CANNABIS AND THE BRAIN: INVESTIGATING THE LONG-TERM EFFECTS OF HIGH-CBD CANNABIS EXPOSURE ON BEHAVIOUR IN THE LONG-EVANS RAT

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For my family.

ABSTRACT

Cannabis, a drug derived from the plant *Cannabis Sativa,* is one of the most commonly used drugs worldwide, with a rich history of medicinal application. The increased use of cannabis in recent history has led to a rapid diversification of cannabis strains, each with unique levels of major cannabinoid constituents including tetrahydrocannabinol (THC), the main psychoactive component of cannabis, and cannabidiol (CBD). As social and legislative attitudes towards cannabis begin to change, more people are seeking out this drug for its purported health benefits, many of which are thought to be attributable to CBD. Using four novel high-CBD, low-THC extracts, this research seeks to explore the long-term effects of chronic cannabis exposure on behaviour in a rodent model. Results from this study demonstrate that there are few long-term effects of chronic, high-CBD cannabis exposure on behaviour and that the changes that were observed were not of detriment.

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LIST OF ABREVIATIONS

2-Ag	2-arachidionyl glycerol
5-HT	Serotonin
5-HT1	Serotonin Receptor Type 1
5-HT3a	Serotonin Receptor Type 3
ACTH	Adrenocorticotropin
AEA	Anandamide
AID	Agranular Insular Cortex
AMP	Adenosine Monophosphate
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BCE	Before Common Era
CA1	Hippocampal Subfield 1
CB1	Cannabinoids Receptor Type 1
CB2	Cannabinoid Receptor Type 2
CBC	Cannabichromene
CBD	Cannabidiol
CBDV	Cannabidivarin
CBE	Cannabielsoin
CBG	Cannabigerol
CBL	Cannabicyclol
CBN	Cannabinol
CBND	Cannainodiol
CBT	Cannabitriol
CHR	Corticotropin-Releasing Hormone
Cg3	Cingulate Cortex
CNS	Central Nervous System
DMTP	Delayed-Matching-to-Place
FAAH	Fatty Acid Amide Hydrolase
GABA	Gamma-Aminobutyric Acid
GPRC	G-Protein Coupled Receptors
HPA Axis	Hypothalamic Adrenal Pituitary Axis
HPC	Hippocampus
LTD	Long Term Depression
MAGL	Monoacylglycerol Lipase
mTORC1	Mammalian Target of Rapamycin Complex 1
MWT	Morris Water Task
NMDA	N-Methyl-D-aspartic acid
PFC	Prefrontal Cortex
THC	Tetrahydrocannabinol
THCV	Tetrahydrocannabivarin
TRP	Transient Receptor Potential Channels
TRPV1	Transient Vanilloid Receptor 1
VR1	Vanilloid Receptor type 1

Chapter 1

General Introduction

A Brief History of Medicinal Cannabis Use

Cannabis Sativa is one of the oldest cultivated plants in human history. This versatile plant has been utilized by different cultures around the world for millennia to make textiles, for food and medicine, and for use in religious and cultural ceremonies. Some of the earliest records of the use of the cannabis plant date back to 4000 BCE in China where the fibrous stalks of the plants, called hemp, were likely cultivated for use in textile manufacturing (Li, 1974). In fact, the cultivation of cannabis was so ubiquitous in ancient China that it is identified in text as one of the "five grains" along with millet, barley, soybeans, and rice (Li 1974). Historians have found reference to the "medicinal" properties of cannabis mentioned in the *Pên-Ts'ao Ching*, the world's oldest pharmacopoeia written in the first or second century C.E., which describes the hallucinogenic properties of cannabis (Li, 1974). In this interpretation, the ancient Chinese text suggests that it was the hallucinogenic properties of cannabis that were desired for medicinal use. Indeed, accounts of cannabis being mixed with wine to be used as anaesthetic during surgery would suggest just that (Li, 1974). I highlight the word medicinal above to draw attention to the observation that, in current medical practise, the hallucinogenic or mind-altering effect of cannabis is not often the primary effect sought by those using medical cannabis.

Moving westward, cannabis was also cultivated for use in ancient Egypt, a society historically recognized for its advanced medical systems (Russo, 2007). Preparation

instructions for the "treatment for the eyes" including cannabis and celery are detailed on medical papyri dating back to 1700 BCE (Russo, 2007). Russo, (2007) provides a detailed exploration of historical references to medical cannabis and draws an interesting comparison between this preparation of cannabis and current treatments for glaucoma. Records of cannabis being used as a therapeutic agent arise in Assyria, as well as in Greek and Roman texts as the cultivation and trade of cannabis expanded from Asia to Europe during the bronze age (Li, 1974). Cannabis is thought to have been introduced to the practice of Western medicine by Irish physician William O'Shaughnessy, who suggested use of the drug in the treatment of a variety of conditions including rheumatism, tinnitus, and cholera (O'Shaughnessy, 1840).

By the beginning of the 20th century, the use of medicinal cannabis was beginning to wane. In reflection, Zuardi (2006) posits this may have been due to the difficulty in producing replicable results as there was no means of controlling the levels of the different constituents of cannabis which we now know to be driving the desired effects, and the efficacy of the drug would depend on its origins and method of preparation. Around this time, medical advancements were also leading to the development of novel drugs and therapeutics capable of treating health issues with a greater precision and higher efficacy (Health Canada, 1972). It is also possible that colonial attitudes towards non-Western medicine influenced this shift as the beginning of the 20th century saw an influx of immigrants to Europe and the Americas swiftly followed by a skepticism of Eastern medicine (Palma, 2018). Public perception of drug use was also changing during this time and cannabis use was increasingly being associated with crime and other ani-social behaviours (Ko et al., 2016). These factors were quickly consolidated as the

Canadian government made cannabis use illegal in 1923 as part of an Act to Prohibit the Improper Use of Opium and other Drugs, (cannabis being one of the other drugs, along with heroin, and codeine).

Beginning in the 1960's recreational cannabis use was on the rise, holding importance in many social groups mostly driven by desire for its hallucinogenic and hedonistic properties. This increase in the social relevance of the drug drove scientific exploration specifically into the constituents of the drug and their potential mechanisms of action in the body. In 1964, Gaoni and Mechoulam isolated what they called the active constituent of cannabis, Δ^9-tetrahydrocannabinol (THC) for the first time, as well as another primary cannabinoid, cannabidiol (CBD), although the structure of this molecule appears to have been elucidated decades earlier by Adams et al., (1940). These discoveries made it possible for researchers to then begin to quantify the effects of the drug as well as to begin to look for a mechanism of action.

The Endocannabinoid System

Cannabinoid Receptors

In 1988, Devane and colleagues described high affinity binding sites for cannabinoids in the brain, which appeared to have particular affinity for THC. Building on this work, Matsuda and colleagues (1990) were able to clone these receptors, which were found throughout the central nervous system (CNS). They also concurred that these receptors appeared to have a stronger activity when binding with psychoactive constituents of cannabis (e.g. THC) than non-psychoactive constituents of cannabis (e.g. CBD). The discovery of this receptor, now known as the cannabinoid receptor type 1

(CB1) provided the first insight into a mechanism of action of cannabis in the brain and a likely source of the psychoactive effects of cannabis. These CB1 receptors are one of the most abundant G-protein-coupled receptors (GPCRs) in the CNS and are found in high density in the basal ganglia, hippocampus, and frontal cortex (Herkenham et al., 1991). Considering the number of non-psychoactive effects of cannabis, researchers began searching for additional mechanisms outside the CNS.

In 1993, Munro and his team isolated and cloned additional cannabinoid receptors located in the periphery, what we now call the cannabinoid receptor type 2 (CB2). These receptors are found throughout the body including in the heart, lung, pancreas, gonadal tissue, and spleen, and are believed to regulate immune function (Galiègue et al., 1995). Like CB1 receptors, CB2 receptors are also GPCRs which modulate the activity of adenylate cyclase, and ion channels including calcium channels, potassium channels, and NMDA associated ion channels (Devane et al., 1988; McAllister & Glass, 2002; Mackie & Hille., 1992; Mackie et al., 1995). Alteration in ion channel function has direct implications on brain function and resulting behaviour. For example, activation of CB1 receptors in hippocampal pyramidal neurons leads to inhibition of calcium influx through NMDA receptors which may be one mechanism by which cannabinoids influence learning and memory processes (Misner & Sullivan 1999).

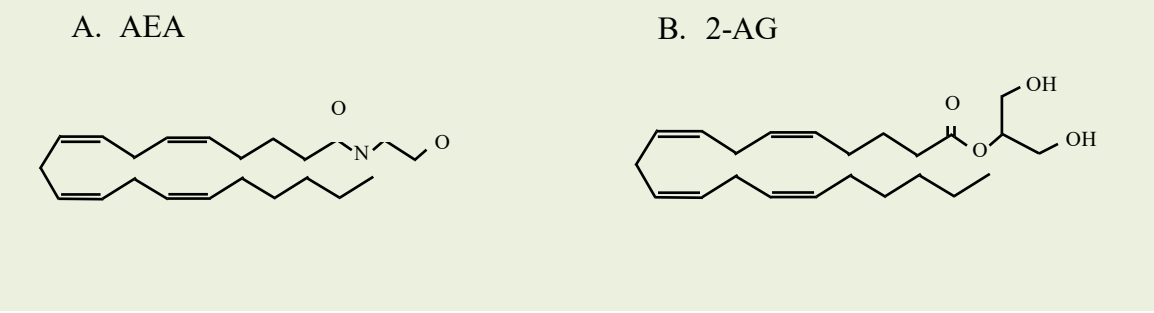
Finally, the cannabinoid system also exerts effects through transient receptor potential channels (TRPs). These receptors, of which there are six subtypes known to be activated by cannabinoids, are pain receptors which are widely expressed in the periphery as well as in the hippocampus (Zygmunt et al., 1999; Muller et al., 2019; Mezey et al., 2000). This mechanism may provide a potential avenue to explore the analgesic and anti-

inflammatory properties of cannabinoids. Indeed, it has been suggested that exposure to cannabinoids can desensitize certain TRP receptors which may be implicated in conditions of chronic pain (De Petrocellis et al., 2011).

Endogenous Cannabinoids

Following the discovery of cannabinoid receptors in the brain, researchers began to explore the possibility of an endogenous system which might utilize these receptors to mediate cognitive function. Indeed, this was the thought process of Devane and colleagues (1992) who identified a ligand capable of displacing a radiolabelled cannabinoid probe, which they called anandamide (AEA). This ligand was shown to have a similar binding affinity to THC and targeted CB1 receptors in the brain. Similarly, research exploring the potential of an endogenous ligand affiliated with the CB2 receptor identified the ligand 2-arachidionyl glycerol (2-AG) located both in the periphery (Mechoulam et al., 1995) as well as in the brain (Sugiura et al., 1995). These two endogenous ligands, AEA, and 2-AG are the primary drivers of endocannabinoid mediated activity in the brain (Fig. 1.1).

Fig. 1.1
Structure of Primary Endogenous Cannabinoids



Note. Figure 1.1A is a structural representation of the endogenous cannabinoid, anandamide (AEA). Figure 1.1B is a structural representation of the endogenous cannabinoid, 2-arachidionyl glycerol (2-AG).

AEA is broken down by the enzyme fatty acid amide hydrolase (FAAH), which is ubiquitous in the brain, and is present in high levels in the aforementioned brain areas which utilize cannabinoid signalling, like the basal ganglia (Tsou et al., 1998). 2-AG is broken down by several enzymes, the primary being monoacylglycerol lipase (MAGL) (Blankman et al., 2007). Knockout models of these two enzymes result in increased concentrations of endogenous cannabinoids AEA and 2-AG in the brain respectively (for a review see Hillard, 2015).

Function of the Endocannabinoid System

There are a couple of features of CB1 receptors which can help us begin to understand the function of the endocannabinoid system in the brain. First is the distribution of cannabinoid receptors throughout the brain. As mentioned previously, there are dense collections of CB1 receptors present in specific areas of the brain including the basal ganglia, the hippocampus, and the cortex, primarily the frontal and temporal cortex. Each of these areas is responsible for specialized control of cognitive function and behaviour and, therefore, the likelihood of some involvement of the endocannabinoid system in the proper functioning of these areas is high. For example, cannabinoids are known to cause changes in motor behaviour, which is largely controlled by the basal ganglia. Both AEA and 2-AG exert a primarily inhibitory influence driven by CB1 receptor activation, primarily in the globus pallidus and substantial nigra, areas of the basal ganglia key for motor control (Di Marzo et al., 2000; Fernández-Ruiz et al., 2002). Endocannabinoids also target the dense collection of CB1 receptors in the hippocampus to mediate plasticity in hippocampal circuits and are critical in memory

processes (Carlson et al., 2002; Chevaleyre & Castillo, 2004). Another method of inferring the involvement of the endocannabinoid system in specialized brain areas is to explore dysfunction. Increased density of CB1 receptors has been demonstrated in the prefrontal cortex of patients with schizophrenia (Dean et al., 2001), as have elevated levels of endogenous cannabinoids in the cerebro-spinal fluid (Leweke et al., 1999). The involvement of the endocannabinoid system in each of the behavioural assays explored in this thesis will be discussed in greater detail in the relevant chapters.

The second observation which gives insight as to the function of the endocannabinoid system in the brain, is the location of the receptors on the neurons themselves. Conventionally, neurotransmitters are released from the synapse when the neuron is depolarized, cross over the synaptic cleft, and affect the post-synaptic neuron in the manner appropriate to the cell type and neurotransmitter released. Endocannabinoids, however, are released from the post-synaptic neuron and travel in a retrograde fashion across the synaptic cleft to the pre-synaptic site where they bind with CB1 receptors to influence neurotransmitter release (Devane & Axelrod, 1994). In fact, endocannabinoids are the primary retrograde neurotransmitter in the brain (Südhof & Malenka, 2008). The discovery of the retrograde action of endocannabinoids came after researchers observed a phenomenon called depolarization-induced suppression inhibition or DSI (Wilson & Nicoll, 2002). Pitler and Alger (1992) observed that a depolarizing stimulus applied to a hippocampal neuron resulted in the suppression of GABA, the brain's primary inhibitory neurotransmitter. The researchers postulated, in agreeance with Llano, Lenresche and Marty (1991) who suggested a similar mechanism in their investigation of cerebellar Purkinje cells, that this effect may be due to "a diffusible second messenger that escapes

from the post-synaptic terminals". We now know those diffusible second messengers to be endocannabinoids. Unlike other classical neurotransmitters, endocannabinoids are typically generated on demand in the post-synaptic site by triggers like depolarization, NMDA receptor activation, or activation of metabotropic glutamate receptors, all of which trigger calcium influx into the post-synaptic cell (Di Marzo et al 1994, Stella et al, 1997; Stella & Piomelli, 2001). This influx in calcium triggers the synthesis of endocannabinoids from lipid precursors, which then move across the synaptic cleft and bind pre-synaptically to CB1 receptors. As was mentioned, the CB1 receptor is a GPCR and its activation triggers two potential mechanisms, either increasing potassium influx into the cell causing hyperpolarization, or inhibiting voltage dependant calcium channels (Hillard, 2015). Both potential pathways result in a decreased likelihood of neurotransmitter release from the pre-synaptic site. These neuronal pathways are thought to be different from the activity of cannabinoids in other cell populations where the CB1 receptor is associated with inhibition of the production of cyclic AMP (Adel & Alexander., 2021, pp. 18). Put plainly, endogenous cannabinoids like AEA and 2-AG, are retrograde neurotransmitters which bind pre-synaptically and act to decrease the likelihood of neurotransmitter release in the brain.

Exogenous Cannabinoids

Constituents of Cannabis

Cannabis sativa contains over 550 chemical compounds (Rock & Parker, 2021). Over 100 of these compounds are identified phytocannabinoids, and according to El Sholy & Gull (2012, pp. 4), can be categorized into 11 types: THC (18 compounds), Δ^8-

THC (2 compounds), cannabichromene (CBC; 8 compounds), CBD (8 compounds), cannabielsoin (CBE; 5 compounds), cannabigerol (CBG; 17 compounds), cannabicyclol (CBL; 8 compounds), cannabinol (CBN; 10 compounds), cannainodiol (CBND; 2 compounds), cannabitriol (CBT; 9 compounds), and miscellaneous cannabinoids (22 compounds). Two of these compounds are of particular interest in this analysis and have been previously introduced, THC and CBD.

As mentioned, the structure of THC was first elucidated in 1964 by Gaoni and Mechoulam (Fig. 1.2A). As the primary psychoactive component of cannabis, THC is responsible for many of the characteristic effects of cannabis consumption including euphoria and relaxation, however THC is also responsible for the hallucinogenic effects of cannabis and has been linked to psychosis. THC acts as a partial agonist at both CB1 and CB2 receptors which is understood to be its main mechanism of action in the brain. Again, the distribution of endocannabinoid receptors in the brain lends weight to this theory, as receptors are densely packed in areas involved in emotional processing (amygdala), learning and memory (hippocampus), and parts of the reward network (striatum, PFC) all of which are functions or processes influenced by THC exposure.

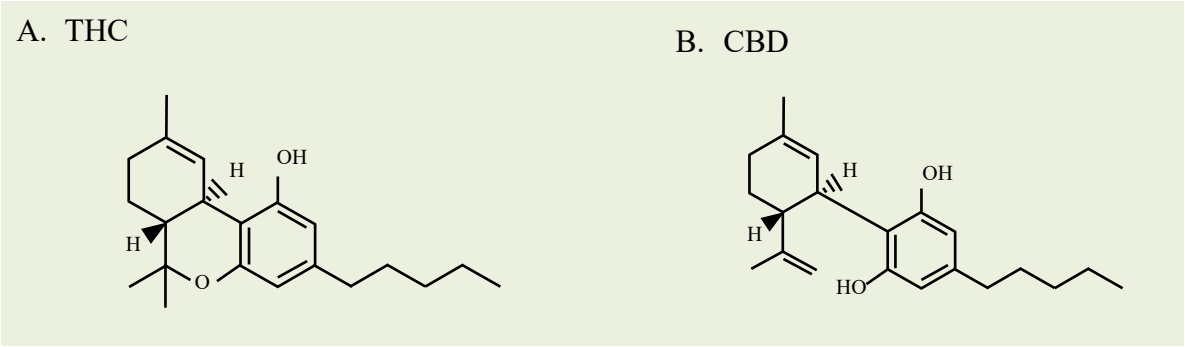
The structure of CBD was first reported by Adams et al., in 1940 (Fig. 1.2B). Unlike THC, CBD does not have a psychomimetic profile and does not appear to have the same potential for abuse (Schoedel et al., 2018). On the contrary, CBD appears to possess many of the health benefits people seek from cannabis, including apparent anti-inflammatory and neuroprotective properties (for a review see Maroon & Bost 2018). CBD has a broad number of complex mechanisms of action in the brain. In fact, a non-comprehensive list compiled by Dos Santos et al., (2021, pp.33), lists 33 targets of CBD

activity, many of which are poorly understood or just beginning to be studied. First, let us review its action at the primary cannabinoid receptors we have discussed thus far. At the CB1 receptor, CBD is considered a non-competitive negative allosteric modulator (Laprairie et al., 2015), which means that CBD binds to a distinct site on the CB1 receptor, different from that of endocannabinoids and THC, and influences the efficacy of those other cannabinoids at the receptor. At the CB2 receptor, CBD acts as an inverse agonist by invoking the opposite effect of endogenous cannabinoids and inhibiting immune cells, a potential mechanism of the anti-inflammatory effects of CBD (Pertwee 2008). CBD has also been shown to interact with the endocannabinoid system by modulating levels of anandamide in the brain through the inhibition of FAAH (Bisogno et al., 2001). CBD also interacts with other neurotransmitter systems like the serotonergic system, both by activating serotonergic receptors (5-HT receptors) and by inhibiting serotonin re-uptake, and the dopaminergic system by inhibiting uptake of dopamine (for a review see Campos et al., 2017).

A final phytocannabinoid of note which will prove relevant to the following experiment, is tetrahydrocannabivarin (THCV), an analogue of THC. This molecule, discovered by Gill and colleagues in 1970, is able to illicit THC-like effects by acting at the CB1 receptor site (Pertwee, 2008). Interestingly however, this cannabinoid can serve as both an agonist and antagonist at the site as it has been shown to inhibit the activation of the CB1 receptor *in vivo* at doses lower than 3 mg/kg and act as a CB1 agonist at doses higher than 10 mg/kg (Thomas et al., 2005), as well as blocking activity of the receptor *in vitro* (Ma et al., 2008). THCV also binds at the CB2 receptor where, *in vitro*, it acts as a partial agonist (Thomas et al., 2005). These apparently contrasting effects highlight the

necessity of testing *in vivo*, as *in vitro* pharmacology might may not be providing a complete picture of how cannabinoids will behave in a living system.

Figure 1.2
Structure of Primary Exogenous Cannabinoids



Note. Figure 1.2A is a structural representation of the exogenous cannabinoid, Δ^9-tetrahydrocannabinol (THC). Figure 1.2B is a structural representation of the exogenous cannabinoid, cannabidiol (CBD).

A Brief Introduction to the Effects of Exogenous Cannabinoids on Behaviour

Peak concentration of THC in the body is reached minutes after exposure and peak intoxication in humans is reached approximately 30 minutes after exposure (Grotenhermen, 2003). In rats exposed to an inhalation model of administration, maximum THC concentration was observed within 10 minutes of them being removed from the administration chamber after having been exposed for 50 minutes and had a half-life of 3.7 hours (Ravula et al., 2018). Other research has suggested that maximum plasma THC concentration is reached 30 minutes following a single exposure to cannabis inhalation in chronic users (Lee et al., 2015). Beyond THC, a number of other cannabinoids including CBD (120 mg/kg), CBDV(60 mg/kg), CBG(120 mg/kg) and Δ^9-THCV (30 mg/kg) were not detectable in plasma or brain tissue after 24 hours (Dieana et

al., 2012). In humans, the half-life of cannabinoids following chronic use is noticeably longer. The half-life of CBD following chronic oral administration ranges from 2-5 days compared to a half-life in the order of hours following single dose administration (Consroe et al., 1991; for a review see Millar et al., 2018). No such data appear to be available in rodent models. Presence of THC and its metabolites can be measured in the plasma and tissue of chronic users for up to several weeks depending on administration rate and concentration (for a review see Grotenhermen, 2003).

The behavioural effects of cannabis exposure are most commonly assessed using a collection of well-validated tests called the tetrad assay (Martin et al. 1991). This collection of tests examines what are thought to be hallmark behavioural indications of cannabinoid activity including hypomotility (locomotor suppression), antinociception, catalepsy and hypothermia. These effects are typically observed following acute exposure and are CB1 receptor dependant. As such, these outcomes are most reliably produced by THC, whereas the acute effects on behaviour of cannabinoids with primary mechanisms of action in the brain separate from the CB1 receptor, can be more variable. Chronic repeated exposure to cannabinoids can manifest slightly differently than a simple one-time exposure. Long and colleagues (2010) attempted to address these differences and observed that both acute (at doses of 10 mg/kg) and chronic (21 days; at doses of one, three and 10 mg/kg) exposure to THC decreased locomotor activity. Contrastingly, only acute exposure to THC elicited hypothermia, and catalepsy. Acute CBD exposure (one or 10 mg/kg) however, had no effect on locomotor activity, catalepsy or nociception and only slight hypothermia suggesting that acute exposure to CBD does not elicit the same changes in behaviour that follow acute THC exposure. Research exploring the long-term

effects of cannabinoid exposure on these behaviours, particularly after a wash-out period during which the drug is no longer being administered, is much less prevalent and will be the primary goal of this thesis. An outline of the acute and long-term effects of the primary cannabinoids of interest, THC and CBD, will be discussed as they pertain to each behavioural assay in the relevant chapter.

Interaction Effects

A crucial piece of this thesis is that all experimentation was conducted using whole plant cannabis extracts. Following the isolation of THC and CBD, much of the research that was conducted assessed the individual effects of these cannabinoids in the brain and on behaviour. However, it is known that administration of these cannabinoids together alters their activity, giving rise to interaction effects. These effects are exceedingly complex and depend on a number of factors including whether THC and CBD are administered together or if their administration is staggered and, if they are staggered, the delay between administrations, the concentrations administered, and so on. For example, Britch et al. (2017) found that pre-treatment with CBD enhanced a THC-induced analgesic effect in rats, but when CBD was administered concurrently with THC, there were no potentiating effects. Some research also suggests that THCV acts as a competitive inhibitory, blocking THC from binding at the CB1 receptor site, which may contribute to the inhibition of some behavioural effects of THC when co-administered with other cannabinoids (Pertwee et al., 2007). In addition to considering interactions between major cannabinoids, these extracts have unique profiles of terpenoids and flavonoids, which are naturally occurring molecules that give plants their taste and smell, and can provide pharmacological effects. In fact, aromatic terpenes in cannabis have been

shown to possess anti-inflammatory action of their own (Gallily et al., 2018). These effects will be taken into consideration in the following analyses.

Withdrawal Effects

Withdrawal symptoms occur during abstinence from drug use, cannabis included. For a long period of time, it was thought that there were no symptoms of cannabis withdrawal, which is a notion that is now being refuted (for a review see Budney et al., 2004). Symptoms of withdrawal can occur as early as 24-48 hours after drug consumption has ceased and linger for up to two weeks depending on the dose and duration of exposure (Budney et al., 2004). In rodent models, precipitated withdrawal experiments, which typically utilize a cannabinoid antagonist like SR141716A to immediately block cannabinoid activity, produce the most consistent withdrawal effects including increased grooming, head shakes, paw shakes, and increased horizontal activity (Tsou et al.,1995; Diana et al., 1998). In contrast to precipitated withdrawal, reports of abrupt cannabis withdrawal symptoms, where administration of the drug is stopped to manifest withdrawal, are more inconsistent and are dependent on the species, and dosing/testing paradigm being used (González et al., 2005). The trend in rat studies appears to suggest that following the administration of chronic THC, there are few behavioural signs of withdrawal (Leite & Carlini, 1974; Diana et al., 1998). However, Wiley and colleagues (2007) did observe a significant reduction in the locomotor activity of rats 24-hours after 10 days of THC exposure suggesting the trend may not always hold. Interestingly, there may be a sex difference in the effects of cannabis on withdrawal symptoms as Harte-Hargrove and Dow-Edwards (2010) observed that only female rats

showed a decrease in locomotor activity the day following the cessation of THC administration; there was no effect of abrupt withdrawal on male animals.

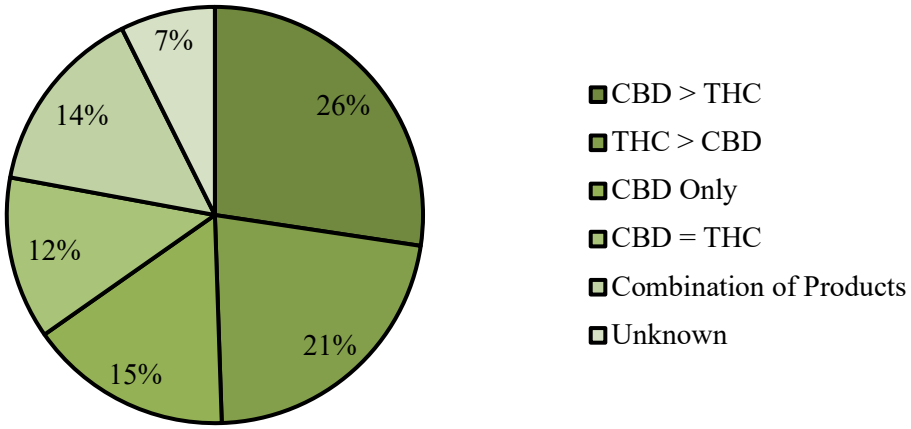
Relevance of the Research and Thesis Objectives

While it is by no means a novel use of cannabis, research into medicinal applications of the drug in western medicine has broadly expanded over the past number of decades as more countries move to decriminalize and legalize its use. Cannabis has been shown to possess a number of desirable health effects including serving as an analgesic (Rog et al., 2005; Hill et al., 2017), anti-inflammatory agent (Nagarkatti, 2009; Namdar & Hinanit, 2018) and antioxidant (Girgih et al., 2011). Due to these and other neuroprotective properties, cannabis is beginning to be utilized in clinical settings for the treatment of a number of diseases and disorders. For example, cannabis treatment has been shown to lessen the damage in the brain following stroke (Ceprián et al., 2017; Lafuente et al., 2011; Hillard, 2008). Cannabis has also been used to supplement medical treatment for inflammatory bowel disease (Ahmed & Katz, 2016), has been reported to lessen symptoms of multiple sclerosis (Consroe et al., 1997; Rog et al., 2005) and improve quality of life for cancer patients (Strasser et al., 2006) as cannabinoids appears to possess broad anti-cancer properties (Kovalchuk & Kovalchuk, 2021).

Considering the apparent potential of CBD to serve a therapeutic role in medical practise, the rise in discourse surrounding medicinal cannabis use is apt. As of March 2021, approximately 332,000 people in Canada were registered with federal health services to use cannabis medicinally (Health Canada, 2021). This number has dropped from 372,00 people in October 2018, just prior to the federal legalization of cannabis in

Canada, likely indicating a rise in people self-medicating with cannabis. Indeed in 2020, 76% of respondents to the Canadian Cannabis Survey (2020) who identified using cannabis for medical purposes, did so without any form of documentation from a healthcare professional. It is also evident that a great number of Canadians using medical cannabis are using products containing relatively high levels of CBD compared to THC to address their health concerns (Fig 1.3), and it would appear this is being done with a certain level of perceived success, as 57% of medical users surveyed reported that using cannabis allowed them to decrease use of other medications.

Figure 1.3
Relative Levels of Major Cannabinoids in Medical Purpose Cannabis in Canada (Health Canada, 2020)



Note. This figure represents the relative levels of major cannabinoid constituents in cannabis products used by medical cannabis users in Canada (Health Canada, 2021).

It is important to remember that the consideration of cannabis as a viable treatment option, and as a supplement to or replacement for traditional medication, must be two-fold. First, cannabis treatment needs to be effective in treating the targeted medical condition. Second, the cannabis treatment should not generate negative side

effects. The cure should not be worse than the disease. For all the social, academic, and scientific interest in pursuing an understanding of medical cannabis, there remains a lack of research into many aspects of this drug including nuances of its effects, its mechanisms, and its applications. Cannabis research is multi-faceted with any number of changeable variables, which highlights the need for research into all aspects of medicinal cannabis treatment: an understanding of both acute and long-term effects, how these effects differ following acute compared to chronic treatment, how different levels of cannabinoids interact and how those interactions may affect the efficacy of the drug or result in changes in behaviour, etc. This research sought to address a part of that complex undertaking.

The objective of this thesis was to explore the long-term effects of chronic, high-CBD cannabis exposure in a rodent model. This foundational research will serve to better inform our understanding of the effects of cannabis on the brain and on behaviour. The extracts used in the experiment were selected for their relatively high concentrations of CBD, and low concentrations of THC as they best represent the kind of cannabis that would have the most therapeutic benefit in a medicinal application. Indeed, they are also representative of the kinds of cannabis Canadians are already using to self-medicate (Fig. 1.3). The use of whole plant cannabis extracts in this experiment allowed for a broader understanding of the interaction between CBD and THC and how relative levels of these cannabinoids may affect behaviour. The findings from this research assessed the potential long-term risks of high-CBD cannabis use and will serve to better inform both the general public seeking health benefits from this promising therapeutic agent as well as

researchers intending to pursue pre-clinical, and clinical trials treating serious human disorder and disease using cannabis.

Research Question and General Hypothesis

This research was based on the theory of neuroplasticity which posits that *the brain has the capacity to change in response to our environment, which drives changes in behaviour in a reciprocal manner.* Bearing in mind this principle, this research will attempt to address two main questions:

1. Does chronic exposure to high-CBD cannabis extract create long-term changes in rodent behaviour?
2. Does chronic exposure to high-CBD cannabis extract create long-term changes in behaviour that are sexually dimorphic?

Based on these questions, I hypothesised:

1. Chronic exposure to high CBD cannabis extract will have minimal long-term impact on rodent behaviour.
2. The effects of chronic, high-CBD cannabis extract on rodent behaviour will produce sexually dimorphic results.

Conditionally, I do predict any changes in behaviour to be small and not necessarily be of detriment to the animals. Specific hypotheses as to the effects of this chronic, high-CBD cannabis exposure on each behavioural outcome will be discussed in the appropriate chapter.

Organization of the Thesis

Chapter 2, which follows, describes the general methodology used. Included in this chapter is an overview of the subjects involved, details of cannabis administration, testing paradigms and general comments on the statistical analyses used in this thesis. In order to thoroughly explore the long-term effects of high-CBD cannabis exposure on behaviour, each behavioural assay will be described in a dedicated a chapter, complete with introduction, specific methodology regarding the behavioural measure, results, discussion, and conclusion. Behavioural assays are included as follows: Chapter 3 – Locomotor Activity, Chapter 4 – Fine Motor Function, Chapter 5 – Anxiety-Like Behaviour, and Chapter 6 – Spatial Learning and Memory. Finally, Chapter 7 provides a general discussion of the main findings of this research, a critical reflection on experimental design, and thoughts as to the contribution of this thesis to the field of pre-clinical neuroscience research.

Chapter 2

General Methodology

Subjects

Experiment One

All procedures were conducted in accordance with the Canadian Council of Animal Care and were approved by the University of Lethbridge Animal Care and Use Committee. This project included 41 female and 49 male animals, all of which were born in the vivarium at the Canadian Centre for Behavioural Neuroscience.

Eleven male and 11 female animals of approximately 70 days old were acquired from Charles River and allowed to acclimatize to the vivarium environment prior to breeding. These 11 breeding pairs yielded a total of 91 pups. Pups were raised with the dam until postnatal day (P) 21 at which point, they were weaned and housed with up to two conspecifics in standard polyethylene shoebox cages. Animals were given ad libitum access to food and water, unless otherwise stated in the protocol, and were maintained on a 12:12 light/dark cycle at 23^{o}C. All animals were weighed daily to monitor their health over the course of the study.

Experiment Two

All procedures were conducted in accordance with the Canadian Council of Animal Care and were approved by the University of Lethbridge Animal Care and Use Committee. This project included 66 female and 49 male animals, all of which were born in the vivarium at the Canadian Centre for Behavioural Neuroscience.

Sixteen male and 16 female animals of approximately 70 days old were acquired from Charles River and allowed to acclimatize to the vivarium environment prior to

breeding. These 16 breeding pairs yielded a total of 115 pups. Pups were raised with the dam until P21 at which point, they were weaned and housed with up to two conspecifics in polyethylene cages with a mezzanine, which allowed for full vertical extension. Animals were given ad libitum access to food and water, unless otherwise stated in the protocol, and were maintained on a 12:12 light/dark cycle at 23°C. All animals were weighed daily to monitor their health over the course of the study.

Behavioural Testing

Early Behavioural Test Battery

An early-life test battery which included maternal care (P8, 12, and 16), open field (P10, 11, 12, 13, and 15), and negative geotaxis testing (P9, and 10), was administered prior to weaning, well before any experimental manipulation, to ensure typical development. The results of these tests will not be discussed in this present thesis.

Adolescent Test Battery

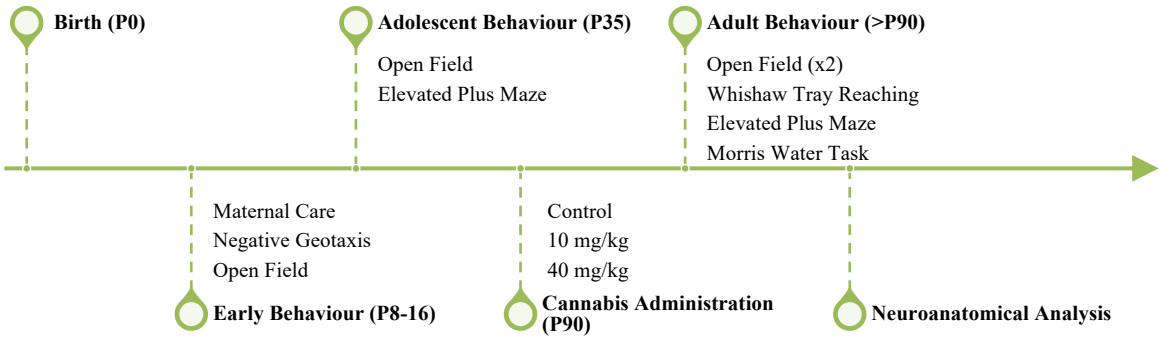
An adolescent test battery consisting of open field testing (a measure of exploratory behaviour and anxiety; P35) and elevated plus maze testing (a measure of anxiety; P36) was administered prior to cannabis administration in order to provide a behavioural baseline from which changes following cannabis administration could be assessed.

Adult Test Battery

The final adult test battery again consisted of open field testing (approx. P110 – 115 in experiment one; approx. P130 – 150 in experiment two) and elevated plus maze testing (approx. P116 in experiment one; approx. P135 – 150 in experiment two), while

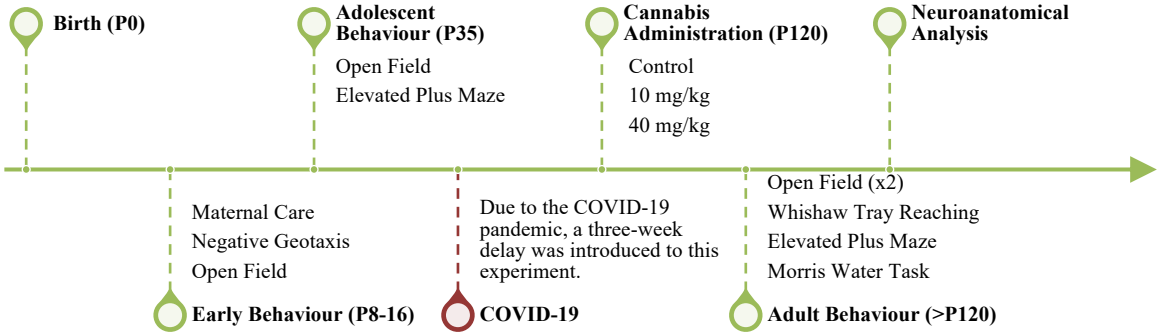
also including the Whishaw tray reaching task (approx. P114 in experiment one; approx. P133 – 148 in experiment two), and the Morris water task (MWT) (approx. P117 - 122 in experiment one). These tests were administered following the completion of cannabis administration in order to assess any resulting changes in behaviour. The timeline of the experimental procedure is represented below in Figures 2.1, and 2.2.

Figure 2.1
A Timeline of the Experimental Design of Experiment One



Note. This figure represents the timeline of experimental methodology followed in experiment one.

Figure 2.2
A Timeline of the Experimental Design of Experiment Two



Note. This figure represents the timeline of experimental methodology followed in experiment two

Cannabis Preparation

Extracts were prepared as previously described with proprietary modifications (Casiraghi et al., 2018; Wang et al., 2020). In brief, each of the four different cannabis cultivars used in these experiments were grown in a licenced facility at the University of Lethbridge. Flowers were harvested from plants and dried before being ground in liquid nitrogen, homogenized in ethyl acetate (10 mg/ml), and centrifuged. The supernatant was dried in a sterile rotor evaporator and reconstituted in sterile, food grade grapeseed oil. These preparations were stored at four degrees Celsius for up to a week prior to administration. Concentrations were based on proprietary cell line model studies. The cannabinoid content of each extract was approximately 40% and the relative ratios of cannabinoids in extracts #129, #81, and #10 were 20:1 CBD to THC. The final extract, #98, contained a ratio of approximately 6:4:1 THCV:THV:CBD. While the inclusion of a high THC extract may seem curious, it is important to note that extract #98 was included with extracts #10 and #81 in a 4:1 ratio, meaning the overall ratio of cannabinoids still heavily favoured the CBD constituents over the THC, THCV, and THV constituents. These combined extracts were selected based on previous *in vitro* experimentation assessing potential medical applications of the lines, specifically their potential to act as an anti-inflammatory agent.

Cannabis Dosing Paradigm

At approximately P85 in experiment one and P115 in experiment two, animals began their pre-dosing regimen. All animals were removed from their home cage, taken to a separate, novel room, and housed individually in standard polyethylene shoebox

cages. Female animals were given one gram of peanut butter while male animals were given two grams of peanut butter. This peanut butter pre-dosing regimen took place over the five days immediately preceding cannabis administration and served to habituate the animals to both the administration cages, and to the peanut butter vehicle. On the first day of cannabis dosing, animals were again brought from their home cages to their individual dosing cages. Animals were then given the appropriate dosage of whole-plant cannabis extract (either 10 mg/kg or 40 mg/kg), which was mixed into the peanut butter vehicle and weighed before administration. Treatment groups were populated as follows for experiment one (Table 2.1) and experiment two (Table 2.2).

Table 2.1
Animals in Experiment One

	Female (n)	Male (n)
Control	9	10
Extract #129, 10 mg/kg	8	10
Extract #129, 40 mg/kg	8	10
Extract #81, 10mg/kg	8	10
Extract #81, 40 mg/kg	8	10

Note. Table depicting the number of animals assigned to each treatment group in experiment one

Table 2.2
Animals in Experiment Two

	Female (n)	Male (n)
Control	12	8
Extracts #10+98, 10 mg/kg	13	9
Extracts #10+98, 40 mg/kg	14	10
Extracts #81+98, 10mg/kg	13	10
Extracts #81+98, 40 mg/kg	15	11

Note. Table depicting the number of animals assigned to each treatment group in experiment two

Once the entirety of the dosage had been consumed, or the allotted time for consumption had expired (one hour), the animals were collected and returned to their home cage. Any remaining peanut butter was then weighed, and the actual amount of the dosage consumed was calculated. This administration continued as described for 10 days.

Perfusion and Tissue Preparation

Upon the conclusion of behavioural testing, all animals were euthanized, and their brains perfused and extracted for anatomical analysis. Animals were administered an overdose of sodium pentobarbital through an i.p. injection and perfused with 200ml of a 0.9% saline solution, intracardially. Body tissues were sampled for molecular analysis and included liver, lung, spleen, small intestine, and large intestine. These tissues were flash frozen in liquid nitrogen before being stored at -80 degrees Celsius prior to processing. The right gonad of each animal was also flash frozen in liquid nitrogen while left gonad was post-fixed in a 4% PFA and sucrose solution. The brains of these animals

were extracted, weighed, and stored in Golgi-Cox solution in a light sensitive environment for 14 days. After the 14-day period, the brains were moved to a 30% sucrose solution where they were stored until the tissue was sectioned. Golgi-Cox-stained brains were cut on a Vibratome at a thickness of 200 μm and mounted on gelatin-coated slides. Staining procedure followed that outlined by Gibb and Kolb (1998).

Subject Inclusion Criteria

Pilot experimentation with these types of whole plant extracts indicated that, at high doses, the terpenes in certain extracts made them rather unpalatable to some animals. The extracts were therefore dissolved accordingly, such that the animal was delivered the smallest amount at the highest concentration possible to best mask the taste of the extract with the vehicle. It was predetermined that for a subject to be included in any of the forthcoming analyses, subjects must have consumed more than 50% of their administered cannabis extract over the course of the 10-day exposure. This resulted in the exclusion of 2 female animals from the extracts #81+98 40 mg/kg group. Another female animal from this same treatment group was excluded from analysis due to an eye infection contracted prior to behavioural testing. All animals in the other seven treatment groups consumed the entirety of their daily administration such that population distributions ahead of statistical analysis were as follows for experiment one (Table 2.3) and experiment two (Table 2.4).

Table 2.3

Final Number of Animals Included in the Analysis of Experiment One

	Female (n)	Male (n)
Control	9	10
Extract #129, 10 mg/kg	8	10
Extract #129, 40 mg/kg	8	10
Extract #81, 10mg/kg	8	10
Extract #81, 40 mg/kg	8	10

Note. This table displays the number of animals included in each treatment group. Based on inclusion criteria, no subjects needed to be excluded from analysis.

Table 2.4

Final Number of Animals Included in the Analysis of Experiment Two

	Female (n)	Male (n)
Control	12	8
Extracts #10+98, 10 mg/kg	13	9
Extracts #10+98, 40 mg/kg	14	10
Extracts #81+98, 10mg/kg	13	10
Extracts #81+98, 40 mg/kg	12	11

Note. This table displays the number of animals included in each treatment group. Based on inclusion criteria, two animals were excluded from analysis. A third female was excluded for medical reasons.

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics 27 software. Graphs and tables were made using Excel 16.25 for Mac. The specific statistical models used in analysis of each behavioural task are discussed in the appropriate chapter. Statistical

analyses of all extracts were run separately such that comparisons were only drawn between control animals, and those receiving 10 mg/kg, or 40 mg/kg of one extract. Typically, a Bonferroni correction would be applied to all alpha values and consideration of significance to account for the repetition of the control data in multiple analyses. As such, in the following analyses an alpha value of $p < .025$ will be required to be considered significant.

Chapter 3

Long-Term Effects of Chronic, High-CBD Cannabis Exposure on Locomotor Activity

Introduction

Locomotor Activity and the Tetrad Assay

The effects of cannabis on locomotor activity are some of the most consistently studied in the literature at large. Cannabis exposure is known to typically induce hypomotility, an effect which is assessed by the aforementioned tetrad assay (Martin et al, 1991). Again, this collection of tests examines what are thought to be hallmark behavioural indications of cannabinoid activity including hypomotility (locomotor suppression), antinociception, catalepsy and hypothermia. The tetrad tests show high correlation to CB1 activity and are therefore accepted as a metric of the influence of cannabis on behaviour.

Effects of Exogenous Cannabinoids on Locomotor Activity: Focus on THC

Much of the research surrounding the effects of cannabis on locomotor activity focuses on the influence of THC only. As previously mentioned, the tetrad assay is treated as a measure of cannabimimetic influence on behaviour because changes in those four behaviours are highly correlated with CB1 receptor activity, however, not every cannabinoid has a high affinity for the CB1 receptor. Rather, because THC is the primary exogenous CB1 agonist in cannabis it is likely driving the main effects observed in the tetrad assay (Varvel et al., 2005). The influence of other cannabinoids alone on this set of behaviours has also been investigated, albeit to a much lesser extent (for a summary see

DeLong, Wolf, Poklis, & Lichtman, 2012; Radwan et al., 2015). Let us consider the effect of other prominent phytocannabinoids. Research regarding the effects of CBD on tetrad behaviours has produced varied results. Considering locomotor behaviour specifically, some research has found that administration of CBD alone produces no cannabimimetic effect at all (El-Alfy et al., 2010; Hayakawa et al, 2008). Alternatively, other research has indicated that administration of CBD alone does influence locomotor activity, increasing activity at doses of 10 mg/kg while decreasing activity at higher doses of 30 mg/kg, when compared to a vehicle (Varvel et al., 2006). An important consideration for this experiment, however, is that because we are utilizing a whole plant extract, we are dealing with not only THC or CBD, but a variety of cannabinoids which can interact with each other to create unique behavioural effects. These additional cannabinoids may also have an influence on locomotor activity. For example, when administered alone, the cannabinoid cannabichomene (CBC), has been shown to elicit a hypolocomotor effect at significant doses of 80 mg/kg or 100 mg/kg (El-Alfy et al., 2010; DeLong, Wolf, Poklis, & Lichtman, 2012). Similarly to THC, THCV has been shown to reduce locomotor activity in a dose dependant manner following acute exposure (Zagzoog et al., 2020). Other research has suggested that THCV administration may reduce the hyperlocomotion characteristic of animal models of psychosis (Cascio et al., 2015). Long-term effects of THCV are less apparent as, again, like THC, THCV is metabolized rather quickly. For example, Deiana and colleagues (2011) observed elimination of the molecule from the brain < 1.5 hrs following oral administration of a 30 mg/kg dose. Additionally, previous research has shown that depending on dose, and time of administration/testing, CBD can potentiate the locomotor supressing effects of THC

(Britch, Wiley, Yu, Clowers, & Craft, 2017; Hayakawa et al., 2008). These findings are not without contradiction, however, as other researchers have observed no potentiation of CBD pre-treatment on the locomotor suppressing effects of THC (Varvel et al., 2006). As we will come across again, it likely that these divergent effects are due to differences in dosage, or timing of administration and testing. This leads us to the second consideration: cannabis exerts a biphasic effect on behaviour.

Biphasic Effect of Cannabis on Behaviour

A biphasic effect is one which drives a dependant variable, in our case a behaviour, in one direction (be it an increase or decrease) at one level of the independent variable, and the opposite direction at another. It is typically observed that low doses of cannabis can increase locomotor activity while high doses elicit the decreased locomotor activity characteristic of the tetrad. This can lead to a semantic grey area in the literature however, as the terms "high" and "low" are rather arbitrary. For example, El-Alfy et al. (2010) demonstrated the biphasic effect of THC on motor behaviour by showing that while "high" doses of 10 mg/kg, 20 mg/kg, and 40 mg/kg of THC supressed locomotor activity, low doses of "1.25" mg/kg significantly increased activity. Comparatively, Sañudo-Peña and colleagues (2002), observed a triphasic effect of THC exposure on locomotor activity as "low" doses of THC (<0.2 mg/kg) decreased locomotor activity, "higher" doses (>1-<2 mg/kg) increased activity, until catalepsy was observed at doses higher than 2.5 mg/kg. Paying attention to the dosage, rather than terminology, provides more clarity. Other researchers, however, have found that doses of 0.1 mg/kg of THC increase activity while doses of 1 mg/kg of THC are enough to decrease activity when tested one to two hours after dosing (Katsidoni, Kastellakis, & Panagis, 2013). These

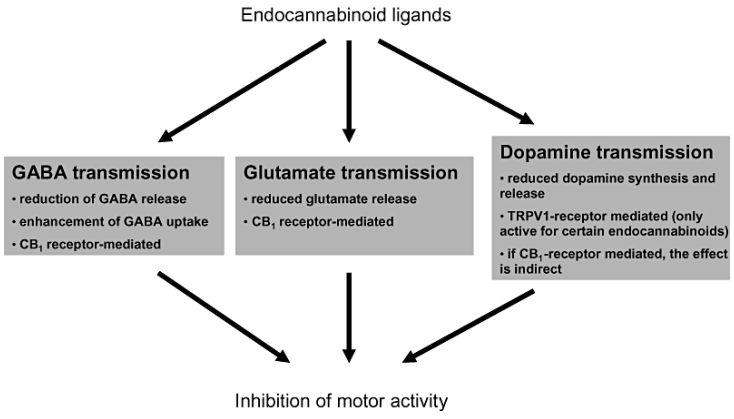
studies suggests that, in addition to the biphasic effects of dosage level, the timing of testing after exposure may also exhibit a similar biphasic influence. Immediately following relatively high doses of cannabis, that is 3 mg/kg or 5 mg/kg, the drug was found to reduce ambulation (a measure of activity levels) when tested three hours following administration, compared to the same group when tested 30 minutes after administration (Drew, Miller, & Wikler, 1972). The researchers here rightfully point out that this is an important consideration, as the time of testing could lead to dramatically different inferences about the effect of the drug. An alternative investigation found that chronic exposure to cannabis smoke led to an increase in activity immediately after exposure, but no difference 24 or 48 hours after exposure (Bruijnzeel et al, 2016).

Effects of Cannabinoids on Locomotor Activity

Cannabinoids affect motor movement through their influence on the endogenous cannabinoid system (Fig. 3.1) and there are high densities of CB1 receptors in the basal ganglia, an area of the brain critical in locomotion (Herkenham et al. 1991, Tsou, Brown, Sañudo-Peña, Mackie, & Walker, 1998; for a review see Garcia-Rill, 1986). The basal ganglia are made up of several nuclei, the largest among them is the striatum. The striatum is in turn made up of the caudate nucleus and the putamen, and forms reciprocal connections with the thalamus and the cortex (for a review see Graybeil, 2000). Damage to, or degradation of, the basal ganglia and related nuclei lead to significant motor disorders including Huntington's disease and Parkinson's disease (Vonsattel et al., 1985; Fearney & Lees, 1991). Broadly speaking, the basal ganglia are posited to facilitate the selection of motor movements and inhibit competing motor movements so the desired movement can occur without competing signals (Mink, 1996). Cannabinoids exert their

influence in this region of the brain by acting upon both the excitatory glutamatergic pathways as well as the inhibitory GABAergic pathways by targeting presynaptically located receptors to affect neurotransmitter release and re-uptake (San, Patrick, Patrick &Walker, 1996; Sañudo-Peña & Walker, 1998; Sañudo-Peña, Tsou, and Walker, 1999; For a review see Fernández-Ruiz, Lastres-Becker, Cabranes, González, & Ramos 2002). It has also been hypothesized that cannabinoids indirectly act on dopaminergic neurons in the caudate putamen thereby exerting effect on locomotor behaviour (Fernández-Ruin & González, 2005). By binding to vanilloid VR1 receptors located on nigrostriatal dopaminergic neurons in the basal ganglia, cannabinoid agonists decrease dopaminergic transmission which creates a depression in activity (de Lago, de Miguel, Lastres-Becker, Ramos & Fernández-Ruiz, 2004). This pathway is utilized by endogenous cannabinoids, like anandamide, but exogenous cannabinoids, like THC do not elicit the same mechanism of action (de Lago, de Miguel, Lastres-Becker, Ramos & Fernández-Ruiz, 2004).

Figure 3.1
The Potential Mechanisms of the Effects of Endogenous Cannabinoids on Locomotor Activity



Note. This figure summarizes the mechanism of action of the endocannabinoid system in moderating motor function in the basal ganglia. Adapted from Fernández-Ruiz, 2009.

Specific Hypothesis

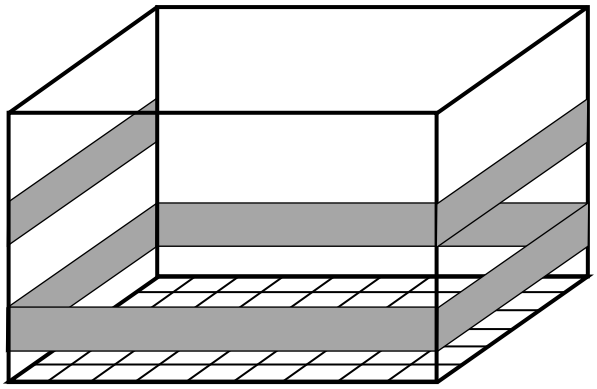
Considering the limited influence of CBD on locomotor behaviour, and the relatively limited amount of THC present in the cannabis extracts used in this experiment, I hypothesize that exposure to high-CBD cannabis extract will exhibit little influence on locomotor activity. If there is to be an influence on activity, I anticipate it will result in lower levels of activity in cannabis exposed groups compared to controls.

Methodology

Testing Paradigm

Locomotor activity was recorded at three time points across the lifespan: once in adolescence (P35) to determine a baseline level of activity, in adulthood, 24-hours (immediately) following the cessation of dosing, and again, two weeks after the cessation of dosing. The open field task measures locomotor and exploratory activity of the animals using Accuscan activity monitoring Plexiglas® boxes (41cm x 41cm x 30.5cm). Activity levels were recorded using VersaMax software, where activity was measured as the number of beam breaks during 10, one minute sampling periods. Total distance covered (cm) in the testing period is used as a metric of total activity.

Figure 3.2
Schematic Representation of the Open Field Apparatus



Note. The image above is a simplified visual representation of the apparatus used to assess locomotor activity.

Statistical Analysis

Analyses of total locomotor activity levels were conducted using a repeated measure, 2-way ANOVA with treatment (control, 10 mg/kg (low dose) or 40 mg/kg (high dose)) and sex as between-subject factors, and time of treatment (adolescence, immediately following dosing, and two weeks following dosing) as a within-subject factor. Planned contrasts were used to further investigate the difference in activity levels between time points, between treatment groups.

Results

Long-Term Effects of Cannabis Extract #129 on Locomotor Activity

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = .886$, $p = .642$, therefore, no corrections were applied to the following statistical observations.

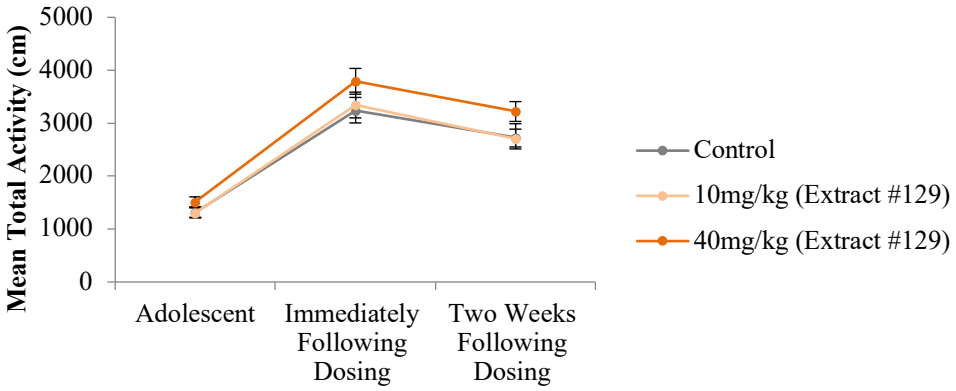
A main effect of time of testing was observed, $F(2, 92) = 143.448$, $p < .001$, $\eta p^2 = .757$, suggesting total activity levels changed significant across the three testing periods. Contrasts revealed a significant difference in total activity between adolescence and immediately following dosing $F(1, 46) = 235.946$, $p < .001$, $\eta p^2 = .837$, as well as between immediately following dosing and two weeks following dosing $F(1, 46) = 22.073$, $p = .003$, $\eta p^2 = .324$, which suggests that the total activity levels of all animals increased between adolescence and immediately following extract exposure, and then decreased two weeks after the cessation of dosing.

There was no significant Time of Testing x Treatment interaction observed in measures of total distance suggesting there was no effect of the cannabis treatment on the changes in total activity observed over the lifespan $F(4, 92) = .435$, $p = .763$, $\eta p^2 = .020$ (Fig. 3.3). There was a significant Time of Testing x Sex interaction observed, suggesting the change in female animals' total activity across the lifespan was different than that of male animals $F(2, 96) = 12.943$, $p < .001$, $\eta p^2 = .209$ (Fig. 3.4). Contrasts reveled this effect is driven by a significantly larger increase in female animals' activity levels from adolescence following dosing, compared to males $F(1, 46) = 18.514$, $p < .001$, $\eta p^2 = .287$. There was no significant Time of Testing x Sex x Treatment interaction observed in total activity suggesting the effect of cannabis treatment on total activity was not dependent on

the sex of the animals, that is it influenced all groups similarly $F(4, 96) = .247$, $p = .911$, $\eta p^2 = .016$ (Fig. 3.5).

Figure 3.3

Changes in Total Activity Levels of Animals Exposed to Cannabis Extract #129 Compared to Controls

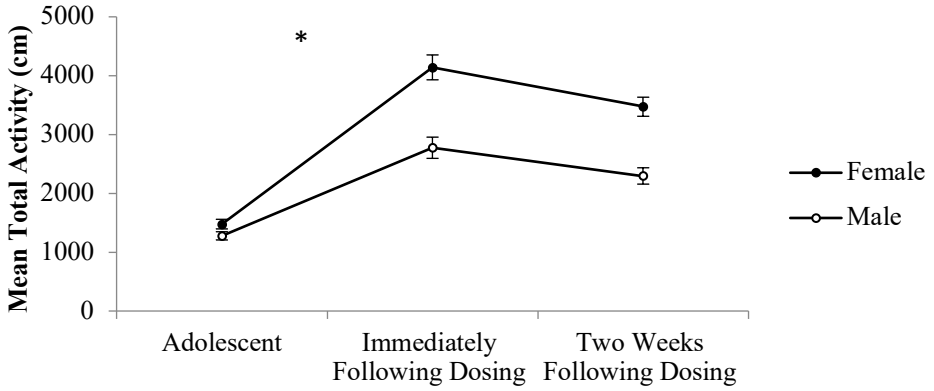


Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10mg/kg) dose, or high (40mg/kg) dose of cannabis extract #129 compared to controls, across three testing periods.

[a]There were no significant differences observed between treatment groups in changes in measures of total activity.

Figure 3.4

Changes in Total Activity Levels of Female and Male Animals



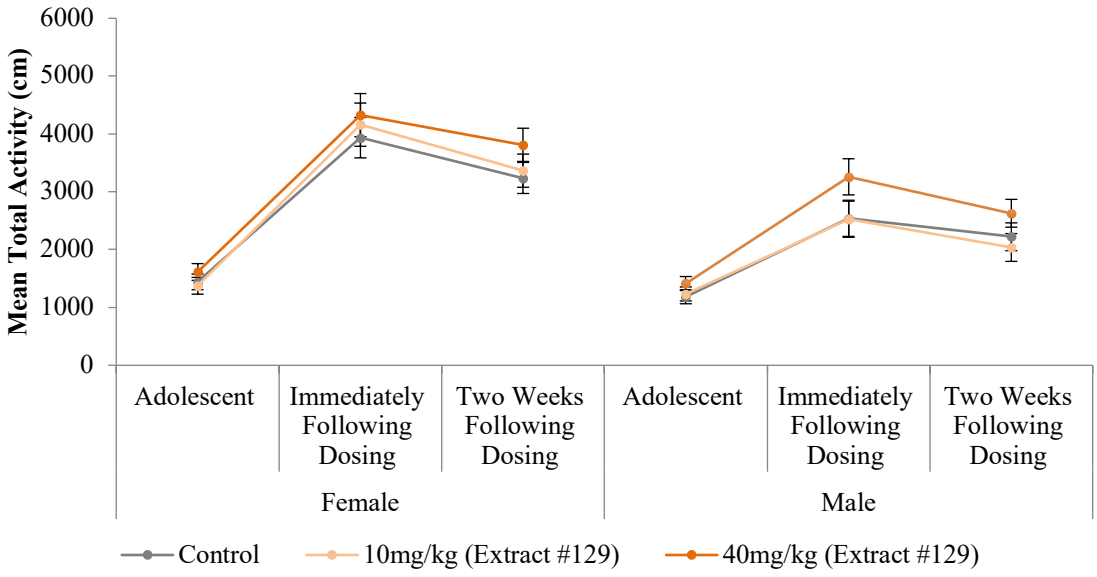
Note. This figure demonstrates the change in total activity levels as a function of sex, across three testing periods.

[a] Female animals showed a significantly larger increase in total activity from adolescence to immediately following dosing than male animals. [b] Both female and male animals showed a similar decrease in activity levels two weeks following dosing.

*$p < 0.025$

Figure 3.5

Changes in Total Activity Levels of Female and Male Animals Exposed to Extract #129



Note. This figure demonstrates the change in total activity levels of female and male animals exposed to extract #129 as a function of treatment group, across three testing periods. [a] Exposure to extract #129 did not significantly alter the changes in total activity levels of female animals across the three points of testing. [b] Exposure to extract #129 did not significantly affect the change in total activity levels of male animals across these three time points.

Long-Term Effects of Cannabis Extract #81 on Locomotor Activity

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 4.034$, $p = .133$, therefore, no corrections were applied to the following statistical observations.

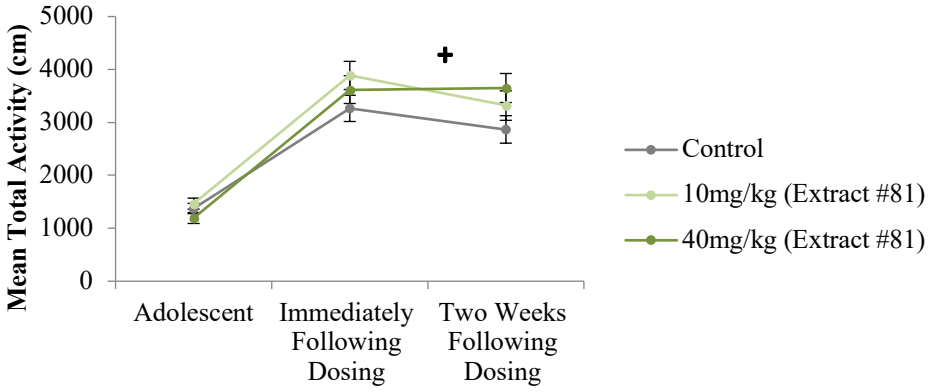
A main effect of time of testing was observed, $F(2, 94) = 210.009$, $p < .001$, $\eta p^2 = .817$, suggesting total activity levels changed significantly across the three testing periods. Contrasts revealed that the overall, total activity levels of all animals increased between adolescence and immediately following extract exposure, $F(1, 47) = 331.709$, p

< .001, ηp^2 = .876, and then decreased two weeks after the cessation of dosing $F(1, 47)$ = 9.603, p =.003, ηp^2 = .170.

There was a significant Time of Testing x Treatment interaction, $F(4, 94)$ = 3.364, p = .013, ηp^2 = .125 suggesting cannabis treatment affected the changes in total activity observed over the lifespan (Fig. 3.6). Contrasts revealed no significant difference between adolescent measures and those immediately following exposure, $F(2, 47)$ = 2.154, p = .127, ηp^2 = .084, and only a trending difference in the change in total activity immediately following exposure and two weeks following exposure $F(1, 47)$ = 3.016, p = .059, ηp^2 = .114. This trending effect appears to be driven by the high dose group which showed an increase in activity levels two weeks following dosing, while the low dose group and controls, showed a decrease in activity levels. There was a significant Time of Testing x Sex interaction observed, $F(2, 94)$ = 11.906, p < .001, ηp^2 = .202 (Fig. 3.7). Contrasts revealed this effect is driven by a significantly larger increase in female animals' activity levels from adolescence following dosing, compared to males $F(1, 47)$ = 16.621, p < .001, ηp^2 = .261, and not by changes from immediately following dosing to two weeks following dosing, which showed no difference based on sex $F(1,47)$ = .000, p = .984, ηp^2 = .000. There was no significant Time of Testing x Treatment x Sex interaction observed in total distance suggesting the change in total activity was similar across all treatment groups and was not dependant on the sex of the animal $F(4, 94)$ = .376, p = .825, ηp^2 = .016 (Fig. 3.8).

Figure 3.6

Changes in Total Activity Levels of Animals Exposed to Cannabis Extract #81 Compared to Controls



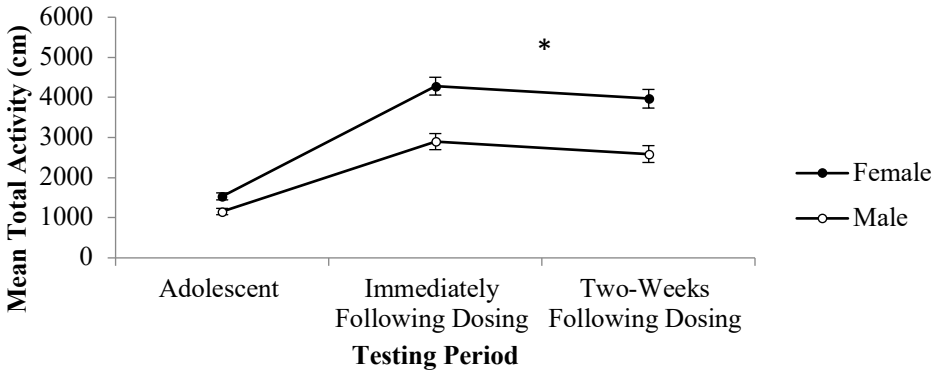
Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10mg/kg) dose, or high (40mg/kg) dose of cannabis extract #81, compared to controls, across three testing periods.

[a] A trending difference was observed between two time points as animals who were given the high dose of extract #81 did not show the same decrease in activity levels from immediately following dosing to two weeks post-dosing, as the low dose and control groups did. In fact, they showed a slight increase in total activity.

$^{+}$ $p < .07$

Figure 3.7

Changes in Total Activity Levels of Female and Male Animals



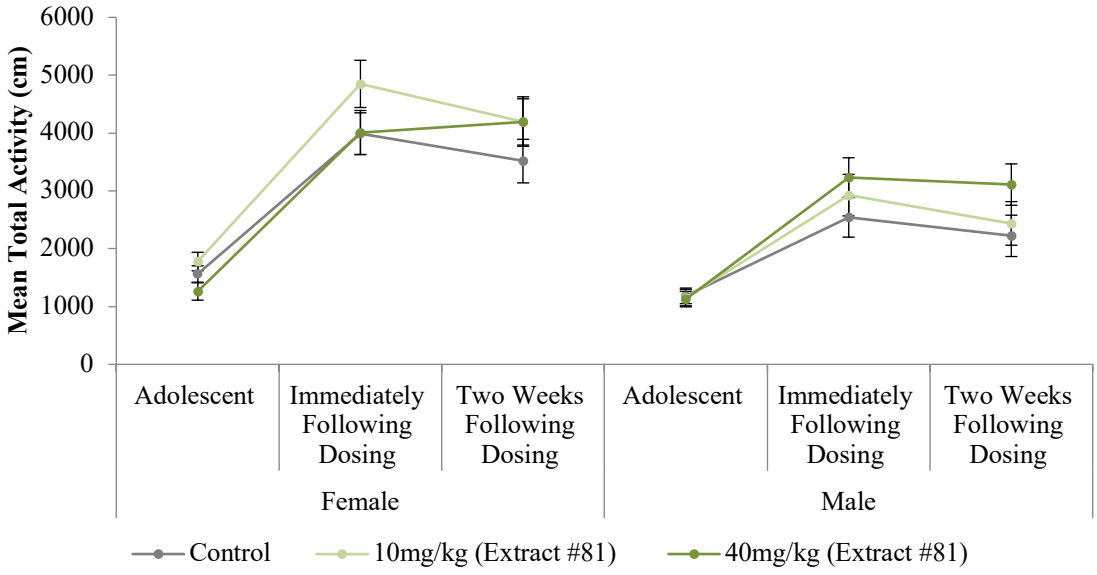
Note. This figure demonstrates the change in total activity levels as a function of sex over, across three testing periods.

[a] Female animals showed a significantly larger increase in total activity from adolescence to immediately following dosing than male animals. [b] Both female and male animals showed a similar decrease in activity levels two weeks following dosing.

*$p < 0.025$

Figure 3.8

Changes in Total Activity Levels of Female and Male Animals Exposed to Extract #81



Note. This figure demonstrates the change in total activity levels of female and male animals exposed to extract #81 as a dose, across three testing periods.
[a] No significant changes were observed in the change in activity levels over the lifespan between groups. The trending Treatment x Time of Testing effect is observable in the female animals as the high dose groups maintained elevated activity levels from immediately after exposure to two weeks following exposure, where the other groups, the low dose group, and controls, both showed a decrease in total activity over that time period. [b] No significant changes were observed in the change in activity levels over the lifespan between groups. Again, the trending Time of Testing x Treatment interaction is observable in the male animals as the high dose groups maintained elevated activity levels from immediately after exposure to two weeks following exposure, where the other groups, the low dose group, and controls, both showed a decrease in total activity.

Long-Term Effects of Cannabis Extracts #10+98 on Locomotor Activity

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 4.044$, $p = .132$, therefore, no corrections were applied to the following statistical observations.

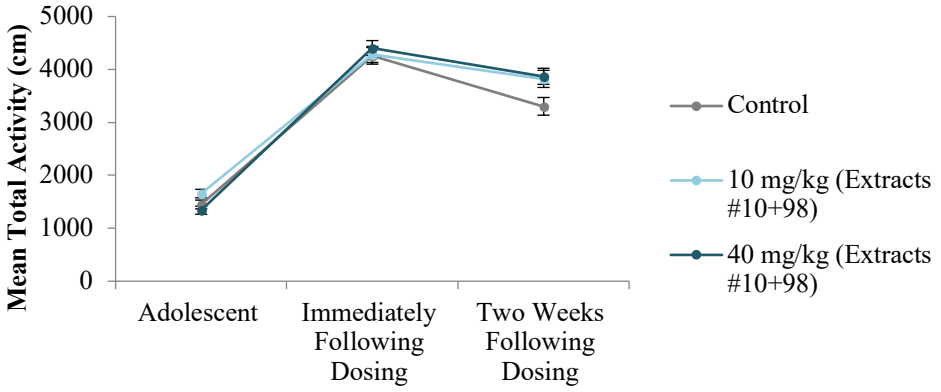
A main effect of time of testing, $F(2, 118) = 277.605$, $p < .001$, $\eta p^2 = .825$ suggesting total activity levels changed significantly across the three testing periods. Contrasts revealed a significant increase in total activity between adolescence and

immediately following dosing, $F(1, 59) = 529.979$, $p < .001$, $\eta p^2 = .900$, as well as significant decrease in total activity from immediately following dosing and two weeks following dosing, $F(1, 59) = 33.145$, $p < .001$, $\eta p^2 = .360$.

There was no significant Time of Testing x Treatment interaction observed in measures of total distance suggesting there was no effect of the cannabis treatment on the changes in total activity observed over the lifespan $F(4, 118) = 1.640$, $p = .169$, $\eta p^2 = .053$ (Fig. 3.9). There was, however, a significant Time of Testing x Sex interaction observed $F(2, 118) = 9.689$, $p < .001$, $\eta p^2 = .141$ (Fig. 3.10). Contrasts reveled this effect is driven by a significantly larger increase in female animals' activity levels from adolescence to immediately following dosing, compared to males $F(2, 59) = 18.488$, $p < .001$, $\eta p^2 = .239$. There was no significant Time of Testing x Treatment x Sex interaction observed in total distance suggesting the change in total activity across the lifespan was similar across all treatment groups and was not dependant on the sex of the animal, $F(4,118) = 1.718$, $p = .151$, $\eta p^2 = .055$ (Fig. 3.11).

Figure 3.9

Changes in Total Activity Levels of Animals Exposed to Cannabis Extract #10+98 Compared to Controls

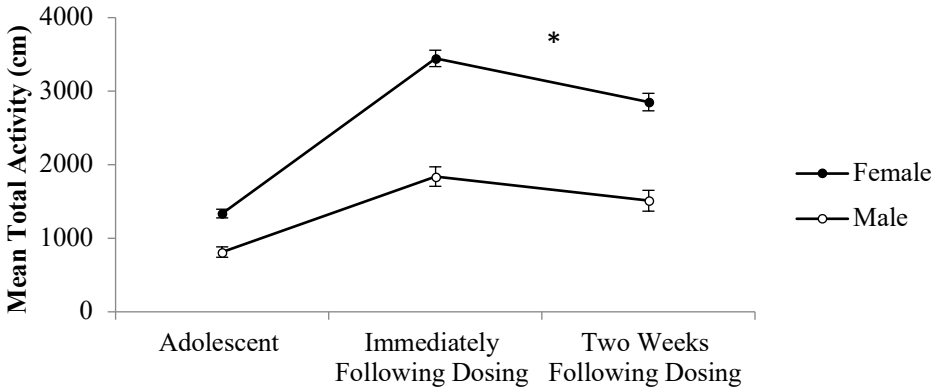


Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10mg/kg) dose, or high (40mg/kg) dose of cannabis extracts #10+98 compared to controls, across three testing periods.

[a] While the total activity of the low dose group appears not to decrease as significantly by two weeks post-dosing compared to the high-dose and control groups, this difference was not statistically significant.

Figure 3.10

Changes in Total Activity Levels of Female and Male Animals Exposed to Extracts #10+98



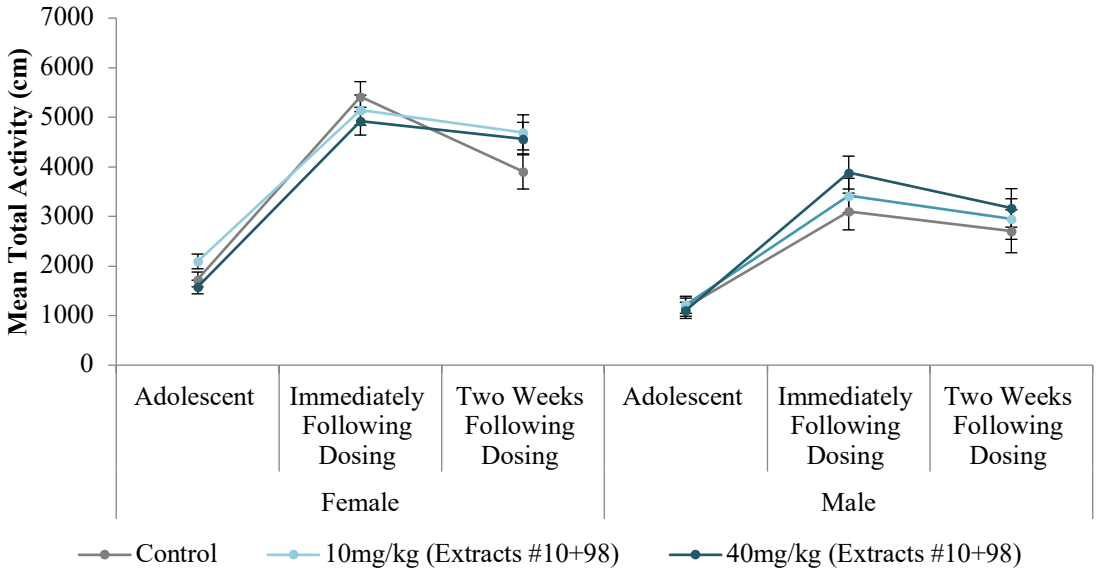
Note. This figure demonstrates the change in total activity levels as a function of sex, across three testing periods

[a]Female animals showed a significantly larger increase in total activity from adolescence to immediately following dosing than male animals. [b] Both female and male animals showed a similar decrease in activity levels two weeks following dosing.

*$p < 0.025$

Figure 3.11

Changes in Total Activity Levels of Female and Male Animals Exposed to Extracts #10+98



Note. **A.** This figure demonstrates the change in total activity levels of female and male animals exposed to extracts #10+98 as a function of treatment group, across three testing periods. [a] While the interaction between Time of Testing x Treatment x Sex was statistically insignificant, it does appear that female animals given extracts 10+98, at any dosage, maintained elevated activity levels from immediately following dosing, to two weeks post-dosing whereas control animals showed a decrease in their total activity over this time frame. When compared with Fig. 3.11, there does appear to be a difference in the way the treatment affected female and male animals. [b] Again, while the Time of Testing x Treatment x Sex interaction was insignificant, the visualization of the data suggest that exposure to extract #10+98 had not affected the total activity levels of exposed male animals, as it had female animals.

Long-Term Effects of Cannabis Extracts #81+98 on Locomotor Activity

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.941$, $p = .139$, therefore, no corrections were applied to the following statistical observations.

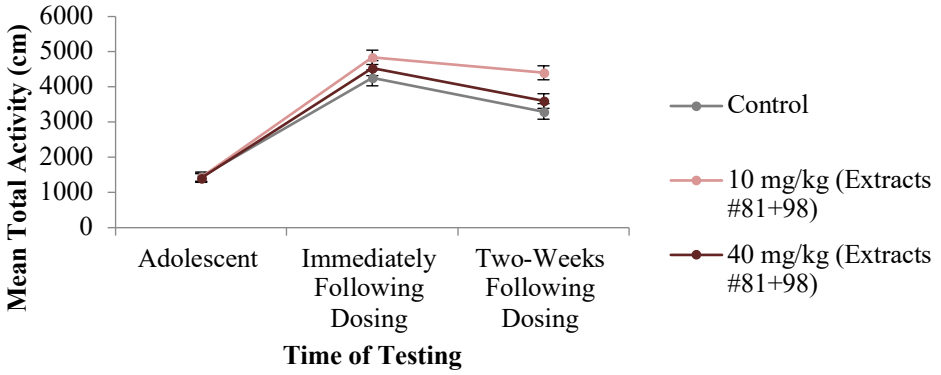
A main effect of Time of Testing was observed, $F(2, 120) = 428.999$, $p < .001$, $\eta p^2 = .877$, suggesting that total activity levels changed across all three testing periods. Contrasts revealed a significant increase in total activity from adolescence to immediately

following dosing $F(1, 60) = 793.667$, $p < .001$, $\eta p^2 = .930$, as well as a significant decrease from immediately following dosing to two weeks following dosing $F(1, 60) = 63.086$, $p < .001$, $\eta p^2 = .513$.

A significant Time of Testing x Treatment interaction, $F(4, 120) = 4.473$, $p = .002$, $\eta p^2 = .130$ suggests there was an effect of the cannabis treatment on the changes in total activity observed over the lifespan (Fig. 3.12). Contrasts revealed no significant difference between adolescent and immediately following exposure, $F(2, 60) = 2.361$, $p = .103$, $\eta p^2 = .073$, but a trending difference in total activity from immediately following exposure to two weeks following exposure $F(1, 60) = 2.991$, $p = .058$, $\eta p^2 = .091$, likely driven by animals given the low dose of extracts #81+98. These animals appear to have a slightly steeper increase in locomotor activity from adolescent to immediately following dosing as well as maintained elevated activity levels two weeks post-dosing, compared to the high dose group and control group which both showed decreases in activity levels across this time. There was also a significant Time of Testing x Sex effect observed, $F(2, 120) = 8.514$, $p < .001$, $\eta p^2 = .124$ (Fig. 3.13). Contrasts reveled this interaction is driven by a significantly larger increase in female animals' activity levels from adolescence to following dosing, compared to males $F(1, 60) = 16.996$, $p < .001$, $\eta p^2 = .221$, and smaller, trending decrease in activity two weeks after dosing $F(1, 60) = 4.264$, $p = .043$, $\eta p^2 = .066$. There was no significant Time of Testing x Treatment x Sex interaction observed in total distance suggesting the change in total activity was similar across all treatment groups and was not dependant on the sex of the animal, $F(4, 120) = 2.205$, $p = .072$, $\eta p^2 = .068$ (Fig. 3.14).

Figure 3.12

Changes in Total Activity Levels of Animals Exposed to Cannabis Extracts #81+98 Compared to Controls

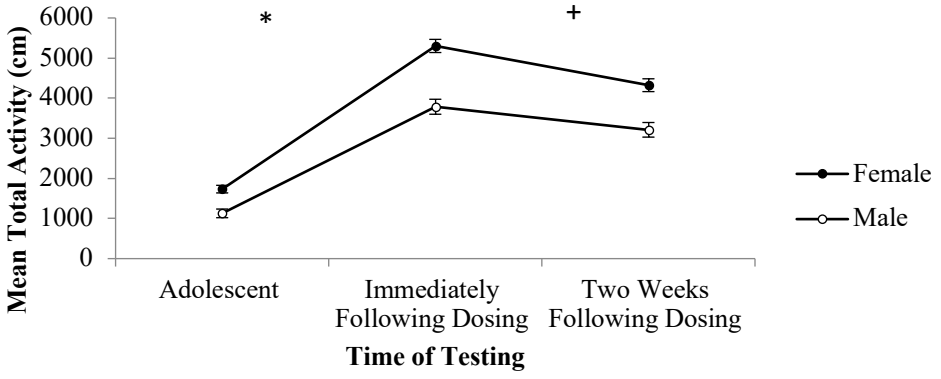


Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10mg/kg) dose, or high (40mg/kg) dose of cannabis extracts #81+98 compared to controls, across three testing periods.

[a] A significant Treatment x Time of testing interaction was observed, however planned contrasts revealed only a trending difference between two time points as animals who were given the low (10 mg/kg) dose of extracts #81+98 did not show the same drop in activity levels from immediately following dosing to two weeks post-dosing, as the high (40 mg/kg) dose and control groups did.

Figure 3.13

Changes in Total Activity Levels of Female and Male Animals Exposed to Extracts #81+98



Note. This figure demonstrates the change in total activity levels as a function of sex and treatment, across three testing periods.

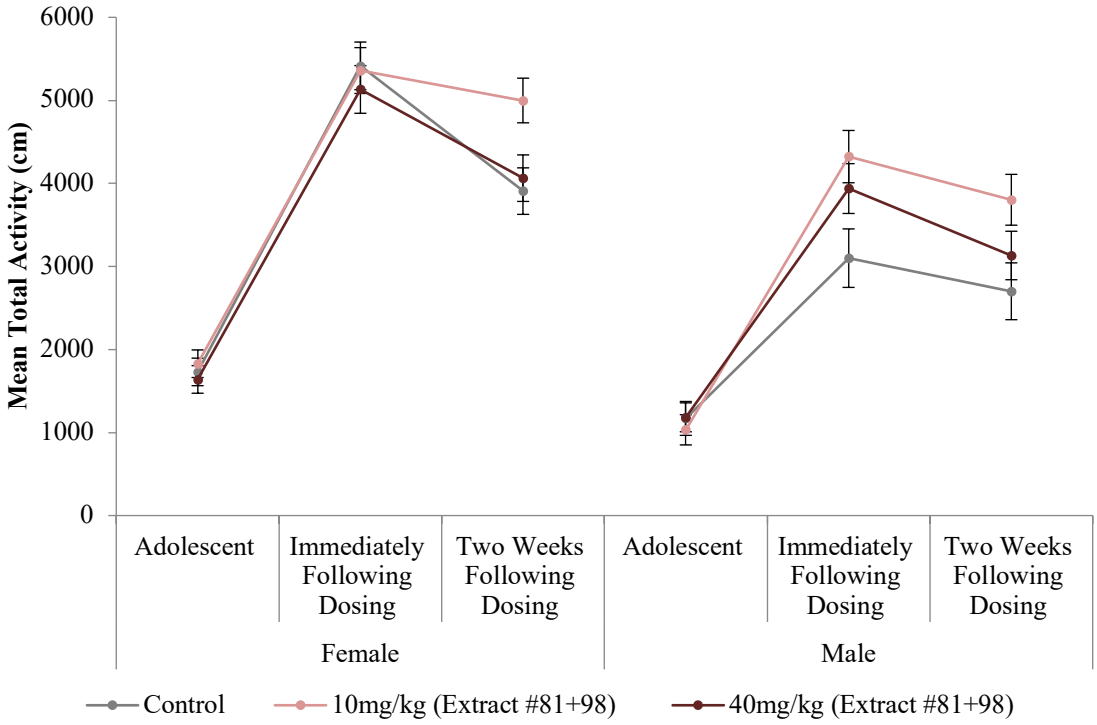
[a] Female animals showed a significantly larger increase in total activity from adolescence to immediately following dosing than male animals. [b] Female animals also displayed a greater decrease in activity from immediately to two weeks post-dosing compared to males.

**p < .025*

+p < .07

Figure 3.14

Changes in Total Activity Levels of Female and Male Animals Exposed to Extracts #81+98



Note. This figure demonstrates the change in total activity levels of female and male animals exposed to extracts #81+98 as a function of treatment group, across three testing periods. [a] While the Time of Testing x Treatment x Sex interaction was insignificant, it does appear that female animals given the low (10 mg/kg) dose of extracts #10+98, showed elevated activity levels, where both high dose (40 mg/kg) and control animals decreased their activity over this time frame; an observation that does not hold true for the male animals as displayed in Fig. 3.14. [b] Again, while the Time of Testing x Treatment x Sex interaction was insignificant, it appears that male animals given extracts #81+98, at any dosage, showed increased activity levels from adolescence to immediately following testing compared to controls. Female animals showed no difference in activity level between groups, between these time points as illustrated in Fig. 3.14.

Discussion

General Discussion

This analysis sought to explore the influence of high-CBD cannabis extract on locomotor activity in Long-Evans rats measured at three time points across the lifespan: in adolescence (prior to cannabis administration), 24 hours following the cessation of dosing (referred to in figure as 'immediately"), and approximately two weeks following the cessation of dosing. Exposure to these high-CBD cannabis extracts appears not to generate drastic differences in locomotor behaviour, however they were not without some influence.

Exposure to extract #129 and #10+98 yielded no effect on measures of total locomotor activity. Exposure to extract #81 and #81+98 did show significant Treatment x Time of Testing interactions, however, follow up testing revealed only trending differences between groups. This trending difference was consistent in timing across the two extracts as exposure caused activity levels of treated animals to remain elevated two weeks after the cessation of dosing compared to controls. The difference between the effect of these two extracts manifested in which treatment group was driving the interaction. Animals treated with the high dose of extract #81 showed an increase in mean total activity levels two weeks post-dosing, compared to the low dose group and the control group, which both showed a decrease in mean activity levels. Comparatively, animals treated with the low dose of extracts #81+98 showed a less significant decrease in total activity from immediately following dosing to two weeks following dosing, compared to the high-dose group and controls.

These results would suggest that it is some effect of extract #81 which is driving the changes as it was administered to all groups exhibiting difference in locomotor activity when compared to controls. The curious effect here is that it was the 40 mg/kg groups which displayed long-term elevated activity levels of the animals administered extract #81, but the 10 mg/kg group which showed these effects in animals administered extracts #81+98. A mentioned in Chapter 2, extracts #81+98 were combined in a 4:1 ratio which would mean that animals given this extract were receiving less of extract #81 than those animals given extract #81 alone. It may be that something about extract #98 was dampening the long-term effects of extract #81 on locomotor activity but only when present in sufficiently high levels. This would mean that animals given 40 mg/kg of the combination extracts #81+98 would not show elevated activity, but that animals given only 10 mg/kg of the combination extracts would. Indeed, it appears that sufficiently high levels of extract #81 are required to elicit the change in locomotor activity observed as it was only seen in the 40 mg/kg group. Additionally, the cannabinoid composition of extract #81 and #129 are similar, with both having a 20:1 ratio of CBD to THC which would suggest the auxiliary profiles of these extracts are a potential driver of the observed effects of exposure on locomotor activity levels.

High-CBD Cannabis Does Not Affect Activity Levels 24-Hours Following Chronic Exposure

First, let us address the effect of high-CBD cannabis exposure based on the time of testing. Exposure to these four cannabis extracts did not appear to significantly affect the mean total activity of exposed animals 24 hours after the final dose was administered. The activity dampening effects of THC are characteristic of acute exposure and, as has

been previously mentioned, THC reaches peak concentrations shortly after administration and is typically eliminated in the order of hours. Therefore, if we were to have seen any influence of THC on locomotor activity it likely would have been observable during this testing period. No physiological measure of cannabinoid concentration was taken during or after administration, which would have provided insight into the exact level of cannabinoids in sampled tissue following chronic exposure. In absence of this concrete evidence there are two approaches that can be made to interpret the observed results. The first is that after 24-hours the amount of THC in the brain, even after 10-day chronic exposure, had been reduced and as such would no longer elicit the typical effects characteristic of acute cannabis exposure. It is likely that the levels of THC, already being at a low level in the selected extracts, were not sufficient to illicit any potential suppression of locomotor activity, and that testing 24 hours after the final dose would miss the window of maximum THC concentration. It also appears that any potential effects of THC on locomotor activity were not exacerbated or potentiated by the higher levels of CBD immediately after exposure. The second approach is that even with residual cannabinoids, most likely mainly CBD, present in the system, there was still no effect on locomotor activity levels 24 hours after exposure. This is a likely occurrence as cannabinoids can remain in the system of chronic users long after the final exposure (for a review see Grotenhermen, 2003). Therefore, we may infer that these cannabis extracts have no effect on locomotor behaviour, immediately following the cessation of chronic exposure.

Chronic Exposure to High-CBD Cannabis Generated Long-Term Hyperlocomotion

Next let us address the second testing time point. The only influence of these cannabis extracts on behaviour manifested as long-term changes in activity observed two weeks after dosing and follow-up testing revealed that changes in locomotor activity from 24 hours after dosing to two-weeks post-dosing were only trending in significance. Any effect observed over this time period was likely an effect of CBD which has been hypothesized to remain in the system longer than other cannabinoids following chronic exposure as it can be stored in fatty tissue (Millar et al., 2018). Harte-Hargrove and Dow-Edwards (2012) observed a similar long-term effect in their examination of adolescent THC exposure which showed that locomotor depression was initially ablated following drug cessation, however, re-appeared following a period of abstinence. What little effects of cannabis exposure on long-term behaviour we observed are curious as again, the vast majority of the literature suggests that exposure to cannabinoids illicit hypolocomotion in animals. Looking at the graphical representation of the data collected here suggests that in not the case. No combination of time of testing, treatment, and sex resulted in a mean total activity lower than that of control animals. In fact, animals exposed to the 40 mg/kg dose of cannabis extract #81 showed an increase in their activity levels two weeks after dosing. Because of the two-week washout period prior to this third testing point, it is possible that chronic exposure to these specific strains of high-CBD cannabis generated structural or functional changes in the brain that persisted past the stage of acute exposure. Chronic cannabis exposure can result in many plastic changes in the brain including increasing grey matter in, and attenuating the functional connectivity of, the basal ganglia (Moreno-Alcázar et al., 2018; Blanco-Hinojo et al., 2017). Chronic

exposure to THC has also been found to significantly reduce CB1 receptor binding in the basal ganglia (Romero et al., 1997). However, again considering the limited amount of THC present in these extracts and the two-week washout period, this is not likely the main mechanism of action.

To explore another potential mechanism, we can look to another functional target of CBD. Research has demonstrated that CBD may also act on serotonergic receptors in the brain to modulate serotonin transmission (Zanelati et al., 2010; Gomes, 2011). The basal ganglia are highly innervated by serotonergic inputs which exert a modulatory role over dopamine release (for a review see Marthur & Lovinger, 2012; Westfall et al., 1982). Research has shown that the effect of CBD on locomotor behaviour is at least in part directed by its interaction with these serotonergic receptors in the basal ganglia (Espejo-Porras et al., 2013). The commonly observed trend holds once again, however, as these researchers noted only decreases in measures of locomotor activity compared to controls. This leads to another potential explanation for the hyperlocomotion observed at two weeks post-dosing. Serotonergic receptors have also been proposed as the target of CBD's anxiolytic effects (Zanelati et al., 2010). An increase in locomotor activity may be indicative of a decrease in anxiety-like behaviour. The effects of high-CBD cannabis exposure on anxiety-like behaviours will be discussed in a subsequent chapter.

Conclusion

These high-CBD cannabis extracts appear to have limited long-term effects on total locomotor activity levels in rats. There were no differences observed in the change in locomotor activity immediately following the cessation of dosing. The only effects of

drug exposure that were observed manifested as a less substantial decrease in activity levels from immediately following dosing to two weeks following dosing in one case (10 mg/kg extracts #81+98) and a marginal increase in activity levels across this time in another (40 mg/kg extract #81). Anxiolytic properties of CBD may be responsible for the apparent elevated levels of activity in these dosing groups compared to controls and will be further explored in Chapter 5.

Chapter 4

Long-Term Effects of Chronic, High-CBD Cannabis Exposure on Fine Motor Function

Introduction

Skilled Motor Function

Rats, like many other mammals, are able to make skillful prehensile movements of the forepaw in order to grasp and manipulate objects in their world. Studies of non-human animals have revealed that there are many motor centres in the brain, each contributing in unique ways to the motor program of the animal. In rats, the motor program which controls forepaw reaching is thought to be a fixed, relatively rigid motor program controlled by a highly organized neural network (Metz & Whishaw, 2000). Included in this network are areas we have previously discussed as being involved in locomotor activity including the basal ganglia, and associated structures like the putamen, and striatum (Whishaw et al., 1986; Wise & Donoghue, 1986 p 250), as well as higher cortical areas like the motor and somatosensory cortices of the frontal and parietal lobes (Woolsey et al., 1952; Wise & Donoghue, 1986 p 248). These cortical areas are critical for integrating motor and sensory inputs and executing the appropriate motor behaviour. Furthermore, people with degenerative diseases of areas like the basal ganglia characteristically have difficulty executing motor movements. Lesions to the caudate putamen, and motor cortex impair skilled reaching in the rat, further suggesting the importance of these two areas in the generation and execution of motor programs (Whishaw et al., 1986).

The Role of the Endocannabinoid System in Motor Function

Many of the areas involved in skilled motor movement contain a high density of cannabinoid receptors. As was discussed in chapter three, the basal ganglia, which contain a dense distribution of cannabinoid receptors, are highly involved in regulating locomotor activity (Herkenham et al., 1991). These receptors are often the target for both endogenous and exogenous cannabinoids to modify the production of both glutamate and GABA (and by extension, dopamine) which influences gross motor activity (For a review see Fernandez-Ruiz et al., 2002). As was discussed in Chapter 1, the frontal cortex also has a high density of cannabinoid receptors. The human primary motor cortex, however, has been shown to have the lowest density of cannabinoid receptors of all motor related brain areas (Glass et al., 1997). This would suggest that it is the structures of the basal ganglia, rather than higher cortical areas, that would be the most likely source of any effect cannabinoids would have on motor function. We may also then consider that the influence of exogenous cannabinoids on motor behaviour is not likely to affect the selection of the motor program, a function of the motor cortex, but rather the execution of the motor program. Recall that the basal ganglia have a critical inhibitory role in ensuring there are not completing motor programs activated while the intended program is being carried out. Again, the degradation of this inhibitory system is thought to be the root of the symptoms of motor dysfunction in several neurodegenerative diseases including Parkinson's and Huntington's disease (For a review see Fernandez-Ruiz, 2009).

Effects of Exogenous Cannabinoids on Motor Function

As has been previously discussed, there is a wealth of literature exploring the effects of exogenous cannabinoids on motor behaviour, with the majority focusing on

simple motor behaviours like locomotion. In these tests, THC appears to have a biphasic, or even triphasic effect, causing hyperlocomotion at low doses and hypolocomotion at higher dosages until catalepsy is reached. The effects of exogenous cannabinoids on skilled motor behaviours, however, is far less researched. Scullion and colleagues (2016) utilized a single pellet reaching task to explore the effects of THC administration on complex motor behaviour and found that exposure to 1.0 mg/kg THC or 2.5 mg/kg THC did not significantly affect the reaching success of treated animals compared to controls. Exposure to these dosages of THC did appear to generate changes in motor map expression in the brain, however, as mentioned, this did not lead to any behavioural deficiency in reaching success. They also found no difference in the components of the motor program that make up the reaching behaviour. While the effects of other cannabinoids like THCV on fine motor function specifically, have yet to be explored, administration of THCV has been suggested to influence the motor system through its action at the CB1 receptor. For example, administration of THCV can enhance motor functioning in animal models of Parkinson's disease (Garcia et al., 2011).

Specific Hypothesis

Based on the limited influence of THC on skilled reaching behaviour, and the relatively little amount of THC in the extracts being administered, in concert with the limited influence of CBD on general locomotor behaviour, I predict there will be no effect of high-CBD cannabis exposure on skilled reaching behaviour.

Methodology

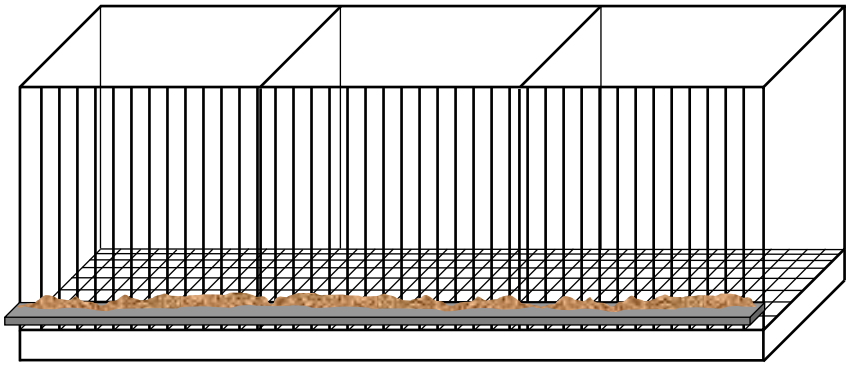
Tray Reaching Testing Paradigm

The tray reaching task is used as a measure of fine motor function as described by Whishaw, O'Connor, and Dunnett (1986) (Fig 4.1). In this task, animals are placed into the apparatus (20cm x 27cm x 19.5cm) where three sides are made of clear Plexiglas®, and the fourth side is made up of thin metal bars (3mm wide and 10mm apart) running vertically across the front of the box. A removable food tray, filled with chicken feed, runs horizontally across the front of the apparatus, flush with the floor. The bottom floor of the box is elevated five cm off the table and is made of a wire mesh such that anything the animal drops is then inaccessible. The testing paradigm began by habituating the animals to the apparatus for 30 minutes a day for five days. Following habituation, the animals are trained for two weeks to reach through the narrow bars at the front of the cage, grasp the food with their paw, and bring it back inside the apparatus to eat the piece of food. During this training period, the animals were food restricted to 90% of their average body weight to encourage reaching. At the end of the two-week training period, after ensuring all animals were performing the task proficiently, the animals were filmed for a 10-minute period to assess how successfully they are able to complete the task. The number of successful reaches, whereby an animal extends their paw through the bars, successfully grabs the piece of food, brings it back through the bars and eats it, are tallied along with any unsuccessful attempts where the food is dropped along the way.

The results are reported as a percent success by dividing the number of successful reaches by the total number of reaches as illustrated below:

$$\%\ Success = \frac{Number\ of\ Successful\ Reaches}{Total\ Number\ of\ Reaches\ (Both\ Successful\ and\ Unsuccessful)}$$

Figure 4.1
Whishaw Tray Reaching Apparatus



Note. A schematic of the Whishaw tray reaching apparatus

Statistical Analysis

Analysis of tray reaching success was conducted using a two-way ANCOVA, controlling for total number of reaches, with treatment (control, 10 mg/kg (low dose) or 40 mg/kg (high dose)) and sex (female or male) as factors.

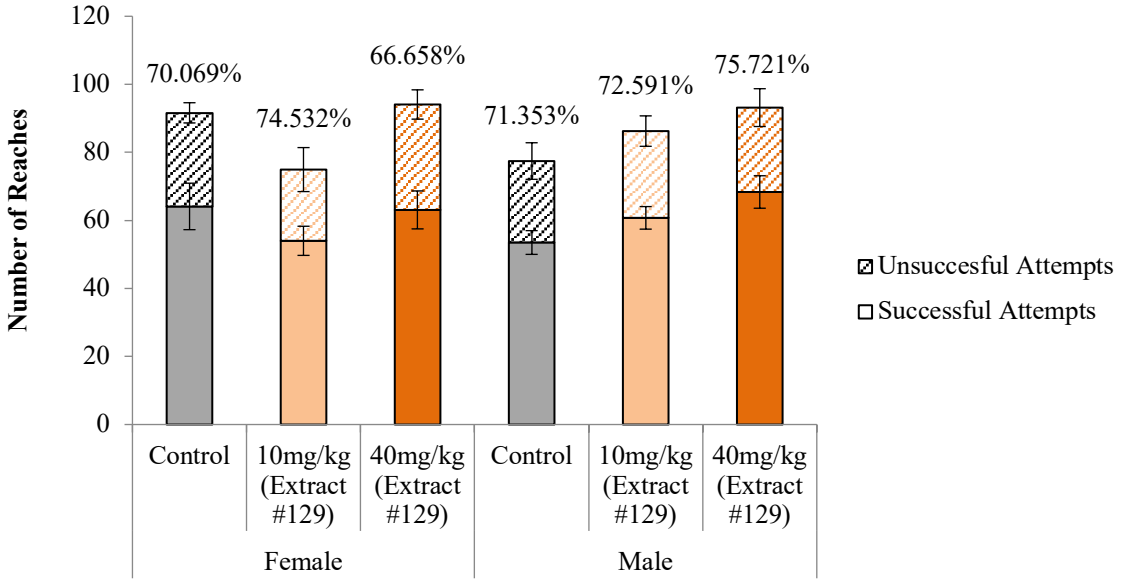
Results

Effect of Cannabis Extract #129 on Reaching Success

The covariate, total number of reaches, was significantly related to the overall reaching success $F(1, 48) = 14.689$, $p < .001$, $\eta p^2 = .234$. There was no effect of exposure to extract #129 observed in measures of reaching success when controlling for total number of reaches, all treatment groups performed equally $F(2, 48) = .493$, $p = .614$ $\eta p^2 = .020$ (Fig. 4.2). There was no observed effect of sex as female and male animals did not differ in reaching success $F(1, 48) = .398$, $p = .531$, $\eta p^2 = .008$. Finally, there was no observed Treatment x Sex interaction suggesting that the effect of the treatment did not differ depending on the sex of the animal, $F(2, 48) = .881$, $p = .421$, $\eta p^2 = .035$.

Figure 4.2

The Effect of Extract #129 on Reaching Success



Note. This figure displays the ratio of successful to unsuccessful reaches, as a function of treatment group and sex. Data labels represent the reaching percent success. Error bars represent the standard error of the mean.

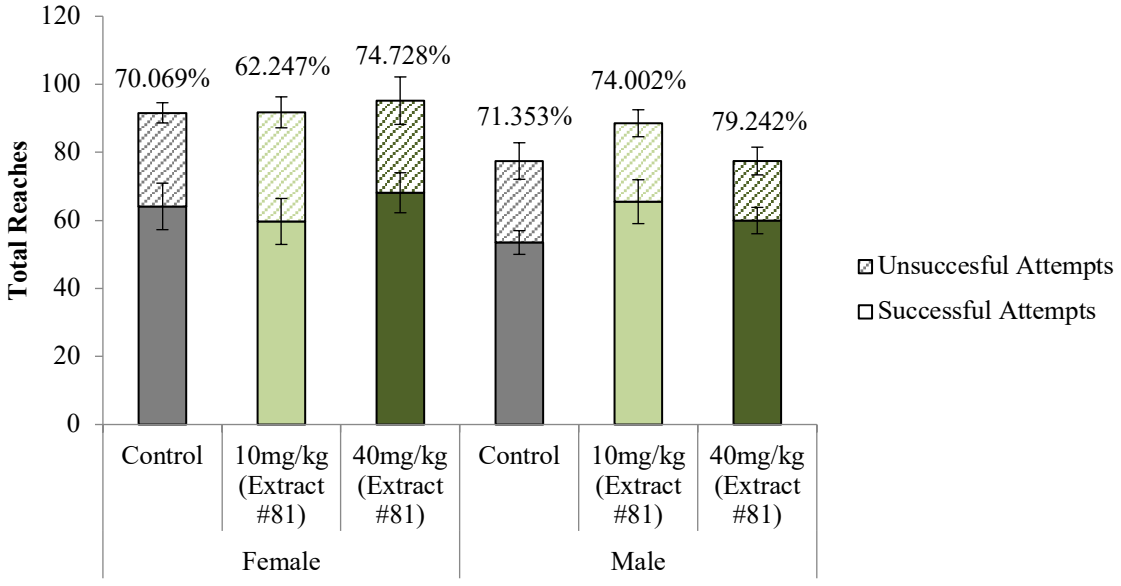
[a] Exposure to extract #129 did not affect the reaching success of exposed animals.

Effect of Cannabis Extract #81 on Reaching Success

The covariate, total number of reaches, was close to being significantly related to the overall reaching success $F(1, 46) = 3.795$, $p = .058$, $\eta p^2 = .076$. There was no significant effect of exposure to extract #81 in measures of reaching success when controlling for total reaches, all treatment groups performed equally $F(2, 46) = 2.265$, $p = .115$ $\eta p^2 = .090$ (Fig. 4.3). There was no effect of sex on reaching success as female and male animals did not differ in reaching success $F(2, 46) = 1.303$, $p = .316$, $\eta p^2 = .028$. There was no significant Treatment x Sex interaction suggesting that the effect of the treatment did not differ depending on the sex of the animal, $F(2, 46) = 1.183$, $p = .316$, $\eta p^2 = .049$

Figure 4.3

The Effect of Extract #81 on Reaching Success



Note. This figure displays the ratio of successful to unsuccessful reaches, as a function of treatment group and sex. Data labels represent the reaching percent success. Error bars represent the standard error of the mean.

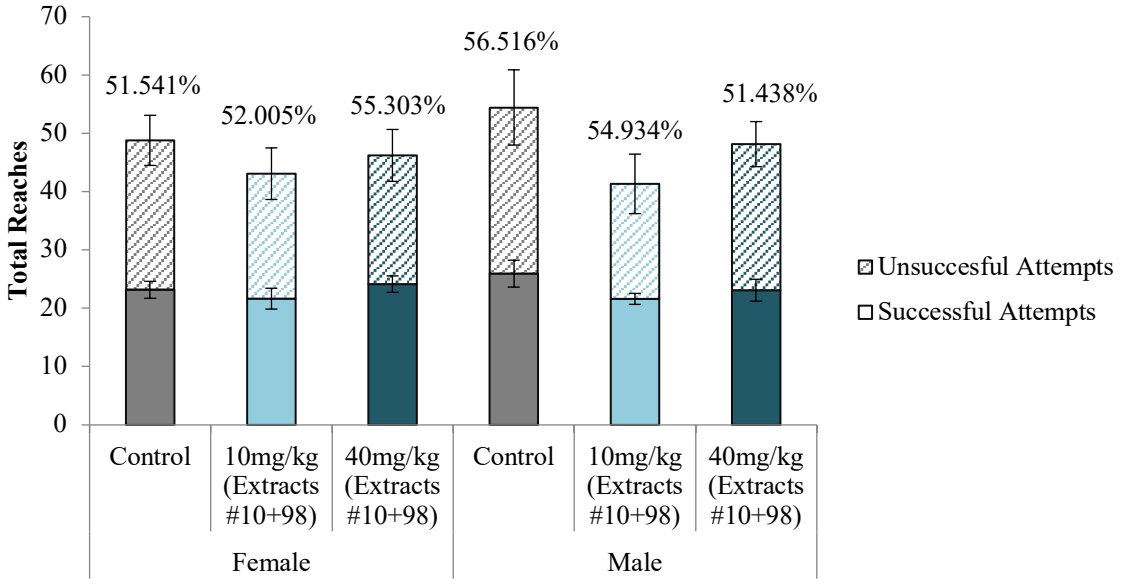
[a] Exposure to extract #81 did not affect the reaching success of exposed animals.

Effect of Cannabis Extracts #10+98 on Reaching Success

The covariate, total number of reaches, was significantly related to the overall reaching success $F(1, 59) = 73.740$, $p < .001$, $\eta p^2 = .556$**.** There was no significant effect of exposure to extracts #10+98 in measures of reaching success when controlling for total reaches, all treatment groups performed equally $F(2, 59) = .029$, $p = .972$., $\eta p^2 = .057$ (Fig. 4.4). There was no effect of sex on reaching success as female and male animals did not differ in reaching success $F(1, 59) = .328$, $p = .569$, $\eta p^2 = .006$. Finally, there was no significant Treatment x Sex interaction suggesting that the effect of the treatment did not differ depending on the sex of the animal, $F(2, 59) = 1.338$, $p = .270$, $\eta p^2 = .043$.

Figure 4.4

The Effect of Extracts #10+98 on Reaching Success



Note. This figure displays the ratio of successful to unsuccessful reaches, as a function of treatment group and sex. Data labels represent the reaching percent success. Error bars represent the standard error of the mean.

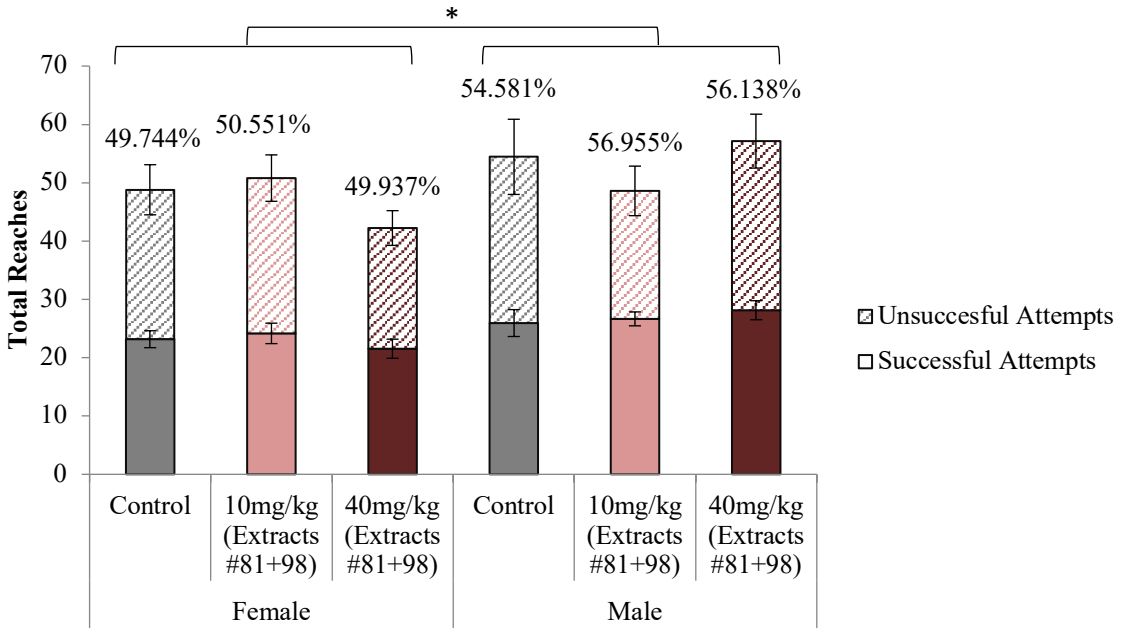
[a] Exposure to extract #10+98 did not affect the reaching success of exposure animals.

Effect of Cannabis Extracts #81+98 on Reaching Success

The covariate, total number of reaches, was significantly related to the overall reaching success $F(1, 59) = 71.200$, $p < .001$, $\eta p^2 = .547$. There was no significant effect of exposure to extracts #81+98 in measures of reaching success when controlling for total reaches, all treatment groups performed equally $F(2, 59) = .176$, $p = .839$, $\eta p^2 = .006$ (Fig. 4.5). There was a significant sex effect observed on reaching success as female had an overall lower success rate than males $F(1, 59) = 7.179$, $p = .010$, $\eta p^2 = .108$. There was no significant Treatment x Sex interaction suggesting that the effect of the treatment did not differ depending on the sex of the animal, $F(2, 59) = .051$, $p = .951$, $\eta p^2 = .002$.

Figure 4.5

The Effect of Extracts #81+98 on Reaching Success



Note. This figure displays the ratio of successful to unsuccessful reaches, as a function of treatment group and sex. Data labels represent the reaching percent success. Error bars represent the standard error of the mean.

[a] Exposure to extracts #81+98 did not affect the reaching success of exposed animals, however there was a significant effect of sex observed whereby female animals appeared overall less successful at reaching than male animals.

*$p < .025$

Discussion

General Discussion

This analysis examined the influence of high-CBD cannabis extract on skilled, fine motor behaviour in the Long-Evans rat. Fine motor control was assessed in conjunction with general locomotor activity to allow us to better gauge the full extent of the impact of exposure to this kind of high-CBD cannabis on motor behaviour. Exposure to high-CBD cannabis extract had no effect on skilled motor behaviour as assessed by the Whishaw tray reaching task. We did observe an effect of sex, only in analysis of animals administered extracts #81+98, whereby female animals had lower reaching success than males. However, there was no Treatment x Sex interaction observed suggesting the treatment did not affect the female and male animals differently and was not driving this effect.

High-CBD Cannabis Does Not Have Long-Term Effects on Skilled Motor Behaviour

First let us address the potential role of the THC component of the whole cannabis extracts in reaching behaviour. As was mentioned in the introduction, even acute exposure to THC appears to have no effect on skilled reaching behaviour (Scullion et al., 2016). This observation was true for both the success rate of reaching behaviour as well as of the individual component behaviours that make up the reaching program (for details of the subcomponents see Whishaw et al., 1993). Considering the relatively low levels of THC in the four cannabis extracts utilized and the approximately two-week delay between the final dosage and the testing session, we did not anticipate there would be a significant influence of THC on skilled reaching behaviour to begin with. Scullion's exploration (2016) does posit an interesting finding; that acute exposure to THC changed

the organization of the motor map in the brain. This has also been shown in other studies which have explored manipulations to neurotransmission in the brain, including the cholinergic, serotoninergic, and dopaminergic systems, which can also result in changes in fine motor skills (Ramanathan et al., 2009; Gharbawie & Whishaw, 2003). This raises an interesting question. As CBD appears to target the serotonergic system as a mechanism of action in the basal ganglia, and the basal ganglia contains significant connections with the motor cortex, could this pathway cause a CBD-driven change in motor mapping while not influencing motor outcomes? It does appear that the cannabinoid system is involved in the development of motor maps in the rodent brain (Li et al., 2009). However, if we recall, Scullion and colleagues (2016) found that the change in motor mapping following THC exposure did not result in a change in reaching success, and was not observable 24 hours after administration, making it unlikely that a change in motor mapping from chronic CBD exposure would result in long-term changes in reaching behaviour. Indeed, it appears that chronic exposure to high-CBD cannabis extract simply has no long-term effects on this measure of fine motor control.

High-CBD Cannabis Does Not Have Long-Term Effects on Skilled Motor Learning

While administration of these high-CBD cannabis extracts appears to not have long-term effects on motor function execution, we may also consider the impact of these extracts on motor learning. Motor learning is classically defined as a type of procedural learning in which motor programs are refined with practise (Hallett, 2006, pp. 89). This process occurs in two stages; the acquisition phase, marked by high error rates and fast improvement in performance, followed by the consolidation phase, marked by attenuating error rates and incremental gains in performance following repetition of the

task (Karni et al., 1998). These stages are marked by structural and neurochemical changes in the motor centres of the cortex and hindbrain (Kleim et al., 1998; Karni et al., 1998; Yin et al., 2009; Miyachi at al., 1997; Miyachi et al., 2002). The Whishaw tray reaching task is a good metric of motor skill learning as the animals are trained to reach through the slatted bars at the front of the cage to acquire food, a motor behaviour which is then reinforced by two weeks of daily training. The endocannabinoid system is intrinsically involved in motor learning as this system has been observed to drive long-term plasticity in relevant brain networks (for a review see El Manira & Kyriakatos, 2010). For example, endocannabinoids-mediated long-term depression (LTD) in the striatum is proposed to play a role in motor control. Striatal LTD is dependent on CB1 and dopaminergic receptor activation, and in mice models of Parkinson's disease, administration of URB597, an inhibitor of FAAH, in combination with dopamine receptor agonists reduce characteristic motor deficits (Kreitzer & Malenka, 2007). In this system, endocannabinoids cause inhibition of GABA release, thereby activating the striatum, and leading to an observable improvement in motor function. In our experiment, motor learning began with reaching training, two days after the cessation of dosing, and continued for two weeks while the animals learned the task. Using the metric of overall reaching success as an indication of how well the animal learned the required motor program, it is evident that chronic exposure to high-CBD cannabis did not affect motor learning as there were no effects of treatment on the outcome variable.

Conclusion

In humans, cannabinoids have been shown to ameliorate negative symptoms of complex movement disorders like spasticity in children (Lizbon et al., 2018), and are thought to be a potential approach to treating motor disorders associated with degradation of the basal ganglia, seen in Parkinson's disease and Huntington's disease (for a review see Fernández-Ruiz, 2009). The preceding analysis showed that there are no long-term effects on motor learning or motor function following chronic high-CBD cannabis use. An encouraging discovery considering the potential future therapeutic applications of this sort of drug. However, future research should consider the potential acute effects of high-CBD extracts on motor function, as well as alternative measures of motor function in case some sort of compensation was at play here (although compensatory reaching mechanisms frequently result in overall lower reaching success (Metz & Whishaw, 2000)). Future research should also consider measuring acquisition rate of the reaching task to confirm the null effect of high-CBD cannabis extract on motor learning as was suggested by the reaching success results provided in this study. In summation, this analysis provides encouraging findings that chronic exposure to high-CBD cannabis extracts does not have long-term repercussions on skilled motor behaviours in the Long-Evans rat.

Chapter 5

Long-Term Effects of Chronic, High-CBD Cannabis Exposure on Anxiety-Like Behaviour

Introduction

Cannabis and Anxiety

The relationship between cannabis and anxiety or stress, is a complex one. Chronic cannabis use in humans has been shown to be positively correlated to a number of mood disorders including anxiety disorders and depression (Kedzior & Laeber, 2014; Degenhardt et al., 2003). However, these findings need to be carefully considered in context as we should be wary of falling into the logical fallacy of 'post hoc ergo propter hoc', or 'after it, therefore, because of it'. This refers to the tendency to assume that correlation implies causation, which is rarely true. As in the case of the relationship between cannabis use and anxiety, there could be many contextual factors that contribute to this correlation including social and genetic factors (Table 5.1).

Table 5.1

Factors Associated with Cannabis Induced Anxiety

Individual and genetic vulnerability
Personality traits
Sex
Frequency of use
Dose and quantity consumed
Proportions and concentrations of cannabinoids (especially of THC and CBD)
History of previous episode
Presence of anxiety disorders/symptoms
Basal anxiety levels
Abstinence states
Environment and context of use

Note. Adapted from Crippa et al., 2009.

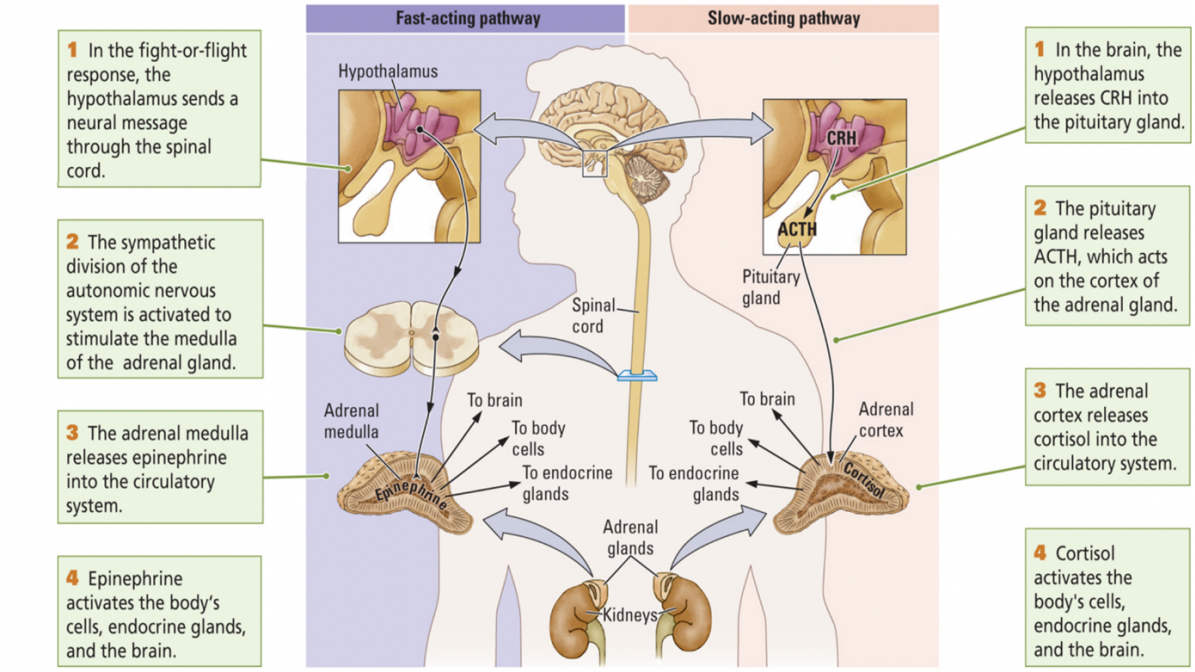
Despite these parallels, many people who use cannabis do so because of the frequently reported anxiolytic, or anxiety reducing properties of cannabis. In the year 2020, three of the top four reasons Canadians gave for increasing their cannabis use during the COVID-19 pandemic included to relax (73%), because of stress (53%) and because of anxiety (53%) (Government of Canada, 2021). Another factor complicating the relationship between cannabinoids and anxiety is that, just as observed in locomotor behaviour, exogenous cannabinoids influence anxiety-like behaviours in a biphasic manner with low doses of cannabis acting to reduce anxiety, while high doses of cannabis appear to increase anxiety (for a review see Moireria et al., 2009).

Stress in the Brain

To better understand how cannabis affects anxiety-like behaviours, let us first discuss the body's internal stress-mediating mechanisms. This system involves both the central and peripheral nervous systems and is primarily mediated by the hypothalamic-pituitary-adrenal axis, or HPA axis (Fig. 5.1). During stressful situations, the paraventricular nucleus of the hypothalamus is signalled by a network of stress-sensing brain regions to secrete the peptide hormone, corticotropin-releasing hormone (CHR) (Herman & Cullinan, 1997). This peptide then signals the anterior pituitary to produce adrenocorticotropin (ACTH), which circulates though the blood stream and triggers the adrenal glands to produce cortisol, the primary stress hormone. There are strong similarities between the stress signalling pathways in both rats and humans although the primary stress hormone in rats is corticosterone. Cortisol has a wide range of acute and chronic effects. The primary role of this hormone is to divert energy away from costly metabolic processes and 'turn-off' body systems that are not immediately required to deal

with the stressful stimuli, like the reproductive and immune systems. Kolb, Whishaw, and Teskey (2019, pp. 205) have differentiated the forementioned stress pathway from an alternative, faster acting pathway. The process of cortisol release can take in the order of hours, however in this fast-acting stress pathway a signal is sent from the hypothalamus via the spinal cord to the adrenal glands which release epinephrine into the body, triggering the classic 'fight or flight' response (2019).

Figure 5.1
The Stress Response



Note. This figure demonstrates the two pathways governing the body's stress response (Kolb, Whishaw & Teskey, 2019, pp. 205)

Typically, cortisol regulates its release via a negative feedback mechanism in the brain. Cortisol receptors in the paraventricular nucleus as well as the hippocampus, which contains significant projections to the hypothalamus, in concert with steroid feedback systems, work to return the body to a state of homeostasis following a stressful event

(Sapolsky, 2005; Herman & Cullinan, 1997). In situations of chronic stress however, constantly elevated cortisol levels can result in a number of damaging effects including muscle wasting or fatigue, growth hormone inhibition and gastrointestinal issues (Kolb, Whishaw and Teskey, 2019). In fact, in both murine and primate models, chronic stress has been shown to cause a number of disadvantageous changes in the brain including reductions in neuronal branching and synaptic connections in the prefrontal cortex, changes in neurotransmitter signalling and atrophy of the hippocampus (Wellman, 2001; see Herman & Cullinan, 1997 for review; Sapolsky, 2005).

The Endocannabinoid System and Anxiety

The brain's endogenous cannabinoid system plays a critical role in regulating the body's stress response. Specifically, activation of the CB1 receptor is integral in the body's stress signalling pathways (Hill, McLaughlin, et al. 2010; Hill, Patel, et al. 2010). These CB1 receptors are abundant in many brain areas critical in perceiving and mediating stressful stimuli including the amygdala, the hypothalamus, the hippocampus, and the prefrontal cortex (Katona et al., 2001; Cristino et al., 2006; Katona et al., 1999; Lisboa et al., 2015). One way to explore the critical role of endocannabinoid signalling in the stress response, is to modulate the levels of endogenous cannabinoids AEA and 2-AG in the brain. Studies exploring the influence of endogenous cannabinoids has shown that genetic deletion of FAAH, the primary AEA-degrading enzyme in the central nervous system, leads to increased AEA levels in the brain which in turn leads to decreases in anxiety-like behaviours (Moirera et al. 2008; Bambico et al., 2010). However, it has recently been suggested that this observed effect may only be relevant when the animal is faced with significantly aversive stimuli and not mildly aversive stimuli (Haller et al.,

2009). Similarly, it has also been shown that decreased levels of 2AG in the brain cause increases in anxiety-like behaviours (Shonesy et al., 2014). To further investigate the target of the endocannabinoid action in relation to anxiety, Rey and colleagues selectively knocked down CB1 receptors in either GABAergic or glutamatergic neurons. What they discovered was the anxiolytic effect of cannabinoids at low doses depends on CB1 receptor activation on cortical glutamatergic neurons, whereas the anxiogenic effect at high doses is mediated by CB1 receptor activation of forebrain GABAergic neurons (2012). Recall that cannabinoids act as retrograde inhibitors in the brain, so the activation of CB1 receptors on glutamatergic neurons will lead to the suppression of glutamate release, and activation of CB1 receptors on GABAergic neurons will lead to the suppression of GABA release. Suppressing glutamate release will result in an overall inhibitory signal or reduced activation, and because these target neurons are in systems responsible for the activation and maintenance of the stress response, an overall anxiolytic effect is observed. The receptors on these neurons differ in both their sensitivity and basal activity as the sensitivity of CB1 receptors is higher on GABAergic neurons than glutamatergic (Ohno-Shosaku et al., 2002; Ruehle et al, 2012). The tonic activity of the endocannabinoid system is also different across different subtypes of neurons. Basal levels of endocannabinoids create stronger inhibition at GABAergic sites than they do at glutamatergic sites which may also contribute to the biphasic effect of cannabinoids on anxiety as low levels of cannabinoids would first affect the glutamatergic neurons (Roberto et al., 2010; Rey et al., 2012).

Exogenous Cannabinoids and Anxiety

Acute exposure to THC also elicits biphasic effects, leading to reduced anxiety-like effects when low doses (5 or 10 ug) are injected into the ventral hippocampus and frontal cortex respectively, whereas high doses of the drug were found to be anxiogenic (Rubino et al., 2008). Interestingly, this profile was not maintained following THC injection in the basolateral amygdala where even low doses increased anxiety-like behaviours and high doses were simply ineffective (Rubino et al., 2008). It has been suggested, and it would appear such given the findings of Rubino and colleagues, that the influence of the endocannabinoid system on fear response (dependent on the basolateral amygdala) is distinct from the HPA axis, and what we are calling the stress response (Kamprath et al., 2009). Cannabidiol shares a response curve similar to that following THC administration in the basolateral amygdala. At low to medium doses, CBD administration results in anxiolytic effects, while at higher doses, the drug becomes ineffective (Zuardi et al., 1981; Guimarães et al., 1990; Onaivi et al., 1990). Functional imaging studies in humans have suggested that these effects are mediated by CBD activity in limbic and paralimbic regions of the brain including the amygdala, hippocampus, and the hypothalamus where administration was shown to decrease activity (Crippa et al., 2004). The mechanism by which CBD exerts its effects on anxiety are dependent on a number of factors including the area of the brain being observed, the kind of behavioural test being administered to assess anxiety levels and the animal's history (for a review see Blessing et al., 2015). However, it is likely that that serotonergic, GABAergic CB1, and TRPV1 receptors in the relevant brain regions are all involved (Fogaça et al., 2014; Onaivi et al., 1990; Bisogno et al., 2001). In a manner similar to

other cannabinoids, administration of THCV has been shown to reduce measures of anxiety in an animal model. Zagzoog and colleagues (2020) observed that a dose of both 1 mg/kg and 10 mg/kg of THCV increased the amount of time mice spent in the central quadrant of the open field test, suggesting an anxiolytic effect. Contrastingly, an exploration of the effect of chronic THCV exposure done by O'Brien et al., (2013) found that on day 1, 7 or 14 of dosing, THCV did not affect anxiety-like behaviour at a dose of 2.5 mg/kg which they attribute to "neutral antagonism" of the CB1 receptor. Recall that at higher doses higher than 10 mg/kg, THCV can act as a CB1 agonist *in vivo*. Perhaps at doses exceeding this level, a profile affecting anxiety-like behaviour similar to THC would arise, but that has yet to be evaluated.

Specific Hypothesis

I hypothesize that chronic exposure to high-CBD cannabis extracts will have little to no effect on anxiety-like behaviours. It is predicted that if any effects are to be observed they will manifest as a reduction in anxiety-like behaviours.

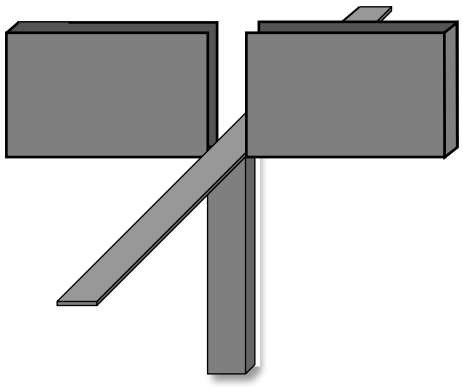
Methodology

Elevated Plus Maze Testing Paradigm

The elevated plus maze (EPM) task utilizes a "+" shaped structure to assess anxiety-like behaviour and exploratory behaviour (Handley & Mithani, 1994). This structure consists of two closed arms, walled-in with black opaque Plexiglas®, and two open arms, which have no walls (Fig. 5.2). The animal is placed in the center square of

the structure and allowed to explore for five minutes while their behaviour is recorded. The time the animal spends in each part of the structure is summed (closed arm, open arm, and center square) as are the number of entries into each section of the maze. Closed arm time, and number of entries into the closed arm are used as a metric of assessing anxiety-like behaviour.

Figure 5.2
Schematic Representation of the Elevated Plus Maze Apparatus



Note. The image above is a simplified visual representation of the apparatus used to assess anxiety-like behaviour.

Open Field Testing Paradigm

The open field apparatus was also used to assess anxiety-like behaviour. The testing paradigm of the open field is detailed in the previous chapter. The marginal area of the open field was defined as the outermost 2.5 cm perimeter of the apparatus and time spent in this area was used as a metric of anxiety-like behaviour.

Statistical Analysis

Analysis of anxiety-like behaviour in the EPM was conducted using a repeated measure, two-way ANOVA with treatment (control, 10 mg/kg (low dose) or 40 mg/kg

(high dose)) and sex (female or male) as between-subject factors, and time of treatment (pre-exposure and post-exposure) as a within-subject factor. Analysis of anxiety-like behaviour in the open field was conducted using a repeated measure, two-way ANOVA with treatment (control, 10 mg/kg (low dose) or 40 mg/kg (high dose)) and sex (female or male) as between-subject factors, and time of treatment (adolescence, immediately following dosing, and two weeks following dosing) as a within-subject factor. Planned contrasts were used to further investigate the difference in activity levels between time points, based on treatment group where appropriate.

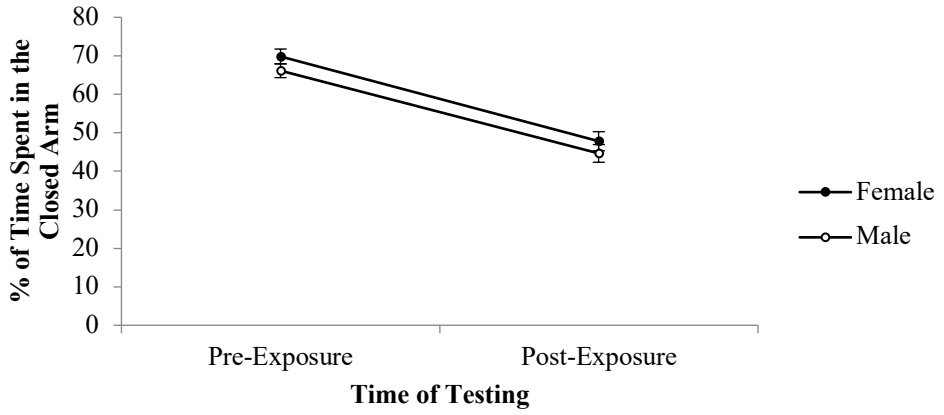
Results

Long-Term Effects of Extract #129 on Anxiety-Like Behaviour in the EPM

Percent of Time Spent in the Closed Arm. There was a significant main effect of time of testing on the percentage of time spent in the closed arm, which decreased following cannabis exposure, $F(1, 48) = 124.312$, $p < .001$, $\eta p^2 = .721$. There were no interaction effects observed between Time of Testing x Sex, $F(1, 48) = .018$, $p = .895$, $\eta p^2 = .000$, suggesting male and female animals showed the same decrease in the percent of time that was spent in the closed arm following cannabis exposure (Fig. 5.3); Time of Testing x Treatment, $F(2, 48) = 2.030$, $p = .143$, $\eta p^2 = .078$, suggesting all treatment groups showed the same decrease in the percentage of time that was spent in the closed arm following cannabis exposure (Fig. 5.4); or Time of Testing x Sex x Treatment, $F(2, 48) = .421$, $p = .659$, $\eta p^2 = .017$, suggesting that the effect of the treatment did not differ depending on the sex of the animal (Fig. 5.5).

Figure 5.3

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #129 as a Function of Time of Testing and Sex

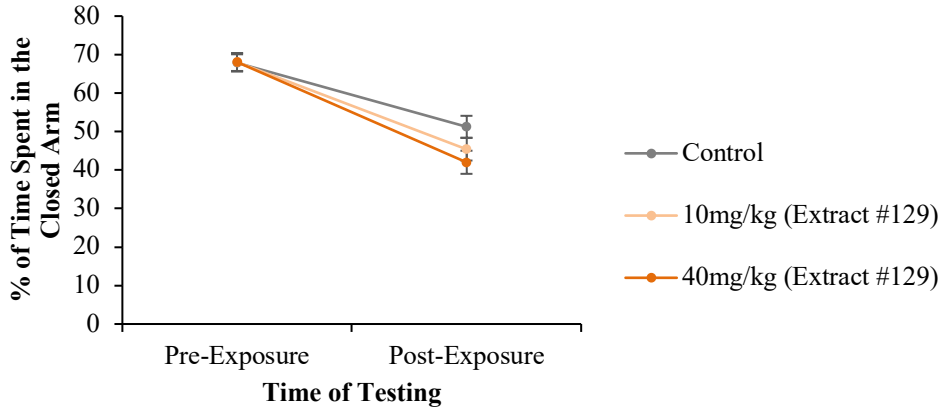


Note. This graph displays the mean change in the percentage of time spent in the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] There were no significant difference observed between male and female animals.

Figure 5.4

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #129 as a Function of Time of Testing and Treatment

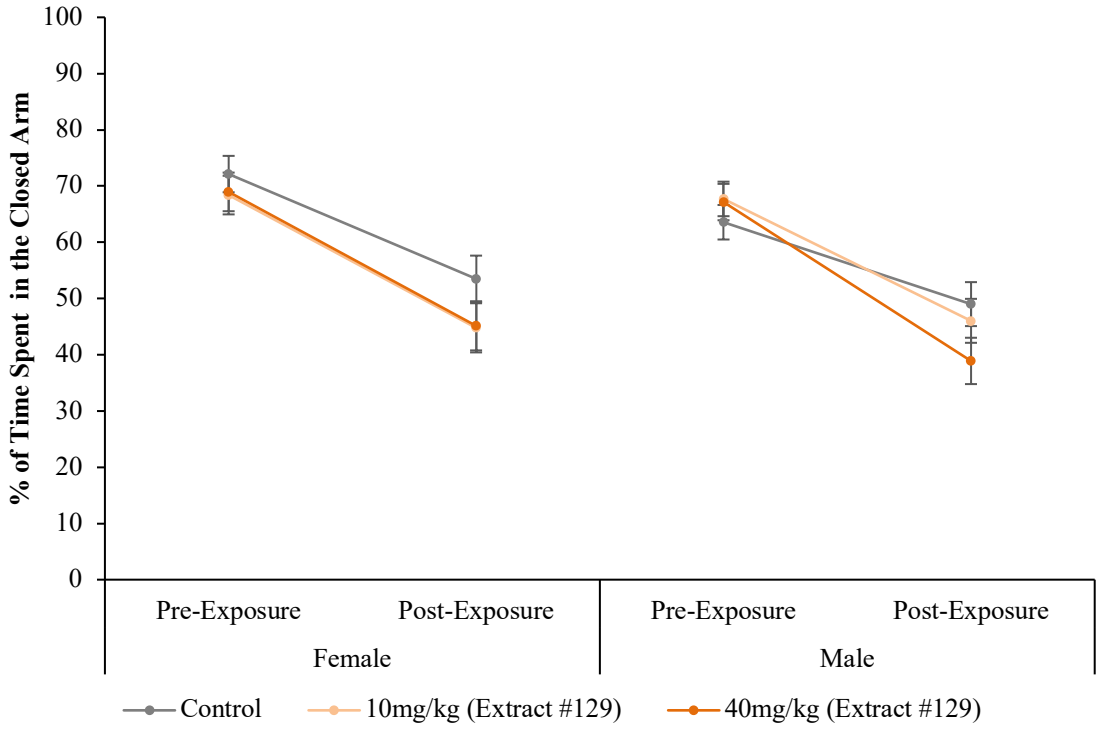


Note. This graph displays the mean change in the percentage of time spent in the closed arm of animals given extract #129, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing.

[a] There were no significant differences observed between treatment groups in the change in the percent of time spent in the closed arm.

Figure 5.5

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #129, as a Function of Time of Testing, Treatment, and Sex



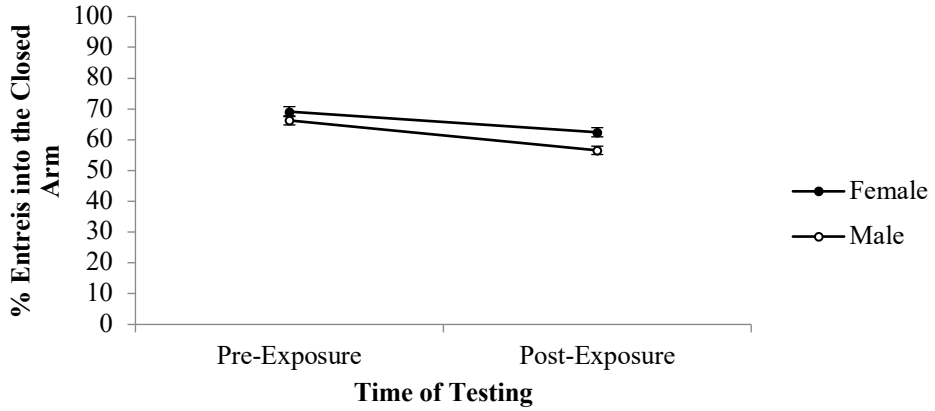
Note. This graph displays the mean percent of time spent in the closed arm of both male and female animals given extract #129, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing.

[a] There were no significant interaction effects observed, only a main effect of time of testing by which all animals showed a similar decrease in time spent in the closed arm from pre-to-post cannabis exposure.

Percentage of Entries Made into the Closed Arm. There was a significant main effect of time of testing on the change in the percentage of entries made into the closed arm suggesting the percentage of closed arm entries of all animals decreased significantly from pre-to-post cannabis exposure $F(1, 47) = 37.245$, $p < .001$, $\eta p^2 = .441$. There were no interaction effects observed between Time of Testing x Sex, $F(1, 47) = 1.260$, $p = .267$, $\eta p^2 = .026$, suggesting male and female animals showed the same change in closed arm entries following cannabis exposure (Fig. 5.6); Time of Testing x Treatment, $F(2, 47) = 1.184$, $p = .315$, $\eta p^2 = .048$, suggesting all treatment groups showed the same change in closed arm time following cannabis exposure (Fig. 5.7); or Time of Testing x Sex x Treatment, $F(2, 47) = 1.096$, $p = .342$, $\eta p^2 = .045$, suggesting that the effect of the treatment did not differ depending on the sex of the animal (Fig. 5.8).

Figure 5.6.

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #129 as a Function of Time of Testing and Sex

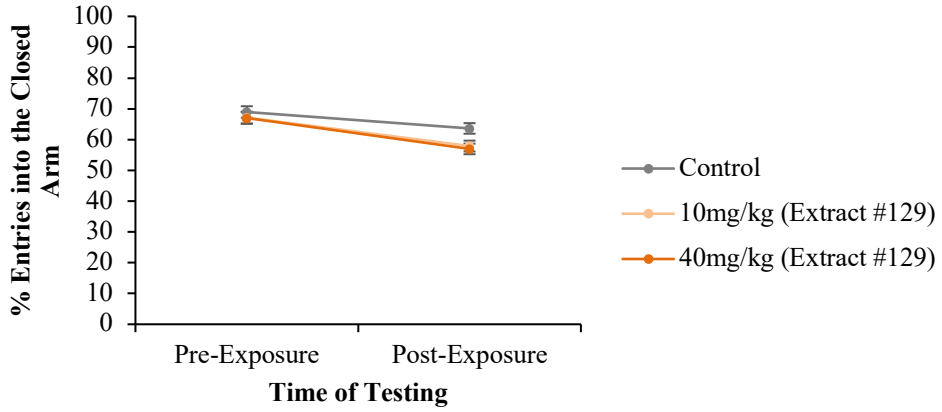


Note. This graph displays the mean change in the percentage of entries made into the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] There was no significant difference observed between male and female animals.

Figure 5.7.

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #129 as a Function of Time of Testing and Treatment

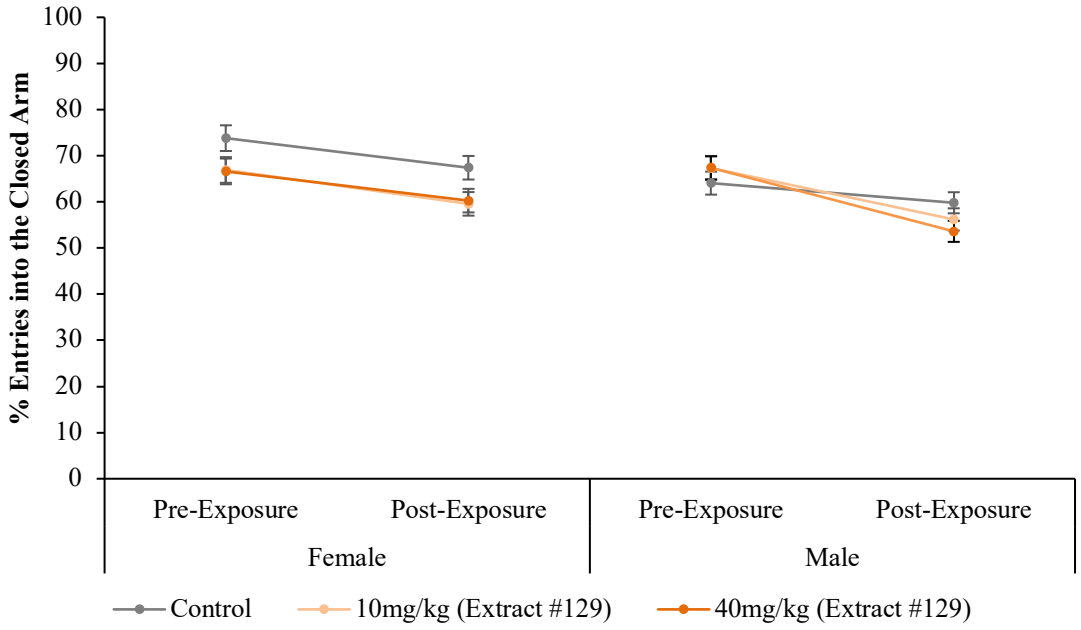


Note. This graph displays the mean change in the percent of entries made into the closed arm of animals given extract #129, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing.

[a] There were no significant differences observed between treatment groups in the change in the percent of entries made into the closed arm

Figure 5.8

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #129, as a Function of Time of Testing, Treatment, and Sex



Note. This graph displays the change in mean percentage of time spent in the closed arm of both male and female animals given extract #129, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing.

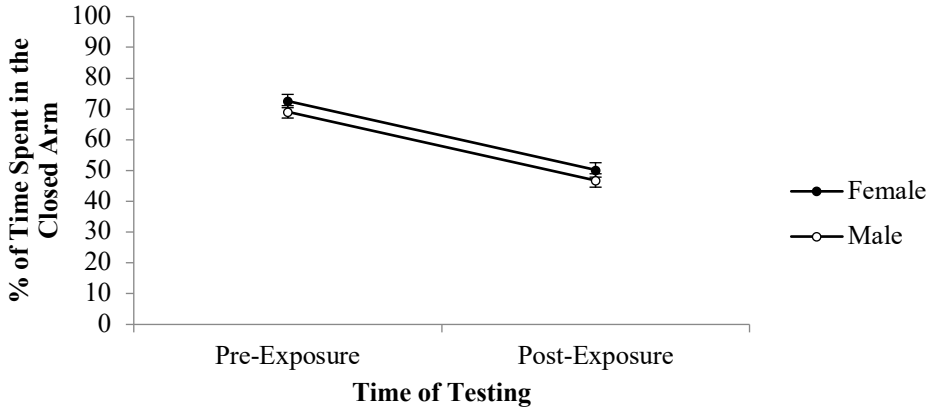
[a] There were no significant interaction effects observed

Long-Term Effects of Extract #81 on Anxiety-Like Behaviour in the EPM

Percent of Time Spent in the Closed Arm. A significant main effect of time of testing suggests that change in the percentage of time spent in the closed arm decreased from adolescence to adulthood $F(1, 48) = 127.380$, $p < .001$, $\eta p^2 = .726$. There was no Time of Testing x Sex interaction $F(1, 48) = .00$, $p = .984$, $\eta p^2 = .000$, suggesting the change in the percentage of time spent in the closed arm was not different between females and males (Fig. 5.9); no significant Time of Testing x Treatment interaction $F(2, 48) = 2.170$, $p = .125$, $\eta p^2 = .083$, suggesting cannabis treatment did not affect the change in time spent in closed arm following cannabis exposure (Fig. 5.10); and no significant Age x Treatment x Sex interaction $F(2,48) = .304$, $p = .739$, $\eta p^2 = .013$, suggesting the effects of cannabis exposure did not differ based on the sex of the animal (Fig. 5.11).

Figure 5.9

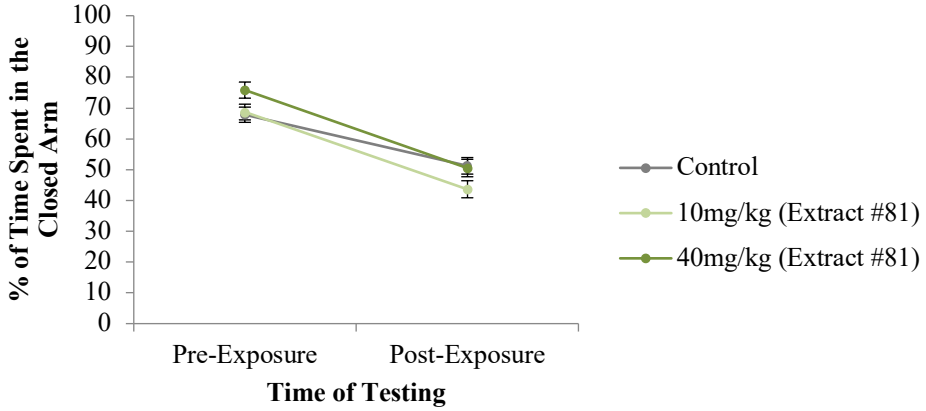
Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #81 as a Function of Time of Testing and Sex



Note. This graph displays the mean change in time spent in the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood.
[a] There was no significant difference observed between male and female animals.

Figure 5.10

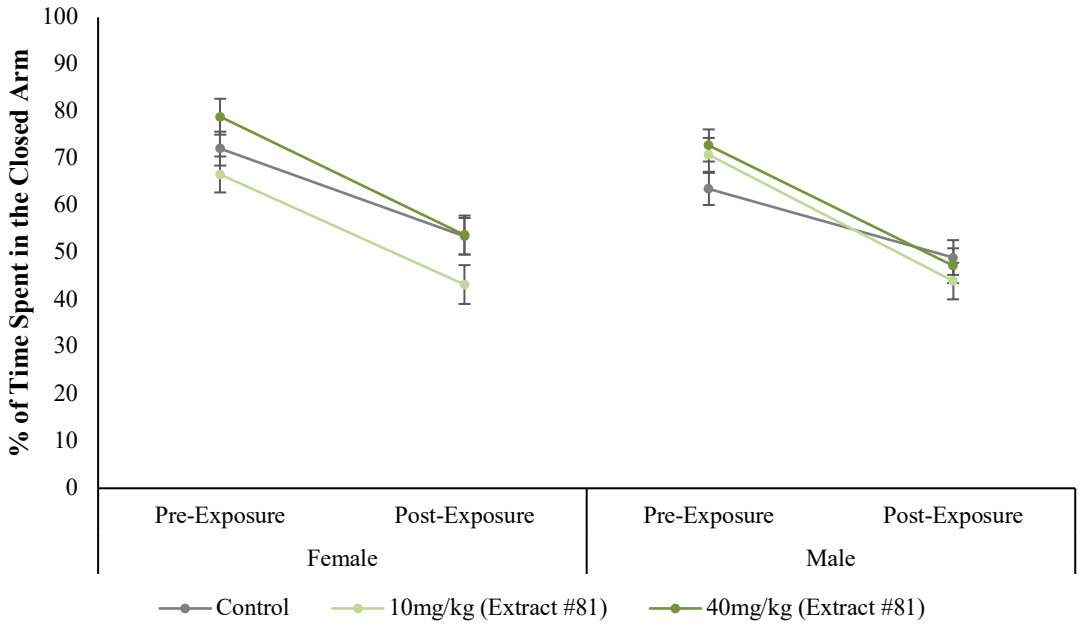
Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #81 as a Function of Time of Testing and Treatment



Note. This graph displays the mean change in the percentage of time spent in the closed arm of animals given extract #81, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.
[a] There were no significant differences observed between treatment groups in the change in the percentage of time spent in the closed arm.

Figure 5.11

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extract #81 as a Function of Time of Testing, Treatment and Sex



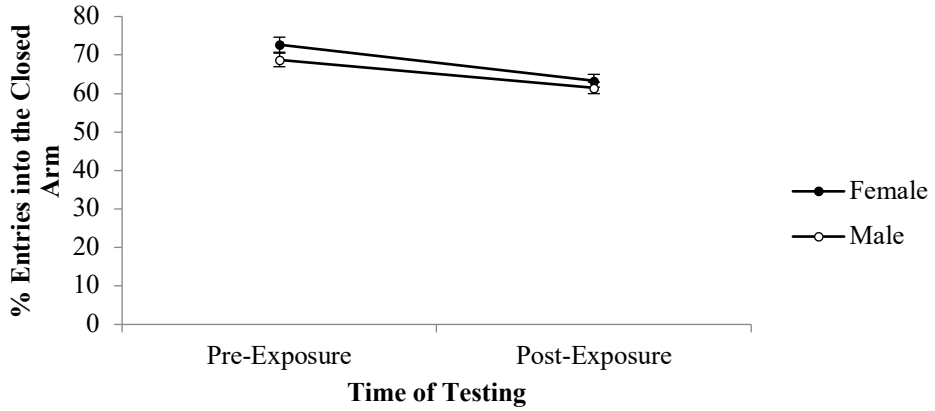
Note. This graph displays the mean closed arm time of both male and female animals given extract #81, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant interaction effects observed, only a main effect of time of testing by which all animals showed a similar decrease in time spent in the closed arm from pre-to-post cannabis exposure. Visualizing the data of this group appears to show a slight difference in male animals as both the low and high dose groups show a sharper decrease in time spent in the closed arm than controls. Recall however, that the Time of Testing x Treatment x Sex interaction was not significant.

Percent of Entries Made into the Closed Arm. There was a significant main effect of time of testing on the change in the percentage of entries made into the closed arm suggesting a significant decrease from pre-to-post cannabis exposure $F(1, 47) = 43.461$, $p < .001$, $\eta p^2 = .480$. There was no significant interaction effect observed between Time of Testing x Sex, $F(1, 47) = .704$, $p = .406$, $\eta p^2 = .015$, suggesting there was no difference in the change in the percentage of closed arm entries between female and male animals (Fig. 5.12); Time of Testing x Treatment, $F(2, 47) = 1.426$, $p = .251$, $\eta p^2 = .057$, suggesting all treatment groups showed the same change in the percentage of entries made into the closed arm from pre-to-post cannabis exposure (Fig. 5.13); or Time of Testing x Treatment x Sex $F(2, 47) = .369$, $p = .693$, $\eta p^2 = .015$, suggesting that the effect of the treatment did not differ depending on the sex of the animal (Fig. 5.14).

Figure 5.12

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #81 as a Function of Time of Testing and Sex

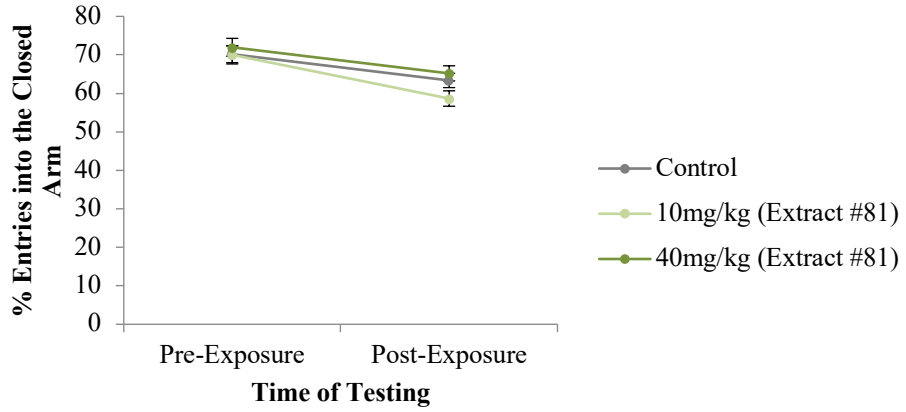


Note. This graph displays the mean change in the percentage of entries made into the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] There was no significant difference observed between male and female animals.

Figure 5.13

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #81 as a Function of Time of Testing and Treatment

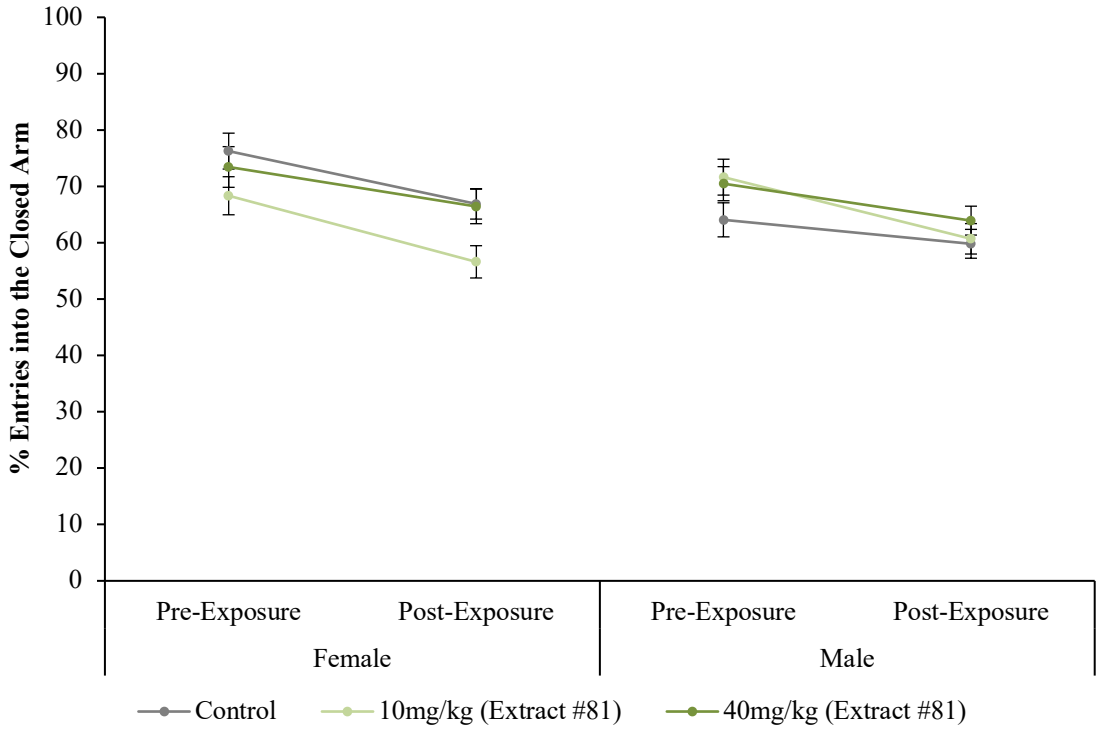


Note. This graph displays the mean change in the percentage of entries made into the closed arm of animals given extract #81, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant differences observed between treatment groups in the change in the percent of closed arm entries.

Figure 5.14

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extract #81, as a Function of Time of Testing, Treatment, and Sex



Note. This graph displays the change in mean percent of entries made into the closed arm of both male and female animals given extract #81, at either a low (10mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

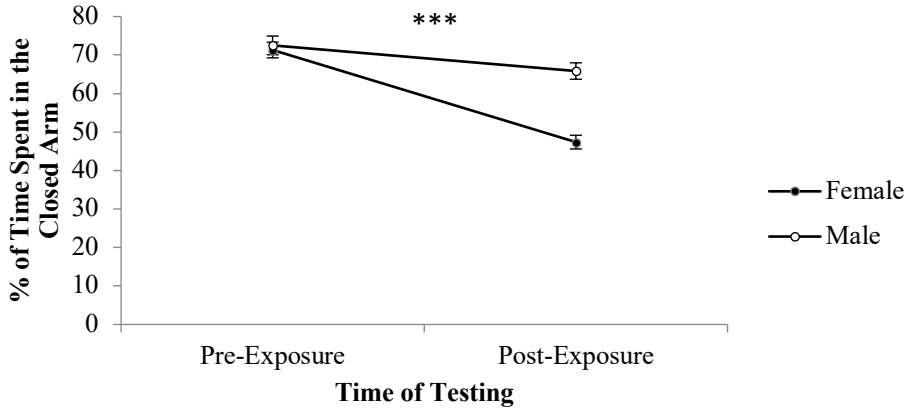
[a] There were no significant interaction effects observed.

Long-Term Effects of Extracts #10+98 on Anxiety-Like Behaviour in the EPM

Percent of Time Spent in the Closed Arm. A significant main effect of time of testing was observed and suggests that the change in the percentage of time spent in the closed arm decreased across all groups following cannabis exposure $F(1, 59) = 83.801$, $p < .001$, $\eta p^2 = .587$. There was a Time of Testing x Sex interaction $F(1, 59) = 26.669$, $p < .001$, $\eta p^2 = .311$, suggesting male animals showed a more significant decrease in the percentage of time spent in the closed arm following cannabis exposure compared to females (Fig. 5.15). There was also a significant Time of Testing x Treatment interaction $F(2, 59) = 5.086$, $p = .009$, $\eta p^2 = .147$, suggesting both cannabis exposed groups showed a more significant decrease in the percentage of time spent in the closed arm compared to controls (Fig. 5.16). There was no significant Time of Testing x Treatment x Sex interaction $F(2,59) = 1.585$, $p = .214$, $\eta p^2 = .051$, suggesting cannabis treatment did not influence the change in closed arm time differently based on the sex of the animal (Fig. 5.17).

Figure 5.15

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #10+98 as a Function of Time of Testing and Sex



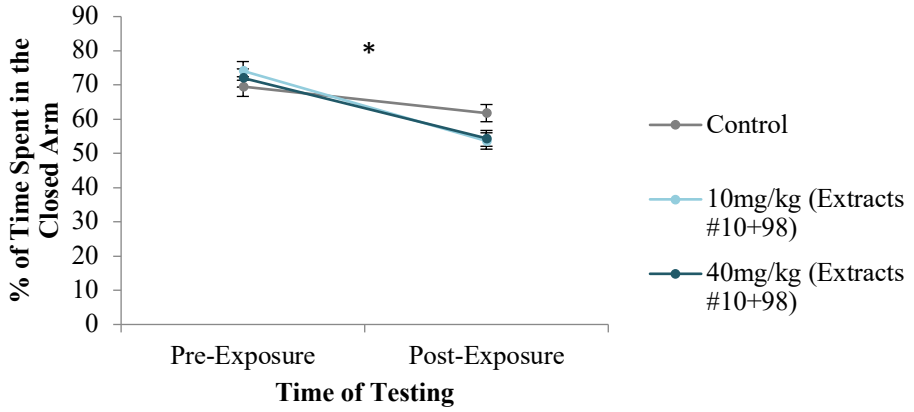
Note. This graph displays the mean change in time spent in the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] Female animals showed a more significant decrease in the change in the percentage of time spent in the closed arm from pre-to-post dosing than did male animals.

***p < .001

Figure 5.16

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #10+98 as a Function of Time of Testing and Treatment



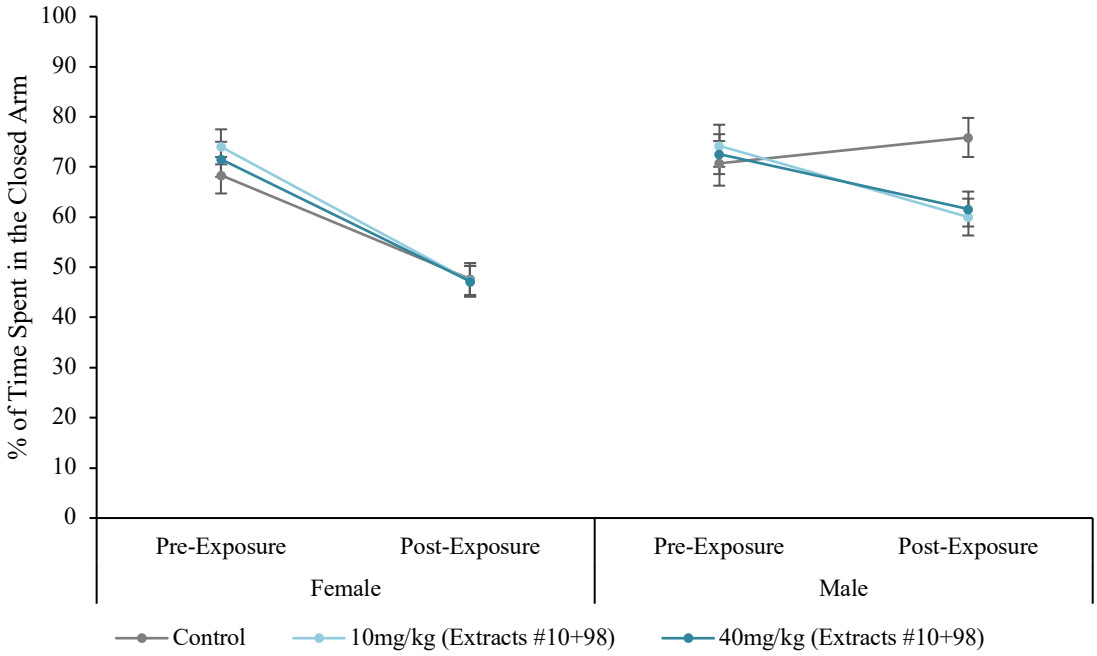
Note. This graph displays the mean change in the percentage of time spent in the closed arm of animals given extracts #10+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There was a significant effect of treatment observed as both the low and high dose group showed a more significant decrease in the time percentage of time spent in the closed arm from pre-to-post dosing, compared to controls.

*p < .025

Figure 5.17

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #10+98 as a Function of Time of Testing, Treatment, and Sex

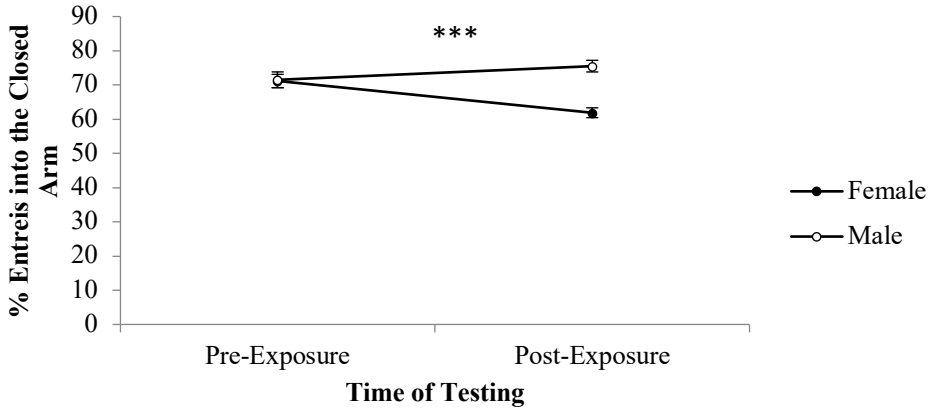


Note. This graph displays the mean closed arm time of both male and female animals given extracts #10+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.
[a] There were no significant interaction effects observed, only a main effect of time of testing by which all animals showed a similar decrease in time spent in the closed arm from pre-to-post cannabis exposure. [b] Of note is the increase in the percentage of time spent in the closed arm of male control animals. This is likely driving the significant Time of Testing x Sex and Time of Testing x Treatment interactions.

Percent of Entries Made into the Closed Arm. There was no significant main effect of time of testing on the percentage of entries made into the closed arm suggesting these values held constant across the two testing periods $F(1, 59) = 2.662$, $p < .108$, $\eta p^2 = .043$. There was a significant interaction effect observed between Time of Testing x Sex, $F(1, 59) = 16.812$, $p < .001$, $\eta p^2 = .222$, as male animals showed an increase in the percentage of entries made into the closed arm following cannabis exposure, while female animals showed a decrease in the percentage of entries made into the closed arm following cannabis exposure (Fig. 5.18). There was no significant interaction of Time of Testing x Treatment, $F(2, 59) = 2.222$, $p = .117$, $\eta p^2 = .070$, suggesting all treatment groups showed the same change in the percentage of entries made into the closed arm following cannabis exposure (Fig. 5.19); or Time of Testing x Treatment x Sex, $F(2, 59) = 1.535$, $p = .224$, $\eta p^2 = .049$, suggesting that the effect of the treatment did not differ depending on the sex of the animal (Fig. 5.20).

Figure 5.18

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #10+98 as a Function of Time of Testing and Sex



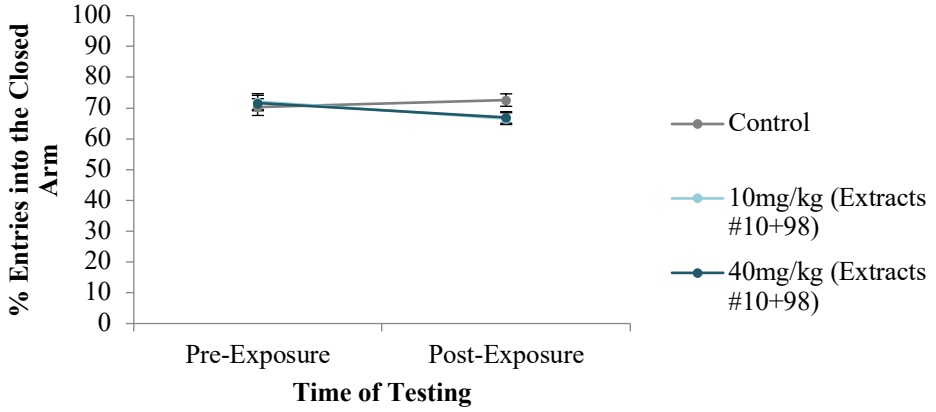
Note. This graph displays the mean change in the percentage of entries made into the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] Female animals showed a decrease in the percentage of entries made into the closed arm from pre-to-post dosing, while male animals increased the percentage of entries made into the closed arm over this time.

***p < .001

Figure 5.19

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #10+98 as a Function of Time of Testing and Treatment

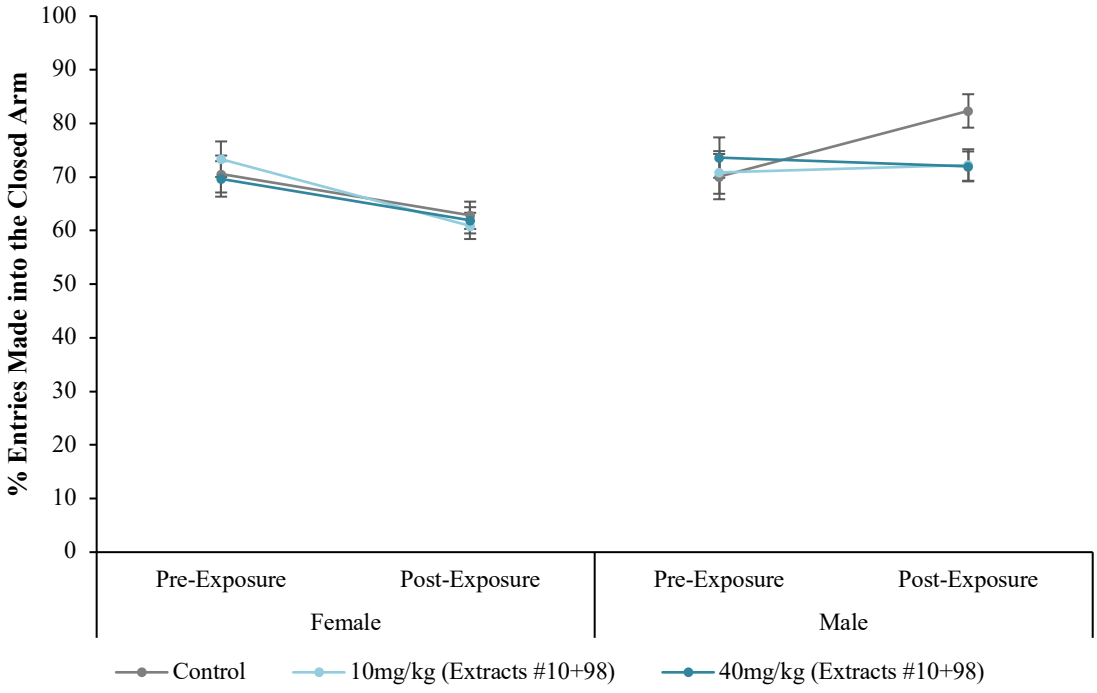


Note. This graph displays the mean change in the percentage of entries made into the closed arm of animals given extracts #10+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant differences observed between treatment groups in the change in the percent of closed arm entries. [b] However, the data does appear to be trending in a way that would suggest cannabis exposed groups showed a reduction in the percentage of entries made into the closed arm compared to controls, which showed a slight increase.

Figure 5.20

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #10+98, as a Function of Time of Testing, Treatment, and Sex



Note. This graph displays the change in mean percentage of time spent in the closed arm of both male and female animals given extracts #10+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

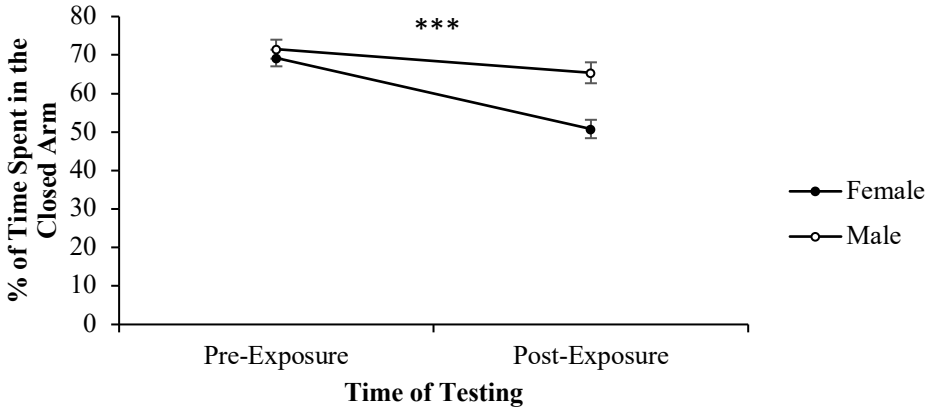
[a] There were no significant interaction effects observed. [b] Of note is the significant increase in the percentage of entries into the closed arm of male control animals. This is likely driving the significant Time of Testing x Sex interaction as well as the trend observed in the graphical representation of the Time of Testing x Treatment interaction.

Long-Term Effects of Extracts #81+98 on Anxiety-Like Behaviour in the EPM

Percent of Time Spent in the Closed Arm. There was a significant main effect of time of testing on the percentage of time spent in the closed arm, which decreased following cannabis exposure $F(1, 60) = 27.485$, $p < .001$, $\eta p^2 = .314$. There was a Time of Testing x Sex interaction observed $F(1, 59) = 6.893$, $p = .011$, $\eta p^2 = .103$, suggesting female animals showed a more significant decrease in the change in percentage of time spent in the closed arm following cannabis exposure compares to males (Fig. 5.21). There were no significant interactions between Time of Testing x Treatment, $F(2, 48) = 2.030$, $p = .143$, $\eta p^2 = .078$, suggesting all treatment groups showed the same decrease in the percentage of time spent in the closed arm following cannabis exposure (Fig. 5.22); or Time of Testing x Treatment x Sex, $F(2, 48) = .421$, $p = .659$, $\eta p^2 = .017$, suggesting that the effect of the treatment did not differ based on the sex of the animal (Fig. 5.23).

Figure 5.21

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #81+98 as a Function of Time of Testing and Sex



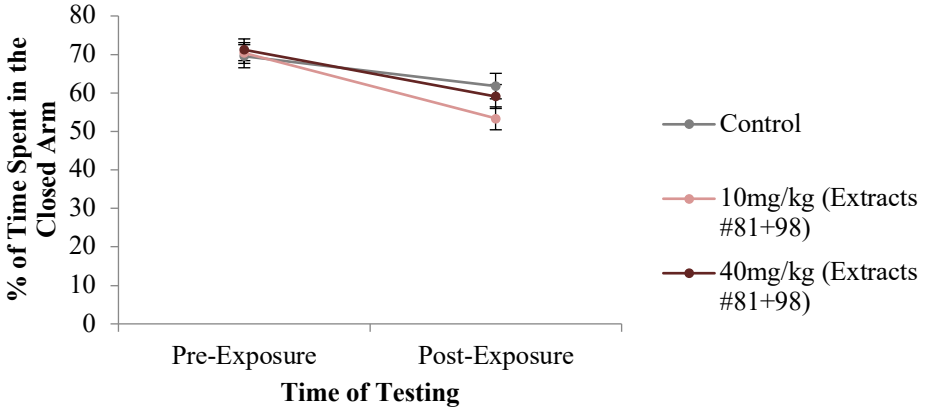
Note. This graph displays the mean change in time spent in the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] Female animals showed a more significant decrease in the change in the percentage of time spent in the closed arm from pre-to-post dosing than did male animals.

***p < .001

Figure 5.22

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #81+98 as a Function of Time of Testing and Treatment

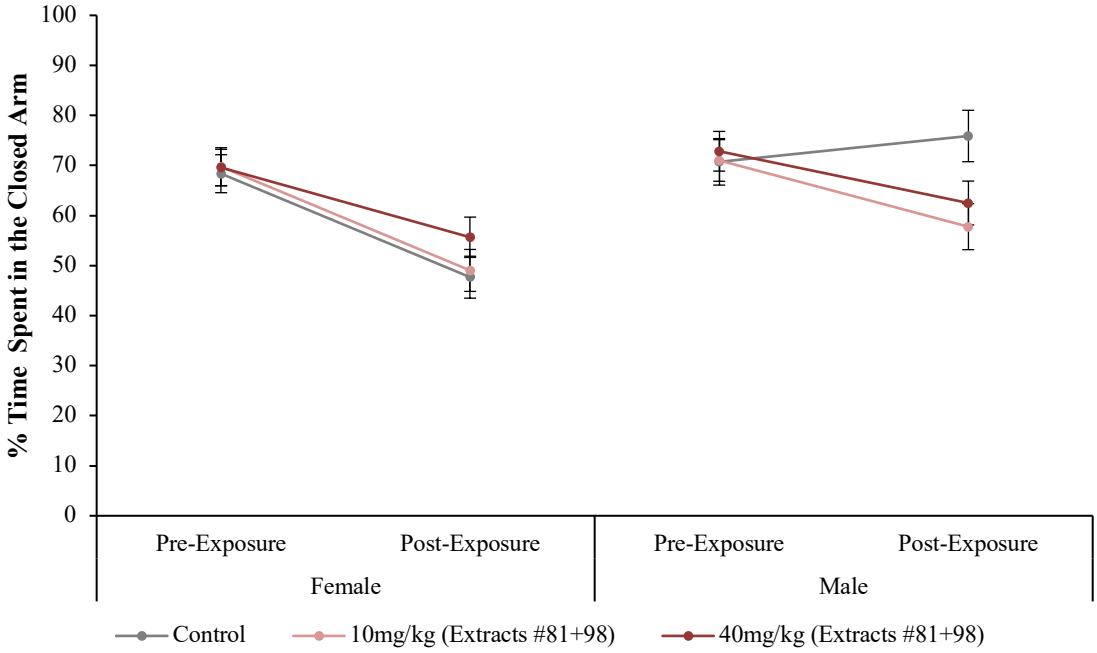


Note. This graph displays the mean change in the percentage of time spent in the closed arm of animals given extracts #81+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant differences observed between treatment groups in the change in the percentage of time spent in the closed arm.

Figure 5.23

Percent of Total Time Spent in the Closed Arm of Animals Exposed to Cannabis Extracts #81+98 as a Function of Time of Testing, Treatment, and Sex



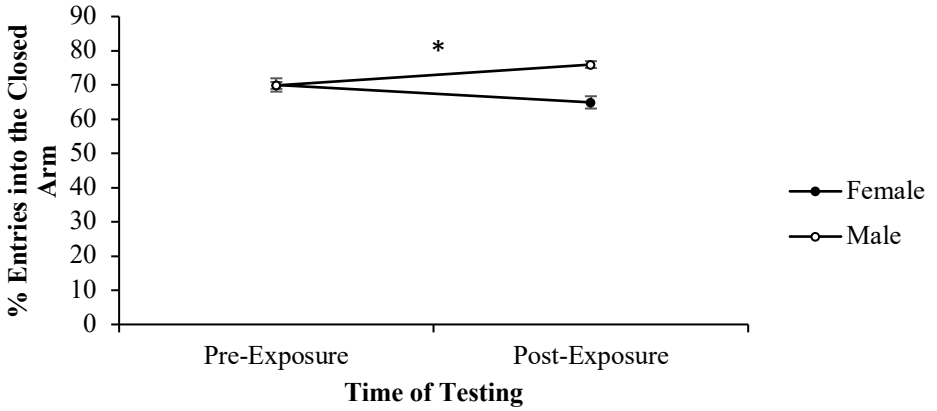
Note. This graph displays the mean closed arm time of both male and female animals given extracts #81+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant interaction effects observed, only a main effect of time of testing by which all animals showed a similar decrease in time spent in the closed arm from pre-to-post cannabis exposure.

Percent of Entries Made into the Closed Arm. There was no significant main effect of time of testing on closed arm entries suggesting the percentage of entries made into the closed arm did not change following cannabis exposure $F(1, 60) = .086$, $p = .771$, $\eta p^2 = .001$. There was a Time of Testing x Sex interaction $F(1, 60) = 10.655$, $p = .002$, $\eta p^2 = .151$, suggesting female animals showed a more significant decrease in closed arm entries from adolescence to post-exposure compared to male animals which increased the percentage of entries made into the closed arms following cannabis exposure (Fig. 5.24). There were no significant interactions between Time of Testing x Treatment, $F(2, 60) = .360$, $p = .699$, $\eta p^2 = .012$, suggesting all treatment groups showed similar changes in the percentage of closed arm entries following cannabis exposure (Fig. 5.25); or Time of Testing x Sex x Treatment, $F(2, 60) = 1.561$, $p = .218$, $\eta p^2 = .049$, suggesting that the effect of the treatment did not differ depending on the sex of the animal (Fig. 5.26).

Figure 5.24

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #81+98 as a Function of Time of Testing and Sex



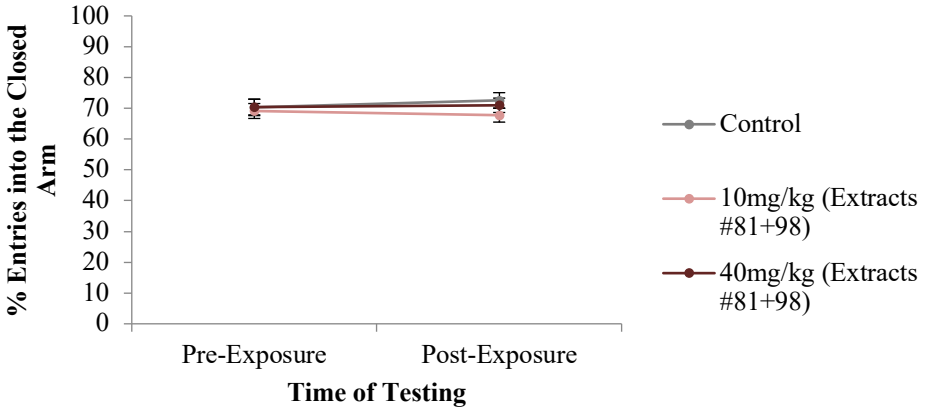
Note. This graph displays the mean change in the percentage of entries made into the closed arm of female and male animals from pre-dosing in adolescence to post-dosing in adulthood. Error bars represent the standard error of the mean.

[a] Female animals showed a decrease in the change in the percentage of time they spent in the closed arm form pre-to-post dosing, while male animals increased the percentage of entries made into the closed arm over this time.

*p < .025

Figure 5.25

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #81+98 as a Function of Time of Testing and Treatment

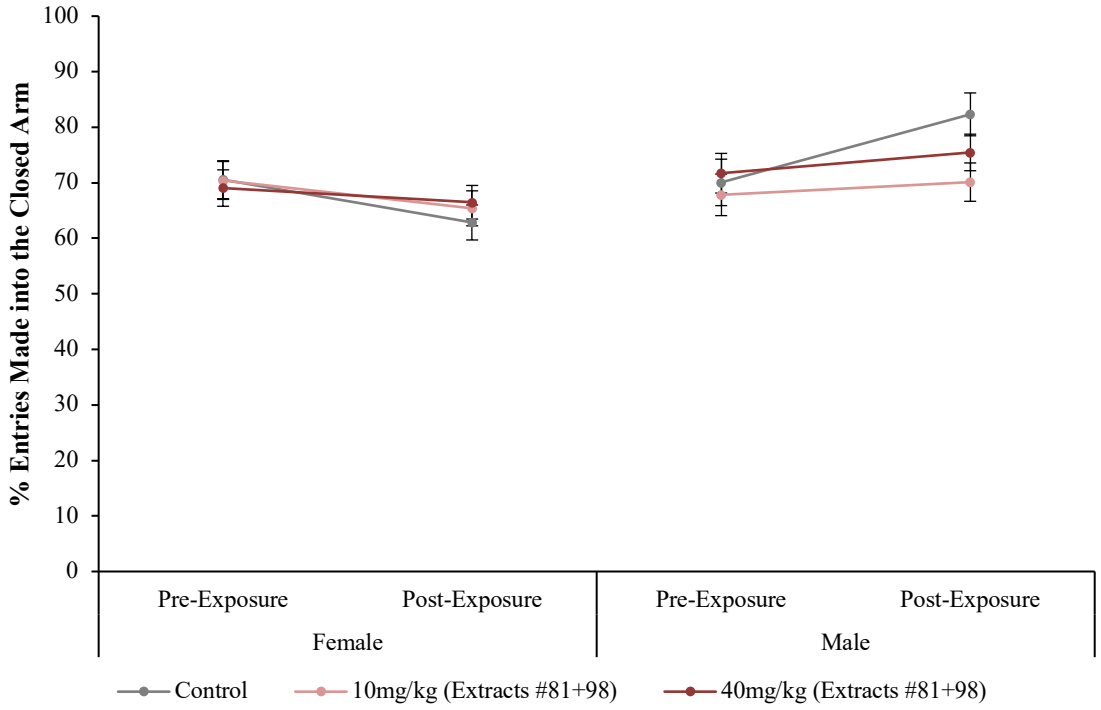


Note. This graph displays the mean change in the percent of entries made into the closed arm of animals given extracts #81+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant differences observed between treatment groups in the change in the percentage of entries made into the closed arm

Figure 5.26

Percent of Entries Made into the Closed Arm of Animals Exposed to Cannabis Extracts #81+98, as a Function of Time of Testing, Treatment, and Sex



Note. This graph displays the change in mean percentage of time spent in the closed arm of both male and female animals given extracts #81+98, at either a low (10 mg/kg) or high (40 mg/kg) dose, compared to controls, as a function of time of testing. Error bars represent the standard error of the mean.

[a] There were no significant interaction effects observed.

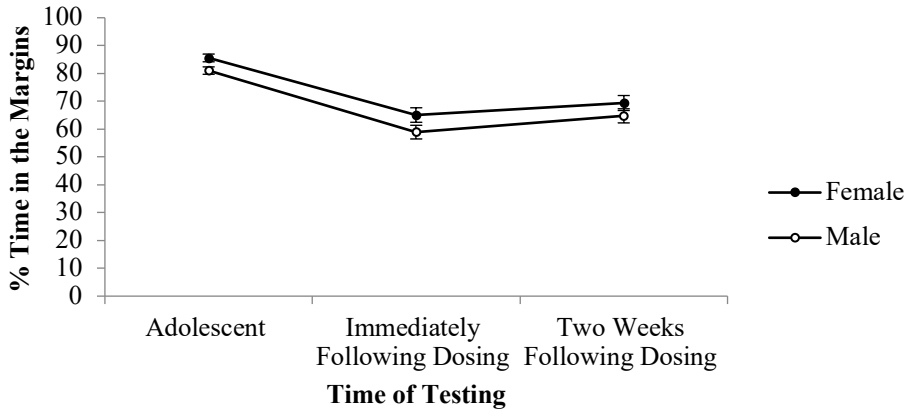
Long-Term Effects of Extract #129 on Anxiety-Like Behaviour in the Open Field

Percent of Time Spent in the Margins of the Open Field. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.814$, $p = .148$, therefore, no corrections were applied to the following statistical observations.

There was a significant main effect of time of testing on the change in the percentage of time spent in the margins of the open field, which decreased following cannabis exposure. $F(2, 94) = 68.758$, $p < .001$, $\eta p^2 = .594$. There were no interaction effects observed between Time of Testing x Sex, $F(2, 94) = .113$, $p = .893$, $\eta p^2 = .002$, suggesting male and female animals showed the same change in the percentage of time spent in the margins of the open field (Fig. 5.27); Time of Testing x Treatment, $F(4, 94) = 1.310$, $p = .272$, $\eta p^2 = .053$, suggesting all treatment groups showed the same change in the percentage of time spent in the margins of the open field (Fig. 5.28); or Time of Testing x Sex x Treatment, $F(4, 94) = 1.685$, $p = .160$, $\eta p^2 = .067$, suggesting that the effect of cannabis exposure did not differ depending on the sex of the animal (Fig. 5.29).

Figure 5.27

Changes in the Percent of Time Spent in the Margins as a Function of Sex

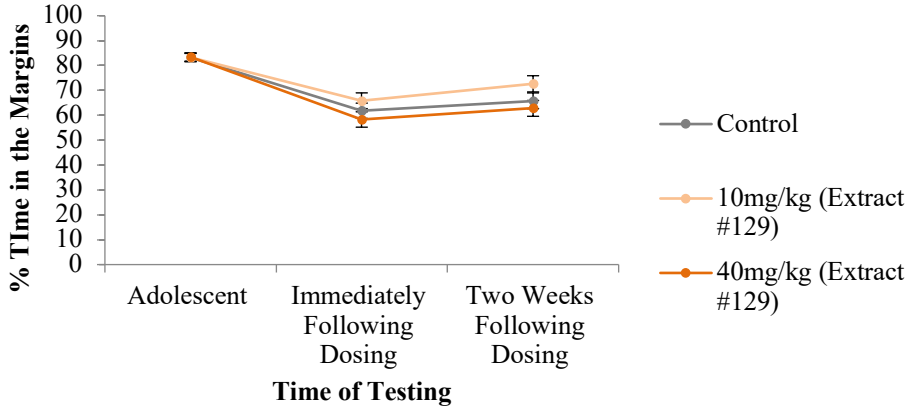


Note. This figure demonstrates the change in total activity levels as a function of sex, across three testing periods. Error bars represent the standard error of the mean.

[a] There was no significant difference in the change in time spent in the marginal area of the open field, between female and male animals

Figure 5.28

Changes in the Percent of Time Spent in the Margins of Animals Exposed to Cannabis Extract #129, Compared to Controls

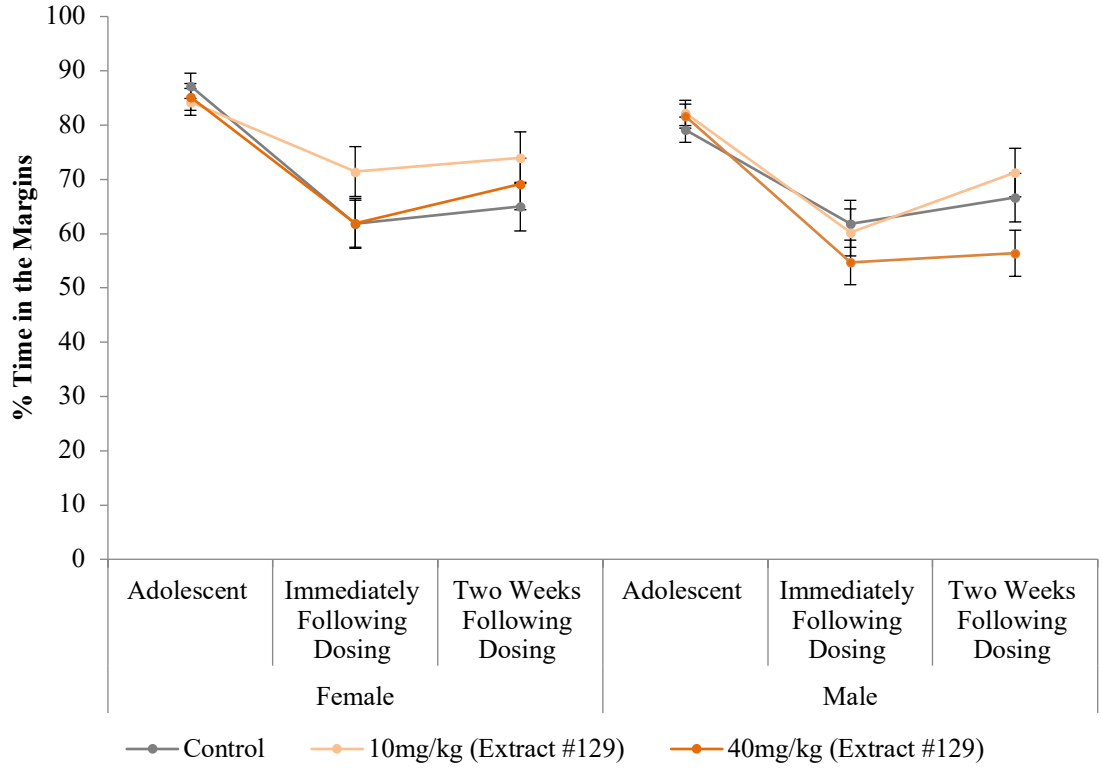


Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10 mg/kg) dose, or high (40 mg/kg) dose of cannabis extract #129 compared to controls, across three testing periods. Error bars represent the standard error of the mean.

[a] There were no significant differences observed in the change in time spent in the margins of the open field between treatment groups.

Figure 5.29

Changes in the Percent of Time of Female and Male Animals Exposed to Extract #129, Compared to Controls



Note. This figure demonstrates the change in the percentage of time spent in the margins of the open field of female and male animals, as a function of treatment group, across three testing periods. Error bars represent the standard error of the mean.

[a] Exposure to extract #129 did not create any significant Time of Testing x Treatment x Sex interactions suggesting the treatment did not affect animals differently depending on sex.

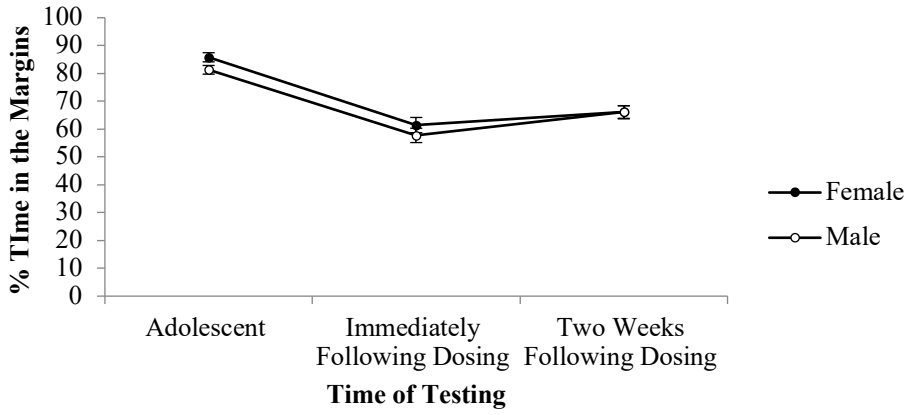
Long-Term Effects of Extract #81 on Anxiety-Like Behaviour in the Open Field

Percent of Time Spent in the Margins of the Open Field. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = .729$, $p = .695$, therefore, no corrections were applied to the following statistical observations.

There was a significant main effect of time of testing on the change in the percentage of time spent in the margins of the open field, which decreased following cannabis exposure. $F(2, 94) = 85.233$, $p < .001$, $\eta p^2 = .645$. There were no interaction effects observed between Time of Testing x Sex, $F(2, 94) = .825$, $p = .442$, $\eta p^2 = .017$, suggesting male and female animals showed the same change in the percentage of time spent in the margins of the open field (Fig. 5.30); Time of Testing x Treatment, $F(4, 94) = .448$, $p = .773$, $\eta p^2 = .019$, suggesting all treatment groups showed the same change in the percentage of time spent in the margins of the open field (Fig. 5.31); or Time of Testing x Sex x Treatment, $F(4, 94) = .985$, $p = .419$, $\eta p^2 = .040$, suggesting that the effect of cannabis exposure did not differ depending on the sex of the animal (Fig. 5.32).

Figure 5.30

Changes in the Percent of Time Spent in the Margins as a Function of Sex

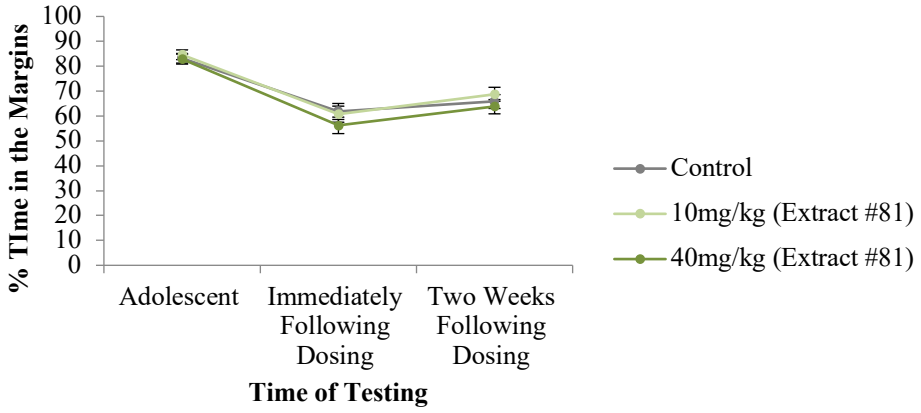


Note. This figure demonstrates the change in total activity levels as a function of sex, across three testing periods. Error bars represent the standard error of the mean.

[a] There was no significant difference in the change in time spent in the marginal area of the open field between female and male animals

Figure 5.31

Changes in the Percent of Time Spent in the Margins of Animals Exposed to Cannabis Extract #81, Compared to Controls

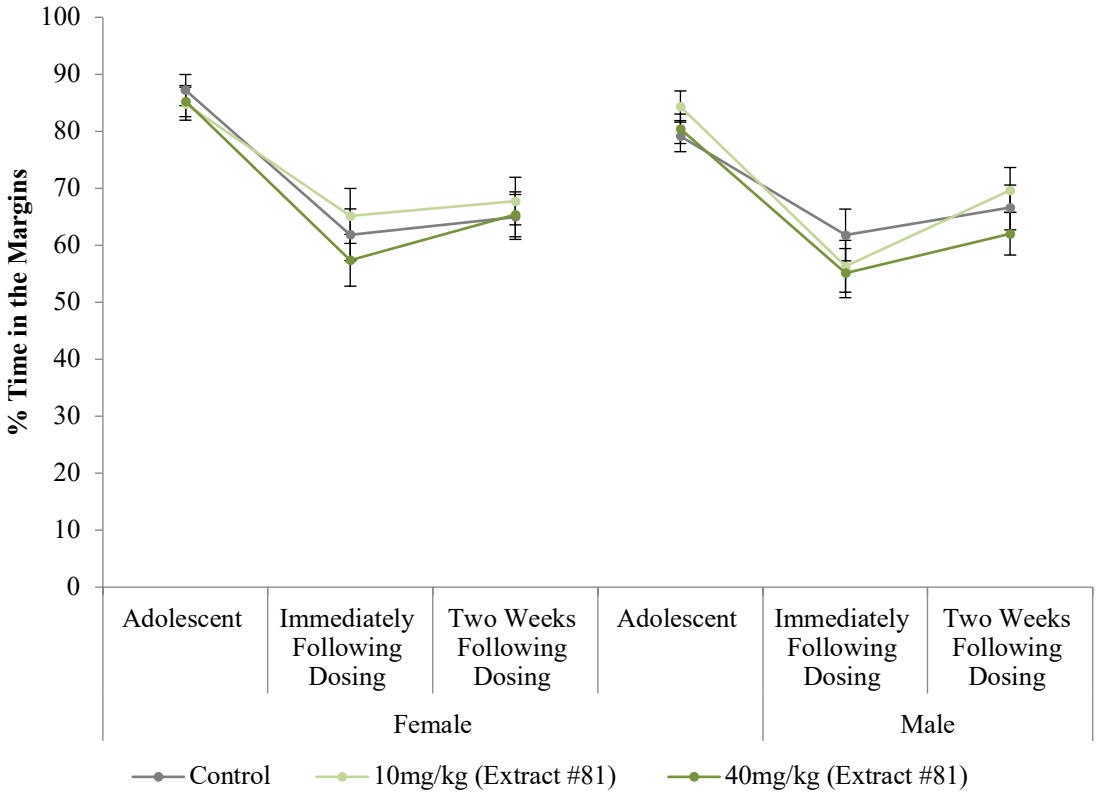


Note. This figure demonstrates the change in the percentage of time spent in the margins of the open field exposed to either the low (10 mg/kg) dose, or high (40 mg/kg) dose of cannabis extract #81 compared to controls, across three testing periods. Error bars represent the standard error of the mean.

[a] There were no significant differences observed in the change in time spent in the margins of the open field between treatment groups.

Figure 5.32

Changes in the Percent of Time Spent in the Margins of Female and Male Animals Exposed to Extract #81, Compared to Controls



Note. This figure demonstrates the change in the percentage of time spent in the margins of the open field of female and male animals, as a function of treatment group, across three testing periods. Error bars represent the standard error of the mean.

[a] Exposure to extract #81 did not create any significant Time of Testing x Treatment x Sex interactions suggesting the treatment did not affect animals differently depending on sex.

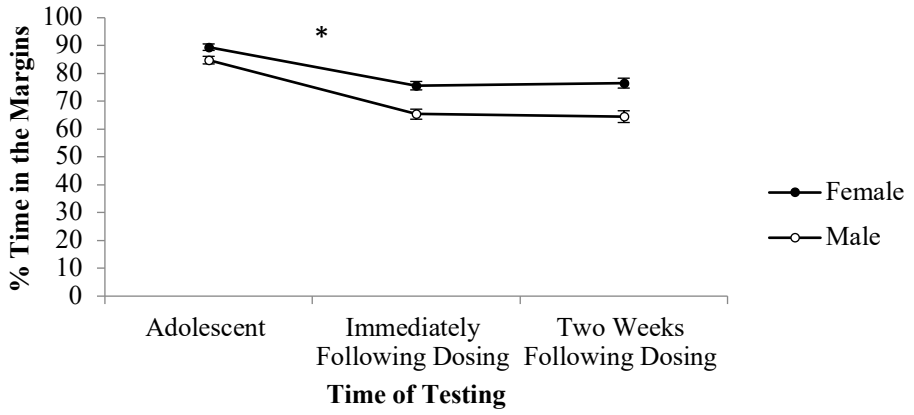
Long-Term Effects of Extracts #10+98 on Anxiety-Like Behaviour in the Open Field

Percent of Time Spent in the Margins of the Open Field. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 10.891$, $p = .004$, therefore, a Huynh-Feldt corrections is applied to the following statistical observations ($\varepsilon = .951$).

There was a significant main effect of time of testing on the change in the percentage of time spent in margins of the open field, which decreased following cannabis exposure. $F(1.902, 112.246) = 101.485$, $p < .001$, $\eta p^2 = .632$. There was a significant Time of Testing x Sex interaction observed, $F(1.902,112.246) = 4.109$, $p = .021$, $\eta p^2 = .098$ (Fig. 5.33). Contrasts revealed this effect was driven by trending difference between adolescent and immediately-post-dosing testing where males showed a more significant decrease in the change in the percentage of time spent in the margins, compared to females $F(1, 59) = 4.672$, $p = .035$. There was a significant Time of Testing x Treatment interaction observed, $F(3.805,112.246) = 3.217$, $p = .017$, $\eta p^2 = .098$ (Fig. 5.34). However, contrasts revealed only a trending difference between groups in the change in the percentage of time spent in the margins from immediately following dosing to two weeks following dosing $F(2, 59) = 2.785$, $p = .070$. This effect appears to be driven by the animals given the 10 mg/kg dosage of extract #10+98 which increased the percentage of time spent in the margins across this time point compared to the 40 mg/kg group and controls. There was no Time of Testing x Sex x Treatment interaction, $F(3.805,112.246) = 1.991$, $p = .104$, $\eta p^2 = .063$, suggesting that the effect of cannabis exposure did not differ depending on the sex of the animal (Fig. 5.35).

Figure 5.33

Changes in the Percent of Time Spent in the Margins as a Function of Sex



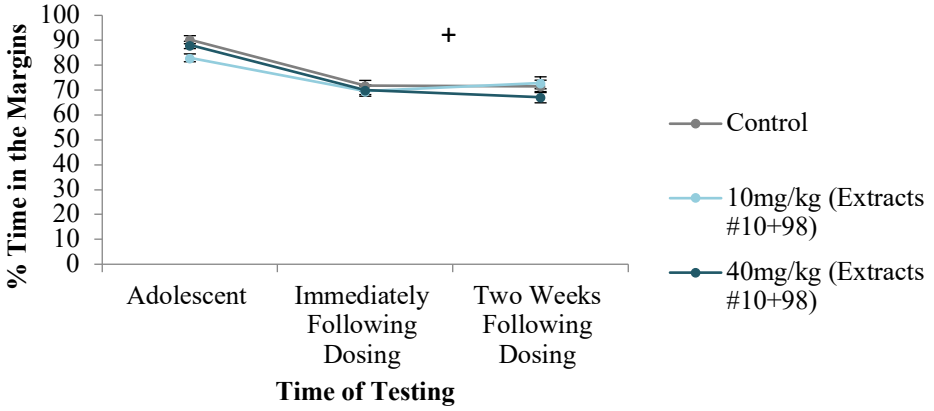
Note. This figure demonstrates the change in total activity levels as a function of sex, across three testing periods.

[a] There was no significant difference in the change in time spent in the marginal area of the open field between female and male animals

** p<.025*

Figure 5.34

Changes in the Percent of Time Spent in the Margins of Animals Exposed to Cannabis Extracts #10+98, Compared to Controls



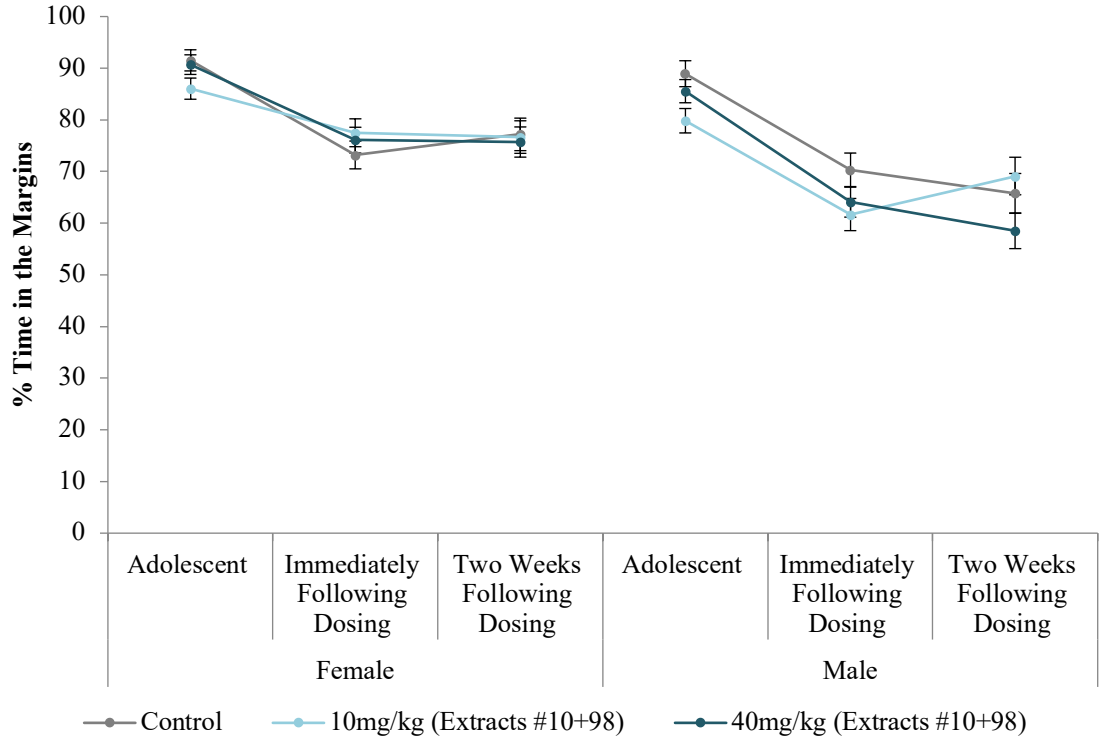
Note. This figure demonstrates the change in total activity levels of animals exposed to either the low (10 mg/kg) dose, or high (40 mg/kg) dose of cannabis extracts #10+98 compared to controls, across three testing periods.

[a] There was a significant Time of Testing x Treatment interaction observed, however contrasts revealed only a trending difference between the change in the percentage of time spent in the margins. The animals exposed to the 10 mg/kg dose of extracts #10+98 appeared to increase the time spent in the margins of the open field from immediately post-dosing to two weeks post-dosing. Both the 40 mg/kg and control animals showed a decrease in the percentage of time spent in the margins across this time.

+ p = .07

Figure 5.35

Changes in the Percent of Time Spent in the Margins of Female and Male Animals Exposed to Extracts #10+98, Compared to Controls



Note. This figure demonstrates the change in the percentage of time spent in the margins of the open field of female and male animals, as a function of treatment group, across three testing periods. Error bars represent the standard error of the mean.

[a] Exposure to extracts #10+98 did not create any significant Time of Testing x Treatment x Sex interactions suggesting the treatment did not affect animals differently depending on sex. [b] It does appear, however, that the male animals contributed to driving the Time of Testing x Treatment effect as the 10 mg/kg group increased the percentage of time spent in the margins two weeks after dosing, while the 40 mg/kg and control groups showed a decrease in the percentage of time spent in the margins at two weeks post-dosing.

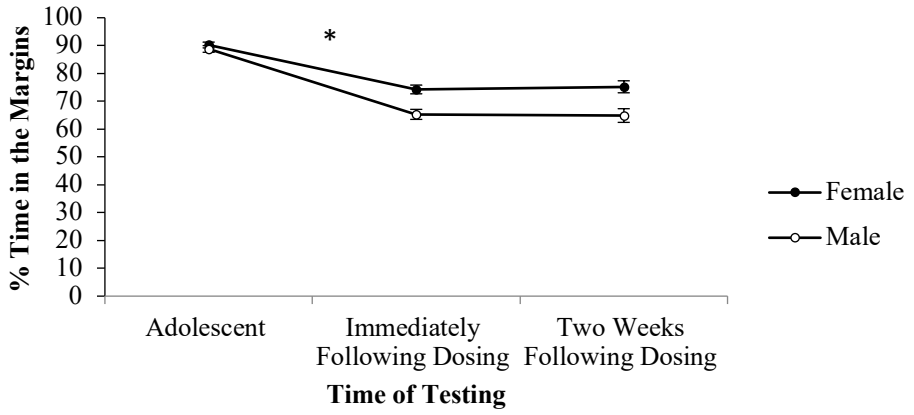
Long-Term Effects of Extracts #81+98 on Anxiety-Like Behaviour in the Open Field

Percent of Time Spent in the Margins of the Open Field. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 18.356$, $p < .001$, therefore, a Huynh-Feldt corrections was applied to the following statistical observations ($\varepsilon = .872$).

There was a significant main effect of time of testing on the change in the percentage of time spent in margins of the open field, which decreased following cannabis exposure. $F(1.743, 101.100) = 125.555$, $p < .001$, $\eta p^2 = .684$. There was a significant Time of Testing x Sex interaction observed, $F(1.743, 101.100) = 5.648$, $p = .007$, $\eta p^2 = .017$ (Fig. 5.36). Contrasts revealed this effect was driven by significant difference between adolescent and immediately post-dosing testing where males showed a more significant decrease in the change in the percentage of time spent in the margins, compared to females $F(1, 58) = .315$, $p = .004$. There was no significant Time of Testing x Treatment interaction observed, $F(3.486, 101.100) = .216$, $p = .910$, $\eta p^2 = .007$, suggesting all treatment groups showed the same change in the percentage of time spent in the margins of the open field (Fig. 5.37); or Time of Testing x Sex x Treatment, $F(3.486, 101.100) = 1.893$, $p = .126$, $\eta p^2 = .061$, suggesting that the effect of cannabis exposure did not differ depending on the sex of the animal (Fig. 5.38).

Figure 5.36

Changes in the Percent of Time Spent in the Margins as a Function of Sex



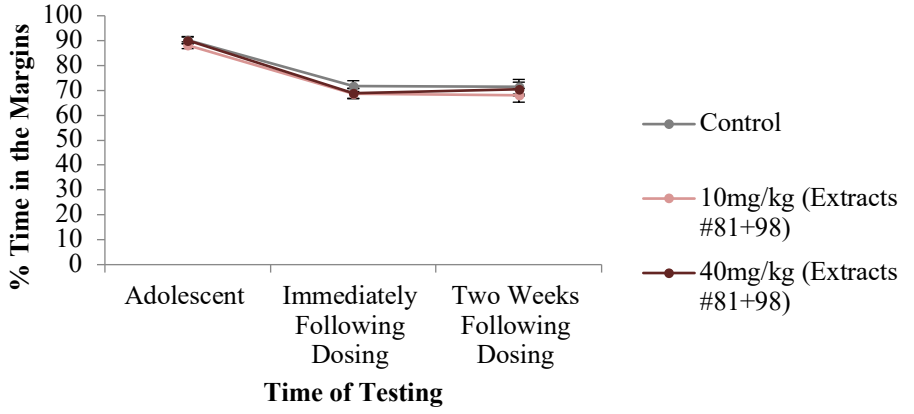
Note. This figure demonstrates the change in the percentage of time spent in the margins of the open field as a function of sex, across three testing periods. Error bars represent the standard error of the mean.

[a] There was no significant difference in the change in time spent in the marginal area of the open field between female and male animals.

**p<.025*

Figure 5.37

Changes in the Percent of Time Spent in the Margins of Animals Exposed to Cannabis Extracts #81+98, Compared to Controls

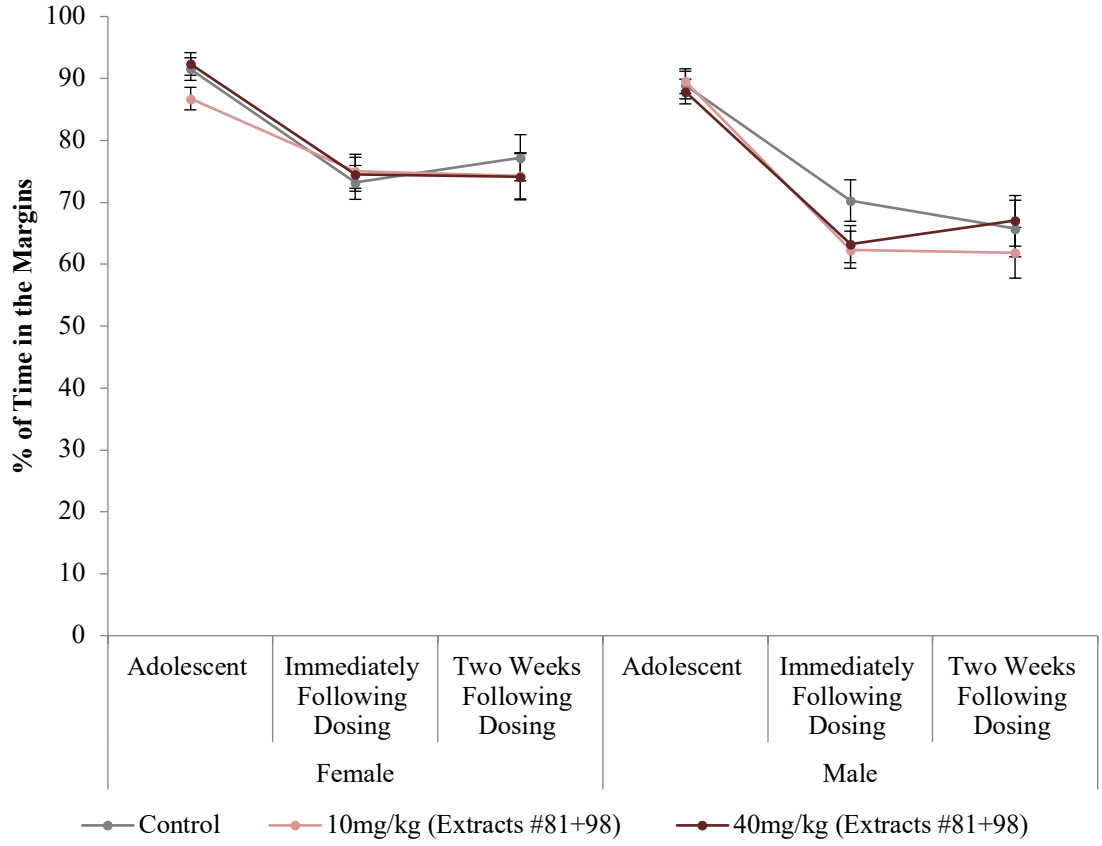


Note. This figure demonstrates the change in percentage of time spent in the margins of the open field of animals exposed to either the low (10 mg/kg) dose, or high (40 mg/kg) dose of cannabis extracts #81+98 compared to controls, across three testing periods. Error bars represent the standard error of the mean.

[a] There were no significant differences observed in the change in time spent in the margins of the open field between treatment groups.

Figure 5.38

Changes in the Percent of Time Spent in the Margins of Female and Male Animals Exposed to Extracts #81+98, Compared to Controls



Note. This figure demonstrates the change in the percent of time spent in the margins of the open field of female and male animals, as a function of treatment group, across three testing periods. Error bars represent the standard error of the mean.

[a] Exposure to extracts #81+98 did not create any significant Time of Testing x Treatment x Sex interactions suggesting the treatment did not affect animals differently depending on sex. [b] While it appears that the 40 mg/kg group increased the time spent in the margins of the open field two weeks following dosing, the interaction was not statistically significant.

Discussion

General Discussion

This analysis sought to explore the influence of chronic, high-CBD cannabis exposure on anxiety-like behaviour in the Long-Evans rat. Rodents are prey animals and exhibit a behaviour called positive thigmotaxis which refers to the tendency of rodents to seek safety next to vertical surfaces. In the EPM, this safety is offered by the security of the closed arms, and in the open field, is offered by the marginal space near the walls of the apparatus. Open, exposed spaces leave rodents vulnerable to predation, making thigmotaxis an adaptive behaviour. Based on this logic, we can infer that less time in the closed arm of the EPM, and in the margins of the open field are indicative of a reduction in species typical, anxiety-like behaviours. In the EPM, animals were tested in adolescence prior to cannabis administration, and again following the cessation of cannabis administration to assess if there were long-term changes in behaviour that would result from this exposure. In the open field, animals were tested in adolescence prior to cannabis exposure, one day following the cessation of dosing to assess any immediate effects of exposure on anxiety-like behaviour, and again two weeks following the cessation of cannabis administration to assess long-term changes in behaviour resulting from this exposure. Overall, there were very few significant changes in anxiety-like behaviour observed and what few effects were observed, were dependent on the strain of cannabis extract used.

High-CBD Cannabis Has Little Effect on Anxiety-Like Behaviours in the EPM

Exposure to extracts #129, #81, and #81+98 had no effect on the change in anxiety-like behaviours from pre-to post-administration, as measured by the percentage of time the animal spent in the closed arm of the EPM. Exposure to extracts #10+98 however, did cause a more significant decrease in the percentage of time the animals spent in the closed arm, compared to controls. This effect appeared to be present at both the low, 10 mg/kg dose, and the high, 40 mg/kg dose. This effect was not accompanied by a change in the percentage of entries made into the closed arm during the animal's time in the apparatus, despite the appearing trend in the visually represented data. In fact, there were no scenarios where cannabis exposure affected the percentage of entries made into the closed arm. Measuring the number of entries made into the closed arm ensures that the animals were indeed entering into the open arm more frequently, and not simply running from one closed arm to the other. These same animals showed a hypolocomotor effect of treatment on total activity levels measured in the open field task, which tells us that these effects are not simply attributable to a change in locomotor activity or motor function but are likely due to a reduction in anxiety-like behaviour. Together, these findings confirm my hypothesis that these high-CBD cannabis extracts would have little long-term effect on anxiety-like behaviour. They also confirm my hypothesis that what little effect they may have, will manifest as a reduction in anxiety-like behaviours.

High-CBD Cannabis Has Little Effect on Anxiety-Like Behaviours in the Open Field

Anxiety-like behaviour was also assessed by measuring the percentage of time the animal spent in the margins of the open field. Again, exposure to extracts #129, #81, and

#81+98 showed no effect of treatment on this measure of anxiety. Exposure to the 10 mg/kg dose of extracts #10+98 however, caused a trending increase in the percentage of time spent in the margins from immediately post-dosing to two weeks following dosing, while the 40 mg/kg and control groups showed a decrease in marginal time across these testing periods. This would suggest an increase in anxiety-like behaviour in this treatment group, in contrast to the effects observed in the EPM. This effect is curious. It may be that because these animals had been exposed to the open field only two weeks prior, and in total, were tested in this task three times, that this is a less accurate measure of anxiety-like behaviour than the EPM. It may also be that rather than staying low and close to the walls of the apparatus, which would be expected of a more anxious animal, these animals were exploring the walls of the apparatus which would increase the percentage of time spent in the margins but not necessarily be suggestive of increased anxiety-like behaviour. To explore this idea, we looked at the amount of time these animals spent vertically exploring the open field. Rats tend to like to rear against vertical surfaces, so vertical exploration in the maze is likely also time spent in the marginal area of the maze. Indeed, in both post-dosing measures, the time spent vertically exploring the open field and the percentage of time spent in the margins are correlated. This was not the case in adolescence when the open field was completely novel and likely a much more anxiety-inducing experience where the animal would predictably keep low against the wall. Animals given the 10 mg/kg, and 40 mg/kg doses appear to show a less significant decrease in vertical exploration in the open field from immediately following dosing to two weeks post-dosing compared to controls. This means cannabis exposed animals were

spending more time than controls exploring vertically, which could account for the increase in the percentage of time spent in the margins observed in the 10 mg/kg group.

High-CBD Cannabis has Few Long-Term Effects on Anxiety-Like Behaviours

The anxiolytic effects of CBD are typically observed as acute effects, where the behavioural measures of anxiety-like behaviours are collected shortly after administration of the drug. There are few studies which explore the effects of chronic cannabis exposure on anxiety-like behaviour. In a recent study, Campos and colleagues found that three weeks of daily exposure to 5 mg/kg of CBD generated an anxiolytic effect in rats tested in a T-maze 40 minutes after the final administration but not when tested three hours after the final administration (2013). Long et al., found that chronic CBD administration in mice had no effect on behaviour in the EPM (2010). There is some research that has shown chronic CBD exposure may produce anxiogenic effects in mice, however this observation may be dependent on the type of behavioural task, as the conditional emotional response task used in this research measures fear responses and as previously discussed, it may be that the fear system is a distinct pathway, within but unique from the HPA axis of our focus (ElBatsh et al., 2012; Kamprath et al., 2009).

The approximately two-week delay between the cessation of dosing and our post-dosing observation and resulting decrease in physiological CBD concentration across that time is likely why the majority of the extracts did not elicit the classic anxiolytic effect expected of CBD administration. However, as was discussed in the previous chapter, cannabinoids can linger in the body following chronic administration depending on the dosage, rate of exposure, and route of administration. While we did not collect measures of cannabinoid concentration at the time of testing, it is possible that extracts #10+98 has

a unique pharmacokinetic profile and that lingering cannabinoids contributed to the observed decrease in anxiety-like behaviour of exposed animals.

An alternate explanation may be that this singular extract caused plastic changes in the brain, thereby altering the stress response circuitry which may generate the anxiolytic effects we observed. These changes would likely be localized in the limbic structures of the brain, including the hippocampus, ventral striatum, and prefrontal cortex. Herman et al., (1997) have hypothesized that the stress response pathway involving these structures is more sensitive to stressors that elicit higher order processing. That is, stimuli that require the processing of multiple sensory modalities and pose no immediate threat to physiological well-being and are therefore stressful only in comparison to previous experience. Recall, this pathway would appear to be very akin to the slow-processing pathway discussed in the introduction. I would argue that the EPM is exactly the sort of stimuli to activate this limbic-sensitive HPA response. The EPM poses no immediate physiological threat, involves an appraisal of the apparatus and situation, and at the time of post-exposure testing the animals had seen the maze before. Therefore, any change in behaviour observed in the EPM is likely to result from changes to these brain areas. Indeed, past research has linked both structural, and functional changes in the HPA axis to abnormal performance on the EPM (Landgraf et al., 1999).

As there are few studies exploring the effects of chronic cannabis exposure on anxiety-like behaviours, there has been little investigation into potential mechanisms of action. It has been shown the chronic administration of cannabinoids, in particular THC, can lead to changes in CB1 receptor binding in limbic structures, and an increase in the endogenous cannabinoid, AEA, in specific limbic forebrain structures (Romero et al.,

1998; Di Marzo et al., 2000). This resulting increase in AEA is posited to be responsible for the 'rewarding' interpretation of the drug and may contribute to the anxiolytic effects. In the aforementioned study exploring the effects of chronic CBD administration on anxiety-like behaviour, Campos et al., propose that chronic administration of CBD may also generate effects through modulation of neurotransmission involving the endocannabinoid system (2013). By inhibiting the enzyme FAAH, CBD could contribute to an increase in activation of serotonergic neurons in the dorsal raphe nucleus, which could in turn contribute to an increase in serotonin signalling in limbic areas (Bambico et al., 2009). To elucidate the exact mechanism of action of this particular cannabis extract, further molecular or anatomical research would be necessary.

As previously mentioned, all animals were tested on the EPM approximately two weeks after the cessation of dosing. This could potentially be within the window in which cannabis withdrawal symptoms may have been observable, as symptoms in humans can extend in the order of weeks depending on the dose and frequency of exposure (Budney et al., 2004). Cannabis withdrawal, however, is typically associated with an increase in anxiety, so if withdrawal was thought to be driving behavioural change, it would have been more likely that the observed results would have been antithetical to what was actually observed. Harte-Hargrove and Dow-Edwards (2012) did observe a decrease in anxiety-like behaviour on the EPM in male, but not female animals during drug abstinence from chronic THC exposure which they contend is due to sexual dimorphism in the distribution and density of CB1 receptors in limbic brain structures. However, no effect was observed in these animals two weeks following the cessation of dosing (Harte-Hargrove & Dow-Edwards, 2012). Withdrawal symptoms often manifest quite quickly

and are typically observable the day following the cessation of dosing (Budney & Hughes, 2004). If we were to have seen withdrawal effects, they likely would have been best observed in the open field measures of anxiety-like behaviour that were collected immediately following the cessation of dosing, and there were no effects of treatment observed over this testing period

Conclusion

In summary, exposure to high-CBD cannabis extract has little long-term effect on anxiety-like behaviour in the Long-Evans rat. Only exposure to extracts #10+98 caused a significant reduction in the percentage of time spent in the closed arm compared to controls, which suggests a reduction in anxiety-like behaviour in the cannabis exposed animals. Measures of anxiety taken from the open field assessment, again showed no change in anxiety-like behaviour of cannabis exposed animals with the exception of those administered the 10 mg/kg dose of extracts #10+98 who increased time spent in the margins two weeks following the cessation of dosing. I do not interpret this effect as an increase in anxiety however as these animals also spent more time vertically exploring the apparatus than did control animals.

Chapter 6

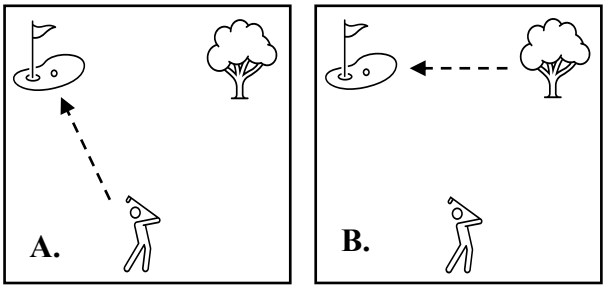
Long-Term Effects on Chronic, High-CBD Cannabis Exposure on Spatial Learning and Memory

Introduction

Spatial Learning and Memory

The ability to effectively and efficiently navigate through the environment is critical to every animal's survival. Spatial cognition refers to our understanding of our environment and generates for us a mental representation of the space we are in. This allows us to determine how to move from place to place, how to locate food and shelter, how to plan out movements in space and how to communicate about space (Kolb et al., 2019, pp. 523). There are many ways to conceptualize space including in reference to ourselves, which is called egocentric space, or in reference to other objects, which is called allocentric space. We are also able to navigate through our world based on these same understandings. For example, I could say, "the hole is five yards ahead of you and to your left", where all instruction for navigation is in reference to your own position in space (Fig. 6.1A). That is egocentric navigation. I could also say "the hole is five yards to the left of the tree", where all instruction is in reference to other object in the space (Fig. 6.1B)

Figure. 6.1
Egocentric vs. Allocentric Navigation



Note. The left pane (**A**.) depicts an egocentric method of understanding space whereby the space is understood in reference to the person/animal themselves. The right pane (**B**.) depicts allocentric navigation whereby space is understood in reference to other objects in that space.

Egocentric navigation is informed by internal cues in the body (internal feedback from the limbs, head direction, etc.). Allocentric navigation is informed by distal cues, or landmarks in the environment, and will be referred to from this point forward as spatial navigation. Intrinsically related to spatial navigation is spatial memory. This refers to the cognitive process of understanding, encoding, and recalling details about the spatial construction of the world around us. We will assess changes in spatial navigation caused by chronic high-CBD cannabis exposure using a well validated behavioural measure called the Morris water task. Details of this test are described later in this chapter.

There are a number of brain regions and networks involved in spatial navigation and memory, including the hippocampus (Morris et al., 1982), the entorhinal (Burgess et al., 2002), retrosplenial (Ekstrom et al., 2014), and parahippocampal (Ekstrom et al., 2003) cortices, as well as cortical areas like the prefrontal cortex (Granon & Poucet, 1995). Lesion studies provide critical insight into the influence of these areas on the process of spatial navigation and memory by illustrating that damage to areas such as the

hippocampus and other structures of the medial temporal lobe impair functions in tests of spatial navigation (Morris et al., 1982; Kuruvilla & Ainge, 2017; Ramos, 2017). In fact, there is broad consensus regarding the importance of the hippocampus in driving spatial navigation in animals, as well as humans, providing the opportunity for a confident translational interpretation of this research.

The Role of the Endocannabinoid System in Spatial Learning and Memory

As we know, the endocannabinoid system is proposed to influence brain plasticity through modulation of synaptic transmission. This is thought to include plasticity processes which underlie processes of spatial learning and memory, as there are high concentrations of both cannabinoid receptors and endogenous cannabinoids present in the hippocampus (Herkenham et al., 1991; Wilson & Nicoll, 2001). Endocannabinoids in the HPC bind more densely in the dorsal HPC than the ventral HPC (Herkenham et al., 1991) which further implicates the role of this system in spatial function as the dorsal and ventral HPC are functionally distinct, with the former being primarily responsible for the aforementioned spatial function (Faneslow & Dong, 2010). As was the case in the basal ganglia, CB1 receptors in the HPC are located primarily on inhibitory GABAergic interneurons (Tsou et al., 1999), and act as a target for the retrograde signalling of endogenous cannabinoids. In this system, cannabinoids are thought to modulate synchronized firing of neurons that when disrupted, could lead to a muddied signal, and potentially disrupt HPC function (Wilson & Nicoll, 2001). Concurrent with this idea, increased levels of GABA in the HPC have been shown to enhance spatial learning (O'Connell et al., 2001; Davies et al., 2002). There are a number of other potential mechanisms by which the endocannabinoid system could influence spatial learning and

memory which include through the modulation of other neurotransmitters like dopamine, or by causing changes to dendritic morphology; these mechanisms are summarized succinctly by Davies and colleagues in a review (2002).

The Effects of Exogenous Cannabinoids on Spatial Learning and Memory

Cannabis has, colloquially, long been known to contribute to memory deficits. Acute administration of THC has repeatedly been shown to impair spatial learning and memory (Lichtman et al. 1995; Mishima et al., 2002; Han et al., 2012). Again, it likely exerts this influence through a CB1 dependant mechanism, as administration of CB1 receptor antagonists appears to prevent the memory deficits caused by THC administration (Wise et al., 2009). THC administration also activates several signalling pathways in the hippocampus including the mammalian target of rapamycin complex 1 (mTORC1) pathway (Puighermanal et al., 2009), and protein kinase-C pathway (Busquets-Garcia et al., 2018) both of which involve NMDA receptor activation, and which appear to be implicated in long-term and short-term memory impairments respectively.

In contrast to the observed effects of acute THC administration, acute administration of CBD does not appear to generate deficits in spatial navigation or memory as measured by the radial arm maze (Lichtman et al., 1995). Similarly, chronic long-term exposure of cannabidiol appears to have the same null effect. Fadda and colleagues (2004) utilized two different kinds of cannabis extracts, one a high-CBD extract and the other a high-THC extract, to assess spatial working memory in a delayed-matching-to-place (DMTP) version of the water task. They found that, as we would predict, the high-THC extract produced noticeable deficits in task performance in a dose-

dependent manner suggesting these animals were experiencing troubles with spatial working memory. In contrast, exposure to up to 50 mg/kg of the high-CBD extract had no effect on spatial working memory. Schleicher and colleagues (2018) explored the effect of long-term exposure to a dosage of 20 mg/kg CBD in adult mice on spatial learning and memory both while the animals were receiving treatment and following a washout period. Similarly to Fadda and colleagues (2004), they found that neither group showed deficits in spatial learning and memory as assessed by the MWT.

Another study found that while THC alone impaired spatial memory, and CBD alone had no effect on spatial memory, a combination of 50 mg/kg CBD and 1 mg/kg of THC, impaired acquisition of spatial memory in a dose-dependent manner (Hayakawa et al., 2008). This suggests that in certain combinations, CBD can exacerbate the spatial memory deficits caused by THC. Interestingly, and while being aware of the different species used between the experiments, a large disparity in the ratio of CBD to THC appears to be necessary to observe these effects as administrations of 0.5, 5, and 10 mg/kg of CBD combined with 2 mg/kg THC did not have any effect on spatial memory (Fadda et al., 2004). These studies highlight the specificity of the effects of cannabinoids and their dependence on the ratio of THC to CBD, the time of administration and testing, and the species being administered the drug. All of these factors are important to consider in determining a profile of behavioural effects of this drug on spatial learning and memory.

Specific Hypothesis

Considering the limited influence of CBD on spatial learning and memory, and the relatively small amount of THC present in the cannabis extracts used in this experiment, I hypothesize that exposure to high-CBD cannabis extract will exhibit little to no influence on spatial learning and memory in the MWT.

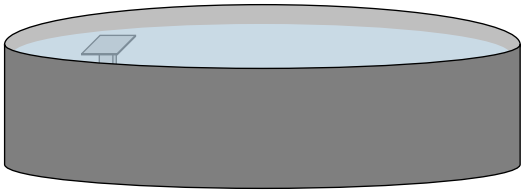
Methodology

Morris Water Task Testing Paradigm

The MWT is a measure of spatial learning and memory as described by Morris (1984). In this standard place version of the task, the animal is trained for five days to swim to find a platform hidden just below the surface of a water-filled pool (1.5 m in diameter) by using distally located cues on the walls of the testing room. The platform (12 cm x 12 cm) sits 1.5 cm below the surface of the water, which is made opaque with white, non-toxic, tempura paint. On each training day, each animal is given four, 60-second trials to find the platform, starting each trial from one of the four cardinal directions. If the animal does not reach the platform in that time, they are guided to the platform by the experimenter and allowed to remain on the platform for 10 seconds, while they observe their surroundings. The placement of the platform remains the same for all animals, between all trials, and between testing days. Once the trial is completed and the animal has found the platform, or is guided to the platform, the animal is removed, towel dried, and returned to a holding cage until their next trial. Animals were tested in groups of no more than 9, leaving no more than 10 minutes between each consecutive trial. On the sixth day of testing, called the probe trial, the platform is

removed, and the animal is given one, 60-second trial, to swim freely in an attempt to locate the missing platform. During all testing days an overhead mounted camera tracks the animal's path, latency, speed, and direction using HSV Image software. Latency to find the platform will be used as the measure of spatial learning and memory in the following analysis.

Figure 6.2
A Schematic Representation of the Morris Water Task



Note. The image above is a simplified visual representation of the Morris Water Task, used to assess spatial learning and memory.

Statistical Analysis

Spatial learning and memory in the Morris water task was analyzed using a repeated measure, two-way ANOVA with treatment (control, 10 mg/kg (low dose) or 40 mg/kg (high dose)) and sex (female or male) as between subject factors, and training days as within-subject factors. Planned contrasts were used to further investigate the difference in activity levels between time points, between treatment groups.

A Note on COVID-19

Constraints resulting from the COVID-19 pandemic resulted in the exclusion of the MWT from the testing protocol of the second experiment. As such there is no data available exploring the effects of extracts #10+98 or extracts #81+98 on spatial learning and memory.

Results

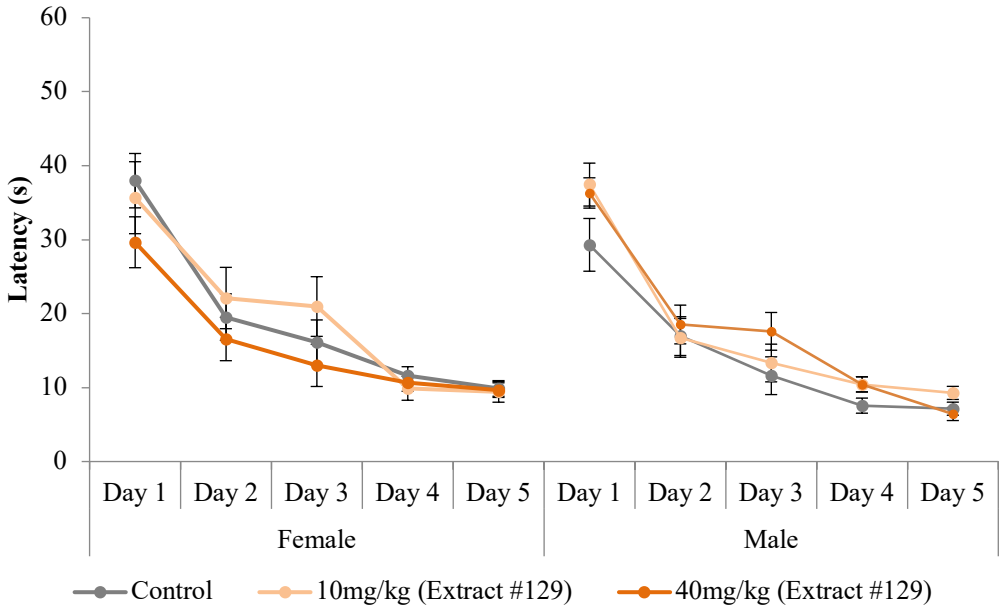
Long-Term Effects of Cannabis Extract #129 on Spatial Learning and Memory

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(9) = 46.197$, $p < .001$, therefore, Greenhouse-Geisser corrections were applied to the following statistical observations ($\varepsilon = .722$)

There was a main effect of Time of Testing, $F(4, 172) = 109.290$, $p < .001$, $\eta p^2 = .718$, suggesting that as training progressed, all animals learned the task and their latency to find the platform decreased in a similar fashion. There was no significant Day x Treatment interaction, suggesting all animals acquired the task at the same rate $F(5.775, 124.152) = .257$, $p = .952$ $\eta p^2 = .012$; no significant Day x Sex interaction, as male and female animals acquired the task equally $F(2.887, 124.152) = .233$, $p = .866$, $\eta p^2 = .005$; and no significant Treatment x Day x Sex interaction observed $F(5.775, 124.152) = 1.563$, $p = .166$, $\eta p^2 = .068$, suggesting the effect of treatment on acquisition of the task was not dependent on the sex of the animal (Fig. 6.3).

Figure 6.3

Acquisition of the MWT by Animals Exposed to Extract #129 Compared to Controls



Note. This figure represents the latency to find the hidden platform of female and male animals across five days of testing as a function of treatment with cannabis extract #129. Error bars represent the standard error of the mean.

[a] There were no significant interactions observed suggesting all animals acquired the task at similar rates regardless of sex or treatment.

Long-Term Effects of Cannabis Extract #81 on Spatial Learning and Memory

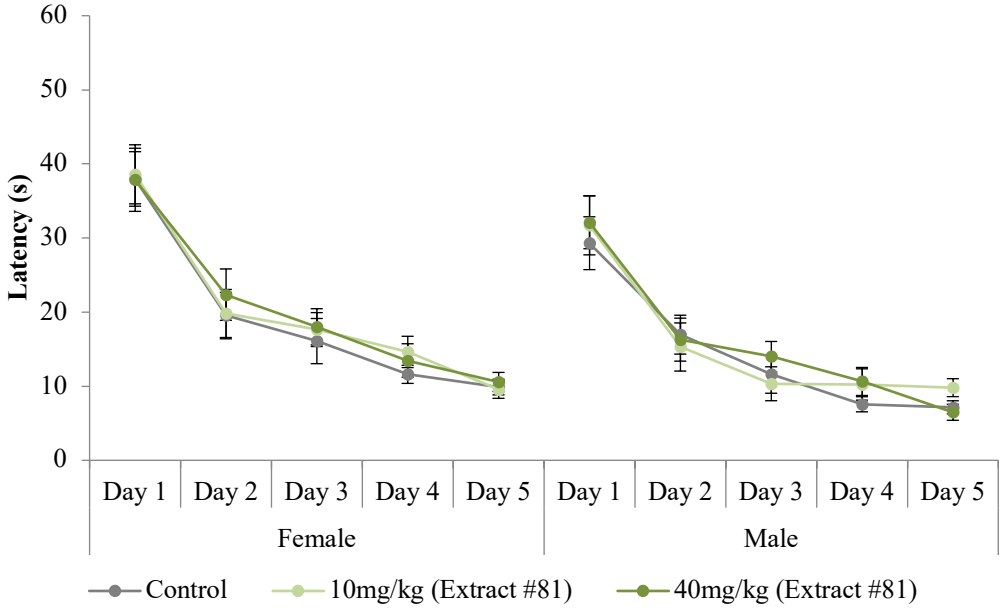
Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(9) = 41.365$, $p < .001$, therefore, Greenhouse-Geisser corrections were applied to the following statistical observations ($\varepsilon = .697$)

There was a main effect of Time of Testing, $F(4, 180) = 91.387$, $p < .001$, $\eta p^2 = .670$, suggesting that as training progressed, all animals learned the task and their latency to find the platform decreased in a similar fashion. There was no significant Day x Treatment interaction, suggesting all animals acquired the task at the same rate $F(5.579, 125.529) = .242$, $p = .955$ $\eta p^2 = .011$; no significant Day x Sex interaction, as female and

male animals acquired the task equally $F(2.790, 125.529) = .310$, $p = .685$, $\eta p^2 = .011$; and no significant Treatment x Day x Sex interaction observed $F(5.579, 125.529) = .310$, $p = .922$, $\eta p^2 = .014$, suggesting the effect of treatment on acquisition of the task was not dependent on the sex of the animal (Fig. 6.4).

Figure 6.4

Acquisition of the MWT by Animals Exposed to Extract #81 Compared to Controls



Note. This figure represents the latency to find the hidden platform of female and male animals across five days of testing as a function of treatment with cannabis extract #81.
[a] There were no significant interactions effects suggesting all animals acquired the task at similar rates, regardless of sex or treatment.

Discussion

General Discussion

This analysis sought to explore the long-term effects of chronic high-CBD cannabis exposure on spatial learning and memory in the Long-Evans rat. Exposure to these high-CBD extracts appear not to generate any negative effects on this kind of behaviour as assessed by performance in the MWT.

Again, only two of the four extracts explored in this thesis were included in this specific analysis due to constraints placed on the second experiment due to the COVID-19 pandemic. Exposure to neither the 10 mg/kg nor the 40 mg/kg dosage of extract #129 had any effect on the exposed animal's performance in the MWT. Affected animals learned the location of the hidden platform at a rate very near controls and measures of the latency to find the platform collected across 5 training days followed a similar curve. The control animals reached asymptote in their learning at approximately day four of training, a feature which was also apparent in the latency curve of the animals exposed to extract #129. Similarly, exposure to neither the 10 mg/kg nor the 40 mg/kg dosage of extract # 81 had any effect on the exposed animal's performance in the MWT. Again, we see that acquisition curve of the affected animals closely matched that of the controls. Animals exposed to extract #81 also appeared to reach asymptote around day four of training, similar to controls and to animals exposed to extract #129.

Chronic Exposure to High-CBD Cannabis Does Not Impair Spatial Learning and Memory

As was predicted, chronic exposure to these high-CBD cannabis extracts does not impair spatial learning and memory in the long-term as assessed by the MWT. There was approximately a three-week delay between administration of the final dosage of the extract and when measures of spatial learning and memory were collected in the MWT. These findings allow us to draw several conclusions.

First, in these extracts, the levels of THC were not sufficient to elicit the deficits in spatial memory commonly observed following both acute and chronic THC exposure. As was mentioned in the introduction, acute exposure to THC consistently produces deficits in spatial learning and memory via a CB1 dependant mechanism (Da Silva & Takahashi, 2002; Hayakawa et al., 2008). These same deficits have been shown to become more advanced in a chronic model where rats were administered 5 mg/kg of THC, six days a week for 90 days, however the impairments in spatial learning and memory appeared to reverse themselves 30 days after use of the drug was discontinued (Nakamur et al., 1991). Similarly, Cha et al., observed no effect on spatial learning and memory in adult rats chronically treated with 5 mg/kg THC for 21 days, 28 days after drug administration was ceased. Our results agree with this previous research, suggesting that the exposure to the low levels of THC in these high-CBD cannabis extracts did not cause long-term plastic changes that have repercussions on spatial behaviour after the drug administration has ceased.

Second, these findings suggest that exposure to CBD does not generate long-term consequences in spatial learning and memory. This finding is again corroborated by

previous research which has shown that CBD exposure does not cause impairments in these behaviours, either with acute exposure (Fadda et al., 2004) or following a delay period of no drug exposure (Schleicher et al., 2019). This was expected as there appear to be few instances of CBD having negative effects on spatial learning and memory. In fact, CBD has been shown to be useful in treating spatial memory deficits in many pre-clinical models including in malaria (Campos et al., 2015), chronic liver disease (Magen et al., 2009), and ischemia (Schiavon et al., 2014). While the exact mechanisms underlying the effects of CBD on memory systems have yet to be elucidated, interactions with the dopaminergic, serotonergic, or endocannabinoid systems may be of future interest (Luciana et al., 1998; Seeman, 2016; Mele et al., 2004; Bisogno et al., 2001). For example, administration of 5-HT3a antagonists has been shown to improve rats' performance in the MWT (Fontana et al., 1995) and CBD has been shown to act as an allosteric modulator of this specific receptor (Yang et la., 2010). While this association is purely speculative, the influence of CBD on these different neurotransmitter systems should be explored in the search for a mechanism of action. Also of importance to note, is that in these combinations, CBD does not exacerbate the deficits in spatial learning and memory typically caused by exposure to THC, as has been previously reported (Hayakawa et al., 2008). As was mentioned in the introduction, this ability of CBD to potentiate the effects of THC appears to be highly specific to certain combinations of THC and CBD.

Third, the findings suggest that exposure to these high-CBD extracts did not negatively affect the brain networks responsible for spatial navigation, specifically, that these extracts do not have detrimental long-term effects in the hippocampus. While

specific anatomical or molecular analysis would be necessary to confirm this inference, and potential mechanisms of compensation should be systematically ruled out, the results of the MWT, which is fine-tuned to observe deficits in function of the hippocampus, should give a reasonable indication of normal hippocampal functioning.

Conclusion

In humans, chronic exposure to cannabis has been shown to produce deficits in a number of memory functions including working, verbal, and spatial memory, which can persist even following abstinence (for a review see Solowji & Battisti et al., 2008). These effects are thought to be produced mainly by THC which acts in the CB1 receptor-dense hippocampus, an area of the brain critical in memory function. Administration of CBD alone, however, appears to have little effect on memory function, be it acute or chronic administration, during administration, or following a period of absence from exposure. In fact, CBD is being explored as a therapeutic agent to ameliorate memory deficits in a number of clinical settings including post-traumatic stress disorder. This analysis explored the long-term effects of high-CBD cannabis exposure on spatial learning and memory in the Long-Evans rat. Exposure to neither 10 mg/kg nor 40 mg/kg of either extract #129 or #81 produced any negative effects on spatial learning and memory as assessed by the MWT. There are a number of future considerations in the interpretation of this research. First, the lack of effects observed in this study are relevant to these specific extracts and their THC to CBD ratios. As has been evidenced in this chapter, the effects of exposure on spatial cognition appear to be dependent on the specific levels of cannabinoids in the extract. Second, future research should also consider the acute effects of chronic exposure to these high-CBD cannabis extracts on spatial learning and memory.

In summation, this analysis provides encouraging findings that chronic exposure to high-CBD cannabis extracts does not have long-term repercussions on spatial learning and memory in the Long-Evans rat.

Chapter 7

General Discussion

The preceding chapters detail the long-term behavioural consequences of chronic cannabis exposure in the Long-Evans rat. This chapter will discuss general trends observed in the results of these experiments, provide final comments about the relation of this research to the literature at large surrounding medicinal cannabis use, critically reflect on the experimental design and discuss limitations and improvements for future research, and finally provide comment on the potential translational impact of this research.

Review of Hypotheses and Predictions

To begin, let us first revisit the research questions guiding these experiments. They were:

1. Does chronic exposure to high-CBD cannabis extract create long-term changes in rodent behaviour?
2. Does chronic exposure to high-CBD cannabis extract create long-term changes in behaviour that are sexually dimorphic?

Based on these questions, I hypothesized:

1. Chronic exposure to high-CBD cannabis extract will have minimal long-term impact on rodent behaviour.
2. The effects of chronic exposure to high-CBD cannabis behaviour will produce sexually dimorphic effects on in rodent behaviour.

Based on the current literature and the constitution of the cannabis extracts selected, I predicted any changes in behaviour would be small and would not necessarily be of detriment to the animals.

Review of Findings by Behaviour

The pivotal finding of this research is that chronic exposure to high-CBD, low-THC cannabis extracts had very little effect on long-term behavioural outcomes in the Long-Evans rat.

Locomotor Activity

There were slight differences resulting from high-CBD cannabis exposure observed in the open field assessment of locomotor activity (Fig. 7.1). There was a significant effect of treatment with both extract #81 and extracts #81+98 on locomotor activity across the lifespan. In this task, the general activity level of animals administered extract #81 appeared to be significantly affected by the treatment when activity data was collected immediately following the cessation of dosing to when activity data was collected again two weeks following dosing. This trending effect was only observed between these two testing periods and appeared to be driven by the 40 mg/kg group which displayed elevated activity levels across this time, compared to the 10 mg/kg group and control group which decreased slightly. The activity levels of animals administered extracts #81+98 also appeared to be significantly affected by the treatment, however planned contrasts again revealed only a trending difference between behaviour collected immediately following dosing and two weeks following dosing. This effect appeared to

be driven by the 10 mg/kg group who exhibited elevated activity levels across this time period while the control group and 40 mg/kg group showed a decrease.

Fine Motor Control

There were no differences observed between groups in the Whishaw tray reaching task. This suggests that there were no long-term effects of these high-CBD cannabis extracts on this measure of fine motor function (Fig. 7.1).

Anxiety-Like Behaviour

There were small differences noted in the elevated plus maze assessment of anxiety-like behaviour (Fig. 7.1). In the elevated plus maze, animals administered both the 10 mg/kg and 40 mg/kg of extracts #10+98 spent less time in the closed arm indicating a reduction in anxiety-like behaviour. Anxiety-like behaviour was also assessed using the open field, where time spent in the margins of the open field was used as a proxy of thigmotaxis. There was a significant effect of treatment resulting in an increase in marginal time in animals exposed to 10 mg/kg of extracts #10+98, which would typically indicate an increase in anxiety. However, this was also accompanied by an increase in rearing behaviour. Assuming animals prefer rearing against vertical surfaces, this increase in marginal time is likely an indication of the animals increased rearing and not and increase in anxiety-like behaviour. As such, the only long-term effect of chronic, high-CBD cannabis exposure was a decrease in anxiety-like behaviour in animals exposed to extracts #10+98.

Spatial Learning and Memory

There were no significant long-term effects of chronic, high-CBD cannabis treatment observed on spatial learning and memory (Fig. 7.1). All animals, regardless of treatment group, showed similar acquisition curves suggesting they learned the task at similar rates and there were no perceivable deficits in spatial learning and memory resulting from this cannabis treatment.

Figure 7.1

A Summary of Behavioural Effects

Behavioural Assessment	Treatment			
	Extract #129	Extract #81	Extracts #10+98	Extracts #81+98
Locomotor Activity				
Immediately Following Dosing	-	-	-	-
Two Weeks Following Dosing	-	⇑ (40mg/kg)	-	⇑ (10mg/kg)
Fine Motor Control	-	-	-	-
Anxiety Like-Behaviour (EPM)				
Closed Arm Time	-	-	⇓ (10mg/kg, 40mg/kg	-
Closed Arm Entries	-	-	-	-
Anxiety-Like Behaviour (Open Field)	-	-	⇑ (10mg/kg)	-
Spatial Learning and Memory	-	-	N/A	N/A

Note. This table summarizes the behavioural effects observed in the two experiments described in this thesis.

[a] Chronic cannabis treatment resulted in hyperlocomotion in animals administered 40 mg/kg of extract #81, and 10 mg/kg of extracts #81+98. [b] Chronic cannabis treatment also resulted in a decrease in anxiety-like behaviour in animals administered 10 mg/kg and 40 mg/kg of extracts #10+98. [c] Finally, chronic cannabis treatment increased the amount of time animals administered 10mg/kg of extracts 10+98 spent in the marginal area of the open field. However, this was also accompanied by an increase in vertical exploration suggesting it may not be the most accurate representation of anxiety-like behaviour.

General Conclusions and Observations

In summary, the lack of long-term effects observed in these experiments are consistent with much of the literature which suggests that CBD exhibits few long-term repercussions on behaviour. A study by Kasten et al. (2019), similar to the experiments presented in this thesis, found that acute exposure to a combination of CBD and THC produced effects on both anxiety-like behaviours and locomotor activity of mice, but that these same effects were not observed following a period of abstinence. This is encouraging, as the results presented here echo these findings in a different rodent model providing more validity to the claim that high-CBD cannabis extracts cause little long-term effect on rodent behaviour.

General trends in the literature suggest that it is mainly acute exposure to cannabinoids that elicit observable changes in behaviour and that THC, rather than CBD, is likely responsible for more significant changes in behaviour. Long-term consequences of cannabis exposure are also dependent on the age at exposure. Adolescence is a critical time in development when the brain is extremely plastic and exposure to cannabinoids during this period, and into early adulthood, can cause dramatic changes in the brain and long-lasting effects on behaviour (Frontes et al., 2011; Gruber et al., 2012; Pope & Yurgelun, 1996;) It is for this reason that the utmost precaution must be taken when considering the use of medicinal cannabis during periods of development.

There were no interactions of cannabis treatment and sex observed in these analyses which suggests treatment affected both female and male animals equally. This effect was surprising considering previous research has observed differences in cannabis activity in the brain and different effects on behaviour depending on the sex of the user,

or animal (for a review see Crane et al., 2012). These differences are driven by differences in both the distribution and the availability of endocannabinoid receptors in the brains of female and males (Laurikainen et al., 2019; Farquhar et al., 2019), and potential hormonal interactions with exogenous and endogenous cannabinoids and their receptors (Paola et al., 2014). Current studies suggest that again, sex differences in the effects of cannabis exposure are primarily observed after acute exposure. Although there are some studies exploring sex differences following chronic cannabis administration, the results are varied and dependant on the dosage, the frequency of use and the behavioural measures collected (for a review see Crane et al., 2012).

There were no apparent biphasic effects in any of the extracts administered in this study as may be expected in an analysis of the acute effects of cannabis exposure on behaviour. Certain treatment groups did appear to be affected differently in the open field task, but the results did not appear to manifest in a biphasic manner as animals exposed to the 10 mg/kg dose of extract #81 showed no effect while the 40 mg/kg dose group did, and the animals exposed to the 10 mg/kg dose of extracts #81+98 showed an effect while the 40 mg/kg dose group did not. It is possible that the dosages utilized in this study fell within the same range, that is 10 mg/kg and 40 mg/kg are not sufficiently different to elicit a biphasic response. Future research in which the dosage level of these extracts is manipulated would provide more insight into these questions although considering neither dose of any extract generated significant detrimental effects, unless relevant to a specific pre-clinical application, this would not be the most pressing future direction of this research.

As was previously mentioned in Chapter 2, the extracts included in the second analysis were a 4:1 combination of one high-CBD low-THC extract (either #10 or #81), and a second extract with higher levels of THCV and THV in a 6:4:1 ratio respectively with CBD. These combinations of extracts had the highest level of non-CBD cannabinoids and, while the overall level of CBD was still far greater than the other constituents, these extracts showed the greatest number of long-term changes in behaviour of the four groups tested. However, that is not to say that these effects were necessarily overwhelming. Animals given the 10 mg/kg dose of extracts #81+98 showed a less significant decrease in locomotor activity across a two week washout period compared to the control and 40 mg/kg dose animals. Both the 10 mg/kg and 40 mg/kg dose of extracts #10+98 generated a reduction in anxiety-like behaviour as measured in the elevated plus maze when compared to controls. The other combined extract had no effect on this behaviour specifically which would suggest that some unique interaction of extracts #10 and #98 was driving this effect as the relative ratios of cannabinoids should have been consistent between both kinds of combined extracts, and no effects were observed in animals administered extracts #81+98. Animals exposed to the 10 mg/kg dose of the combination of extracts #81+98 showed a less significant decrease in locomotor activity across the two week washout period than did the 40 mg/kg and control groups. Overall, each combined extract only affected one of three behavioural outcomes tested and none of the effects, neither the decrease in anxiety-like behaviour nor the slightly elevated activity levels would likely be of detriment to the animal. In fact, these findings may lend favour to the potential use of these extracts in in combating conditions of anxiety, or neurological conditions characterized by hypomotility. So while these

combined extracts had the potential to illicit behavioural change due to the higher levels of THCV in extract #98 (Zagzoog et al., 2020; Garcia et al., 2011; O'Brien et al., 2013), they did not generate broad, long-term effects on a number of behaviours. Much like the high-CBD extracts alone, the observed effects were dependant on the behavioural assay being conducted and the specific dose of the extract. Again, this may be due to the relatively quick elimination of THCV from the system, or due to a unique interaction whereby the CBD ameliorates any long-term effects of the THCV, an idea which has yet to be explored.

Limitations and Suggestions

This research suggests that these specific high-CBD cannabis extracts do not exert any negative, long-term effects on the behaviours assessed by the selected test battery. However, there are potential refinements in methodology which would lend further support to this conclusion. First, collecting biological samples to assess cannabinoid levels in the body of animals following exposure would have provided helpful insight into the action of these extracts in the brain and served to lend support for some of the conclusions drawn in this thesis. Biological tissue samples were collected at the time of perfusion which, when analysed, may begin to provide additional evidence as to the long-term molecular effects of these high-CBD extracts on gene expression, but an interim measure during and immediately following cannabis exposure would be of benefit.

Second, the collection of a few more behavioural observations would lend further support to the conclusions drawn from these experiments. Including an observational measure of withdrawal symptoms would ensure that there were no negative behavioural effects of withdrawal from the selected cannabis extracts as the majority of the

behavioural data was collected more than two weeks following the cessation of dosing, likely after withdrawal symptoms would be the most apparent. As mentioned in Chapter 4, chronic high-CBD cannabis exposure did not affect motor learning as assessed by the Whishaw tray reaching task. To lend further weight to this observation, data could be sampled throughout the training period to confirm all animals were acquiring the task at the same rate.

Finally, the inclusion of another treatment group administered an extract with higher levels of THC than CBD, would have served to provide further confirmation as to the lack of effects resulting from high-CBD cannabis exposure. Predictable changes in behaviour resulting from the high-THC cannabis exposure contrasted with the apparent lack of effects resulting from the high-CBD cannabis extracts would provide confirmation that the lack of effects observed was indeed because the high-CBD extracts were not having a negative influence and not that there was some detail of the experimental methodology that was impeding the effects from being observed. Along the same vein, a separate analysis of extracts #10 and #98 would also provide more background information on effects of the combined extracts.

Future Directions

The future directions and potential applications of this research are extensive. There are several questions of basic research that should be addressed prior to considering further medicinal applications of cannabis. First, future research should seek to clarify the mechanism of action of CBD in the brain. All potential mechanisms of action proposed in this thesis were speculative and each behavioural assay should be

considered in turn when assessing the potential targets of CBD in the brain. This task would sound simple, but as has been explored briefly in the contents of this thesis, the molecular targets, pathways, and potential effects of CBD on the brain are diverse and broad reaching. Second, further characterization of the interactions and entourage effects of cannabinoids is pertinent. The diversity of effects on behaviour observed simply by changing levels of major cannabinoids in the system is evidence enough that medical cannabis should be developed with a profile of tightly regulated cannabinoids. This would ensure that the levels of all major constituents of cannabis; CBD, THC, THCV, and other cannabinoids, as well as terpenoids and flavonoids would be such that there would be no adverse behavioural or cognitive effects on those seeking to use cannabis for health reasons.

Speaking to a broader application, this research has encouragingly demonstrated that, at least with these four extracts and their specific cannabinoid profiles, cannabis treatment may indeed be a viable alternative or supplementary treatment, with few long-term effects. As has been mentioned previously, cannabis, specifically CBD, has a number of neuroprotective effects including, but not limited to, acting as an anti-inflammatory agent, an antioxidant, and reducing excitotoxicity in the brain (Mori et al., 2017; Marsicano et al., 2002; Hampson et al., 1998). This would suggest that cannabis may be a worthwhile treatment for any number of neurodegenerative disorders and diseases characterized by neuroinflammation or excessive oxidative stress. In fact, our research group is currently exploring the use of these extracts as therapeutic agents for their ability to improve behavioural recovery following stroke.

In recent years, public opinion has become more critical of traditional drugs, such as opioids. This, combined with a growing distrust in the zeitgeist of so called “big pharma”, has many people beginning to seek out alternative modalities in the treatment of health conditions. Cannabis offers an appealing alternative as it is a naturally occurring product with numerous health benefits and, as this research proposes, few long-term behavioural side effects. Statistics would suggest however, people seeking to use cannabis as an alternative or supplementary medicine are often doing so without input from a healthcare provider. This makes it even more critical to be able to disseminate research about medicinal cannabis to the general public so they are able to make the most informed decisions they can in regard to their health.

There are also a number of economic considerations to be made in regard to medicinal cannabis treatment. In countries where citizens do not have access to universal pharma care, cannabis treatment may offer an economically accessible course of treatment for pervasive medical issues for which traditional treatment is exceedingly costly. In cases where cannabis treatment is used in addition to traditional courses of treatment, the economic repercussions are more complex. An economic review of cannabis prescribed in addition to a traditional course of treatment in patients with multiple sclerosis suggests that whether cannabis treatment is cost-effective for patients is dependent on the country in which the survey was being conducted (Herzog et al., 2017). Four of the five industry-sponsored assessments included in this review concluded that cannabis treatment was cost-effective by using a cost-benefit analysis. The authors of this review suggest there are caveats these conclusions however, finding issue with the assessment of health-related quality of life, and economic evaluations of the studies

included in their analysis. Alternative analyses of the cost-effectiveness of cannabis treatment in patients with neuropathic pain showed that cannabis treatment was cost effective in populations who had not achieved success with one or more standard therapy (Griffin et al., 2019). This suggests that perhaps cannabis treatment is most economically advantageous for people who do not respond to traditional courses of treatment. The uncertainty of the reviews on this subject lead to the conclusion, any future clinical medical cannabis research should include an assessment of its cost-effectiveness. A study of the "willingness to pay" of a Canadian population found that when presented with two courses of MS treatment, 51% of the population would choose the course which included cannabis treatment. This further confirms that the interest in medical cannabis treatment is real but puts onus on researchers to make sure that the repercussions of medicinal cannabis use, including the clinical, behavioural, and economic repercussions, are explored fully to best serve the interests of the general public.

Conclusion

The research presented in this thesis explores the long-term effects of chronic high-CBD cannabis extract exposure on behaviour in the Long-Evans rat. Most of the hypotheses outlined in this thesis were confirmed as it was observed that there are few behavioural consequences resulting from chronic exposure to these four unique high-CBD, low-THC cannabis extracts. Broadening the literature regarding the long-term effects of cannabis is a much-needed resource, and these experiments have contributed to narrowing the gap in understanding the potential effects of this increasing popular drug. Overall, this research project will contribute significantly to our understanding of the

behavioural effects of high-CBD cannabis extract and serve to inform research regarding potential medical and health applications.

References

Adams, R., Hunt, M., & Clark, J. H. (1940). Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp. I. *Journal of the American Chemical Society, 62*(1), 196-200. https://doi.org/10.1021/ja01858a058

Ahmed, W., & Katz, S. (2016). Therapeutic use of cannabis in inflammatory bowel disease. *Gastroenterology & Hepatology, 12*(11), 668.

Blanco-Hinojo, L., Pujol, J., Harrison, B. J., Macià, D., Batalla, A., Nogué, S., Torrens, M., Farré, M., Deus, J., & Martín-Santos, R. (2017). Attenuated frontal and sensory inputs to the basal ganglia in cannabis users. *Addiction Biology, 22*(4), 1036-1047. https://doi.org/https://doi.org/10.1111/adb.12370

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chemical Biology, 14*(12), 1347-1356. https://doi.org/10.1016/j.chembiol.2007.11.006

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., Moriello, A. S., Davis, J. B., Mechoulam, R., & Di Marzo, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: Effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *British Journal of Pharmacology, 134*(4), 845-852. https://doi.org/10.1038/sj.bjp.0704327

Britch, S. C., Wiley, J. L., Yu, Z., Clowers, B. H., & Craft, R. M. (2017). Cannabidiol-Δ(9)-tetrahydrocannabinol interactions on acute pain and locomotor activity. *Drug and Alcohol Dependence, 175*, 187-197. https://doi.org/10.1016/j.drugalcdep.2017.01.046

Budney, A. J., Hughes, J. R., Moore, B. A., & Vandrey, R. (2004). Review of the validity and significance of cannabis withdrawal syndrome. *American Journal of Psychiatry, 161*(11), 1967-1977. https://doi.org/10.1176/appi.ajp.161.11.1967

Burgess, N., Maguire, E. A., & O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. *Neuron, 35*(4), 625-641. https://doi.org/10.1016/S0896-6273(02)00830-9

Busquets-Garcia, A., Gomis-González, M., Salgado-Mendialdúa, V., Galera-López, L., Puighermanal, E., Martín-García, E., Maldonado, R., & Ozaita, A. (2018). Hippocampal protein kinase C signaling mediates the short-term memory impairment induced by delta9-tetrahydrocannabinol. *Neuropsychopharmacology, 43*(5), 1021-1031. https://doi.org/10.1038/npp.2017.175

Campos, A. C., de Paula Soares, V., Carvalho, M. C., Ferreira, F. R., Vicente, M. A., Brandão, M. L., Zuardi, A. W., Zangrossi, H., & Guimarães, F. S. (2013). Involvement of serotonin-mediated neurotransmission in the dorsal periaqueductal gray matter on cannabidiol chronic effects in panic-like responses in rats. *Psychopharmacology, 226*(1), 13-24. https://doi.org/10.1007/s00213-012-2878-7

Campos, A. C., Fogaça, M. V., Scarante, F. F., Joca, S. R. L., Sales, A. J., Gomes, F. V., Sonego, A. B., Rodrigues, N. S., Galve-Roperh, I., & Guimarães, F. S. (2017). Plastic and neuroprotective mechanisms involved in the therapeutic effects of cannabidiol in psychiatric disorders. *Frontiers in Pharmacology, 8*(269). https://doi.org/10.3389/fphar.2017.00269

Carlson, G., Wang, Y., & Alger, B. E. (2002). Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neuroscience, 5*(8), 723-72. https://doi.org/10.1038/nn879

Cascio, M. G., Zamberletti, E., Marini, P., Parolaro, D., & Pertwee, R. G. (2015). The phytocannabinoid, Δ9-tetrahydrocannabivarin, can act through 5-HT1A receptors to produce antipsychotic effects. *British Journal of Pharmacology, 172*(5), 1305-1318. https://doi.org/https://doi.org/10.1111/bph.13000

Casiraghi, A., Roda, G., Casagni, E., Cristina, C., Musazzi, U. M., Franzè, S., Rocco, P., Giuliani, C., Fico, G., & Minghetti, P. (2018). Extraction method and analysis of cannabinoids in cannabis olive oil preparations. *Planta Medica, 84*(04), 242-249. https://doi.org/10.1055/s-0043-123074

Ceprián, M., Jiménez-Sánchez, L., Vargas, C., Barata, L., Hind, W., & Martínez-Orgado, J. (2017). Cannabidiol reduces brain damage and improves functional recovery in a neonatal rat model of arterial ischemic stroke. *Neuropharmacology, 116*, 151-159. https://doi.org/10.1016/j.neuropharm.2016.12.017

Chen, J., Paredes, W., Lowinson, J. H., & Gardner, E. L. (1991). Strain-specific facilitation of dopamine efflux by Δ9-tetrahydrocannabinol in the nucleus accumbens of rat: An in vivo microdialysis study. *Neuroscience Letters, 129*(1), 136-140. https://doi.org/10.1016/0304-3940(91)90739-g

Chevaleyre, V., & Castillo, P. E. (2004). Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron, 43*(6), 871-881. https://doi.org/10.1016/j.neuron.2004.08.036

Consroe, P., Kennedy, K., & Schram, K. (1991). Assay of plasma cannabidiol by capillary gas chromatography/ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. *Pharmacology Biochemistry and Behavior, 40*(3), 517-522. https://doi.org/https://doi.org/10.1016/0091-3057(91)90357-8

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

Davies, S. N., Pertwee, R. G., & Riedel, G. (2002). Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology, 42*(8), 993-1007. https://doi.org/https://doi.org/10.1016/S0028-3908(02)00060-6

Dean, B., Sundram, S., Bradbury, R., Scarr, E., & Copolov, D. (2001). Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience, 103*(1), 9-15. https://doi.org/10.1016/S0306-4522(00)00552-2

Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., Platt, B., & Riedel, G. (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour. *Psychopharmacology, 219*(3), 859-873. https://doi.org/10.1007/s00213-011-2415-0

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

Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S., & Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology, 34*(5), 605.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science, 258*(5090), 1946-1949. https://doi.org/10.1126/science.1470919

Devane, W. A., & Axelrod, J. (1994). Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid receptor, by brain membranes. *Proceedings of the National Academy of Sciences, 91*(14), 6698. https://doi.org/10.1073/pnas.91.14.6698

Di Marzo, V., Berrendero, F., Bisogno, T., González, S., Cavaliere, P., Romero, J., Cebeira, M., Ramos, J. A., & Fernández-Ruiz, J. J. (2000). Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ9-tetrahydrocannabinol-tolerant rats. *Journal of*

Neurochemistry, 74(4), 1627-1635. https://doi.org/https://doi.org/10.1046/j.1471-4159.2000.0741627.x

Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.-C., & Piomelli, D. (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature, 372*(6507), 686-691. https://doi.org/10.1038/372686a0

Di Marzo, V., Hill, M. P., Bisogno, T., Crossman, A. R., & Brotchie, J. M. (2000). Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *Federation of American Societies for Experimental Biology Journal, 14*(10), 1432-1438. https://doi.org/10.1096/fj.14.10.1432

El Batsh, M. M., Assareh, N., Marsden, C. A., & Kendall, D. A. (2012). Anxiogenic-like effects of chronic cannabidiol administration in rats. *Psychopharmacology, 221*(2), 239-247. https://doi.org/10.1007/s00213-011-2566-z

El Manira, A., & Kyriakatos, A. (2010). The role of endocannabinoid signaling in motor control. *Physiology, 25*(4), 230-238. https://doi.org/10.1152/physiol.00007.2010

El Sohly, M., & Gul, W. (2014). Constituents of Cannabis sativa. In R. G. Pertwee (Eds.), *Handbook of cannabis* (pp. 3-22). Oxford University Press. Oxford, UK.

Ekstrom, A. D., Kahana, M. J., Caplan, J. B., Fields, T. A., Isham, E. A., Newman, E. L., & Fried, I. (2003). Cellular networks underlying human spatial navigation. *Nature, 425*(6954), 184-188. https://doi.org/10.1227/01.neu.0000309603.15208.48

Ekstrom, A. D., Arnold, A. E. G. F., & Iaria, G. (2014). A critical review of the allocentric spatial representation and its neural underpinnings: Toward a network-based perspective. *Frontiers in Human Neuroscience, 8*(803). https://doi.org/10.3389/fnhum.2014.00803

Espejo-Porras, F., Fernández-Ruiz, J., Pertwee, R. G., Mechoulam, R., & García, C. (2013). Motor effects of the non-psychotropic phytocannabinoid cannabidiol that are mediated by 5-HT1A receptors. *Neuropharmacology, 75*, 155-163. https://doi.org/https://doi.org/10.1016/j.neuropharm.2013.07.024

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

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron*, *65*(1), 7–19. https://doi.org/10.1016/j.neuron.2009.11.031

Farquhar, C. E., Breivogel, C. S., Gamage, T. F., Gay, E. A., Thomas, B. F., Craft, R. M., & Wiley, J. L. (2019). Sex, THC, and hormones: Effects on density and sensitivity of CB(1) cannabinoid receptors in rats. *Drug and Alcohol Dependence, 194*, 20-27. https://doi.org/10.1016/j.drugalcdep.2018.09.018

Fernández-Ruiz, J. (2009). The endocannabinoid system as a target for the treatment of motor dysfunction. *British Journal of Pharmacology, 156*(7), 1029-1040. https://doi.org/10.1111/j.1476-5381.2008.00088.x

Fernandez-Ruiz, J., Lastres-Becker, I., Cabranes, A., Gonzalez, S., & Ramos, J. (2002). Endocannabinoids and basal ganglia functionality. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 66*(2-3), 257-267. https://doi.org/10.1054/plef.2001.0350

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry, 198*(6), 442-447. https://doi.org/10.1192/bjp.bp.110.077479

Galiègue, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., Bouaboula, M., Shire, D., LE Fur, G., & Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *European Journal of Biochemistry, 232*(1), 54-61. https://doi.org/https://doi.org/10.1111/j.1432-1033.1995.tb20780.x

Gallily, R., Yekhtin, Z., & Hanuš, L. O. (2018). The anti-inflammatory properties of terpenoids from cannabis. *Cannabis and Cannabinoid Research, 3*(1), 282-290. https://doi.org/10.1089/can.2018.0014

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society, 86*(8), 1646-1647. https://doi.org/10.1021/ja01062a046

García, C., Palomo-Garo, C., García-Arencibia, M., Ramos, J., Pertwee, R., & Fernández-Ruiz, J. (2011). Symptom-relieving and neuroprotective effects of the phytocannabinoid Δ9-THCV in animal models of Parkinson's disease. *British Journal of Pharmacology, 163*(7), 1495-1506. https://doi.org/https://doi.org/10.1111/j.1476-5381.2011.01278.x

Gibb, R. & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *Journal of Neuroscience Methods,* 79(1), 1-4. https://doi.org/10.1016/s0165-0270(97)00163-5

Gill, E. W., Paton, W. D. M., & Pertwee, R. G. (1970). Preliminary Experiments on the Chemistry and Pharmacology of Cannabis. *Nature, 228*(5267), 134-136. https://doi.org/10.1038/228134a0

Girgih, A. T., Udenigwe, C. C., & Aluko, R. E. (2011). In vitro antioxidant properties of hemp seed (Cannabis sativa L.) protein hydrolysate fractions. *Journal of the American Oil Chemists' Society, 88*(3), 381-389. https://doi.org/10.1007/s11746-010-1686-7

Glass, M., Faull, R., & Dragunow, M. (1997). Cannabinoid receptors in the human brain: A detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience, 77*(2), 299-318. https://doi.org/10.1016/S0306-4522(96)00428-9

Gomes, F. V., Resstel, L. B., & Guimarães, F. S. (2011). The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology, 213*(2), 465-473.

Granon, S., & Poucet, B. (1995). Medial prefrontal lesions in the rat and spatial navigation: Evidence for impaired planning. *Behavioral Neuroscience, 109*(3), 474. https://doi.org/10.1037//0735-7044.109.3.474

Grotenhermen, F. (2003). Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics, 42*(4), 327-360. https://doi.org/10.2165/00003088-200342040-00003

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gönenc, A., & Killgore, W. D. (2012). Age of onset of marijuana use impacts inhibitory processing. *Neuroscience Letters, 511*(2), 89-94. https://doi.org/10.1016/j.neulet.2012.01.039

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (−)Δ9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences, 95*(14), 8268. https://doi.org/10.1073/pnas.95.14.8268

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., Koehl, M., Abrous, Djoher N., Mendizabal-Zubiaga, J., Grandes, P., Liu, Q., Bai, G., Wang, W., Xiong, L., Ren, W., Marsicano, G., & Zhang, X. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell, 148*(5), 1039-1050. https://doi.org/10.1016/j.cell.2012.01.037

Harte-Hargrove, L. C., & Dow-Edwards, D. L. (2012). Withdrawal from THC during adolescence: Sex differences in locomotor activity and anxiety. *Behavioural Brain Research, 231*(1), 48-59. https://doi.org/10.1016/j.bbr.2012.02.048

Hayakawa, K., Mishima, K., Hazekawa, M., Sano, K., Irie, K., Orito, K., Egawa, T., Kitamura, Y., Uchida, N., Nishimura, R., Egashira, N., Iwasaki, K., & Fujiwara, M. (2008). Cannabidiol potentiates pharmacological effects of Δ9-tetrahydrocannabinol via CB1 receptor-dependent mechanism. *Brain Research, 1188*, 157-164. https://doi.org/10.1016/j.brainres.2007.09.090

Health Canada. (1973). *Final report of the commission of inquiry into the non-medical use of drugs* (Catalogue number H21-5370/2E). Retrieved from https://publications.gc.ca/site/eng/9.699765/publication.html

Health Canada. (2021). *Data on cannabis for medical purposes*. Retrieved from https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/medical-purpose.html

Health Canada. (2021). *Canadian cannabis survey 2020: summary.* Retrieved from https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/canadian-cannabis-survey-2020-summary.html

Herkenham, M., Lynn, A. B., de Costa, B. R., & Richfield, E. K. (1991). Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Research, 547*(2), 267-274. https://doi.org/10.1016/0006-8993(91)90970-7

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: A quantitative in vitro autoradiographic study. *Journal of Neuroscience, 11*(2), 563-583. https://doi.org10.1523/JNEUROSCI.11-02-00563.1991

Herzog, S., Shanahan, M., Grimison, P., Tran, A., Wong, N., Lintzeris, N., Simes, J., Stockler, M., & Morton, R. L. (2018). Systematic review of the costs and benefits of prescribed cannabis-based medicines for the management of chronic illness: Lessons from multiple sclerosis. *PharmacoEconomics, 36*(1), 67-78. https://doi.org/10.1007/s40273-017-0565-6

Hill, K. P., Palastro, M. D., Johnson, B., & Ditre, J. W. (2017). Cannabis and pain: A clinical review. *Cannabis and Cannabinoid Research, 2*(1), 96-104. https://doi.org/10.1089/can.2017.0017

Hillard, C. J. (2008). Role of cannabinoids and endocannabinoids in cerebral ischemia. *Current Pharmaceutical Design, 14*(23), 2347-2361. https://doi.org/10.2174/138161208785740054

Hložek, T., Uttl, L., Kadeřábek, L., Balíková, M., Lhotková, E., Horsley, R. R., Nováková, P., Šíchová, K., Štefková, K., & Tylš, F. (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.

Huestis, M. A., Mitchell, J. M., & Cone, E. J. (1996). Urinary excretion profiles of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in humans after single smoked doses of marijuana. *Journal of Analytical Toxicology, 20*(6), 441-452.

Kamprath, K., Plendl, W., Marsicano, G., Deussing, J. M., Wurst, W., Lutz, B., & Wotjak, C. T. (2009). Endocannabinoids mediate acute fear adaptation via glutamatergic neurons independently of corticotropin-releasing hormone signaling. *Genes, Brain, and Behaviour, 8*(2), 203-211. https://doi.org/10.1111/j.1601-183X.2008.00463.x

Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1998). The acquisition of skilled motor performance: Fast and slow experience-driven changes in primary motor cortex. *Proceedings of the National Academy of Sciences, 95*(3), 861-868. https://doi.org/10.1073/pnas.95.3.861

Kasten, C. R., Zhang, Y., & Boehm, S. L. (2019). Acute cannabinoids produce robust anxiety-like and locomotor effects in mice, but long-term consequences are age- and sex-dependent. *Frontiers in Behavioral Neuroscience, 13*(32). https://doi.org/10.3389/fnbeh.2019.00032

Kleim, J. A., Barbay, S., & Nudo, R. J. (1998). Functional reorganization of the rat motor cortex following motor skill learning. *Journal of Neurophysiology, 80*(6), 3321-3325. https://doi.org/10.1152/jn.1998.80.6.3321

Ko, G. D., Bober, S. L., Mindra, S., & Moreau, J. M. (2016). Medical cannabis - the Canadian perspective. *Journal of Pain Research, 2016(9)*, 735-744. https://doi.org/10.2147/jpr.S98182

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

Kreitzer, A. C., & Malenka, R. C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature, 445*(7128), 643-647. https://doi.org/10.1038/nature05506

Kuruvilla, M. V., & Ainge, J. A. (2017). Lateral entorhinal cortex lesions impair local spatial frameworks. *Frontiers in Systems Neuroscience, 11*(30). https://doi.org/10.3389/fnsys.2017.00030

Lafuente, H., Alvarez, F. J., Pazos, M. R., Alvarez, A., Rey-Santano, M. C., Mielgo, V., Murgia-Esteve, X., Hilario, E., & Martinez-Orgado, J. (2011). Cannabidiol reduces brain damage and improves functional recovery after acute hypoxia-ischemia in newborn pigs. *Pediatric Research, 70*(3), 272-277. https://doi.org/10.1203/PDR.0b013e3182276b11

Landgraf, Wigger, Holsboer, & Neumann. (1999). Hyper-Reactive Hypothalamo-Pituitary-Adrenocortical Axis in Rats Bred for High Anxiety-Related Behaviour. *Journal of Neuroendocrinology, 11*(6), 405-407. https://doi.org/https://doi.org/10.1046/j.1365-2826.1999.00342.x

Laprairie, R. B., Bagher, A. M., Kelly, M. E. M., & Denovan-Wright, E. M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *British Journal of Pharmacology, 172*(20), 4790-4805. https://doi.org/10.1111/bph.13250

Latash, M. L., & Lestienne, F. (2006). *Motor Control and Learning*. Springer. New York. https://doi.org/10.1007/0-387-28287-4

Laurikainen, H., Tuominen, L., Tikka, M., Merisaari, H., Armio, R.-L., Sormunen, E., Borgan, F., Veronese, M., Howes, O., Haaparanta-Solin, M., Solin, O., & Hietala, J. (2019). Sex difference in brain CB1 receptor availability in man. *NeuroImage, 184*, 834-842. https://doi.org/https://doi.org/10.1016/j.neuroimage.2018.10.013

Lee, D., Bergamaschi, M. M., Milman, G., Barnes, A. J., Queiroz, R. H. C., Vandrey, R., & Huestis, M. A. (2015). Plasma cannabinoid pharmacokinetics after controlled smoking and ad libitum cannabis smoking in chronic frequent users. *Journal of Analytical Toxicology, 39*(8), 580-587. https://doi.org/10.1093/jat/bkv082

Leweke, F. M., Giuffrida, A., Wurster, U., Emrich, H. M., & Piomelli, D. (1999). Elevated endogenous cannabinoids in schizophrenia. *NeuroReport, 10*(8), 1665-1669. https://doi.org/10.1097/00001756-199906030-00008

Li, H. L. (1974). An archaeological and historical account of Cannabis in China. *Economic Botany, 28*(4), 437-448. https://doi.org/10.1007/bf02862859

Li, L., Bender, K. J., Drew, P. J., Jadhav, S. P., Sylwestrak, E., & Feldman, D. E. (2009). Endocannabinoid signaling is required for development and critical period plasticity of the whisker map in somatosensory cortex. *Neuron, 64*(4), 537-549. https://doi.org/10.1016/j.neuron.2009.10.005

Libzon, S., Schleider, L. B.-L., Saban, N., Levit, L., Tamari, Y., Linder, I., Lerman-Sagie, T., & Blumkin, L. (2018). Medical cannabis for pediatric moderate to severe complex motor disorders. *Journal of Child Neurology, 33*(9), 565-571. https://doi.org/10.1177/0883073818773028

Lichtman, A. H., Dimen, K. R., & Martin, B. R. (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology, 119*(3), 282-290. https://doi.org/10.1007/BF02246292

Long, L. E., Chesworth, R., Huang, X.-F., McGregor, I. S., Arnold, J. C., & Karl, T. (2010). A behavioural comparison of acute and chronic Δ9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *International Journal of Neuropsychopharmacology, 13*(7), 861-876. https://doi.org/10.1017/s1461145709990605

Ma, Y. L., Weston, S. E., Whalley, B. J., & Stephens, G. J. (2008). The phytocannabinoid Delta(9)-tetrahydrocannabivarin modulates inhibitory neurotransmission in the cerebellum. *British Journal of Pharmacology, 154*(1), 204-215. https://doi.org/10.1038/bjp.2008.57

Mackie, K., & Hille, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proceedings of the National Academy of Sciences, 89*(9), 3825. https://doi.org/10.1073/pnas.89.9.3825

Mackie, K., Lai, Y., Westenbroek, R., & Mitchell, R. (1995). Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *The Journal of Neuroscience, 15*(10), 6552. https://doi.org/10.1523/JNEUROSCI.15-10-06552.1995

Malone, D. T., & Taylor, D. A. (1999). Modulation by fluoxetine of striatal dopamine release following Δ9-tetrahydrocannabinol: A microdialysis study in conscious rats. *British Journal of Pharmacology, 128*(1), 21-26. https://doi.org/10.1038/sj.bjp.0702753

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

Marsicano, G., Moosmann, B., Hermann, H., Lutz, B., & Behl, C. (2002). Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *Journal of Neurochemistry, 80*(3), 448-456. https://doi.org/https://doi.org/10.1046/j.0022-3042.2001.00716.x

Mathur, B. N., & Lovinger, D. M. (2012). Serotonergic action on dorsal striatal function. *Parkinsonism & Related Disorders, 18*, S129-S131.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature (London), 346*(6284), 561-564. https://doi.org/10.1038/346561a0

McAllister, S. D., & Glass, M. (2002). CB1 and CB2 receptor-mediated signalling: A focus on endocannabinoids. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 66*(2), 161-171. https://doi.org/https://doi.org/10.1054/plef.2001.0344

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R., Pertwee, R. G., Griffin, G., Bayewitch, M., Barg, J., & Vogel, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology, 50*(1), 83-90. https://doi.org/https://doi.org/10.1016/0006-2952(95)00109-D

Metz, G. A. S., & Whishaw, I. Q. (2000). Skilled reaching an action pattern: Stability in rat (Rattus norvegicus) grasping movements as a function of changing food pellet size. *Behavioural Brain Research, 116*(2), 111-122. https://doi.org/10.1016/S0166-4328(00)00245-X

Mezey, E., Tóth, Z. E., Cortright, D. N., Arzubi, M. K., Krause, J. E., Elde, R., Guo, A., Blumberg, P. M., & Szallasi, A. (2000). Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proceedings of the National Academy of Sciences of the United States of America, 97*(7), 3655-3660. https://doi.org/10.1073/pnas.060496197

Mishima, K., Egashira, N., Matsumoto, Y., Iwasaki, K., & Fujiwara, M. (2002). Involvement of reduced acetylcholine release in Δ9-tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. *Life Sciences, 72*(4-5), 397-407. https://doi.org/10.1016/s0024-3205(02)02274-9.

Miyachi, S., Hikosaka, O., & Lu, X. (2002). Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Experimental Brain Research, 146*(1), 122-126. https://doi.org/10.1007/s00221-002-1213-7

Miyachi, S., Hikosaka, O., Miyashita, K., Kárádi, Z., & Rand, M. K. (1997). Differential roles of monkey striatum in learning of sequential hand movement. *Experimental Brain Research, 115*(1), 1-5. https://doi.org/10.1007/pl00005669

Millar, S. A., Stone, N. L., Yates, A. S., & O'Sullivan, S. E. (2018). A systematic review on the pharmacokinetics of cannabidiol in humans. *Frontiers in Pharmacology, 9*(1365). https://doi.org/10.3389/fphar.2018.01365

Misner, D. L., & Sullivan, J. M. (1999). Mechanism of Cannabinoid Effects on Long-Term Potentiation and Depression in Hippocampal CA1 Neurons. *The Journal of Neuroscience, 19*(16), 6795. https://doi.org/10.1523/JNEUROSCI.19-16-06795.1999

Moreno-Alcázar, A., Gonzalvo, B., Canales-Rodríguez, E. J., Blanco, L., Bachiller, D., Romaguera, A., Monté-Rubio, G. C., Roncero, C., McKenna, P. J., & Pomarol-Clotet, E. (2018). Larger gray matter volume in the basal ganglia of heavy cannabis users detected by voxel-based morphometry and subcortical volumetric analysis. *Frontiers in Psychiatry, 9*(175). https://doi.org/10.3389/fpsyt.2018.00175

Mori, M. A., Meyer, E., Soares, L. M., Milani, H., Guimarães, F. S., & de Oliveira, R. M. W. (2017). Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 75*, 94-105. https://doi.org/https://doi.org/10.1016/j.pnpbp.2016.11.005

Morris, R. G., Garrud, P., Rawlins, J. a., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683. https://doi.org/10.1038/297681a0

Morris, R. (1984). Developments of a water maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods,* 11, 47-60.

Muller, C., Morales, P., & Reggio, P. H. (2019). Cannabinoid ligands targeting TRP channels. *Frontiers in Molecular Neuroscience, 11*(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

Namdar, D., & Koltai, H. (2018). Medical Cannabis for the treatment of inflammation. *Natural Product Communications, 13*(3), 1934578X1801300304.

Nelovkov, A., Philips, M.-A., Kõks, S., & Vasar, E. (2003). Rats with low exploratory activity in the elevated plus-maze have the increased expression of limbic system-associated membrane protein gene in the periaqueductal grey. *Neuroscience Letters, 352*(3), 179-182. https://doi.org/https://doi.org/10.1016/j.neulet.2003.08.061

O'Brien, L. D., Wills, K. L., Segsworth, B., Dashney, B., Rock, E. M., Limebeer, C. L., & Parker, L. A. (2013). Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. *Pharmacology Biochemistry and Behavior, 103*(3), 597-602. https://doi.org/https://doi.org/10.1016/j.pbb.2012.10.008

O'Connell, A. W., Fox, G. B., Kjøller, C., Gallagher, H. C., Murphy, K. J., Kelly, J., & Regan, C. M. (2001). Anti-ischemic and cognition-enhancing properties of NNC-

711, a γ-aminobutyric acid reuptake inhibitor. *European Journal of Pharmacology, 424*(1), 37-44. https://doi.org/10.1016/S0014-2999(01)01116-5

O'Shaughnessy, W. B. (1843). On the preparations of the Indian hemp, or Gunjah: Cannabis indica their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *Provincial Medical Journal and Retrospect of the Medical Sciences, 5*(123), 363-369.

Palma, P. (2018). Unexpected healers: Chinese medicine in the age of global migration (Lima and California, 1850-1930). *História, Ciências, Saúde-Manguinhos, 25*(1), 13-31. https://doi.org/10.1590/s0104-59702018000100002

Pertwee, R. G., Thomas, A., Stevenson, L. A., Ross, R. A., Varvel, S. A., Lichtman, A. H., Martin, B. R., & Razdan, R. K. (2007). The psychoactive plant cannabinoid, Δ9-tetrahydrocannabinol, is antagonized by Δ8- and Δ9-tetrahydrocannabivarin in mice in vivo. *British Journal of Pharmacology, 150*(5), 586-594. https://doi.org/10.1038/sj.bjp.0707124

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *British Journal of Pharmacology, 153*(2), 199-215. https://doi.org/10.1038/sj.bjp.0707442

Paola Castelli, M., Fadda, P., Casu, A., Sabrina Spano, M., Casti, A., Fratta, W., & Fattore, L. (2014). Male and female rats differ in brain cannabinoid CB1 receptor density and function and in behavioural traits predisposing to drug addiction: Effect of ovarian hormones. *Current Pharmaceutical Design, 20*(13), 2100-2113.

Pope, H. G., Jr, & Yurgelun-Todd, D. (1996). The residual cognitive effects of heavy marijuana use in college students. *Journal of the American Medical Association, 275*(7), 521-527. https://doi.org/10.1001/jama.1996.03530310027028

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., & Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nature Neuroscience, 12*(9), 1152-1158. https://doi.org/10.1038/nn.2369

Ramanathan, D., Tuszynski, M. H., & Conner, J. M. (2009). The basal forebrain cholinergic system is required specifically for behaviorally mediated cortical map plasticity. *Journal of Neuroscience, 29*(18), 5992-6000. https://doi.org/10.1523/jneurosci.0230-09.2009

Ravula, A., Chandasana, H., Setlow, B., Febo, M., Bruijnzeel, A. W., & Derendorf, H. (2018). Simultaneous quantification of cannabinoids tetrahydrocannabinol, cannabidiol and CB1 receptor antagonist in rat plasma: An application to characterize pharmacokinetics after passive cannabis smoke inhalation and co-

administration of rimonabant. *Journal of Pharmaceutical and Biomedical Analysis, 160*, 119-125. https://doi.org/10.1016/j.jpba.2018.07.004

Rock E.M., Parker L.A. (2021) Constituents of *Cannabis Sativa*. In Murillo-Rodriguez E., Pandi-Perumal S.R., Monti J.M. (Eds., Vol. 1264*) Cannabinoids and Neuropsychiatric Disorders*. *Advances in Experimental Medicine and Biology*. Springer, NY.

Rog, D. J., Nurmikko, T. J., Friede, T., & Young, C. A. (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology, 65*(6), 812-819. https://doi.org/10.1212/01.wnl.0000176753.45410.8b

Romero, J., Berrendero, F., Manzanares, J., Pérez, A., Corchero, J., Fuentes, J. A., Fernández-Ruiz, J. J., & Ramos, J. A. (1998). Time-course of the cannabinoid receptor down-regulation in the adult rat brain caused by repeated exposure to Δ9-tetrahydrocannabinol. *Synapse, 30*(3), 298-308. https://doi.org/10.1002/(sici)1098-2396(199811)30:3<298::aid-syn7>3.0.co;2-6

Romero, J., Garcia-Palomero, E., Castro, J. G., Garcia-Gil, L., Ramos, J. A., & Fernandez-Ruiz, J. J. (1997). Effects of chronic exposure to Δ9-tetrahydrocannabinol on cannabinoid receptor binding and mRNA levels in several rat brain regions. *Molecular Brain Research, 46*(1), 100-108. https://doi.org/https://doi.org/10.1016/S0169-328X(96)00277-X

Russo, E. B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. *Chemistry & Biodiversity, 4*(8), 1614-1648. https://doi.org/https://doi.org/10.1002/cbdv.200790144

Schoedel, K. A., Szeto, I., Setnik, B., Sellers, E. M., Levy-Cooperman, N., Mills, C., Etges, T., & Sommerville, K. (2018). Abuse potential assessment of cannabidiol (CBD) in recreational polydrug users: A randomized, double-blind, controlled trial. *Epilepsy & Behavior, 88*, 162-171. https://doi.org/https://doi.org/10.1016/j.yebeh.2018.07.027

Südhof, T. C., & Malenka, R. C. (2008). Understanding synapses: Past, present, and future. *Neuron, 60*(3), 469-476. https://doi.org/https://doi.org/10.1016/j.neuron.2008.10.011

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., & Waku, K. (1995). 2-Arachidonoylgylcerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochemical and Biophysical Research Communications, 215*(1), 89-97. https://doi.org/https://doi.org/10.1006/bbrc.1995.2437

Stella, N., Schweitzer, P., & Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature, 388*(6644), 773-778. https://doi.org/10.1038/42015

Stella, N., & Piomelli, D. (2001). Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *European Journal of Pharmacology, 425*(3), 189-196. https://doi.org/10.1016/S0014-2999(01)01182-7

Strasser, F., Luftner, D., Possinger, K., Ernst, G., Ruhstaller, T., Meissner, W., Ko, Y.-D., Schnelle, M., Reif, M., & Cerny, T. (2006). Comparison of orally administered cannabis extract and delta-9-tetrahydrocannabinol in treating patients with cancer-related anorexia-cachexia syndrome: a multicenter, phase III, randomized, double-blind, placebo-controlled clinical trial from the Cannabis-In-Cachexia-Study-Group. *Journal of Clinical Oncology, 24*(21), 3394-3400. https://doi.org/10.1200/JCO.2005.05.1847

Thomas, A., Stevenson, L. A., Wease, K. N., Price, M. R., Baillie, G., Ross, R. A., & Pertwee, R. G. (2005). Evidence that the plant cannabinoid Δ9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. *British Journal of Pharmacology, 146*(7), 917-926. https://doi.org/https://doi.org/10.1038/sj.bjp.0706414

Tsou, K., Nogueron, M. I., Muthian, S., Sañudo-Peña, M. C., Hillard, C. J., Deutsch, D. G., & Walker, J. M. (1998). Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neuroscience Letters, 254*(3), 137-140. https://doi.org/https://doi.org/10.1016/S0304-3940(98)00700-9

Tyree, G. A., Sarkar, R., Bellows, B. K., Ellis, R. J., Atkinson, J. H., Marcotte, T. D., Wallace, M. S., Grant, I., Shi, Y., Murphy, J. D., & Grelotti, D. J. (2019). A cost-effectiveness model for adjunctive smoked cannabis in the treatment of chronic neuropathic pain. *Cannabis and Cannabinoid Research, 4*(1), 62-72. https://doi.org/10.1089/can.2018.0027

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*, *12*(22), 22425–22444. https://doi.org/10.18632/aging.202225

Westfall, T. C., & Tittermary, V. (1982). Inhibition of the electrically induced release of [3H]dopamine by serotonin from superfused rat striatal slices. *Neuroscience Letters, 28*(2), 205-209. https://doi.org/10.1016/0304-3940(82)90153-7

Whishaw, I. Q., O'Connor, W. T., & Dunnett, S. B. (1986). The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain, 109*(5), 805-843. https://doi.org/10.1093/brain/109.5.805

Wilson, R. I., & Nicoll, R. A. (2002). Endocannabinoid signaling in the brain. *Science, 296*(5568), 678-682. https://doi.org/doi:10.1126/science.1063545

Wise, S. P., & Donoghue, J. P. (1986). Motor cortex of rodents. *Sensory-motor areas and aspects of cortical connectivity*, Springer, Boston, MA, 243-270.

Woolsey, C. N., Settlage, P. H., Meyer, D. R., Sencer, W., Pinto Hamuy, T., & Travis, A. M. (1952). Patterns of localization in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. *Association for Research in Neural and Mental Diseases, 30*, 238-264.

Yin, H. H., Mulcare, S. P., Hilário, M. R., Clouse, E., Holloway, T., Davis, M. I., Hansson, A. C., Lovinger, D. M., & Costa, R. M. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience, 12*(3), 333-341. https:doi.org/ 10.1038/nn.2261

Zagzoog, A., Mohamed, K. A., Kim, H. J. J., Kim, E. D., Frank, C. S., Black, T., Jadhav, P. D., Holbrook, L. A., & Laprairie, R. B. (2020). In vitro and in vivo pharmacological activity of minor cannabinoids isolated from Cannabis sativa. *Scientific Reports, 10*(1), 20405. https://doi.org/10.1038/s41598-020-77175-y

Zanelati, T., Biojone, C., Moreira, F., Guimarães, F., & Joca, S. R. (2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *British Journal of Pharmacology, 159*(1), 122-128.

Zilles, K. (1985). *The cortex of the rat: A stereotaxic atlas*. Springer-Verlag, Belin, Germany.

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H.-h., Sørgård, M., Di Marzo, V., Julius, D., & Högestätt, E. D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature, 400*(6743), 452-457. https://doi.org/10.1038/22761