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Differential effects of cannabis vapour constituents on brain connectivity: Exploring the long-term effects of adolescent exposure

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Abstract

Cannabis use is common in adolescence and there is evidence for sex differences regarding the long-term effect of cannabis use. We aimed to investigate how exposure to 3 types of cannabis vapour in adolescent rats impacts brain development using magnetic resonance imaging. Male and female Sprague Dawley rats were divided into four groups and exposed to high-CBD, high-THC, balanced CBD + THC, or air at post-natal days 28-42 using a vaporizer. In adulthood, rats underwent diffusion and functional MRI. Results indicated sex-dependent differences in the long-term effects of cannabis exposure in the adult brain. In male rats, we found a single network with altered functional connectivity amongst the four groups and two networks with altered structural connectivity amongst the four groups. In female rats, MRI results indicated no altered structural or functional networks. Adolescent cannabis vapour exposure can lead to long-lasting effects in adulthood, with males possibly being more vulnerable.

Keywords

Cannabis, Adolescence, Development, MRI.

Summary for Lay Audience

As more countries legalize cannabis, there is a great availability of cannabis products, with new equipment and routs of administration. For instance, there has been a dramatic increase in cannabis vaping consumption in North America, especially among adolescents. Adolescence is a critical period for brain development and cannabis usage during this period of life might have long-term detrimental effects. Thus, this study aimed to investigate how vapor exposure to 3 types of cannabis in adolescent rats impacts brain development. We employed a technique called magnetic resonance imaging (MRI), that allows us to investigate the functionality and structure of the brain. We administered cannabis to male and female adolescent rats using a vaporizer, to simulate the rout of administration used by humans. Upon reaching adulthood, we performed MRI analysis on the rats. We found that the adult male rats exposed to cannabis in adolescence had altered brain functionality and structure in many brain regions related to cognition and emotion. On the other hand, adult female rats showed no altered brain functionality or structure. We conclude adolescent cannabis vapour exposure is related to long-term brain alterations, with males being more vulnerable than females.

Co-Authorship Statement

This thesis is a result of a collaboration of students in Dr. Khokhar's Lab.

Jaiden Smith and Dr. Hakan Kayir performed the cannabis exposures and the behavioural experiments briefly described in this thesis.

I worked in collaboration with Dr. Jude Frie and Dr. Patrick McCunn in order to perform the MRI analysis.

Dr. Hakan Kayir performed the cannabinoid levels blood analysis.

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Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

CBD, cannabidiol

THC, Δ9-tetrahydrocannabinol

ES, endocannabinoid system

CB1, cannabinoid receptor 1

CB2, cannabinoid receptor 2

2-AG, 2-arachidonoyl glycerol

GPCR, G protein-coupled receptor

5HT1a, serotonin 1A receptor

TRPV1, transient receptor potential cation channel

CUD, cannabis use disorder

VTA, ventral tegmental area

IQ, intelligence quotient

MRI, Magnetic Resonance Imaging

fMRI, functional MRI

BOLD, Blood Oxygen Level Dependent

rsfMRI, resting-state fMRI

RSFC, resting state functional connectivity

NBS, Network Based Statistics

dMRI, diffusion MRI

DMN, default mode network

PCC, posterior cingulate cortex

PND, postnatal day

MS, mass spectrometry

ESI, electrospray ionization

PRM, parallel-reaction monitoring

HCD, higher-energy C-trap dissociation

PPI, prepulse inhibition

IP, intraperitoneal

CRI, constant rate infusion

CFMM, Centre for Functional and Metabolic Mapping

EPI, echo-planar-imaging

RABIES, Rodent Automated Bold Improvement of EPI Sequences

FWHM, full-width at half maximum

FSL, fMRI Software Library

iFOD2, Integration over Fiber Orientation Distributions

FWE, family-wise error

ANOVA, analysis of variance

PET, positron emission tomography

Chapter 1

1 Introduction

1.1 Cannabis and the endocannabinoid system

Cannabis is a psychoactive plant used medically and recreationally by human populations for thousands of years (Mechoulam & Parker, 2013). It has two major compounds, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), with the latter being responsible for its main psychoactive effects (Lafaye et al., 2017). Cannabis is also the third most consumed controlled substance worldwide after alcohol and tobacco, and it is estimated that about 3.9% of the global adult population have used cannabis in the previous year (Hasin et al., 2013; World Drug Report, 2020; Connor et al., 2021). Specifically in North America, it is estimated that 8% of the United States population uses cannabis daily (Ritchay et al., 2021) and 18.7% of the United States population and 26% of the Canadian population reported cannabis use in the past 12 months (Substance Abuse and Mental Health Services Administration, 2020; Canadian Cannabis Survey, 2024).

THC and CBD exert their action in the central nervous system by interaction with the endocannabinoid system (ES). The ES mainly comprises the cannabinoid receptor 1 (CB1), cannabinoid receptor 2 (CB2), the endogenous ligands arachidonoylethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG), and the enzymes responsible for their synthesis and hydrolysis (Pacher et al., 2020). The inhibitory and retrograde signaling nature of the ES allows for the modulation of many other neurotransmitter systems within the brain, including dopamine and glutamate (Mechoulam & Parker, 2013). In fact, CB1 is the most abundant G protein-coupled receptor (GPCR) in the brain, is expressed in neurons and astrocytes, and pharmacological activity in this receptor exerts profound effects on downstream signaling pathways and networks (Mechoulam & Parker, 2013; Lu & Mackie, 2021).

Despite having about 100 cannabinoids with diverse actions, THC and CBD are responsible for the most psychoactive and behavioural effects of cannabis (Zeyl et al., 2020). The “high” that follows cannabis use is caused by the interaction of THC with the

CB1 in the brain. Common subjective effects of acute THC use include euphoria, relaxation, and feelings of detachment; long-term effects of excessive use might also include panic, anxiety, depression, and psychosis (Johns, 2001; Levine et al., 2017; Connor et al., 2021). Even though the complete binding profiles for CBD are yet to be identified, the two main targets are believed to be the serotonin 1A receptor (5HT1a) and transient receptor potential cation channel (TRPV1), and possibly cannabinoid and opioid receptors (de Almeida & Devi, 2020). CBD has gained much attention in recent years due to its clinical effects on a variety of conditions, including psychosis, epilepsy, inflammation, anxiety, depression, and chronic pain (Muller & Reggio, 2020; Peng et al., 2022).

Despite the common belief that cannabis does not produce dependence or withdrawal symptoms, the acute “high” triggered by cannabis consumption can lead to a desire for repetitive use and some users might progress to cannabis use disorder (CUD) (Connor et al., 2021). The condition is characterized by the persistence of cannabis use despite negative consequences that promote distress or impairment in functioning (Sherman & McRae-Clark, 2016). A recent meta-analysis of epidemiological studies showed that up to 22% of people who use cannabis meet the criteria for CUD. It was also found that daily and weekly users, as well as young individuals, are at greater risk for developing CUD (Leung et al., 2020). Currently, there are no pharmacological treatments available for CUD, and psychosocial-based interventions are the first-line treatment used, even though the vast majority of users remain untreated (Sherman & McRae-Clark, 2016; Connor et al., 2021).

1.2 Adolescence and cannabis use

Adolescence is a critical time for brain development. During this developmental period, the brain creates more robust neuronal pathways, through the maintenance of useful neurons and synapses while others are pruned and eliminated; this process results in an overall reduction in grey matter and an increase in whole-brain white matter (Giorgio et al., 2008; Blest-Hopley et al., 2020). This fine-tuning in brain pathways during adolescence leads to the maturation of many brain systems and is accompanied by behavioural changes, such as increased risk-taking, impulsivity, and drug initiation

(Blest-Hopley et al., 2020). Unfortunately, due to the ongoing maturation process, the adolescent brain is highly vulnerable to exogenous insults, such as drug use, with possible long-lasting effects (Salmanzadeh et al., 2020).

Similarly to other drugs, it is during adolescence when most cannabis use begins, with an average age of onset between 18 and 19 years old (Degenhardt et al., 2016). Unfortunately, studies have shown that regular cannabis use during this developmental stage is associated with a higher likelihood of negative consequences when compared to regular use in adulthood and is an important contributing factor for psychiatry vulnerability, especially the development of psychotic symptoms (Volkow et al., 2016; Rubino & Parolaro, 2014). Additionally, as legalization increases worldwide, the perceived risk associated with cannabis consumption is decreasing, which could lead to an even larger increase in adolescent cannabis users in the future (Volkow et al., 2016). Data indicates that the perceived risk of cannabis use by adolescents has also been decreasing in the last decade (Lorenzetti et al., 2020).

In addition to that, studies have shown that the cannabinoid system undergoes substantial changes throughout adolescence. Endocannabinoid levels such as 2-AG are reported to decrease throughout development while anandamide levels are reported to be higher in the nucleus accumbens and prefrontal cortex in mid- and late-adolescence (Thorpe et al., 2020). In line with these fluctuations, the expression of cannabinoid receptors is also reported to have marked changes in adolescence. In general, both CB1 and CB2 protein expression are higher in the adult prefrontal cortex and nucleus accumbens compared to adolescent expression, even though CB1 synaptic inhibition in the prefrontal cortex pyramidal neurons of rats decreases (Thorpe et al., 2020).

Other studies have addressed the effects of cannabis use during adolescence on neurotransmitter profiles in the rat brain. Adolescent THC exposure lowers the protein expression of CB1 receptors in the ventral tegmental area (VTA) and prefrontal cortex up to 24 hours after administration, with some evidence showing that this reduction might persist into adulthood. Adolescent THC exposure also increases anandamide in the nucleus accumbens of rats, and adolescent exposure to the synthetic cannabinoid agonist

WIN 55,212-2 results in increased levels of anandamide in adulthood. These effects on anandamide levels might be related to impairment in short and long-term plasticity and reward processing and, therefore, underlie the behavioural changes observed upon adolescent THC exposure (Thorpe et al., 2020).

As cannabis legalization becomes more widespread worldwide, there is a greater availability of cannabis products, devices, and new routes of administration. In recent years, there has been a dramatic increase in cannabis vaping (Hopfer, 2014). As such, the number of adolescents using cannabis vaping products has also risen dramatically (Hopfer, 2014). For example, adolescent cannabis vaping has increased from 7.2% in 2017 to 13.2% in the year of 2020 (Lim et al., 2022). Thus, it is essential to research the impact that cannabis vapor exposure has on the developing adolescent brain and if it is linked to any long-term implications in adulthood.

1.3 Human studies on the impact of adolescent cannabis use on cognition

Much attention is given to the effects of cannabis on mood. However, its effects on cognition might be the most severe and enduring, especially on memory function (Levine et al., 2017). Solowij et al. (2011) conducted a neuropsychological study to investigate if cannabis use is related to learning and memory impairment in adolescents aged 16-20 years old. Adolescents who use cannabis performed worse on verbal learning and memory tasks compared to non-users. The cognitive impairment was also associated with the duration, quantity, frequency, and onset age of cannabis use (Solowij et al., 2011). Another study compared 14-17 year-old adolescent cannabis users with healthy controls on multiple cognitive domains. Adolescent cannabis use was associated with short-term recall memory (Dougherty et al., 2013). Finally, Gruber et al. (2012) compared heavy cannabis users with non-users controls on multiple cognitive tasks, including many memory measurements. Chronic, heavy cannabis users performed significantly worse in several measures of cognition, especially those related to executive function. Also, individuals who started cannabis use before the age of 16 had more prominent impairment compared to those who started using cannabis after 16 years old (Gruber et al., 2012).

Due to the vulnerability of the brain during adolescence, acute cannabis exposure might have long-term consequences on cognition. Longitudinal studies have the capability of understanding the long-term effects of adolescent cannabis use in humans (Lorenzetti et al., 2020). Morin et al. (2019) investigated the effects on cognition of cannabis consumption in 3826 seventh-grade students for four years in Canada. The average frequency of cannabis use predicted lower performance in working memory, perceptual reasoning, and inhibition. In addition to that, the within-subject analysis showed that a further increase in cannabis consumption frequency was associated with impairment in delayed recall memory (Morin et al., 2019). Another longitudinal study conducted by Tait et al. (2011) evaluated the impact of cannabis consumption on cognitive function following 2404 adolescents and young adults for a period of eight years. Through the use of self-report, authors defined six groups of cannabis usage, ranging from “never” to “remain heavy”. Even though there were significant differences in cognitive measures at baseline between cannabis groups, only immediate recall measures showed evidence for improvement associated with prolonged cannabis abstinence in past heavy users. For all other cognitive measures, authors found no significant differences related to cannabis consumption (Tait et al., 2011). Finally, a prospective longitudinal study administered neuropsychological tests on pre-teens aged 9-12 as a measurement of pre-drug exposure (Fried et al., 2005). During the ages of 17-21, they investigated if cannabis use was associated with cognitive impairment. Cannabis use was associated with lower intelligence quotient (IQ), immediate and delayed memory performance (Fried et al., 2005).

Since most human studies are not able to assess cognitive functioning before cannabis use initiation, and therefore exclude the possibility that poor cognition in cannabis users might precede heavier and prolongated cannabis use, studies in twins are performed to evaluate if any difference in cognitive performance in cannabis users is related to a genetic vulnerability or the cannabis use (Lorenzetti et al., 2020). In a co-twin design study, Meier et al. (2018) followed individuals from age 5-18 and assessed the frequency of cannabis use, IQ, and executive function (attention, vigilance, and working memory) throughout this timeline. Adolescents who used cannabis at the age of 18 had lower IQ before cannabis initiation (in childhood) and at 18 years old. In addition to that, even

though cannabis use was associated with low IQ and poorer executive function at the age of 18, these associations were not evident in twins from the same family. Therefore, the authors conclude that there is no causal relationship between cannabis use and IQ decline or executive function impairment in adolescence, even at the level of heavy use or dependence (Meier et al., 2018). Another co-twin study prospectively assessed adolescent cannabis use and outcomes in early adulthood in psychiatric and cognitive domains. Even though adolescent cannabis use was associated with poorer academic performance, there was no evidence of a causal effect on mental health and cognition in early adulthood (Schaefer et al., 2021).

In a scenario of contradictory results regarding the neuropsychological effects of cannabis use in adolescence, meta-analysis can provide a quantitative synthetizes of studies being conducted on the topic. One meta-analysis conducted by Scott et al. (2018) with 69 cross-sectional studies compared cognitive functioning between cannabis users and non-users in a sample of adolescents and young adults. Results indicated reduced cognitive functioning in adolescent and youth adults associated with frequent or heavy cannabis use, even though authors report a small effect size and no association with the age of cannabis use onset (Scott et al., 2018). This study goes in opposition to a previous systematic review that analyzed 105 investigations reporting the cognitive effects of acute and chronic cannabis use. Authors conclude that impairment in a range of cognitive domains (especially memory and attention) can persist in adolescents after acute cannabis intoxication, especially in frequent users (Broyd et al., 2016).

1.4 Animal studies on the impact of adolescent cannabis use on cognition

Inherent to clinical and epidemiological human studies is the limitation for controlling confounding factors. These include the wide variety of cognitive tests employed, the heterogeneous history of cannabis use, inconsistent neurodevelopmental stages, genetic background, psychopathology, polydrug use, and the range of cannabinoid compounds. Therefore, these factors likely explain the mixed evidence regarding the long-term effect of cannabis use as well as the inability to establish causal connections (Broyd et al., 2016; Levine et al., 2017). In this scenario, well-controlled and valid animal models have been

successfully applied to study the effects of cannabis use on adolescence and adulthood (Levine et al., 2017).

Extensive research has been conducted on assessing the impact of cannabis exposure on reward and reward-related cue learning and memory function (Stringfield & Torregrossa, 2021). Hamidullah et al. (2021) showed that THC consumption by rats during adolescence was associated with impairment on a reward-mediated memory test in adulthood. Ellner et al. (2021) measured reward-related behaviour in adulthood using Pavlovian auto-shaping after exposing adolescent rats to SR144528, an antagonist/inverse agonist of the CB2 receptor. Modulating CB2 circuitry in adolescence had a long-lasting effect on reward behaviour since adult rats presented less sign-tracking compared to controls. On the other hand, voluntary oral THC consumption during adolescence was found to increase sign-tracking behaviour during adulthood in rats (Kruse et al., 2019). However, this effect was present only in males. The type of behavioural test could contribute to the variability.

For instance, upon assessing learning and memory through the object recognition test, several studies reported impaired memory performance on the test when performed in adulthood after previous exposure to THC in adolescence (Zamberletti et al., 2012; Renard et al., 2013; Blest-Hopley et al., 2020). Kasten et al. (2017) injected THC in two strains of male mice in adolescence and assessed object discrimination in adulthood. In both strains, adolescent THC administration resulted in impairment in object recognition in adulthood, with indication or upregulation of CB1 expression five weeks after the last exposure compared to animals treated in adulthood (Kasten et al., 2017). Similarly, Quinn et al. (2008) exposed adolescent and adult male rats and assessed behaviour in adulthood. Only adolescent-treated animals showed impaired object recognition memory. Furthermore, the authors report several proteins that were differentially expressed in the hippocampus of adolescent-treated rats compared to adults. This evidence possibly indicates the hippocampus as a locus for the long-term impairment of memory function upon adolescent cannabis use. Although there are contradictory findings, the Morris water maze, fear conditioning, and passive avoidance appear to be less sensitive tests

compared to the radial arm maze, novel object recognition, and active avoidance for the long-lasting effects of cannabis (Kayir et al., 2022).

1.5 Sex differences on the impact of adolescent cannabis use on cognition

Strong evidence suggests sex differences in cannabis consumption, physiological effects, progression to dependence, and co-occurring psychiatric comorbidities (Cuttler et al., 2016; Calakos et al., 2017; Cooper & Craft, 2018; Noorbakhsh et al., 2020). Men use cannabis more frequently (Substance Abuse and Mental Health Services Administration, 2020), present earlier usage onset (Pope et al., 2003), meet more criteria for cannabis abuse (Khan et al., 2013), and have more than double the prevalence of CUD compared to women as to the DSM-V (Hasin et al., 2016). On the other hand, women with CUD are at greater risk for psychiatric comorbidities (Khan et al., 2013), tend to present a telescoping progression from first use to cannabis dependence (Schepis et al., 2011; Khan et al., 2013), and seek treatment earlier than men (Hernandez-Avila et al., 2004).

Very few human studies on the effects of adolescent cannabis exposure on cognition have included female participants and those who have included failed to analyze the results with sex as a main effect (Calakos et al., 2017; Levine et al., 2017). Most studies investigating the long-term effects of cannabis use on multiple cognitive domains find no sex differences (Pope et al., 2003; Fried et al., 2005; Solowij et al., 2011; Meier et al., 2012). Another study investigated the relationship between the age of cannabis initiation and neuropsychological performance in young adults. Contrary to the previous studies, initiation of cannabis use at an earlier age was associated with poorer episodic memory in females, but not in males (Crane et al., 2015).

Even though human studies consistently show important sex differences regarding cannabis outcomes, few pre-clinical research has included sex as a variable when investigating the long-term effects of adolescent cannabis exposure (Rubino & Parolaro, 2015; Stringfield & Torregrossa, 2021). Keeley et al. (2015) injected THC into two strains of male and female rats upon puberty onset and assessed learning in adulthood. THC impaired discriminative fear behaviour in Long Evans females only, followed by

smaller hippocampal, dentate gyrus, and CA1 volumes, while only Wistar males exhibited active avoidance in adulthood. Another study found that early adolescent exposure to THC impaired active place avoidance in male and female rats, with the performance of females being superior, though (Harte & Dow-Edwards, 2010). Freels et al. (2023) found that only females who self-administered THC vapour in adolescence displayed disrupted behavioural flexibility in adulthood in an attentional set-shifting task. Finally, chronic THC exposure during adolescence did not affect spatial learning through the water maze test in both male and female Sprague–Dawley rats four weeks after the last exposure (Cha et al., 2007).

1.6 Magnetic resonance imaging

1.6.1 Functional Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a type of non-invasive imaging technique that employs nuclear magnetic resonance coupled with gradients in a magnetic field to generate images with different contrasts (Glover, 2011). Over the years, different modalities of MRI were developed and are now applied in research and clinical settings to investigate a wide range of biological processes (Grover et al., 2015).

One modality of MRI is called functional MRI (fMRI). fMRI was developed to investigate regional and time-varying changes in brain metabolism. When one brain region is activated due to a task or an unregulated process in the resting brain, there is a local increase in energy demands and, therefore, increased blood flow to provide oxygen. Thus, the hemodynamic response to neuronal activation results in a local increase in oxygenated hemoglobin and a decrease in deoxygenated hemoglobin (Glover, 2011). In fMRI, the ratio of oxygenated and deoxygenated hemoglobin is explored to produce time-dependent images of brain metabolism using the Blood Oxygen Level Dependent (BOLD), the most popular contrast used in neuroimaging (Logothetis, 2008).

The modality of fMRI that analyzes spontaneous fluctuations in BOLD signal between brain regions at rest (without performing a specific task) is called resting-state fMRI (rsfMRI). The temporal correlation in BOLD signals in different parts of the brain is believed to represent the magnitude of communication between these regions (Jones,

2010). The statistical analysis that investigates synchronicity in the activation of brain regions led to the concept of resting state functional connectivity (RSFC): reproducible functional connectivity networks within the brain that are active at rest. In this case, RSFC is believed to represent an intrinsic, frequently coordinated organization of the brain and has been found altered in multiple human conditions (Biswal et al., 1995; Raichle et al., 2001; Zalesky et al., 2010; Frie, 2024).

One of the reasons rsfMRI gained popularity in translational neuroscience research has to do with some of its advantages compared to other imaging/recording modalities. First, as a non-invasive technique, rsfMRI can be used to generate analogous datasets of brain functionality in both human and non-human animal models. Second, instead of localized recording of brain activity in cortical and subcortical regions, rsfMRI allows investigators to assess whole-brain connectivity with relatively homogeneous resolution. Finally, the comparison of human and non-human datasets has demonstrated that many of the functional networks are evolutionarily conserved across mammals, allowing for high translation modeling of human conditions and pharmacological interventions (Frie, 2024).

One approach to investigate the functional connections within the brain is using Network Based Statistics (NBS) (Zalesky et al., 2010). This statistical analysis is based on graph theory, and it reduces the complexity of the brain by looking at the interactions between its basic parts. When applied to MRI analysis, nodes represent each functional region and edges the temporal varying statistical connection between regions. The collection of pairwise nodes and edges can reconstruct biologically relevant functional networks in the brain that can be compared between species, groups, and time points (Frie, 2024).

1.6.2 Diffusion Magnetic Resonance Imaging

Given our understanding of the structure and function of the nervous system, the connections between cell bodies of neurons in different functional grey matter areas are mediated by predominantly myelinated axons, known as white matter. Since the advent of functional connectivity analysis, researchers have given little attention to the physical connections that mediate information transfer between non-contiguous grey matter

regions. This little effort to characterize white matter fibers was partially due to time-consuming neuroanatomy tracer methods that did not allow for the study of whole brain white matter in vivo (Jones, 2010).

The advent of diffusion MRI (dMRI) overcame most of the previous limitations and brought the possibility of visualizing white matter microstructure. dMRI primarily measures the diffusion of water molecules in different directions. Since the axons restrict the diffusion of water molecules in a specific direction, dMRI can provide local estimates of water diffusion orientation and indirectly indicate white matter microstructure. A local estimate of a single digital white matter fiber pathway is called streamline. The collection of these local estimates of bundles of axons can be put together to recreate whole brain structural connectome or tractograms that are believed to reflect the structural connections within the brain (Assaf et al., 2019; Chandio et al., 2023).

1.6.3 Neuroimaging studies on the effects of cannabis use on the brain

Considering the critical stage of brain development during adolescence and early adulthood, dMRI studies in humans can demonstrate the effects of cannabis use on structural development in the brain of individuals in this stage of life (Stringfield & Torregrossa, 2021). These dMRI studies have mainly analyzed the implications of cannabis use in adolescence on white matter integrity. In that regard, white matter microstructure alterations were found in adolescents with CUD, with reduced fractional anisotropy in many regions, including frontal-parietal circuitry, especially the inferior frontal region, splenium of the corpus callosum, postcentral gyrus, and left superior longitudinal fasciculus (Bava et al., 2009); left inferior fronto-occipital fasciculus (Epstein et al., 2014); bilateral posterior internal capsule/thalamic radiation, left middle temporal gyrus, and right superior temporal gyrus (Ashtari et al., 2009); and left inferior longitudinal fasciculus (Epstein & Kumra, 2015). These previous results in adolescents indicate a possible effect of cannabis use on the integrity of white matter microstructure.

rsfMRI analysis demonstrated these structural changes in functional alterations in brain connectivity in adolescent cannabis users. Functional abnormalities have been described on the default mode network (DMN), where cannabis use was related to a set of increases

and decreases in connectivity within the DMN, especially in the posterior cingulate cortex (PCC) with other DMN areas (Ritchay et al., 2021). Further rsfMRI studies investigating other networks in adolescents exposed to cannabis identified decreased functional connectivity between the caudal anterior cingulate cortex and the orbitofrontal cortex (Camchong et al., 2016), reduced interhemispheric connectivity in the pyramids of the cerebellum and the superior frontal gyrus (Orr et al., 2013) and abnormal functional connectivity between the striatum and frontal cortex and sensory cortex (Blanco-Hinojo et al., 2017). Together, these findings on human subjects indicate a possible effect of cannabis exposure during adolescence on brain functionality.

Cannabis, especially CBD, has gained attention in recent years as a potential therapeutic for many human conditions (Muller & Reggio, 2020;Peng et al., 2022). Studies have also implicated alterations in functional connectivity in the therapeutic effects of CBD in psychosis (Bhattacharyya et al., 2018; van Boxel, et al., 2023). These findings lead to the question of if these functional alterations in the brain seen upon cannabis consumption could be beneficial, at least in some populations (Jenkins & Khokhar, 2021). It is expected that some of the therapeutical effects of cannabis compounds would alter functional connectivity in specific brain regions and that might be what some of these studies are capturing.

MRI studies in rodents have a strong potential to investigate the structural and functional changes induced by cannabis exposure during the developmental stages. Increasing evidence from animal literature has also been implicating cannabis exposure in adolescence to changes in adulthood using MRI techniques. In fact, BOLD MRI analysis reveals that rodent brain is intensely impacted by cannabis exposure, especially with activation of areas rich in CB1 receptors (e.g., olfactory system, cortex, and amygdala) (Farra et al., 2020). These prominent activations result in structural and functional alterations within the rodent brain. Coleman et al. (2022) exposed male and female adolescent mice to cannabis for 28 days, followed by structural and functional MRI analysis. Females exhibited alteration in fractional anisotropy in the forebrain and hindbrain, and males showed functional abnormalities in areas of the thalamus, hypothalamus, and brainstem reticular activating system.

Given the differential effects of THC and CBD on the brain, studies comparing the different effects of these two compounds may help to assess their contribution to brain damage, if any. Sadaka et al. (2021) exposed adult male mice to different concentrations of CBD, followed by rsfMRI. It was found that CBD alone was responsible for activation (prefrontal cortex) and deactivation (brainstem and cerebellum) in different brain regions. The study also reported that CBD exposure was responsible for a decrease in connectivity of regions in the hindbrain and midbrain (Sadaka et al., 2021). Another study investigated the effects of a high and low THC concentration on BOLD signals in rats. In general, the lowest dose was responsible for a greater increase in positive BOLD response (e.g., central amygdala, parafascicular thalamus, insular cortex, and CA1 and CA3 areas of hippocampus) and a greater negative BOLD response (e.g., raphe, periaqueductal gray and retrosplenial rostral cortex) when compared to control and the highest dose (Madularu et al., 2017).

1.7 Objectives and Hypothesis

This study aimed to investigate the long-term differential effects of adolescent exposure to vaporized cannabis flower containing varying amounts of THC and CBD, in adulthood in male and female rats using functional and diffusion MRI.

Based on previous literature, it was hypothesized that adolescent cannabis exposure would result in structural and functional changes to the hippocampus and that the high-THC-exposed group would show increased functional connectivity in the somatosensory cortex and visual cortex (Quinn et al., 2008; Winton-Brown et al., 2011; Klumpers et al., 2012; Madularu et al., 2017; Orihuel et al., 2023). Additionally, there is evidence that adolescent cannabis exposure results in changes in brain connectivity and biochemical features within areas of the brain responsible for motivation, emotion, and cognition (Hurd et al., 2014; Jager & Ramsey, 2008; Jacobus & Tapert, 2014; Peters et al., 2021; Ertl et al., 2024). Finally, based on current literature (Craft et al., 2013;Lee et al., 2014), we also predicted that these alterations would be sex-specific, where male and female cohorts would display differential neurocircuitry alterations, with males having more prominent effects.

Chapter 2

2 Material and Methods

2.1 Animals and Housing

The animal protocol was approved by the University of Guelph and the University of Western Ontario Animal Care Committees and was conducted in accordance with the Canadian Council on Animal Care. Male (n=28) and female (n=32) Sprague-Dawley pups were delivered to the Central Animal Facility at the University of Guelph and were weaned, during adolescence, on postnatal day (PND) 28. All rats were triad or pair housed and kept in a temperature-controlled room (22 ± 2 °C and humidity 50–70%) on a 12-hour light-dark cycle (0700 h lights on). The rats had ad libitum access to food and water during cannabis exposure and two weeks post-exposure. The procedures described in this study were carried out separately in different cohorts of male and female animals, as indicated below. A scheme of the procedures carried out in this study can be found in Figure 1.

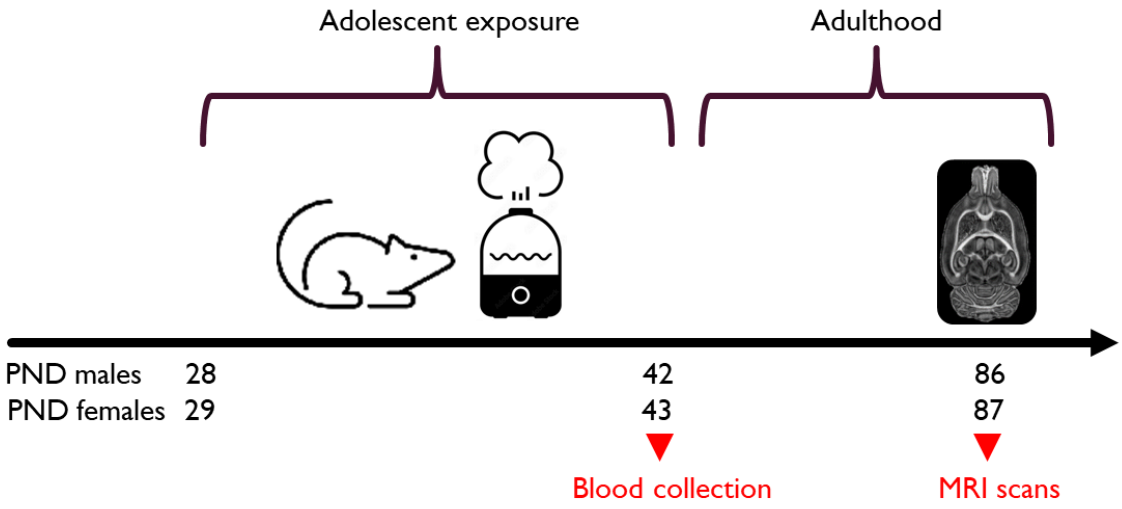

Figure 1: Schematic with the timeline of adolescent exposure to cannabis products or air in male and female rats and subsequent timelines for blood collection and MRI scans.

2.2 Drugs

Rats were randomly divided into 4 groups (7 in each group for males and 8 in each group for females) and assigned to either a control group or one of three treatment groups. Treatment consisted of either high-CBD (Pure Sunfarms BC Grown-Pure Sun CBD dried flower; 159 mg/g CBD and 6 mg/g THC), high-THC (Truro Wedding Mint dried flower; 284.451 mg/g THC and 1.097 mg/g CBD), or balanced CBD/THC (Twd. Balanced THC+CBD dried flower; 107 mg/g CBD and 80 mg/g THC). The amount of dried cannabis flower administered in this experiment is aligned with the mass of one standard joint unit found in Kögel et al. (2017) study.

2.3 Equipment

A Volcano® Vaporizer (Storz and Bickel, GmbH and Co., Tuttlingen, Germany) was used to administer cannabis vapor as described previously (Hazekamp et al., 2006). Dried cannabis flower (0.250 g) was ground and loaded into the vaporizer and vaporized at 200°C. The fan of the vaporizer was then turned on, and the cannabis vapor was pumped through a tube into an enclosed box (48×39×20 cm). The box contained a small breathing hole for the animals and a plastic divider. The divider split the box into 4 quadrants which were used to keep the rats separated from one another. All rats of an assigned exposure group were exposed simultaneously. The box and divider were cleaned between each exposure. For the control group, the vaporizer was left empty, but the fan of the vaporizer was still turned on to deliver heated clean air with separate, clean tubing delivered the air to the control group in an identical clean cage. The fan ran for 5 minutes, filling the box with either cannabis vapor or air. After 5 minutes, the fan was turned off, and the rats sat in the cannabis vapor or air for an additional 10 minutes. Every rat was in the box, exposed to either cannabis vapor or air for 15 minutes daily. Exposures ran for 14 consecutive days (PND 28-42 for males and PND 29-43 for females) at the same hours of the day (between 1000 and 1200 h). The discrepancy in exposure start dates was unfortunately caused by a shipment error. For this reason, no direct comparisons will be made between male and female rats.

2.4 Blood collection

Blood samples were collected immediately before and after air and cannabis exposure on the final day of exposure (day 14; PND 42 for males and PND 43 for females). Since animals were undergoing functional and diffusion MRI analysis in adulthood and, therefore, the necessity to keep their brains intact, we opted for the analysis of cannabinoid levels in the blood, instead of cannabinoid levels in the brain. The hind legs of the rats were shaved before starting the exposures. They were placed on heated pads for 5 min, Vaseline was applied to the area matching the saphenous vein, and a 22G needle was used to punctuate the vein. The blood drops were collected with a capillary blood collection tube with a maximum volume of 300 μL (Microvette CB300, Sarstedt, Germany). The samples were kept in ice and were centrifuged at 8000 RPM for 5 min. The supernatant serum was transferred to the cryovials and stored in a −80°C freezer until analysis. Cannabinoid serum levels from male and female control groups were also collected and analyzed for method validation.

2.4.1 Serum THC, CBD, and 11-OH-THC quantification

Reference standards of THC, CBD, and 11-OH-THC, and their deuterated internal standards THC-D3, CBD-D3, and 11-OH-THC-D3 were purchased from Sigma-Aldrich Canada (Oakville, ON). To extract considered cannabinoids from rat serum, Captiva enhanced matrix removal lipid (EMR-Lipid) 96-well plate was used (Agilent, Santa Clara, CA, USA). Briefly, 250 μL of acetonitrile (acidified with 1% formic acid) was added to each well, then 50 μL of rat serum and 20 μL of internal standard solution were added. After the sample passed through under positive pressure at 3 psi, the extraction plate was washed with 150 μL of a mixture of water/acetonitrile (1:4, v:v) solution. The effluent evaporated under nitrogen at 40 °C, and the residual was reconstituted with the mobile phase for subsequent LC-MS/MS analysis. Calibration Standards (2-1000 ng/mL) and quality controls (3 ng/mL and 800 ng/mL) were prepared on the day of analysis by spiking standard working solutions into blank rat serum. The liquid chromatography separation was achieved on a Thermo Scientific Vanquish Flex UHPLC system. Five microliters of plasma extracts were injected and separated on an ACQUITY UPLC BEH C18 Column (1.7 μm, 2.1 mm × 50 mm; Waters, Ireland) connected with a VanGuard

UPLC BEH C18 Pre-Column (Waters, Ireland). The auto sampler was kept at 4 °C and column temperature was at 35 °C. The mobile phase consisted of A: 10 mM ammonium formate with 0.1% formic acid aqueous solution, and B: acetonitrile with 0.1% formic acid. The flow rate was 400 μL/min under a gradient mode. The gradient conditions were sustained as follows: Mobile phase B linearly ramped up from 40% to 95% from 0.1 to 4 min and maintained at 95% for 2 min, then ramped back to 40%. 11-OH-THC, CBD and THC were eluted at 3.5, 4.0 and 4.6 minutes, respectively, with a total run time of 7 min. Mass spectrometry (MS) analysis was conducted with a Thermo Q Exactive Focus Orbitrap mass spectrometer equipped with an Ion Max source in positive electrospray ionization (ESI) mode. The source conditions were optimized as the spray voltage of 3.5 kV, the capillary temperature of 300 °C, and aux gas heater temperature of 425 °C. Data were acquired and processed in parallel-reaction monitoring (PRM) mode using Thermo Scientific™ TraceFinder™ software. In this PRM mode, protonated 11-OH-Δ9-THC ion (m/z 331.23), CBD ion (m/z 315.23) and Δ9-THC ions (m/z 315.23) were selected as precursors, then fragmented in the higher-energy C-trap dissociation (HCD) cell at collision energy of 20 eV for 11-OH-THC and 25 eV for CBD and THC. The resulting MS/MS product ions were detected in the Orbitrap at a resolution of 17,500 (FWHM at m/z of 200) with AGC target set at 1e5. The most abundant fragments from the MS/MS spectra (m/z 313.22 for 11-OH-THC and m/z 193.12 for CBD and THC) were selected as the quantifying ions. Other specific fragments, m/z 193.12 for 11-OH-THC and m/z 259.17 for CBD and THC, were selected as the confirming ions. The resulting chromatograms were extracted and reconstructed with a mass accuracy of 5 ppm for quantification and confirmation. The optimized MS/MS compound parameters are summarized in Table 1.

Analyte & Internal Standard	**Precursor ion (*m/z*)**	**CE**	**Quantitation ion (*m/z*)**	**Confirming ion (*m/z*)**	**RT (min)**
11-OH-Δ^9-THC	331.23	20	313.22	193.12	3.5
11-OH-Δ^9-THC–D3	334.24	20	316.23	196.14	3.5
CBD	315.23	25	193.12	259.17	4.0
CBD-D3	318.25	25	196.14	262.19	4.0
Δ^9-THC	315.23	25	193.12	259.17	4.5
Δ^9-THC–D3	318.25	25	196.14	262.19	4.5

Table 1: Optimized LC-MS/MS compound parameters for quantitation of 11-OH-THC, CBD, and THC using PRM mode (CE: collision energy, m/z: mass/charge ratio, RT: retention time).

2.5 Behavioral experiment

The behavioural component of this study consisted of male (n=28) and female (n=32) Sprague-Dawley rats. Animals were randomly divided into 4 groups (7 in each group for males and 8 in each group for females) and assigned to either a control group or one of three treatment groups, as described earlier. Dried cannabis flower (0.250 g) was ground and loaded into the vaporizer and vaporized at 200°C. Every rat was in the box, exposed to either cannabis vapor or air for 15 minutes daily. Exposures ran for 14 consecutive days (PND 28-42 for males and PND 29-43 for females) at the same hours of the day (between 1000 and 1200 h). In adulthood (PND 56-84 for males and PND 57-81 for females), rats underwent Pavlovian autoshaping, active avoidance, and prepulse

inhibition (PPI) of the acoustic startle reflex. Results of behavioural experiments can be found in the Appendices. After completion of the behavioural tests, the same animals underwent functional and behavioural MRI analysis, which is the focus of this thesis and described in detail below.

2.6 Neuroimaging

The methods applied in this neuroimaging study closely followed recent studies in our laboratory for functional and diffusion MRI analysis and can be found in Frie et al. (2024) and Aziz (2024).

2.6.1 Subjects

Animal procedures used in this study were approved by the University of Western Ontario Animal Use Subcommittee and followed guidelines established by the Canadian Council on Animal Care. Before initiation of the scans, animals were placed in an induction chamber with 4-5% isoflurane and an oxygen flow rate of 1-1.5 L/min to initiate anesthesia. Following induction, animals were kept on 2.0-2.5% isoflurane along with an oxygen flow rate of 1-1.5 L/min using a custom-built nose cone. Once the animal was positioned in the MRI scanner, it was administered an intraperitoneal (IP) injection of 0.018 mg/kg/hr dexmedetomidine at a constant rate infusion (CRI) for the duration of the experiment (Wallin et al., 2021). Once dexmedetomidine infusion was initiated, isoflurane was reduced over a period of 15 minutes from between 2.0-2.5% to between 0.8-1.0% and an oxygen flow rate of 1-1.5 L/min. An air heater system was used to keep the rectal temperature at 37.0 ± 0.5 ºC. For the analysis of the MRI data, male and female cohorts were analyzed independently due to an upgrade in the imaging system between the male and female cohorts.

2.6.2 Acquisition

Images were acquired using a 9.4 T Bruker small animal MRI scanner at the Centre for Functional and Metabolic Mapping (CFMM) located within the Robarts Research Institute at the University of Western Ontario.

2.6.3 Anatomical images

T2 Anatomical images were acquired for each subject at the beginning of each session using a T2-weighted TurboRARE pulse sequence (Hennig et al., 1986) (8 averages, 35 slices, slice thickness = 400 mm, FOV 38.4 x 38.4 mm, matrix size 192 x 192, in-plane resolution = 200 x 200 mm, TE = 44.0 ms, TR = 7.0 s, Echo Spacing = 11.00 ms, Rare Factor 8, total acquisition time = 14 min).

2.6.4 Functional

rsfMRI images were acquired using a gradient-echo echo-planar-imaging (EPI) sequence (400 volumes, TE = 15.0 ms, TR = 1.5 s, FOV 38.4 x 38.4 mm, matrix size 96 x 96, isotropic resolution = 400 mm, bandwidth 280 kHz).

2.6.5 Diffusion

Diffusion images were acquired using a multi-shot, spin echo, EPI acquisition pulse sequence (4 shots, 32 slices, slice thickness = 500 mm, FOV 40 x 40 mm, matrix size, 160 x 160, in-plane resolution = 250 × 250 mm, TE = 26.71 ms, TR = 2.5 s). The diffusion scheme used was previously described in detail and was shown to produce reproducible and reliable results at 9.4 Tesla (McCunn et al., 2019). Shell one: 30 directions, b-value = 1000 s/mm2, gradient (G) = 172.85 mT/m, time between the first and second diffusion pulse (Δ) = 14 ms, duration of diffusion pulse (δ) = 4.5 ms. Shell two: 60 directions, b-value = 2000 s/mm2, gradient strength (G) = 345.70 mT/m, time between the first and second diffusion pulse (Δ) = 14 ms, duration of diffusion pulse (δ) = 4.5 ms.). Ten b = 0 s/mm2 shells were interspersed evenly throughout the acquisition. Four averages were used to ensure an adequate signal-to-noise ratio in the higher b-value shell. A single reverse phase encoded b=0 volume was also acquired before the diffusion sequence for subsequent use in image processing to correct image distortions.

2.6.6 fMRI image processing

The processing of fMRI images was conducted using the open-source Rodent Automated Bold Improvement of EPI Sequences (RABIES) software (https://github.com/CoBrALab/RABIES) (Desrosiers-Gregoire et al., 2022). For both the

anatomical and functional images, extra space around the brain was automatically cropped, and temporal spikes were corrected for at each voxel (Cox, 1996). Dummy scans were automatically detected and removed from each EPI. If dummy scans are detected, the median of these volumes provides a volumetric EPI image as a reference, given their higher anatomical contrast. Otherwise, a volumetric EPI image was derived using a trimmed mean across the EPI frames, after an initial motion realignment step. Using this volumetric EPI as a target, the head motion parameters were estimated by realigning each EPI frame to the target using a rigid registration. To conduct common space alignment, structural images were corrected for inhomogeneities and then registered together to allow the alignment of different MRI acquisitions. This registration was conducted by generating an unbiased data-driven template through the iterative nonlinear registration of each image to the dataset consensus average, where the average gets updated at each iteration to provide an increasingly representative dataset template (https://github.com/CoBrALab/optimized_antsMultivariateTemplateConstruction) (Avants et al., 2011).

The finalized template after the last iteration provides a representative alignment of each MRI session to a template that shares the acquisition properties of the dataset, which makes it a stable registration target for cross-subject alignment. After aligning the MRI sessions, this newly generated unbiased template was then itself registered, using a nonlinear registration, to the SIGMA rat brain template (Barrière et al., 2019). To correct for EPI susceptibility distortions, the volumetric EPI was also subjected to inhomogeneity correction and then registered using a nonlinear registration to the anatomical scan from the same MRI session (Wang et al., 2017).

Finally, after calculating the transformations required to correct for head motion and susceptibility distortions, transforms were concatenated into a single resampling operation (avoiding multiple resampling) which is applied at each EPI frame, generating the preprocessed EPI time series in native space (Esteban et al., 2019). Preprocessed time series in common space were also generated by further concatenating the transforms allowing resampling to the reference atlas, at a voxel resolution of 0.3x0.3x0.3 mm.

Confound correction was executed on the EPI time series resampled to common space. Voxelwise linear detrending was first applied to remove first-order drifts and the average image. Motion sources were then automatically removed using a modified version of the ICA-AROMA classifier (Pruim et al., 2015) where classifier parameters and anatomical masks are instead adapted for rodent images. Next, high-pass filtering (0.01Hz) and lowpass filtering (0.1Hz) were applied (Abraham et al., 2014). Estimated nuisance time courses during preprocessing were then used for confound regression. More specifically, using ordinary least square regression, the 6 rigid motion parameters, the mean signal from the white matter and cerebrospinal fluid masks, and the global signal were modeled at each voxel and regressed from the data. Before analysis, a spatial Gaussian smoothing filter (Abraham et al., 2014) was applied at 0.3mm full-width at half maximum (FWHM).

Next, for each animal, it was generated whole-brain connectivity matrices in common space using the SIGMA functional template (59 Regions of Interest) by extracting the seed time course for every parcel and then measuring the cross-correlation (Pearson's r) between every region pair. The correlation values obtained were transformed using the Fisher z-transformation, thereby creating a whole-brain matrix representing the 'connectivity strength' between every corresponding region pair for each animal. Since the regions of interest in the atlas are functionally defined, some areas are just represented in one hemisphere, while others are interhemispheric (Barrière et al., 2019).

2.6.7 dMRI image processing

Images were preprocessed using the fMRI Software Library (FSL, v. 6.0.4) and MRtrix (v. 3.0.2) (Tournier et al., 2019). Gibbs Ringing Removal (Kellner et al., 2016) followed by PCA denoising (Veraart et al., 2016) was performed first in MRtrix. TOPUP (Smith et al., 2004; Andersson et al., 2003) followed by EDDY (Andersson & Sotiropoulos, 2016) was used to correct for eddy current-induced distortions as well as susceptibility-induced distortions. Tractography was then performed using the MRtrix software package. Response functions for single-fiber white matter, as well as grey matter and cerebrospinal fluid, were estimated from the data themselves using an unsupervised method (dhollander) (Dhollander et al., 2016). Fiber orientation distribution images were calculated using multi-tissue spherical deconvolution (msmt_csd) (Tournier et al., 2004;

Jeurissen et al., 2014) followed by images undergoing multi-tissue informed log-domain intensity normalization. Whole brain tractograms were generated using second-order Integration over Fiber Orientation Distributions (iFOD2) (Tournier et al., 2019) with 10 million streamlines, followed by filtering of tractograms (Smith et al., 2015).

Diffusion data was registered in the same way as the functional data above with one difference: inverse transformation matrices were used to bring the SIGMA atlas regions of interest into diffusion space for final analysis. This was done due to the unique spatial nature of diffusion imaging, and the potential for confounding effects due to resampling and registration of the diffusion data to common space. Finally, using the SIGMA ROIs in diffusion space, single subject connectomes weighted by Streamline Count were produced.

2.6.8 Statistical Analysis

NBS (Zalesky et al., 2010) was used to identify statistically significantly different subnetworks (clusters of nodes and edges) between groups. Briefly, NBS first identifies edges that surpass a given threshold (suprathreshold links), followed by identification of connected nodes within this subnetwork, and finally, permutation testing to assign a p-value (controlled for the family-wise error (FWE)) to each subnetwork based on its size. Using NBS, all matrices were entered into a one-way analysis of variance (ANOVA) ($p = 0.05$, F-threshold =6). Statistically significant networks ($p < .05$) were extracted for post hoc analysis of between-group differences using two-sample one-tailed t-tests ($p = .004$, T-threshold = 2.5). Post-hoc statistical significance was Bonferroni corrected (4 groups in each cohort, twelve contrasts =0.05/12 = 0.004).

Chapter 3

3 Results

3.1 Blood Levels of THC, CBD, and 11-OH-THC

3.1.1 Males cannabinoid levels

The serum concentrations of THC, CBD and 11-OH-THC were measured immediately after the vapour exposure of the three different cannabis strains. In males, there were comparable differences between the serum THC, CBD and 11-OH-THC levels of the treatment groups in accordance with the type of cannabis used [$F(2,15)= 5.376$; $p= 0.017$, $F(2,13)= 7.106$; $p= 0.008$, $F(2,8)= 14.020$; $p= 0.002$, respectively]. Post hoc Tukey's test indicated that the THC level in the group treated with high-THC cannabis, and the CBD level in the high-CBD cannabis treated groups was higher than the other two groups (p values< 0.05, Fig.1A). Also, the 11-OH-THC level was higher in high-THC cannabis treated group compared to high-CBD and balanced cannabis groups ($p< 0.05$, Fig.1A).

3.1.2 Females cannabinoid levels

Unlike the male rats, there were no comparable differences between the serum THC, CBD and 11-OH-THC levels of the female rats in accordance with the type of cannabis used [$F(2,21)= 2.709$; $p= 0.009$, $F(2,21)= 0.453$; $p= 0.642$, $F(2,21)= 0.013$; $p= 0.987$, respectively, Fig 1B].

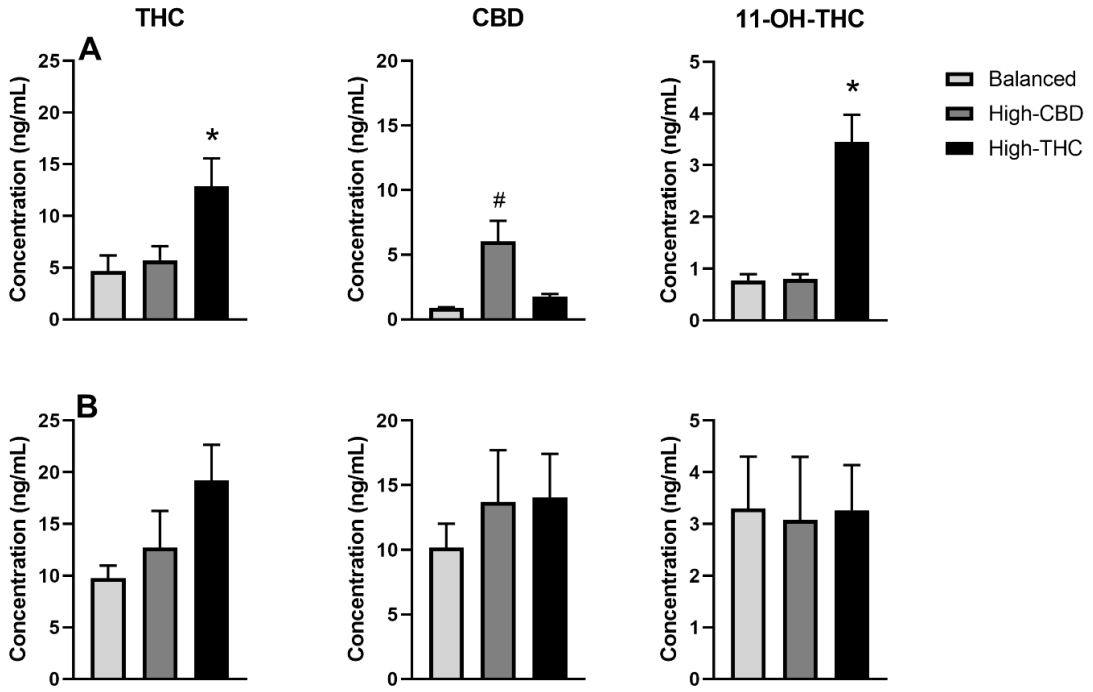

Figure 2: The serum concentrations of THC, CBD, and 11-OH-THC of male (A) and female (B) rats treated with high-THC, high-CBD and balanced cannabis strains. Data are mean ± standard error of mean (*p< 0.05, compared to balanced and high-CBD cannabis groups; #p< 0.05, compared to balanced and high-THC cannabis groups, Tukey's tests).

3.2 MRI results

Data was successfully collected from 28 male and 28 female rats. Figure 2 shows a representative single subject T2 anatomical image, diffusion b=0 image, and the first volume of an fMRI dataset.

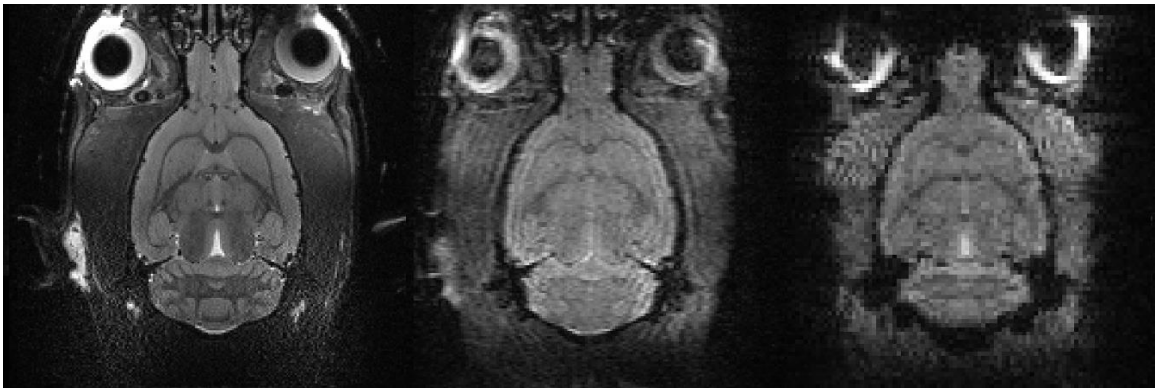

Figure 3: Representative single subject T2 anatomical image (left), diffusion b=0 image, (middle) and the first volume of an fMRI dataset (right).

Group	**Sex**	**n**	**Weight ± SD (g)**
Control	Male	7	337 ± 24
CBD	Male	7	306 ± 31
Balanced	Male	7	298 ± 11
THC	Male	7	312 ± 17

Table 2: Male MRI Subject Demographics

Group	Sex	n	Weight ± SD (g)
Control	Female	7	288 ± 27
CBD	Female	8	291 ± 16
Balanced	Female	7	302 ± 21
THC	Female	7	281 ± 22

Table 3: Female MRI Subject Demographics

3.2.1 Functional MRI results

3.2.1.1 Males fMRI results

NBS statistics revealed a single network with altered functional connectivity amongst the four male groups using an ANOVA ($p = 0.016$, 5 edges, 5 nodes, Figure 3). The affected connections comprised the Primary Somatosensory Cortex (Left), Primary and Secondary Visual Cortex (Left), Retrosplenial Granular Cortex (Interhemispheric), RSD/RSGa (Retrosplenial system) (Right), and the Raphe/Median (paramedian) Pontine Reticular Nucleus (Right).

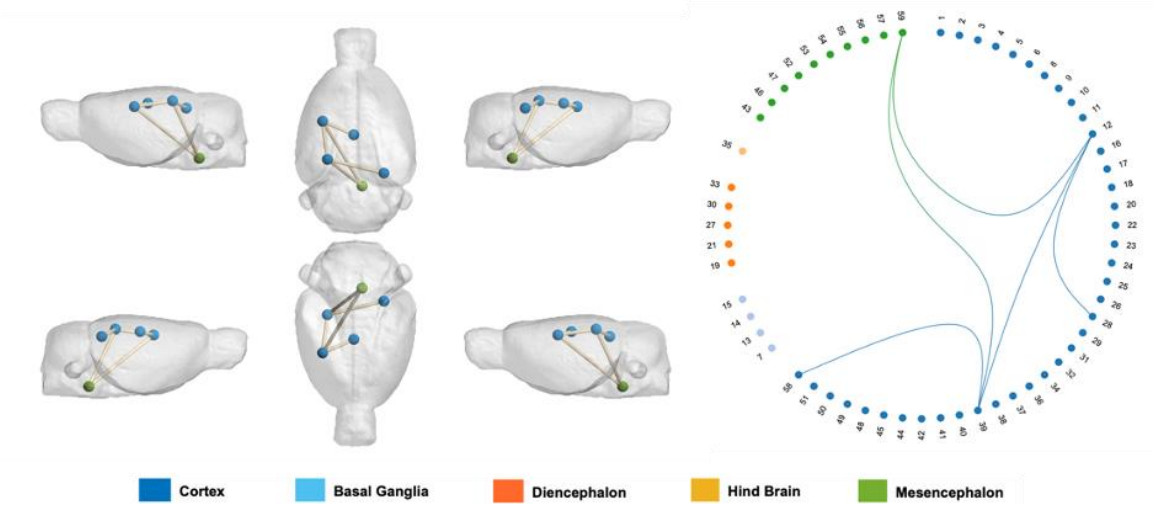

Figure 4: NBS statistics revealed a single network with altered functional connectivity (p = 0.016, 5 edges, 5 nodes). The affected connections comprised the Primary Somatosensory Cortex (Left), Primary and Secondary Visual Cortex (Left), Retrosplenial Granular Cortex (Interhemispheric), RSD/RSGa (Retrosplenial system) (Right), and the Raphe/Median (paramedian) Pontine Reticular Nucleus (Right).

Post-hoc t-tests revealed a single network with increased functional connectivity in the CBD group in comparison with the control group ($p < 0.001$, 4 edges, 5 nodes, Table 4). Similarly, t-tests revealed also a single network with increased functional connectivity in the CBD group in comparison to the THC group ($p = 0.002$, 3 edges, 4 nodes, Table 5).

Node 1	**Hemisphere**	**Node 2**	**Hemisphere**
Primary Somatosensory Cortex	Left	Retrosplenial Granular Cortex	Interhemispheric
Primary Somatosensory Cortex	Left	Primary and Secondary Visual Cortex	Left
Primary and Secondary Visual Cortex	Left	RSD/RSGa (Retrosplenial system)	Right
Primary Somatosensory Cortex	Left	Raphe/Median (paramedian) Pontine Reticular Nucleus	Right

Table 4: All connections identified by NBS statistics to have statistically significantly higher functional connectivity in the CBD group as opposed to the control group. ($p < 0.001$, 4 edges, 5 nodes).

Node 1	Hemisphere	Node 2	Hemisphere
Primary Somatosensory Cortex	Left	Primary and Secondary Visual Cortex	Left
Primary and Secondary Visual Cortex	Left	RSD/RSGa_R	Right
Primary Somatosensory Cortex	Left	Raphe/Median (paramedian) Pontine Reticular Nucleus	Right

Table 5: All connections identified by NBS statistics to have statistically significantly higher functional connectivity in the CBD group as opposed to the THC group. (p = 0.002, 3 edges, 4 nodes).

3.2.1.2 Females fMRI results

NBS statistics revealed a single network with altered functional connectivity amongst the four female groups using an ANOVA (p = 0.047, 4 edges, 5 nodes). The affected connections comprised the Primary and Secondary Motor to Right Parietal Cortex (Auditory), Left Dorsal Hippocampus to Right Parietal Cortex (Auditory), Primary and Secondary Motor to Raphe/Median (paramedian) Pontine Reticular Nucleus, and RSD/RSGa to Raphe/Median (paramedian) Pontine Reticular Nucleus. However, post-hoc Bonferroni corrected t-tests revealed no significant network with functional connectivity difference among the 4 treatment groups.

3.2.2 Diffusion MRI results

3.2.2.1 Males dMRI results

NBS statistics revealed two networks with altered structural connectivity amongst the four groups using an ANOVA. In Network 1 (p = 0.016, 3 edges, 4 nodes, Figure 4), the affected connections comprised the Striatum (Left), Insular Cortex 2 (Right), Endo/Piriform Cortex (Left) and Ventral Thalamus (Interhemispheric). In Network 2 (p = 0.014, 3 edges, 4 nodes, Figure 5), the affected connections comprised the Intermedial Entorhinal Cortex (Right), Pontine Nuclei (Interhemispheric), Interpeduncular Nucleus (Interhemispheric), and Raphe/Median (paramedian) Pontine Reticular Nucleus (Right).

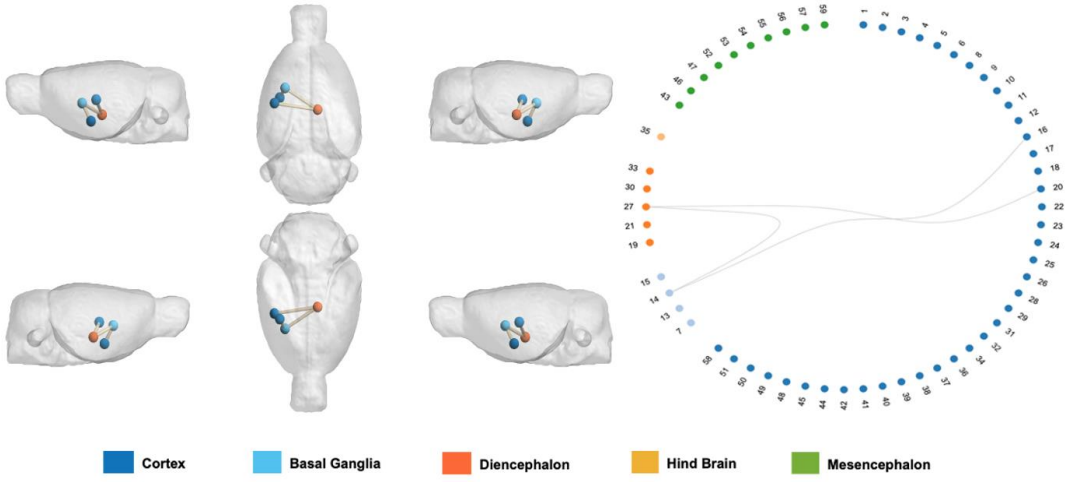

Figure 5: NBS statistics revealed statistically significantly lower streamline counts in the Striatum (Left), Insular Cortex 2 (Right), Endo/Piriform Cortex (Left) and Ventral Thalamus (Interhemispheric). Network 1 (p = 0.016, 3 edges, 4 nodes).

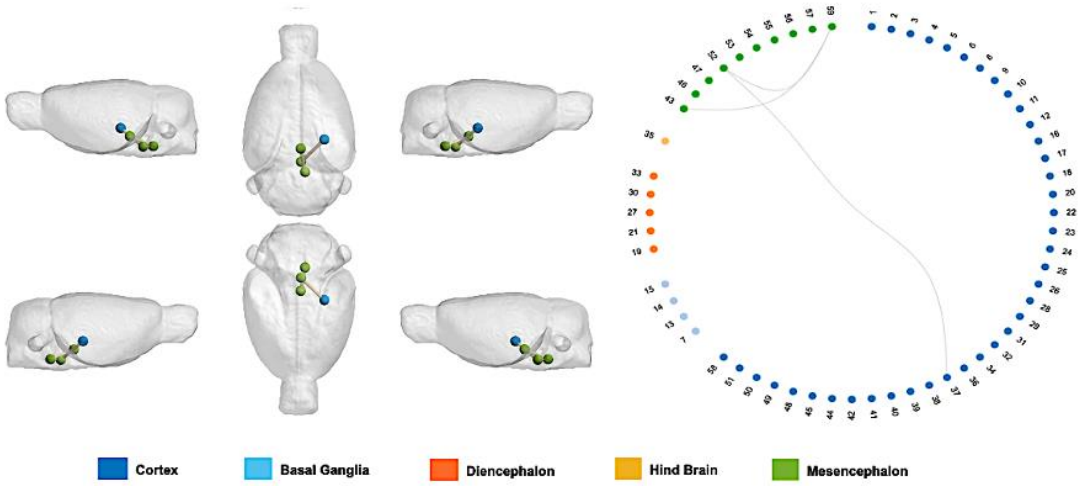

Figure 6: NBS statistics revealed statistically significantly higher streamline count in the Intermedial Entorhinal Cortex (Right), Pontine Nuclei (Interhemispheric), Interpeduncular Nucleus (Interhemispheric), and Raphe/Median (paramedian) Pontine Reticular Nucleus (Right). Network 2 (p = 0.014, 3 edges, 4 nodes).

Post-hoc t-tests of Network 1 revealed statistically significantly lower streamline count in the control group as compared to the CBD, Balanced, and THC groups ($p < 0.001$ for all, 3 edges, 4 nodes, Table 6).

Node 1	**Hemisphere**	**Node 2**	**Hemisphere**
Striatum	Left	Endo/Piriform Cortex	Left
Striatum	Left	Ventral Thalamus	Interhemispheric
Insular Cortex 2	Right	Ventral Thalamus	Interhemispheric

Table 6: All connections identified by NBS statistics to have statistically significantly lower streamline count (3 edges, 4 nodes) in the control group as opposed to the CBD ($p < 0.001$), Balanced ($p < 0.001$), and THC ($p < 0.001$).

Post-hoc t-tests of Network 2 revealed statistically significantly higher streamline count in the control group as compared to the CBD, Balanced, and THC groups ($p < 0.001$ for all, 3 edges, 4 nodes, Table 7).

Node 1	**Hemisphere**	**Node 2**	**Hemisphere**
Intermedial Entorhinal Cortex	Right	Pontine Nuclei	Interhemispheric
Interpeduncular Nucleus	Interhemispheric	Raphe/Median (paramedian) Pontine Reticular Nucleus	Right
Pontine Nuclei	Interhemispheric	Raphe/Median (paramedian) Pontine Reticular Nucleus	Right

Table 7: All connections identified by NBS statistics to have statistically significantly higher streamline count (3 edges, 4 nodes) in the control group as opposed to the CBD ($p < 0.00$), Balanced ($p < 0.001$), and THC ($p < 0.001$).

3.2.2.2 Females dMRI results

NBS statistics revealed no networks with altered structural connectivity amongst the four female drug groups using an ANOVA.

Chapter 4

4 Discussion

4.1 Summary

This study investigated the effects of exposure to three different types of vaporized cannabis flower (high-THC, high-CBD, and balanced) during adolescence on brain function and structure (e.g. diffusion MRI and functional MRI) in adulthood. Based on the serum plasma levels on exposure day 14, all rats received detectable levels of cannabinoid exposure. Our fMRI results in male animals indicated one single network with increased functional connectivity in the high-CBD exposed group compared to the control group, and one single network with increased functional connectivity in the high-CBD exposed group compared to the high-THC group. Female animals showed one single network with altered functional connectivity, but these findings did not survive post-hoc testing and multiple-comparison collection. Regarding our dMRI results, in male animals, we found two networks presenting altered structural connectivity: Network 1 with a lower streamline count in the control group compared to all other three cannabis-exposed groups and Network 2 with a higher streamline count in the control group compared to all other three groups. Female animals showed no significant differences in structural connectivity. The divergent results of adolescent high-CBD and high-THC cannabis vapour exposure in adult male rats suggest the potential long-term effects of different constituents of cannabis during adolescence and the sex differences that might exist in the exposure to, and effects of, cannabis vapour.

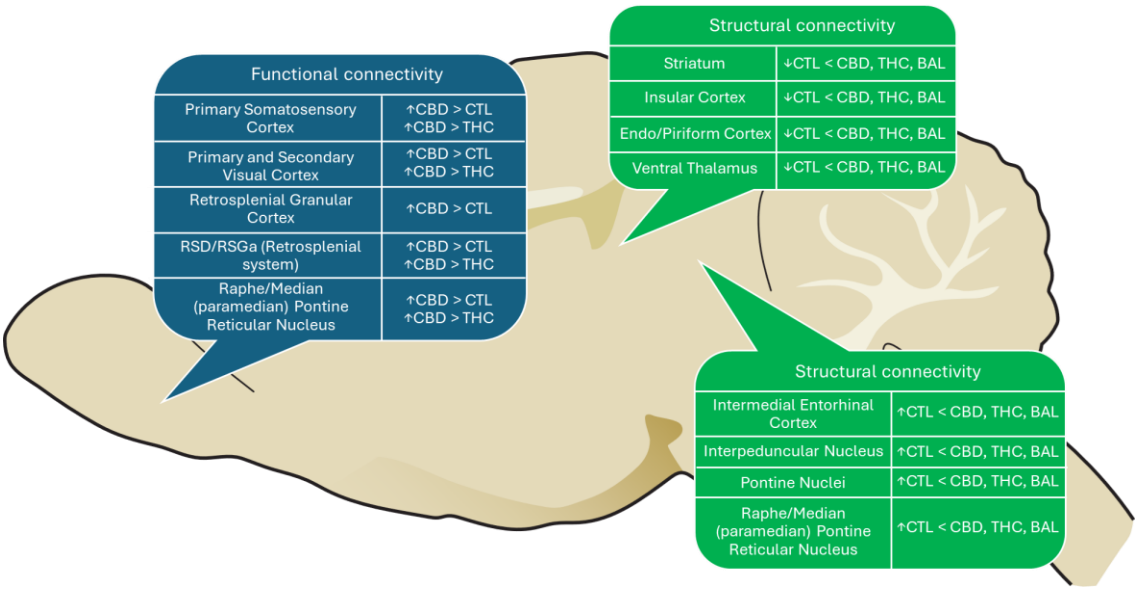

Figure 7: Summary of brain regions found to have functional or structural connectivity changes in adulthood upon adolescent cannabis exposure in their respective groups. Arrows (↑ ↓) indicate increase or decrease in functional or structural connectivity on the indicated group.

4.2 Serum cannabinoid quantification

Similar to the previous reports, blood levels of THC, CBD, and 11-OH THC showed variability in male and female rats exposed to different cannabis strains (Craft et al., 2013; Wiley & Burston, 2014; Ruiz et al., 2021); they were consistent with the exposed cannabis strain in male rats, but not in female rats. The understanding of the pharmacokinetics in the context of cannabis exposure is crucial since it might explain the sex differences observed in the behavioural effects upon cannabis exposure (Tseng et al., 2004).

Consistent evidence supports the idea of sex differences in the pharmacokinetics of cannabinoids in rats (Ruiz et al., 2021). Female rats frequently display higher cannabinoid levels, as well as a greater conversion of THC into its active metabolite 11-OH-THC (Wiley & Burston, 2014; Ruiz et al., 2021; Baglot et al., 2021; Torrens et al.,

2020). It has been proposed that differential expression of liver cytochrome enzymes might underlie the differential THC metabolism in male and female rats, with females preferentially converting THC to its active metabolite 11-OH-THC, while males metabolize THC into several other compounds (Tseng et al., 2004). Another hypothesis is related to the percentage of fat tissue in male and female animals. Adult males display a greater percentage of fat tissue compared to adult females (Tseng et al., 2004) and, since THC is highly lipophilic, its sequestration in fat would result in different distribution and metabolism profiles (Huestis, 2007).

4.3 Functional MRI

Many of the brain areas found to have altered functionality in our study can be related to the physiological and behavioral alterations seen upon cannabis consumption. The Primary Somatosensory Cortex and Primary and Secondary Visual Cortex in males and the Parietal Cortex (Auditory) in females are areas in the brain that receive and integrate sensory information from many body parts, including skin and eyes, and the appropriate processing of environmental information within these areas with other areas in the brain allows for planning, execution, and control of motor behaviors (Grill-Spector & Malach, 2004; Borich et al., 2015; Yao et al., 2020). Similarly, the motor cortex, found altered in female animals, integrates, plans and executes motor behaviour (Li et al., 2015) and is found to be altered in cannabis users (Pillay et al., 2004; Ward et al., 2023). The altered functional connectivity of these brain regions might indicate persistent changes in sensory and motor processing after adolescent cannabis exposure.

In this study, the CBD group in males was the only group that showed increased functional connectivity, compared to both the THC and control groups. Grimm et al. (2018) found a similar pattern of results, where CBD increased the fronto-striatal connectivity in comparison to the placebo group, while THC showed no difference. van Boxel et al. (2023) also reported increased functional connectivity in the default mode network followed by 28 days of CBD administration in recent-onset psychosis patients. Interestingly, our findings contradict another human study that investigated the effects of THC through fMRI, where THC was responsible for a significant reduction in functional connectivity between the nucleus accumbens and areas of the limbic lobe, medial

prefrontal cortex, striatum, and thalamus (Ramaekers et al., 2016). Importantly, the individuals in that study were regular drug users and, since the study did not include healthy controls, it's impossible to conclude if their brains already had functional alterations due to frequent drug use.

CB1 receptors are reported to have high density across the somatosensory cortex and visual cortex. The effect of cannabis on these areas explains the visual hallucinogenic and sensory intensification effects found after cannabis consumption (Bloomfield et al., 2019). One rsfMRI study with human subjects reported altered functional connectivity in sensorimotor and visual networks upon acute THC administration, consistent with mood and alertness symptoms, and subjective feelings of "high" reported by the users (Klumpers et al., 2012). Interestingly, in our study rodents exposed to CBD (and not THC) were the ones that expressed higher functional connectivity in these areas. However, our observations were during adulthood after adolescent exposure, long after the last exposure to cannabis, and may reflect a compensatory response to the cannabis exposure.

Our study provides one of the few imaging study data on the differential effects of CBD and THC in the brains of non-human animals. Bhattacharyya et al. (2010) conducted a functional imaging study in human subjects also comparing the differential effects of CBD and THC. The study indicated some areas in which oral THC and CBD had opposite functional effects, including the striatum, anterior cingulate and prefrontal cortex, parahippocampal gyrus, amygdala, and areas of the temporal and occipital cortices when subjects were performing cognitive tasks. Another study from the same group also found differential modulation of THC and CBD on prefrontal, striatal, and medial temporal cortex function (Bhattacharyya, 2012). In our study, this differential effect was also evident, with different areas implicated, though. One possible explanation for that is that, in addition to utilizing different models (humans as opposed to rodents) and different routes of administration (oral vs. vapour), the studies conducted by (Bhattacharyya et al., 2010; Bhattacharyya, 2012) investigated BOLD activation in these areas while performing cognitive tasks, while our study investigated resting state functional connectivity.

Pavlovian appetitive and conditioned avoidance learning were the main behavioural outcomes assessed in this study. While cognitive function is known to be affected by cannabis use (Prini et al., 2020), with evidence that exposure during adolescence results in long-lasting impairments (De Felice et al., 2023), we had hypothesized that the hippocampus would show altered structure and functionality in the treatment groups. In our Pavlovian conditioned approach task, male animals in the control and balanced CBD+THC treated groups acquired Pavlovian conditioned learning normally, while the high-CBD and high-THC groups displayed learning impairments in this task. All female animals acquired the Pavlovian conditioned learning, indicating a learning impairment in this task in males only (Appendix 1). On the other hand, all three male cannabis-treated groups showed impaired operant learning in an active avoidance test, whereas treatment did not seem to affect females (Appendix 2). Interestingly, our imaging data reports no indication of altered functional or structural connectivity of the hippocampus, indicating that these learning impairments in our rodent model may not be hippocampus-dependent. Our findings contrast with many imaging studies performed with cannabis users where they report impairment in different domains of learning and memory at the functional and structural levels (Bloomfield et al., 2019). In most of these fMRI studies conducted in humans, the hippocampus or parahippocampal regions are often found to be altered, probably due to the high density of CB1 receptors in that area (Bhattacharyya et al., 2018; Rabinak et al., 2014; Carey et al., 2015; Sneider et al., 2013). Finally, the retrosplenial system (RSD/RSGa) was also found to have altered functional connectivity in male and female animals. This is an area of the neocortical system involved in the sense of visuospatial imagery, spatial learning, and navigation (Mitchell et al., 2018), and could be related to the learning impairments found in males in cannabis-treated groups.

4.4 Diffusion MRI

In our study, we also assessed structural connectivity changes through dMRI. The investigation of white matter structure is important since the brain is reported to continue to develop throughout adolescence and may be implicated in the long-term consequences of cannabis consumption (Lebel et al., 2012; Becker et al., 2015). Our results indicated two networks in males that presented altered structural connectivity: Network 1

comprising the Striatum, Insular Cortex 2, Endo/Piriform Cortex and Ventral Thalamus, and Network 2 comprising the Intermedial Entorhinal Cortex, Pontine Nuclei, Interpeduncular Nucleus, and the Raphe/Median Pontine Reticular Nucleus.

The two networks with altered structural connectivity comprise areas that control different aspects of cognition and behavior, such as emotional and sensory processing, physiological regulation, and memory, indicating that the consequences of adolescent exposure to cannabis can be widespread in the adult brain (Beck et al., 2004; Chen et al., 2014; Takehara-Nishiuchi, 2014; Yager et al., 2015; Evrard, 2019; Wolff & Vann, 2019). This is also evident by the observation of altered structural connectivity in one particular area, the thalamus, which is a heterogeneous structure that functions as the core for the integration of sensory and motor information in the brain before being directed to the cerebral cortices (Wolff & Vann, 2019). In our study, this area was found to have persistently altered structural connectivity in adulthood upon cannabis use in adolescence. Rather than comprising a single cognitive or behavior domain as it would be expected for many other areas, because of its integrative nature, altered structural connectivity in the thalamus may present detrimental effects related to many brain regions (Wolff & Vann, 2019).

The Raphe and Pontine Reticular Nucleus were the only areas affected both functionally and structurally by adolescent cannabis exposure in males. Furthermore, this area also showed altered functional connectivity in female animals. Specifically, the raphe nuclei are a set of structures in the midbrain interconnected with many functionally distinct brain areas, that is best known for being the locus of the serotoninergic system and their projections to the limbic system can be related to cognitive and emotional processing and psychiatric conditions (Hornung, 2003; Beck et al., 2004; Luo et al., 2015; Commons, 2015; Huang et al., 2019). Our results for the functional and structural alterations of these brain regions add to the emerging consensus that adolescent cannabis consumption is implicated in psychiatric vulnerability (Rubino et al., 2012; Stringfield & Torregrossa, 2021). As the neurobiology of cannabis-induced psychiatric vulnerability is not elucidated (Rubino & Parolaro, 2016), our study adds to a possible spatial locus for the implication of cannabis and psychiatric conditions, and future studies could clarify the

molecular and cellular mechanisms of the serotonin system in psychiatric conditions upon cannabis exposure (Viñals et al., 2015; Ibarra-Lecue et al., 2022). On the other hand, the Pontine Reticular Nucleus has been implicated in PPI response (Fendt et al., 2001; Cano et al., 2021). Since in our study, treatment in male and female animals resulted in no altered PPI response in adulthood (Appendix 3), it is possible that an underlying change in this brain region was not sufficient to trigger a behavioural alteration.

Despite these considerations, it is still not clear how cannabis consumption is implicated in several mental illnesses. One hypothesis is that the endocannabinoid tone is essential for coordinating neurodevelopment and adolescent cannabis use might disrupt the endocannabinoid system and lead to changes implicated in the development of mental illness, such as depression, schizophrenia and bipolar disorder (Jenkins & Khokhar, 2021). Another less explored possibility is the role of CB2 in the association of cannabis use and mental illness. CB2 is expressed in the mesocorticolimbic signaling pathway and modulates the firing of dopaminergic neurons (Zhang et al., 2017). A few studies have demonstrated the modulating effects of CB2 on the rewarding effects of many drugs, including alcohol, nicotine and cocaine, suggesting CB2 plays a role in drug addiction (Ellner et al., 2021).

The striatum was one of the regions found to have affected structural connections between the control group and cannabis-exposed groups in male animals. This area has been previously associated with dopaminergic signaling, schizophrenia and drug addiction (Yager et al., 2015). Cannabis has also been linked to striatal activation (Zhou et al., 2019; Bossong et al., 2009), and another neuroimaging study found disrupted dopaminergic signaling in the striatum among cannabis users using positron emission tomography (PET) (Leroy et al., 2012). The findings in our study can potentially be linked to aberrant neurodevelopment that would result in vulnerability to drug addiction and psychosis in adulthood (Godin & Shehata, 2022). Further studies could address this issue by investigating striatum abnormalities in adolescent exposed animals and their impact on addictive behaviors in adulthood.

As mentioned above, male rats treated with cannabis during adolescence presented long-lasting learning impairments in adulthood. In our dMRI results, the entorhinal cortex was found to have altered structural connectivity. The entorhinal cortex is an area adjacent to the hippocampus well known for its role in memory consolidation that serves as a bridge between the hippocampus and neocortical regions (Takehara-Nishiuchi, 2014). Since the association between cannabis use and learning impairment in humans is well documented (Solowij & Battisti, 2008; Abdel-Salam et al., 2013), future studies should further investigate the consequences of adolescent cannabis use on the neurobiology of the entorhinal cortex and its association with cannabis-induced learning impairments.

Our dMRI data provides an evaluation of the impact of adolescent cannabis consumption on brain structural connectivity in adulthood. We could demonstrate that many areas of the adult rodent brain are structurally differentiated due to cannabis effects. Considering the dramatic morphological changes the brain undergoes during adolescence, our findings go in line with current literature for non-human animals (Rubino & Parolaro, 2016) and humans (Stringfield & Torregrossa, 2021) on the vulnerability of the adolescent brain to cannabis use. Specifically, two studies performed a longitudinal analysis of the impact of cannabis use in human adolescents. Becker et al. (2015) investigated axonal fibers organization within a two-year interval in regular young adult cannabis users who initiated consumption in adolescence. Chronic cannabis use was associated with changes in white matter structure in many regions, especially the superior longitudinal fasciculus, superior frontal gyrus, corticospinal tract, and anterior thalamic radiation. Since the imaging results correlated with the quantity and frequency of cannabis consumption, the authors suggest cannabis is associated with aberrant patterns of brain development (Becker et al., 2015). A second study also investigated the impact of cannabis use on adolescents over 18 months (Epstein & Kumra, 2015). The findings indicated a reduced fractional anisotropy in the left inferior longitudinal fasciculus. The authors also report that these changes in this region are correlated with the number of days of exposure to cannabis within the interscan interval.

In our study, some brain regions showed structural connectivity alterations without functional connectivity alterations. In fact, structural connectivity is highly correlated

with, and contributes to, the observed functional connectivity, and lesioned or weakened structural connectivity can lead to weakened functional connectivity (Achard et al., 2012; Benitez Stulz et al., 2024). Studies investigating the relationship of these two entities have suggested that the brain has compensatory mechanisms that can potentially reorganize and keep the homeostasis of functional connectivity after alterations in structural connectivity (Benitez Stulz et al., 2024).

4.5 Limitations

One important limitation of our study is the uniform cannabis exposure in adolescence for male and female animals (Aziz, 2024). It has been demonstrated that male and female animals exhibit different timing of puberty onset, with females initiating adolescence earlier than males (Spear, 2000). The marked rise in sex hormones in this developmental period might differentially modulate the effects of psychoactive drugs and our study might have failed to capture the impact of these differences.

Another limitation of our study is related to the passive administration of cannabis vapour. Even though the concentration of cannabis strains and the amount loaded is predetermined, not all the vapour in the chamber is being inhaled by the end of the exposure duration. Additionally, differences within subjects and between male and female animals related to breathing rate or location in the cage with respect to the vapour port could lead to different inhalation profiles (Frie et al., 2020). To address and try to control for this issue, this study employed cannabinoid serum level quantification.

Regarding the MRI analysis, the brain atlas used in this study contains 59 brain regions defined according to neuronal activity near anatomical neural landmarks (Barrière et al., 2019; Aziz, 2024). This is a general division/labeling that might not cover the multiple neuronal activities that could happen in a single brain region. Additionally, 59 brain regions do not represent all functional regions found in the rat brain.

Finally, in this study dexmedetomidine was used as the main anesthesia drug during the MRI scans. Even though dexmedetomidine has been shown to mildly disrupt functional networks (Grandjean et al., 2014; (Wallin et al., 2021) and is believed to mirror the

natural sleeping state, it should be acknowledged that the brain is still under the effect of a drug that alters neurotransmitter levels.

4.6 Future directions

It has been consistently shown in scientific literature a strong correlation between cannabis use, especially in adolescence, and the development of psychosis and schizophrenia (Casadio et al., 2011; Godin & Shehata, 2022). However, it is yet to be elucidated if this is a causal correlation and its underlying neurobiology. In our study, the use of cannabis in adolescence was associated with structural changes in the striatum, a key brain region involved in psychosis and schizophrenia. In addition to that, there are evidence for high expression of CB1 receptors in the striatum that could hypothetically be affected by cannabis use (Van Waes et al., 2012). Therefore, future studies should investigate the long-term effects of adolescent cannabis use on the neurobiology of the striatum and the effects of different cannabis compounds in this brain region on a schizophrenia model.

As mentioned earlier, adolescence onset varies in male and female rats (Spear, 2000). Additionally, it is hypothesized that sex hormones influence sex differences in behavioural outcomes upon cannabis consumption (Tseng et al., 2004; Ruiz et al., 2021). Therefore, future studies should investigate the roles of sex hormones throughout adolescence in the context of cannabis exposure and whether the different chronology in puberty plays a role in the long-term effects of cannabis use in adolescence.

4.7 Conclusions

In conclusion, the findings of this study support the notion that cannabis consumption during adolescence can lead to lasting impacts on brain development (functionally and structurally) in adulthood, with males possibly being more vulnerable than females. However, more research needs to be conducted to fully understand the results collected. At both the functional and structural level we found indication of changes in the brain of male adult animals that we previously exposed to cannabis. Our data goes in line with human data discussed above regarding the longitudinal effects of cannabis exposure and

in demonstrating the vulnerability of the adolescent brain to drug use. However, the translation to human studies must be taken with caution, since most of these studies are more susceptible to confounding effects, such as co-use with other drugs, especially alcohol (Lubman et al., 2015). Due to the sex differences in outcomes related to cannabis consumption, the study of its neurobiology might help to establish differential therapeutical strategies for men and women with CUD and associated psychiatric conditions.

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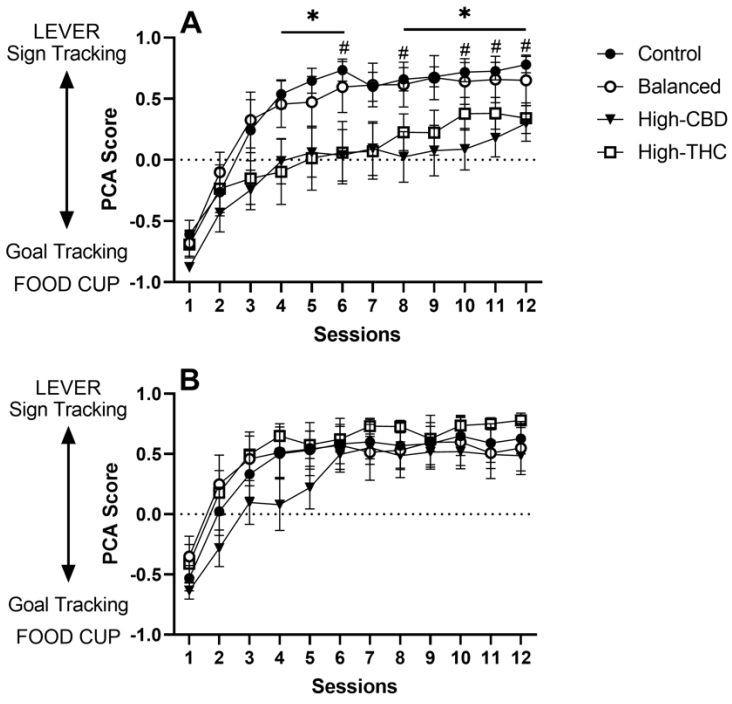
Appendices

Appendix 1: Pavlovian Conditioned Approach (PCA) scores of adult male (A) and female (B) rats treated with three different cannabis strains (high-CBD, high-THC, and balanced) or just air for two weeks during adolescence. Data are mean ± standard error of mean (*p< 0.05, control vs high-CBD; #p< 0.05, control vs high-THC, Bonferroni test).

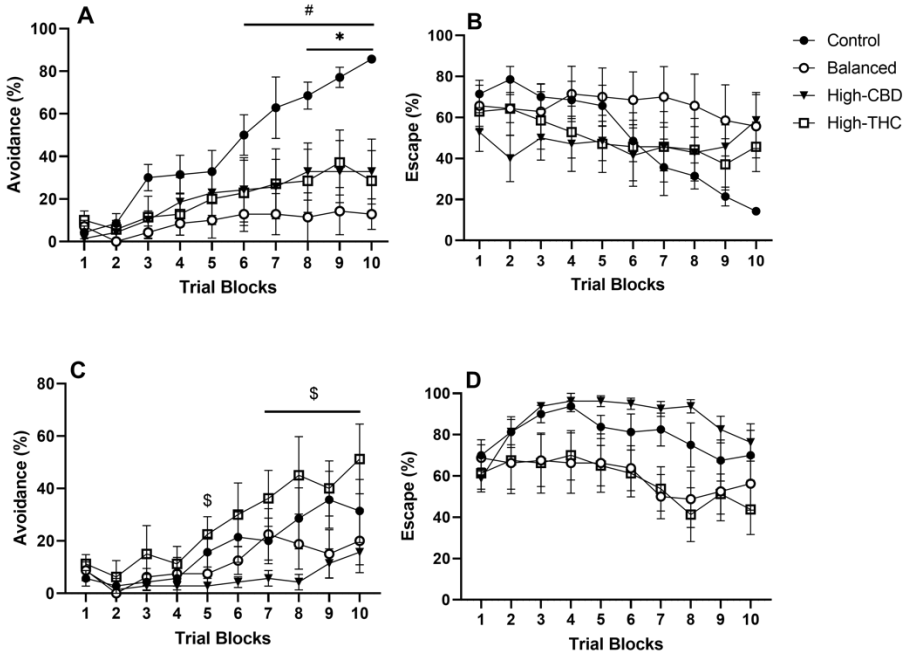


A two-way ANOVA for repeated measures indicated a significant effect of adolescent cannabis exposure on PCA scores during adulthood in male rats [treatment effect: $F(3,24)= 3.077$; $p=0.047$, Appendix 1A]. PCA scores significantly changed over the sessions [time effect: $F(11,264)= 55.201$; $p<0.0001$], but the effects of treatment did not interact with those changes over the sessions [interaction: $F(33,264)= 1.195$; $p=0.223$]. Post hoc analyses revealed that male rats exposed to high-THC or high-CBD cannabis during adolescence had less lever-directed behaviour (sign-tracking) after the 3rd session

(p values< 0.05). However, balanced cannabis exposure had no effect on PCA score compared the control group (p values> 0.05).

In female rats, there was no significant effect of adolescent cannabis exposure on PCA scores during adulthood [treatment effect: F(3,28)= 0.737; p=0.539, Appendix 1B]. PCA scores significantly changed over the sessions [time effect: F(11,308)= 48.21; p<0.0001], but the effects of treatment did not interact with those changes over the sessions [interaction: F(33,308)= 0.885; p=0.652].

Appendix 2: Rates of avoidance (A and C) and escape (B and D) behaviours per trial blocks during the active avoidance task in adult male (A and B) and in female (C and D) rats. Data are mean ± standard error of mean (#p< 0.05, control vs balanced cannabis treated group; *p< 0.05, control vs high-CBD or high-THC treated group; $p< 0.05, high-THC vs high-CBD, Bonferroni test).

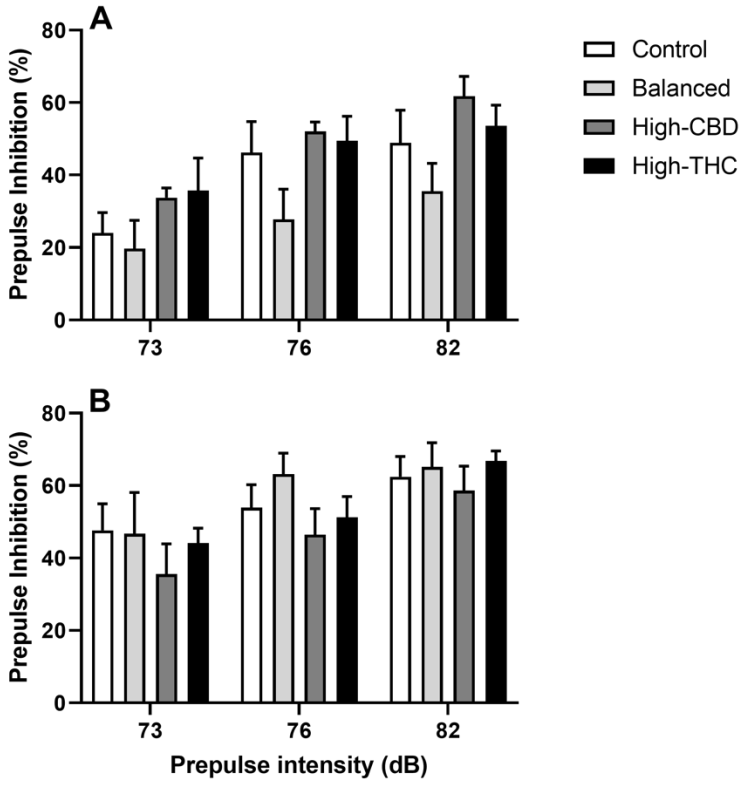


A two-way ANOVA for repeated measures indicated significant changes in avoidance ratio of male rats through the trials in active avoidance task, and those changes modulated

by cannabis treatment [time effect: $F(9,216)= 16.93$; $p< 0.0001$; treatment effect: $F(3,24)= 3.591$, $p= 0.029$, Appendix 2A]. The effects of cannabis treatment on avoidance ratio interacted with the avoidance trial blocks [interaction: $F(27,216)= 2.799$; $p< 0.0001$, Appendix 2A]. Post hoc analyses indicated that balanced cannabis treated group dissociated from control group starting with 6th trial block while high-CBD and high-THC treated groups differed with control group during the final three trials (p values< 0.05). Escape behaviours also showed difference over the trial blocks [time effect: $F(9,216)= 4.255$; $p= 0.022$, Appendix 2B] and treatment groups showed different responses from each other during the trials [interaction: $F(27,216)= 2.135$; $p= 0.002$, Appendix 2B]. However, overall treatment effect did not reach a statistically significant level [$F(3,24)= 0.724$; $p> 0.05$, Appendix 2B].

In female rats, significant changes in avoidance ratio through the trials were observed [time effect: $F(9,234)= 8.838$; $p< 0.0001$, Appendix 2C], and the cannabis treatment had a significant effect on avoidance ratio [treatment effect: $F(3,26)= 2.985$, $p= 0.049$]. However their effects were independent from each other [interaction: $F(27,234)= 1.077$; $p= 0.369$]. Post hoc analyses indicated that high-THC group had higher avoidance ratios compared to high-CBD group at 5th and after 7th trial block (Appendix 2C). Escape behaviours also showed difference over the trial blocks [time effect: $F(9,252)= 4.119$; $p= 0.017$, Appendix 2D] and treatment groups showed distinct escape ratios [treatment effect: $F(3,28)= 3.875$; $p= 0.019$, Appendix 2D]. However, treatment effect did not change over the trial blocks [interaction: $F(27,252)= 1.106$; $p> 0.05$, Appendix 2D].

Appendix 3: Prepulse inhibition rates (%) of the adult male (A) and female (B) rats from different treatment groups at 73, 76, and 82 dB prepulse intensity levels. Data are mean±standarts error of mean.



Cannabis treatment during adolescence in male rats did not produce any statistically significant effect on PPI measured during adulthood [treatment effect: $F(3,24)= 2.224$; $p> 0.05$, Appendix 3A]. As expected, PPI rates changed by the increased prepulse intensity levels [$F(2,48)= 47.19$: $p< 0.0001$, Appendix 3A], but the observed changes did not interact with the treatment groups [$F(6,48)= 1.270$; $p> 0.05$, Appendix 3A].

Like males, cannabis treatment during adolescence in female rats did not produce any statistically significant effect on PPI measured during adulthood [treatment effect: $F(3,28)= 0.605$; $p> 0.05$, Appendix 3B]. As expected, PPI rates changed by the increased prepulse intensity levels [$F(2,56)= 30.69$: $p< 0.0001$, Appendix 3B], but the observed changes did not interact with the treatment groups [$F(6,56)= 0.927$; $p> 0.05$, Appendix 3B].

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