INVESTIGATING THE BEHAVIOURAL IMPACTS OF CHRONIC HIGH-CBD CANNABIS CONSUMPTION IN ADOLESCENT LONG-EVANS RATS

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ABSTRACT

Cannabis sativa, or 'cannabis', is an herbaceous plant that possesses hallucinogenic and medicinal properties. The primary constituents found within cannabis are CBD and THC, both of which are capable of eliciting behavioural and physiological effects in mammals. CBD has shown promise in controlling the adverse effects of THC. THC and CBD together have shown promising synergistic and medicinal effects. With the increased use of cannabis as a medicine, it is imperative we determine the safety of consumption in an adolescence. Using two high-CBD cannabis extracts at two dosing levels, adolescent animals were dosed for 14 days. Following dosing, animals were tested on a behavioural test battery looking for altered anxiety and learning and memory skills. Overall, there were no major impacts of sex, treatment or Treatment x Sex Differences in behaviour. In conclusion, there are no lasting behavioural impacts of consuming high-CBD cannabis extract.

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2-AG	2-arachidionyl glycerol
A1R	Adenosine-1 receptors
AC	Adenylyl cyclase
AD	Alzheimer's Disease
AEA	Anandemide
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
ANOVA	Analysis of Variance
Ca^{2+}	Calcium ions
CB_1	Cannabinoid type 1
CB_1R	Cannabinoid type 1 receptor
CB_2	Cannabinoid type 2
CB_2R	Cannabinoid type 2 receptor
CBC	Cannabichromene
CBD	Cannabidiol
CBN	Cannabinol
CNS	Central Nervous System
DAGLα	Diacylglycerol lipase alpha
eCB	Endocannabinoid
eCBR	Endocannabinoid receptor
EPM	Elevated Plus Maze
GABA	Gamma-Aminobutyric acid
K^+	Potassium ions
LTD	Long-term depression
LTP	Long-term potentiation
mGluR	Metabotropic glutamate receptor
MS	Multiple sclerosis
MWT	Morris Water Task
PKA	Protein kinase A
PLCβ	Phospholipase C beta
THC	Δ^9-tetrahydrocannabinol
Δ^9-THC	Δ^9-tetrahydrocannabinol
Δ^8-THC	Δ^8-tetrahydrocannabinol
TRPV	Transient receptor potential vanilloid
TRPV1	Transient receptor potential vanilloid subtype 1
TRPV1R	Transient receptor potential vanilloid subtype 1 receptor
VGCC	Voltage gated calcium channels

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 What is Cannabis

Cannabis sativa, or 'cannabis', is an herbaceous plant that possesses hallucinogenic and medicinal properties. Cannabis has been used for centuries by a variety of cultures for both its medicinal and intoxicating properties; currently cannabis is recognized as the world's most frequently used illicit drug and is among the oldest drugs known to man (Nunes 2012). Understanding the extent of cannabis' effects has been pivotal in the use of cannabis as a medicine. To date, cannabis has been used to treat a variety of ailments including nausea, inflammation, cancer, Multiple Sclerosis (MS), Alzheimer's Disease (AD), childhood seizure disorders and many more (Tramer et al., 2001; Croxford & Yamamura, 2005; Chong et al., 2006; Velasco, Sánchez & Guzmán, 2016; Karl, Garner & Cheng, 2017; Dale et al., 2019).

1.1.2 Cannabis constituents

Cannabis is comprised of over 150 individual constituents. These constituents are responsible for a variety of medicinal and recreational effects including increased appetite, euphoria, and anti-inflammatory properties (Mechoulam, 2005; Russo & Guy, 2006). With the vast number, each constituent is organized into one of 18 classifications of chemicals, including: nitrogenous compounds, carbohydrates, fatty acids and, the two we will discuss as they pertain to the variety of effects cannabis elicits, cannabinoids and terpenes/terpenoids (Russo, 2011).

1.1.3 Cannabinoids

Cannabis contains over 100 discovered individual cannabinoids. Cannabinoids in general are the constituents capable of eliciting effects in mammals. The variety of effects is still being researched and is not yet fully understood. What is known is that cannabinoids induce behavioural effects in mammals by binding to receptors within the brain (Vann et al., 2008). The cannabinoid classification is further subdivided into three broad categories: Endocannabinoids phytocannabinoids, and synthetic cannabinoids.

Endogenous cannabinoids (or endocannabinoids) are cannabinoids synthesized within the human body. These cannabinoids are responsible for maintaining your body's homeostatic state as well as many cellular processes (Zhu, 2006; Katona & Freund, 2012; Mechoulam & Parker, 2013). The existence of naturally occurring endocannabinoids within our bodies is the reason we possess receptors that are capable of binding phyto- and synthetic cannabinoids.

Synthetic cannabinoids are cannabinoids artificially synthesized in a lab (Klein & Cabral, 2006). These cannabinoids will not be discussed in detail within this review. For more information on synthetic cannabinoids see Vandrey et al. (2012) and Castenato et al. (2014).

The third classification is phytocannabinoids. Phytocannabinoids are cannabinoids that are organically synthesized outside of the human body such as those found within the cannabis plant. These are the cannabinoids people are most familiar with as this category includes Δ9-tetrahydrocannabinol and cannabidiol which are commonly known as THC and CBD respectively. Phytocannabinoids, which we will hence forth refer to as cannabinoids, are known to affect sleep, temperature regulation, food intake, arousal, and

pain perception by binding to receptors within brain regions that support these body functions (Vann et al., 2008).

1.1.4 Terpenes

Terpenes are a parent category of terpenoids. Terpenoids are components of essential oils that are naturally occurring in plants and commonly found in a wide range of products from food to cosmetics, to pharmaceuticals and more specifically, cannabis. [For more details see a review by Russo (2011)]. Simply, terpenoids are the molecule responsible for the unique aromas of each cannabis strain. The terpenoids most frequently found within cannabis are limonene, myrcene, and pinene. Independently, terpenes are pharmaceutically diverse; they can interact with enzymes, cell membranes, ion channels, neurotransmitter receptors, and second messenger systems (Ben-Shabat et al., 1998; Gurgel do Vale et al., 2002; Nuutinen, 2018). Beyond that, terpenes are thought to have some impacts to the effects of cannabis. This will be discussed later along with other 'entourage effects'.

1.2 The Endocannabinoid System

As previously mentioned, cannabinoids elicit behavioural and physiological effects in mammals by binding to receptors in a variety of places within the body and brain, hence, directly interacting with the endocannabinoid (eCB) system. In a sense, one can think of the endocannabinoid system as the body's natural cannabinoid system. The eCB system is comprised of at least two receptors known as cannabinoid type 1 and cannabinoid type 2 receptors (CB_1R and CB_2R). These G protein-coupled receptors can bind all three classes of cannabinoids listed above. Within the central nervous system

(CNS), eCBs are neuroactive lipids that are involved in plasticity and memory, motivation, emotional control, and potentially the reward response (Gardner et al., 1988; Hampson, Heyser & Deadwyler, 1993; Hampson & Deadwyler, 2000; Bossong et al., 2010; Busquets-Garcia et al., 2011; García-Gutiérrez & Manzanares, 2011). Dysregulation of the eCB system has been linked to psychiatric disorders, anxiety, and depression (Hillard, Weinlander & Stuhr, 2012; Mechoulam & Parker, 2013).

1.2.1 Cannabinoid Receptors

CB_1R are expressed abundantly throughout the brain. This expression has been related to involvement in various brain areas and the cognitive, motor, emotional, and physiological functions they support. Specifically, CB_1Rs are located in the prefrontal cortex (executive functions and mental skills), basal ganglia and cerebellum (control of fine motor activity), pons and medulla (autonomic functions) as well as the limbic system including the hippocampus (memory), amygdala (emotional control), thalamus (sensory perception), and the hypothalamus (endocrine control). CB_1R are also expressed in the peripheral nervous system in the terminus of nerves, eyes, and testes (Herkenham et al., 1991; Porter & Felder, 2001; Howlett, 2002; Vann et al., 2008). CB_2R are expressed throughout the body and are thought to play a role in modulating inflammation, immune response, and injury recovery (Pertwee, 2008; Turcotte et al., 2016).

1.2.2 Endocannabinoids

The two most extensively studied endocannabinoids are anandamide (AEA) and 2-arachidonylglycerol (2-AG) (Mechoulam et al., 2014). Both act upon CB_1 and CB_2 receptors. Specifically, within the CNS these eCBs have been linked to learning and

memory directly through their impacts to long-term potentiation (LTP) and long-term depression (LTD) (Castillo et al., 2012). eCB synaptic regulation occurs via retrograde messengers supressing neurotransmitter release in both excitatory and inhibitory synapses (Chevaleyre et al., 2007). Beyond signalling in mature synaptic systems, the eCB system has been linked to synaptic formation and neurogenesis (Harkany, Mackie & Doherty, 2008). Endocannabinoids AEA and 2-AG as well as CB1 and CB2 receptors have been found in the dentate gyrus and hippocampal regions of both developing and adult individuals suggesting the relation of the eCB system to neurogenesis (Harkany et al., 2007; Goncalves et al., 2008). This neurogenesis is abolished in CB1 deficient mice, deepening our understanding of the eCB's relation to neurogenesis (Jiang et al., 2005). Similar to endocannabinoids, phytocannabinoids are capable of binding to both CB_1R and CB_2R throughout the body and brain (Mechoulam et al., 2014). For the purposes of this study, we will primarily focus on CB_1 receptors due to their presence in the CNS and their implications in cognition, and learning and memory.

1.3 Endocannabinoid Signalling

Endocannabinoids regulate synaptic functioning through retrograde signaling, non-retrograde signalling and neuron-astrocyte signalling (Navarrete & Araque, 2008). CB_1R are localized at the terminus of neurons suggesting that they specifically play an important role in synaptic functioning via short-term and long-term plasticity (Heifets & Castillo, 2009; Regehr, Carey & Best, 2009).

1.3.1 Retrograde Signalling

Retrograde signalling occurs when postsynaptic activation leads to eCBs being released into the synaptic cleft to travel backwards to bind to cannabinoid receptors on the presynaptic surface hence supressing neurotransmitter release (Regehr, Brown & Brenowitz, 2003). Short-term plasticity occurs when CB_1R are activated for only a few seconds. This postsynaptic activity triggers Ca^{2+} influx via voltage gated Ca^{2+} channels (VGCC). Increasing post synaptic Ca^{2+} content results in a cascade that stimulates the release of 2-AG from the post-synaptic neuron which then binds to pre-synaptic CB_1R. Stimulation of the CB_1R inhibits VGCC receptors, reducing the amount of presynaptic Ca^{2+} present. This reduction in presynaptic Ca^{2+} reduces the amount of neurotransmitter being released into the synaptic cleft (Kreitzer & Regehr, 2001; Regehr, Brown & Brenowitz, 2003).

During long-term plasticity, the endocannabinoid system is only involved in the initial induction on long-term depression (eCB-LTD) (Chevaleyre & Castillo, 2003; Heifets & Castillo, 2009). Presynaptic release of glutamate (the primary excitatory neurotransmitter) stimulates postsynaptic metabotropic glutamate receptors (mGluRs) that unbind phospholipase-Cβ (PLCβ) and diacylglycerol lipase (DAGLα) that then promote the synthesis and release of 2-AG from the post synaptic terminus (Maejima et al., 2001; Tanimura et al., 2010). The released 2-AG targets and binds to presynaptic CB_1R. A Gαi/o-dependent reduction in adenylyl cyclase (AC) and protein kinase A (PKA) activity suppresses neurotransmitter release (Marinelli et al., 2008). This occurs on both excitatory and inhibitory presynaptic neurons (Lovinger, Gerdeman & Ronesi, 2002; Chevaleyre & Castillo, 2003).

Retrograde signaling is essential for the development, maintenance, and activity-dependent modification of synapses. In adolescent, eCB signalling plays an integral role in regulating stress and anxiety (Hill et al., 2009). The eCB system regulates the activity of local and circuit populations of neurons and is particularly important for mediating the balance between excitatory and inhibitory neurotransmission during adolescence via these retrograde signalling pathways (Dow-Edwards & Silva, 2017; Meyer, Lee & Gee, 2018).

1.3.2 Nonretrograde Signalling

There is growing evidence that shows transient receptor potential cation channel subfamily V member 1 (TRPV1) also participates in eCB signaling (De Petrocellis & Di Marzo, 2010; Pertwee et al., 2010). TRPV1 receptors are found both in the peripheral and central nervous systems and can bind lipophilic substances including AEA (Di Marzo et al., 2002; Cristino et al., 2006). Activation of mGluR and Ca^{2+} release from intracellular stores promotes the synthesis of AEA that activates TRPV1 channels (Liu et al., 2008). AEA acting on TRPV1 causes increased postsynaptic Ca^{2+} levels which results in the endocytosis of the glutamate cation channel receptors, AMPAR, mediating postsynaptic eCB-LTD (Grueter, Brasnjo & Malenka, 2010). This supports the concept that AEA acts as an intracellular messenger (van der Stelt et al., 2005). Unlike eCB receptor signalling, there is no definitive proof that TRPV1 signalling acts on inhibitory neurons (Liu et al., 2008).

Nonretrograde signalling can also occur following elevated Ca^{2+} levels in the cell resulting in the synthesis of AEA and 2-AG, as a result of sustained neuronal activity (Prince, Bacci, & Huguenard, 2004). Repetitive activation of specific GABAergic neurons triggers a CB_1R-dependent postsynaptic hyperpolarization (Jung et al., 2007).

This slow self-inhibition results from increasing K^+ channel conductance, hyperpolarizing the cell which reduces the cells excitability (Prince, Bacci & Huguenard, 2004; Marinelli et al., 2008).

1.3.3 Neuron-Astrocyte and Other eCB Signalling

The presence of eCBR on glia, specifically astrocytes has recently been demonstrated (Gutiérrez-Rodríguez et al., 2018). Postsynaptic activity generates endocannabinoid release that binds to CB_1R on the astrocytic surface activating astrocyte Ca^{2+} signaling, which releases adenosine and activates adenosine-1 receptors (A1Rs) on the presynaptic surface, decreasing inhibitory neurotransmitter gamma-aminobutyric acid (GABA) release (Stella, 2009; Castillo et al., 2012; Hablitz et al., 2020). In hippocampal cells, it was shown that astrocytes potentiate synaptic efficacy by the same mechanism of increased Ca^{2+} stimulation by endocannabinoids (Navarrete & Araque, 2008; 2010). Both endo- and phytocannabinoids are capable of binding to eCB and TRPV receptors making them a good target for medicinal research (Muller, Morales & Reggio, 2019). Beyond that, the eCB system has been linked to microglia cells. The presence of CB1 and CB2 recpetors on microglial cells has been linked to chemokine and cytokine expression, this is thought to be linked to modulation of part of the inflammatory response (Cabral, 2005). *In vitro* microglia cells have been shown to be capable of producing both 2-AG and AEA (Carrier et al., 2004). The relationship of this endocannabinoid production and synaptic transmission is unknown.

1.3.4 Exocannabinoids (THC and CBD)

Exogenous- or exocannabinoids are cannabinoids synthesized outside of the human body such as phyto- and synthetic cannabinoids. Beyond THC and CBD there are many other cannabinoids that have been studied including cannabichromene (CBC), cannabinol (CBN) and other variants of THC (Δ^8-THC) (Thompson et al., 1973; Izzo et al,. 2012; Hill et al., 2021). THC produces the effects commonly associated with cannabis intoxication; the "stoned" feeling. THC administered alone elicits dose dependent impairments to delayed verbal memory and psychosis symptoms reminiscent of schizophrenia (Ranganathan & D'Souza, 2006; Broyd et al., 2016). Expanding on that, THC administration has been associated with increases in positive and negative schizophrenia-like symptoms, increased anxiety, euphoria, altered perception, and disruptions to recall and working memory (D'Souza et al., 2004; D'Souza et al., 2008; Morgan et al., 2018). Furthermore, recent data demonstrate that THC increases striatal glutamate levels which can be associated with the expressed psychosis (Colizzi et al., 2020). This risk of psychosis is higher in chronic and adolescent cannabis users who use cannabis that is high in THC and low in CBD (Zuardi, Rodrigues & Cunha 1991; D'Souza, Sewell & Ranganathan, 2009; Di Forti et al., 2012; Broyd et al 2016; Lorenzetti et al., 2016). CBD lacks the psychoactive effects that THC possesses (Zuardi, Rodrigues & Cunha, 1991). The lack of psychoactive effects has led to research into CBD as a therapeutic agent. Currently, it has been suggested that CBD can be used as a therapy against anxiety, certain types of cancers, AD, and in conjunction with other medications to treat Dravet Syndrome, a drug resistant epilepsy (McAllister et al., 2010; Das et al., 2013; Devinsky et al., 2017; Karl, Garner & Cheng, 2017). It was demonstrated that CBD can increase neurogenesis in adult mice without impacting learning whereas THC

impaired learning without impacting neurogenesis, further reinforcing that the two molecules function very differently (Wolf et al., 2010).

1.4 Strains, Constituent Interactions, and Entourage Effects

There are a multitude of different names for cannabis reflecting both the history of cannabis as a drug and variations in genetics and constituent content. Cannabis strains possess differences in the relative amounts of constituents within cannabis, resulting in the variety of smells, tastes, and potencies (Ilan et al., 2005). As previously mentioned, cannabis constituents, specifically, cannabinoids and terpenes, can interact resulting in the unique properties possessed by each strain. Additionally, this means that some combinations elicit more pain relief while others have larger neuroactive effects that can lead to higher levels of intoxication or even memory impairments (Fadda et al., 2004; Morgan et al., 2010; Darkovska-Serafimovska et al., 2018). Many of these constituents are found in trace amounts in cannabis, but the effects when taken together is what researchers are interested in. The possibilities for cannabis use and research are unprecedented as more data about different constituent ratios and individual strains are being published daily.

Cannabinoids and terpenes can interact with one another in a variety of ways. Each cannabinoid or terpene can elicit an individual effect as well as an additive, an antagonistic, or a synergistic effect (Chaudhary et al., 2012; Finlay et al., 2020). Additive effects occur when the effect of the compounds together is equal to the summation of the individual compound's effects. Antagonistic effects occur when the overall effect is equal to less than the summation of the individual effects. Synergistic effects are often the ones

most scientists are interested in, and they occur when the effect of the compounds together is equal to more than the summation of the individual compound's effects.

1.4.1 Cannabinoid Interactions

Shortly after the identification of various cannabinoids, research began on how they may impact one another. This research has primarily focused on how THC's psycholytic effects are impacted by other non-psychoactive cannabinoids. Previously, the idea was that CBD was able to reduce the negative side effects of THC, but data have been conflicting on whether this is the case or not (Karniol & Carlini, 1972; Hložek et al., 2017). Data frequently suggest that there are many factors including age, sex, predispositions for drug use and psychosis, dose, and route of administration that impact this complex relationship (Varvel et al., 2006; Morgan et al., 2010; Hillard, Weinlander & Stuhr, 2012; Raup-Konsavage et al., 2020). Research on consumption of CBD in conjunction with THC has varied greatly over the last 50 years and has thus provided no definitive proof of the relationship between THC and CBD. An overview of this literature leads to the conclusion that CBD can alter a variety of THC's effects under some circumstances (Bhattacharyya et al., 2010; Morgan et al., 2010; Englund et al., 2013; Taffe, Creehan & Vandewater, 2015; Morgan et al., 2018). In a study done in 2013, participants given CBD prior to THC showed a reduction in paranoid symptoms and hippocampal memory impairments (Englund et al., 2013). THC is known to reduce social play in rodents; this was reversed when it was administered in conjunction with CBD (Malone, Jongejan & Taylor, 2009). Beyond that, historical data demonstrated the potential of THC consumed alone to be a sufficient analgesic, but more modern data have shown this is not the case. Rather, THC analgesia only occurs when consumed in

conjunction with CBD either in Sativex (an oral mucosal spray of a 1:1 ratio of CBD and THC) or in orally or inhaled cannabis (Noyes et al., 1975; Ware et al., 2010; Andreae et al., 2015; Romero-Sandoval, Kolano & Alvarado-Vázquez, 2017; Darkovska-Serafimovska et al., 2018).

1.4.2 Terpene Interactions

In addition to cannabinoid interactions, it has been suggested that terpenes are able to enhance the psychoactive and medicinal properties of cannabis. (Gurgel do Vale et al., 2002; Nuutinen, 2018). As it is important to consider the interaction terpenoids may have with the cannabinoids present, researchers have now begun to focus their attention on these aromatic molecules. The phenomenon of potentiation by lesser phytocannabinoids and phytoterpenoids coined “entourage effect” was first described in a study done by Ben-Shabat et al. (1998) using endogenous glycerol esters to increase target affinity of the endogenous cannabinoids 2-AG and AEA. The term entourage effect is more recently used to refer to the idea that whole cannabis plant extracts may possess greater therapeutic potential than the individual constituents (Russo, 2011; Finlay et al., 2020). The idea of entourage effects has been recently disputed in a study done by Finlay et al. (2020) where 5 common terpenes were administered in vitro alone or with either THC, CBD, or 2-AG and no definitive increase in therapeutic potential was observed. Although direct interactions between terpenoids and binding affinity may not exist there are likely other mechanisms mediating the changes observed in the historical data (Ben-Shabat et al., 1998; Blasco-Benito et al., 2018; Santiago et al., 2019; Finlay et al., 2020). Taken together, this information provides insights into why research into whole plant cannabis extracts beyond the constituents alone is required. Further research, including

the research reported in this thesis, will provide deeper insight into what secondary constituent relationships may exist.

1.5 Adolescent Exposure

1.5.1 Adolescent Exposure and Use

As mentioned, cannabis is the most frequently used illicit drug worldwide, with over 20% of Canadians beginning their use by age 15. Adolescent use is an important consideration not only for the medicinal benefits but for recreational concerns as well (Currie, 2016). “Street cannabis” tends to have a higher percentage of THC leading to concerns with increases in positive and negative schizophrenia-like symptoms, increased anxiety, reduced movement, euphoria, altered perception, and disruptions to recall and working memory in adult humans (D’Souza et al., 2004; D’Souza et al., 2008; Morgan et al., 2018). Cannabis consumption in adolescence may interfere with brain development (Schneider, 2008; Squeglia, Jacobus & Tapert, 2009). A review of a collection of studies looking at cannabis use in adolescence and its effects on the brain and ultimately behaviour, demonstrated an association between cannabis use and hyperactivity of parietal and frontal networks but in most studies, there were little to no apparent behavioural changes (Lorenzetti et al., 2016). Behavioural changes documented in this review also indicate a parallel between what is observed in adult and adolescent use (Lorenzetti et al., 2016). An important consideration related to this review is that many of the studies included cannabis users who consumed their choice of street cannabis. This review did not attempt to differentiate between the strains consumed and therefore the relative CBD and THC content in the cannabis studied in each publication was not accounted for. There are various other reports of adolescent consumption of exogenous

cannabinoids resulting in behavioural changes including locomotor activity, play and overall impaired cognitive abilities (Rubino et al., 2015; Renard et al., 2016; VanRyzin et al., 2019). As previously mentioned, the relative amounts of the cannabis constituents may thus have varied a great deal from study to study. More research, including this thesis, will attempt to resolve some of the informational gaps that remain.

1.5.2 Adolescent Sexually Dimorphic Cannabis Effects

A variety of different publications have shown a higher density of CB_1 receptors observed in males compared to females in almost all the cerebral regions analyzed (Rubino, et al., 2008; Burston et al., 2010; Riebe et al., 2010; Mateos et al., 2011). There is evidence to suggest sexually dimorphic changes in endocannabinoid levels that accounts for differences in pain perception experienced by each sex (Tseng & Craft, 2001; Bradshaw et al., 2006). In the study done by Tseng and Craft, 3 different cannabinoid agonists produced greater effects in female rats than their male counterparts. These relative levels are impacted by hormones and change throughout different phases of the hormonal cycle in females (Scorticati et al., 2003; Riebe et al., 2010). Both the differences in receptor density and relative hormone levels have been shown to be true in adults, adolescent, and neonatal animals and humans and seem to have lasting impacts (Burston et al., 2010; Krebs-Kraft et al., 2010; Riebe et al., 2010; Long et al., 2012; Renard et al., 2016; Meyer, Lee & Gee, 2018; VanRyzin et al., 2019). Taken together, considering both sexes individually including developmental differences and general behavioural differences, is imperative to gain a full understanding the effect of cannabis on the brain.

1.6 Medicinal Applications of Cannabis

Cannabinoids can impact the human body and brain in a variety of ways, but this review will now focus on how cannabis can be utilized for its medicinal rather than recreational properties. An eCB system that fails to maintain a homeostatic state can result in the development of a variety of diseases. An example of this is the upregulation of CB_1 and CB_2 receptors and an increase in endocannabinoids that has been observed in neurodegenerative and cardiovascular diseases as well as in cancer (Miller & Devi, 2011; Kovalchuk & Kovalchuk, 2020). Understanding the mechanisms of action in the eCB system is therapeutically relevant for cannabis as a medicine as it allows scientists better knowledge on how to mediate upregulation of receptors and efficacy of agonists. There are many more medicinal uses for cannabis that are currently being researched but the remainder of this review will focus on the use of cannabis as it relates to treatment of diseases that impact the brain.

1.6.1 Cancer

The impacts that cannabinoids, specifically THC and CBD have on cancer (defined as the uncontrolled division of cells) has been vigorously researched. Cannabinoids were shown to reduce tumor growth in mice and were later shown to inhibit tumor growth by modulating different cellular pathways of a variety of cancers (McAllister et al., 2010). Recently, this data has been disputed, and one study even reported the opposite effects (Hart, Fischer & Ullrich, 2004; McKallip, Nagarkatti & Nagarkatti, 2005; Raup-Konsavage et al., 2020). It does seem as though cannabinoids can help in very specific instances including breast, prostate, and colorectal cancers (McAllister et al., 2010; Morell et al., 2016; Cherkasova, Kovalchuk & Kovalchuk,

2021). Beyond that, cannabinoids have been shown to reduce the nausea experienced as a side effects of standard cancer treatments and chemotherapy (Tramer et al., 2001). Based on the cumulative evidence, continued research into the use of cannabis as cancer treatment appear to be a good prospect.

1.6.2 Inflammation

With regards to inflammation, cannabinoids work by inducing apoptosis, preventing cell proliferation, reducing cytokine production, and enhancing T-regulatory cells (Nagarkatti et al., 2009; Suryavanshi, Kovalchuk & Kovalchuk, 2021). Cannabinoids have been shown to trigger apoptosis in malignant immune cells (such as those in leukemia or lymphoma) in vivo, effectively modulating immune cell function (Rieder et al., 2010). Taking that information into account, research began to focus attention on how both endo- and exogenous cannabinoids impact hepatitis. In a study done by Hegde et al., (2008), both THC and AEA treatment slowed the liver injury caused by hepatitis. Studies have also demonstrated that CBD can increase adenosine signaling which is thought be a mechanism by which CBD can reduce inflammation (Carrier, Auchampach & Hillard, 2006). The data hint at the complex relationship between endo- and exogenous cannabinoids and inflammation.

1.6.3 CBD and Neuroinflammatory Disease

Chronic neuroinflammation contributes to the development of AD (Akiyama et al., 2000; Wenk et al., 2000). Neuroinflammation promotes neuronal death, powering a vicious cycle responsible for the progression of AD, by interfering with the molecules responsible for neuroinflammation. In a rat model CBD acts as a neuroprotective

molecule and slows the progression of AD (Esposito et al., 2011). CBD has also been shown to increase neurogenesis which has implications for cognitive recovery from AD in mice (Wolf et al., 2010). CBD also promotes microglial cell migration both *in vitro* with human cell lines and in mice which is shown to be beneficial in the treatment of AD (Lunn et al., 2006; Wolf et al., 2010; Karl, Garner & Cheng, 2017; Tagne et al., 2019). Beyond CBD, high CBD cannabis extracts showed more potent microglial migration than CBD alone indicating there is beneficial interactions between the other constituents within cannabis *in vitro* on AD pathology (Tagne et al., 2019). In both rats and mice CBD has been shown to reduce the neuronal damage that occurs with AD without impairing spatial learning and memory (Esposito et al., 2011; Martín-Moreno et al., 2011). This is understandable when related to a meta-analysis by Watt & Karl (2017) that compared the effects of THC+CBD together on AD. These authors showed that not only did the combination therapy work better, but the CBD content limited the adverse effects of THC consumption making it an ideal treatment option for AD.

The pathologic changes of MS include neuroinflammation, excitotoxicity, demyelination, and neurodegeneration (Maroon & Bost, 2018). In an early study, MS patients reported a reduction in symptoms and relapses when smoking cannabis (Consroe et al., 1997). In more recent work Sexton and colleagues (2014) detected a significant increase AEA in serum from individuals with MS compared to control subjects thereby indicating a relationship between the eCB system and MS (Centonze et al., 2007; Sexton et al., 2014). Moving forward, research has focused on enhancement of the eCB system as a means to promote neuroprotection and therapeutic effects for MS (Loria et al., 2010; Zajicek et al., 2012).

1.6.4 Epilepsy

Despite the existence of antiepileptic drugs, many people with epilepsy continue to have seizures (Brodie et al., 2012). Use of cannabinoids to treat epilepsy first stemmed from the idea that AEA can control neuronal excitability by binding to the TRPV1R (Pertwee, 2008). Patients with epilepsy have been shown to have lower amounts of AEA in cerebrospinal fluid and less expression of CB_1R. There was also a reduction in DAGLα which, as mentioned above, is involved in the synthesis of 2-AG in post-synaptic terminals (Tanimura et al., 2010). Administration of CBD was shown to restabilize the homeostatic state of neuronal networks by altering neuronal excitability by binding to TRPVR which antagonizes a G-protein–coupled receptor, leading to decreased presynaptic release of glutamate, activating 5-hydroxytryptophan 1A receptors, and inhibiting adenosine reuptake (Carrier, Auchampach & Hillard, 2006; De Petrocellis et al., 2011; Campos, Ferreira, & Guimarães, 2012; Sylantyev et al., 2013). Thus, CBD is presented as a prospective treatment for generalized or partial seizures (Consroe et al., 1982; Friedman & Devinsky, 2015). Treatment of temporal lobe seizures and penicillin model of partial seizures (an experimental model of generalized spike-and-wave discharges occurring during clinical absence attacks) with CBD showed a significant decrease in the frequency of the most severe seizures (Jones et al., 2012). CBD administered as a supplement to standard epileptic treatment led to a reduction in the frequency of convulsive seizures. Unfortunately, it was also shown to have adverse interactions with other medications (Devinsky et al., 2017). Research is now being conducted into how to manage these additional side effects, including research into the safety of using high CBD cannabis extracts in adolescence.

1.7 Purpose of Study and Thesis Overview

Consumption of multiple constituents of cannabis at a time demonstrates the likelihood of entourage effects. These entourage effects have the capacity to both enhance the medicinal benefits of individual constituents as well as reduce the adverse side effects of cannabis consumption, precisely those related to THC. This thesis reports the impact that acute consumption of high CBD cannabis strains elicited on adolescent behavioural development. Understanding the potential for the therapeutic use of cannabis in adolescence, while monitoring for negative behaviour consequences will broaden our understanding of the utility and effectiveness of cannabis as a medical treatment during development.

With all of this in mind, this project sought to resolve the concerns of safety of adolescents chronically exposed to high CBD cannabis extracts by assessing the following questions:

1. Does adolescent exposure to high CBD cannabis extract result in lasting behavioural changes in a rodent?
2. Are there long-term sex differences in behavior that arises from exposure to high CBD cannabis extracts in adolescent rodents?

Based on these questions, I hypothesized:

1. Exposure to high CBD cannabis extract will have little to no long-term impact on adolescent rodent behaviour.
2. The if the effects of high CBD cannabis extract on adolescent rodent behaviour occur, they will be sexually dimorphic in nature.

Consequently, I do predict that any behavioural impacts will be minimal. Male and female Long-Evans rats were evaluated on a battery of behavioural tasks assessing anxiety, and learning and memory after excretion of cannabis metabolites. Despite the differences in pharmacology of high CBD whole plant extracts and pure CBD it was predicted there would be no behavioural difference between the two. Based on the profile of CBD, no withdrawal symptoms were expected. Based on inherent differences, it was predicted that males would outperform females on spatial navigation tasks (memory) and there would be no apparent difference in anxiety. Chapter 2 will delve into the details of methodology used. The project at hand will help preclinically determine the safety of using high CBD cannabis extracts in an adolescent model. This will in turn provide a natural alternative to the standard medical treatments available to adolescents.

CHAPTER 2: METHODS

2.1 Animals

Female and male Hooded Long-Evans rats were obtained from Charles River and were allowed to acclimate to the University of Lethbridge animal housing rooms for approximately 1 month until post-natal (P)day 90. Rats were paired and allowed to breed for 7 days. Animals were returned to their previous home cages until approximately 2 days before parturition where females were separated. 10 breeding pairs yielded a total of 127 pups. At P7 all animals were weighed daily to monitor health and acclimate to handling. All pups were weaned at P21 and placed into sex-matched pairs or triplets. All rats were housed in standard laboratory conditions (21˚C and 35% relative humidity; 12D:12L) in double decker laboratory housing units with libitum access to food and water unless otherwise indicated. A total of 116 animals were used for this experiment; extra animals were transferred off the animal handling protocol. All rats handling and procedures were done in accordance with the University of Lethbridge's Animal Welfare Committee and the Canadian Council on Animal Care guidelines.

At P21 rats were pseudorandomly assigned into testing cohorts were housed in a home cage with subjects of the same treatment group. All housing units were multi-level and equipped with a PVC tube for play and enrichment (Kolb, Gibb & Gorny, 2003; Sutherland, Gibb & Kolb, 2010). A total of 10 females and 10 males were assigned to each extract x dose cohort and a total of 10 females and 10 males comprised the control animals. As per University of Lethbridge Animal Welfare Guidelines, a total of 16 (8 male, 8 female) animals were housed in standard conditions but did not undergo any treatments as a control for proper growth comparison of the test subjects.

2.2 Cannabis Preparation

Extracts were prepared as described (Casiraghi et al., 2018; Wang et al., 2020). Two different cannabis cultivars were used in these experiments and were grown in a licenced facility at the University of Lethbridge. The cultivars were labeled #81 for extract 1 and CD10 for extract 2. Both cultivars are considered to have a high CBD: low THC content with varying amounts of other constituents, with approximate levels of CBD and THC to be 40% and 2%, respectively. Flowers were harvested, dried and ground before being mixed in liquid nitrogen, and 10mg/mL of ethyl acetate, then centrifuged. The supernatant was dried in a sterile rotor evaporator and suspended in food grade grapeseed oil to create extract at concentration of 100mg/mL and allowed to dissolve for approximately 24 hours then filtered. The preparations were then stored at four degrees Celsius until administration. At administration the extract was combined with powdered rat chow and 0.4g of peanut butter to create simulated protein balls that we denoted as "chow balls". Individual chow balls were prepared immediately prior to administration.

2.3 Exposure

Animals were pseudorandomly assigned to 1 of 5 groups: Administration of 10mg/kg of body weight of Extract 1, 40mg/kg of body weight of Extract 1, 10mg/kg of body weight of Extract 2, 40mg/kg of body weight of Extract 2 or control (vehicle only).

Animals were dosed with cannabis-free "blank" chow balls for 3 days in their home cages followed by 2 days in the dosing chambers. For the first 5 days of dosing animals were food deprived from approximately 7:45 – 12:00. Food hoppers were returned post dosing and water was available *ad libitum* for the duration of the experiment. Pre-exposure occurred from P35-39.

The animals were removed from their home cage and put into an isolated dosing chamber for 1.0 hour during administration. Animals were dosed daily at 12:00 for 14 consecutive days. Dosing occurred under standard laboratory conditions (21˚C and 35% relative humidity; 12D:12L) in Plexiglas® shoebox cages (46cm x 25cm x 20cm). During dosing, animals did not have access to food or water. The chow balls were deposited into each dosing chamber prior to the animals. Extract doses were calculated based on their individual weight recorded at 8:00 that day. Dosing occurred from P40-54.

2.4 Controls

10 female and 10 male control animals were given blank chow balls for the 5 pre-exposure days and the 14 exposure days. Dosing was administered under the same conditions as test subjects. Individual blank chow balls were mixed immediately prior to administration.

2.5 Behavioural Analysis and Data Recording

2.5.1 Morris Water Task

Apparatus - The Morris Water Task (MWT) described by Sutherland, Whishaw & Kolb (1983) is a test designed to determine a rodent's spatial memory abilities and locomotion. The apparatus consisted of a large circular pool with a diameter of 1.4m that was filled to a depth of 40cm with 21˚C water mixed with white non-toxic acrylic paint (CraftSmart, Rajasthan, India) to render the pool opaque. A clear Plexiglas® square platform (13cm X 13cm) was placed in the pool approximately 3.0cm below the water surface. Extra maze cues, including posters, the computer, the experimenter, animal

holding chambers and the water hose placement remained stationary throughout the acquisition and experimental period.

Trials - Trials began 36 hours following dosing to control for remaining cannabis metabolites. MWT training was done as described previously. Starting positions labeled N, S, E, W were determined in a quasi-random fashion such that all starting positions were used every day. For all trial days 1-5, rats were placed in the pool facing the wall and allowed 60s to reach the submerged platform. If they did not reach the platform after 60s, they were guided to it by the experimenter. Once rats had mounted the platform, they were to remain there for 15s. For all trials on days 1-5, the platform was in a consistent position in the centre of the NE quadrant. On day 6, the platform was removed for a probe trial, and rats were allowed to freely swim for 60s before being removed from the pool. The probe starting position was from W and remained consistent for all probe trials. The animals were tested in groups of 8 or less with an interim interval of 300-600s excluding November 2nd in which a medical emergency resulted in 5 male (3 40 mg/kg #81 and 2 10 mg/kg CD10) animals with half (2) of their trials run 3 hours after their other trials. Task acquisition from days 1-5 was analysed, latency and heading direction was analysed from probe day (day 6).

2.5.2 The Elevated Plus Maze

Apparatus - The elevated plus-maze (EPM) protocol as described by Guimeres et al. (1990) is a test that provides a direct measure of the animal's anxiety during testing. Each elevated plus maze session was video recorded for a duration of 5 minutes. The base of the apparatus was 94cm high off the floor and was made of black Plexiglas®. Both open and closed arms measured 10cm in width and 40cm in length. The walls of the

closed arms were 40cm in height. A video camera was placed at the end of one of the open arms at a 40˚ angle looking downward to record behaviour.

Trials – Trials occurred 7 days post dosing to control for residual cannabis metabolites. All trials were run with the lights on and began at approximately 15:15. Rats were placed in the centre square of the apparatus facing the open arm (and camera) and were allowed to freely explore for 5.0 minutes. Experimenters would deposit the subject onto the apparatus and leave the room as to not bias the animal's response. Following testing, rats were removed from the maze and immediately placed in their home cage. Only 1 animal could be run on the apparatus at a time and the testing apparatus was thoroughly cleaned between each subject. Quantification of behaviour was done through video scoring after the experiment. The number of arm entries and the amount of time spent in open and closed arms were recorded.

2.5.3 The Activity Box (Open Field) Test

Apparatus - The Activity Box, described by Seibenhener & Wooten (2015) is a test to determine a rodent's locomotion, exploratory behaviour, and anxiety in a novel environment. The apparatus was composed of 8 Accuscan activity monitoring Plexiglas® chambers (41cm x 41cm x 30.5cm) allowing for 8 animals to be simultaneously monitored during each test. Each chamber has 4 infrared bars creating a beam along the bottom of the chamber, as well as two upper beams to count the number of times each subject reared. Activity was measured as the number of beam breaks during 10, one minute sample periods that were recorded using VersaMax software. The total distance covered (cm) during the testing period was used as the metric of total activity.

Trials – Trials occurred 7 days post dosing to control for residual cannabis metabolites. Animals were deposited into the testing chamber at 15:00, and the experimenter left the room as to not bias the animal's response. Each chamber was thoroughly cleaned between subjects. The open-field test was run for a duration of 10 minutes, divided into 10 1-minute datums. Rearing and movement were analysed for each subject. Rearing is when the rats stand on their hind legs as to get a better look around the space, a behaviour associated with reduced anxiety levels (Guimaraes et al., 1990). Following the activity box, subjects were immediately transported to the EPM test.

2.6 Physiological Analysis

The day following final behavioural testing day, all rats body weights were recorded, then deeply anesthetized using isoflurane followed by 100 mg/kg of body weight sodium pentobarbital injected into the intraperitoneal space. Animals were whole body transcardially perfused using a 0.9% saline solution as to not contaminate the sera and organ tissues. Following perfusion, animals were be decapitated and brains extracted immediately. The whole sample brains were then weighed and analysed for brain weight and stored for future analysis.

2.7 Statistics

Analysis of spatial learning and memory was conducted using the Morris Water and was analyzed using a repeated measure contrast of day with treatment (control, 10 mg/kg or 40 mg/kg) and sex (female or male) as between subject factors, and training days as within-subject factors. Analysis of anxiety-like behaviour in the Elevated Plus Maze was conducted using a one-way ANOVA paradigm with treatment (control, 10

mg/kg, or 40 mg/kg) and sex (female or male) as between subject factors. EPM data was double scored, and a correlation was run to demonstrate significance between scorings. Analysis of anxiety-like behaviour and locomotion in the Activity Box was conducted using a one-way ANOVA paradigm with treatment (control, 10 mg/kg, or 40 mg/kg) and sex (female or male) as between subject factors.

All data were analyzed through IBM SPSS Statistics 27 software. All data were reported using alpha level of .025 as the control animals were used for comparison of multiple analyses.

CHAPTER 3: RESULTS

3.1 MWT and Spatial Learning and Memory

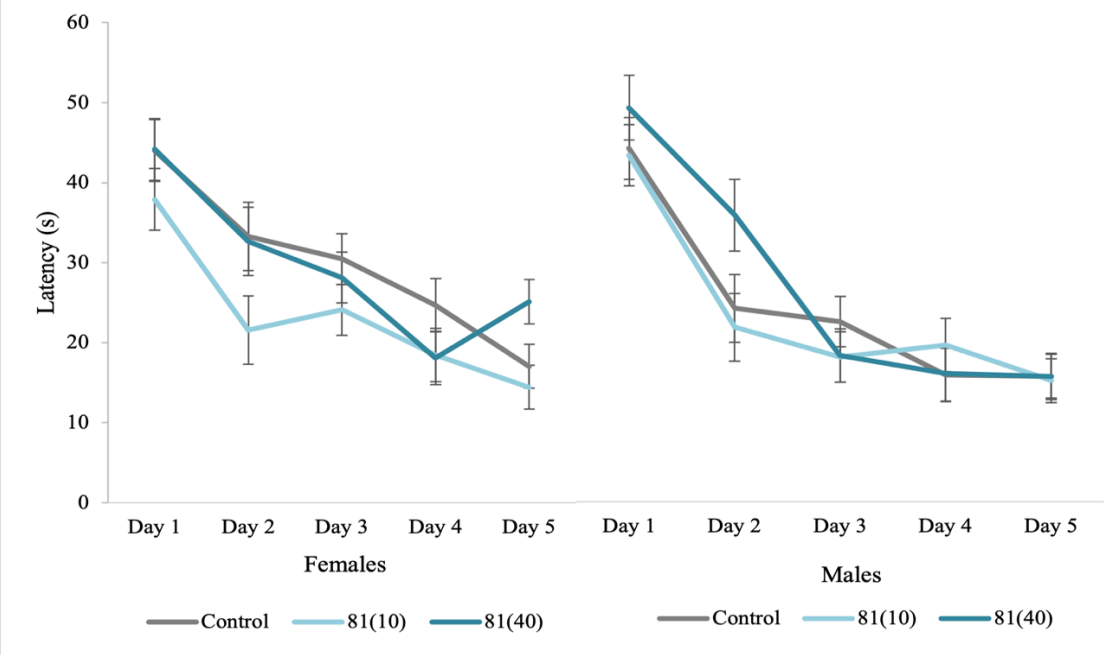
3.1.1 Extract #81 and Task Acquisition

Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated, χ^2 (9) = 16.910, $p < .001$, therefore, Huynh-Feldt corrections were applied to the following statistical observations ($\varepsilon = 1.000$).

There was a main effect of Testing Day [$F(4, 50) = 73.171$, $p < .001$], suggesting that each sequential day the animals performed the task, their latency to locate the platform decreased. There was a trending toward significant Day x Sex interaction, $F(8, 50) = 2.750$, $p = .029$, suggesting that although males and females were not statistically different, males had acquired the task somewhat faster than females. There was no significant Day x Treatment interaction $F(4, 100) = 1.830$, $p = .073$, suggesting all animals were able to acquire the task equivalently. There was no significant Day x Sex x Treatment interaction $F(8, 100) = 0.892$, $p = .524$, suggesting that the effect of treatment on the acquisition of the task was not reliant on the sex of the subject.

Figure 3.1.1

Acquisition of the MWT by animals exposed to cannabis extract #81 as compared to controls.



Note. This figure is a graphical depiction of the mean latency male and female animals took to find the hidden platform during 5 days of testing following exposure to either 10 mg/kg [81(10)] or 40 mg/kg [81(40)] of cannabis extract #81.

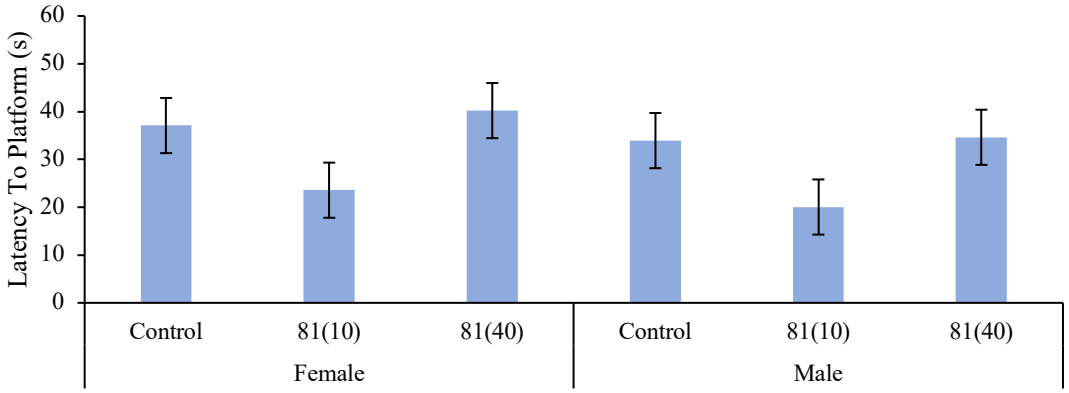
[a] There was a significant effect of day, there was no significant effect of treatment in males or females. Animals were able to learn the information of the task at a similar rate regardless of treatment group. Error bars represent the SEM.

3.1.2 Extract 81 and Latency to Platform

ANOVAs were conducted using Bonferroni's post-hoc test. There was no significant effect of Treatment on the time it took the animals to make contact with the outline of the removed platforms previous location, $F(2, 54) = 2.761$, $p = .072$, suggesting that the animals were able to learn the location of the platform equivalently regardless of treatment. There was no significant effect of sex on the time it took the animals to find the location of the removed platform, $F(1, 54) = 0.476$, $p = .493$, suggesting that the animals were able to learn the location of the platform equivalently regardless of sex. There was no significant Sex x Treatment interaction on the time it took the animals to find the

location of the removed platform, $F(2, 54) = 0.016$, $p = .984$, suggesting that the animals were able to learn the location of the platform statistically equivalently regardless of the combined effects of their sex and treatment.

Figure 3.1.2
Latency of animals exposed to cannabis extract #81 to reach the location of the removed platform in the MWT



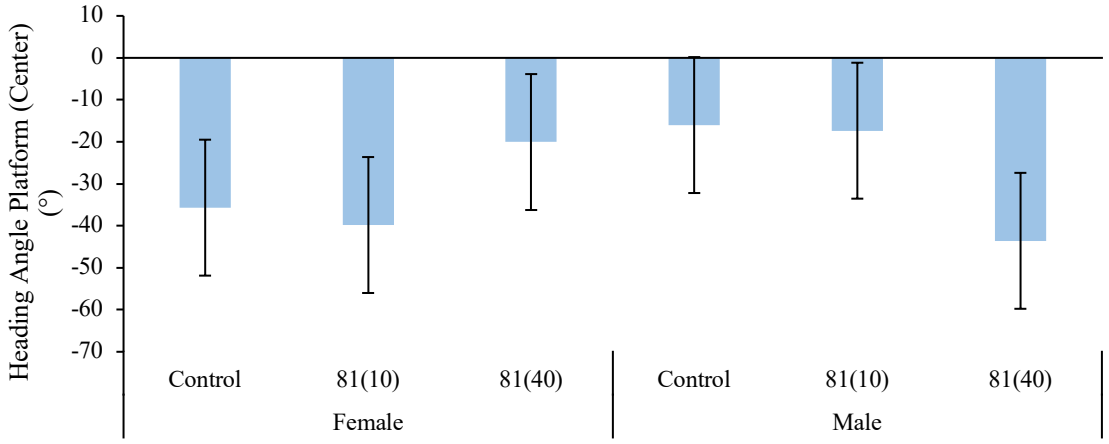
Note. Graphical depictions of the latency in seconds it took for animals to make contact with the outlined position of the removed platform on probe day (day 6) of testing by animals exposed to 10 mg/kg [81(10)] or 40 mg/kg [81(40)] cannabis extract #81.
[a] There was no significant effect of treatment, sex or Treatment x Sex interactions observed in the latency to find the location of the removed platform. Error bars represent the SEM.

3.1.3 Extract 81 and Initial Heading Direction

ANOVAs were conducted using Bonferroni's post-hoc test. There was no significant effect of Sex on the degree of heading angle relative to platform centre, $F(1, 54) = 0.220$, $p = .641$, suggesting that the animals were able to learn the location of the platform equivalently regardless of sex. There was no significant effect of treatment on the degree of heading angle relative to platform centre, $F(2, 54) = 0.068$, $p = .934$, suggesting that the animals were able to learn the location of the platform equivalently

regardless of treatment. There was no significant Sex x Treatment interaction on the degree of heading angle relative to platform centre, $F(2, 54) = 1.269$, $p = .289$, suggesting that the animals were able to learn the location of the platform statistically equivalently regardless of the combined effects of their sex and treatment.

Figure 3.1.3
Heading angle of animals exposed to cannabis extract #81 relative to the centre of the location of the removed platform in the MWT



Note. Graphical depictions of the angle relative to centre of the platform of initial movement animals took locate the position of the removed platform on probe day (day 6) of testing by animals exposed to 10 mg/kg [81(10)] or 40 mg/kg [81(40)] cannabis extract #81.
[a] There was no significant effect of treatment, sex or Treatment x Sex interactions observed in the heading angle to the location of the removed platform. Error bars represent the SEM.

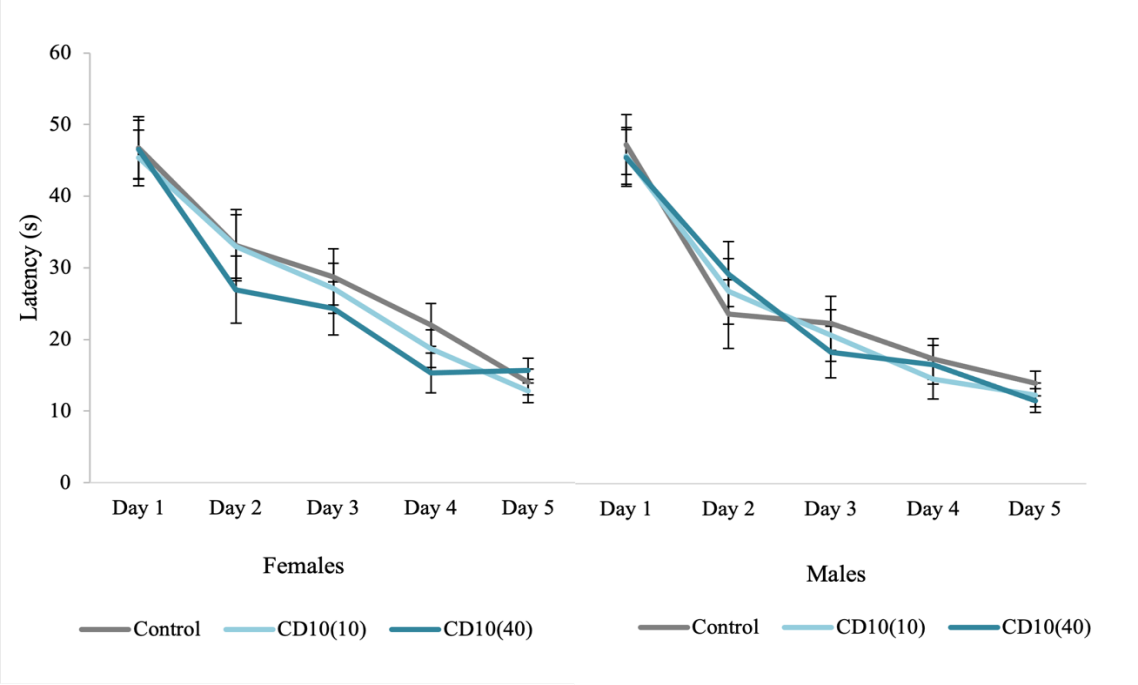
3.1.4 Extract CD10 and Task Acquisition

Mauchly's Test of Sphericity indicated that the assumption of sphericity was violated, χ^2 (9) = 43.902, $p < .001$, therefore, Huynh-Feldt corrections were applied to the following statistical observations ($\varepsilon = 1.000$).

There was a main effect of testing day $F(3.474, 47) = 101.233$, $p < .001$, suggesting that each sequential day the animals performed the task, their latency to locate the platform decreased. There was no significant Day x Treatment interaction $F(3.474, 47) = 0.294$, $p = .955$, suggesting all animals were able to acquire the task equivalently. There was no significant Day x Sex interaction, $F(6.949, 94) = 0.864$, $p = .474$, suggesting males and females performed statistically equivalently. There was no significant Day x Sex x Treatment interaction $F(6.949, 94) = 0.577$, $p = .773$, suggesting that the effect of treatment on the acquisition of the task was not reliant on the sex of the subject.

Figure 3.1.4

Acquisition of the MWT by animals exposed to cannabis extract CD10 as compared to controls.



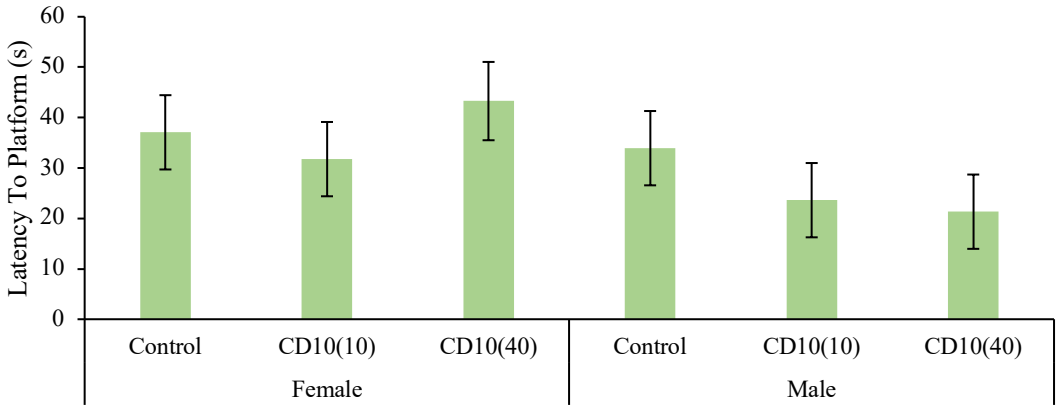
Note. This figure is a graphical depiction of the mean latency male and female animals took to find the hidden platform during 5 days of testing following exposure to either 10 mg/kg [CD10(10)] or 40 mg/kg [CD10(40)] of cannabis extract CD10.
[a] There was a significant effect of day, there was no significant effect of treatment in males or females. Animals were able to learn the information of the task at a similar rate regardless of treatment group. Error bars represent the SEM.

3.1.5 Extract CD10 and Latency to Platform

ANOVAs were conducted using Bonferroni's post-hoc test. There was no significant effect of treatment on the time it took the animals to make contact with the outline of the removed platforms prior location, $F(2, 53) = 0.569$, $p = .070$, suggesting that the animals were able to learn the location of the platform equivalently regardless of treatment. There was no significant effect of sex on the time it took the animals to find the location of the removed platform, $F(1, 53) = 3.327$, $p = .570$, suggesting that the animals were able to learn the location of the platform equivalently regardless of sex. There was

no significant Sex x Treatment interaction on the time it took the animals to find the location of the removed platform, $F(2, 53) = 0.844$, $p = .436$, suggesting that the animals were able to learn the location of the platform statistically equivalently regardless of the combined effects of their sex and treatment.

Figure 3.1.5
Latency of animals exposed to cannabis extract CD10 *to reach the location of the removed platform in the MWT*



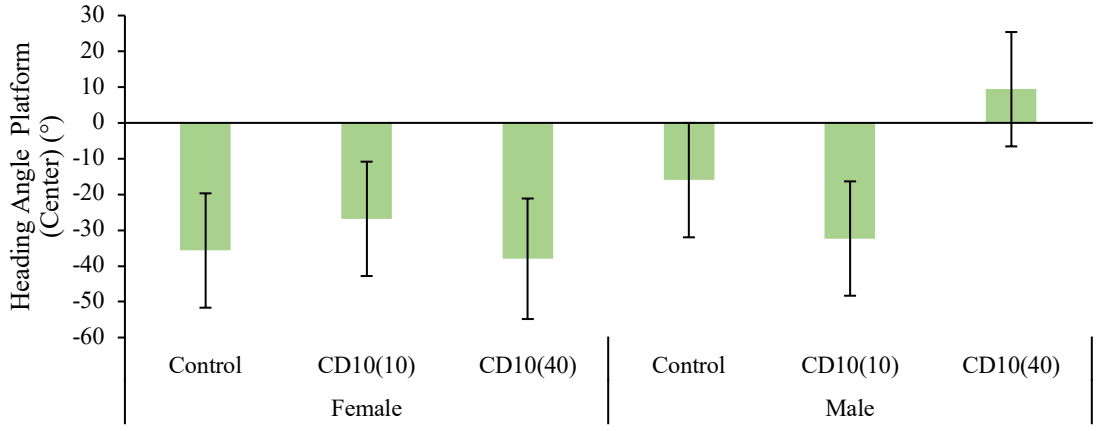
Note. Graphical depictions of the latency in seconds it took for animals to make contact with the outline of the removed platform on probe day (day 6) of testing by animals exposed to 10 mg/kg [CD10(10)] or 40 mg/kg [CD10(40)] cannabis extract CD10.
[a] There was no significant effect of Treatment, Sex or Treatment x Sex interactions observed in the latency to find the location of the removed platform. Error bars represent the SEM.

3.1.6 Extract CD10 and Initial Heading Direction

ANOVAs were conducted using Bonferroni's post-hoc test. There was no significant effect of sex on the degree of heading angle relative to platform centre, $F(1, 53) = 2.428$, $p = .125$, suggesting that the animals were able to learn the location of the platform equivalently regardless of sex. There was no significant effect of treatment on the degree of heading angle relative to platform centre, $F(2, 53) = 0.481$, $p = .621$,

suggesting that the animals were able to learn the location of the platform equivalently regardless of treatment. There was no significant Sex x Treatment interaction on the degree of heading angle relative to platform centre, $F(2, 53) = 1.333$, $p = .272$, suggesting that the animals were able to learn the location of the platform statistically equivalently regardless of the combined effects of their sex and treatment.

Figure 3.1.6
Heading angle of animals exposed to cannabis extract CD10 relative to the centre of the location of the removed platform in the MWT



Note. Graphical depictions of the angle relative to centre of the platform of initial movement animals took locate the position of the removed platform on probe day (day 6) of testing by animals exposed to 10 mg/kg [CD10(10)] or 40 mg/kg [CD10(40)] cannabis extract CD10.
[a] There was no significant effect of treatment, sex or Treatment x Sex interactions observed in the heading angle to the location of the removed platform. Error bars represent the SEM.

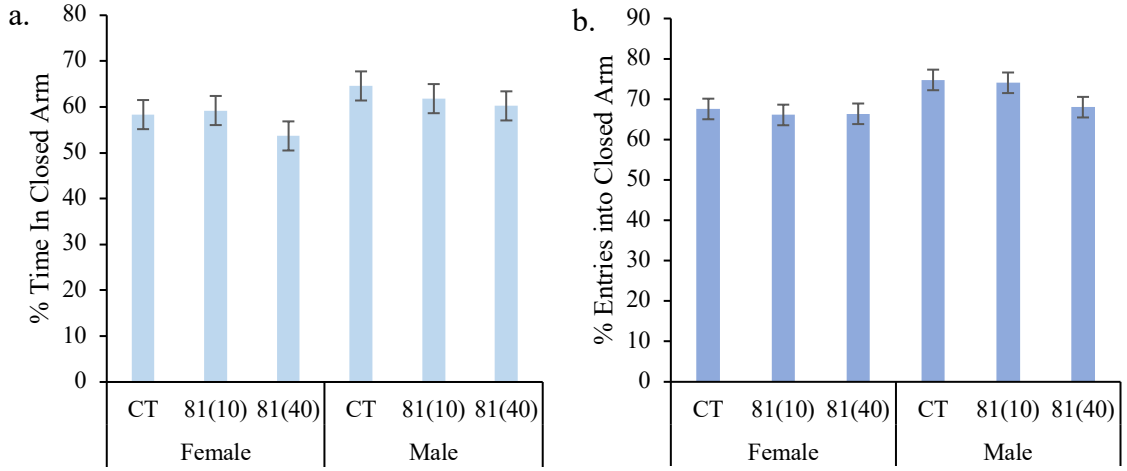
3.2 EPM and Anxiety-Like Behaviour

3.2.1 Extract #81

ANOVAs were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on the percent of entries into the closed arm, $F(2, 54) = 7.245$, p

= .009, suggesting male entries into the closed arm accounted for higher percentage of their entries. There was no significant effect of treatment on the percent of entries into the closed arm, $F(1, 54) = 1.296$, $p = .282$, suggesting the animals entered the closed arm at an equal ratio regardless of treatment. There was no Sex x Treatment interactions observed with regards to percent of entries into the closed arm, $F(2, 54) = 0.917$, $p = .406$, suggesting the effect of the treatment was not influenced by the subject's sex. There was no significant effect of treatment on the percent of time spent in the closed arm, $F(1, 54) = 1.119$, $p = .334$ suggesting the animals spent comparable time in the closed arm regardless of treatment. There was no significant effect of sex on the percent of time spent in the closed arm, $F(2, 54) = 3.917$, $p = .053$, suggesting the animal's sex did not impact the time spent in the closed arm. There was no Sex x Treatment interactions on the percent of time spent in the closed arm, $F(2, 54) = 0.241$, $p = .717$

Figure 3.2.1
Anxiety-like behaviour during the EPM of animals exposed to cannabis extract #81 as compared to controls



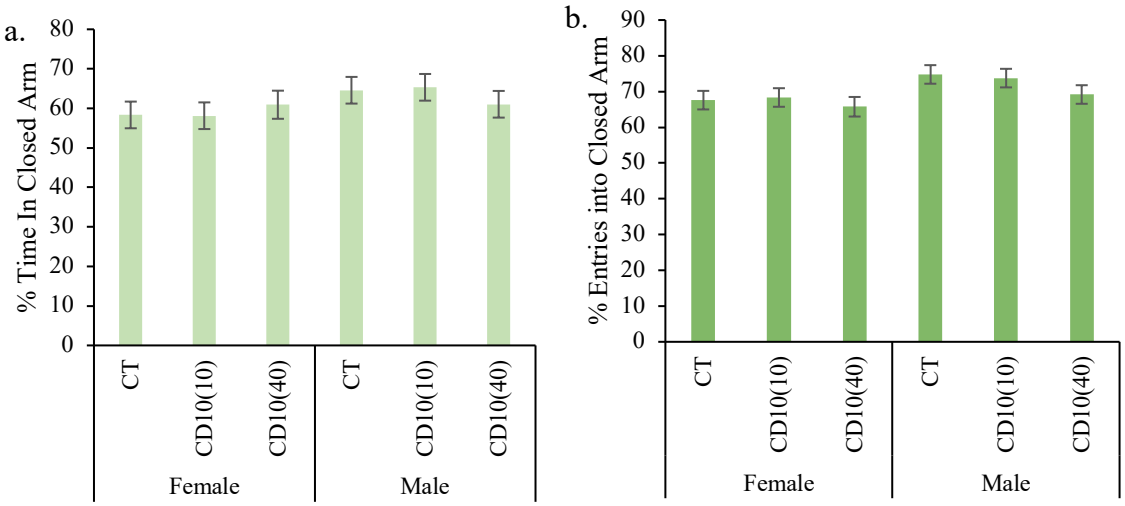
Note. Graphical depictions of a. the percent of time spent in the closed arm of the EPM, and b. the percent of total entries that were into the closed arm of the EPM by animals exposed to 10 mg/kg [81(10)] or 40 mg/kg [81(40)] cannabis extract #81.
[a] There was no significant effect of treatment, sex or Treatment x Sex interactions observed in the time spent in the closed arm of the apparatus.
[b] There was an effect of sex on the percent number of entries into the closed arm overall, there was no effect of treatment on the percent number of entries. Error bars represent the SEM.

3.2.2 Extract CD10

ANOVAs were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on the percent of entries into the closed arm, $F(2, 53) = 6.240$, $p = .016$, suggesting male entries into the closed arm accounted for higher percentage of their total entries. There was no significant effect of Treatment on the percent of entries into the closed arm, $F(1, 53) = 1.275$, $p = .288$, suggesting the animals entered the closed arm at an equal ratio regardless of treatment. There was no Sex x Treatment interactions observed with regards to percent of entries into the closed arm, $F(2, 53) = 0.256$, $p = .775$,

suggesting the effect of the treatment was not influenced by the subject's sex. There was no significant effect of treatment on the percent of time spent in the closed arm, $F(1, 53) = 0.025$, $p = .975$, suggesting the animals spent comparable time in the closed arm regardless of treatment. There was no significant effect of sex on the percent of time spent in the closed arm, $F(2, 53) = 2.639$, $p = .110$, suggesting the animal's sex did not impact the time spent in the closed arm. There was no Sex x Treatment interactions on the percent of time spent in the closed arm, $F(2, 53) = 0.625$, $p = .539$.

Figure 3.2.2
Anxiety-like behaviour during the EPM of animals exposed to cannabis extract CD10 as compared to controls



Note. Graphical depictions of a. the percent of time spent in the closed arm of the EPM, and b. the percent of total entries that were into the closed arm of the EPM by animals exposed to 10 mg/kg [CD10(10)] or 40 mg/kg [CD10(40)] cannabis extract CD10.
[a] There was no significant effect of treatment, sex or Treatment x Sex interactions observed in the time spent in the closed arm of the apparatus.
[b] There was an effect of sex on the percent number of entries into the closed arm overall, there was no effect of treatment on the percent number of entries. Error bars represent the SEM.

3.3 Activity Box and Anxiety-Like Behaviour

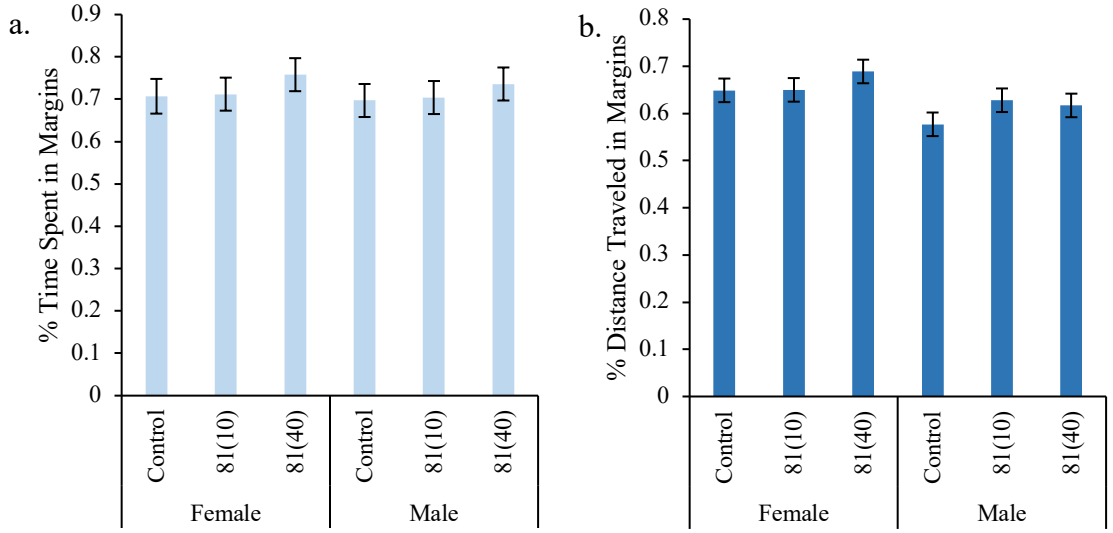
3.3.1 Extract #81 Marginal Time and Distance

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was no significant effect of sex on percent time spent in the margins of the chamber $F(1, 53) = 0.166$, $p = .685$, suggesting males and females spent equivalent time in the margins of the chamber. There was no significant effect of treatment on percent time spent in the margins of the chamber, $F(2, 53) = 0.082$, $p = .454$, suggesting animals spent equivalent time in the margins of the chamber and the lack of increased anxiety. There was no Sex x Treatment interactions $F(2, 53) = 0.019$, $p = .982$, suggesting that the effect of treatment was not influenced by the subject's sex.

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on percent distance travelled in the margins of the chamber $F(1, 54) = 7.144$, $p = .010$, suggesting males travelled less of their distance in the margins of the chamber. There was no significant effect of treatment on percent distance travelled in the margins of the chamber, $F(2, 54) = 1.279$, $p = .287$, suggesting animals traveled equivalent percent distances in the margins of the chamber. There was no Sex x Treatment interactions $F(2, 54) = 0.643$, $p = .530$, suggesting that the effect of treatment was not influenced by the subject's sex.

Figure 3.3.1

Percent time spent in the marginal region of the Activity Box of animals exposed to extract #81 as compared to controls



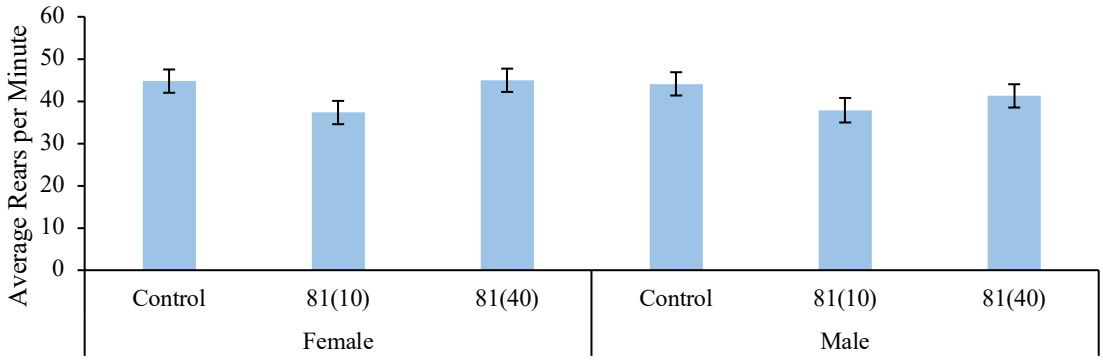
Note. A visual depiction of the mean percent time and distance travelled of each group of animals exposed to cannabis extract #81 spent in the margins of the activity box.
[a] There was no significant effect of treatment on time spent in the margins of the apparatus. Error bars represent the SEM.
[b] There was a significant effect of sex with males traveling less of their total distance in the margins. There was no significant effect of treatment on percent distance traveled in the margins of the apparatus. Error bars represent the SEM.

3.3.2 Extract #81 on Rearing

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was no significant effect of treatment on number of rears per minute, $F(2, 53) = 3.344$, $p = .043$, suggesting animals reared equivalently regardless of treatment indicating no significant increase in anxiety. There was no significant effect of sex on number of rears per minute $F(1, 53) = 0.311$, $p = .579$, suggesting males and females reared statistically equivalent amounts. There was no Sex x Treatment interactions $F(2, 53) = 0.310$, $p = .735$, suggesting that the effect of treatment was not influenced by the subject's sex.

Figure 3.3.2

Average rears per minute in the Activity Box of animals exposed to extract #81 as compared to controls



Note. A visual depiction of the average rears per minute over 10 minutes for each group of animals exposed to cannabis extract #81 in the activity box. Error bars represent the SEM.

[a] There was no significant effect of treatment on average rears per minute. Error bars represent the SEM.

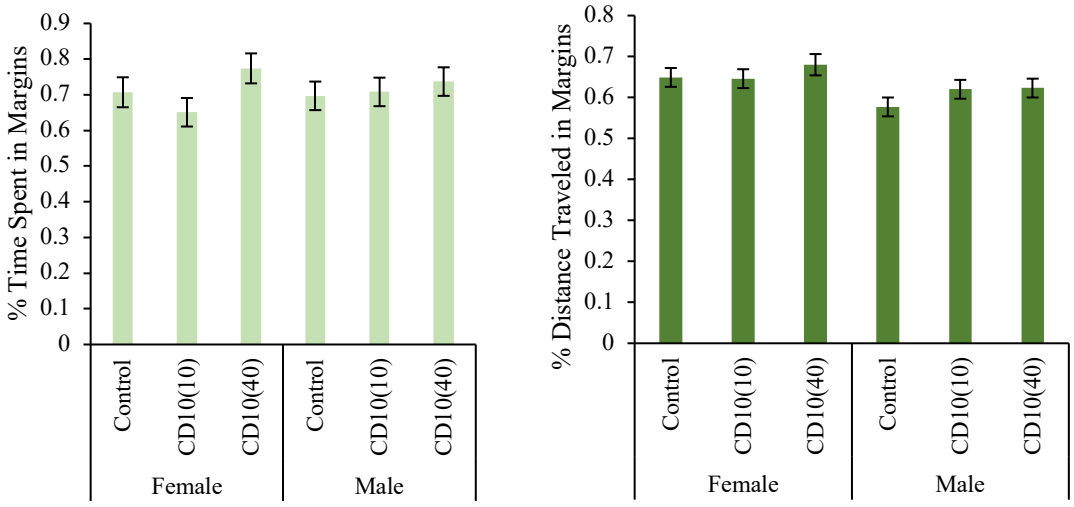
3.3.3 Extract CD10 Marginal Time and Distance

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was no significant effect of sex on percent time spent in the margins of the chamber $F(1, 52) = 0.011$, $p = .918$, suggesting animals spent equivalent time in the margins of the chamber regardless of their sex indicating no significant increase in anxiety. There was no significant effect of treatment on percent time spent in the margins of the chamber, $F(2, 52) = 1.826$, $p = .171$, suggesting animals spent equivalent time in the margins of the chamber. There was no Sex x Treatment interactions $F(2, 52) = 0.712$, $p = .496$, suggesting that the effect of treatment was not influenced by the subject's sex.

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on percent distance travelled in the margins of the chamber $F(1, 52) = 7.151$, $p = .010$, suggesting males travelled less of their distance in the margins of the chamber. There was no significant effect of treatment on percent distance travelled in

the margins of the chamber, $F(2, 52) = 1.324$, $p = .275$, suggesting animals traveled equivalent percent distances in the margins of the chamber. There was no Sex x Treatment interactions $F(2, 52) = 0.517$, $p = .600$, suggesting that the effect of treatment was not influenced by the subject's sex.

Figure 3.3.3
Percent time spent in the marginal region of the Activity Box of animals exposed to extract CD10 as compared to controls



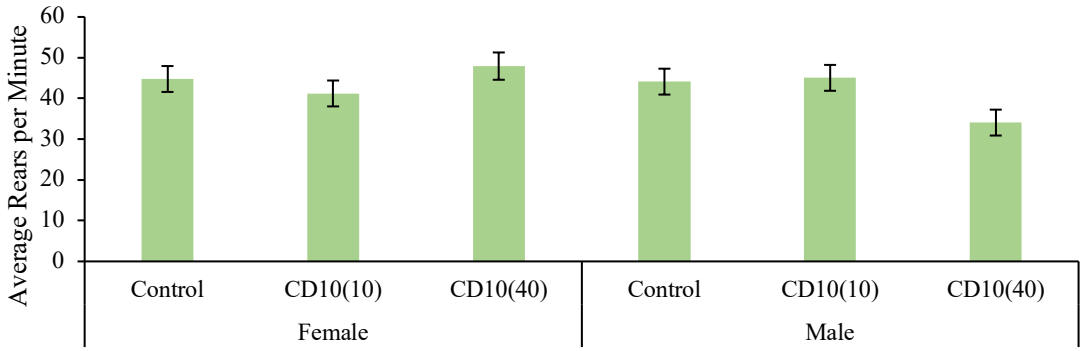
Note. A visual depiction of the mean percent time and distance travelled of each group of animals exposed to cannabis extract CD10 spent in the margins of the activity box.
[a] There was no significant effect of sex on time spent in the margins of the apparatus. There was no effect of treatment. Error bars represent the SEM.
[b] There was a significant effect of treatment on percent distance traveled in the margins of the apparatus with males travelling less of their total distance in the margins. Error bars represent the SEM.

3.3.4 Extract CD10 on Rearing

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was significant Sex x Treatment interactions $F(2, 53) = 4.049$, $p = .023$, suggesting that the effect of treatment was influenced by the subject's sex, with males exposed to a 40mg/kg dose of CD10 rearing significantly less. There was no significant effect of sex on number of rears per minute $F(1, 53) = 1.894$, $p = .180$, suggesting males and females reared statistically equivalent amounts. There was no significant effect of treatment on number of rears per minute, $F(2, 53) = 0.580$, $p = .564$, suggesting animals reared equivalently regardless of treatment.

Figure 3.3.4

Average rears per minute in the Activity Box of animals exposed to extract CD10 as compared to controls



Note. A visual depiction of the average rears per minute over 10 minutes for each group of animals exposed to cannabis extract #CD10 in the activity box. Error bars represent the SEM.

[a] There was a significant Sex x Treatment effect of average rears per minute. There was no significant effect of treatment or sex on average rears per minute. Error bars represent the SEM.

3.4 Physiological Analysis

3.4.1 Extract #81

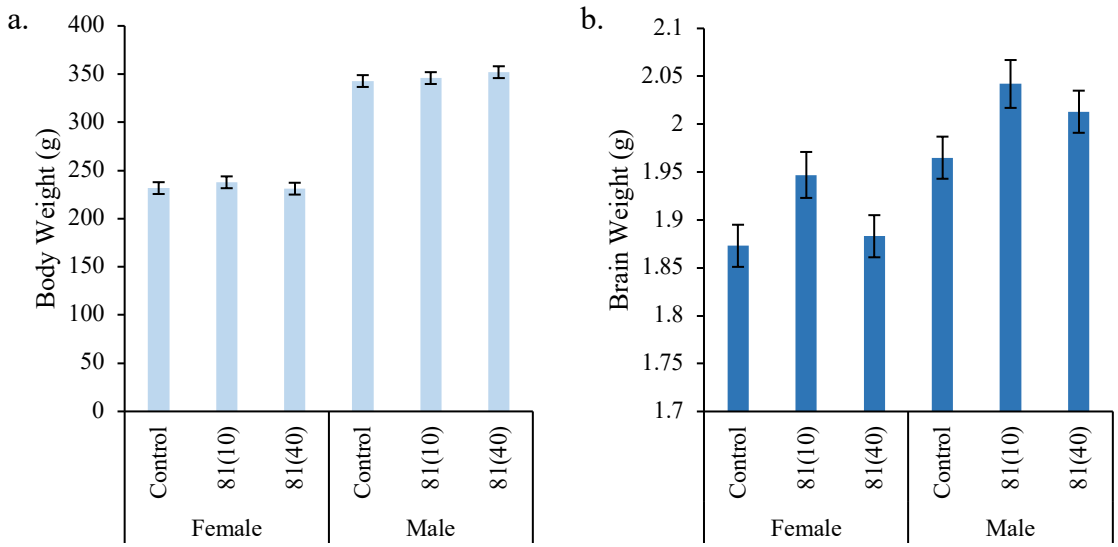
Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on body weight of the animal $F(1, 54) = 512.141$, $p < .001$, males are statistically larger than females. There was no significant effect of treatment on body weight, $F(2, 54) = 3.48$, $p = .708$, suggesting animals body growth was statistically normal regardless of treatment. There was no significant Sex x Treatment interactions $F(2, 54) = 0.591$, $p = .557$, suggesting that the effect of treatment was not influenced by the subject's sex.

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on brain weight of the animal $F(1, 51) = 35.257$, $p < .001$, males are statistically larger average brain size than females. There was a significant effect of treatment on brain weight, $F(2, 51) = 5.239$, $p = .009$, suggesting animals exposed specifically to the 10mg/kg dose of extract #81 had a larger average brain size. There was no significant Sex x Treatment interactions $F(2, 51) = 0.453$, $p = .639$, suggesting that the effect of treatment was not influenced by the subject's sex.

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on brain weight as a percent of body weight of the animal $F(1, 51) = 346.327$, $p < .001$; females have a statistically larger average brain size than males when controlled for body weight. There was no significant effect of treatment on brain weight, $F(2, 51) = 0.590$, $p = .558$, suggesting the subject's brain weights as controlled for body weight are not impacted by consumption of extract #81. There was no significant Sex x Treatment interactions $F(2, 51) = 0.131$, $p = .877$, suggesting that the effect of treatment was not influenced by the subject's sex.

Figure 3.4.1

Average body and brain weight of animals exposed to extract #81 as compared to controls

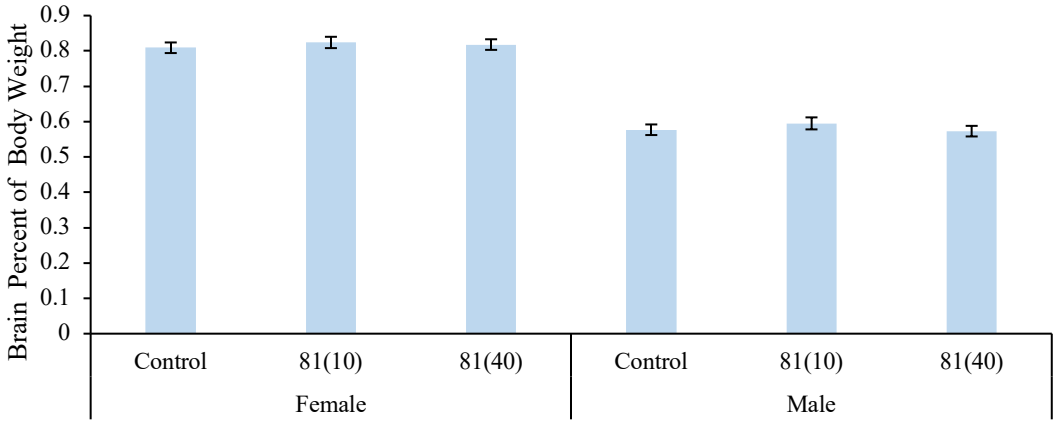


Note. A visual depiction of the mean raw body and brain weights of each group of animals exposed to cannabis extract #81.

[a] There was a significant sex effect, with males being larger. There was no effect of treatment on body size. Error bars represent the SEM.

[b] There was a significant effect of sex with males having larger brains. There was a significant effect of treatment on with the 10mg/kg groups having significantly larger brains. Error bars represent the SEM.

Figure 3.4.2
Average brain weight as a percent of body weight of animals exposed to extract #81 as compared to controls



Note. A visual depiction of the mean brain weight as controlled for body weight of each group of animals exposed to cannabis extract #81.
[a] There was a significant sex effect, with females having a proportionally larger brain as compared to their body weight. There was no effect of treatment on brain size as relation to body weight. Error bars represent the SEM.

3.4.2 Extract CD10

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on body weight of the animal $F(1, 54) = 512.141$, $p < .001$, males are statistically larger than females. There was no significant effect of treatment on body weight, $F(2, 54) = 3.48$, $p = .708$, suggesting animals body growth was statistically normal regardless of treatment. There was no significant Sex x Treatment interactions $F(2, 54) = 0.591$, $p = .557$, suggesting that the effect of treatment was not influenced by the subject's sex.

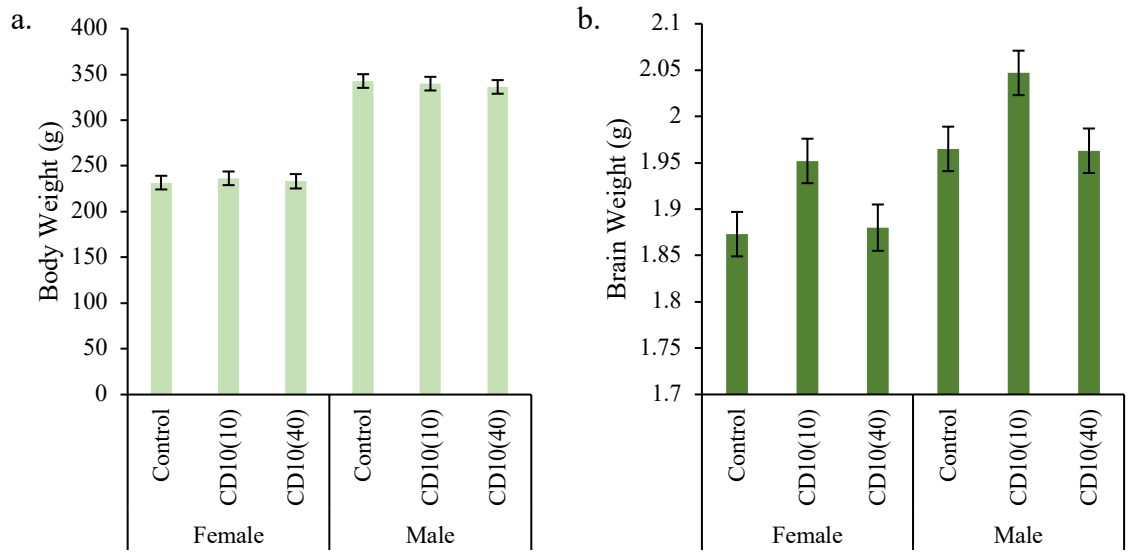
Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on brain weight of the animal $F(1, 53) = 20.557$, $p < .001$, males have statistically larger average brain size than females. There was a significant effect of

treatment on brain weight, $F(2, 53) = 7.158$, $p = .002$, suggesting animals exposed specifically to the 10mg/kg dose of extract #81 had a larger average brain size. There was no significant Sex x Treatment interactions $F(2, 53) = 0.029$, $p = .971$, suggesting that the effect of treatment was not influenced by the subject's sex.

Post hoc analyses were conducted using Bonferroni's post-hoc test. There was a significant effect of sex on brain weight as a percent of body weight of the animal $F(1, 53) = 237.170$, $p < .001$, females have a statistically larger average brain size than males when controlled for body weight. There was no significant effect of treatment on brain weight, $F(2, 53) = 1.295$, $p = .282$, suggesting the subject's brain weights as controlled for body weight are not impacted by consumption of extract CD10. There was no significant Sex x Treatment interactions $F(2, 53) = 0.043$, $p = .958$, suggesting that the effect of treatment was not influenced by the subject's sex.

Figure 3.4.3

Average body and brain weight of animals exposed to extract CD10 as compared to controls



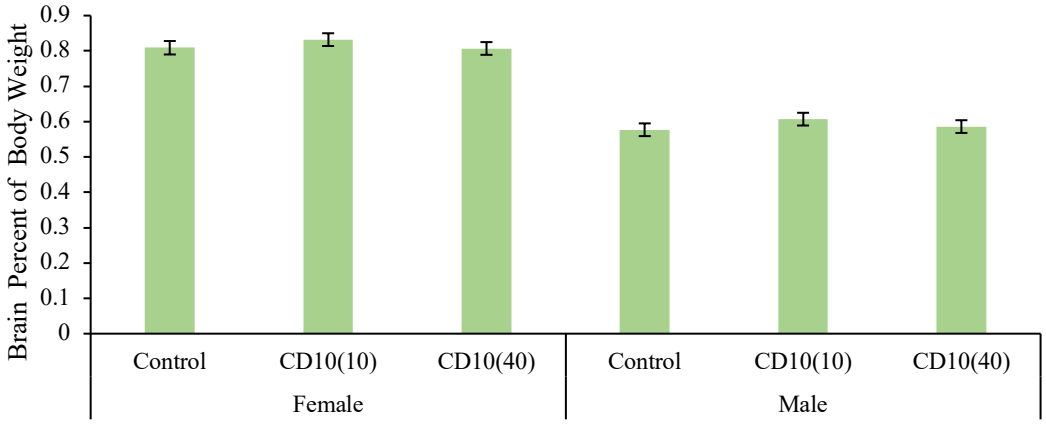
Note. A visual depiction of the mean body and brain weights of each group of animals exposed to cannabis extract CD10.

[a] There was a significant sex effect, with males being larger. There was no effect of treatment on body size. Error bars represent the SEM.

[b] There was a significant effect of sex with males having larger brains. There was a significant effect of treatment on with the 10mg/kg groups having significantly larger brains. Error bars represent the SEM.

Figure 3.4.4

Average brain weight as a percent of body weight of animals exposed to extract CD10 as compared to controls



Note. A visual depiction of the mean brain weight as controlled for body weight of each group of animals exposed to cannabis extract CD10.

[a] There was a significant sex effect, with females having a proportionally larger brain as compared to their body weight. There was no effect of treatment on brain size as relation to body weight. Error bars represent the SEM.

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 General Discussion

As cannabis consumption has been linked to increases in anxiety and deficits in learning in memory it was important to explore how these extracts may be related to these behaviours (Ranganathan & D'Souza, 2006, Vann et al., 2008). As well, these effects are exaggerated when looking at an adolescent model (Rubino et al., 2008; Schneider, 2008; Renard et al., 2016; Meyer, Lee & Gee, 2018). Much of the available literature demonstrates that THC is the primary culprit in producing these effects. Much research has been conducted on the effects of THC but the contribution of CBD to observed physiological and behavioral changes remains relatively unknown. As a result, there has been a change in gears to CBD as a primary research candidate (Bhattacharyya et al., 2010; Taffe, Creehan & Vandewater, 2015). Although CBD demonstrates novel medicinal properties, we would be remiss to not discuss the variety of medicinal properties of THC (Noyes et al., 1975; El-Alfy et al., 2010; Karl, Garner & Cheng, 2017). Beyond the individual constituents, whole-plant cannabis extract may be able to produce synergistic effects beyond those of the constituents alone (Fernandes et al., 1974). It has been shown that, in addition to the divergent effects between an adolescent brain and an adult brain, there are sex differences in how cannabis is metabolized in a mammalian system resulting in added complexities in the effects of cannabis consumption (Tseng & Craft, 2001; Bradshaw et al., 2006; Krebs-Kraft et al., 2010). Demonstrating the lack of significant behavioural impacts that cannabis containing both THC and CBD opens new avenues of research regarding utilizing both constituents in conjunction; this was done through unpublished data in our lab. Expanding beyond that, determining that there are no

significant sex differences in these effects and that there are no novel effects that occur when looking at a developing brain are imperative to preclinical and clinical research of cannabis.

As there were no treatment dependent nor Sex x Treatment effects in this specific study indicates that there is likely minimal to no significant behavioural impacts following exposure to either a low dose (10 mg/kg of body weight) or a high dose (40 mg/kg of body weight) of the extracts that was used. This exposure was done as a chronic/long-term exposure, such as what would be recommended when attempting to control a chronic illness. A major difference between this study and much of the currently available research is the timing of behavioural testing, for the MWT there was over 36-hour rest period between final dosing and testing, and for the EPM and Activity Box tests, there was an 8-day delay. This break allowed for all cannabis constituents to be metabolized as the study aimed specifically to look for lasting impacts of high-CBD cannabis extract exposure. The analyses performed were designed to explore the effects of long-term oral consumption of high-CBD whole plant cannabis extracts on adolescent behaviour. Specifically, the test battery used was designed to reveal any behavioural impacts to anxiety, locomotion and spatial learning and memory in the Long-Evans rat after consumption has ceased.

4.2 Behavioural Analysis Discussion

4.2.1 Morris Water Task and Spatial Learning and Memory

Consumption of either extract #81 or CD10 demonstrated no lasting impairments to learning and memory in the Long-Evans rat. Treated animals were able to acquire information about the hidden platform at a statistically equivalent rate as non-treated

animals. Acquisition curves for animals treated with 40mg/kg of body weight, 10mg/kg of body weight and controls followed a comparable curve that plateaued at approximately Day 4 of acquisition. Unpublished data from our laboratory showed that adult animals treated with extract #81 for 10 days demonstrated no significant in acquisition curves and learning and memory effects, data comparable to what was seen here.

These data are reinforced by the lack of significant findings on the probe day trials for heading direction and latency to cross the location of the removed platform. There were no sex differences in effects, there were no Sex x Treatment effects observed in this task indicating high-CBD cannabis consumption is presumably safe for both developing males and females. The data presented in this thesis demonstrates the lack of lasting learning and memory impairments following chronic consumption of high-CBD cannabis. This opens the possibility of using these extracts as an additional treatment option in conjunction with a patients current medicinal regime for a variety of chronic illnesses that impact adolescents such as rheumatoid arthritis, specific cancers, and epilepsy.

4.2.2 Elevated Plus Maze and Anxiety-Like Behaviour

Consumption of either extract #81 or CD10 demonstrated no lasting increases to anxiety behaviour with regards to time spent in the closed, “safe” arm. There was also no significant effect of treatment with either extract indicating no significant increases in anxiety following treatment with either extract.

There was an overall significant effect in both treatment groups of percentage of total entries that were into the closed arm. This shows that overall, the males in this experiment preferred entering the closed arms but still spent equivalent time in the open and closed arms. Therefore, although they entered the closed arms with a higher

frequency, each individual entrance to the open arm was associated with a longer stay in the open arm and each entrance into the closed arm was associated with a short stay in the closed arm. We therefore do not believe that this significance in percentage of entrances into the closed arm by the male animals holds any relation to anxiety rates in the males. This will be further explained when compared to the Activity Box data.

4.2.3 Activity Box and Anxiety-Like Behaviour

Consumption of extract #81 or CD10 resulted in no significant effects of sex, treatment, or Sex x Treatment interactions. Overall, there were no significant effects on percentage of time spent in the marginal region or percentage of distance travelled in the marginal region of the chamber. This coincides with the data on rearing that only depicted significance in the high dose CD10 males. These data, when taken in conjunction with the other data depicting no significant increase in anxiety levels, could likely be a result of these animals choosing not to rear as much per minute. This behaviour is associated with decreased anxiety of a rat (Slawecki, 2005; Turner & Burne, 2014).

Taking into account rearing behaviour, percent distance traveled (did they enter the marginal space and hide, still, against a wall) and percent time in each region gives us a better understanding of the relationship high-CBD cannabis has to anxiety. If a decrease in the rate of rearing had occurred, there could have been an association of increased anxiety when compared to the significant amount of time spent in the margins of the activity box which may also be associated to the significant number of entries the males made into the closed arm of the EPM.

4.3 Physiological Analysis Discussion

Physiologically, we would expect that males have a larger body size and brain size than females overall as rats are sexually dimorphic in their size. This is what we saw clearly presented through the significant sex differences observed. The most interesting data point is that that the low dose groups in both extracts had significantly larger average brain size raw data, a significance that was lost when accounting for the individual animal's body weight. This is explainable through basic genetics, although pseudorandomly organized, the females coming from litters that were predominantly male would likely have a larger brain size, as well, some animals will in general have a larger brain (Wolf et al., 2002). When brain weight was accounted for as a percent of body weight this significance disappears as the animal's overall size controls for the differences in brain size, indicating that high CBD cannabis is not likely impacting overall brain size.

4.4 Final Remarks and Conclusion

4.4.1 Overall Adolescent Effects

Although behaviour is imperative to our understanding and diagnosis of an appropriate developmental trajectory, it is critical that further research is conducted into examining the brain tissue of these animals. Developmental alterations in cell density and number of connections in regions of the prefrontal cortex and hippocampus can be associated with changes in learning and memory, and anxiety behaviours that may be being compensated for in these examinations (Farrell et al., 2016; de Melo et al., 2018). This research creates the steppingstone to continue research into the field of the lasting impacts of consumption of high-CBD cannabis extracts.

It was expected that there would be changes to anxiety levels in the adolescent animals, increases from the THC or decreases from the CBD, yet no alterations were observed in this study (Guimarães et al., 1990; Bhattacharyya et al., 2010; Campos, Ferreira & Guimarães, 2012). There may then be hidden effects that are not severe enough to be presenting themselves as behavioural impacts. In a realistic medicinal setting, lack of behavioural impacts is ideal as the drug, therefore, should not impact the day-to-day life of an adolescent patient. Furthermore, this data reinforces the idea that there are major interactions between constituents within cannabis as there were no observable behavioural changes. It is important to note that much of the data available related to adolescent consumption of cannabis focusses primarily on illicit consumption or consumption of street cannabis, these, as mentioned above, are strains specifically high in THC and low in CBD (Scheider, 2008; Squeglia, Jacobus & Tapert, 2009; Lorenzetti et al., 2016). Understanding the difference in these strains will aid researchers in providing the best quality data on cannabis as a medicine further aiding practitioners with the tools required for recommending personalized treatment options.

4.4.2 Overall Sex Differences

Expanding on personalized treatment, it is important to focus on the differences between male and females. Cannabis not only impacts males and females differently, it impacts those of the same sex differently at different time points (estrus, season, etc.) (Scorticati et al., 2003; Riebe et al., 2010). Within this study there were sexually dimorphic results on brain and body size but overall, no observable sexually dimorphic treatment effects. Further anatomical data of cell counts, dendritic spine density, thalamic volume and cortical thickness currently being processed in our lab will aid us in

determining any underlying effects that did not present through behavioural testing. Another interesting tidbit of data to touch on is the females having a generalized larger brain when body weight is accounted for. This can likely be attributed to the developmental stage of the animals as the females are developing faster than the males (Hernandez et al., 2020). As mentioned above, behavioural development, learning and memory and overall cognition are associated with areas of the prefrontal cortex and hippocampus. Examining the associated areas will give us better insight into if there is a lack of significant structural changes that resulted in the lack of lasting behavioral changes.

4.4.3 Future Directions of Research

This research is topical to current medicinal research as it demonstrates that cannabis consumption for a chronic period in adolescence may not be inducing lasting impacts to behaviour. Expanding beyond that, learning and memory are extraordinarily important during adolescence as the frontal cortex is undergoing immense structural changes that will have lasting impacts (Rubino et al., 2008; Oliveira-da-Silva et al., 2009; Hehar et al., 2015; Renard et al., 2016). It is known that THC impacts learning and memory, this is especially true in adolescents (Schneider, 2008). Using the MWT, we were able to demonstrate that there are no lasting impairments to learning and memory following chronic use of high-CBD cannabis extract (which still contains THC). It thus appears to be important to consider the use of whole plant cannabis extracts as a treatment for chronic illness.

As previously stated, research on the medicinal use of cannabis is showing positive outcomes for patients battling many inflammatory diseases, neuroinflammatory

diseases, cancers, and more (Esposito et al., 2011; Lowin, Schneider & Pongratz, 2019; Kovalchuk & Kovalchuk, 2020; Cherkasova, Kovalchuk & Kovalchuk, 2021). Allowing for adolescents battling these diseases to use novel, natural medicinal products, such as cannabis, allows for larger options for personalized treatment. Specifically with regards to epilepsy, CBD has been shown to restabilize the homeostatic state of an overexcited brain but has strange interactions with other drug-resistant epilepsy controls resulting in unfortunate side effects (Devinsky et al., 2017; Laux et al., 2019). Researching and approving the use of whole plant cannabis extracts allow for a potential new therapy in addition to CBD that may reduce the side effects experienced by patients. Using whole-plant cannabis also allows for synergistic interactions from other constituents present in cannabis. This has already been shown through the interactions of THC and CBD as CBD reduces some negative effects of THC, allowing for patients to gain the medicinal benefits of both THC and CBD with fewer side effects.

There is currently a lack of data available regarding the differences in consumption of cannabis via different routes of administration. High-CBD cannabis inhaled, injected, topically applied, or orally administered has different absorption and metabolizing rates, which may result in different behavioural impacts (Phillips, Turk & Forney, 1971). Specifically, data are needed to compare consumption of high-CBD cannabis taken orally versus inhaled as these are the two most common routes of administration of cannabis, with inhalation having a faster onset of effects with other strains. Using oral ingestion and expanding upon the methods in this study, it would be interesting to conduct a comparative study using these specific cannabis strains to those that have equal CBD and THC content and furthermore to those that have higher THC

than CBD content to better understand what the critical THC/CBD ratio is where the behavioural impacts begin to outweigh the medicinal benefits.

Blood was taken from these animals at 3 time points, pre-dosing, immediately post-dosing, and at time of euthanasia and tissue samples were taken from the animals at endpoint. Analysis of inflammatory biomarkers in these tissues will provide deeper insight into the lasting effects of chronic high-CBD cannabis consumption in adolescence. Analyzing cell density in the brain and dendritic connections in the prefrontal cortex and hippocampus of sampled brains will also reinforce our understanding of the underlying impacts that may not be presented through behavioural changes. All these data are currently being analysed.

4.4.4 Limitations

There were numerous limitations within this study. First and foremost, it is imperative to recognize that these were living organisms with ethics associated with their use. Using adolescent animals (which are more susceptible to additional stressors such as testing time, time away from housing unit, handling experience) limits the number of tests that can be conducted on each animal within a given time frame. Therefore, we had to limit the number of behavioural tests conducted as the animals needed down time to recover from excess stress. Due to the age of the animals and the nature of the tests, it was imperative that tests be conducted at the same post-natal day and with accelerated aging in a rodent model, there were limitations associated with the timing of conducting dosing and behavioural testing. Originally, we were to conduct an additional social behaviour test to analyse the effects of high-CBD cannabis on social interaction during adolescence but we unable to do so with the limited time frame during this study and the nature of this

specific test being a repeated measures model requiring baseline testing. With such a young age, we had to push back dosing because of the stressors caused from weaning and litter separation that occurred at P21 and P22-25, therefore we were unable to test baseline behaviours for even the tests used in this test battery. Replicating this experiment in an adult model yields this additional ability, a method done in unpublished data from our lab.

Beyond behaviour, we only had access to high-CBD cannabis. Using a larger variety of strains and in particular those with different CBD:THC ratios would provide more insight into individual strain differences. Using living organisms also limits the *N* of our test populations, replicating this study with more animals, an extended dosing regimen, different strains of cannabis with different constituent content will most definitely yield the most promising data. Overall, this experiment was conducted to the best of our abilities and has provided deeper insight into the lasting behavioural effects of high-CBD cannabis in adolescence.

4.4.5 Conclusion

Understanding the extent of cannabis' effects has been pivotal in the use of cannabis as a medicine. Historically there has been a debate over the interaction of THC and CBD in the literature (Malone, Jongejan & Taylor, 2009; Zuardi, Hallak & Crippa, 2012; Taffe, Creehan & Vandewater, 2015; Hložek et al., 2017). Both THC and CBD are able to independently elicit medicinal effects ranging from analgesia, and antiemesis, anti-inflammation and antitumor properties (Noyes et al., 1975; Tramer et al., 2001; Kovalchuk & Kovalchuk, 2020; Cherkasova, V., Kovalchuk & Kovalchuk; 2021). Utilizing both constituents in conjunction, whole-plant cannabis extract may produce

synergistic effects beyond those of the constituents alone (Fernandes et al., 1974). Accordingly, this study aimed to investigate the sexually dimorphic behavioural impacts of consuming high-CBD cannabis extract. Overall, there were no significant behavioural impacts of consuming high-CBD cannabis extract at either dosing level. Neither were there sexually dimorphic behavioral impacts of treatment. This is consistent with available data on consumption of CBD (Hložek et al., 2017). In conclusion, this study demonstrates that adolescent consumption of high-CBD cannabis lacks long-term or lasting behavioural impacts. Further research into the anatomical and physiological impacts are recommended to reinforce the safety of these products.

References

Andreae, M. H., Carter, G. M., Shaparin, N., Suslov, K., Ellis, R. J., Ware, M. A., Abrams, D. I., Prasad, H., Wilsey, B., Indyk, D., Johnson, M., & Sacks, H. S. (2015). Inhaled cannabis for chronic neuropathic pain: A meta-analysis of individual patient data. *The Journal of Pain, 16*(12), 1221-1232. https://doi.org/10.1016/j.jpain.2015.07.009

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M.-H., Vogel, Z., Bisogno, T., De Petrocellis, L., Di Marzo, V., & Mechoulam, R. (1998). An entourage effect: Inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *European Journal of Pharmacology, 353*(1), 23–31. https://doi.org/10.1016/S0014-2999(98)00392-6

Bhattacharyya, S., Morrison, P., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O'Carroll, C., Seal, M., Allen, P., Mehta, M., Stone, J., Tunstall, N., Giampietro, V., Kapur, S., Murray, R., Zuardi, A., Crippa, J., Atakan, Z., McGuire, P. (2010). Opposite effects of Δ-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology, 35*(3), 764-774. doi:10.1038/npp.2009.184

Blasco-Benito, S., Seijo-Vila, M., Caro-Villalobos, M., Tundidor, I., Andradas, C., García-Taboada, E., Wade, J., Smith, S., Guzmán, M., Pérez-Gómez, E., Gordon, M., & Sánchez, C. (2018). Appraising the "entourage effect": Antitumor action of a pure cannabinoid versus a botanical drug preparation in preclinical models of breast cancer. *Biochemical Pharmacology, 157*, 285-293. https://doi.org/10.1016/j.bcp.2018.06.025

Bradshaw, H. B., Rimmerman, N., Krey, J. F., & Walker, J. M. (2006). Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 291*(2), R349-R358. https://doi.org/10.1152/ajpregu.00933.2005

Brodie, M. J., Barry, S. J. E., Bamagous, G. A., Norrie, J. D., & Kwan, P. (2012). Patterns of treatment response in newly diagnosed epilepsy. *Neurology, 78*(20), 1548-1554. https://doi.org/10.1212/WNL.0b013e3182563b19

Bossong, M. G., Jager, G., van Hell, H. H., Zuurman, L., Jansma, J. M., Mehta, M. A., van Gerven, J. M. A., Kahn, R. S., Ramsey, N. F. (2012). Effects of Δ9-tetrahydrocannabinol administration on human encoding and recall memory function: A pharmacological fMRI study. *Journal of Cognitive Neuroscience, 24*(3), 588-599. doi:10.1162/jocn_a_00156

Broyd, S. J., van Hell, H. H., Beale, C., Yücel, M., & Solowij, N. (2016). Acute and chronic effects of cannabinoids on human cognition—A systematic review.

Biological Psychiatry (1969), 79(7), 557-567. https://doi.org/10.1016/j.biopsych.2015.12.002

Burston, J. J., Wiley, J. L., Craig, A. A., Selley, D. E., & Sim-Selley, L. J. (2010). Regional enhancement of cannabinoid CB1 receptor desensitization in female adolescent rats following repeated Δ9-tetrahydrocannabinol exposure. *British Journal of Pharmacology, 161*(1), 103-112. https://doi.org/10.1111/j.1476-5381.2010.00870.x

Busquets-Garcia, A., Puighermanal, E., Pastor, A., de la Torre, R., Maldonado, R., & Ozaita, A. (2011). Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. *Biological Psychiatry, 70*(5), 479-486. https://doi.org/10.1016/j.biopsych.2011.04.022

Cabral, G. A. (2005). Cannabinoid receptors in microglia of the central nervous system: Immune functional relevance. *Journal of Leukocyte Biology, 78*(6), 1192-1197. https://doi.org/10.1189/jlb.0405216

Campos, A. C., Ferreira, F. R., & Guimarães, F. S. (2012). Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: Possible involvement of 5HT1A receptors. *Journal of Psychiatric Research, 46*(11), 1501-1510. https://doi.org/10.1016/j.jpsychires.2012.08.012

Carrier, E., Kearn, C., Barkmeier, A., Breese, N., Yang, W., Nithipatikom, K., Pfister, S., Campbell, W., & Hillard, C. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Molecular Pharmacology, 65*(4), 999-1007. https://doi.org/10.1124/mol.65.4.999

Carrier, E. J., Auchampach, J. A., & Hillard, C. J. (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: A mechanism of cannabinoid immunosuppression. *Proceedings of the National Academy of Sciences, 103*(20), 7895-7900. https://doi.org/10.1073/pnas.0511232103

Casiraghi, A., Roda, G., Casagni, E., Cristina, C., Musazzi, U. M., Franze, S., Rocco, P., Giuliani, C., Fico, G., Minghetti, P., & Gambaro, V. (2018). Extraction method and analysis of cannabinoids in cannabis olive oil preparations. *Planta Medica, 84*(4), 242-249. https://doi.org/10.1055/s-0043-123074

Castaneto, M. S., Gorelick, D. A., Desrosiers, N. A., Hartman, R. L., Pirard, S., & Huestis, M. A. (2014). Synthetic cannabinoids: Epidemiology, pharmacodynamics, and clinical implications. *Drug and Alcohol Dependence, 144*, 12-41. https://doi.org/10.1016/j.drugalcdep.2014.08.005

Castillo, P., Younts, T., Chávez, A., & Hashimotodani, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron, 76*(1), 70-81. https://doi.org/10.1016/j.neuron.2012.09.020

Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., De Chiara, V., Battistini, L., Bernardi, G., Bernardini, S., Martino, G., & Maccarrone, M. (2007). The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain,130*(10), 2543-2553. https://doi.org/10.1093/brain/awm160

Chaudhary, S., Siddiqui, M., Athar, M., & Alam, M. S. (2012). d-limonene modulates inflammation, oxidative stress and ras-ERK pathway to inhibit murine skin tumorigenesis. *Human & Experimental Toxicology, 31*(8), 798-811. https://doi.org/10.1177/0960327111434948

Cherkasova, V., Kovalchuk, O., & Kovalchuk, I. (2021). Cannabinoids and endocannabinoid system changes in intestinal inflammation and colorectal cancer. *Cancers, 13*(17), 4353. https://doi.org/10.3390/cancers13174353

Chevaleyre, V., & Castillo, P. E. (2003). Heterosynaptic LTD of hippocampal GABAergic synapses: A novel role of endocannabinoids in regulating excitability. *Neuron*, *38*(6), 997-997. https://doi.org/10.1016/S0896-6273(03)00351-9

Chevaleyre, V., Heifets, B. D., Kaeser, P. S., Sudhof, T. C., & Castillo, P. E. (2007). Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1[alpha]. *Neuron, 54*(5), 801. https://doi.org/10.1016/j.neuron.2007.05.020

Chong, M. S., Wolff, K., Wise, K., Tanton, C., Winstock, A., & Silber, E. (2006). Cannabis use in patients with multiple sclerosis. *Multiple Sclerosis, 12*(5), 646-651. https://doi.org/10.1177/1352458506070947

Colizzi, M., Weltens, N., McGuire, P., Lythgoe, D., Williams, S., Van Oudenhove, L., & Bhattacharyya, S. (2020). Delta-9-tetrahydrocannabinol increases striatal glutamate levels in healthy individuals: Implications for psychosis. *Molecular Psychiatry, 25*(12), 3231-3240. https://doi.org/10.1038/s41380-019-0374-8

Consroe, P., Benedito, M. A., Leite, J. R., Carlini, E. A., & Mechoulam, R. (1982). Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. *European Journal of Pharmacology, 83*(3-4), 293.

Consroe, P., Musty, R., Rein, J., Tillery, W., & Pertwee, R. (1997). The perceived effects of smoked cannabis on patients with multiple sclerosis. *European Neurology, 38*(1), 44-48. https://doi.org/10.1159/000112901

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., & Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience, 139*(4), 1405-1415. https://doi.org/10.1016/j.neuroscience.2006.02.074

Croxford, J. L., & Yamamura, T. (2005). Cannabinoids and the immune system: Potential for the treatment of inflammatory diseases? *Journal of Neuroimmunology, 166*(1), 3-18. https://doi.org/10.1016/j.jneuroim.2005.04.023

Currie, D. (2016). Growing up unequal: gender and socioeconomic differences in young people's health and well-being. Health Behaviour in School-aged Children (HBSC) study: International report from the 2013/2014 survey (No. 7). *World Health Organization*. Retrieved September 22nd, 2020 from https://www.euro.who.int/en/health-topics/Life-stages/child-and-adolescent-health/health-behaviour-in-school-aged-children-hbsc/hbsc-international-reports/growing-up-unequal.-hbsc-2016-study-20132014-survey

Dale, T., Downs, J., Olson, H., Bergin, A. M., Smith, S., & Leonard, H. (2019). Cannabis for refractory epilepsy in children: A review focusing on CDKL5 deficiency disorder. *Epilepsy Research, 151*, 31-39. https://doi.org/10.1016/j.eplepsyres.2019.02.001

Darkovska-Serafimovska, M., Serafimovska, T., Arsova-Sarafinovska, Z., Stefanoski, S., Keskovski, Z., & Balkanov, T. (2018). Pharmacotherapeutic considerations for use of cannabinoids to relieve pain in patients with malignant diseases. *Journal of Pain Research, 11*, 837-842. https://doi.org/10.2147/JPR.S160556

Das, R. K., Kamboj, S. K., Ramadas, M., Yogan, K., Gupta, V., Redman, E., Curran, H. V., & Morgan, C. J. A. (2013). Cannabidiol enhances consolidation of explicit fear extinction in humans. *Psychopharmacology, 226*(4), 781-792. https://doi.org/10.1007/s00213-012-2955-y

de Melo, S. R., de David Antoniazzi, Caren Tatiane, Hossain, S., & Kolb, B. (2018). Neonatal stress has a long-lasting sex-dependent effect on anxiety-like behavior and neuronal morphology in the prefrontal cortex and hippocampus. *Developmental Neuroscience, 40*(2), 93-103. https://doi.org/10.1159/000486619

De Petrocellis, L., & Di Marzo, V. (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: Focus on G-protein-coupled receptors and transient receptor potential channels. *Journal of Neuroimmune Pharmacology, 5(*1), 103-121. https://doi.org/10.1007/s11481-009-9177-z

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allarà, M., Bisogno, T., Petrosino, S., Stott, C. G., & Di Marzo, V. (2011). Effects of cannabinoids and cannabinoid-enriched cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology, 163*(7), 1479-1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x

Devinsky, O., Cross, J. H., Laux, L., Marsh, E., Miller, I., Nabbout, R., Scheffer, I. E., Thiele, E. A., Wright, S., Cannabidiol Dravet Syndrome Study, & Cannabidiol in

Dravet Syndrome Study Group. (2017). Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *The New England Journal of Medicine, 376*(21), 2011-2020. https://doi.org/10.1056/NEJMoa1611618

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., Sirianni, M., La, C. C., Stilo, S. A., Marques, T. R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A. S., Morgan, C., Powell, J., Murray, R. M. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biological Psychiatry, 72*(10), 811-816. doi:10.1016/j.biopsych.2012.06.020

Di Marzo, V., De Petrocellis, L., Fezza, F., Ligresti, A., & Bisogno, T. (2002). Anandamide receptors. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 66*(2-3), 377-391. https://doi.org/10.1054/plef.2001.0349

Dow-Edwards, D., & Silva, L. (2017). Endocannabinoids in brain plasticity: Cortical maturation, HPA axis function and behavior. *Brain Research, 1654*(Pt B), 157-164. https://doi.org/10.1016/j.brainres.2016.08.037

D'Souza, D., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y., Braley, G., Gueorguieva, R., & Krystal, J. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: Implications for psychosis. *Neuropsychopharmacology (New York, N.Y.), 29*(8), 1558-1572. https://doi.org/10.1038/sj.npp.1300496

D'Souza, D. C., Ranganathan, M., Braley, G., Gueorguieva, R., Zimolo, Z., Cooper, T., Perry, E., & Krystal, J. (2008). Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology (New York, N.Y.), 33*(10), 2505-2516. https://doi.org/10.1038/sj.npp.1301643

D'Souza, D. C., Sewell, R. A., & Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: Human studies. *European Archives of Psychiatry and Clinical Neuroscience, 259*(7), 413-431. https://doi.org/10.1007/s00406-009-0024-2

El-Alfy, A. T., Ivey, K., Robinson, K., Ahmed, S., Radwan, M., Slade, D., Khan, I., ElSohly, M., & Ross, S. (2010). Antidepressant-like effect of Δ 9-tetrahydrocannabinol and other cannabinoids isolated from cannabis sativa L. *Pharmacology, Biochemistry and Behavior, 95*(4), 434-442. https://doi.org/10.1016/j.pbb.2010.03.004

Englund, A., Morrison, P. D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., Stone, J. M., Reichenberg, A., Brenneisen, R., Holt, D., Feilding, A., Walker, L., Murray, R. M., & Kapur, S. (2013). Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *Journal of Psychopharmacology, 27*(1), 19-27. https://doi.org/10.1177/0269881112460109

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Cipriano, M., Carratù, M. R., Iuvone, T., & Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PloS One, 6*(12), e28668. https://doi.org/10.1371/journal.pone.0028668

Fadda, P., Robinson, L., Fratta, W., Pertwee, R. G., & Riedel, G. (2004). Differential effects of THC- or CBD-rich cannabis extracts on working memory in rats. *Neuropharmacology, 47*(8), 1170-1179. https://doi.org/10.1016/j.neuropharm.2004.08.009

Farrell, M. R., Holland, F. H., Shansky, R. M., & Brenhouse, H. C. (2016). Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats. *Behavioural Brain Research, 310*, 119-125. https://doi.org/10.1016/j.bbr.2016.05.009

Fernandes, M., Schabarek, A., Coper, H., & Hill, R. (1974). Modification of Δ^9-THC-actions by cannabinol and cannabidiol in the rat. *Psychopharmacologia, 38*(4), 329-338. https://doi.org/10.1007/BF00429130

Finlay, D. B., Sircombe, K. J., Nimick, M., Jones, C., & Glass, M. (2020). Terpenoids from cannabis do not mediate an entourage effect by acting at cannabinoid receptors. *Frontiers in Pharmacology, 11*, 359-359. https://doi.org/10.3389/fphar.2020.00359

Friedman, D., & Devinsky, O. (2015). Cannabinoids in the treatment of epilepsy. *The New England Journal of Medicine, 373*(11), 1048-1058. https://doi.org/10.1056/NEJMra1407304

García-Gutiérrez, M. S., & Manzanares, J. (2011). Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *Journal of Psychopharmacology (Oxford), 25*(1), 111-120. https://doi.org/10.1177/0269881110379507

Gardner, E. L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., & Morrison, D. (1988). Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. *Psychopharmacology, 96*(1), 142-144. https://doi.org/10.1007/BF02431546

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology, 3*(7), 408-414. https://doi.org/10.1038/nchembio.2007.5

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J., Xie, X., Altmann, K., Karsak, M., & Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings*

of the National Academy of Sciences, 105(26), 9099-9104. https://doi.org/10.1073/pnas.0803601105

Goncalves, M. B., Suetterlin, P., Yip, P., Molina-Holgado, F., Walker, D. J., Oudin, M. J., Zentar, M. P., Pollard, S., Yáñez-Muñoz, R. J., Williams, G., Walsh, F. S., Pangalos, M. N., & Doherty, P. (2008). A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. *Molecular and Cellular Neurosciences, 38*(4), 526-536. https://doi.org/10.1016/j.mcn.2008.05.001

Grueter, B. A., Brasnjo, G., & Malenka, R. C. (2010). Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nature Neuroscience, 13*(12), 1519-1525. https://doi.org/10.1038/nn.2685

Guimaraes, F., Chiaretti, T., Graeff, F., & Zuardi, A. (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology, 100*(4), 558-559. https://doi.org/10.1007/BF02244012

Gurgel do Vale, T., Couto Furtado, E., Santos, J. G., & Viana, G. S. B. (2002). Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from lippia alba (mill.) N.E. brown. *Phytomedicine, 9*(8), 709-714. https://doi.org/10.1078/094471102321621304

Gutiérrez-Rodríguez, A., Bonilla-Del Río, I., Puente, N., Gómez-Urquijo, S. M., Fontaine, C. J., Egaña-Huguet, J., Elezgarai, I., Ruehle, S., Lutz, B., Robin, L. M., Soria-Gómez, E., Bellocchio, L., Padwal, J. D., van der Stelt, M., Mendizabal-Zubiaga, J., Reguero, L., Ramos, A., Gerrikagoitia, I., Marsicano, G., & Grandes, P. (2018). Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. *Glia, 66*(7), 1417-1431. https://doi.org/10.1002/glia.23314

Hablitz, L. M., Gunesch, A. N., Cravetchi, O., Moldavan, M., & Allen, C. N. (2020). Cannabinoid signaling recruits astrocytes to modulate presynaptic function in the suprachiasmatic nucleus. *Eneuro, 7*(1), ENEURO.0081-19.2020. https://doi.org/10.1523/ENEURO.0081-19.2020

Hampson, R. E., Heyser, C. J., & Deadwyler, S. A. (1993). Hippocampal cell firing correlates of delayed-match-to-sample performance in the rat. *Behavioral Neuroscience, 107*(5), 715-739. https://doi.org/10.1037/0735-7044.107.5.715

Hampson, R. E., & Deadwyler, S. A. (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *The Journal of Neuroscience, 20*(23), 8932-8942. https://doi.org/10.1523/JNEUROSCI.20-23-08932.2000

Harkany, T., Guzmán, M., Galve-Roperh, I., Berghuis, P., Devi, L. A., & Mackie, K. (2007). The emerging functions of endocannabinoid signaling during CNS

development. *Trends in Pharmacological Sciences, 28*(2), 83-92. https://doi.org/10.1016/j.tips.2006.12.004

Harkany, T., Mackie, K., & Doherty, P. (2008). Wiring and firing neuronal networks: Endocannabinoids take center stage. *Current Opinion in Neurobiology, 18*(3), 338-345. https://doi.org/10.1016/j.conb.2008.08.007

Hart, S., Fischer, O., & Ullrich, A. (2004). Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Research, 64*(6), 1943-1950. https://doi.org/10.1158/0008-5472.CAN-03-3720

Heblinski, M., Santiago, M., Fletcher, C., Stuart, J., Connor, M., McGregor, I. S., & Arnold, J. C. (2020). Terpenoids commonly found in cannabis sativa do not modulate the actions of phytocannabinoids or endocannabinoids on TRPA1 and TRPV1 channels. *Cannabis and Cannabinoid Research, 5*(4), 35-317. https://doi.org/10.1089/can.2019.0099

Hegde, V. L., Hegde, S., Cravatt, B. F., Hofseth, L. J., Nagarkatti, M., & Nagarkatti, P. S. (2008). Attenuation of experimental autoimmune hepatitis by exogenous and endogenous cannabinoids: Involvement of regulatory T cells. *Molecular Pharmacology, 74*(1), 20-33. https://doi.org/10.1124/mol.108.047035

Hehar, H., Yeates, K., Kolb, B., Esser, M. J., & Mychasiuk, R. (2015). Impulsivity and concussion in juvenile rats: Examining molecular and structural aspects of the frontostriatal pathway. PloS One, 10(10), e0139842-e0139842. https://doi.org/10.1371/journal.pone.0139842

Heifets, B. D., & Castillo, P. E. (2009). Endocannabinoid signaling and long-term synaptic plasticity. *Annual Review of Physiology, 71*(1), 283-306. https://doi.org/10.1146/annurev.physiol.010908.163149

Herkenham, M., Groen, B. G., Lynn, A. B., De Costa, B. R., & Richfield, E. K. (1991). Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. *Brain Research, 552*(2), 301.

Hernandez, A. R., Truckenbrod, L. M., Campos, K. T., Williams, S. A., & Burke, S. N. (2020). Sex differences in age-related impairments vary across cognitive and physical assessments in rats. *Behavioral Neuroscience, 134*(2), 69-81. https://doi.org/10.1037/bne0000352

Hill, M. N., McLaughlin, R. J., Morrish, A. C., Viau, V., Floresco, S. B., Hillard, C. J., & Gorzalka, B. B. (2009). Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology (New York, N.Y.), 34*(13), 2733-2745. https://doi.org/10.1038/npp.2009.114

Hill, V. A., Schaffer, M. I., Paulsen, R. B., & Stowe, G. N. (2021). Cannabinoids tetrahydrocannabinol, cannabinol, cannabidiol, tetrahydrocannabivarin and 11-nor-9-carboxy-Δ9-THC in hair. *Journal of Analytical Toxicology*, https://doi.org/10.1093/jat/bkab068

Hillard, C. J., Weinlander, K. M., & Stuhr, K. L. (2012). Contributions of endocannabinoid signaling to psychiatric disorders in humans: Genetic and biochemical evidence. *Neuroscience, 204*, 207-229. https://doi.org/10.1016/j.neuroscience.2011.11.020

Hložek, T., Uttl, L., Kadeřábek, L., Balíková, M., Lhotková, E., Horsley, R. R., Nováková, P., Šíchová, K., Štefková, K., Tylš, F., Kuchař, M., & Páleníček, T. (2017). Pharmacokinetic and behavioural profile of THC, CBD, and THC+CBD combination after pulmonary, oral, and subcutaneous administration in rats and confirmation of conversion in vivo of CBD to THC. *European Neuropsychopharmacology, 27*(12), 1223-1237. https://doi.org/10.1016/j.euroneuro.2017.10.037

Howlett, A. C. (2002). The cannabinoid receptors. *Prostaglandins & Other Lipid Mediators, 68*, 619-631. https://doi.org/10.1016/S0090-6980(02)00060-6

Ilan, A., Gevins, A., Coleman, M., ElSohly, M., & de Wit, H. (2005). Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behavioural Pharmacology, 16*(5-6), 487-497. https://doi.org/10.1097/00008877-200509000-00023

Izzo, A. A., Capasso, R., Aviello, G., Borrelli, F., Romano, B., Piscitelli, F., Gallo, L., Capasso, F., Orlando, P., & Di Marzo, V. (2012). Inhibitory effect of cannabichromene, a major non-psychotropic cannabinoid extracted from cannabis sativa, on inflammation-induced hypermotility in mice. *British Journal of Pharmacology, 166*(4), 1444-1460. https://doi.org/10.1111/j.1476-5381.2012.01879.x

Jiang, W., Zhang, Y., Xiao, L., Van Cleemput, J., Ji, S., Bai, G., & Zhang, X. (2005). Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *The Journal of Clinical Investigation, 115*(11), 3104-3116. https://doi.org/10.1172/JCI25509

Jones, N. A., Glyn, S. E., Akiyama, S., Hill, T. D. M., Hill, A. J., Weston, S. E., Burnett, M. D. A., Yamasaki, Y., Stephens, G. J., Whalley, B. J., & Williams, C. M. (2012). Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure (London, England), 21*(5), 344-352. https://doi.org/10.1016/j.seizure.2012.03.001

Jung, K., Astarita, G., Zhu, C., Wallace, M., Mackie, K., & Piomelli, D. (2007). A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent

endocannabinoid mobilization. *Molecular Pharmacology, 72*(3), 612-621. https://doi.org/10.1124/mol.107.037796

Karl, T., Garner, B., & Cheng, D. (2017). The therapeutic potential of the phytocannabinoid cannabidiol for Alzheimer's disease. *Behavioural Pharmacology, 28*(2 and 3-Spec Issue), 142.

Karniol, I. G., & Carlini, E. A. (1972). The content of Δ9-trans-tetrahydrocannabinol (Δ9-THC) does not explain all biological activity of some Brazilian marihuana samples. *Journal of Pharmacy and Pharmacology, 24*(10), 833–835. https://doi.org/10.1111/j.2042-7158.1972.tb08897.x

Katona, I., & Freund, T. F. (2012). Multiple functions of endocannabinoid signaling in the brain. *Annual Review of Neuroscience, 35*(1), 529-558. https://doi.org/10.1146/annurev-neuro-062111-150420

Klein, T. W., & Cabral, G. A. (2006). Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. *Journal of Neuroimmune Pharmacology, 1*(1), 50-64. doi:10.1007/s11481-005-9007-x

Kolb, B., Gibb, R., & Gorny, G. (2003). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory, 79*(1), 1-10. https://doi.org/10.1016/S1074-7427(02)00021-7

Kovalchuk, O., & Kovalchuk, I. (2020). Cannabinoids as anticancer therapeutic agents. *Cell Cycle (Georgetown, Tex.), 19*(9), 961-989. https://doi.org/10.1080/15384101.2020.1742952

Krebs-Kraft, D. L., Hill, M. N., Hillard, C. J., & McCarthy, M. M. (2010). Sex difference in cell proliferation in developing rat amygdala mediated by endocannabinoids has implications for social behavior. *Proceedings of the National Academy of Sciences, 107*(47), 20535-20540. https://doi.org/10.1073/pnas.1005003107

Kreitzer, A. C., & Regehr, W. G. (2001). Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron (Cambridge, Mass.), 29*(3), 717-727. https://doi.org/10.1016/S0896-6273(01)00246-X

Laux, L. C., Bebin, E. M., Checketts, D., Chez, M., Flamini, R., Marsh, E. D., Miller, I., Nichol, K., Park, Y., Segal, E., Seltzer, L., Szaflarski, J. P., Thiele, E. A., Weinstock, A., on behalf of CBD EAP study group, CBD EAP Study Grp, & CBD EAP study group. (2019). Long-term safety and efficacy of cannabidiol in children and adults with treatment resistant Lennox-Gastaut syndrome or Dravet syndrome: Expanded access program results. *Epilepsy Research, 154*, 13-20. https://doi.org/10.1016/j.eplepsyres.2019.03.015

Liu, J., Wang, L., Harvey-White, J., Huang, B. X., Kim, H., Luquet, S., Palmiter, R. D., Krystal, G., Rai, R., Mahadevan, A., Razdan, R. K., & Kunos, G. (2008). Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology, 54*(1), 1-7. https://doi.org/10.1016/j.neuropharm.2007.05.020

Long, L. E., Lind, J., Webster, M., & Weickert, C. S. (2012). Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. *BMC Neuroscience, 13*(1), 87-87. https://doi.org/10.1186/1471-2202-13-87

Lorenzetti, V., Alonso-Lana, S., Youssef, G. J., Verdejo-Garcia, A., Suo, C., Cousijn, J., Takagi, M., Yucel, M., & Solowij, N. (2016). Adolescent cannabis use: What is the evidence for functional brain alteration? *Current Pharmaceutical Design, 22*(42), 6353-6365. https://doi.org/10.2174/1381612822666160805155922

Loría, F., Petrosino, S., Hernangómez, M., Mestre, L., Spagnolo, A., Correa, F., Di Marzo, V., Docagne, F., & Guaza, C. (2010). An endocannabinoid tone limits excitotoxicity in vitro and in a model of multiple sclerosis. *Neurobiology of Disease, 37*(1), 166-176. https://doi.org/10.1016/j.nbd.2009.09.020

Lovinger, D. M., Gerdeman, G. L., & Ronesi, J. (2002). Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neuroscience, 5*(5), 446-451. https://doi.org/10.1038/nn832

Lowin, T., Schneider, M., & Pongratz, G. (2019). Joints for joints: Cannabinoids in the treatment of rheumatoid arthritis. *Current Opinion in Rheumatology, 31*(3), 271-278. https://doi.org/10.1097/BOR.0000000000000590

Lunn, C., Fine, J., Rojas-Triana, A., Jackson, J., Fan, X., Kung, T., Gonsiorek, W., Schwarz, M., Lavey, B., Kozlowski, J., Narula, S., Lundell, D., Hipkin, R., & Bober, L. (2006). A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment in vivo. *The Journal of Pharmacology and Experimental Therapeutics, 316*(2), 780-788. https://doi.org/10.1124/JPET.105.093500

Maejima, T., Hashimoto, K., Yoshida, T., Aiba, A., & Kano, M. (2001). Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron (Cambridge, Mass.), 31(*3), 463-475. https://doi.org/10.1016/S0896-6273(01)00375-0

Malone, D. T., Jongejan, D., & Taylor, D. A. (2009). Cannabidiol reverses the reduction in social interaction produced by low dose Δ9-tetrahydrocannabinol in rats. *Pharmacology Biochemistry and Behavior, 93*(2), 91–96. https://doi.org/10.1016/j.pbb.2009.04.010

Marinelli, S., Pacioni, S., Bisogno, T., Di Marzo, V., Prince, D. A., Huguenard, J. R., & Bacci, A. (2008). The endocannabinoid 2-arachidonoylglycerol is responsible for

the slow self-inhibition in neocortical interneurons. *The Journal of Neuroscience, 28*(50), 13532-13541. https://doi.org/10.1523/JNEUROSCI.0847-08.2008

Maroon, J., & Bost, J. (2018). Review of the neurological benefits of phytocannabinoids. *Surgical Neurology International, 9*(1), 91-91. https://doi.org/10.4103/sni.sni_45_18

Martín-Moreno, A. M., Reigada, D., Ramírez, B. G., Mechoulam, R., Innamorato, N., Cuadrado, A., & de Ceballos, M. L. (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: Relevance to Alzheimer's Disease. *Molecular Pharmacology, 79*(6), 964.

Mateos, B., Borcel, E., Loriga, R., Luesu, W., Bini, V., Llorente, R., Castelli, M., & Viveros, M. (2011). Adolescent exposure to nicotine and/or the cannabinoid agonist CP 55,940 induces gender-dependent long-lasting memory impairments and changes in brain nicotinic and CB1 cannabinoid receptors. *Journal of Psychopharmacology, 25*(12), 1676-1690. https://doi.org/10.1177/0269881110370503

McAllister, S. D., Murase, R., Christian, R. T., Lau, D., Zielinski, A. J., Allison, J., Almanza, C., Pakdel, A., Lee, J., Limbad, C., Liu, Y., Debs, R. J., Moore, D. H., & Desprez, P. (2011). Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation, invasion, and metastasis. *Breast Cancer Research and Treatment, 129*(1), 37-47. https://doi.org/10.1007/s10549-010-1177-4

McKallip, R., Nagarkatti, M., & Nagarkatti, P. (2005). Delta-9-tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. *The Journal of Immunology, 174*(6), 3281-3289. https://doi.org/10.4049/jimmunol.174.6.3281

Mechoulam, R. (2005). Plant cannabinoids: A neglected pharmacological treasure trove. *British Journal of Pharmacology, 146*(7), 913-915. https://doi.org/10.1038/sj.bjp.0706415

Mechoulam, R., & Parker, L. A. (2013). The endocannabinoid system and the brain. *Annual Review of Psychology, 64*(1), 21-47. https://doi.org/10.1146/annurev-psych-113011-143739

Mechoulam, R., Hanus, L. O., Pertwee, R., & Howlett, A. C. (2014). Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nature Reviews. Neuroscience, 15*(11), 757-764. https://doi.org/10.1038/nrn3811

Meyer, H. C., Lee, F. S., & Gee, D. G. (2018). The role of the endocannabinoid system and genetic variation in adolescent brain development. *Neuropsychopharmacology (New York, N.Y.), 43*(1), 21-33. https://doi.org/10.1038/npp.2017.143

Miller, L. K., & Devi, L. A. (2011). The highs and lows of cannabinoid receptor expression in disease: Mechanisms and their therapeutic implications. *Pharmacological Reviews, 63*(3), 461-470. https://doi.org/10.1124/pr.110.003491

Morell, C., Bort, A., Vara, D., Ramos-Torres, A., Rodriguez-Henche, N., & Diaz-Laviada, I. (2016). The cannabinoid WIN 55,212-2 prevents neuroendocrine differentiation of LNCaP prostate cancer cells. *Prostate Cancer and Prostatic Diseases, 19*(3), 248-257. https://doi.org/10.1038/pcan.2016.19

Morgan, C. J. A., Schafer, G., Freeman, T. P., & Curran, H. V. (2010). Cannabidiol attenuates the appetitive effects of Δ9-tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology, 35*(9), 1879-1885. https://doi.org/10.1038/npp.2010.58

Morgan, C. J. A., Schafer, G., Freeman, T. P., & Curran, H. V. (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: Naturalistic study. *British Journal of Psychiatry, 197*(4), 285-290. https://doi.org/10.1192/bjp.bp.110.077503

Morgan, C. J. A., Freeman, T. P., Hindocha, C., Schafer, G., Gardner, C., & Curran, H. V. (2018). Individual and combined effects of acute delta-9-tetrahydrocannabinol and cannabidiol on psychotomimetic symptoms and memory function. *Translational Psychiatry, 8*(1), 181-10. https://doi.org/10.1038/s41398-018-0191-x

Muller, C., Morales, P., & Reggio, P. H. (2019). Cannabinoid ligands targeting TRP channels. *Frontiers in Molecular Neuroscience, 11*, 487-487. https://doi.org/10.3389/fnmol.2018.00487

Nagarkatti, P., Pandey, R., Rieder, S. A., Hegde, V. L., & Nagarkatti, M. (2009). Cannabinoids as novel anti-inflammatory drugs. *Future Medicinal Chemistry, 1*(7), 1333-1349. https://doi.org/10.4155/FMC.09.93

Navarrete, M., & Araque, A. (2008). Endocannabinoids mediate neuron-astrocyte communication. *Neuron, 57*(6), 883-893. https://doi.org/10.1016/j.neuron.2008.01.029

Navarrete, M., & Araque, A. (2010). Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron, 68*(1), 113-126. https://doi.org/10.1016/j.neuron.2010.08.043

Noyes, J., R, Brunk, S. F., Baram, D. A., & Canter, A. (1975). Analgesic effect of delta-9-tetrahydrocannabinol. *Journal of Clinical Pharmacology, 15*(2-3), 139. Retrieved January 19, 2022, from: https://accp1.onlinelibrary.wiley.com/doi/epdf/10.1002/j.1552-4604.1975.tb02348.x

Nunes, E. D. (2012). United Nations office on drugs and crime (UNODC). Global study on homicide: Trends, context, data. Vienna: UNODC; 2011. *Ciência & Saúde Coletiva, 17*(12), 3447-3449. doi:10.1590/S1413-81232012001200029

Nuutinen, T. (2018). Medicinal properties of terpenes found in cannabis sativa and humulus lupulus. *European Journal of Medicinal Chemistry, 157*, 198-228. https://doi.org/10.1016/j.ejmech.2018.07.076

Oliveira-da-Silva, A., Vieira, F. B., Cristina-Rodrigues, F., Filgueiras, C. C., Manhães, A. C., & Abreu-Villaça, Y. (2009). Increased apoptosis and reduced neuronal and glial densities in the hippocampus due to nicotine and ethanol exposure in adolescent mice. *International Journal of Developmental Neuroscience, 27*(6), 539-548. https://doi.org/10.1016/j.ijdevneu.2009.06.009

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: [delta]9-tetrahydrocannabinol, cannabidiol and [delta]9-tetrahydrocannabivarin. *British Journal of Pharmacology, 153*(2), 199. https://doi.org/10.1038/sj.bjp.0707442

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., Di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB1 and CB2. *Pharmacological Reviews, 62*(4), 588-631. https://doi.org/10.1124/pr.110.003004

Phillips, R. N., Turk, R. F., & Forney, R. B. (1971). Acute toxicity of Δ9-tetrahydrocannabinol in rats and mice 1. *Proceedings of the Society for Experimental Biology and Medicine, 136*(1), 260-263. https://doi.org/10.3181/00379727-136-35241

Porter, A. C., & Felder, C. C. (2001). The endocannabinoid nervous system. *Pharmacology & Therapeutics (Oxford), 90*(1), 45-60. https://doi.org/10.1016/S0163-7258(01)00130-9

Prince, D. A., Bacci, A., & Huguenard, J. R. (2004). Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature, 431*(7006), 312-316. https://doi.org/10.1038/nature02913

Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: A review. *Psychopharmacology, 188*(4), 425-444. https://doi.org/10.1007/s00213-006-0508-y

Raup-Konsavage, W. M., Carkaci-Salli, N., Greenland, K., Gearhart, R., & Vrana, K. E. (2020). Cannabidiol (CBD) oil does not display an entourage effect in reducing

cancer cell viability in vitro. *Medical Cannabis and Cannabinoids, 3*(2), 95-102. https://doi.org/10.1159/000510256

Regehr, W. G., Brown, S. P., & Brenowitz, S. D. (2003). Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. *Nature Neuroscience, 6*(10), 1048-1057. https://doi.org/10.1038/nn1126

Regehr, W. G., Carey, M. R., & Best, A. R. (2009). Activity-dependent regulation of synapses by retrograde messengers. *Neuron, 63*(2), 154-170. https://doi.org/10.1016/j.neuron.2009.06.021

Renard, J., Vitalis, T., Rame, M., Krebs, M., Lenkei, Z., Le Pen, G., & Jay, T. M. (2016). Chronic cannabinoid exposure during adolescence leads to long-term structural and functional changes in the prefrontal cortex. *European Neuropsychopharmacology, 26*(1), 55-64. https://doi.org/10.1016/j.euroneuro.2015.11.005

Riebe, C. J. N., Hill, M. N., Lee, T. T. Y., Hillard, C. J., & Gorzalka, B. B. (2010). Estrogenic regulation of limbic cannabinoid receptor binding. *Psychoneuroendocrinology, 35*(8), 1265-1269. https://doi.org/10.1016/j.psyneuen.2010.02.008

Rieder, S. A., Chauhan, A., Singh, U., Nagarkatti, M., & Nagarkatti, P. (2010). Cannabinoid-induced apoptosis in immune cells as a pathway to immunosuppression. *Immunobiology, 215*(8), 598-605. doi:10.1016/j.imbio.2009.04.001

Romero-Sandoval, E. A., Kolano, A. L., & Alvarado-Vázquez, P. A. (2017). Cannabis and cannabinoids for chronic pain. *Current Rheumatology Reports, 19*(11), 1-10. https://doi.org/10.1007/s11926-017-0693-1

Rubino, T., Vigano', D., Realini, N., Guidali, C., Braida, D., Capurro, V., Castiglioni, C., Cherubino, F., Romualdi, P., Candeletti, S., Sala, M., & Parolaro, D. (2008). Chronic delta(9)-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: Behavioral and biochemical correlates. *Neuropsychopharmacology, 33*(11), 2760-2771. https://doi.org/10.1038/sj.npp.1301664

Rubino, T., Prini, P., Piscitelli, F., Zamberletti, E., Trusel, M., Melis, M., Sagheddu, C., Ligresti, A., Tonini, R., Di Marzo, V., & Parolaro, D. (2015). Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. *Neurobiology of Disease, 73*, 60-69. https://doi.org/10.1016/j.nbd.2014.09.015

Russo, E., & Guy, G. W. (2006). A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Medical Hypotheses, 66*(2), 234-246. https://doi.org/10.1016/j.mehy.2005.08.026

Russo, E. B. (2011). Taming THC: Potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology, 163*(7), 1344-1364. https://doi.org/10.1111/j.1476-5381.2011.01238.x

Santiago, M., Sachdev, S., Arnold, J. C., McGregor, I. S., & Connor, M. (2019). Absence of entourage: Terpenoids commonly found in cannabis sativa do not modulate the functional activity of Δ9-THC at human CB1 and CB2 receptors. *Cannabis and Cannabinoid Research, 4*(3), 165-176. https://doi.org/10.1089/can.2019.0016

Schneider, M. (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addiction Biology, 13*(2), 253-263. https://doi.org/10.1111/j.1369-1600.2008.00110.x

Scorticati, C., Mohn, C., De Laurentiis, A., Vissio, P., Solari, J., Seilicovich, A., McCann, S., & Rettori. (2003). The effect of anandamide on prolactin secretion is modulated by estrogen. *Proceedings of the National Academy of Sciences - PNAS, 100*(4), 2134-2139. https://doi.org/10.1073/pnas.0437924100

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments*, (96), e52434-e52434. https://doi.org/10.3791/52434

Sexton, M., Cudaback, E., Abdullah, R. A., Finnell, J., Mischley, L. K., Rozga, M., Lichtman, A. H., & Stella, N. (2014). Cannabis use by individuals with multiple sclerosis: Effects on specific immune parameters. *Inflammopharmacology, 22*(5), 295-303. https://doi.org/10.1007/s10787-014-0214-z

Slawecki, C. J. (2005). Comparison of anxiety-like behavior in adolescent and adult Sprague-Dawley rats. *Behavioral Neuroscience, 119*(6), 1477-1483. https://doi.org/10.1037/0735-7044.119.6.1477

Squeglia, L. M., Jacobus, J., & Tapert, S. F. (2009). The influence of substance use on adolescent brain development. *Clinical EEG and Neuroscience, 40*(1), 31-38. https://doi.org/10.1177/155005940904000110

Stella, N. (2009). Endocannabinoid signaling in microglial cells. *Neuropharmacology, 56*(Suppl 1), 244-253. https://doi.org/10.1016/j.neuropharm.2008.07.037

Suryavanshi, S., Kovalchuk, I., & Kovalchuk, O. (2021). Cannabinoids as key regulators of inflammasome signaling: A current perspective. *Frontiers in Immunology, 11*, 613613-613613. https://doi.org/10.3389/fimmu.2020.613613

Sutherland, R. J., Whishaw, I. Q., & Kolb, B. (1983). A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. *Behavioural Brain Research, 7*(2), 133-153. https://doi.org/10.1016/0166-4328(83)90188-2

Sutherland, R., Gibb, R., & Kolb, B. (2010). The hippocampus makes a significant contribution to experience-dependent neocortical plasticity. *Behavioural Brain Research, 214*(1), 121-124. https://doi.org/10.1016/j.bbr.2010.05.051

Sylantyev, S., Jensen, T. P., Ross, R. A., & Rusakov, D. A. (2013). Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. Proceedings of the National Academy of Sciences*, 110*(13), 5193-5198. https://doi.org/10.1073/pnas.1211204110

Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., Kita, Y., Hashimoto, K., Shimizu, T., Watanabe, M., Sakimura, K., & Kano, M. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. *Neuron,65*(3), 320-327. https://doi.org/10.1016/j.neuron.2010.01.021

Tagne, A. M., Marino, F., Legnaro, M., Luini, A., Pacchetti, B., & Cosentino, M. (2019). A novel standardized cannabis sativa L. extract and its constituent cannabidiol inhibit human polymorphonuclear leukocyte functions. *International Journal of Molecular Sciences, 20*(8), 1833. https://doi.org/10.3390/ijms20081833

Taffe, M. A., Creehan, K. M., & Vandewater, S. A. (2015). Cannabidiol fails to reverse hypothermia or locomotor suppression induced by Δ9-tetrahydrocannabinol in Sprague-Dawley rats. *British Journal of Pharmacology, 172*(7), 1783-1791. doi:10.1111/bph.13024

Thompson, G. R., Rosenkrantz, H., Schaeppi, U. H., & Braude, M. C. (1973). Comparison of acute oral toxicity of cannabinoids in rats, dogs and monkeys. *Toxicology and Applied Pharmacology, 25*(3), 363. https://doi.org/10.1016/0041-008X(73)90310-4

Tramèr, M. R., Carroll, D., Campbell, F. A., Reynolds, D. J. M., Moore, R. A., & McQuay, H. J. (2001). Cannabinoids for control of chemotherapy induced nausea and vomiting: Quantitative systematic review. *BMJ, 323*(7303), 16-21. https://doi.org/10.1136/bmj.323.7303.16

Tseng, A. H., & Craft, R. M. (2001). Sex differences in antinociceptive and motoric effects of cannabinoids. *European Journal of Pharmacology, 430*(1), 41-47. https://doi.org/10.1016/S0014-2999(01)01267-5

Turcotte, C., Blanchet, M., Laviolette, M., & Flamand, N. (2016). The CB2 receptor and its role as a regulator of inflammation. *Cellular and Molecular Life Sciences, 73*(23), 4449. doi: https://doi-org.ezproxy.uleth.ca/10.1007/s00018-016-2300-4

Turner, K. M., & Burne, T. H. J. (2014). Comprehensive behavioural analysis of Long Evans and Sprague-Dawley rats reveals differential effects of housing conditions

on tests relevant to neuropsychiatric disorders. *PloS One, 9*(3), e93411. https://doi.org/10.1371/journal.pone.0093411

van der Stelt, M., Trevisani, M., Vellani, V., De Petrocellis, L., Schiano Moriello, A., Campi, B., McNaughton, P., Geppetti, P., & Di Marzo, V. (2005). Anandamide acts as an intracellular messenger amplifying Ca2+ influx via TRPV1 channels. *The EMBO Journal, 24*(19), 3517-3518. https://doi.org/10.1038/sj.emboj.7600839

Vandrey, R., Dunn, K. E., Fry, J. A., & Girling, E. R. (2012). A survey study to characterize use of spice products (synthetic cannabinoids). *Drug and Alcohol Dependence, 120*(1), 238-241. https://doi.org/10.1016/j.drugalcdep.2011.07.011

Vann, R. E., Gamage, T. F., Warner, J. A., Marshall, E. M., Taylor, N. L., Martin, B. R., & Wiley, J. L. (2008). Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of Δ9 -tetrahydrocannabinol. *Drug and Alcohol Dependence, 94*(1), 191-198. doi:10.1016/j.drugalcdep.2007.11.017

VanRyzin, J. W., Marquardt, A. E., Argue, K. J., Vecchiarelli, H. A., Ashton, S. E., Arambula, S. E., Hill, M. N., & McCarthy, M. M. (2019). Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. *Neuron, 102*(2), 435-449.e6. https://doi.org/10.1016/j.neuron.2019.02.006

Varvel, S. A., Wiley, J. L., Yang, R., Bridgen, D. T., Long, K., Lichtman, A. H., & Martin, B. R. (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology, 186*(2), 226-234. https://doi.org/10.1007/s00213-006-0356-9

Velasco, G., Sánchez, C., & Guzmán, M. (2016). Anticancer mechanisms of cannabinoids. *Current Oncology, 23*(2), 23-S32. doi:10.3747/co.23.3080. https://doi.org/10.3747/co.23.3080

Wang, B., Kovalchuk, A., Li, D., Rodriguez-Juarez, R., Ilnytskyy, Y., Kovalchuk, I., & Kovalchuk, O. (2020). In search of preventive strategies: Novel high-CBD cannabis sativa extracts modulate ACE2 expression in COVID-19 gateway tissues. *Aging (Albany, NY.), 12*(22), 22425-22444.

Ware, M. A., Wang, T., Shapiro, S., Robinson, A., Ducruet, T., Huynh, T., Gamsa, A., Bennett, G. J., & Collet, J. (2010). Smoked cannabis for chronic neuropathic pain: A randomized controlled trial. *Canadian Medical Association Journal, 182*(14), E694-E701. https://doi.org/10.1503/cmaj.091414

Watt, G., & Karl, T. (2017). In vivo evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's Disease. *Frontiers in Pharmacology, 8*, 20-20. https://doi.org/10.3389/fphar.2017.00020

Wenk, G. L., McGann, K., Mencarelli, A., Hauss-Wegrzyniak, B., Del Soldato, P., & Fiorucci, S. (2000). Mechanisms to prevent the toxicity of chronic neuroinflammation on forebrain cholinergic neurons. *European Journal of Pharmacology, 402*(1), 77-85. https://doi.org/10.1016/S0014-2999(00)00523-9

Wolf, C., Hotchkiss, A., Ostby, J., LeBlanc, G., & Gray, L. (2002). Effects of prenatal testosterone propionate on the sexual development of male and female rats: A dose-response study. *Toxicological Sciences, 65*(1), 71-86. https://doi.org/10.1093/toxsci/65.1.71

Wolf, S. A., Bick-Sander, A., Fabel, K., Leal-Galicia, P., Tauber, S., Ramirez-Rodriguez, G., Mueller, A., Melnik, A., Waltinger, T. P., Ullrich, O., & Kempermann, G. (2010). Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Communication and Signaling, 8*(1), 12-12. https://doi.org/10.1186/1478-811X-8-12

Zajicek, J. P., Hobart, J. C., Slade, A., Barnes, D., Mattison, P. G., MUSEC Res Grp, MUSEC Research Group, & on behalf of the MUSEC Research Group. (2012). MUltiple sclerosis and extract of cannabis: Results of the MUSEC trial. *Journal of Neurology, Neurosurgery and Psychiatry, 83*(11), 1125-1132. https://doi.org/10.1136/jnnp-2012-302468

Zhu, P. J. (2006). Endocannabinoid signaling and synaptic plasticity in the brain. *Critical Reviews in Neurobiology, 18*(1-2), 113. doi: 10.1615/CritRevNeurobiol.v18.i1-2.120

Zuardi, A., Rodrigues, J., & Cunha, J. (1991). Effects of cannabidiol in animal-models predictive of antipsychotic activity. *Psychopharmacology, 104*(2), 260-264. https://doi.org/10.1007/BF0224418

Zuardi, A. W., Hallak, J. E. C., & Crippa, J. A. S. (2012). Interaction between cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC): Influence of administration interval and dose ratio between the cannabinoids. *Psychopharmacology (Berlin, Germany), 219*(1), 247-249. https://doi.org/10.1007/s00213-011-2495-x