever presses ($F_{(5, 110)} = 13.98$; $P<.0001$) but no effect of adolescent diet ($F_{(1, 22)} = .80$; $P=.38$) (Fig 3B). Similarly, there was a main effect of training day on pellets earned ($F_{(4.392, 96.62)} = 24.64$; $P<.0001$), with no effect of adolescent diet on pellets earned ($F_{(1, 22)} = .0008$; $P=.977$) (Fig 3C). Efficiency scores were calculated using the equation in Fig 3D. There was a main effect of training day on efficiency score ($F_{(4.332, 95.30)} = 18.3$; $P<.0001$), but no effect of adolescent diet ($F_{(1, 22)} = .0008$; $P=.977$).

3.4 DRL Test Day

After the 14 days of DRL training concluded, Rats were tested for one day on the DRL task during non-fasted conditions, where impulsive responding for food is expected to be lower. Results revealed that even under non-fasted conditions, there were no group differences in efficiency score, active lever presses, or pellets earned in the DRL task (Fig 4A-C).

3.5 Edible cannabinoid receptor effects on eating behaviors

Rats given an edible cannabinoid receptor agonist, CP55940, showed significant increases in food intake (main effect of drug, ($F_{(1, 20)} = 8.371$; P=.0090). Post hoc Fishers LSD analyses revealed a significant increase in chow intake in animals on CP55940 that had consumed a WD during adolescence (P=.03), with a non-significant trend toward a drug effect to increase food intake in the animals fed chow during adolescence (P=.097) (Fig 5A).

Our prior studies show that this dose of edible CP55940 increases impulsive responding for food intake in female rats, therefore we next tested whether edible CP55940 would increase impulsive responding for food in these male rats. Male rats given edible CP55940 prior to testing in DRL 20 did not have more active lever presses, earn more pellets, or have a difference in efficiency score compared to rats who received a vehicle edible (Fig 5 B-D).

Discussion

We hypothesized that a WD consumption during adolescence would increase impulsive responding for food during adulthood. Contrary to our hypothesis, we observed no differences in impulsive responding for food in male rats fed a WD during adolescence when compared with rats fed standard chow during adolescence. Prior research in our lab found that female rats fed a WD displayed increased impulsive action for a sucrose pellet in the DRL task. However, when this same procedure is replicated in male rats, we observed no differences in impulsive behavior. Thus, our findings reveal a sex difference in the effects of adolescent dietary factors on brain development. Notably, others have investigated the effects of a WD in impulsive behavior previously. For example a study conducted by Marwitz et al. (2015) found that WD rats showed

increased hyperactivity and impulsivity [32]. When testing for impulsive choice, researchers found that chronic exposure to WD increased impulsive behavior[51]; and it is important to note that other studies found these increases in impulsive behavior are not reversible through exercise interventions[52]. An interesting finding in impulsive choice is that when rats were fed either high sucrose concentrations or HFD and compared to a control diet, no group differences were found in this aspect of impulsive behavior [53, 54]. Importantly, a major difference with our study is that the WD feeding period was confined to the adolescent phase of development.

This is an important distinction, as during the adolescent phase, the brain is in a critical stage of development, making it the most susceptible to environmental factors such as diet[1]. Changes caused during the critical period of development can have long-term consequences that cannot be reversed in adulthood[3, 6, 24]. Our findings show that adolescent consumption of WD results in increased caloric intake in the first week of introduction to the diet. This significant difference in caloric consumption does not last throughout all of adolescence, however it does result in an increase in body weight within the WD group, that does not correct itself after both groups switch to the chow diet. Beyond the increased consumption of calories in the WD group, WD rats consumed roughly 25%[48], but the extent to how this affects male adult behavior is less understood. The DRL task we conducted revealed the presence of sex differences in how the WD affects the brain during adolescence. (Fig 3).

One of the hallmarks of impulsivity is the preference for a smaller immediate reward over a larger delayed reward. A study conducted by Steele et al. (2017) quantified impulsive behavior through the delayed discounting (DD) task[55]. Steele et al. (2017) demonstrated that rats fed a WD displayed increased delayed discounting, which means they were less willing to wait for a larger reward demonstrated that adult rats fed WD for 8 weeks between PND 127-182 preferred

the smaller immediate reward, even after returning to a standard chow diet during the second phase of DD testing between PND 268 through the end of experiments [13]. This suggests long lasting effects of WD on decision making and impulsivity. In addition to this, Gomez-Martinez et al. (2022) examined impulsivity in human participants and found that individuals with higher impulsivity scores had lower adherence to healthy diets and a stronger preference for WD[56]. Over the course of a three year follow up period, impulsivity was found to be positively correlated with a higher consumption of processed foods and saturated fats [14]. This bidirectional relationship highlights that impulsive individuals are more likely to consume WD, and in turn, this WD consumption exacerbates impulsive tendencies, a finding that goes hand in hand with the "vicious cycle" proposed by Hargrave et al[37]. These studies results show that WD consumption alters reward processing and executive function, making it harder for individuals to delay instant gratification for a larger reward. [57] However, our studies suggest that the effects of the WD on impulsive behavior which have been reported by others do not persist once the animals are no longer on the diet, at least when the exposure period is confined to adolescence, in male rats.

A secondary aim of our experiment was to determine the impact of cannabinoids on food intake and impulsive action in animals on either a WD of control chow diet during adolescence. The general function of the ECS has been established to play a role in energy homeostasis, metabolism, eating behaviors, and various physiological processes[16, 44]. However, increasing evidence suggests that ECS function differs significantly between sexes, which can be explained by hormonal regulation, receptor expression, and metabolic processing of cannabinoids[58, 59]. These differences, which are not thoroughly explored, may have critical implications for cannabis use, cannabis-based therapeutics, and understanding the broader effects of cannabinoids

on both male and female physiology. We observed no effect of a cannabinoid receptor agonist on impulsive behavior in the DRL task in male rats, and this was in contrast to a prior report from Lord et al., who showed that that edible administered CB1 receptor agonist CP55940 when given to female rats increased impulsive responding for sucrose pellets in the DRL task. When male rats given the same hyperphagic dose of .12mg/kg they did not act more impulsively for a sucrose pellet (Fig 5B, Fig 5C, Fig 5D). One interesting result was that the cannabinoid hyperphagic effect was more pronounced in WD fed rats (Fig 5A), however these findings are preliminary and will need to be replicated with a larger sample size. Future studies could investigate whether rats who consume a WD during adolescence are more sensitive to the effects of cannabinoids during adulthood, and whether this reflects differences in signaling of the endocannabinoid system[16, 44][58, 59].

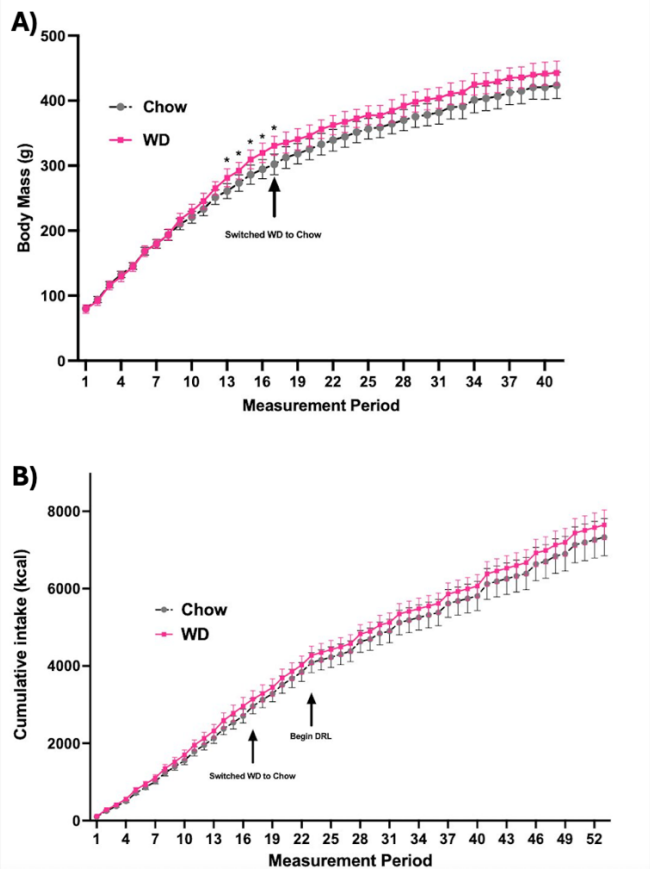

Fig 2.1: Changes in Body Mass and Food Intake Over Time. Body mass (A) and cumulative food intake (B) in chow fed (CHOW) or western diet fed (WD). For body weight there was a time x diet interaction for body weight gain in WD group ($F_{(40, 880)} = 9.648$; P<.0001) with a main effect of diet ($F_{(1, 22)} = 10.48$; P=.0038) and time ($F_{(1.86, 40.94)} = 4642$; P<.0001). Post hoc analyses revealed that body weights were significantly heavier in the WD-fed animals on measurements 13-17, which occurred during the last measurement days while animals were still on the WD (Fig 1A). For cumulative caloric intake, there was a significant time x diet interaction for calorie intake ($F_{(52, 1144)} = 2.008$; P<.0001) with main effect of time ($F_{(1.159, 25.5)} = 6799$; P<.0001) (Fig 1B).

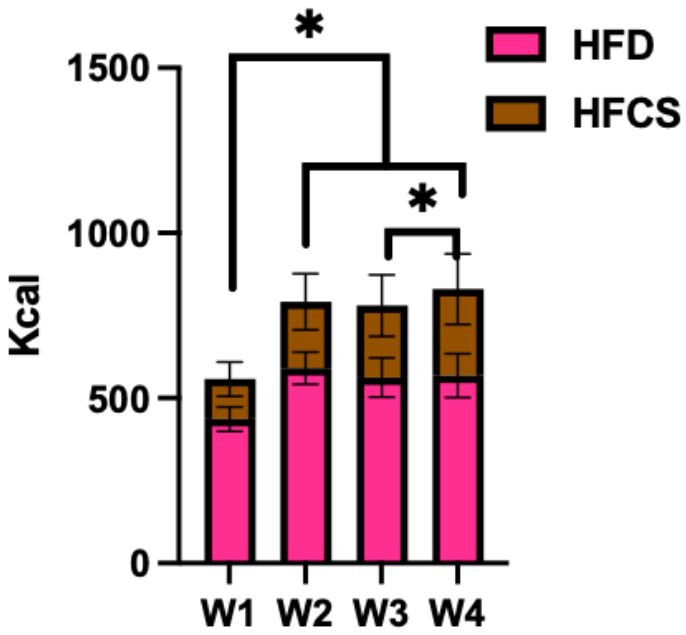

Fig 2.2: Western Diet Source of Kcal Intake

Animals consumed more average kcals of both high fat, high sugar diet (HFHS) and 11% high fructose corn syrup solution (HFCS) during weeks 2-4 (W2-W4) when compared with W1. Rats also showed an increase in consumption of kcals from the sugar sweetened beverage during W4 when compared with W3. Data are means +/- SEM; *P<.05.

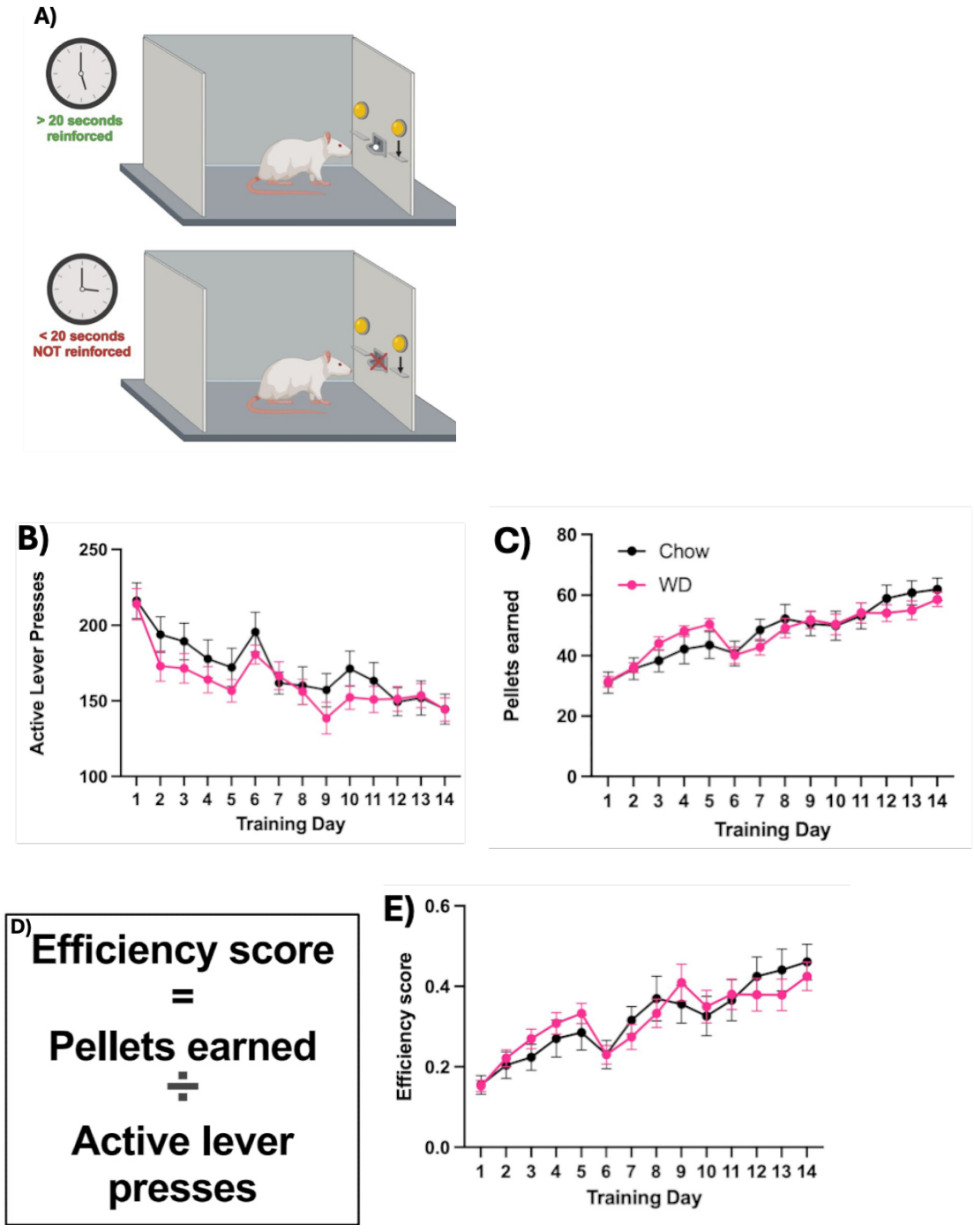

Fig 2.3: Impulsive action measured via the differential reinforcement of low rates of responding (DRL) 20 task during training days 1-14. DRL was utilized to measure impulsive behavior; a schematic of the task shows the binary outcome of either pressing the lever after the 20-second interval or prematurely pressing the lever (A). There were no differences in active lever presses

(B), or pellets earned(C) between the two diet groups. From these measurements, an efficiency score is calculated by the equation in (D). No difference in efficiency score (E) was calculated. All behavior was examined during the dark cycle. Data are means +/- SEM; *$p < 0.05$

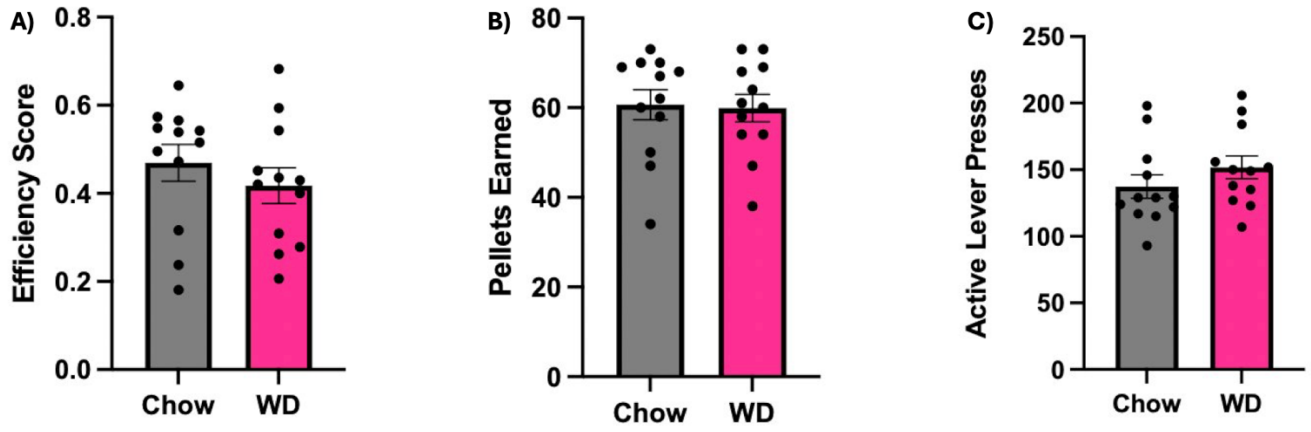

Fig 2.4: Test Day of the DRL task: No difference in efficiency score (A) was calculated. There were no differences in or pellets earned (B) active lever presses (C) between the two diet groups. All behavior was examined during the dark cycle. Data are means +/- SEM; *$p < 0.05$

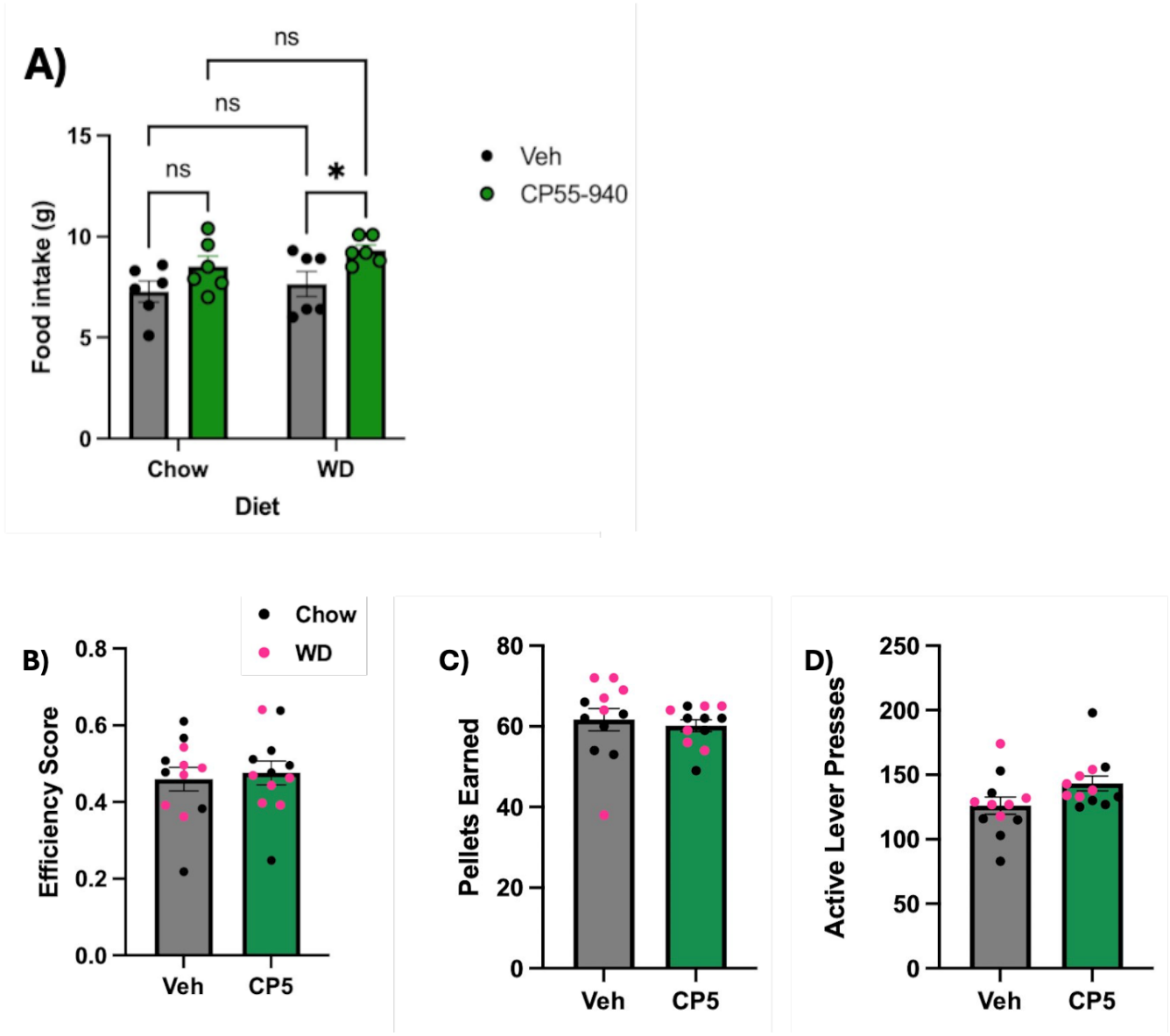

Fig 2.5: Food intake and DRL data following voluntary ingestion of edible CP55940 consumption in chow (n=12) and WD (n=12) rats. There was a main effect of drug ($F_{(1, 20)}$ = 8.371; P=.0090) where WD (A) rats had increased standard chow intake over the first two hours of the dark cycle compared to the three other groups. No difference in efficiency score (B) was calculated There were no differences in pellets earned (C), or active lever presses(D) between the two diet groups in the DRL task. All behavior was examined during the dark cycle. Data are means +/- SEM; *p < 0.05

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Chapter 3

CONCLUSION

Research has established that consumption of a WD both during adolescence and adulthood has adverse health and neurological effects, and particularly negatively impacts the function of brain regions that are important for learning and memory function. However, these brain regions, such as the hippocampus and prefrontal cortex also are important for regulating higher order aspects of eating behavior. Furthermore, these brain regions are still developing during adolescence, and it was previously unknown how a WD consumption at only the adolescent stage impacts some of these higher order aspects of eating behavior, such as food impulsive behavior. Further research has shown that the WD effects on the brain and eating behavior has a connected role with the endocannabinoid system, but the full mechanism behind these interactions remains unclear. Therefore, the aim of this research was to investigate the effects WD consumption during adolescence on adult behavior, and as a secondary outcome to determine how these effects relate to the cannabinoid systems role in impulsivity and cannabinoid induced hyperphagia.

Within one week of introduction to the WD, the WD rats had a significant increase in caloric intake between measurement days 1-2 and 4-5, paired with a significantly higher body weight between measurement days 13-17. This gap between the WD and Chow fed group never corrected itself, with the WD group still weighing more and having a higher cumulative caloric

intake at the conclusion of experiments. Despite these differences, there was no effect of an adolescent WD consumption on impulsive action for sucrose pellets in the DRL task in male rats.

Voluntary consumption of a cannabinoid receptor agonist-containing edible by male rats produced acute hyperphagia of standard chow, and these effects were only significant in the post hoc analysis in animals fed a WD during adolescence. As the sample size was small in this experiment, further research should investigate the effects of an adolescent WD on the cannabinoid system and cannabinoid sensitivity. Cannabinoid-containing edibles did not increase impulsive responding for sucrose in male rats, which is in contrast to our previous finding that a similar dose of the cannabinoid receptor agonist increased impulsive responding for sucrose in female rats. The gelatin-based edible model of cannabinoid consumption simulates the human experience of edible consumption with a high degree of integrity and enhances translational relevance in the mechanistic study of cannabinoids. These preclinical findings demonstrate several behavioral modifications and sex differences that may be relevant to clinical populations considering cannabinoid-based therapeutics.

This research project provided insights into the presence of sex differences with the effects of adolescent consumption of WD on adult behavior. Overall, the findings of this thesis contribute significantly to the understanding of the impact of adolescent exposure to the WD on adult behavior and provide a basis for further research in this area.