Effects of voluntary ingestion of delta-9-tetrahydrocannabinol on the hedonic value of rewarding and aversive substances

by

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Abstract

Human studies indicate a relationship between Δ-9-tetrahydrocannabinol (THC) and alcohol use, yet research on their co-dependency remains limited. Rodent models have shown that cannabinoids can enhance the incentive salience and hedonic value of sucrose and alcohol, but results vary, especially with high doses of THC. This study addresses these discrepancies by using a translational model with lower, human-relevant doses of synthetic THC (Dronabinol). Male Long-Evans rats consumed either a vehicle or THC-containing (0.5 mg/kg) cookie before testing in a taste reactivity paradigm. Results show that THC increased hedonic reactions to both sucrose (0.1M & 0.5M) and alcohol (10% & 40% ETOH) compared to control. Comparatively, THC reduced aversion to alcohol (10% & 40%) and quinine. THC dose dependently downregulated cannabinoid receptor 1 receptor expression in the dorsal hippocampus and increased pERK expression in the same region, while no significant changes were observed in the Nucleus Accumbens or Amygdala. These findings suggest that oral THC enhances the hedonic value of rewarding and aversive substances and provide insights into the neurobiological mechanisms that may contribute to the co-morbidity of alcohol and THC use disorders.

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Dedication

This thesis is dedicated to my most kind, loving, and supportive family members—my mother, Shanne, my sister, Cierra, and especially to my wife, Katherine, and my daughter, Magnolia. Your unwavering love and encouragement have been the foundation of my journey, and for that, I am eternally grateful.

Chapter 1 - Introduction

Models of Cannabis Use

Cannabis use has been documented for centuries (Russo, 2007), but it has now become the most frequently used illicit drug among users in the United States (Schulenberg et al., 2018). Foods that are infused with cannabis (edibles) have been the trending method of human consumption during the rise of legalization in the United States (Barrus et al., 2016). Researching the effects of cannabis use in human models has become limited by ethical and federal barriers, as well as a lack of control over subjects' prior exposure (Smoker, Hernandez, et al., 2019; Smoker, Mackie, et al., 2019). Recently, literature has cited a disconnect between human cannabis use and pre-clinical models and has attempted to create rodent models utilizing edible THC. These recent studies have been able to display cognitive deficits as well as behavioral changes induced by ingesting THC (Nelson et al., 2019; Rohleder et al., 2020; Smoker, Hernandez, et al., 2019; Smoker, Mackie, et al., 2019). However, these primarily used high doses unrepresentative of human patterns of consumption.

With the recent increase in the legalization of cannabis and the growing popularity of THC-infused edibles, preclinical models have sought to explore the cognitive and behavioral effects associated with oral consumption (Barrus et al., 2016). However, these models have largely neglected to investigate the impact of ingesting low doses that are more reflective of typical human use. Significant differences exist between the pharmacokinetics of cannabinoids absorbed through digestion compared to those absorbed via inhalation. When inhaled, cannabinoids typically produce effects within minutes, which subside within two to three hours. In contrast, the effects of ingested cannabis are delayed, usually manifesting within 30 to 90 minutes, and can persist for up to eight hours (Grotenhermen, 2003; Russo, 2007). Δ-9-

tetrahydrocannabinol (THC), the principal psychoactive compound in cannabis, is absorbed through the pulmonary system when inhaled. However, when consumed orally, THC undergoes first-pass metabolism in the liver, where it is converted into 11-hydroxy-tetrahydrocannabinol (OH-THC), 11-nor-carboxy-THC, and other minor metabolites. Notably, OH-THC is also psychoactive, which underscores the differences in both the potency and duration of intoxication between oral and inhaled cannabinoids (Favrat et al., 2005; Grotenhermen, 2003; Huestis, 2007).

Co-morbidity Prevalence of THC in Humans

Recent legalization and acceptance of cannabis use in the U.S. has greatly increased its availability across all ages (Russo, 2007; Schulenberg et al., 2018). Recent studies have shown that exposure to cannabis increases the reinforcing effects of psychostimulants, narcotics, and non-drug rewards (Barrus et al., 2018; Verendeev & Riley, 2012). Further, throughout more than a decade of research, cannabis use has been correlated with the use of other illicit substances, depicting marijuana as a "gateway drug" (Baggio et al., 2018; Hasin & Walsh, 2021; Jorgensen & Wells, 2022). Importantly, the National Epidemiology Survey on Alcohol and Related Conditions (NESARC) has found that roughly 50% of people with cannabis use disorder (CUD), also meet the diagnostic criteria of alcohol use disorder (AUD) (Hasin & Walsh, 2021; Stinson et al., 2006). However, there is still a gap in the pre-clinical literature on cannabis exposure and its effect on alcohol consumption. The omission of this research has resulted in unknown information on cannabis exposure's influence on alcohol use and therefore results in a critical barrier for the ability to design effective treatment and therapeutic intervention for the comorbid use of cannabis and alcohol and their respective use disorders.

THC and Alcohol

Due to human studies indicating a correlation between THC use and alcohol use (Hasin & Walsh, 2021; Stinson et al., 2006), preclinical models have begun to model this phenomenon in rodents. However, research on the development of co-dependency of cannabis and alcohol use is limited. There are a few pre-clinical studies designed to look at co-exposure to cannabis and alcohol. Many are designed to characterize behavioral effects, where researchers utilize extremely high doses and methods of administration that do not match human use. Despite these translational confounds, results have been intriguing. In one study, researchers gave rats an intraperitoneal injection of combined THC and alcohol and found that it altered their preference for familiar and novel objects. Rats receiving THC and alcohol combined showed a lack of memory of familiar objects, whereas this effect was not observed in rats exposed to THC or alcohol alone (Swartzwelder et al., 2012). A previous study has also found that injections of exogenous cannabinoids dose-dependently increased operant responding for both sucrose and low doses of alcohol (Gallate et al., 1999). Another study found that microinjections of the exogenous cannabinoid WIN55,212-2 into the nucleus accumbens increased operant responding for ethanol (Malinen & Hyytiä, 2008). A recent study employed vapor chamber exposure to THC and allowed rats free access to alcohol. Interestingly, on days rats received vapor THC exposure, they did not drink any more or less than rats with control vapor exposure. However, on days with no vapor exposure, THC rats drank more than control rats, indicating a possibility of a substitution effect, where rats appeared to replace the effect of THC with alcohol (Hamidullah et al., 2021). Importantly, this effect has also been reported in humans (Allsop et al., 2014). On the other hand, one study found that rats with free access to THC edibles reduced ethanol drinking (Nelson et al., 2019). However, Nelson et al., utilized THC doses above 5mg/kg, causing overall aversion including some rats refusing to eat drug-laced cookies in the remainder of the

experiment (2019). Despite evidence of THC altering alcohol consumption, pre-clinical models studying THC and co-occurring alcohol use have primarily utilized open access or operant paradigms for alcohol. Importantly, these paradigms only translate to one aspect of addiction, whereas we know that the incentive motivation to take drugs is characterized by two distinct parts, measured by unique tests. Therefore, the current project aimed to identify changes in alcohol "liking" modulated by THC using a translational model of consumption.

Synthetic delta-9-tetrahydrocannabinol

As cannabis legalization has risen across the United States, a large amount of cannabis research both clinical and pre-clinical has been designed around understanding cannabinoids. Currently, the CDC states that we know of over 100 cannabinoids in the cannabis plant (What We Know About Marijuana, 2022). Within these cannabinoids, there are units of tetrahydrocannabinol (THC) which has psychoactive properties, along with cannabidiol (CBD) which has not been cited to cause any cognitive impairments (Rosenberg et al., 2015). As our knowledge has grown about THC, we have discovered the primary psychoactive cannabinoid, Δ-9-tetrahydrocannabinol. To further investigate the therapeutic efficacy of Δ-9-tetrahydrocannabinol by itself, synthetic analogs have been widely utilized in clinical trials (Barrus et al., 2016). The most prescribed and studied synthetic Δ-9-tetrahydrocannabinol is Dronabinol, which received FDA approval in 1985 (O'Donnell et al., 2022). Because of its prevalence in clinical trials and treatment, preclinical trials have also been designed utilizing Dronabinol to study the effects of Δ-9-tetrahydrocannabinol. These studies have shown profound behavioral effects including hypolocomotion and impaired working memory (Carrica et al., 2023; Nelson et al., 2019). Importantly, these studies have also performed liquid chromatography tandem mass spectrometry to quantify levels of Δ-9-tetrahydrocannabinol and 11-hydroxy-THC

and confirm the metabolism of ingested cannabinoids in rodents resembles human metabolism (Carrica et al., 2023; Miller et al., 2018). Further, Dronabinol has been shown to bind to CB1 with high affinity in both rodents and humans (Pertwee, 2006; Wedenoja et al., 2010) While it is known that cannabis contains over 100 cannabinoids, the proposed experiment is focused on changes induced by main psychoactive compound, delta-9-tetrahydrocannabinol. Therefore, the synthetic isolate, Dronabinol, was used in the current experiments.

Incentive Motivation

The theory of incentive motivation has now been widely cited in addiction research to break down the motivation to respond for reinforcers into two categories, "liking" (hedonic value) and "wanting" (inventive salience). The willingness to respond to stimuli, wanting, is primarily mediated by mesocorticolimbic dopamine transmission (Berridge & Robinson, 2017). Pleasureful experiences from interaction with stimuli, liking, are mediated by 'hedonic hotspots' primarily comprised of Mu opioid receptors (MORs) in the mesocorticolimbic system (Berridge, 2000, Peciña, Smith, & Berridge, 2006). The proposed study is focused on the effects of THC on hedonic value. If increased "liking" (or decreased disliking/aversion) to alcohol is mediated by THC, it could provide substantial evidence to consider THC as a "gateway" drug to alcohol. To date, the extant literature has primarily examined the biological and behavioral effects of THC, claiming a potential for co-morbid drug use. However, most of these studies have primarily used operant paradigms, which only measure incentive salience.

Taste Reactivity

The taste reactivity paradigm has evolved into the gold standard for measuring hedonic value. However, it is not a novel method in preclinical research. Standards for measuring hedonic and aversive reactions were set in 1978 by Grill and Norgren. Fluids directly infused

into the mouth via the intraoral fistula cause orofacial reactions that are broken down into two groups, aversive and hedonic. Gapes, forelimb flails, headshakes, passive drips, and fluid expulsions are classified as aversive behaviors, whereas tongue protrusions, lateral tongue protrusions, and paw licks are distinguished as hedonic behaviors (Grill and Norgren, 1978). The taste reactivity paradigm has had minor revisions throughout its years of use. One example of this is redacting mouth movements as a hedonic response (Kiefer, 1995), to keep its pedigree as the optimum test to measure hedonic and aversive behaviors. When analyzing taste reactivity trials, increased liking or aversion can be seen by an increase in omitted responses. Together, these hedonic and aversive responses make up "palatability". Most preclinical models of drug use utilize operant paradigms, where researchers can measure palatability and incentive salience concurrently, and more so incentive salience independently. To date, the taste reactivity paradigm is the only preclinical method to solely investigate hedonic responding ("liking") without concurrently measuring appetitive behaviors correlating to incentive salience ("wanting") (Berridge, 2000). Currently, there are only a couple of studies involving cannabinoids utilizing the taste reactivity paradigm. In two separate studies, the investigation of the endogenous cannabinoid anandamide and its role in enhanced liking has been assessed. Both studies utilized the same method of administration, an intracranial injection of anandamide into the nucleus accumbens (NAc). Importantly, both studies found that administration of the endogenous cannabinoid significantly increased orofacial "liking" of sucrose in a taste reactivity paradigm (Mahler et al., 2007; Mitchell et al., 2018). Despite these results, there has yet to be a study investigating changes in the hedonic value of rewarding or aversive substances induced by exogenous cannabinoids utilizing a translatable method of drug administration. The present

experiments are the first to investigate this gap in the literature, importantly, by utilizing a translatable model of ingesting THC.

THC Modulating Palatability of Reinforcing and Aversive Substances

While it has been widely stereotyped that humans get the "munchies" after consuming cannabis, multiple studies report an increased appetite for sweet food shortly after using the drug (Foltin et al., 1988; Iversen, 2001; Mattes et al., 1994). However, there is very little literature researching the effects of exogenous cannabinoids on the palatability of rewarding and aversive substances in preclinical models. The overall premise of the proposed experiment is built from two papers in THC literature. Jarrett et al. (2005), first showed THC's effect on sucrose palatability where they found that injections of a moderate dose (2.5mg/kg) of THC increased hedonic responses to sucrose (2%, 10%, and 32%), two hours post-injection. Importantly, this study was replicated and followed up by Jarret et al. (2006), where the researchers showed increased hedonic responses to sucrose from injections of THC and reversed this effect by administering the CB1 antagonist AM251. Further, this study was the first to show THC's effects on aversive substances. At 30min up to 240min THC injections at a moderate dose (2.5mg/kg) decreased aversive response to quinine (0.05%). While these studies greatly expand our understanding of THC's impact on hedonic value, there are important factors to be addressed. In the proposed study, I attempted to replicate these findings by utilizing a translatable method of consumption of voluntary ingestion of THC edibles. To increase the robustness of the experiment, I also tested a range of THC doses to capture the effects at low and high doses of THC ingestion. Further, while these studies show the increase in hedonic response to sucrose and decreased aversion to quinine induced by THC, the effect of THC on alcohol palatability has not been assessed. As previously stated, injections of dose-dependently increased operant responding

for sucrose and low doses of alcohol (Gallate et al., 1999). Therefore, given the relationship between cannabinoids on alcohol incentive salience, the current experiment investigates the effects of voluntary edible THC consumption on the hedonic value of alcohol at low and high concentrations.

Cannabinoid Receptors

Current literature has analyzed how THC use alters cannabinoid receptor 1 (CB1) expression throughout the brain. Both human and preclinical literature cites that chronic use of THC can significantly reduce the number of CB1 expressed throughout the brain (Kruse et al., 2019; Lazenka et al., 2014; Villares, 2007; Zamberletti et al., 2016). While it is known that CB1s play a critical role in neuronal development, they also help institute synaptic connections and maintain gene expression (Kano et al., 2009). Importantly, it has also been shown that endocannabinoids and CB1s modulate incentive behaviors for reinforcers (Barrus et al., 2018; Gallate et al., 1999; Mahler et al., 2007; Mitchell et al., 2018). Particularly, stimulation of the CB1's in the nucleus accumbens plays an important role in modulating hedonic value (Mahler et al., 2007; Mitchell et al., 2018). As previously stated, literature shows that activation of the CB1 via binding of the endogenous agonist anandamide (a partial agonist to CB1) increases hedonic value for reinforcers (Mahler et al., 2007; Mitchell et al., 2018). CB1 is also bound to and activated by the partial agonist, exogenous ligand Δ-9-THC (Huestis, 2007; Romero et al., 1997). Therefore, in addition to the taste reactivity behavior assessment, the proposed experiment will also investigate changes in CB1 expression within the mesocorticolimbic system induced by ingestion of THC at low or high doses. While previous edible studies have shown little change in CB1 expression in the nucleus accumbens, these studies have also shown small behavioral changes, identifying possible dosage issues. To investigate the differences between low and high

doses, the proposed study evaluates CB1 expression differences in the NAc. Further, it is known that the amygdala plays a large role in taste preferences as well as alcohol-drinking behavior (Berridge, 1996; McBride, 2002; Shinohara & Yasoshima, 2019; Warlow & Berridge, 2021). Although human data shows little difference in CB1 expression between heavy cannabis users and non-users (Schacht et al., 2012), pre-clinical studies have shown a correlation between THC and downregulation of CB1 in the central amygdala (CeA) (Lazenka et al., 2014). In addition to the NAc, the current study examines changes in CB1 induced by low or high doses of edible THC in the amygdala (Amyg). The current experiment follows a novel combination of the route of administration and dosing methodology for THC, which has previously resulted in the downregulation of CB1 in the CA1 region of the hippocampus (Laux et al., in review). Additionally, studies have replicated THC-induced down regulation of CB1 in the dorsal hippocampus (dHipp) using other routes of administration (Lazenka et al., 2014). Along with regions that are specific to the behavioral task, the current study also examined CB1 expression in the dHipp to confirm active THC metabolites in the central nervous system by drug-induced downregulation of CB1 in the brain.

ERK & pERK

Extra-cellular regulated kinase (ERK) can be described as a regulator for cell proliferation and differentiation that is found intracellularly and responds to extracellular stimuli (Seger & Krebs, 1995; Sun et al., 2016). When ERK becomes active, it undergoes phosphorylation, allowing it to translocate to the nucleus (Chen et al., 1992; Sun et al., 2016). Phosphorylated ERK (pERK) in the nucleus can result in the expression, promotion, and transcription of immediate early genes (IEGs) (Davis et al., 2000; Hill et al., 1993; Sun et al., 2016; Treisman, 1996). The signaling of ERK in the mesocorticolimbic system is modulated by a

long list of drugs of abuse (Sun et al., 2016). Regarding cannabis, discoveries have been made in synaptic transmission, as well as signal transduction pathways regulated by CB1s in decision-making, learning and memory, and reward processing. Importantly, activating CB1 results in potassium channels opening, and calcium channels closing, which can modulate protein kinase including ERK (Howlett et al., 2002; Sun et al., 2016). In-vitro, ex-vivo, and in-vivo studies have all shown similar results, in that stimulation of CB1 in the hippocampus activates ERK, resulting in the expression of immediate-early gene expression (Bouaboula et al., 1995; Wartmann et al., 1995; Derkinderen et al., 2003; Massi et al., 2013). Preclinical studies have found that acute injections of THC (1mg/kg) can increase the expression of pERK in mesocorticolimbic structures (Daigle et al., 2011; Zamora-Martinez & Edwards, 2014; Sun et al., 2016). Comparatively, acute injections of THC at high doses (>10mg/kg), result in an increase in pERK expression in the striatum and cerebellum (Daigle et al., 2011; Rubino et al., 2004, 2005; Sun et al., 2016). Chronic THC injections (1mg/kg) have been shown to form THC-induced conditioned place preference (CPP). The use of a MAP/ERK kinase (MEK) inhibitor causes attenuation of the THC-induced CPP, pointing to the involvement of ERK-regulated signaling in THC's rewarding effects (Sun et al., 2016; Valjent et al., 2001). Despite our knowledge of ERK/pERK changes induced by THC exposure and related involvement in reward processing, there are very few preclinical studies investigating changes in ERK/pERK expression induced by ingestion of low or high doses of THC. Therefore, the current project examines the expression changes of ERK/pERK altered by low and high doses of edible THC in rats. Particularly, expression changes in the mesocorticolimbic pathway, specifically, the NAc, Amyg, and dHipp were measured via western blot.

Chapter 2 - Methods and Materials

Animal Husbandry

Male Long-Evans rats (n=27 (24 used for data analysis due to loss of headcap)) were obtained from Charles River Laboratories and arrived at Kansas State University at approximately 250 grams. Animals were pair-housed in plastic shoebox cages (20x43x20 cm). Cages had bedding, and wire tops, and rats were handled weekly during scheduled cage changes. All animals were housed under a 12-hour light/dark cycle and had ad libitum access to food and water throughout the experiment. The colony room was maintained at approximately 22°C, with humidity ranging from 30% to 45%. All behavioral testing was conducted during the light portion of the rats' cycle. All experimental procedures were conducted by the Institutional Animal Care and Use Committee at Kansas State University and the NIH guidelines (Council 2011).

Apparatus

Taste reactivity testing occurred in a tall (27 cm) trapezoidal chamber (Base widths: 27 cm front × 7.25 cm back × 18.55 cm sides; Top widths: 14.6 cm front × 8 cm back × 6.10 cm sides). The chamber consisted of clear polycarbonate front and floor panels and three mirrored interior walls tapering in towards the top and angled at approximately 70° towards the floor of the chamber. A mirror was placed below the chamber, angled at approximately 45° towards the front and floor. A high-definition video camera (59.94 frames per second in 1080p; Cannon Vixia HF R800) faced the chamber with all visible angles typically within the camera frame. Solutions were administered via a syringe pump (78-0100, KDScientific, Hollison, MA) connected to a 10-mL syringe. Solutions were infused at a rate of 1 mL/min through a leash and

swivel arrangement comprised of a polyethylene supply tube encased in vinyl tubing with a captive collar to secure the unit to the head mount (Plastics One; Roanoke, VA).

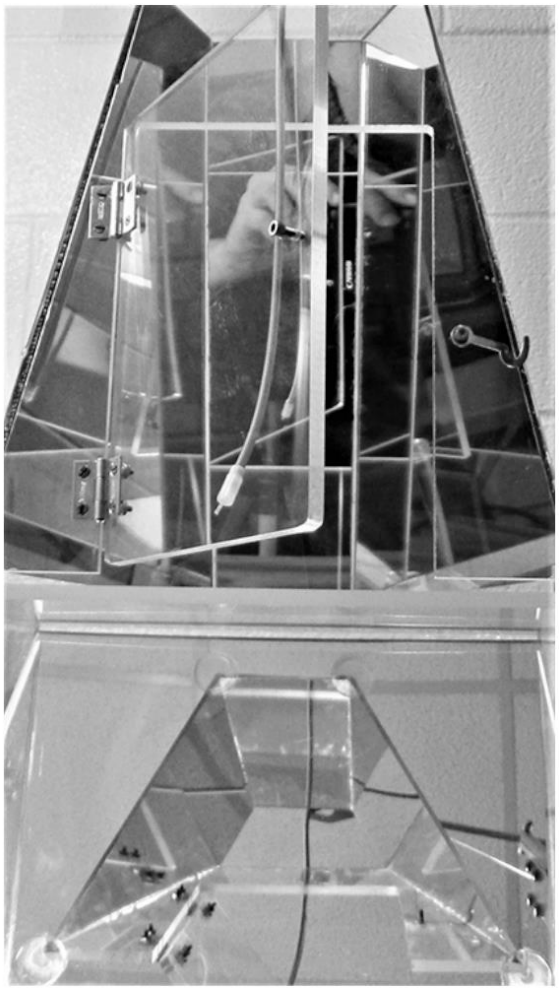

Figure 1 Display of taste reactivity chamber.

Surgical Procedures

Intraoral fistula implantation was necessary to perform the taste reactivity paradigm. All the following procedures had been previously confirmed (Wukitsch et al., 2020a; Wukitsch et al., 2020b; Wukitsch & Cain, 2021). Before surgical procedures beginning, rats were anesthetized using Ketamine (60-80mg/kg, IP) and Diazepam (5mg/kg, IP). Pain withdrawal reflexes were tested by pinching the animal's toes, tail, and palpebral oculogyric reflex. The

righting reflex was also tested. For surgical procedures to begin, no reflexes could occur. Once proper anesthesia was confirmed, fur was shaved on the scalp. The shaved region was aseptically prepared by scrubbing with chlorhexidine surgical scrub followed by 70% isopropyl alcohol rinse; scrubbed regions could air dry. The buccal region near the first maxillary molar was swabbed with a dilute chlorhexidine solution (1:10) and then rinsed with sterile saline. An incision was made on the top of the head to expose the skull. Dilute chlorhexidine surgical scrub was applied to the skull. The exposed skull was dried with sterile gauze and/or sterile cotton-tipped applicators. The fistula, made of 90-gauge polyethylene (PE-90) tubing, was inserted with a needle lateral to the first maxillary molar. After insertion, the needle was directed subcutaneously along the side of the rat's face to the top of the scalp, where it exited the incision. A head stage cannula connector (Plastics One) was then inserted into the tubing and secured using friction. The fistula, with the head stage attached, was mounted to the skull using 3-4 self-tapping small jeweler's screws and dental acrylic cement. A stainless-steel acorn bolt (Small Parts) was used to cover the top of the fistula to prevent bedding or other debris from entering the fistula. After the dental acrylic was dry, chlorhexidine surgical scrub, followed by triple antibiotic ointment, was applied along the edges of the dental cement that contacted the skin to prevent infections. Rats received Meloxicam antibiotic treatment immediately following the surgery (2.0 mg/kg, SC). 24 hours following surgery, rats received one-half dose (1mg/kg, SC) of Meloxicam. To ensure the oral fistula stayed unobstructed, the fistula was flushed with sterile water followed by air at least once daily.

Drug Preparation & Administration

The stock solution was made by extracting synthetic delta-9-tetrahydrocannabinol from a fresh Dronabinol® capsule, mixed into sesame oil (purchased at a local grocery store) for

dilution and vehicle purposes, and stored in a refrigerator. Solution was extracted out of the stock and applied in doses of 0.05 and 0.5 mg/kg to a single Nabisco Mini-Oreo® wafer (no Oreo® filling). Rats were randomly assigned to one of three groups, control (vehicle), 0.05 mg/kg and 0.5mg/kg. Drug condition and dosing did not alter for the entirety of the experiment. Rats were removed from their pair-housed home cage and placed in an individual cage (no bedding or food and water access) for no more than one hour for drug ingestion. The wafer was placed into a small clean plastic tray, and then directly into each rat's individual cage for consumption. Immediately after the wafer was ingested, the tray was removed from the feeding cage and rats were put directly back into their home cage before being transported for behavioral testing. Rats were given one mini wafer per day, with either vehicle (control) or delta-9-THC. Previous literature with edible THC has presented issues with developed taste aversion in rodents (Barrus et al., 2018; Nelson et al., 2018; Smoker et al., 2019). Our lab explored this issue and found that alternating days of THC consumption allowed rats to continue ingesting the drug for 2 weeks (Laux et al., in review), equating to seven total drug exposures before taste reactivity sessions. Therefore, THC consumption followed by behavioral testing occurred every other day. On days off, all rats received Nabisco Mini-Oreo® wafer cookies with vehicle only.

Vehicle

Dronabinol® capsules contain sesame oil as well as glycerin and other products. Due to the capsules already containing sesame oil, we used sesame oil as our vehicle for drug mixing and dilution of delta-9-THC. For the vehicle-only control group, Sesame oil was applied directly to a single wafer cookie and placed in each home cage using the same methods as the -tetrahydrocannabinol groups.

Sucrose

Sucrose solutions including low (0.1M) and high (0.5M) concentrations for taste reactivity were mixed in sterile water for sessions and stored at 4 degrees Celsius.

Alcohol & Quinine

Alcohol solutions including low (10% ETOH), high (40% ETOH) (volume/volume) & quinine (0.1mM) for taste reactivity were mixed in sterile water for sessions and stored at 4 degrees Celsius.

Taste Reactivity Sessions

Following laced cookie consumption and 90 minutes allotted for THC digestion, rats were transported to run taste reactivity behavioral trials. The first 3 trials (Day 1) consisted of habituation to the chamber and passive oral infusions. Habituation sessions consisted of two air trials (5 min) followed by one water trial (3 min). After habituation trials (Day 2&3), each test solution was administered in a 90-second trial. The first and last scored trial for every animal was water used as a control to confirm hedonic or aversive shifts in sucrose and alcohol. All testing trials were counterbalanced between animals utilizing a Latin-Square design. Trials for each solution (sucrose and alcohol) were performed on separate days, at the same trial length (90 seconds). Each concentration of solution was performed on the same day. Between trials, a small bout (>.5ml) of water and air was flushed through the line and fistula, along with a minimum of 10 minutes for solution concentration changes to wash out previous taste effects. Ethanol sessions always ended with a quinine (0.1mM) trial as a control to confirm hedonic or aversive changes to alcohol were induced by taste. Further behavioral trial explanation can be found in Figure 3.

Scoring of Taste Reactivity Data

Each taste reactivity trial resulted in a 60-second video recording that was scored. The following methods of scoring trials had been previously confirmed (Grill & Norgren, 1978, Kiefer, 1995; Berridge & Robinson, 2009). Each video was scored frame-by-frame by a trained rater blind to the experimental conditions for 1 min from the first appearance of one of the following behaviors: tongue protrusions, lateral tongue protrusions, paw licking, gapes, head shakes, forelimb flails, fluid expulsions, and passive drips (Grill & Norgren, 1978; Kiefer, 1995). Tongue protrusions (including lateral tongue protrusions) and paw licking (that was not followed by forelimb flails or headshakes) were considered hedonic responses, while gapes, chin rubs, head shakes, forelimb flails, passive drips, and fluid expulsions were considered aversive responses (Grill & Norgren, 1978; Kiefer, 1995). Scoring consisted of noting each instance of a behavior and time logging it at the first frame in which the behavior occurred. Behaviors were tallied into totals for each behavior for each animal for each concentration. The totals of the behaviors were then combined into their respective hedonic or aversive response totals to be analyzed. Multiple, well-trained scorers with established reliability scored all hedonic and aversive responses. A subset of 10 randomly selected videos was scored and correlated between all raters to assess interrater reliability. To confirm the reliability between blind video scorers, a random subset of videos was scored by all scorers. A Correlations comparison (r) of the number of behaviors scored between scorers was analyzed.

Western Blotting

To examine ERK/pERK and CB1 expression differences in the NAc and Amyg, the analytic technique, western blotting, was performed. On the final drug consumption day, rats were euthanized via rapid decapitation, following the guidelines presented by the American Veterinary Medical Association (AVMA). Samples were collected 60 minutes after THC or

vehicle consumption to account for THC availability. Brains were removed, and flash-frozen on dry ice. Coronal sections (1mm) containing the mesocorticolimbic area were taken, and bilateral microdissections of the NAc and Amyg were extracted and placed in aliquots. Lysis buffer [0.15M NaCl, 1% TX-100, 10% glycerol, 1mM EDTA, 50mM TRIS pH = 7.4, and phosphate + HALT protease inhibitor cocktail (Thermo Fisher Scientific)] was added to aliquots at 20µL/mg of tissue and homogenized at 4°C. Homogenates were centrifuged at 10,000g for 20min at 4°C and stored at -20°C for western blotting. The protein concentration of homogenized samples was quantified via pierce BCA assay (Thermo Fisher Scientific). Sample protein (determined by PiercePierce BCA) and loading buffer (2x Laemmli loading buffer) were loaded into SDS-PAGE gels. Samples were subjected to electrophoresis in a Bio-Rad Western blotting apparatus with Tris/glycine/SDS run buffer at 125 V for 1.5 hours. Following electrophoresis, gels were transferred to the PVDF membrane via Bio-Rad Trans-blot apparatus with a Tris/glycine/methanol transfer buffer at 75 V for 1 hour. 5% nonfat dry milk (NFDM) in TBS-T for blocking was applied to the PVDF membrane for 1 hour at room temp while rocking. Following blocking, membranes were incubated in a solution containing 5 NFDM in TBS-T with antibody directed against CB1(1:1000) (Cell Signaling #93815), ERK1&2 (1:1000) (Cell Signaling #4695), pERK1&2 (1:1000) (Cell Signaling #9101), overnight at 4°C while rocking. Following the primary antibody solution, membranes were washed in TBS-T three times for 15min. Next, a secondary antibody (HRP-conjugated) (1:10000) (ab97051) in TBS-T was applied at room temperature for 1 hour while rocking. Three final TBS-T washes for 15min were applied followed by the application of chemiluminescence (Bio-Rad) and imaging via LI-COR mini digital imaging scanner and software. Following each protein of interest, blots were stripped with an HCl/Glycine/SDS solution for 8min and rinsed with five TBS-T washes for

25min. After stripping, blots were re-probed for total ERK followed by total pERK expression. CB1, ERK, and pERK expression were analyzed with normalization to GAPDH (1:2500) (Cell Signaling #2118) control. Finally, blots were analyzed via densitometric analysis within ImageJ software (ImageJ, Rasband, W.S.).

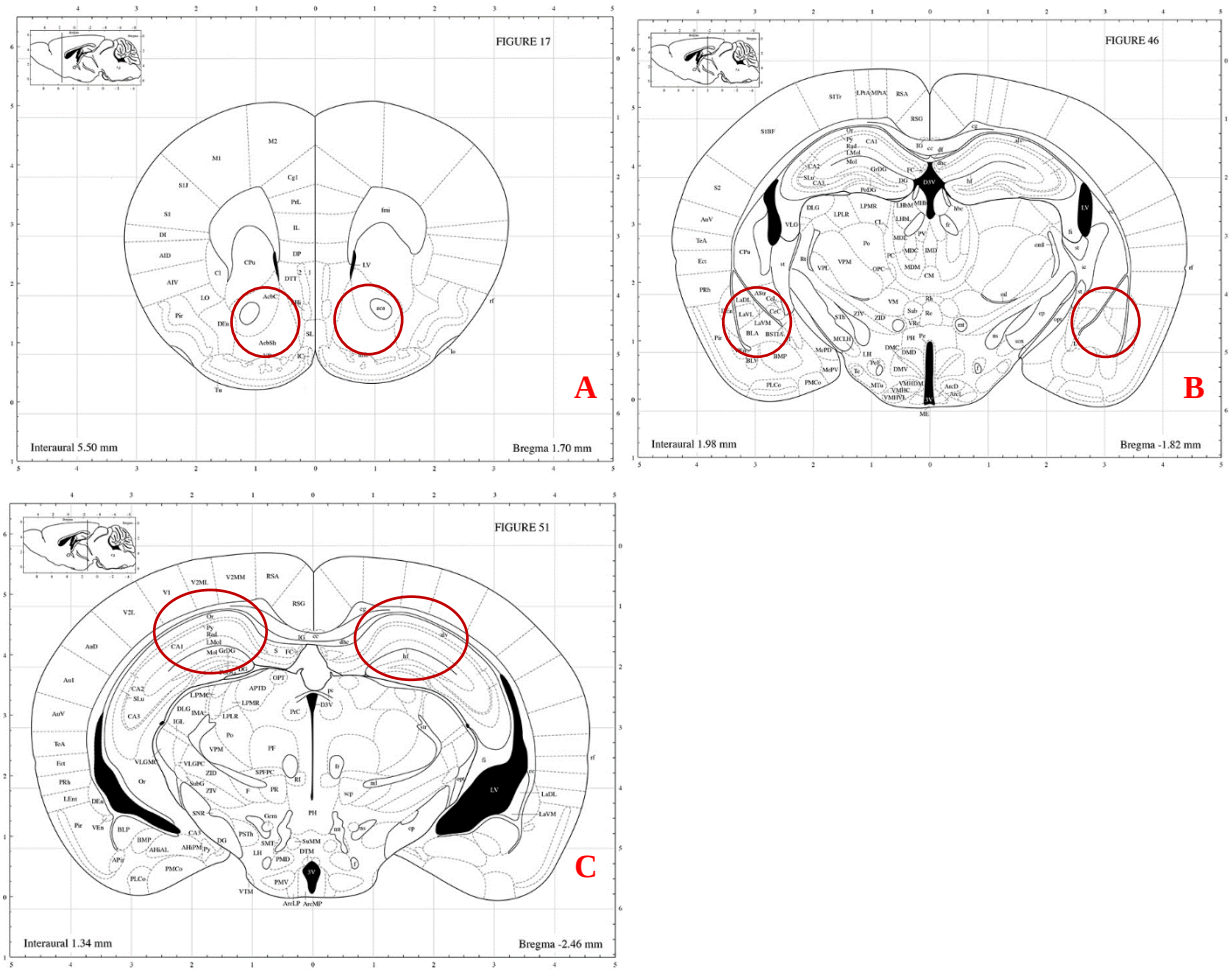

Figure 2 Anatomical location of tissue collection. A) Nucleus Accumbens. B) Amygdala. C) dorsal hippocampus**.**

Data analysis

Taste Reactivity

For taste reactivity analysis, separate statistical models were utilized for hedonic (tongue protrusions, lateral tongue protrusions, and paw licks) and aversive (gapes, forelimb flails,

headshakes, passive drips, and fluid expulsions) behaviors. Each set of behaviors was combined into one of the two categories. Before statistical testing, order effects of taste reactivity substance were probed, and each test was run after confirming no order effect was present in trials and cohorts. Separate linear mixed-effects models predicting each hedonic or aversive taste reaction count were performed for each substance (water, ethanol, quinine, and sucrose) with the fixed effects of solution concentration (Concentration; ethanol: 10 & 40%, quinine: 0.1mM, sucrose: 0.1M & 0.5M) as well as THC dose (vehicle, .5mg/kg & 5mg/kg). Additionally, the interaction of THC and TR substance was probed using simple effects (Bonferroni-corrected). The model also included the random effects of the intercept and the interaction of intercept and concentration to credit within subjects' changes for each subject across taste reactivity sessions, thus creating a repeated measures analysis. All analyses were subject to determination through an alpha of .05. Analyses for aversive behaviors in response to sucrose were analyzed; however, given that aversive responses to sucrose are historically near zero, we confirmed this and excluded them from any further analyses. Similarly, analyses for hedonic behaviors to quinine and alcohol were analyzed; however, given that hedonic responses to the bitter substances are historically low, we confirmed and excluded them from further analysis. Statistical models utilizing different random effect structures (e.g., intercept or intercept and concentration) were compared using Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) values to determine the optimal model. The post-hoc test of Tukey's HSD pairwise comparisons was used where appropriate.

Western Blotting

Comparison of total ERK, total pERK, and CbR1 expression, normalized to the loading control, GAPDH, was conducted via one way ANOVA followed by Tukey's post-hoc test,

comparing the three drug treatments (vehicle, .5mg/kg & 5mg/kg THC). Comparison of the pERK to ERK ratio consisted of three separate analyses; normalized ERK (% of the vehicle), normalized pERK (% of the vehicle), and the ratio between pERK to ERK both normalized to the vehicle. The ratio of pERK to ERK was calculated by division, pERK / ERK. Separate ANOVAs were used for each brain area (NAc or Amyg) and protein (ERK/pERK or CbR1) with main effects of THC (vehicle, 0.5, 5.0 mg/kg) and protein expression normalized to GAPDH. Each THC dose and individual brain area were pooled into two samples to run in each blot as replicates. Analyses were subject to determination through an alpha of .05. The post-hoc test of Tukey's HSD pairwise comparisons was used when appropriate.

Hypotheses

Taste Reactivity: Sucrose

Based off prior literature (Jarrett et al., 2005) I hypothesized that oral THC regardless of dose would increase hedonic reactions to both sucrose concentrations in comparison to the vehicle group. Conversely, I hypothesized that THC would not alter aversive reactions to either concentration of sucrose when compared to the vehicle group.

Taste Reactivity: Quinine

I hypothesized that oral THC regardless of dose would not alter hedonic reactions to quinine when compared to the vehicle, consistent with previous literature. Consistent with previous literature, I hypothesized that oral THC would dose dependently decrease aversive reactions to quinine, with 0.05mg/kg THC having no effect compared to the vehicle and 0.5mg/kg THC decreasing aversive reactions compared to the vehicle.

Taste Reactivity: Alcohol

While THC's propensity to modulate the hedonic value of alcohol has not been studied, based on evidence of THC increasing incentive salience of alcohol (Allsop et al., 2014; Hamidullah et al., 2021) I hypothesized that THC would dose dependently increase hedonic reactions to alcohol. Specifically, 0.05mg/kg oral THC would not alter hedonic responding to alcohol while 0.5mg/kg oral THC would increase hedonic responding to alcohol compared to the vehicle. Like quinine, I hypothesized that THC would dose dependently decrease aversive reactions to alcohol, with 0.05mg/kg THC having no effect compared to the vehicle and 0.5mg/kg THC decreasing aversive reactions compared to the vehicle.

Taste Reactivity: Water

I hypothesized that THC would not alter hedonic or aversive reactions to water from the first to final session

Western Blotting: CB1

I hypothesized that chronic oral THC would dose dependently decrease CB1 expression in the NAc, Amyg, and dHipp. Specifically, 0.5mg/kg oral THC decreasing CB1 expression compared to the control group while 0.05mg/kg would not alter expression.

Western Blotting: ERK, pERK, pERK:ERK

I hypothesized that chronic oral THC would dose dependently increase pERKexpression in the NAc, Amyg, and dHipp, with 0.5mg/kg oral THC increase pERK expression compared to the control group while 0.05mg/kg would not alter expression. Therefore, only high doses of oral THC would increase the pERK : ERK ratio in these brain areas.

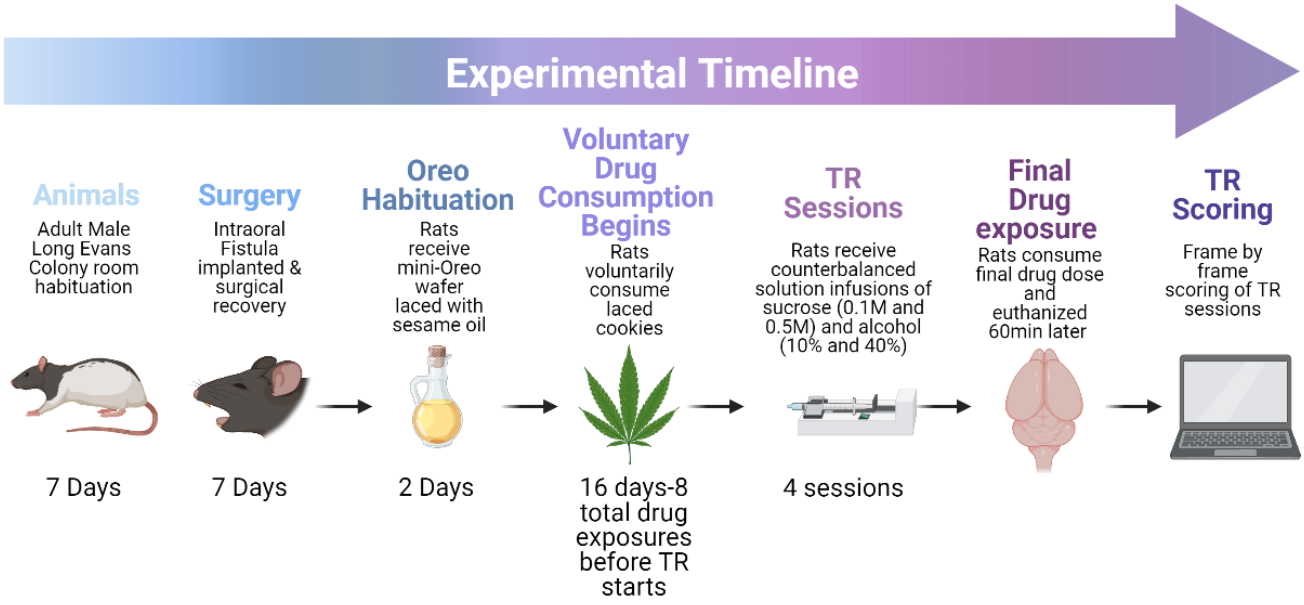

Figure 3 Experimental timeline of procedures.

Chapter 3 - Results

Taste Reactivity

Sucrose

Hedonic behaviors

Rats consuming THC had more hedonic responses to sucrose than rats receiving the vehicle. The analysis of hedonic responses to sucrose revealed a significant fixed effect of THC dose, $F(2,21) = 5.58$, $p < 0.05$ (Fig. 4) (Table 2). Post hoc analysis indicated low (0.05mg/kg) and high (0.5mg/kg) THC rats elicited more hedonic behaviors than the control group $t(21) = -2.51$, $p = 0.05$; $t(21) = -3.1$, $p < 0.05$. The fixed effect of sucrose concentration approached significance $F(1,21) = 3.93$, $p = 0.06$. The interaction of drug dose and sucrose concentration was not significant ($p > .05$). These results indicate that THC affects overall hedonic responses to sucrose regardless of sucrose concentration.

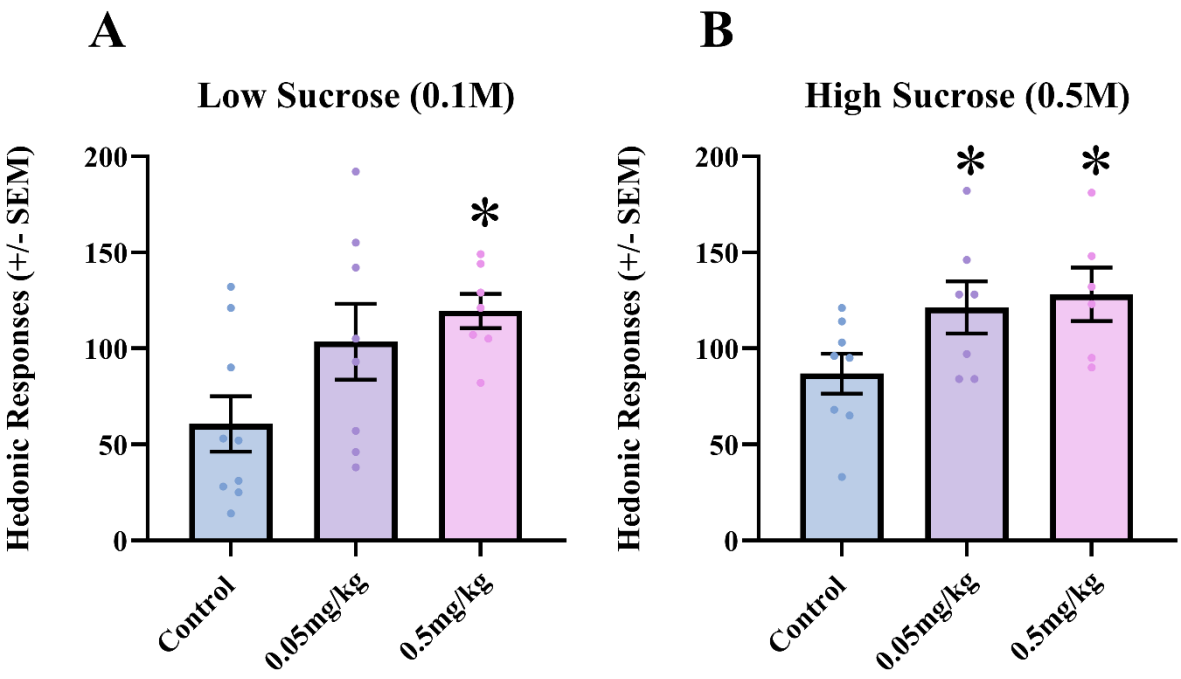

Figure 4 Mean (+/- SEM) hedonic responses to sucrose. A) Results indicated that 0.5mg/kg THC increased hedonic responses to 0.1M sucrose compared to the control ($p < 0.05$). B) Results indicated that 0.05mg/kg and 0.5mg/kg THC increased hedonic responses to 0.5M sucrose compared to the control ($p < 0.05$).

Aversive behaviors

As predicted, the number of aversive reactions to sucrose was low and not affected by THC. The analysis (Table 2) for aversive behaviors to sucrose revealed no significant ($p > .05$) main effects or interactions between drug dose or sucrose concentration, indicating no impact on sucrose aversion.

Quinine

Hedonic behaviors

As expected, the analysis for hedonic behaviors to quinine produced no significant ($p > .05$) main effect of THC dose (Fig. 5) (Table 2). These results show that THC does not influence changes in hedonic responses to quinine.

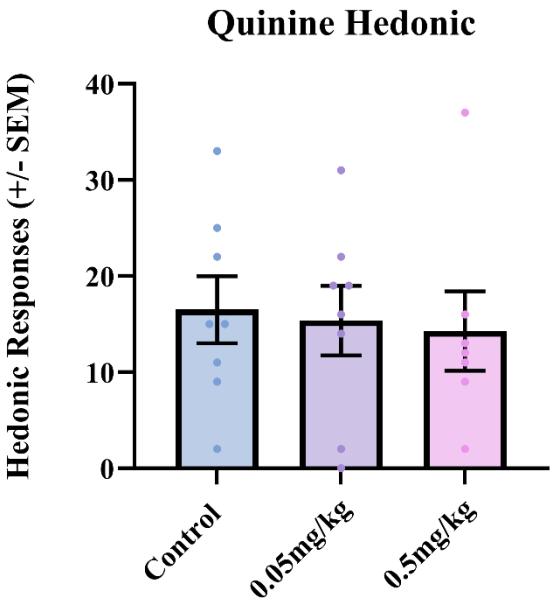

Figure 5 Mean (+/- SEM) hedonic responses to quinine. Results indicated that THC did not alter hedonic responding compared to the control group.

Aversive behaviors

The results reveal a negative relationship between THC dose and aversive reactions to quinine. The analysis for aversive reactions to quinine revealed a significant main effect of THC

dose. Post hoc analysis indicated both low (0.05mg/kg) and high (0.5mg/kg) THC doses resulted in fewer aversive responses to quinine than the vehicle $t(15) = 5.35$, $p < 0.01$; $t(15) = 5.73$, $p < 0.01$ (Fig. 6) (Table 2). These results indicate that THC influences the mitigation of aversive responses to quinine.

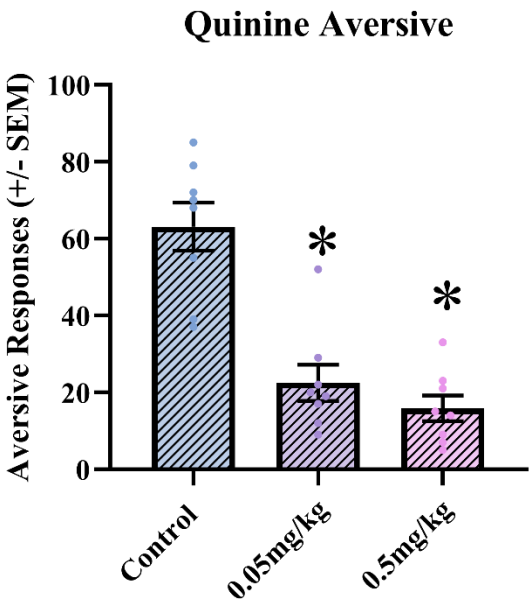

Figure 6 Mean (+/- SEM) aversive responses to quinine. Results indicated that both 0.05mg/kg and 0.5mg/kg significantly reduced aversive behaviors compared to the control ($p < 0.05$).

Ethanol

Hedonic behaviors

Rats ingesting THC had more hedonic responses to ethanol than rats receiving the vehicle. The analysis of hedonic responses to ethanol revealed a significant fixed effect of THC dose, $F(2,21) = 3.74$, $p < 0.05$ (Fig. 7) (Table 2). Post hoc analysis indicated high (0.5mg/kg) THC rats had more hedonic behaviors than the control group, $t(21) = -2.71$, $p < 0.05$. Neither the fixed effect of ethanol concentration nor the interaction between THC dose and concentration

were significant ($p > .05$). These results suggest that high doses of oral THC affect hedonic responses to ethanol across low and high concentrations.

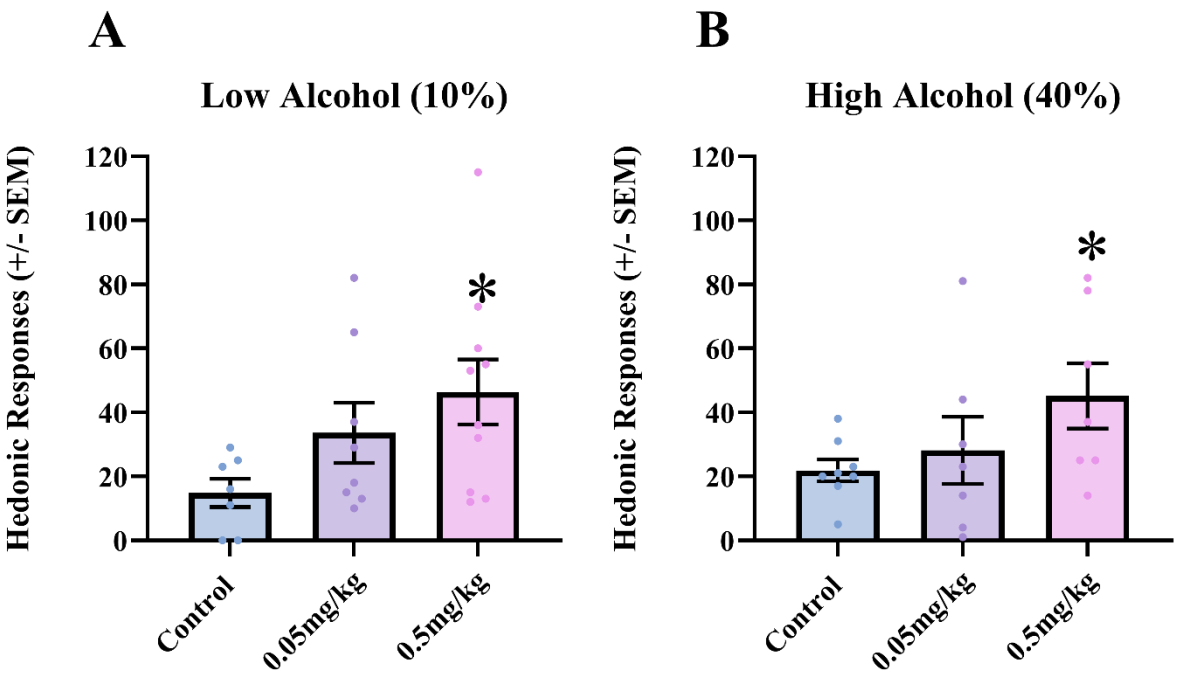

Figure 7 Mean (+/- SEM) hedonic responses to alcohol. Results indicated that 0.5mg/kg THC increased hedonic responses to (A) 10% alcohol and (B) 40% alcohol compared to the control ($p < 0.05$).

Aversive behaviors

Rats consuming oral THC had less aversive responses to ethanol than the control group. Interestingly, the control group had an increase in aversive responses as ethanol concentration increased, whereas THC did not shift aversive responses across concentrations. The analysis for aversive behaviors to ethanol revealed a significant fixed effect of THC dose, $F(2,26) = 17.39$, $p < 0.05$ (Fig. 8) (Table 2). Post hoc analysis indicated both low (0.05mg/kg) and high (0.5mg/kg) THC doses resulted in less aversive responses than the vehicle, $t(26) = 4.89$, $p < 0.01$; $t(26) = 5.39$, $p < 0.01$. The fixed effect of ethanol concentration was significant, $F(1,25) = 13.67$, $p <$

0.01, with an increase in aversive responses for the high concentration, $t(25) = -3.70$, $p < 0.01$. Importantly, the interaction of THC dose and ethanol concentration was significant, $F(2,25) = 3.58$, $p < 0.05$. Post hoc analysis revealed that the control group had significantly more aversive responses as ethanol concentration increased, $t(25) = -4.26$, $p_{adjusted} < 0.001$ whereas low and high THC groups do not (Table 1). These results suggest that oral THC at low and high doses alters aversive reactions to ethanol across low and high concentrations. Further, THC prohibits the increase of aversive reactions at high concentrations of ethanol.

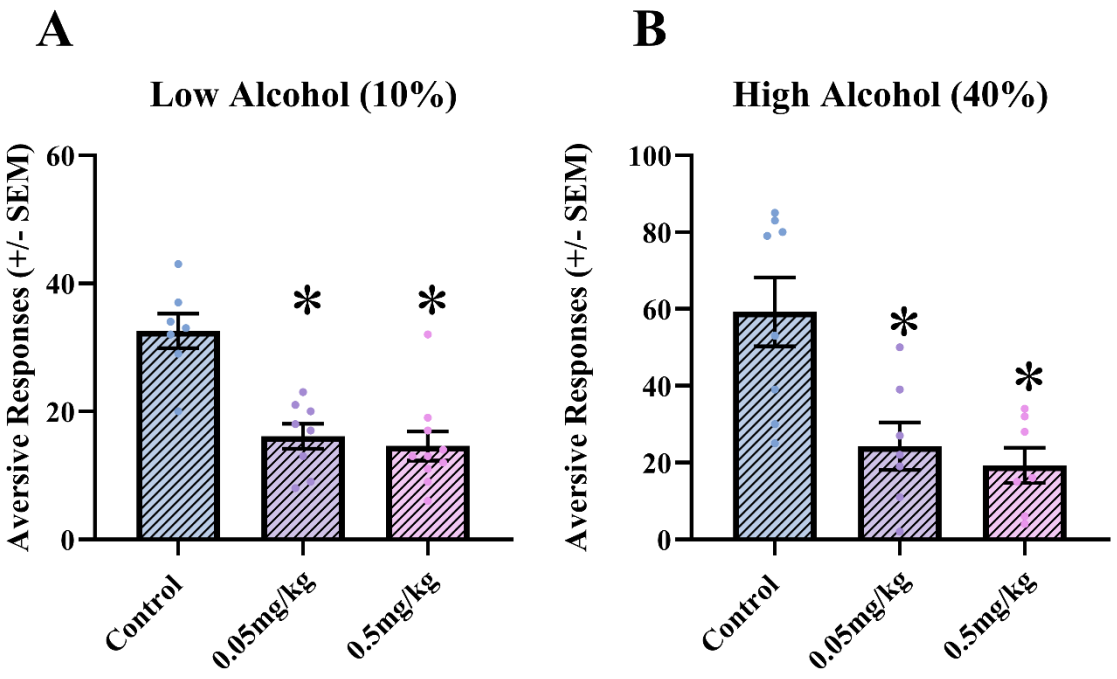

Figure 8 Mean (+/- SEM) aversive responses to alcohol. A) Results indicated that 0.05mg/kg and 0.5mg/kg THC reduced aversive behaviors to (A) 10% alcohol and (B) 40% alcohol compared to the control ($p < 0.001$).

Table 1 ***Post Hoc Comparisons of Drug Condition by Ethanol Concentration for Aversive Reactions to Ethanol***

Treatment Group (THC + etoh)	Comparison Group (THC + etoh)	*B*	SE	*t*	$p_{adjusted}$	Lower 95% CI	Upper 95% CI
Control + Low etoh	Control + High etoh	-25.89	6.08	-4.26	< 0.001 *	-44.66	-7.12
Control + Low etoh	Low THC + Low etoh	17.68	6.80	2.60	0.14	-3.34	38.70
Control + Low etoh	Low THC + High etoh	9.58	7.30	1.31	0.77	-12.96	32.12
Control + Low etoh	High THC + Low etoh	18.76	6.73	2.79	0.09	-2.03	39.55
Control + Low etoh	High THC + High etoh	14.15	7.30	1.94	0.41	-8.41	36.70
Control + High etoh	Low THC + Low etoh	43.57	6.56	6.65	< 0.001 *	23.31	63.82
Control + High etoh	Low THC + High etoh	35.47	7.07	5.02	< 0.001 *	13.65	57.29
Control + High etoh	High THC + Low etoh	44.65	6.48	6.89	< 0.001 *	24.64	64.66
Control + High etoh	High THC + High etoh	40.04	7.07	5.66	< 0.001 *	18.20	61.87
Low THC + Low etoh	Low THC + High etoh	-8.10	6.10	-1.33	0.77	-26.95	10.75
Low THC + Low etoh	High THC + Low etoh	1.08	6.19	0.17	1.00	-18.04	20.20
Low THC + Low etoh	High THC + High etoh	-3.53	6.81	-0.62	0.99	-24.56	17.49
Low THC + High etoh	High THC + Low etoh	9.18	6.73	1.36	0.75	-11.60	29.96
Low THC + High etoh	High THC + High etoh	4.56	7.30	0.63	0.99	-17.98	27.11
High THC + Low etoh	High THC + High etoh	-4.61	5.90	-0.78	0.97	-22.85	13.63

Note. N = 28, 49 total observations, * represents significance of $p < 0.001$.

Water

Hedonic behaviors

Hedonic responses to water on the first and final session were similar across THC groups. The analysis of hedonic responses to water resulted in the fixed effects of THC dose, trial, and

their interaction being insignificant ($p > .05$) (Fig. 9) (Table 2). These results suggest that THC does not influence hedonic responding to water across in both the first and final presentation.

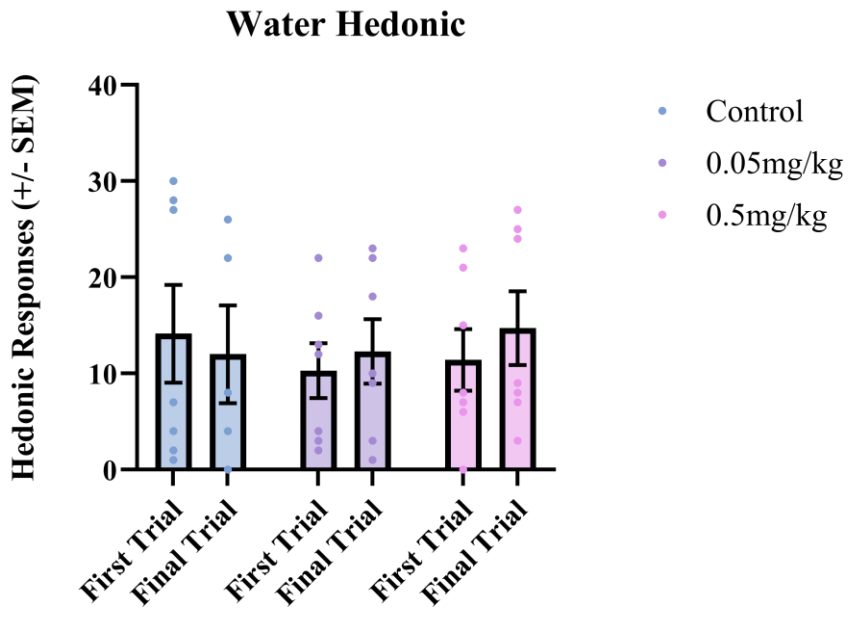

Figure 9 Mean (+/- SEM) hedonic responses to water, comparing the first and final taste reactivity session. Results indicated that THC did not alter hedonic responses to water, regardless of dose or session.

Aversive behaviors

Aversive reactions to water stayed consistent from the first and final presentations. The analysis of aversive responding to water found no significant fixed effects of THC dose, trial, or their interaction (Fig. 10) (Table 2). These results suggest that the THC dose does not affect the aversive responding to water in the first or last session.

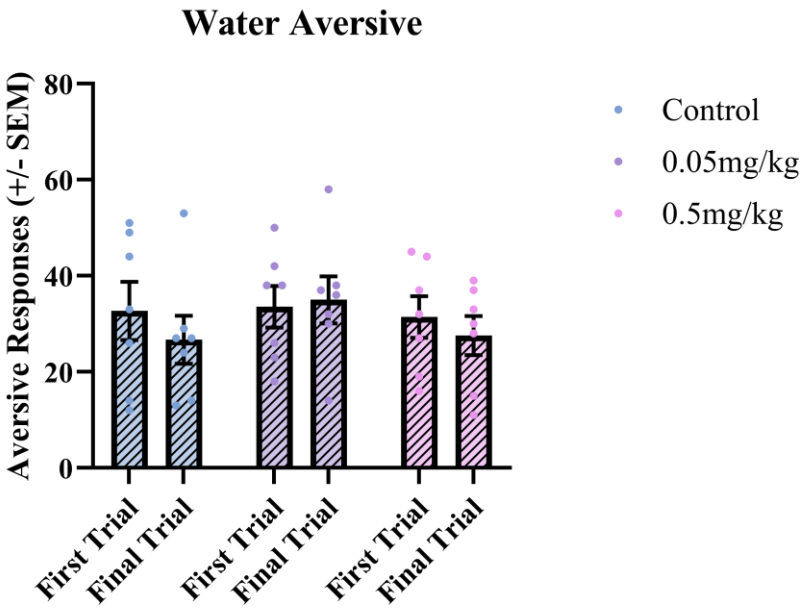

Figure 10 Mean (+/- SEM) aversive responses to water, comparing the first and final taste reactivity session. Results indicated that THC did not alter aversive responses to water, regardless of dose or session.

Table 2 *Model Fit Statistics for Analysis of Taste Reactivity Solutions*

	p	R^2	$R^2_{adjusted}$	*RMSE*	*AIC*	*BIC*
Sucrose (Hedonic)	< .05*	0.65	0.60	32.12	469	479
Sucrose (Aversive)	> .05	0.05	0.23	5.94	245	253
Quinine (Hedonic)	< .05*	0.99	0.99	2.25	180	182
Quinine (Aversive)	< .05*	0.63	0.59	15.22	197	200
Ethanol (Hedonic)	< .05*	0.48	0.42	21.51	465	475
Ethanol (Aversive)	< .05*	0.77	0.75	11.59	407	418
Water (Hedonic)	< .05*	0.29	0.20	14.86	325	334
Water (Aversive)	< .05*	0.23	0.13	11.98	361	371

Note: Significance is indicated by asterisk

Interrater Reliability

Separate Pearson's correlations were used to analyze interrater reliability for each class of taste response (hedonic or aversive) for each substance. Analyses were run for separate, randomly selected videos (N=8 per class/substance, 64 total) as well as select time intervals. The average correlations of hedonic reactions (average r = .93) as well as aversive reactions (average r = .92) were high, suggesting concordant scoring between raters across taste reactivity sessions, substances, and THC doses.

Western Blotting

CB1

Nucleus Accumbens

THC dose did not impact CB1 expression measured via western blot in the NAc. There was no significant effect of THC dose on CB1 protein expression levels, $F(2,5) = 1.00$, $p > 0.05$; Fig. 11. These results suggest that neither low nor high oral THC impact CB1 receptor expression in the NAc.

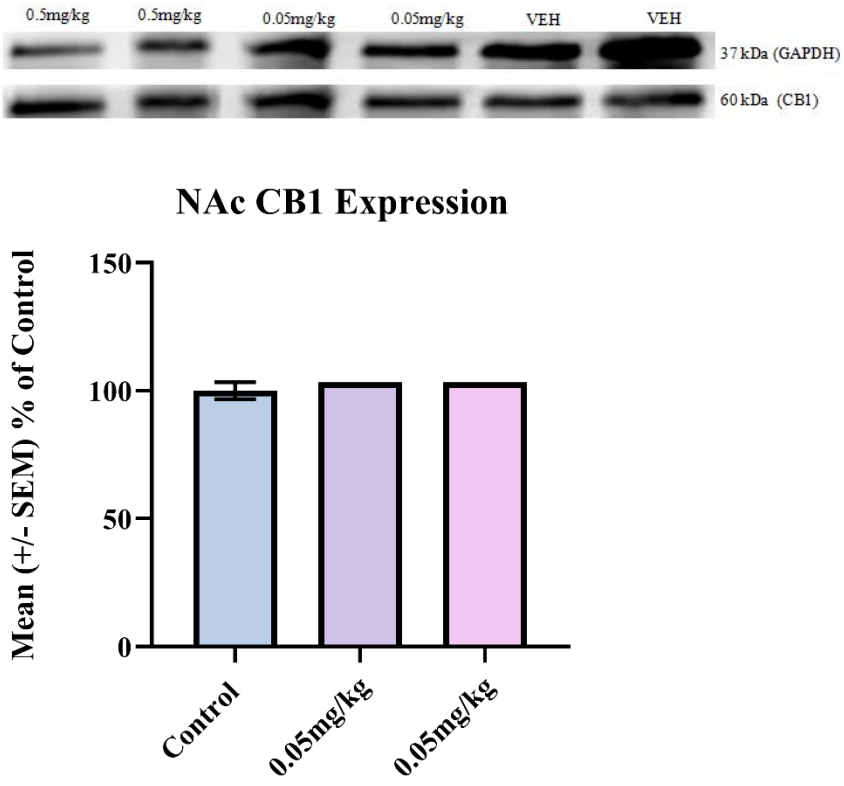

Figure 11 Mean normalized expression (+/-SEM) of CB1 in the NAc. THC did not alter CB1 expression regardless of dose.

Amygdala

THC had no impact on CB1 expression in the Amyg. There was no significant effect of THC dose on CB1 protein expression levels, $F(2,5) = 1.00$, $p > 0.05$; Fig. 12. These results suggest that neither low nor high oral THC impact CB1 receptor expression in the Amyg.

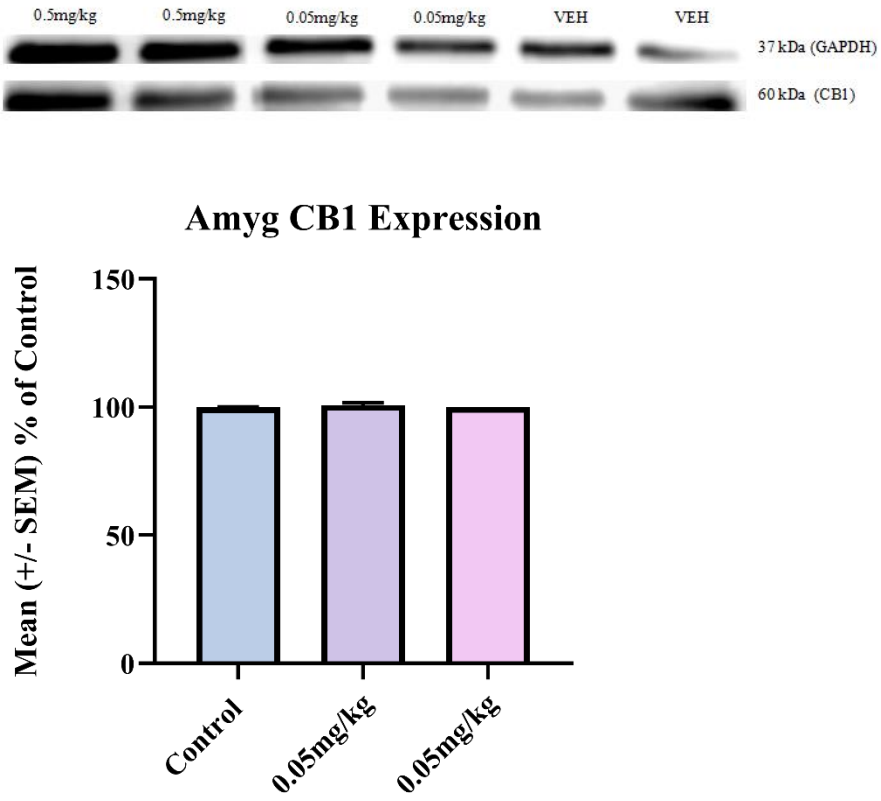

Figure 12 Mean normalized expression (+/-SEM) of CB1 in the Amyg. THC did not alter CB1 expression regardless of dose.

Hippocampus

THC significantly affected CB1 protein expression levels in the dHipp. The analysis, $F(2,5) = 9.79$, $p < 0.05$; Fig. 13, revealed a significant main effect of THC dose. Post hoc comparisons indicated that mean CB1 receptor expression for the control group was significantly different ($p < 0.05$) from the high (0.5mg/kg) THC group. However, the differences between the control group compared to low THC, and low THC compared to high THC were insignificant ($p > 0.05$). This suggests that higher doses of oral THC significantly reduce CB1 protein levels in the dHipp, while lower doses do not.

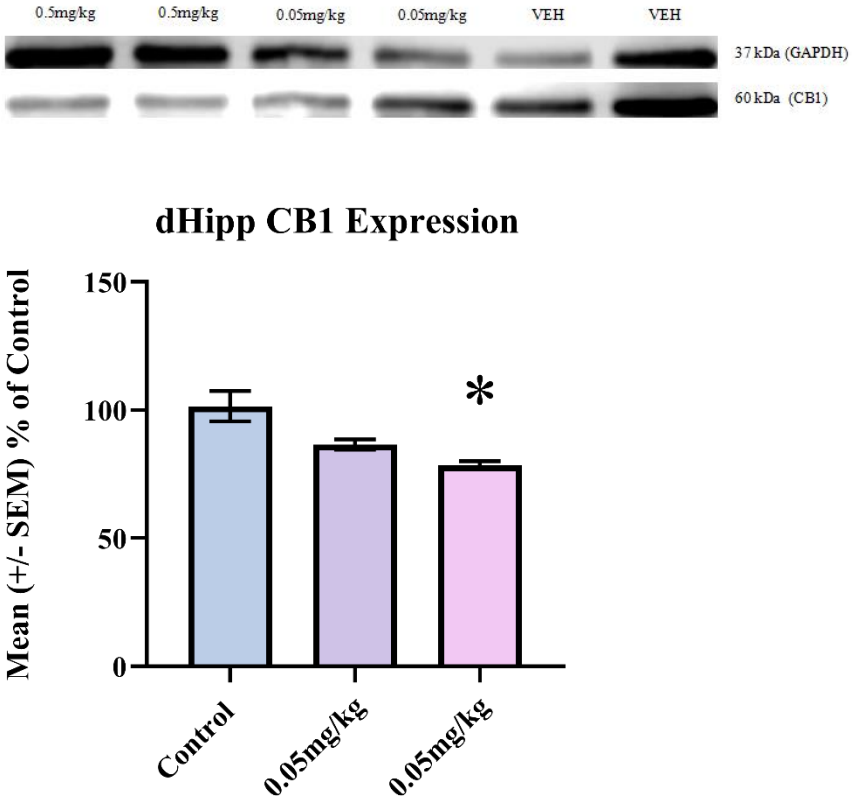

Figure 13 Mean normalized expression (+/-SEM) of CB1 in the dHipp. Results indicated that 0.5mg/kg decreased CB1 expression compared to the control ($p < 0.05$).

ERK

Nucleus Accumbens

THC dose did not change ERK1&2 expression in the NAc. There was no significant effect of THC dose on ERK1&2 protein expression levels, $F(2,5) = 2.35$, $p > 0.05$; Fig. 14A. These results suggest oral THC does not significantly impact ERK1&2 protein expression levels in the NAc.

Amygdala

Similarly, THC dose did not affect ERK1&2 expression in the Amyg. There was no significant effect of THC dose on ERK1&2 protein expression levels, $F(2,5) = 1.49$, $p > 0.05$;

Fig. 15A. These results suggest oral THC does not change ERK1&2 protein expression levels in the Amyg.

Hippocampus

THC did not alter ERK1&2 expression in dHipp. There was no significant effect of THC dose, $F(2,5) = 2.45$, $p > 0.05$; Fig. 16A, suggesting oral THC does not change ERK1&2 protein expression levels in the dHipp.

pERK

Nucleus Accumbens

THC did not alter pERK1&2 expression in the NAc. There was no significant effect of THC dose on pERK1&2 protein expression levels, $F(2,5) = 3.61$, $p > 0.05$; Fig. 14B. These results suggest oral THC does not significantly impact pERK1&2 protein expression levels in the NAc.

Amygdala

Similarly, THC did not change pERK1&2 expression in the Amyg. There was no significant effect of THC dose on pERK1&2 protein expression levels, $F(2,5) = 1.43$, $p > 0.05$; Fig. 15B. These results suggest oral THC does not significantly impact pERK1&2 protein expression levels in the Amyg.

Hippocampus

THC did alter pERK1&2 expression in the dHipp. There was a significant effect of THC dose on pERK1&2, $F(2,5) = 360.42$, $p < 0.05$; Fig. 16B. Post-hoc analysis revealed 0.5mg/kg oral THC had significantly ($p < 0.05$) different expression levels of pERK1&2 than 0.05mg/kg THC and the control. These results suggest that 0.5mg/kg THC but not 0.05mg/kg THC impacts pERK expression in the dHipp.

pERK: ERK ratio

Nucleus Accumbens

THC did not change the ERK1&2:pERK1&2 expression ratio in the NAc. There was no significant effect of THC dose on ERK1&2:pERK1&2 protein expression levels, $F(2,5) = 1.54$, $p > 0.05$; Fig. 14C. These results suggest oral THC does not significantly impact ERK1&2:pERK1&2 protein expression levels in the NAc.

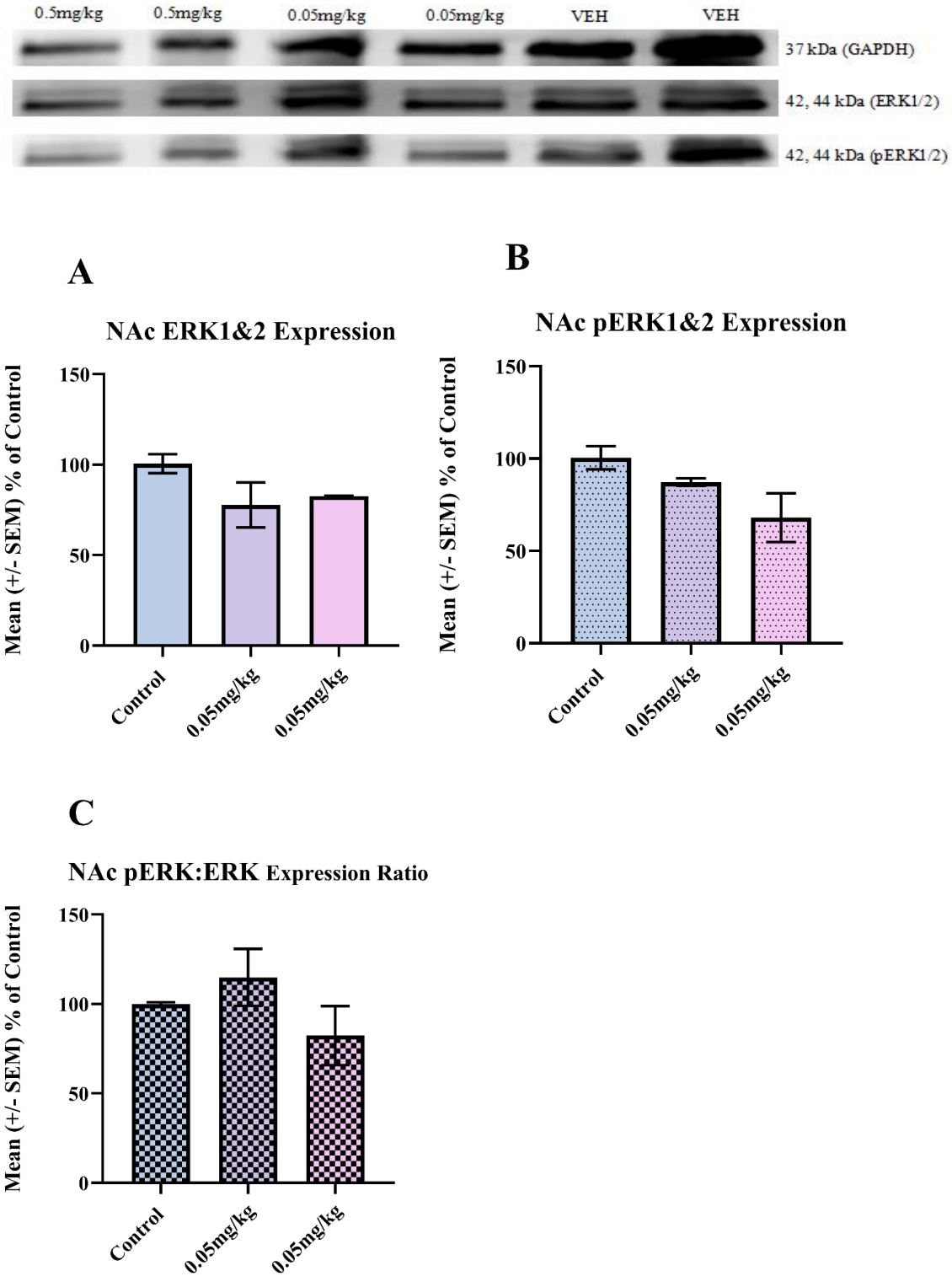

Figure 14 Mean normalized expression (+/-SEM) of A) ERK, B) pERK, C) pERK:ERK in the NAc. Results indicated THC did not alter ERK or pERK expression in the NAc regardless of dose.

Amygdala

Similarly, THC did not change the ERK1&2:pERK1&2 expression ratio in the Amyg. There was no significant effect of THC dose on ERK1&2:pERK1&2 protein expression levels, $F(2,5) = 1.43$, $p > 0.05$; Fig. 15C. These results suggest oral THC does not significantly impact ERK1&2:pERK1&2 protein expression levels in the Amyg.

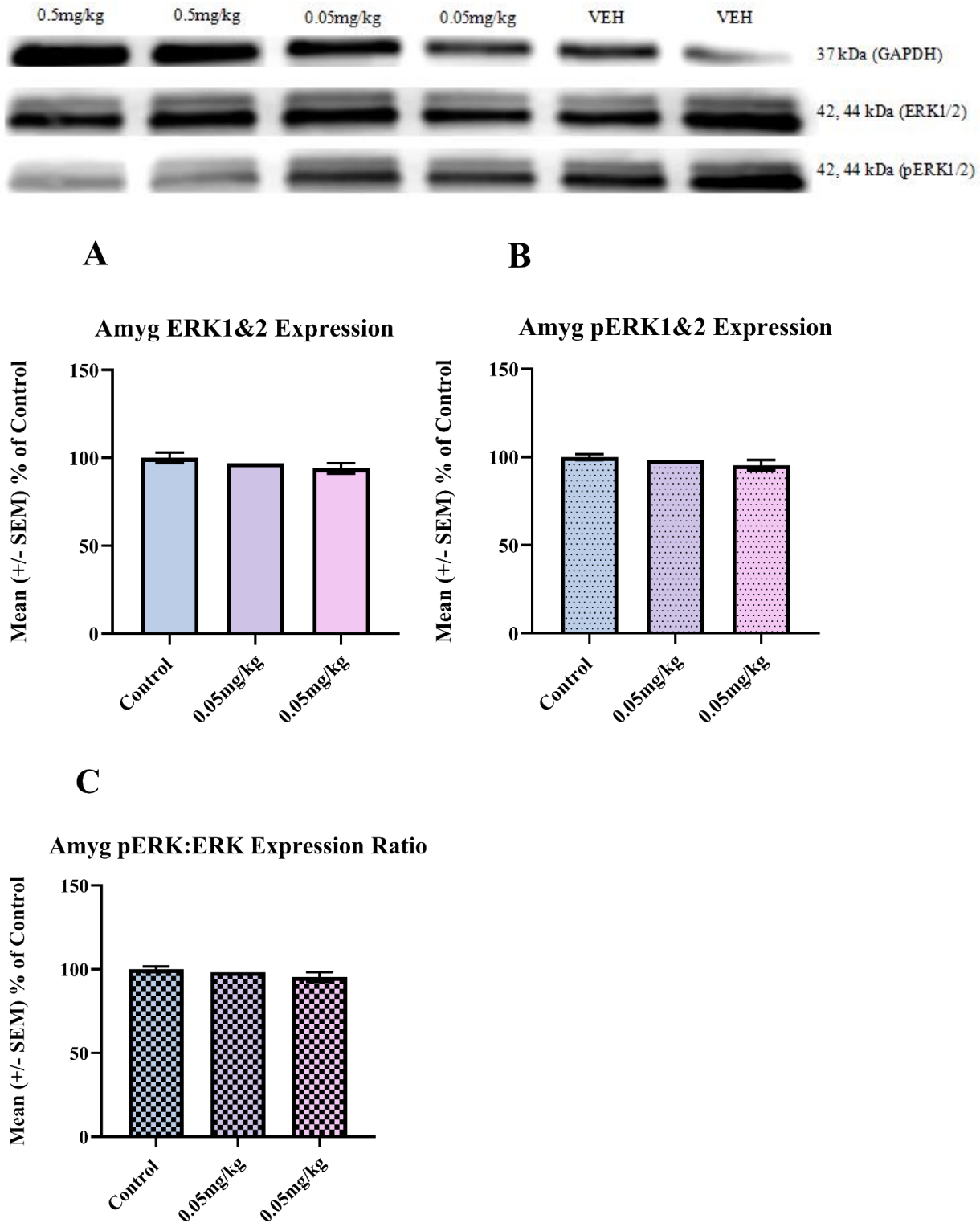

Figure 15 Mean normalized expression (+/-SEM) of A) ERK, B) pERK, C) pERK:ERK in the Amyg. Results indicated THC did not alter ERK or pERK expression in the Amyg regardless of dose.

Hippocampus

THC did alter the expression ratio of pERK1&2:ERK1&2 in the dHipp. THC dose had a significant effect on the expression ratio $F(2,5) = 1.43$, $p > 0.05$; Fig. 16C, with post-hoc analysis revealing that 0.5mg/kg oral THC significantly ($p < 0.05$) altered the expression ratio of pERK: ERK compared to 0.05mg/kg THC and the control. These results suggest that 0.5mg/kg but not 0.05mg/kg oral THC or the control modulates dHipp ERK1&2 phosphorylation.

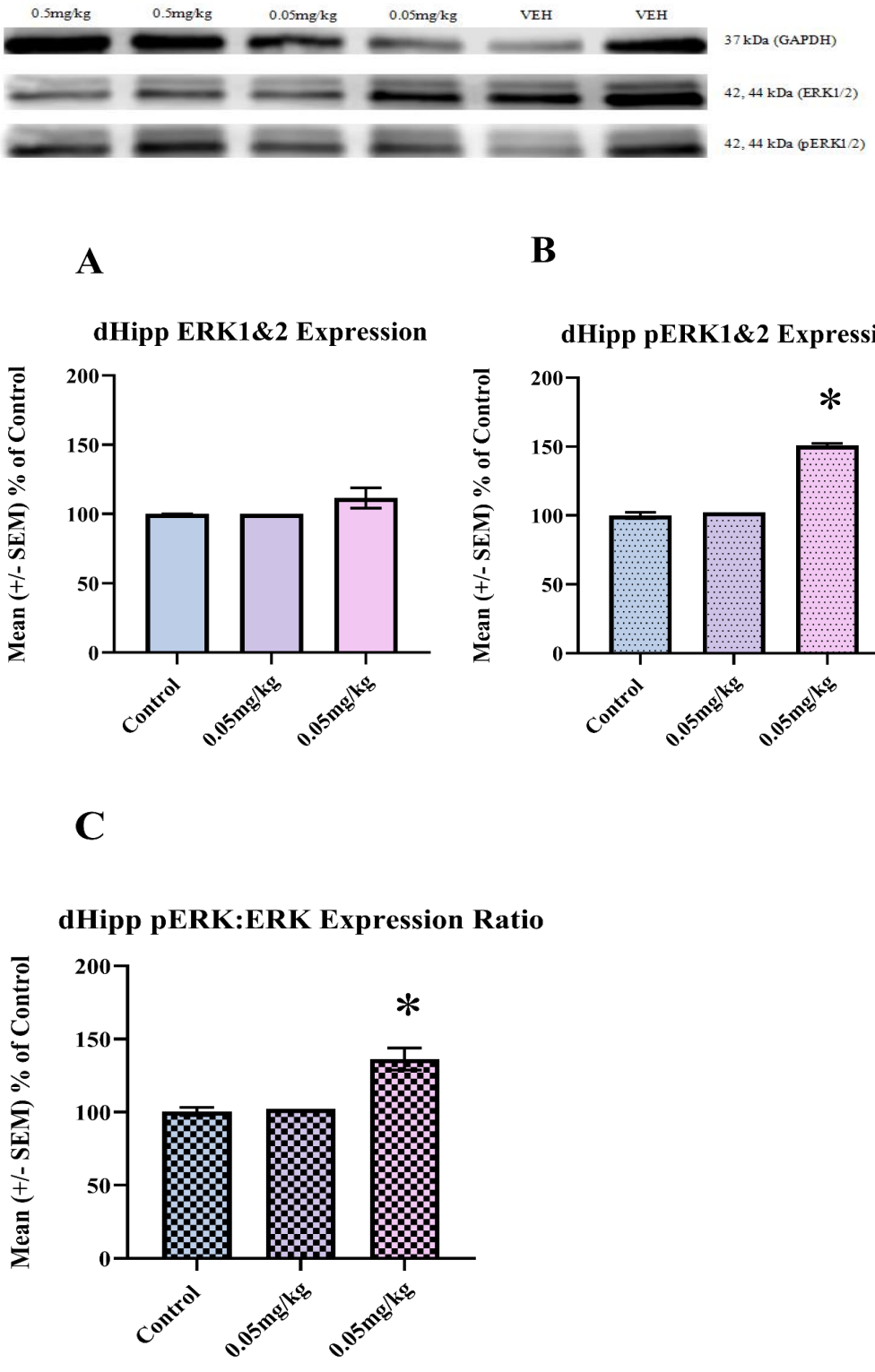

Figure 16 Mean normalized expression (+/-SEM) of A) ERK, B) pERK, C) pERK:ERK in the dHipp. A) THC did not alter ERK expression in the dHipp. B) Results indicated that 0.5mg/kg THC increased pERK expression in the dHipp compared to the control ($p < 0.05$). C) 0.5mg/kg THC significantly increased the pERK to ERK ratio in the dHipp ($p < 0.05$).

Chapter 4 - Discussion

Taste Reactivity

The results revealed that oral THC alters taste reactivity to sucrose, ethanol, and quinine solutions. When compared to the control, 0.5mg/kg oral THC increased hedonic responding across both sucrose concentrations, while having no impact on aversion to sucrose. Similarly, the lower concentration of oral THC (0.05mg/kg) increased hedonic responding to the high (0.5M) sucrose concentration. THC dose-dependently increased hedonic response to ethanol when compared to the control, but only at low (10%) concentrations. Neither 0.05mg/kg nor 0.5mg/kg oral THC altered hedonic response to high (40%) ethanol when compared to the control. Interestingly, both 0.5mg/kg and 0.05mg/kg oral THC reduced aversive behaviors across low and high ethanol concentrations when compared to the control. While THC did not alter hedonic responses to quinine, both 0.5mg/kg and 0.05mg/kg oral THC decreased aversive behaviors to quinine when compared to the control. Importantly, THC did not alter hedonic or aversive responses to water between the first and final trials when compared to the control. These results suggest that oral THC enhances the hedonic value of the rewarding substance, sucrose, and low concentrations of ethanol. Importantly, THC also reduces aversion to the commonly aversive substances, quinine and ethanol.

Sucrose

Previous studies investigating THC and other cannabinoid agonists have shown that the cannabinoid system modulates appetitive behaviors and consumption of sweet foods (Arnone et al., 1997; Koch, 2001). While this research on cannabinoid modulation and food has utilized a range of appetitive measures and consumption paradigms, Jarret et al., found that injections of THC increased the palatability of sucrose, in a time-dependent manor, by utilizing the TR

paradigm (2005). While previous research tested behavioral effects at various time points, the present study adds to the literature by showing consistency in timing and results while utilizing a translation model of drug consumption. Jarret et al. (2005) found that injections of 0.5mg/kg THC increased hedonic behaviors for 2%, 10%, and 32% sucrose solutions, occurring only 120min and not 30min or 60min post-injection. The current experiment found that oral consumption of 0.05mg/kg or 0.5mg/kg THC increased hedonic behaviors to sucrose, independent of sucrose concentration (0.1M & 0.5M or 3% & 17%) at 90min post-drug consumption. Despite the route of administration, the combined results suggest THC starts modulating hedonic value after 60 minutes. Importantly, differences in drug metabolism through route of administration should be addressed. Previous research in rats has shown that injections of 10mg/kg of THC result in peak blood (ng/ml) and brain tissue (ng/g) levels of THC at 15min post-administration, whereas oral administration results in peak levels at 2 hours post-administration (Hložek et al., 2017). Notably, injections of THC result in no OH-THC recorded in the blood, and very low levels in the brain; while oral THC results in low levels of OH-THC in the blood, and high levels in the brain (Hložek et al., 2017). Therefore, the present study shows evidence for an optimal time range for THC modulating the hedonic value of sucrose, contingent on the route of administration. Alternatively, the results may suggest a potential for OH-THC impacting palatability for hedonic substances differently than THC.route of administration. Alternatively, the results may suggest a potential for OH-THC impacting palatability for hedonic substances differently than THC. While the psychoactive effects of OH-THC do not significantly vary from those of delta-9-THC, the shift from delta-9-THC to OH-THC plays an important role in THC modulating behavior due to its increased and prolonged psychoactive effects (Grotenhermen, 2003; Hložek et al., 2017; Huestis, 2007; Russo, 2007). The

increased and prolonged effects from oral THC plays a significant role in investigating the properties of oral THC while highlighting the importance of dosage and timing of behavioral and neurobiological testing.

Quinine

Previous research on THC modulating the palatability of commonly aversive substances has shown that injections (0.5mg/kg) reduce the number of aversive behaviors to quinine (0.01%) while having no effect on hedonic responses (Jarret et al., 2007). This effect was seen at a range of times from 30min until the final test of 3 hours post-drug administration (Jarret et al., 2007). Similarly, the current study revealed that oral THC, 0.05mg/kg and 0.5mg/kg, reduces aversive behaviors to quinine (0.1mM or 3%) 90min post drug consumption. Despite minor discrepancies in drug administration and quinine concentration, the replication of results suggests that THC's effect on modulating the palatability of substances extends from rewarding substances to bitter tastants and is not limited to THC or OH-THC pharmacokinetic profile (Hložek et al., 2017; McGilveray, 2005). While previous research nor the present study found that THC increases hedonic behaviors, it is important to note that quinine is commonly distasteful, and results in very low hedonic behaviors in the TR paradigm (Wukitsch et al., 2020). This 'floor effect' may give a reason why THC only reduced aversion and did not increase hedonic value.

Ethanol

Preclinical studies investigating cannabinoids affecting alcohol drinking have produced mixed results. While some research shows lower doses of THC can increase alcohol incentive salience in an operant paradigm (Gallate et al., 1999), other studies have shown that vaping high doses of THC does not increase free access to alcohol drinking (Hamidullah et al., 2021), and in some cases, high doses of THC edibles can decrease alcohol drinking (Nelson et al., 2019).

While there are notable differences in the route of administration for THC and access to alcohol, these studies only investigate how THC can modulate the incentive salience of alcohol. Importantly, studies that assess ethanol consumption are confounded by both the taste of ethanol and its post-ingestive effects (Kiefer, 1995; Kiefer & Dopp, 1989). Therefore, the present experiment was designed to test the effects of THC on the hedonic value of alcohol utilizing the TR paradigm.

It is important to note that in TR, ethanol produces a combination of both aversive and hedonic reactions (Wukitsch et al., 2020). As oral exposure to alcohol increases, hedonic reactions increase (Kiefer et al., 1994) and aversive reactions decrease (Kiefer & Dopp, 1989). To combat this effect, rats in the present experiment were presented ethanol concentrations in randomized order via Latin-Square design. Like quinine, the results indicated that when compared to the control, both 0.05mg/kg and 0.5mg/kg oral THC reduced aversive reactions to ethanol, independent of concentration. These results suggest that THCs ability to decrease aversion is not limited to quinine, but more likely bitter tastes in general. These similarities suggest that future research on cannabinoids and the hedonic value should explore the disparities between bitter tastants.

Interestingly, as ethanol concentration increased, aversive responding in control animals increased, while THC animals had no change in aversive responses. Comparatively, 0.5mg/kg oral THC increased hedonic responding to ethanol independent of concentration, while 0.05mg/kg THC did not alter hedonic response compared to the control. These results suggest that oral THC significantly alters the palatability of ethanol, by not only increasing the hedonic value but decreasing aversion as well. Further, these results could lead to a better understanding of the high prevalence of co-morbid CUD and AUD in humans (reviewed in Hasin & Walsh,

2021; Stinson et al., 2006). While the scope of the present study is limited to adult rats, it is important to note the animals were alcohol naïve. As previously stated, alcohol exposure prior to testing can impact alcohol preference (Kiefer et al., 1994). Further, human studies indicate nearly 60% of adolescents who consume alcohol also report using cannabis (Martin et al., 1996). The results of the present study suggest that THC use may contribute to increased alcohol drinking before AUD diagnosis. Consequently, it is imperative to gain a better understanding of how THC use can influence alcohol consumption in both adolescents and adults.

Water

As predicted, THC did not affect hedonic or aversive reactions to water across the two sessions. These results suggest THC does not enhance novelty response, as there was no difference between THC or control in the first or final TR session. Recently, this hypothesis was tested in memory tasks using novelty preference in rats, where THC did not influence novelty preference in either odor or object-based tasks (Barnard et al., 2023). While the present study utilized a different sensory mechanism (taste), it adds to the understanding of THC and its impact on shifting preferences.

Behavior Conclusion

While the dose range of oral THC and concentrations of ethanol are limited, the results of the present study suggest that the cannabinoid system modulates the hedonic value of both rewarding and aversive substances. The results are translationally important for cannabis users. during the first exposure to alcohol with no flavor additives. As previous research has shown, as alcohol exposures increase, the incentive motivation to alcohol also increases (Kiefer et al., 1994; Kiefer & Dopp, 1989). Therefore, the result of the present study suggests that oral THC

increases alcohol hedonic value at first exposure, while continued exposure of THC and alcohol may increase liking even greater over time.

Further, it must be taken into consideration that an effect on hedonic value does not directly correlate with incentive salience or ‘wanting’. The neurobiological pathways involved in "wanting" are extensively distributed across the mesocorticolimbic circuits and are largely driven by midbrain dopamine projections to the forebrain (Berridge, 1996; Berridge, 2009; Berridge & Kringelbach, 2013; Berridge & Robinson, 1998; Robinson & Berridge, 2003). In contrast, "liking" is regulated by specific hedonic hotspots, which are smaller regions within larger structures, primarily located in the limbic system (Berridge, 2019; Peciña & Berridge, 2005; Peciña et al., 2006; Richard et al., 2013). Notably, while stimulation of these hotspots with opioids and other neurotransmitters can increase liking responses, dopamine does not produce the same effect (Berridge & Kringelbach, 2013; Berridge et al., 2009; Peciña & Berridge, 2000; Peciña & Berridge, 2005; Peciña & Berridge, 2013). Dopamine's role appears to be confined to the domain of wanting (Berridge & Kringelbach, 2013; Berridge, 2007; Smith & Berridge, 2005). However, recent studies have revealed that stimulation of opioid receptor hot spots not only enhances liking but amplifies food intake and reward value as well (Welsch & Kieffer, 2021). These results suggest that while dopamine does not play a role in liking, the opioid system plays a role in both liking and wanting.

Investigations of THC and its effect on dopamine are thorough, and it has been shown in humans (Bossong et al., 2009, 2015) and rats (French, 1997; Tournier et al., 2016) that THC increases dopamine transmission and receptor expression in a region-specific manner. Similarly, previous studies have shown THC has a direct effect within the opioid system, and strong evidence for interactions between the cannabinoid and opioid systems (Corchero et al., 2004;

Fattore et al., 2004; Kirkham & Williams, 2001; Manzanares et al., 1999). While the present study did not measure THC's effects on the opioid system, the behavior results are correlated to previous research linking the cannabinoid and opioid systems. These similarities suggest that future THC research targets the interaction between the cannabinoid and opioid system, measuring the modulation of hedonic value and incentive salience of both rewarding and aversive tastants.

Western Blotting

CB1

The findings of this study contribute to the broader understanding of how oral THC affects CB1 receptor expression in different brain regions, complementing existing literature. Previous research has established that chronic but not acute THC use can lead to significant downregulation of CB1 receptors throughout the brain (Kruse et al., 2019; Lazenka et al., 2014; Villares, 2007; Zamberletti et al., 2016). The present study investigated CB1 expression changes after chronic oral THC use at two different doses for over two weeks. The CB1 receptors play a critical role in various neural functions, including synaptic connectivity and gene expression (Kano et al., 2009). However, in contrast to the expected downregulation associated with chronic THC use, the present study found that oral THC, particularly at 0.5mg/kg, only significantly reduced CB1 receptor expression in the dHipp, with no significant changes observed in the NAc s or amyg. The lack of CB1 downregulation in the NAc is particularly interesting given its well-established role in modulating hedonic value and incentive behaviors through CB1 receptor activation (Mahler et al., 2007; Mitchell et al., 2018). However, this finding aligns with prior studies that reported minimal changes in CB1 expression in the NAc following THC exposure, suggesting that the effects of THC on CB1 expression in this region may not be present

regardless of dose or route of administration. The current results underscore the complexity of THC's impact on the mesocorticolimbic system, suggesting that the relationship between CB1 receptor expression and behavioral outcomes may not be linear. However, a limitation of the current experiment is the combined analysis of tissue from the Nucleus Accumbens Core (NAcC) and Shell (NAcS). Previous research has shown that hedonic responses to rewarding substances are primarily controlled by mu opioid receptors located in the NAcS, but not in the NAcC (Castro & Berridge, 2014; Peciña et al., 2006). Similarly, modulating CB1 receptor function has been shown to affect the NAcC but not the NAcS (Mahler et al., 2007; Mitchell et al., 2018). As a result, the lack of observed changes in receptor expression in the combined NAc region may be due to the distinct behavioral roles of each sub-region, limiting the specificity of our findings.

In the Amyg, where the cannabinoid system is implicated in taste preferences and alcohol consumption behaviors (Schwerdtfeger et al., 2023) the absence of significant changes in CB1 expression following oral THC administration contrasts with some preclinical studies that have shown CB1 downregulation (Lazenka et al., 2014). However, preclinical research utilizing oral THC has expanded the dose range much higher than human consumption of oral THC (Schlienz et al., 2020). This discrepancy highlights the importance of considering both the dosing and route of THC administration when interpreting its effects on the brain as we continue utilizing translational models to advance our understanding of cannabinoids. It is important to note that previous studies on CB1 receptors in the amygdala have shown differences between sub-regions. Specifically, THC administration has been found to reduce CB1 expression in the basolateral amygdala (BLA), but not in the lateral amygdala (Lazenka et al., 2014). This distinction highlights a limitation of the current experiment, where the entire amygdala region

was collected and analyzed as a whole. Notably, the BLA is part of the circuitry involved in alcohol drinking (Koob, 2001; Koob et al., 1998), which also extends to the NAcS (McBride, 2002). Therefore, it is crucial to identify changes within the cannabinoid system specifically in the subdivisions of the amygdala, as these could play a key role in the modulation of alcohol consumption.

The observed downregulation of CB1 receptors in the dHipp following high-dose oral THC administration is consistent with previous studies that reported similar effects using a range of doses and routes of THC administration (Laux et al., in review; Lazenka et al., 2014).This finding supports the notion that the hippocampus is particularly sensitive to THC-induced CB1 downregulation. Importantly, the heterogeneity of hippocampal connections suggests that CB1 receptors may influence both hedonic value and incentive salience. The dorsal hippocampus (dHipp) projects to a variety of brain regions, notably the NAcS and several amygdala sub-regions (Ibrahim et al., 2024; Tannenholz et al., 2014). Although the present study did not find differences in CB1 expression in the NAc or Amyg as a whole, the specificity of sub-regions and the role of the dHipp provide evidence that CB1 receptors are involved in modulating hedonic value. This may have implications for understanding the role of cannabis and alcohol use. Overall, these results suggest that the impact of oral THC on CB1 receptor expression is both region-specific and dose dependent.

While the effect of CB1 downregulation is isolated to the dHipp in the current experiment, there is evidence of THC induced downregulation of CB1 in regions correlated to hedonic behaviors (Lazenka et al., 2014). The results of the current experiment can be interpreted as a disconnect from CB1 expression reduction and hedonic value changes, however, it is important to note two alternatives. First, the lack of CB1 downregulation within the NAc is

particularly surprising considering the specificity of behavioral control in the taste reactivity task. However, previous research has outlined a large role of the CB1 controlling hedonic value within the dorsal medial NAcS, but not elsewhere in the NAcS or in the NAcC (Mahler et al., 2007; Mitchell et al., 2018). This particular subregion location of the CB1 modulating hedonic value could be the reason why we did not observe downregulation in the western blot results, We analyzed receptor expression within the entire NAc and not the particular subregion that is specific to the taste reactivity task. Second, the downregulation of CB1 found in the current experiment is isolated to the dHipp, which is an important finding when investigating the roles of the mesolimbic system particular to incentive motivation. The dHipp plays a key role in stimulus integration and projecting reward information to the NAcS for different reward related behaviors (Ibrahim et al., 2024; Tannenholz et al., 2014). Therefore, the results of the present experiment may suggest that THC induced downregulation of CB1 in the dHipp could be the cause of behavioral change in taste reactivity. Consequently, it cannot be ruled out that the present neurobiological results are disconnected from the behavioral results. Further, the selective effect on CB1 expression in the brain underscores the need for further research to fully understand the nuances of THC's action, particularly the risks associated with chronic use as well as CUD and co-morbid use disorders.

ERK, pERK

The results of this study provide important insights into the effects of oral THC on ERK and pERK expression across different brain regions, particularly the NAc, Amyg and dHipp. These findings contribute to the existing body of research on the modulation of ERK signaling by cannabinoids, highlighting both region-specific and dose-dependent effects.

ERK, a crucial regulator of cell proliferation, differentiation, and response to extracellular stimuli (Seger & Krebs, 1995; Sun et al., 2016), is modulated by CB1 receptor activation, which can influence synaptic transmission and various signal transduction pathways (Howlett et al., 2002; Sun et al., 2016). Previous studies have demonstrated that CB1 activation in the hippocampus can stimulate ERK, leading to the expression of immediate early genes (Bouaboula et al., 1995; Wartmann et al., 1995; Derkinderen et al., 2003; Massi et al., 2013). However, the current study found that oral THC, irrespective of dose, did not significantly alter ERK1&2 or pERK1&2 expression in the NAc or Amyg, suggesting that these regions may be less responsive to THC's effects on ERK signaling when administered orally. The absence of significant changes in ERK1&2 and pERK1&2 expression in the NAc contrasts with some preclinical studies that reported ERK activation in response to acute and chronic THC exposure in mesocorticolimbic structures (Daigle et al., 2011; Sun et al., 2016; Valjent et al., 2001). This discrepancy could be attributed to sub-region specificity and differences in THC dosing and administration methods, with previous studies often employing high-dose injections rather than oral delivery, which could lead to different pharmacokinetic profiles (Hložek et al., 2017), and subsequent effects on ERK signaling.

The region-specific ERK phosphorylation observed in the current study may be attributed to distinct intracellular pathways associated with hedonic value. Previous research has shown that stimulation of the mu opioid receptor in the NAcS increases liking responses to sucrose (Berridge, 2000; Peciña, Smith, & Berridge, 2006). Similarly, activation of delta and kappa opioid receptors within the NAcS also enhances sucrose liking (Castro & Berridge, 2014). It is hypothesized that the cannabinoid and opioid systems are functionally linked (Kirkham & Williams, 2001; Manzanares et al., 1999), and evidence suggests that THC affects mu, kappa,

and delta opioid receptors in reward processing (Ghozland et al., 2002; Solinas & Goldberg, 2005). Notably, activation of all three opioid receptor types leads to ERK activation. However, when considering the lack of ERK phosphorylation changes in the NAc observed in this study, it is crucial to note that the enhancement of liking triggered by activation of these opioid receptors is localized to the same rostral cubic millimeter within the NAcS (Castro & Berridge, 2014a, 2014b). In contrast, stimulation of these receptors in the caudal portion of the NAcS reduces liking (Castro & Berridge, 2014a, 2014b). If THC enhances liking through interaction with these opioid receptors, ERK activation might be confined to the rostral portion of the NAcS. However, since the present study examined the entire NAc, this may explain the lack of observed effects in regions specifically associated with hedonic value. Future studies could investigate the rostral versus caudal portions of the NAcS using immunohistochemistry rather than western blotting to gain a more detailed understanding of these effects.

The current study found a significant effect of high-dose oral THC (0.5 mg/kg) on pERK1&2 expression and the pERK ratio in the dHipp, but not at the lower dose (0.05 mg/kg) or in the control group. This finding is consistent with previous research indicating that the hippocampus is particularly sensitive to THC's effects on ERK signaling (Daigle et al., 2011; Rubino et al., 2004, 2005; Sun et al., 2016). The increase in pERK expression and the altered pERK ratio at the higher THC dose suggests enhanced ERK phosphorylation, which may have implications for hippocampal-dependent processes such as memory and learning, areas where ERK signaling is known to play a critical role (Davis et al., 2000; Hill et al., 1993; Treisman, 1996).

Overall, these results indicate that while oral THC does not significantly impact ERK or pERK expression in the NAc or amyg, it does induce significant changes in the dHipp at higher

doses. These region-specific effects highlight the complex nature of THC's action on ERK signaling pathways and suggest that the hippocampus is a particularly sensitive target for THC-induced modulation of ERK activity. Further research is needed to explore the functional consequences of these changes, particularly in relation to cognitive and reward-related behaviors, and to clarify the mechanisms underlying the differential effects of THC across brain regions.

Western Blot Conclusion:

Chronic oral THC at 0.5mg/kg reduced CB1 expression and increased ERK phosphorylation in the dHipp, but not in the NAc or Amyg. While sub-region discrepancies were previously addressed, the method of tissue sample preparation remains. Tissue samples were combined by drug dose and each brain region was pooled together to create two replicates per variable. While this method has been previously established (Hudson et al., 2019; Taylor & Posch, 2014), it does limit statistical power and the potential to see effects with larger degrees of freedom from testing each rat individually. However, the results of the present experiment follow trends from previous THC research with region dependent differences in both CB1 and ERK expression differences (Hudson et al., 2019; Lazenka et al., 2014; Laux et al., In Review). Overall, the hypotheses were supported for CB1 and phosphorylation of ERK but limited to the dHipp while NAc and Amyg showed no differences regardless of THC dose. These results suggest that chronic oral THC dose dependently alters CB1 expression and ERK phosphorylation in a region-specific manor.

Conclusion

In conclusion, the present study demonstrates that oral THC produces nuanced effects on both taste reactivity and region-specific molecular signaling pathways within the brain. The enhancement of hedonic responses to sucrose and low concentrations of ethanol, coupled with a

reduction in aversive behaviors to quinine and ethanol, highlights THC's ability to modulate the hedonic value of both rewarding and aversive substances. These behavioral outcomes, together with the observed dose-dependent changes in CB1 receptor expression and ERK phosphorylation, underscore the complex interplay between THC and the neurobiological mechanisms underlying reward and aversion. The differential impact of THC on these systems, particularly in the dHipp, suggests that the cannabinoid's effects are highly contingent on both dosage and brain region. Considering the dHipp's connections with sub-regions of the NAc and Amyg, along with the observed THC-induced changes in CB1 expression and ERK phosphorylation, may provide insights into the role of these neural circuits in incentive motivation. Additionally, the presence of behavioral effects despite the absence of significant neurobiological changes in the NAc and Amyg suggests a division of functional processing within the sub-regions of these brain areas. This has potential implications for understanding the neurobiological basis of substance use disorders. These findings not only contribute to the growing body of research on cannabinoids but also emphasize the importance of considering both the route of administration and dosage when evaluating the effects of THC in preclinical models. Future studies should aim to further elucidate the specific mechanisms by which THC modulates these pathways and explore their relevance to conditions such as Cannabis Use Disorder (CUD) and co-morbid substance use disorders.

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