Investigating resting-state functional connectivity differences in cannabis use disorder and exploring their mitigation through brief mindfulness-based intervention

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Investigating Resting-State Functional Connectivity Differences in Cannabis Use Disorder and Exploring Their Mitigation Through Brief Mindfulness-Based Intervention

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Abstract

Cannabis Use Disorder (CUD) affects 22 million people globally and can lead to adverse psychosocial outcomes, including failed attempts to cut down/quit despite the experience of mental health and cognitive problems. Such problems have been (partly) ascribed to neurobiological alterations within pathways of the addiction neurocircuitry and high in cannabinoid receptors type 1 (CB_1R), to which delta-9-tetrahydrocannabinol binds to exert its psychoactive effects. Emerging functional neuroimaging (fMRI) evidence show that cannabis users vs controls showed altered brain function while resting (i.e., without performing cognitively demanding tasks), measured via resting-state functional connectivity (rsFC); the evidence has not been synthesised systematically.

Study 1 is a PROSPERO pre-registered systematic literature review of 21 studies examining rsFC differences between 737 cannabis users and 659 controls, and their associations with metrics of cannabis exposure and related problems. Cannabis users vs controls showed altered rsFC in fronto-frontal, frontostriatal, and fronto-temporal region pairings, and selected brain pathways correlated with cannabis exposure metrics. Methodological limitations precluded a detailed understanding of the nature of rsFC alterations. For example, it was unclear if rsFC changes were driven by dependent use/CUD, because no study had measured if cannabis users endorsed a CUD using current diagnostic tools (i.e., DSM-5). Furthermore, if rsFC alterations are specific to cannabis use was unclear due to inconsistent accounting for key demographics and substance use/mental health confounders entrenched with cannabis use known to affect brain function. Finally, the behavioural significance of rsFC alterations remained unclear, as it has seldom been examined in relation to cannabis exposure and related problems.

Study 2 aimed to address the limitations of the literature, via examining 107 people aged 18-56 years (35 female) via fMRI scanning, socio-demographic, substance use, mental health, and cognitive testing. The primary aim was to compare rsFC between 65 individuals with a moderate-to-severe CUD who had tried to cut down or quit, and 42 controls, controlling for age, sex, alcohol and nicotine exposure, and depression symptom scores. Regions implicated in addiction neurocircuitry, dense in CB_1R, and implicated in cognitive processes altered in cannabis users were selected as regions-of-interest (ROIs). Associations between CUD group rsFC changes and metrics of cannabis

exposure and related problems were explored. People with a CUD vs controls showed greater rsFC between the following region pairings: nucleus accumbens (NAc)-frontal; pallidum-occipital/occipito-parietal, in correlation with CUD severity and cannabis use days/month; and putamen-occipito-parietal, in correlation with an earlier age of cannabis use onset; and lower hippocampus-occipital rsFC. Thus far, it is unclear if altered rsFC in CUD can be mitigated using psychological interventions.

Study 3 examined if altered rsFC shown in the CUD group in Study 2 (n=56, aged 18-51 years), could be mitigated using one of the following ~2-week interventions: a mindfulness-based intervention (MBI, n=19), *active placebo* (relaxation; n=18), and *passive placebo* (daily monitoring; n=19). It used a double-blind, pseudo-randomised design based on age and sex. The primary aim was to examine intervention-group-by-time effects on rsFC in *a priori* ROIs with altered rsFC identified in Study 2 (i.e., NAc, putamen, pallidum, hippocampus), and how changes in rsFC correlated with those in cannabis exposure and related variables. Pre-to-post MBI, putamen-superior frontal gyrus (SFG)/frontal pole rsFC decreased; and hippocampus-anterior cingulate rsFC increased (correlated with more cannabis use days). Pre-to-post *active placebo,* putamen-frontal pole rsFC increased, correlated with decreased cannabis grams; putamen-SFG/cerebellum/brainstem rsFC increased; and pallidum-anterior superior temporal gyrus (aSTG) rsFC decreased. Pre-to-post *passive placebo*, putamen-frontal pole rsFC increased, correlated with less cannabis use days; pallidum-aSTG rsFC increased; and hippocampus-anterior cingulate and putamen-SFG/cerebellum/brainstem rsFC decreased.

Findings from the thesis demonstrated rsFC alterations in cannabis users and confirmed existence of such alterations in CUD, and that alterations can be mitigated with a brief MBI, as well as relaxation and daily monitoring. rsFC alterations may reflect cannabis exposure or related problem (or both), or a neurobiological vulnerability predating the onset of cannabis use/CUD. Future fMRI studies with larger samples are required to confirm findings and to track over time if continuation of MBI, active and passive placebo interventions consolidate the effects reported herein. The results from this thesis expand upon neuroscientific theories of addiction validated in substances other than

cannabis, by confirming partially overlapping alterations in CUD, and by showing that brief psychological interventions can target brain dysfunction in CUD.

Research Outputs

Published Peer-Review Paper as Chapter of the Thesis (*Chapter 2*)

Thomson, H., Labuschagne, I., Greenwood, L., Robinson E., Sehl, H., Suo, C., & Lorenzetti, V., (2022) Is resting-state functional connectivity altered in regular cannabis users? A systematic review of the literature. *Psychopharmacology* https://doi.org/10.1007/s00213-021-05938-0

Dissemination of Research – Conference Presentations and Awards

Short talk

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Winner of 'President's Poster Prize'

Short talk

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Poster

Thomson, H., Labuschagne I., Robinson, E., & Lorenzetti, I. (October 2020) *Resting-state functional connectivity in cannabis users versus non-using controls: A systematic review of the literature.* Biological Psychiatry Australia 10th Annual Scientific Meeting, Online, Australia.

Short talk

Thomson, H., Labuschagne I., Robinson, E., & Lorenzetti, I. (October 2020) *Resting-state functional connectivity in cannabis users versus non-using controls: A systematic review of the literature.* ACU Psychology Research Conference, Online, Australia.

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Guest Presentation (invited speaker)

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Declaration

This thesis contains no material that has been extracted in whole or part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution. No other person's work has been used without due acknowledgment in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required).

Signed: 

Date: 27/02/2023

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List of Abbreviations and Symbols

Δ	Change
11-OH-THC	11-Hydroxy-Tetrahydrocannabino
3T	3-Tesla
5FMQ	Five-Facet Mindfulness Questionnaire
7T	7-Tesla
α	Alpha
ACBD	Adolescent Brain Cognitive Development study
ACC	Anterior Cingulate Cortex
AIHW	Australian Institute of Health and Welfare
ANOVA	Analysis of Variance
APA	American Psychiatric Association
ART	ARtifact-Detection Tools
aSTG	Anterior Superior Temporal Gyrus
AUDIT	Alcohol Use Disorder Identification Test
B-H	Benjamini-Hochberg
BDI-II	Beck Depression Inventory - II
BIDS	Brain Imaging Data Structure
BOLD	Blood Oxygen Level-Dependent
CB_1R	Cannabinoid Receptor 1
CB_2R	Cannabinoid Receptor 2
CBD	Cannabidiol
CBT	Cognitive Behavioural Therapy
CM	Contingency Management
CNS	Central Nervous System
COVID SS	COVID Stress Scale
CT	Computerized Tomography
CUD	Cannabis Use Disorder
CUDIT-R	Cannabis Use Disorder Identification Test - Revised
CUI	Cannabis Use Interview
CWS	Cannabis Withdrawal Scale
DCM	Dynamic Causal Modelling
DICOM	Digital Imaging and Communications In Medicine
dlPFC	Dorsolateral Prefrontal Cortex

dmPFC	Dorsomedial Prefrontal Cortex
DNM	Default Mode Network
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EC	Effective Connectivity
EEG	Electroencephalogram
EHI-SF	Edinburgh Handedness Inventory – Short Form
EMA	Ecological Momentary Assessment
fALFF	Fractional Amplitude of Low-Frequency Fluctuations
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FSIQ	Full Scale Intelligence Quotient
FTND	Fagerström Test of Nicotine Dependence
GABA	Gamma-Aminobutyric Acid
GLM	General Linear Model
GPCR	G-Protein-Coupled Receptor
HRF	Haemodynamic Response Function
Hx	History
ICA	Independent Components Analysis
ICC	Intrinsic Connectivity Contrast
lFCD	Local Functional Connectivity Density
K	Number of Voxels per Cluster
M	Mean
MBI	Mindfulness-Based Intervention
MBSR	Mindfulness-Based Stress Reduction
MCQ-SF	Marijuana Craving Questionnaire - Short Form
MET	Motivation Enhancement Therapy
mFD	Mean Framewise Displacement
MINI	The MINI International Neuropsychiatric Interview 7.0.2
ML	Marijuana Ladder
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MVPA	Multivoxel Pattern Analysis
n	Sample Size (subgroup)
N	Sample Size (full sample)

NAc	Nucleus Accumbens
NIH	National Institute of Health
OFC	Orbitofrontal Cortex
p	p-value (Significance)
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PhD	Doctor of Philosophy
PIL	Participant Information Letter
PSS	Perceived Stress Scale
QA	Quality Assurance
ROI	Region of Interest
rsFC	resting-state Functional Connectivity
SCID-5-RV	Structured Clinical Interview of DSM-5 – Research Version
SD	Standard Deviation
SLR	Systematic Literature Review
SPECT	Single-Photon Emission Computerized Tomography
STAI-Y	State Trait Anxiety Inventory-Y Form
SUD	Substance Use Disorder
SVM	Support Vector Machine
T1w	T1-Weighted
TGA	Therapeutic Goods Administration
THC	Delta-9-Tetrahydrocannabinol / (Δ9- Tetrahydrocannabinol)
THC-COOH	11-Nor-9-Carboxy-Tetrahydrocannabinol
THCV	Tetrahydrocannabivarin
TLFB	Timeline Follow Back
UNODC	United Nations Office on Drugs and Crime
VMHC	Voxel Mirrored Homotropic Connectivity
vmPFC	Ventromedial Prefrontal Cortex
VS	Versus
WASI-II	Wechsler Abbreviated Scale of Intelligence – Second Edition
WHO	World Health Organisation

Note: This list is not exhaustive and contains only important or common abbreviations mentioned in text.

"I experimented with marijuana a time or two and I didn't like it. I didn't inhale."

~ Bill Clinton, 1992 ~

"*When I was a kid, I inhaled frequently. That was the point."*

~ Barack Obama, 2016 ~

CHAPTER 1:

Thesis Introduction and Overview

Chapter Guide

This thesis aims to examine the neurobiological underpinnings of regular cannabis use, both by way of a Systematic Literature Review of the current field of literature and by way of an empirical investigation. For this reason, the current chapter begins with a comprehensive overview of cannabis, relating to its history, the prevalence of use, and associated outcomes. The compounds of cannabis and the mechanisms of action by which cannabis impacts the brain and elicits the psychoactive effects for which it is known are detailed. This is followed by an exploration of the neural correlates of cannabis use, with a focus on the functional magnetic resonance imaging (fMRI) technique, resting-state functional connectivity (rsFC), which is the primary outcome measure across this thesis. This chapter then explores Cannabis Use Disorder and currently available treatment options.

This thesis additionally aims to investigate the neurobiological underpinnings of a promising treatment modality, brief Mindfulness-Based Intervention, in individuals with a moderate-to-severe cannabis use disorder (CUD). Therefore, this chapter then details current evidence relating to the treatment efficacy of MBI and its known neural correlates. Finally, the limitations which are touched on throughout the chapter are then specifically highlighted, which are to be addressed by the remainder of the thesis. The objectives of the thesis and aims are outlined, and this chapter concludes with a description of the overall thesis structure.

This thesis has been prepared with a 'person first' approach in mind i.e., the placement of the person before the condition. The emphasis on putting the person first is thought to destigmatize conditions and emphasize the person's value. Therefore, the clinical population within the two empirical experiments (Study 2 and 3) will be referred to as 'people with a moderate-to-severe CUD' where possible. In an effort to balance this approach with a succinct style of writing, this population will also at times be referred to as 'the CUD group'. Regrettably, the Systematic Literature Review (*Chapter 2*) was prepared prior to the adoption of this approach by the author, and the manuscript since published, hence within that chapter the clinical population is referred to as 'regular cannabis users'.

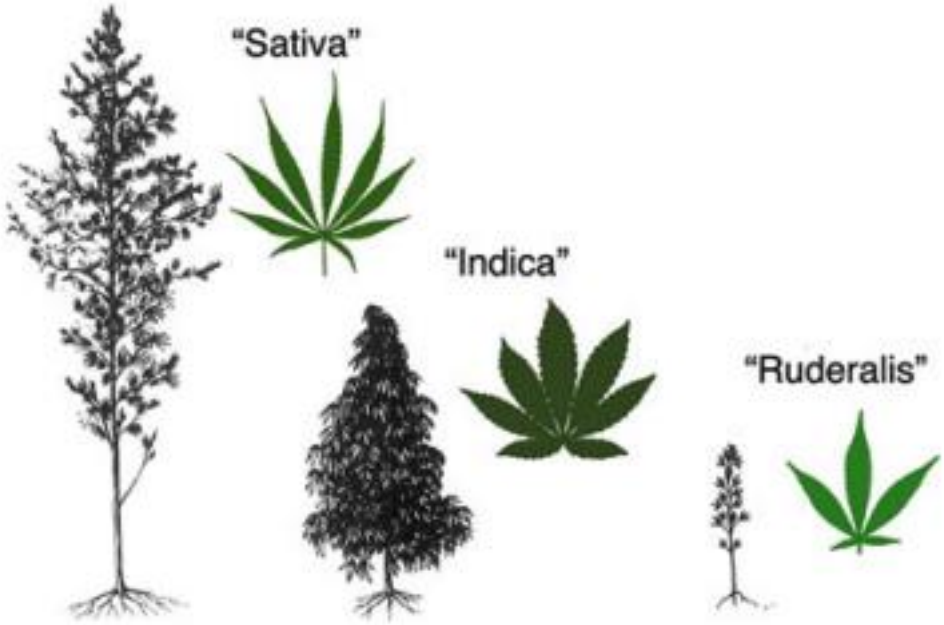
1.1 Origin, History, and Nomenclature of Cannabis

Cannabis sativa L, is an herbaceous species of flowering plant in the cannabaceae family, with evidence of use dating as far back as early Neolithic times (12,000 years BCE; Ren et al., 2021). It is long thought to be one of the oldest crops domesticated and cultivated by humankind and has been commonly purported to originate in Central Asia (Pisanti & Bifulco, 2019). Recent investigations have managed to pinpoint a specific location of the origin of cannabis; the Qinghai Lake region of the north-eastern Tibetan Plateau (McPartland et al., 2019). Cultivation of different varieties of cannabis were dispersed across Russia and Europe, and throughout China; it is thought to have been independently cultivated in parallel in several locations. The word '*sativa*' is the Latin translation of 'cultivated' (Collins Dictionaries, 2020), with the designation *Cannabis sativa* first applied to the species in 1542 by Leonhard Fuchs (Classen, 2001). Further work investigating *Cannabis sativa* by Swedish botanist Carl Linnaeus led to the evolution of its denomination, becoming *Cannabis sativa* L in 1753 (Bonini et al., 2018; Watts, 2006).

Cannabis sativa L, belonging to the genus *cannabis* (Small & Cronquist, 1976), is one of 170 species in the cannabaceae, or hemp, family (McPartland, 2018). The taxonomic division of *Cannabis sativa* L into multiple or sub-species has been long debated. Although various proponents have made arguments for the existence of three separate species (Anderson, 1980; Emboden, 1974; Hillig, 2005; Schultes et al., 1975), in current literature, it is more commonly accepted that *Cannabis sativa* L is monotypic, and variations represent sub-species (Barcaccia et al., 2020; Small & Beckstead, 1973; Small & Cronquist, 1976). See Figure 1.1 for an image of the sub-species. The primary two sub-species are *C. sativa* L and *C. indica* Lam, which can be distinguished based on morphology, phytochemistry, and original geographic range (Hillig, 2005; Lamarck, 1786; Small & Cronquist, 1976). *C. sativa* L is taller with a fibrous stalk, it contains a greater proportion of the phytocannabinoid delta-9-tetrahydrocannabinol (also written Δ^9-tetrahydrocannabinol; THC) than the phytocannabinoid cannabidiol (CBD), and it originated in Europe. In contrast, *C. indica* Lam (named for French naturalist Jean-Baptiste Lamarck who first differentiated it from *Cannabis sativa* L; Lamarck, 1786) is shorter with a woody stalk, contains a lower proportion of THC than CBD, and originated in Asia (McPartland, 2018). *C. ruderalis* Jan, named for Russian botanist Dmitrij

Erastovich Janischevsky (Janischevsky, 1924; Watts, 2006) is the sometimes included putative third variety; it is short and bushy, containing a lower still proportion of THC and thought to originate in Russia (Anderson, 1980; Emboden, 1974).

Figure 1.1. *Cannabis sativa L sub-species*



Cannabis sativa L sub-species line drawing by Anderson (1980).

Cannabis sativa L, hereby referred to as cannabis, has been utilised across history and in modern times for a variety of purposes including as a source of nutrition and fuel, and as a material fibre with which to make cloth, rope, paper, and canvas (Piluzza et al., 2013; Pisanti & Bifulco, 2019). It has also long been consumed for its psychoactive and medicinal purposes (Bonini et al., 2018). Documentation of the medicinal use of cannabis goes as far back as 2,700 years BCE, used by legendary Chinese Emperor Shen Nung (depicted in Figure 1.2), who is known as the Father of Chinese Medicine (Pain, 2015; Pisanti & Bifulco, 2019). The earliest evidence of cannabis used for its psychoactive properties dates back 750 years BCE. This was in light of the discovery made in the late

80s of a well-preserved ‘stash’ of cannabis along with a pestle and mortar in the Gobi Desert, China (Jiang et al., 2006; Russo et al., 2008). Based on the cannabis remains, which were high in the phytocannabinoid THC but not suitable for use as food, fibre, or oil, it was concluded that the cannabis was used for ritual and/or medicinal purposes (Jiang et al., 2006).

Figure 1.2. *Depiction of Chinese Emperor Shen Nung, with cannabis plant*

Source: The Devine Farmer’s Materia Medica, A Translation of the Shen Nong Ben Cao, author Shou-Zhong Yang

1.1.1 Cannabis Control During the 20th Century

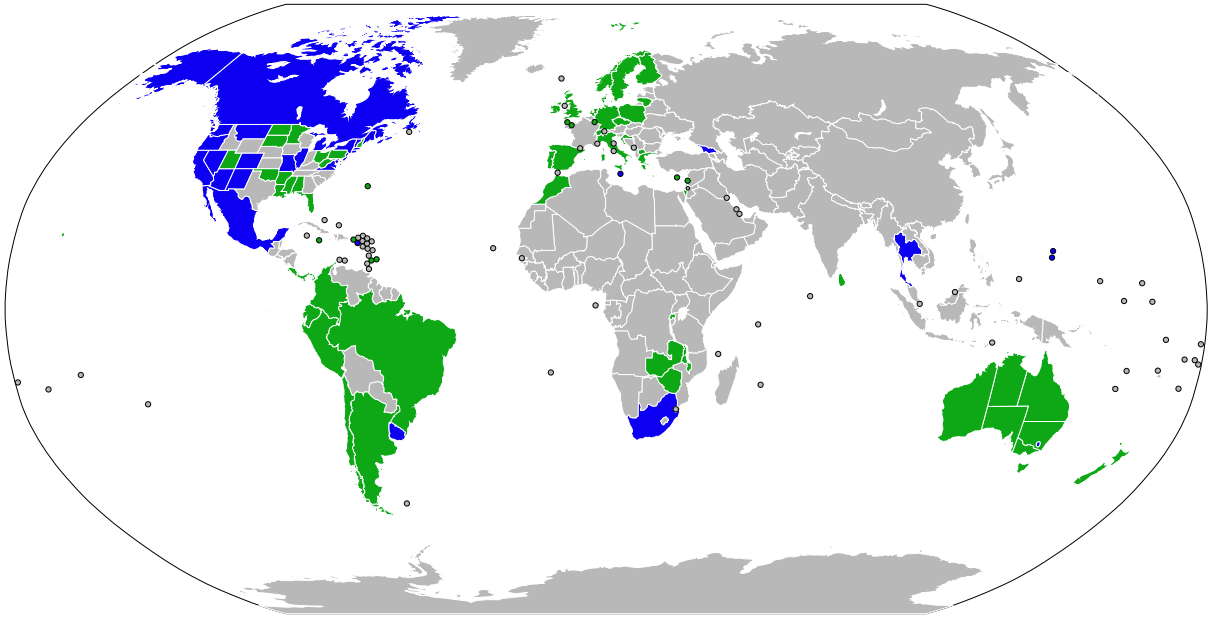
Prior to the 20th century, cannabis was largely used worldwide without restriction, medically and recreationally (Szasz, 2003). Across the early 20th century, the use of cannabis significantly decreased, thought to be in part due to difficulties in obtaining it (Zuardi, 2006). Within the United States of America (USA), a ‘Marijuana Tax Act’ was passed in 1937, largely rendering the growth or distribution of cannabis illegal (McKenna, 2014). In 1960, cannabis was definitively classified as a substance of abuse (Pisanti & Bifulco, 2017). Regardless, the 1960s saw a rise in cannabis use, predominately by ‘hippies’, i.e., college students and anti-Vietnam-war protesters (McKenna, 2014).

The Nixon administration developed the Controlled Substances Act in the late 1960s to further limit use (Drug Enforcement Administration, 2018); First Lady Nancy Reagan later lead anti-cannabis sentiment with her "Just Say No" public campaign during the 1980s (Reagan, 1986). Despite the best efforts of governing bodies, in the time since, public support in America for the legalisation of cannabis use has steadily risen from 12% in 1969, to 34% in the early 2000s, and up to 68% in 2020 (Brenan, 2020).

1.1.2 Recent Evolution of Cannabis Legality

Around the world, many countries are moving toward the decriminalisation of cannabis for personal use, and the legalisation of medical cannabis (Sznitman & Bretteville-Jensen, 2015; Wilkinson et al., 2016). Please see Figure 1.3 for a snapshot of cannabis legality status worldwide. The legality of cannabis use varies between countries, as well as between various states within a country (United Nations Office on Drugs and Crime [UNODC], 2021). In Australia, in 2016, amendments were made to the Narcotics Drug Act (1967), to update cannabis from being exclusively considered to be an illegal narcotic, to instead making some medicinal cannabis products available for specific patient groups under strict medical supervision. This act also permitted its cultivation for medicinal purposes and related research (Freckelton, 2021; Gleeson, 2019). Patients in Australia are currently able to receive a prescription for cannabis products from their general practitioners, who are typically required to lodge an application to the Therapeutic Goods Administration (TGA) to gain approval to prescribe (MacPhail et al., 2022). The Australian capital, Canberra, has also somewhat recently legalised individual possession of up to 50g of dry cannabis for personal use (Mannheim & Lowrey, 2020, Jan 31).

Figure 1.3. *Snapshot of the legal status of cannabis worldwide, as of 2018*



Note: Blue countries indicate where cannabis is legal for any use (no prescription required)
Green countries indicate where cannabis is legal as authorized by a physician

Source: public domain

1.1.3 Current Perceptions of Cannabis

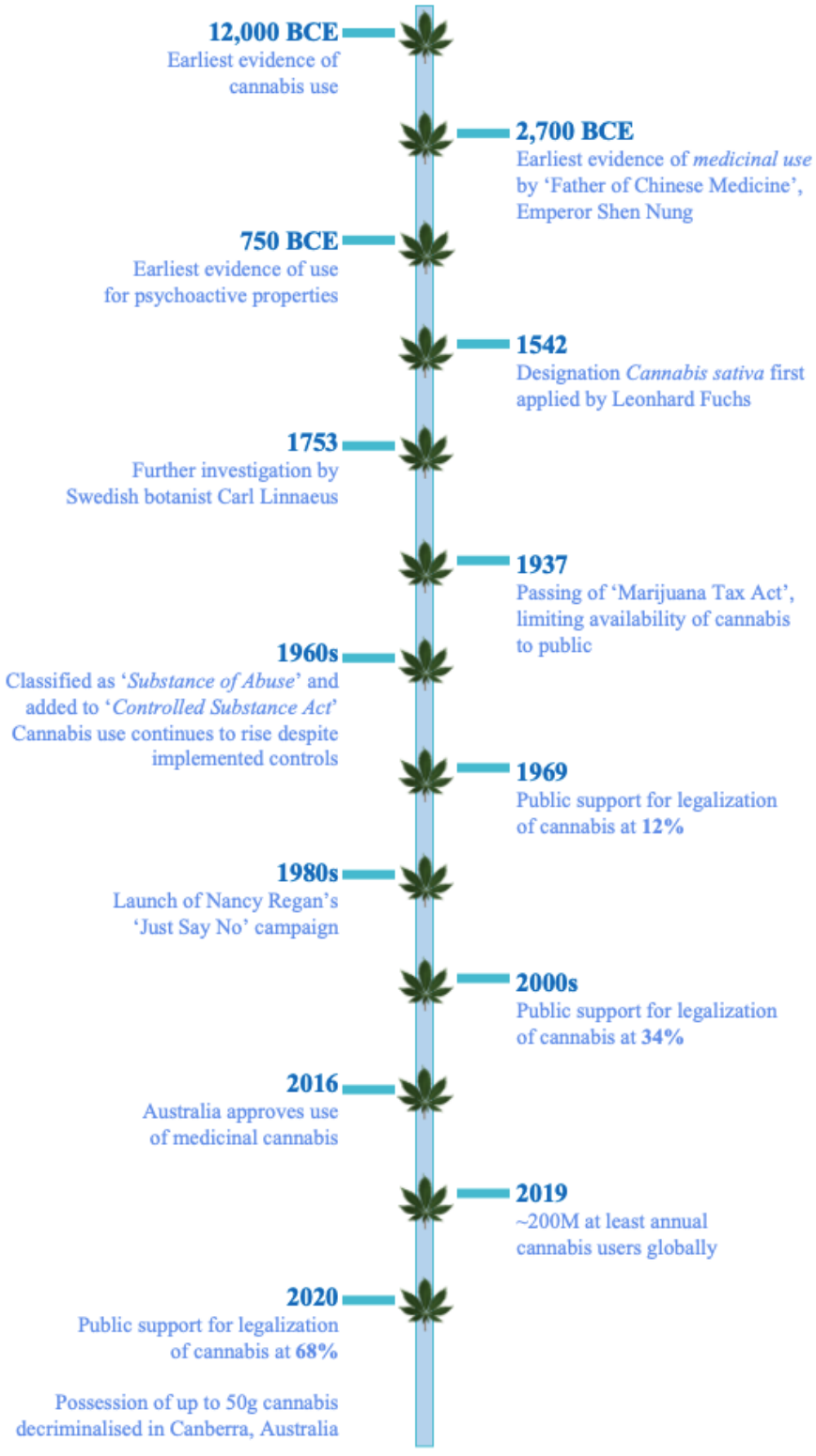
Attitudes towards cannabis and the frequency of cannabis use have continued to evolve over recent years (Australian Institute of Health and Welfare [AIHW], 2020; UNODC, 2019). Patterns of use have trended upwards and attitudes towards cannabis have typically moved in a more 'favoured' direction (Poulton et al., 2001; Sznitman & Bretteville-Jensen, 2015). The recognition of cannabis as an official medicinal substance, as well as the decriminalisation and legalisation of recreational cannabis use and possession, has increased the perception amongst the general public that cannabis is not harmful (Sarvet et al., 2018). There are now approximately 600,000 people in Australia who report past year use of cannabis for medical purposes (AIHW, 2020). There exists a widely held, yet erroneous belief (by both general populations and physicians) that cannabis is not an 'addictive' substance (McKenna, 2014). Furthermore, since the early 2000s, adult and adolescent perception of cannabis use as risky has decreased (Carliner et al., 2017). The perception of risk or harm from

regularly smoking cannabis declined by a quarter between 1995 and 2019, accompanied by an increase in past month cannabis use (UNODC, 2019).

1.1.4 Current Prevalence of Cannabis Use

Cannabis is known to be the most widely cultivated illicit crop worldwide, and the most commonly used of all illicit substances (UNODC, 2019). Recent estimates have placed the number of past-year cannabis users, of people aged 15-64, to be as high as approximately 200 million people worldwide (UNODC, 2019), of which approximately 3 million people are located in Australia (AIHW, 2020). The latter corresponds to approximately 4% of the global adult population, and approximately 15% of the Australian adult population respectively. In America, over 50% of young people report having tried cannabis (Substance Abuse and Mental Health Services Administration, 2014), and in Europe cannabis is now the most commonly taken substance by people utilising specialist addiction services (overtaking heroin; European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2015). As many as 37% of Australian respondents reported use at a weekly or higher frequency (AIHW, 2020). Please see Figure 1.4 for a timeline overview of events described in this chapter thus far.

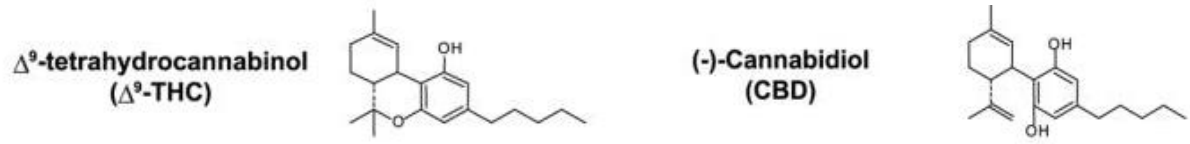
Figure 1.4. *Timeline of cannabis-related events*



1.2 Phytocannabinoids and the Endocannabinoid System

The cannabis plant contains 565 natural compounds. These natural compounds include 120 phytocannabinoids (ElSohly et al., 2017), which are a unique group of C21 terpenophenolic compounds (a class of naturally occurring organic chemicals) specific to cannabis (Chandra et al., 2017). The remaining non-phytocannabinoid compounds include terpenes (responsible for the distinctive smell associated with cannabis), flavonoids, alkaloids, non-cannabinoid phenols, and others (Radwan et al., 2021). Phytocannabinoid is the name given to cannabinoids that are naturally occurring and has been coined to distinguish these compounds from those which are created synthetically (Pate, 1999). Two phytocannabinoids of particular interest are the aforementioned THC and CBD (Radwan et al., 2017). Please see Figure 1.5 for the chemical structure of THC and CBD.

Figure 1.5. *Chemical structure of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD)*



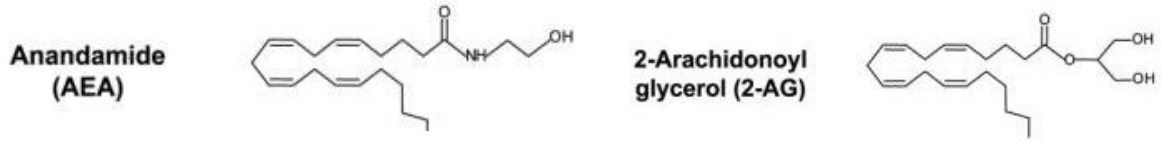
Source: Pacher et al. (2006)

THC has long been the primary focus of cannabis research, as it is considered to be the main psychoactive ingredient of cannabis (Radwan et al., 2017; Small & Marcus, 2002). More recently, focus has also included CBD, largely due to its properties as an antiepileptic/anticonvulsant tool in intractable paediatric epilepsy (Devinsky et al., 2014), as well as its non-intoxicating, antioxidant, anti-inflammatory, neuroprotective, and anxiolytic properties (Pellati et al., 2018). The structure of CBD was first demonstrated in 1963 (Mechoulam & Shvo, 1963) and the structure of THC reported shortly thereafter in 1964 (Gaoni & Mechoulam, 1964).

1.2.1 Endocannabinoid System and Endogenous Cannabinoids

Following the discovery of THC, the existence of two endogenous cannabinoid receptor agonists (called cannabinoids) were identified: *N*-arachidonoylethanolamine (commonly called anandamide) and 2-arachidonoylglycerol (2-AG; Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). These endocannabinoids are largely thought to prevent the development of excessive neuronal activity in the central nervous system (CNS) to maintain homeostasis via agonism of brain cannabinoid receptor 1 (CB_1R) and 2 (CB_2R; Pertwee, 2008). The understanding of endocannabinoids and the endocannabinoid system was significantly refined across the 1990s (De Petrocellis et al., 2004; Lee, 2012; Pisanti & Bifulco, 2019). The endocannabinoid system includes endocannabinoids, CB_1R and CB_2R, and the enzymes and proteins responsible for their biosynthesis, degradation, and re-uptake (Lu & Mackie, 2021; Wu, 2019). Please see Figure 1.6 for the chemical structure of anandamide and 2-AG.

Figure 1.6. *Chemical structure of N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG)*



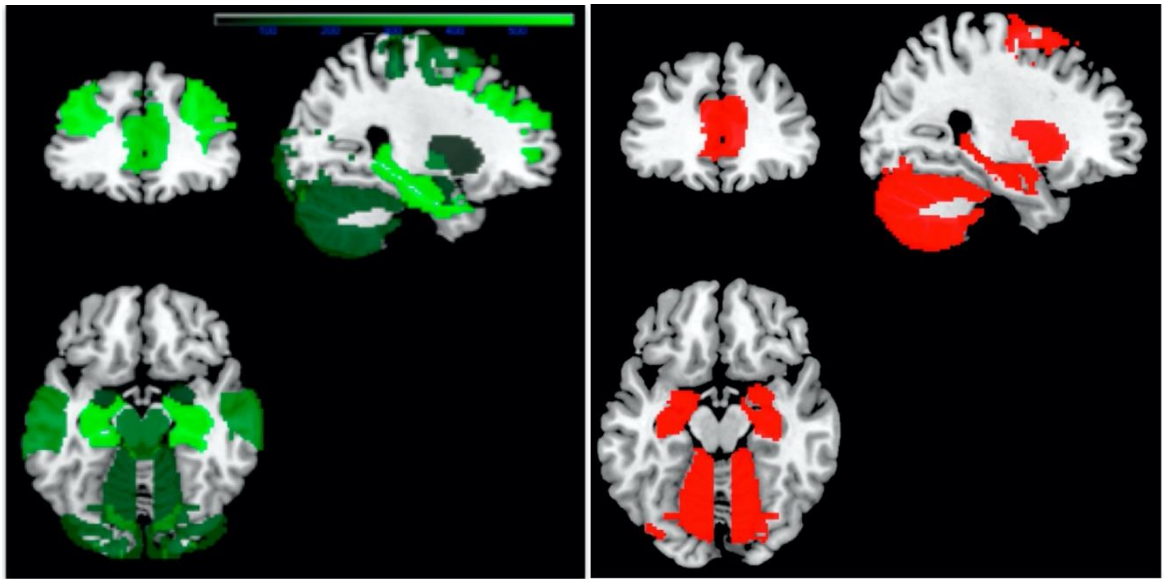
Source: Pacher et al. (2006)

1.2.1.1 Cannabinoid Receptors: CB_1R and CB_2R

CB_1R and CB_2R are both members of the G-protein-coupled receptor (GPCR) family; GPCRs are a class of membrane proteins (Alexander et al., 2021), and are known to be essential nodes of communication between the internal and external environments of cells (Rosenbaum et al., 2009). CB_1Rs are among the most abundant GPCRs in the brain (Mechoulam & Parker, 2013), and are most densely expressed throughout the CNS, in particular on certain GABAergic (gamma-aminobutyric acid-ergic) interneurons (Bodor et al., 2005). The pattern of their distribution throughout the CNS is

consistent with their effect on cognitive processes including memory, their control of motor function, and their analgesic properties (Pertwee, 2008). Specifically, CB_1Rs are found in the allocortex (including hippocampus, entorhinal cortex, and amygdala), the neocortex (including frontal gyri, Wernicke's area, and cingulate gyri), substantia nigra, and cerebellar cortex (Glass et al., 1997; Mackie, 2005). The basal ganglia (including globus pallidus, and dorsal and ventral striatum), secondary motor and sensory areas, occipitotemporal cortex, deep cerebellar nuclei, hypothalamus, and thalamus also have a moderately high prevalence of CB_1Rs (Glass et al., 1997). Please see Figure 1.7 for an overview of the distribution of CB_1R within the brain, and their overlap with regions shown to display neuroanatomical alterations in people who regularly use cannabis.

Figure 1.7. *Distribution of CB_1R within the brain, and their overlap with regions displaying neuroanatomical alterations in people who regularly use cannabis*



Note: dark to light green demonstrates range from 40-1680 density of receptor binding sites (measured via autoradiographic techniques), lighter colours indicate greater receptor density; red illustrates overlap between regions high in CB_1R that also show neuroanatomical alterations in people who regularly use cannabis.

Source: The Role of Cannabinoids in Neuroanatomic Alterations in Cannabis Users. *Biological Psychiatry*. Lorenzetti et al. (2016)

CB_2Rs are most abundantly located in immune cells and peripheral tissue (cardiovascular system, gastrointestinal tract, liver etc.; Mackie, 2005). CB_2Rs were previously thought to be an exclusively 'peripheral' cannabinoid receptor, however more recently CB_2Rs have been identified throughout the CNS, commonly in postsynaptic neuronal tissue, and are now thought to have neuroprotective properties (Mechoulam & Parker, 2013; Wu, 2019).

1.2.2 Endocannabinoid System and Exogenous Cannabinoids

Exogenous cannabinoid refers to the compounds extracted from herbal cannabis (phytocannabinoids including but not limited to THC and CBD), in addition to synthetic cannabinoids (Boggs et al., 2018). THC exerts its psychoactive effects via its action as a *partial agonist* for CB_1R and CB_2R (Mechoulam & Parker, 2013; Pertwee, 2008; Zou & Kumar, 2018), meaning that the receptors are activated, but with only partial efficacy relative to a full agonist. THC is one of only two phytocannabinoids which bind with a high affinity to CB_1R and CB_2R; the other being tetrahydrocannabivarin (THCV), CBD does not bind to either of these receptors (Di Marzo & Piscitelli, 2015).

1.2.2.1 Exogenous Cannabinoids and Addiction

CB_1Rs (as well as the endogenous ligands: anandamide and 2-AG) are abundant in dopaminergic pathways, including the striatum (Herkenham et al., 1991). THC alters the signalling of the endocannabinoid system; agonism of CB_1Rs has been shown to inhibit GABA and glutamate release from the presynaptic terminal (termed retrograde signalling; Gerdeman & Lovinger, 2001; Katona et al., 1999), which in turn modulates dopamine transmission (Bloomfield et al., 2016). Importantly, alterations to glutamate specifically are thought to contribute to the cycle of addiction due to the role that glutamate plays in mediating inhibitory control and drug-seeking behaviour (Kalivas, 2009). Dopamine, given its role in reward, motivation, and goal-directed behaviour, is also thought to play a key role in addiction related processes (Ferland & Hurd, 2020). Acute THC use in cannabis naïve individuals, has been shown to *increase* striatal dopamine release (Bossong et al., 2009; Gardner, 2005), which stems from its inhibition of glutamate, and is thought to be related to the rewarding effects of THC (Bloomfield et al., 2016). Contrastingly, THC use over time has been

shown to down regulate (suppress) CB_1R availability (Ceccarini et al., 2015; D'Souza et al., 2016; Hirvonen et al., 2012), and to *reduce* striatal dopamine synthesis (Bloomfield et al., 2016; Bloomfield et al., 2014). As frontal, temporal and striatal regions are innervated with dopamine projections, repeated THC exposure in cannabis users may alter dopaminergic transmission in these areas; and thus, alters their spontaneous blood oxygen level-dependent (BOLD) fluctuations (see section below *1.3.3 Functional Magnetic Resonance Imaging* for more on this; Meck, 2006; Paus, 2001; Volkow & Fowler, 2000), and the cognitive functions these regions are ascribed to e.g., disinhibition (Volkow et al., 2010).

1.3 Neuroimaging Evidence on the Neurobiology of Cannabis Exposure

1.3.1 A Brief Introduction to Neuroimaging

Magnetic Resonance Imaging (MRI) and functional Magnetic Resonance Imaging (fMRI) are imaging techniques which rely on 'magnetic resonance' to capture information about the brain and body. In MRI, this is achieved by applying pulses of magnetic field, which then enables detection of the direction of protons within the body. Under normal conditions these protons are aligned in a random direction, however when the magnetic field is applied, the protons align parallel to the direction of the main magnetic field, which creates magnetization of the affected tissue (Sanelli et al., 2016). This therefore enables the MRI to generate images based on the detected proton configuration. In fMRI, brain activity is measured by detecting changes in blood flow/oxygenation using the BOLD contrast method (Sanelli et al., 2016). This relies on the principle that there is increased blood flow to areas of neuronal activation (Huettel et al., 2014).

1.3.2 Preclinical Studies

Chronic use of THC has been associated with a host of neurobiological changes, which persist beyond the period of acute intoxication, also known as residual changes. Animal studies have shown that exposure to THC results in neurotoxic changes in brain regions which are known to be high in CB_1R, including the hippocampus, amygdala, and cerebral cortex (Chan et al., 1998; Downer et al., 2001). The first study to utilize resting-state functional connectivity (rsFC) in a preclinical study of

exposure to inhaled THC (for a description of rsFC see below, section 1.3.3.2), found that following one month of daily vaporised cannabis (containing THC) exposure in male mice, decreased and increased rsFC was observed (Coleman et al., 2022). Specifically, hypoconnectivity was reported in a network of regions including the hypothalamus, thalamus, and ascending reticula activating system, whilst hyperconnectivity was reported between the hippocampus and brainstem, linked to deficits in object recognition. Multimodal neuroimaging research, also examining mice following one month of daily exposure to vaporized cannabis (containing THC) revealed midbrain dopaminergic volume and grey matter volume decrease (Sadaka et al., 2023).

1.3.3 Volumetric Studies

MRI has been used to examine volumetric alterations in human studies, both on a whole brain level and exclusive to specific structures (Bloomfield et al., 2019). Whilst results in human studies are less unanimous in their findings than preclinical studies (Batalla et al., 2013), some overlap with the regions susceptible to THC related alterations in animals have been identified. Pattern of volumetric abnormalities in the hippocampus, specific cortical regions (e.g., orbitofrontal cortex and anterior cingulate cortex), and amygdala have been reported, as well as in the parahippocampus, NAc, and basal ganglia, (Ashtari et al., 2011; Chye et al., 2017; Demirakca et al., 2011; Filbey et al., 2015; Hill et al., 2016; Lorenzetti et al., 2010; Lorenzetti et al., 2015; Matochik et al., 2005; Moreno-Alcazar et al., 2018; Yücel et al., 2008). A number of theories have been proposed to explain the volumetric alterations associated with ongoing cannabis use, which include processes of neuroinflammation and/or gliosis (Moreno-Alcazar et al., 2018). Furthermore, it has been posited that volumetric alterations may be driven by a subset of cannabis users who meet the diagnostic criteria for Cannabis Use Disorder (CUD; American Psychiatric Association [APA], 2013; Chye et al., 2017; Lorenzetti & Cousijn, 2016; Lorenzetti et al., 2016).

1.3.4 Functional Magnetic Resonance Imaging

1.3.4.1 Task-Based Activation

Task-based fMRI examines the activation of unique brain regions whilst the individual completes specific tasks within the scanner, by measuring the BOLD signal, considered to be a proxy for neuronal function (Bandettini et al., 1992; Ogawa et al., 1990). Recent meta-analyses and review of task-based fMRI studies showed that cannabis users compared to controls demonstrated alterations in brain regions (i.e., basal ganglia, amygdala, and prefrontal cortex) associated with addiction-related cognitive functions (i.e., reward processing, executive function, and stress; Blest-Hopley et al., 2018; Yanes et al., 2018; Zehra et al., 2018) and across multiple brain networks, including the reward network, executive network, and habit and memory networks (Zilverstand et al., 2018).

A recent comprehensive review of neuroimaging studies in cannabis users (Bloomfield et al., 2019) identified links between alterations in the activation of specific brain regions and deficits in performance on certain cognitive tasks. This includes executive dysfunction linked to the orbitofrontal cortex, insula, and superior temporal gyrus (Cousijn et al., 2013), reduced attention and working memory deficits linked to the prefrontal cortex (Abdullaev et al., 2010; Becker et al., 2010b; Chang et al., 2006; Colizzi et al., 2015; Jager et al., 2010; Kanayama et al., 2004; Tervo-Clemmens et al., 2018), and impaired inhibition linked to the anterior cingulate cortex (Gruber & Yurgelun-Todd, 2005; Hester et al., 2009). Reductions in the performance of chronic cannabis users in tasks of learning and memory were also identified, with links to hippocampal and anterior cingulate cortex hypoactivation, and parahippocampal and medial temporal lobe dysfunction (Becker et al., 2010a; Carey et al., 2015; Jager et al., 2007; Nestor et al., 2008; Riba et al., 2015; Sneider et al., 2013). Finally, during various tasks of reward processing, multiple studies identified either hyperactivity or blunting throughout the striatum (Enzi et al., 2015; Jager et al., 2013; Martz et al., 2016; Nestor et al., 2010; van Hell et al., 2010; Yip et al., 2014), the inferior frontal gyrus (Enzi et al., 2015), and the insula (Nestor et al., 2010).

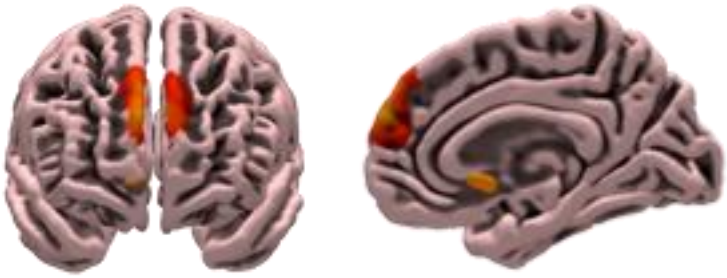
Of note however, as these studies have focused primarily on task-based fMRI, one is limited in interpreting whether group differences reflect underlying cannabis-specific brain function alterations or cognitive confounds such as fluctuations in task performance, strategy, effort, and

adaptation (Fox & Greicius, 2010; Philippi et al., 2020). To circumvent and minimise such confounds, specific imaging methodologies can be used, including rsFC fMRI.

1.3.4.2 Resting-State Functional Connectivity

The seminal paper on functional connectivity using fMRI was published in 1995 (Biswal et al., 1995). rsFC is a putative measure of brain integrity, used to explore the intrinsic organisation of the brain, and can be used as an alternative to task-based fMRI. rsFC techniques can be used to provide evidence of alterations of communication between and within widespread brain circuits. rsFC measures spontaneous fluctuations of brain function (via BOLD signal) in the absence of overt task performance, whilst the participant remains at rest but awake in the scanner (Greicius et al., 2008; van de Ven et al., 2004). Importantly, participants do not engage in a task during this time period, therefore largely eliminating specific cognitive demands. The BOLD signal of functionally distinct and, often, spatially remote brain regions is measured and those which correlate are considered to be functionally connected; please see Figure 1.8 for an example of a rsFC pairing, demonstrating rsFC between the NAc and frontal pole. A distinction is made between positive and negative (anti-) correlations. Positive correlation indicates an increase *or* decrease to the BOLD signal of two regions simultaneously, whereas a negative (or anti-) correlation indicates an increase in the BOLD signal to one area, with a simultaneous decrease to another (van de Ven et al., 2004). rsFC fMRI techniques have been widely applied in healthy and clinical populations (Lv et al., 2018).

Figure 1.8. *An example of a resting-state functional connectivity pairing*



Note: Left is an anterior view; right is an interior sagittal view of the right hemisphere. This figure visually represents resting-state functional connectivity between the right nucleus accumbens and bilateral frontal pole.
Source: excerpt taken from *Chapter 4* of this thesis; section 4.4.2.2 Seed-Based Functional Connectivity.

1.3.4.2.1 rsFC in Normative and Clinical Samples. rsFC patterns in normative samples have indicated that coherent intrinsic brain activity is a key feature of healthy brain function (Fox & Raichle, 2007; van den Heuvel et al., 2009). rsFC has been used to identify the brain's functional architecture in normative samples and fundamental alterations underlying disease. Moreover, rsFC has been used to map how brain integrity changes as a function of fundamental processes such as aging (Ferreira & Busatto, 2013). Patterns of rsFC have been shown to differ between control groups and clinical populations (e.g. Alzheimer's and Parkinson's disease) and various psychopathologies including depression, schizophrenia, and bipolar disorder (Badhwar et al., 2017; Kuhn & Gallinat, 2013; Li et al., 2019; Vargas et al., 2013; Wang et al., 2012; Wolters et al., 2019). The repeated establishment of rsFC relationships with clinical symptoms demonstrates its utility in identifying potential biomarkers or treatment targets of specific conditions.

1.3.4.2.2 rsFC in Substance Users. Distinct patterns of rsFC have also been observed when comparing samples with and without substance use/addiction disorders, including cocaine dependence (Zhang & Li, 2018), and amphetamine use (Anouk Schrantee et al., 2016; A. Schrantee et al., 2016). rsFC patterns have shown utility in predicting the likelihood of relapse in both abstinent cocaine users

and alcohol dependent individuals (Adinoff et al., 2015; Berlingeri et al., 2017; McHugh et al., 2013; Zakiniaeiz et al., 2017). Recent meta-analyses examining patterns of network abnormalities in the rsFC of various substance use disorders identified a pattern of rsFC hyperconnectivity in the putamen, caudate, and middle frontal gyrus (Tolomeo & Yu, 2022), as well as alterations to the limbic, salience, and frontoparietal networks (Taebi et al., 2022) relative to healthy controls. rsFC has consistently been used to identify alterations to frontostriatal pathways across a variety of substance use disorders, including opioids, nicotine, alcohol, and mixed substances or combined samples (Hong et al., 2009; Klugah-Brown et al., 2020; Luciana, 2020; Ma et al., 2010; Motzkin et al., 2014; Sullivan & Pfefferbaum, 2019).

1.3.4.2.3 rsFC in Cannabis Users. The use of rsFC to detect change in regular cannabis users has only occurred across the past decade; the earliest published paper appearing in 2013 (Houck et al., 2013). Evidence from rsFC fMRI studies comparing regular cannabis users and controls supports functional alterations in frontostriatal region pairings (i.e., anterior cingulate cortex/middle frontal gyrus with putamen/caudate/basal ganglia; Blanco-Hinojo et al., 2017). Frontostriatal projections are important for cognitive and behavioural flexibility implicated in goal-directed behaviour (Morris et al., 2016; Vaghi et al., 2017) and inhibitory control (Ersche et al., 2020; Morein-Zamir & Robbins, 2015) underlying addiction. Overlapping brain regions have also been implicated in addiction neurocircuitry (Koob & Volkow, 2010; Volkow et al., 2016) and may inform mechanisms involved in the transition to a maintenance of CUD, as well as potentially informing novel treatment targets to prevent relapse. Specifically, these regions include striatal regions (i.e., NAc, putamen, pallidum, caudate), medial temporal regions (i.e., hippocampus, amygdala), and key cortical regions (i.e., ACC, and precentral gyrus). However, the body of work has thus far revealed heterogeneous results, with alterations of distinct measures of rsFC (i.e., *positive rsFC* and *negative rsFC*), and inconsistent strength (i.e., *higher* or *lower*), as well as the reported location of group differences. rsFC alterations in cannabis users have been preliminarily associated with greater chronicity of cannabis use, worse mental health symptoms, and poorer cognitive ability.

A recent Systematic Literature Review (SLR) examining individuals who regularly use cannabis specifically in comparison to people who do not, identified that cannabis users typically

displayed greater positive rsFC in frontal-frontal, frontotemporal, and frontostriatal region pairings, implicated in disinhibition and reward processing (Thomson et al., 2022); as presented in *Chapter 2*. It was posited that the functional connectivity disturbance identified in regular cannabis users may be secondary to chronic THC exposure (Thomson et al., 2022). Chronic THC exposure, known to result in alterations to dopaminergic transmission, may therefore alter the spontaneous BOLD fluctuations in the identified brain regions (Meck, 2006; Paus, 2001; Volkow & Fowler, 2000), and associated cognitive functions (Volkow et al., 2010). Patterns of the correlations between rsFC alterations and cannabis use level also indicated that greater chronicity of use may drive identified alterations (Thomson et al., 2022).

Importantly, no research to date utilising rsFC in non-intoxicated people who regularly use cannabis examined if the participants in their sample endorsed a Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5**;** APA, 2013) diagnosis of a CUD (see Figure 1.9 for diagnostic criteria), creating a major gap between the current system of diagnosis and the existing literature. The most common classification system used in past research examining regular cannabis users was from the DSM-IV i.e., 'cannabis dependence'. The DSM-5 introduced a dimensional approach (i.e., degrees of severity) rather than a categorical approach employed by the DSM-IV (i.e., dependence present; yes or no). Thus, the fourth and fifth edition of the DSM lack full agreement (Livne et al., 2021), and that the evidence in the published literature on cannabis dependence does not translate to the new diagnostic criteria for a CUD. It remains unclear if identified changes to rsFC in regular cannabis users are driven by individuals with a CUD alone, or by both CUD and recreational users. As postulated by prominent addiction theories, neuroadaptations characterise the transition from recreational/occasional use to dependent use (Koob & Volkow, 2010; Volkow, Koob, et al., 2016).

Figure 1.9. *The Diagnostic Criteria for Cannabis Use Disorder. Excerpt taken from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*

DSM-5 Cannabis Use Disorder (CUD) Diagnostic Criteria

A problematic pattern of cannabis use leading to clinically significant impairment or distress, as manifested by **at least two of the following**, occurring within a 12-month period:

1. Cannabis is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control cannabis use.
3. A great deal of time is spent in activities necessary to obtain cannabis, use cannabis, or recover from its effects.
4. Craving, or a strong desire or urge to use cannabis.
5. Recurrent cannabis use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued cannabis use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of cannabis.
7. Important social, occupational, or recreational activities are given up or reduced because of cannabis use.
8. Recurrent cannabis use in situations in which it is physically hazardous.
9. Cannabis use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by cannabis.
10. Tolerance, as defined by either of the following:
    a. A need for markedly increased amounts of cannabis to achieve intoxication or desired effect.
    b. Markedly diminished effect with continued use of the same amount of cannabis.
11. Withdrawal, as manifested by either of the following:
    a. The characteristic withdrawal syndrome for cannabis.
    b. Cannabis (or a closely related substance) is taken to relieve or avoid withdrawal symptoms.

Specify if:
In early remission: After full criteria for cannabis use disorder were previously met, none of the criteria for cannabis use disorder have been met for at least 3 months but for less than 12 months (with the exception that Criterion A4, "Craving, or a strong de-sire or urge to use cannabis," may be met).
In sustained remission: After full criteria for cannabis use disorder were previously met, none of the criteria for cannabis use disorder have been met at any time during a period of 12 months or longer (with the exception that Criterion A4, "Craving, or a strong desire or urge to use cannabis," may be present).
Specify if:
In a controlled environment: This additional specifier is used if the individual is in an environment where access to cannabis is restricted.
Specify current severity*:*
305.20 (f12.10) Mild: Presence of 2-3 symptoms
305.30 (f12.20) Moderate: Presence of 4-5 symptoms
305.30 (f12.20) Severe: Presence of 6 or more symptoms

Note. Reprinted with permission from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (Copyright © 2013). American Psychiatric Association. All Rights Reserved.
Please see *Appendix 1* for confirmation of permission to reprint.

1.4 Cannabis Use Disorder

Of individuals who use cannabis with some regularity, it has been estimated that between 20% (Leung et al., 2020) and 30% (Hasin et al., 2015; Marel et al., 2019) will go on to develop a CUD. CUD, defined in the DSM-5 (APA, 2013) is characterised by cognitive, behavioural, and physiological changes. Individuals who meet diagnosis exhibit an inability to voluntarily cease consumption of cannabis, despite an underlying desire to do so, and/or in the face of related physical or psychological harms (APA, 2013; World Health Organisation [WHO], 2018). The severity of the disorder is determined by the number of diagnostic criteria met by the individual. Endorsement of 2-3 criteria equates to a 'mild' disorder, 4-5 equates to a moderate disorder, and 6 or more equates to a severe disorder. The frequency at which one uses cannabis has been established as the strongest risk factor for development of a CUD, especially in conjunction with duration of use (i.e., heavy cannabis use, particularly over an extended period of time, increases the likelihood that one will endorse a higher number of CUD symptoms; Binkowska et al., 2022; Curran et al., 2019).

As mentioned above (section *1.1.3 Current Perceptions of Cannabis*), public opinion maintains a consensus that cannabis is a 'low risk' substance. Regular use however remains associated with adverse psychosocial and health outcomes (Hall & Degenhardt, 2009; Volkow et al., 2014) that includes reduced cognitive ability (Solowij & Battisti, 2008; Volkow, Swanson, et al., 2016), engagement in risk-taking behaviour (e.g. smoking while driving; AIHW, 2020), increased use of other illicit substances (Volkow et al., 2014), and increased frequency of mood and psychotic disorders including psychosis, addiction, depression, suicidality, and amotivation (Hall & Degenhardt, 2009; Lev-Ran et al., 2014). Many of the negative outcomes associated with regular cannabis use (including increased antisocial behaviour, use of other illicit substances, legal trouble, unemployment, and mood disorders [depression and anxiety]) have been shown to be significantly worse for regular users who *do* meet diagnostic criteria for CUD than for regular users who *do not* (Foster et al., 2018; van der Pol, Liebregts, de Graaf, Ten Have, et al., 2013). For this reason, it is important to explore the underpinnings of CUD, in order to best develop treatment targets for individuals most impacted, rather than 'recreational' users more broadly.

1.4.1 Costs and Required Supports

Broadly, the burden of cannabis use is felt in healthcare settings, where the largest health related costs of CUD are estimated to be for out-of-hospital care (i.e., primary care and specialist drug treatment services; Whetton et al., 2020). As rates of CUD increase, demand for efficacious CUD treatments have also risen (UNODC, 2019; WHO, 2016). However, it has been estimated that at least 85% of individuals with a CUD do not seek treatment (Hasin et al., 2016) or inversely – only 8% of adults with a CUD reported receiving recent cannabis-specific treatment (Wu et al., 2017). Taken together, this demonstrates the need for readily available and easily accessible treatment modalities.

1.4.2 Treatment

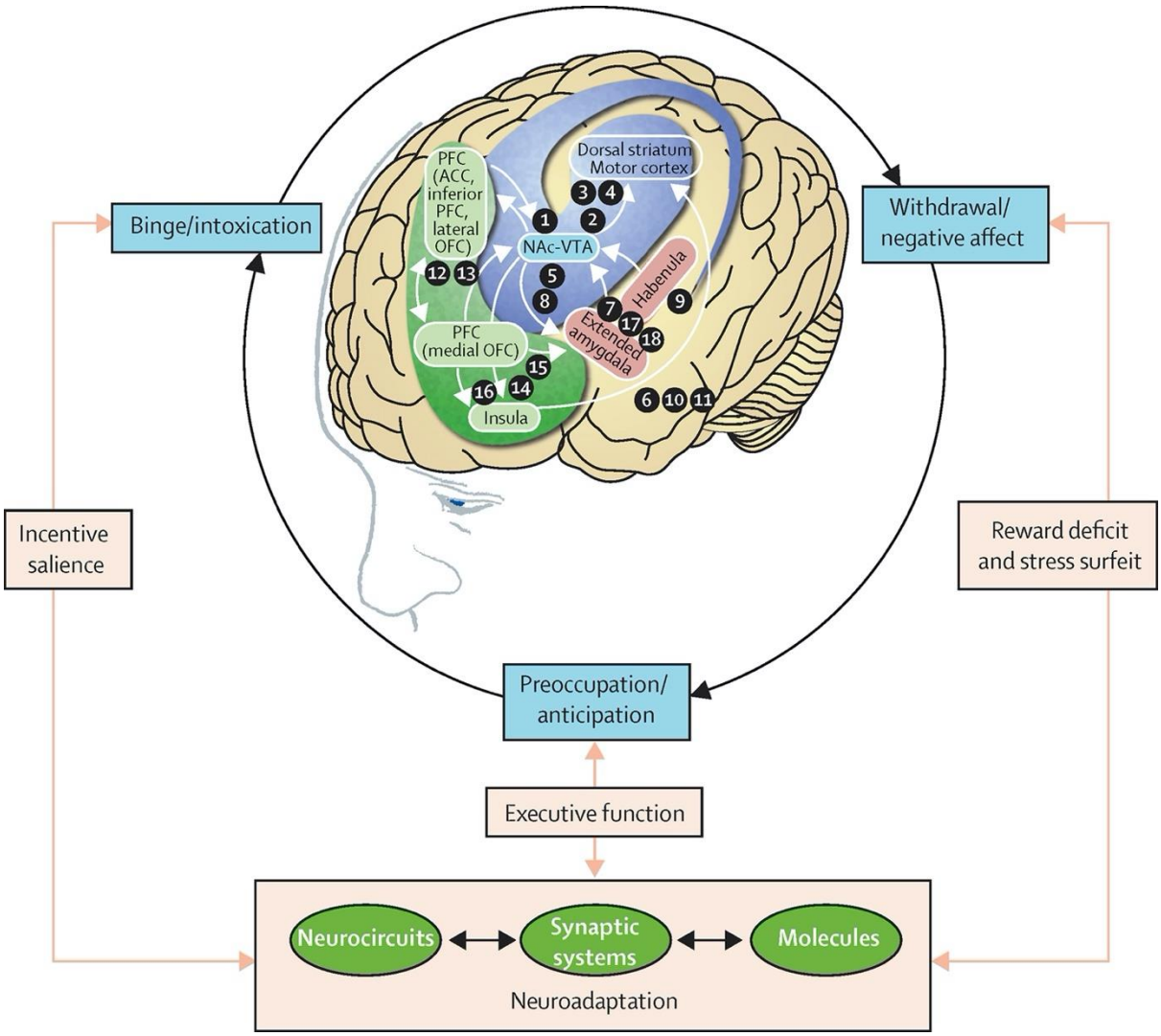
At present, face-to-face psychotherapies (including cognitive behavioural therapy [CBT], motivational enhancement therapy [MET], and contingency management [CM]) have been shown to most effectively treat CUD (i.e., associations with reduced symptomatology, reduced cannabis use, increased cannabis abstinence; Gates et al., 2016; Lees et al., 2021), with the majority of pharmacotherapies thought to be ineffective (Bahji et al., 2021; Kondo et al., 2020; Nielsen et al., 2019). Effective psychotherapies are often lengthy, typically running between 1 and 6 months, and can be prohibitively expensive for individuals with a CUD (Lees et al., 2021). Additionally, poor treatment availability and admission difficulties have been cited as two of the major barriers to treatment seeking (Rapp et al., 2006). It has also been suggested that digitally facilitated interventions may appeal to non-treatment seeking individuals with a CUD, due to a reported desire to be self-reliant and a preference for informal services (van der Pol, Liebregts, de Graaf, Korf, et al., 2013).

1.4.3 Cannabis Use Disorder in the Context of Neurobiological Theory of Addiction

The neurobiology of substance use disorders has been explored in relation to a prominent neuroscientific theory of addiction, first proposed by Koob and Volkow (2010). The Koob and Volkow (2010) neuroscientific theory of addiction postulates that drug addiction is a “chronically relapsing disorder”, driven by neural changes underlying three stages: (1) the binge/intoxication stage, (2) the withdrawal/negative affect stage, and (3) the preoccupation/anticipation stage (Koob &

Volkow, 2016; Volkow et al., 2016; Volkow et al., 2019). Figure 1.10 contains a visual depiction of the neurocircuitry underlying these three stages (Koob & Volkow, 2016).

Figure 1.10. *The Three Stage Neuroscientific Model of Addiction, Koob and Volkow (2010)*



Note: The overall neurocircuitry domains correspond to three functional domains: binge/intoxication (reward and incentive salience: basal ganglia [blue]), withdrawal/negative affect (negative emotional states and stress: extended amygdala and habenula [red]), and preoccupation/anticipation (craving, impulsivity, and executive function: PFC, insula, and allocortex [green]).
ACC = anterior cingulate cortex; NAc = nucleus accumbens; OFC = orbitofrontal cortex; PFC = prefrontal cortex; VTA= ventral tegmental area.
Source: Neurobiology of Addiction: A Neurocircuitry Analysis. *Lancet Psychiatry.* Koob and Volkow (2016). Modified from Koob and Volkow (2010)

The three stages within the Koob and Volkow (2010) neurobiological theory of addiction are thought to be characterised by disturbances in three major neural networks, driven by exposure to substances and related behavioural changes. Specifically, the binge/intoxication stage, which is characterised by impulsivity/compulsivity to use substances, involves the basal ganglia and mesocorticolimbic dopamine reward pathway. The withdrawal/negative affect stage, individuals demonstrate a loss of motivation towards non-substance rewards, plus impaired emotion regulation, is thought to be driven by the extended amygdala, the NAc, and the caudate and putamen. Finally, the preoccupation/anticipation stage, which is marked by experiences of substance cravings and impaired behavioural inhibition, is driven by the PFC. Koob and Volkow (2016) have further identified 18 subsystems demonstrating neuroadaptations associated with disordered substance use, which includes the mesocorticolimbic dopamine system. Neurobiological changes underpinning the three stages of addiction are thought to promote habit formation and drive pathological drug seeking, via promotion of incentive salience (of previously neutral stimuli) and dysregulation of reward and stress function (Koob & Volkow, 2016; Volkow et al., 2016; Volkow et al., 2019).

Research exploring how the three-stage neurobiological theory of addiction applies to cannabis and CUD has been thoroughly synthesized in a previous review (Zehra et al., 2018). Following an extensive review of studies examining the acute and long-term effects of cannabis, it was concluded that CUD largely adhered to changes reported within Koob and Volkow model, however the identified features of CUD within each stage were less robust than other substances of abuse. Importantly, as described above in section *1.3.3.2.3 rsFC in Cannabis Users*, frontostriatal dysfunction has been reported in association with ongoing use of cannabis, which is in line with neurobiological changes reported in the 'binge/intoxication' stage of addiction. Furthermore, chronic cannabis use was associated with symptoms of cravings (a link to the preoccupation/anticipation stage), and with withdrawal and affect dysregulation (in line with the withdrawal/negative affect stage). As put forward by the authors (Zehra et al., 2018), the findings of the review demonstrated an urgent need for future research which further explores the neurobiological changes associated with CUD, to increase understanding of how CUD fits within this prominent addiction theory.

1.5 Mindfulness Based Interventions

One promising treatment modality, readily adaptable for cost-effective, and digital/remote delivery (Garrison et al., 2020; Kamboj et al., 2017), is grounded in the practice of mindfulness. Mindfulness, rooted in Buddhist traditions, has received increasing attention in Western research and medicine over recent decades (see Goldberg et al. (2022) and Zhang et al. (2021) for review). Mindfulness has been defined as "the awareness that emerges through paying attention on purpose, in the present moment, and non-judgmentally to the unfolding of experiences moment by moment" (Kabat-Zinn, 1991). Two of the key practices encompassed by a variety of mindfulness-based interventions (MBIs) include 'focused attention' and 'open monitoring' (Sezer et al., 2022). Focused attention primarily consists of the process of focusing one's attention on a chosen stimulus, be it internal (i.e., the breath or heart rate) or external (i.e., a ticking clock or burning candle). When the individual becomes aware that their thoughts have wandered, they purposefully redirect attention back to the chosen stimulus. Open monitoring entails a process of meta-awareness of the present-moment, primarily focused on thoughts, feelings, emotions, and bodily sensations. Via this process of meta-awareness, the individual is encouraged to accept their inner state without judgement (Lutz et al., 2015). Many MBIs emphasise either one practice or the other, or alternatively consist of a combination of the two (i.e., the object of the focused attention becomes the meta-awareness of the present-moment).

One of the most widely applied MBIs is a Mindfulness-Based Stress Reduction (MBSR) training program, first introduced by Dr Jon Kabat-Zinn in 1979 (Kabat-Zinn, 2003). Whilst effective, this program and many similar others are limited by lengthy durations (multiple months) and associated expenses. Irrespective, MBIs have been linked to mental and physical health benefits in both clinical and non-clinical samples, including in areas of improved cognitive processes, stress-management, social cognition, and general well-being (Campanella et al., 2014; Campos et al., 2019; Chiesa et al., 2011; Chiesa & Serretti, 2009; Gallant, 2016; Howell et al., 2008; Malinowski, 2013; Smith et al., 2015; Tan et al., 2014). Furthermore, MBIs have been linked to reduced symptoms severity in anxiety, post-traumatic stress disorder, attention-deficit/hyperactivity disorder, eating

disorder, and major depressive disorder (Boyd et al., 2018; Hofmann et al., 2010; Piet & Hougaard, 2011; Poissant et al., 2019; Wanden-Berghe et al., 2011).

1.5.1 Mindfulness-Based Intervention Treatment

1.5.1.1 Of Substance Use Disorders

In the context of addiction, it has been posited that mindfulness practice raises awareness of and subsequent control over cravings, affect, and behaviour (Brewer et al., 2013). MBIs are thought to increase control over habitual behaviours, in addition to increasing attention and responsivity to natural rewards (Garland et al., 2014). Moreover, MBIs are thought to facilitate the ability to 'ride out' or 'surf' the urge to use a substance, by bringing one's attention to the experience of the present moment (Korecki et al., 2020). By 'observing' rather than 'reacting to' aversive body/mind states (such as cravings), MBI can foster the replacement of habitual reactions with adaptive responses (Houlihan & Brewer, 2016). It has been suggested that MBIs may restore natural reward processes among substance addicted individuals (Garland et al., 2014) and may have potential to target the neurobiological mechanisms associated with substance use disorders (Kirlic et al., 2021).

Interest in MBIs as treatment for substance use disorders has grown exponentially in recent years. To date, at least 11 SLRs/meta-analyses have been published examining the efficacy of various MBIs in the treatment of substance use disorders (Cavicchioli et al., 2018; Chiesa & Serretti, 2014; Grant et al., 2017; Katz & Toner, 2013; Korecki et al., 2020; Li et al., 2017; Priddy et al., 2018; Ramadas et al., 2021; Sancho et al., 2018; Zgierska et al., 2009), including one Cochrane review (Goldberg et al., 2021). All but one review (Grant et al., 2017) largely supported their utility; of note the highly rigorous Cochrane review concluded that whilst more evidence supporting the utility of MBI is required, MBIs are tentatively efficacious in treatment of substance use disorder (Goldberg et al., 2021). Notable observations included MBI's utility in reducing substance cravings, decreasing frequency of use, and improving depressive symptoms (Cavicchioli et al., 2018; Chiesa & Serretti, 2014; Korecki et al., 2020; Ramadas et al., 2021). The majority of interventions reviewed examined within the identified SLRs involved lengthy, face-to-face programs, indicating limited availability of

and research into *brief* MBI. Furthermore, within the published reviews, only one included paper examined CUD specifically (de Dios et al., 2012).

1.5.1.2 Of Cannabis Use Disorder

Despite the surge of research in recent decades examining MBIs, there remains a dearth of research examining MBIs which focus on CUD (with only one pilot RCT uncovered; de Dios et al., 2012). de Dios et al. (2012) found preliminary evidence for the feasibility and efficacy of two face-to-face sessions of MBI (in conjunction with motivational interviewing), delivered to women with a CUD. Specifically, participants decreased their days of cannabis use per month (reduced by ~6 days at 1-month-follow-up, ~8 days at 2-month-follow-up, and ~7 days at 3-month-follow-up), compared to participants in the control intervention (reduced by ~1 day at 1-month-follow-up, and increased their use at 2- and 3-month-fillow-up).

As called for by van der Pol, Liebregts, de Graaf, Korf, et al. (2013), individuals with CUD may benefit from digitally facilitated interventions, due to a reported desire to be self-reliant and a preference for informal services. The utility of brief (5-15 minutes per day; 1-3 weeks) and remotely delivered (smart phone-based or written) MBI has been tentatively established, in both cigarette smokers (Garrison et al., 2020) and heavy drinkers (Kamboj et al., 2017). Findings demonstrated preliminarily that brief MBI could lessen the association between substance use and cravings (Garrison et al., 2020), and reduce the amount of the substance used (Kamboj et al., 2017). To validate the efficaciousness of brief MBI in CUD, and gain a greater understanding of associated neurobiological underpinnings, further investigation is required.

1.5.2 Neurobiological Underpinnings of Mindfulness Based Interventions

The neurobiological underpinnings of a variety of MBIs have been investigated in mindfulness-naïve, ‘healthy’ participants, using rsFC. MBIs are thought to integrate multiple neurological systems, which regulate attention, working memory, and emotion (Hölzel et al., 2011). Individuals who completed 8-week, intensive programs including weekly group sessions and daily home practice demonstrated increased rsFC following the MBI in auditory and visual networks, and between regions associated with attentional processes and their respective sensory network

(Kilpatrick et al., 2011). It was concluded that increased rsFC between these networks was indicative of enhanced sensory processing and better attentional resource allocation with more consistent attentional focus (Kilpatrick et al., 2011). A later study utilising a similar 8-week MBI, reported rsFC alterations following MBI in executive networks (posterior cingulate cortex [PCC] with dorsolateral prefrontal cortex [dlPFC]), associated with self-reports of improved attention including decreased mind-wandering (Kral et al., 2019).

Two additional studies found increased rsFC between the anterior cingulate cortex (ACC) and dorsomedial prefrontal cortex (dmPFC) following MBIs of varying durations; 40 days (Yang et al., 2016) and 4 (intensive) days (Kwak et al., 2019). The increased rsFC between the ACC and dmPFC was thought to be linked to increased resilience (Kwak et al., 2019) and reduced depression/anxiety scores (Yang et al., 2016). Additionally, parallels have been drawn between rsFC associated with MBI in mindfulness-naïve, healthy populations, and rsFC alterations specific to trait mindfulness (Sezer et al., 2022). Taken together, these results demonstrate the utility of MBIs (of varying durations and intensities) to target brain networks implicated in attentional control, and emotion regulation, and may be linked to antidepressant and anxiolytic outcomes.

1.5.2.1 Neurobiological Underpinnings of Mindfulness Based Interventions in Substance Use

As described above in section *1.4.3 Cannabis Use Disorder in the context of Neurobiological Theory of Addiction,* there are a set of neurobiological alterations consistently observed in conjunction with substance use disorders (Koob & Volkow, 2010), however few interventions have specifically targeted this addiction neurocircuitry. It has been proposed that MBIs have potential to target dysregulated neurocognitive processes which underlie disordered substance use (Garland et al., 2014; Houlihan & Brewer, 2016). As reviewed above in section *1.5.1.1 Mindfulness-Based Intervention Treatment of Substance Use Disorders*, there is a steadily increasing body of evidence demonstrating therapeutic effects following MBI in populations with substance use disorders, however the neurobiological underpinnings of these MBIs remain underexplored. Garland et al. (2014) have proposed a framework by which MBIs may affect neurobiological change by strengthening of functional connectivity (i) within a metacognitive attentional control network (including the PFC, ACC, and parietal regions) and (ii) between the metacognitive attentional control network with habit,

craving, and affect circuits. It is thought that this process is facilitated by disengaging attention from substance-related stimuli, increasing meta-cognitive awareness to de-automate substance seeking behaviours, and improving 'top-down' cognitive control processes. Furthermore, MBIs have been proposed to restore natural reward processes, via neuroplastic alterations of frontostriatal-limbic circuitry.

The neurobiological underpinnings of MBI when treating substance use disorder remain poorly understood, with only 7 studies identified to date which explore MBI (against a control condition) using a variety of fMRI techniques (Fahmy et al., 2019; Froeliger et al., 2017; Janes et al., 2019; Kober et al., 2017; Kragel et al., 2019; Tang et al., 2013; Westbrook et al., 2011). A recent SLR which reviewed this body of work (Lorenzetti et al., *under review*) noted neurobiological changes in pathways relevant for mindfulness and reward processing. On the whole, the reviewed MBIs were effective in reducing substance use (with which brain functional changes were associated) and in reducing substance cravings. Of interest, two of the identified studies utilised rsFC; one in nicotine users (Froeliger et al., 2017) and one in opioid users (Fahmy et al., 2019).

Research examining rsFC in conjunction with MBIs in other substance use disorders has shown promising results (Fahmy et al., 2019; Froeliger et al., 2017). Nicotine dependent users in an MBI condition versus no intervention displayed increased rsFC between the rostral ACC and the orbital frontal cortex (OFC), correlated with smoking reduction (Froeliger et al., 2017). Opioid dependent users in an MBI condition versus treatment as usual (Fahmy et al., 2019) displayed a reduction in rsFC between the superior frontal gyrus (SFG) and the anterior default mode network (DMN; a functional brain network, typically shown to exhibit greater activity at rest or during internally directed/self-related cognition than when engaged in a task; Zhang & Volkow, 2019). The weakening of rsFC between the SFG and anterior DMN was correlated with an increase in subjective mindfulness (Fahmy et al., 2019). These preliminary findings require further investigation and replication however, as studies were limited by small sample sizes (N=13; Froeliger et al., 2017; N=28; Fahmy et al., 2019). Although it has been suggested that MBI may target the neurobiological mechanisms associated with substance use disorders more broadly (Kirlic et al., 2021), the effect that MBI may have on the neurobiological underpinnings of CUD remains to be elucidated.

1.6 Overview Limitations from Literature Examining rsFC in Regular Cannabis Users

1.6.1 Pertaining to Resting-State Functional Connectivity Research in Regular Cannabis Users

Prior to the commencement of this thesis, the body of literature comparing rsFC using fMRI of regular cannabis users versus controls was yet to by synthesised (see *Chapter 2* for the resultant SLR). Previously, the literature had thus far revealed heterogeneous results, with alterations of distinct measures of rsFC (i.e., positive rsFC and negative (or anti-) rsFC, as well as inconsistent strength (higher or lower than controls), and location of group differences. rsFC alterations in cannabis users had been preliminarily associated with greater chronicity of cannabis use (Behan et al., 2014; Cheng et al., 2014; Lopez-Larson et al., 2015), worse mental health symptoms (Shollenbarger et al., 2019; Subramaniam et al., 2018) and poorer cognitive ability (Pujol et al., 2014), however up until the commencement of this thesis, this correlational evidence was yet to be systematically integrated. It remained unclear whether altered rsFC in cannabis users was driven by cannabis users with more chronic use, increased mental health symptoms, and/or reduced cognitive performance.

1.6.2 Pertaining to Resting-State Functional Connectivity Research in Cannabis Use Disorder

At present, there are no studies published which examine rsFC alterations associated with CUD versus controls, utilising the application of the current DSM-5 diagnostic criteria for CUD. This creates a major gap between the published body of literature, and the individuals who are currently meeting the diagnosis. Within the body of research examining regular cannabis users, inconsistent cannabis use metrics and cut offs were applied to determine cannabis user group inclusion (ranging from weekly- to daily-use, with only a third of published studies enforcing a DSM-IV cannabis use dependency criteria; Thomson et al., 2022 [*Chapter 2*]). Furthermore, a number of studies used samples who were abstinent from cannabis at the time of data collection. The interpretation of whether specific cannabis user groups show distinct differences was therefore hindered (e.g., are differences driven by presence of CUD; if so, there may be a ‘wash out’ effect of the inclusion of studies which do not enforce this diagnostic criteria).

Across the body of literature examining rsFC in regular cannabis users, heterogeneous rsFC methodologies have been applied: i.e., a combination of data- and hypothesis-driven approaches. It

has been recommended that in order to effectively build upon the current literature, a hypothesis-driven approach be implemented, based upon the findings of the literature as it stands (Thomson et al., 2022 [*Chapter 2*]). Finally, within the field of research, over half of the published studies used small sample sizes (i.e., less than 30 per group), meaning that the literature may be underpowered to detect subtle but significant differences.

1.6.3 Pertaining to Resting-State Functional Connectivity Research Investigating Brief Mindfulness Based Intervention for Cannabis Use Disorder

At present, there have been no studies published which examine the neurobiological underpinnings (utilising rsFC or other) of a brief MBI for individuals with a CUD. As such, the utility of MBI in treatment of CUD, especially as it relates to neurobiological alterations and neurobiological treatment targets, remains poorly understood. Comparable extant studies which have examined the neurobiological underpinnings of MBIs in other substance use disorders (nicotine and opioids), have demonstrated promising results, which should therefore be replicated in individuals with a CUD (Fahmy et al., 2019; Froeliger et al., 2017). Additionally, this emerging field has largely utilised small samples (N=18-28), reducing the statistical power to detect effects.

1.7 Overall Objectives and Summary of the Thesis

This thesis aimed to map the neurobiological underpinning (using rsFC in fMRI) associated with cannabis use, initially in regular cannabis users within the existing literature, and then more specifically within a sample of individuals who meet the diagnostic criteria for moderate-to-severe CUD. Additionally, this thesis aimed to explore the potential mitigation or targeting of observed rsFC alterations by way of the implementation of a brief MBI. Therefore, three studies were to be completed.

First, an SLR (Study 1) was conducted which examined the field to date, of research which utilises rsFC to map differences associated with regular cannabis use, compared to controls. Results from the SLR were then used as the basis for the hypotheses of the next study (Study 2), a comparison of the rsFC of individuals who meet current DMS-5 diagnosis for CUD compared to controls.

Identified group differences were then used as treatment targets in the third and final study (Study 3), which examined the rsFC before and after a brief MBI (compared to *active placebo* [relaxation] and *passive placebo* [no intervention]), completed by the same group of individuals with moderate-to-severe CUD.

The integration of the identification of rsFC alterations (associated with regular cannabis use, with CUD, and with brief MBI in CUD), along with behavioural correlates, is hoped to aid in a deeper understanding of the potential neurobiological effects of cannabis. It is also hoped to further the understanding of a novel and easily administered intervention (which is also cost and time effective). This is in order to benefit individuals from the general community with a moderate-to-severe CUD, as well as to benefit the broader community which at present, bears the majority of the cost associated with cannabis within a treatment / healthcare setting.

1.7.1 Overview of Study Aims

1.7.1.1 Study 1: Systematic Literature Review

- Aim 1. The primary aim of the SLR was to summarise the findings to date on rsFC differences between regular cannabis users and controls.
- Aim 2. The secondary aim of the SLR was to systematically synthesise the evidence on the associations between rsFC in cannabis users and cannabis use levels (e.g., duration, dosage), cognitive performance (e.g., executive function), and mental health symptoms (e.g., depression), to shed light on whether specific subgroups of cannabis users may be more vulnerable to greater alterations of brain function in the absence of overt task performance.

1.7.1.2 Study 2: Cannabis Users vs Controls

- Aim 1: To compare rsFC for the first time between people with a diagnosis of moderate-to-severe CUD and who had recently tried to cut down or quit cannabis and non-cannabis-using controls, whilst accounting for age, sex, and variables that differed between the two groups (i.e., alcohol and nicotine exposure, and depression symptom scores).

- Aim 2: To explore how rsFC differences identified in the CUD group vs controls would be associated with cannabis use exposure and related behaviours.

1.7.1.3 Study 3: Pre-to-Post Brief MBI (& *Active* & *Passive Placebo* Controlled Intervention) in CUD

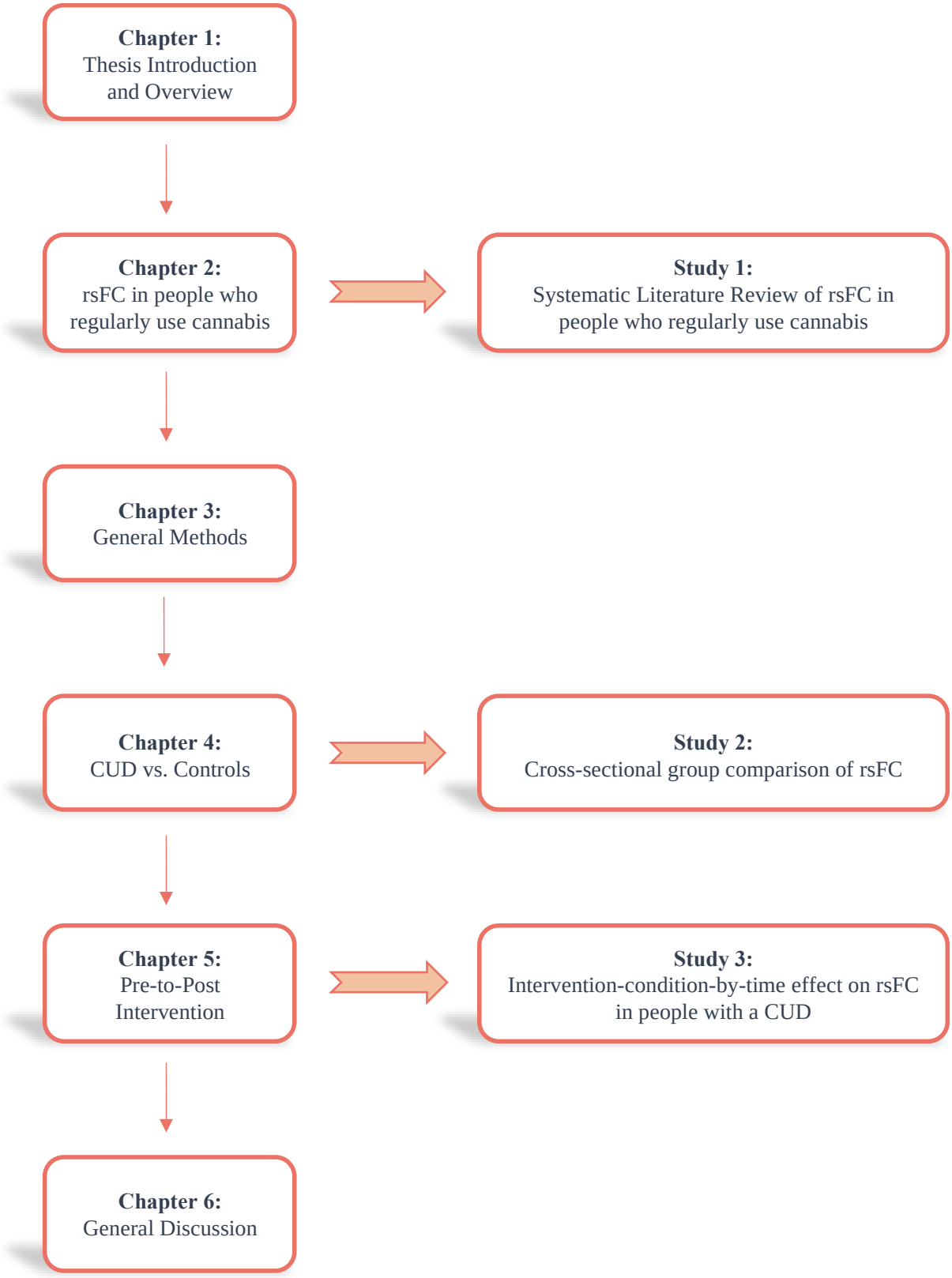
- Aims 1: To examine for the first time how a brief MBI reduced brain dysfunction – measured with rsFC fMRI – in people with a current moderate-to-severe CUD, who had tried to cut down or quit their use in the previous 2 years, compared to *active* and *passive placebo* control intervention conditions.
- Aim 2: To explore if changes in rsFC pre-to-post MBI were associated with changes in cannabis exposure (e.g., grams, use days) and cannabis-use related problems (e.g., withdrawal), as well as psychological measures (e.g., COVID-related-stress, and mindfulness levels).

1.8 Outline of the Thesis Structure

There are a total of six chapters within the current thesis, outlined in Figure 1.11. *Chapter 1* (this chapter) provides a general overview and introduction to the key topics, outlining the thesis rationale, and culminating in the thesis aims. *Chapter 2* (Study 1: SLR) provides a systematic review of the body of literature to date examining rsFC in regular cannabis users, as well as an examination of brain-behaviour correlations. Following on from the introductory chapters, *Chapter 3* (General Methods) comprehensively details the general methods relevant to the two empirical chapters of the current thesis, including the recruitment and resultant samples, the measures including all testing, questionnaires and neuroimaging, the procedures, and finally the analyses on both behavioural and neuroimaging data. *Chapter 4* (Study 2: Experiment 1) outlines the first experiment, which compares rsFC between individuals with a moderate-to-severe CUD and controls, as well as examining behavioural correlates associated with established differences. *Chapter 5* (Study 3: Experiment 2) outlines the second experiment, which examines the neural correlates of a brief MBI (compared to *active*- and *passive-placebo* control interventions), as well as examining behavioural correlates associated with established differences. Finally, *Chapter 6* (General Discussion) outlines the general

discussion of the thesis; it summarises the key findings, it acknowledges the study limitations, and it provides important implications and suggestions for future research.

Figure 1.11. *Outline of Thesis Structure*



CUD = cannabis use disorder; rsFC = resting-state functional connectivity

CHAPTER 2:

Systematic Literature Review

Chapter Guide

The following chapter presents a systematic literature review (SLR) of 21 studies investigating resting-state functional connectivity (rsFC) in people who regularly use cannabis, compared to controls. The studies included herein utilised functional Magnetic Resonance Imaging (fMRI) to measure of rsFC. The included studies stipulated their own definitions of what constituted a 'regular' cannabis user. All included studies examined regular cannabis use groups while they were not acutely intoxicated at the time of rsFC scan acquisition, and all had extensive and largely exclusive histories of cannabis use. This was to examine the chronic residual effects of cannabis use on rsFC. The ***primary aim*** of the review was to summarise the findings to date on rsFC differences between people who regularly use cannabis and controls. The ***secondary aim*** was to systematically synthesise evidence on associations between rsFC in people who regularly use cannabis and cannabis use levels, cognitive performance, and mental health symptoms.

This review was pre-registered with PROSPERO (2020 CRD420220181355). Please see *Appendix 2* for the complete PROSPERO registration document. This review has been published in *Psychopharmacology* (https://doi.org/10.1007/s00213-021-05938-0) and has been included in this chapter without any alterations. Given the manuscript word limit required for publication, relevant information regarding methods and results are presented in the *Supplementary Information*, which are included immediately following the manuscript. Please see *Appendix 3* for authorship contributions to the published manuscript.

Citation:

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Is Resting-State Functional Connectivity Altered in Regular Cannabis Users? A Systematic Review of the Literature

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2.1 Abstract

Rationale: Regular cannabis use has been associated with brain functional alterations within frontal, temporal, and striatal pathways assessed during various cognitive tasks. Whether such alterations are consistently reported in the absence of overt task performance needs to be elucidated to uncover the core neurobiological mechanisms of regular cannabis use.

Objectives: We aim to systematically review findings from studies that examine spontaneous fluctuations of brain function using functional Magnetic Resonance Imaging (fMRI) resting-state functional connectivity (rsFC) in cannabis users *versus* controls, and the association between rsFC and cannabis use chronicity, mental health symptoms, and cognitive performance.

Methods: We conducted a PROSPERO registered systematic review following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines and searched eight databases.

Results: 21 studies were included for review. Samples comprised 1,396 participants aged 16-to-42 years, of which 737 were cannabis users and 659 were controls. Most studies found *greater positive rsFC* in cannabis users compared to controls between frontal-frontal, frontostriatal, and fronto-temporal region pairings. The same region pairings were found to be preliminarily associated with varying measures of cannabis exposure.

Conclusions: The evidence to date shows that regular cannabis exposure is consistently associated with alteration of spontaneous changes in Blood Oxygenation Level-Dependent signal without any explicit cognitive input or output. These findings have implications for interpreting results from task-based fMRI studies of cannabis users, which may additionally tax overlapping networks. Future longitudinal rsFC fMRI studies are required to determine the clinical relevance of the findings and their link to the chronicity of use, mental health, and cognitive performance.

Keywords: functional magnetic resonance imaging, fMRI, brain, cannabis, resting-state functional connectivity, rsFC, connectivity, seed-based connectivity, systematic review

2.2 Introduction

Cannabis is the most commonly used illicit drug worldwide, with 192 million global users (United Nations Office on Drugs and Crime [UNODC], 2020). Prolonged and more regular cannabis use is associated with adverse psychosocial outcomes including increased risk of developing cannabis use disorders (CUDs; Chen et al. 2005; Volkow et al. 2014) and mental health disorders (Hall and Degenhardt 2009; Lev-Ran et al. 2014; Volkow et al. 2014), reduced cognitive performance (e.g., in tasks of verbal learning, memory, and attention; Figueiredo et al. 2020; Lovell et al. 2020), and greater risk-taking behaviour (e.g., driving and operating machinery while intoxicated; Australian Institute of Health and Welfare [AIHW], 2019; Volkow et al. 2014). The adverse psychosocial outcomes associated with regular cannabis use have been ascribed to underlying neurobiological alterations in brain structure and function (Bloomfield et al. 2019).

Recent meta-analyses of functional Magnetic Resonance Imaging (fMRI) studies show that cannabis users compared to controls demonstrate alterations in brain regions associated with addiction-related cognitive functions such as reward processing, executive function, and stress (Blest-Hopley et al. 2018; Yanes et al. 2018; Zehra et al. 2018). Notably, this body of work used task-based fMRI to identify changes in blood oxygen level-dependent (BOLD) signals when engaging in a task. Thus, one is limited in interpreting from these studies whether group differences between cannabis users versus controls reflect an underlying cannabis-specific brain functional alteration or cognitive confounds such as task performance, strategy, effort, and task adaptation (Fox and Greicius 2010; Philippi et al. 2020). To circumvent and minimise such confounds that may undermine the interpretation of task-based studies (Fox and Greicius 2010; Philippi et al. 2020), resting-state functional connectivity (rsFC) fMRI methods have been developed.

rsFC measures spontaneous fluctuations of brain function in the absence of overt task performance, as participants are at rest but awake in the scanner without completing any cognitively demanding tasks (Greicius et al. 2008; van de Ven et al. 2004). rsFC measures the degree to which the function of distinct regions (even those that are spatially remote) is temporally correlated, and whether the direction is strongly (i.e. *positive rsFC*) or poorly (i.e. *negative rsFC* or "*anti-correlation*") correlated (van de Ven et al. 2004). rsFC has been used to identify the brain's functional architecture

in normative samples and fundamental alterations underlying disease (Fox and Greicius 2010; Philippi et al. 2020). In addition to overcoming the described confounds associated with task-based fMRI, rsFC fMRI methods may inform alterations in large-scale neural networks underlying adverse clinical cognitive and behavioural outcomes associated with cannabis use. The first study that examined rsFC in cannabis users compared to controls was published less than 10 years ago (Houck et al. 2013).

Evidence from rsFC fMRI studies comparing regular cannabis users and controls supports functional alterations in frontal-striatal region pairings (Blanco-Hinojo et al. 2017; Filbey et al. 2014; Lopez-Larson et al. 2015; Subramaniam et al. 2018). Frontal-striatal projections are important for cognitive and behavioural flexibility implicated in goal-direct behaviour (Morris et al. 2016; Vaghi et al. 2017) and inhibitory control (Ersche et al. 2020; Morein-Zamir and Robbins 2015) underlying addiction. These brain regions are implicated in addiction neurocircuitry and may inform mechanisms involved in the transition to a maintenance of CUD, as well as potentially informing novel treatment targets to prevent relapse. However, the body of work has thus far revealed heterogeneous results, with alterations of distinct measures of rsFC (i.e., *positive rsFC* and *negative rsFC*), and inconsistent strength (i.e., *higher* or *lower*), and location of group differences. Notably, the literature on rsFC fMRI studies comparing cannabis users and controls is yet to be systematically integrated. rsFC alterations in cannabis users have been preliminarily associated with greater chronicity of cannabis use, worse mental health symptoms, and poorer cognitive ability. The evidence from correlational studies is yet to be systematically integrated. Thus, it remains unclear whether altered rsFC in cannabis users is driven by cannabis users with more chronic use, increased mental health symptoms, and/or reduced cognitive performance.

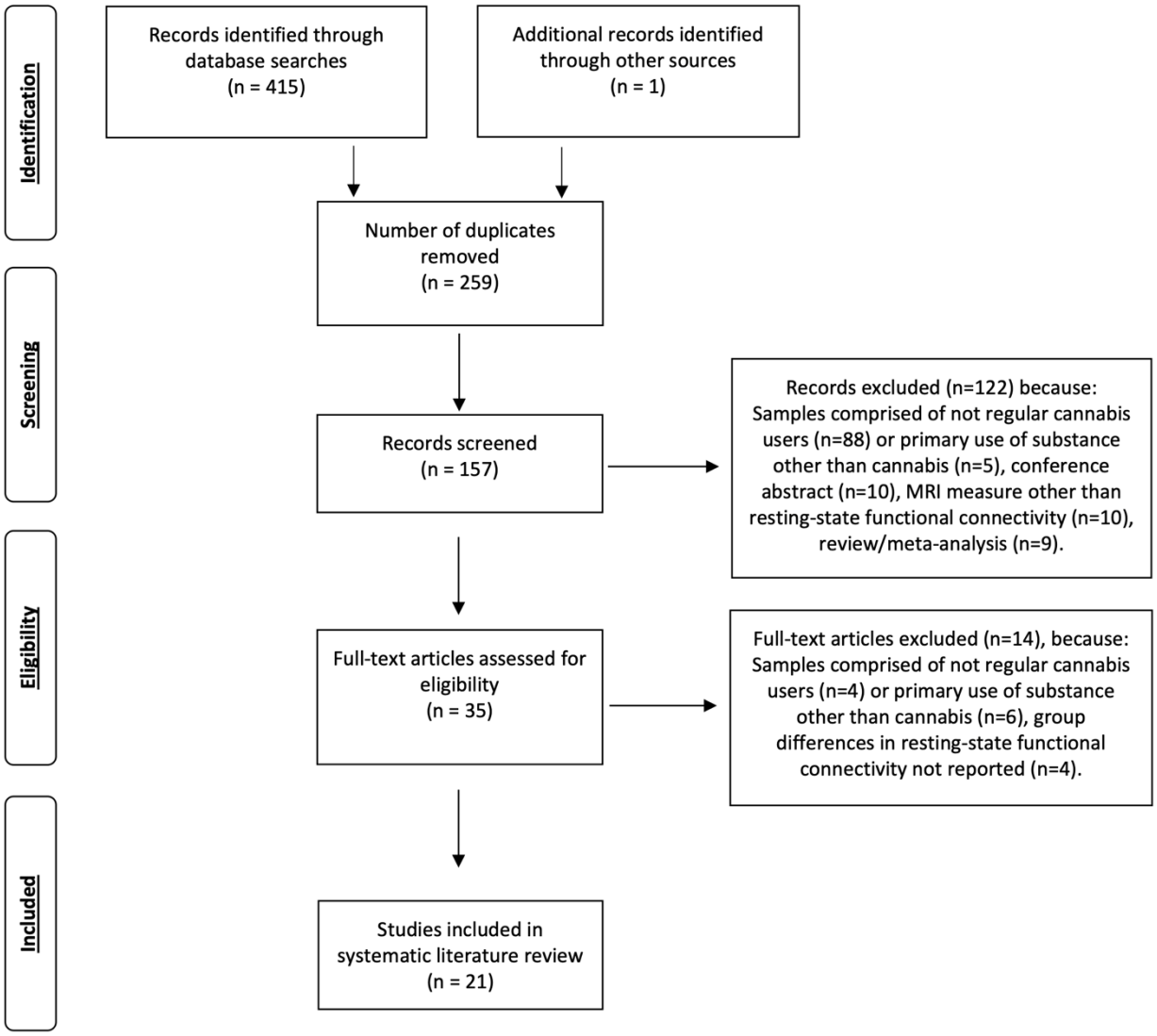
The primary aim of this systematic review was to summarise the findings to date on rsFC differences between regular cannabis users and controls. The secondary aim of this review was to systematically synthesise the evidence on the associations between rsFC in cannabis users and cannabis use levels (e.g., duration, dosage), cognitive performance (e.g., executive function), and mental health symptoms (e.g., depression), to shed light on whether specific subgroups of cannabis

users may be more vulnerable to greater alterations of brain function in the absence of overt task performance.

2.3 Methods

This systematic literature review was pre-registered with PROSPERO (2020 CRD420220181355; submitted on 5/05/2020 and approved on 10/07/2020). As shown in Figure 2.1, the literature search was run following recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009).

Figure 2.1. *PRISMA flowchart outlining study selection for systematic review*



Studies included in systematic literature review: (Behan et al. 2014; Blanco-Hinojo et al. 2017; Camchong et al. 2017; Cheng et al. 2014; Demiral et al. 2019; Filbey et al. 2014; Filbey et al. 2018; Houck et al. 2013; Kim et al. 2019; Lopez-Larson et al. 2015; Manza et al. 2018; Newman et al. 2019; Orr et al. 2013; Pujol et al. 2014; Shollenbarger et al. 2019; Subramaniam et al. 2018; Sweigert et al. 2019; Thijssen et al. 2017; Wetherill et al. 2015; Zhou et al. 2018; Zimmermann et al. 2018).

2.3.1 Search Strategy

A systematic search of the literature was conducted on the 29th of October 2020, using eight databases: CINAHL, Cochrane Library, Embase, Medline, PsychINFO, PubMed, Scopus, and Web of Science. The following search terms were used: ‘‘cannabis’ OR ‘marijuana’ OR ‘hashish’ OR ‘THC’ OR ‘tetrahydrocannabinol’’ AND ‘‘resting state’ OR ‘resting-state’ OR ‘at rest’ OR ‘resting’’ AND ‘‘connect*’ OR ‘funct*’’ AND ‘‘magnetic resonance imaging’ OR ‘MRI’ OR ‘functional magnetic resonance imaging’ OR ‘fMRI’ OR ‘BOLD’ OR ‘blood oxygen level dependent’’. All terms were searched within title, abstract, subject heading, and/or keywords as appropriate. The reference lists of included studies were also searched for cross referencing. Title and abstract screening were completed using the software Covidence (www.covidence.org).

2.3.2 Inclusion and Exclusion Criteria

Studies’ inclusion criteria were: (i) assessment of human participants; (ii) comparison of regular cannabis users with controls (as defined in each study); (iii) measuring functional connectivity using resting-state fMRI; (iv) article written in English; and (v) published in peer-reviewed journals. Studies’ exclusion criteria were: (i) measurement of brain integrity using imaging techniques other than fMRI (e.g., electroencephalogram [EEG], computerized tomography [CT], positron emission tomography [PET], single-photon emission computerized tomography [SPECT], structural MRI); (ii) non-peer-reviewed, non-published, or non-empirical work (e.g., dissertations, corrigendum, editorials, single case reports, book chapters, or conference abstracts, reviews or meta-analyses); (iii) inclusion of a sample with the primary use of drugs other than cannabis (e.g., cocaine, methamphetamines); and (iv) regular cannabis use groups with a primary clinical diagnosis other than CUD (e.g. psychosis). No restrictions were placed on the age of participants or the duration or frequency of cannabis use.

2.3.3 Data Screening and Extraction

Screening of articles using titles, abstracts, and full texts, was completed by two independent raters (H.T., H.S.), who then resolved any discrepancies via discussion with one another. The following data were extracted: (i) key sample characteristics, i.e., sample size, gender composition,

and age; (ii) cannabis use levels i.e., age of onset, duration, frequency, dosage, abstinence; (iii) rsFC analysis methods (and targeted brain area if applicable); (iv) results on group differences for rsFC (cannabis vs controls); and (v) results on correlations between rsFC in cannabis users and measures of cannabis use level, mental health symptoms, and cognitive performance. Additional data was extracted and is presented in the *Supplementary Information* and *Table S2.1 and Table S2.2*: (i) study inclusion/exclusion criteria for both cannabis using and control samples, (ii) cannabis use in the control sample, (iii) recruitment strategies, (iv) handedness, (v) MRI magnet strength (tesla) and brand; and (vi) MRI imaging protocol.

2.3.4 Additional Handling of Data

One study reported an rsFC comparison between a 'high cannabis use' group and 'low cannabis use' group (rather than cannabis users compared to controls; Houck et al. 2013); this study was retained in the review as the 'low cannabis use' group were comparable in cannabis use levels to the control groups of other studies. Three repeated measure studies were included, of which two studies comprised assessment at baseline and after 28 days of controlled abstinence (Blanco-Hinojo et al. 2017; Pujol et al. 2014). For these studies, we extracted data from baseline testing as per inclusion criterion "regular cannabis use". The third study included rsFC at baseline and again after 18-months of unrestricted cannabis use (in the cannabis group only; Camchong et al. 2017). As the current review focused on rsFC in regular cannabis users and associations with cannabis use parameters, data were extracted from the follow-up time-point to maximise the sample representativeness of 'regular' cannabis users. Studies that utilised the same participant dataset but applied distinct methods to analyse rsFC, or recruited additional participants, were considered as independent studies for inclusion in this review.

2.3.5 Risk of Bias

The National Institutes of Health (NIH), National Heart, Lung and Blood Institute's Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies tool was used to perform the quality assessment of the reviewed studies using 14 distinct criteria, each of which was scored 'yes',

'no', or 'not applicable'. The results from the quality assessment are summarised in *Supplementary Information* and *Table S2.3*.

2.4 Results

2.4.1 Included Studies

A total of 21 studies published between 2013 and 2019 were included in this review (Behan et al. 2014; Blanco-Hinojo et al. 2017; Camchong et al. 2017; Cheng et al. 2014; Demiral et al. 2019; Filbey et al. 2014; Filbey et al. 2018; Houck et al. 2013; Kim et al. 2019; Lopez-Larson et al. 2015; Manza et al. 2018; Newman et al. 2019; Orr et al. 2013; Pujol et al. 2014; Shollenbarger et al. 2019; Subramaniam et al. 2018; Sweigert et al. 2019; Thijssen et al. 2017; Wetherill et al. 2015; Zhou et al. 2018; Zimmermann et al. 2018). The 21 included studies examined 18 samples of participants; 3 studies re-analysed rsFC of previously published samples. Studies who utilised the same dataset were Behan et al. (2014) and Orr et al. (2013), Blanco-Hinojo et al. (2017) and Pujol et al. (2014), and Subramaniam et al. (2018) and Lopez-Larson et al. (2015). The participant group analysed by Zhou et al. (2018) included 16 cannabis users and 16 controls, previously reported by Zimmermann et al. (2018), but Zhou et al. (2018) recruited 20 additional participants (8 cannabis users and 12 controls) to increase power and due to the inclusion of additional participants, their sample was considered unique and was therefore the 18th sample.

2.4.2 Sample Characteristics

2.4.2.1 Socio-Demographic Characteristics

The demographic and cannabis use characteristics of the reviewed samples are summarised in Table 2.1. Samples included a total of 1,396 participants aged ~23 years (range 16-to-42 years). Of these, 737 were cannabis users (169 female), and 659 were controls (194 female). Males were overrepresented and comprised about two-third of the participants, with five studies including males only.

Table 2.1. *Summary of sample size, gender, age, and cannabis use characteristics of the reviewed samples*

Author (yr)	**Sample Size, *N* total (female)**		**Age, *years* mean (SD)**		**Cannabis use level mean (SD)**				
	Cannabis	**Control**	**Cannabis**	**Control**	**Age onset, *years***	**Duration, *years***	**Frequency /month**	**Dosage**	**Abstinence, *days***
Houck et al. (2013)	36 (13)	33 (10)	16 (1)	16 (1)	11 (2)	5 (-)[b]	15 (11) *days*	-	-
Orr et al. (2013)	17 (1)	18 (1)	17 (0)	16 (1)	13 (0)	4 (0)	-	43 (10) *joint past wk*	-
Behan et al. (2014)	17 (1)	18 (1)	17 (0)	16 (1)	13 (0)	4 (-)[b]	-	42 (10) *joint past wk*	-
Cheng et al. (2014)	12 (0)	13 (0)	19 (1)	22 (4)	16 (2)	3 (2)	-	13 (11) *joint past wk*	-
Filbey et al. (2014)	48 (15)	62 (23)	28 (8)	30 (7)	18 (3)	10 (8)	44 (4) *events*	-	-
Pujol et al. (2014)	28 (0)	29 (0)	21 (2)	22 (3)	15 (1)	6 (3)	-	17 (13) *joint/wk*	-
Lopez-Larson et al. (2015)	43 (3)	31 (7)	18 (1)	17 (1)	15 (1)	3 (-)[b]	60 (60) *events*	-	-
Wetherill et al. (2015)	19 (9)	24 (10)	28 (7)	31 (9)	19 (5)	9 (5)	27 (4) *days*	14 (11) *gram/wk*	0.7 (-)
Blanco-Hinojo et al. (2017)	28 (0)	29 (0)	21 (2)	22 (3)	14 (1)	6 (3)	-	17 (11) *joint/wk*	-
Camchong et al. (2017)	22 (8)	43 (20)	19 (2)	17 (3)	13 (2)	4 (-)[b]	22 (13)[c] *days*	-	7 (-)
Thijssen et al. (2017)	120 (0)	47 (0)	17 (1)	17 (1)	13 (-)[a]	4 (2)	-	-	30-180[e] (-)
Filbey et al. (2018)	53 (15)	30 (16)	42 (8)	29 (8)	35 (-)[a]	7 (6)	27 (5) *days*	-	-
Manza et al. (2018)	30 (8)	30 (10)	29 (3)	30 (8)	-	-	-	-	-
Subramaniam et al. (2018)	43 (3)	31 (7)	18 (1)	17 (1)	15 (1)	3 (-)[b]	60 (60) *events*	-	-
Zhou et al. (2018)	24 (0)	28 (0)	24 (4)	23 (3)	15 (1)	7 (3)	28 (5) *days*	4 (-)[c] *gram/wk*	30 (-)
Zimmerman et al. (2018)	19 (2)	18 (2)	24 (3)	24 (3)	15 (1)	6 (3)	27 (6) *days*	-	167 (-)
Demiral et al. (2019)	13 (7)	16 (8)	27 (8)	28 (5)	16 (3)	11(7)	28 (4) *days*	35 (21) *joint/wk*	-
Kim et al. (2019)	37 (20)	31 (18)	21 (4)	22 (4)	16 (2)	5 (-)[b]	33 (25) *events*	-	-
Newman et al. (2019)	23 (14)	23 (14)	21 (3)	21 (2)	16 (2)	5 (-)[b]	30 (25) *events*	-	-

Shollenbarger et al. (2019)	79 (37)	80 (35)	23 (3)	23 (2)	-	-	-	15 (14) *grams past wk*	2-3[d] (-)
Sweigert et al. (2019)	26 (13)	25 (12)	26 (4)	26 (5)	17 (5)	4 (3)	-	3 (2) cannabis *gram/wk* 126 (91) *THC mg/wk* 77 (21) *CBD mg/wk*	4 (-)

[a]*Age of onset* computed by running 'Age' minus 'Duration'; [b]*Duration* calculated as 'Age' minus 'Age onset'; [c]Calculated by dividing 'lifetime use' by 'duration of use';

[d]mode provided only; [e]range provided only; / = per (i.e., typical consumption in specified time period); CBD = cannabidiol; mg = milligrams; N = number; past = consumption in most recent block of specified time period; SD = standard deviation; THC = delta-9-tetrahydrocannabinol; wk = week

2.4.2.2 Cannabis Use Levels

The average *age of cannabis use onset* was 16 (range 11-to-35). Most studies however (18 of the 21 studies), examined samples with a mean age of onset of 15 years (range 11-to-19 years), whilst a single study tested participants with a notably older age of onset of 35 years. The average *duration* of cannabis use was 5.5 years (range 3-to-11 years). The *frequency of cannabis use* in the reviewed samples was an average of 25 days per month (range 15-to-28 days) and an average of 45 smoking events per month (from 30-to-60 monthly events). Cannabis dosage levels were heterogeneous across the reviewed samples, with an average of 32 joints over the *past* week (range 13-to-43 joints) or an average of 23 joints per *typical* week (ranged 17-to-35 joints). The average duration of *abstinence* from cannabis prior to fMRI testing was 35 days but varied widely between studies (from 1 day to 167 days). Additionally, one study reported a range of abstinence days typically between 30 and 180 days. An overview of the number of studies reporting different cannabis use levels is provided in the *Supplementary Information*.

2.4.2.3 Other Characteristics

Figure 2.2 shows that in the literature to date, key demographic, clinical, cognitive, and other outcomes were inconsistently measured across studies, poorly matched between groups (i.e., significant differences between cannabis users and controls), and seldom controlled for in the analyses.

Figure 2.2. *Heat map representing the number of studies (out of 21) that examined confounding variables and their influence on brain function*

DSM = Diagnostic and Statistical Manual, K-SADS-PL = Kiddie-Schedule for Affective Disorders and Schizophrenia for School Aged Children-Present and Lifetime, N = number

2.4.3 Methodological Characteristics

2.4.3.1 Analysis Approach to rsFC

A description of methods using rsFC / rs-fMRI analysis is shown in Figure 2.3. As shown in Table 2.2, Seed-Based Connectivity Analysis was the most consistently used method to analyse rsFC data (*n*=16 studies, of which 4 examined seed-to-seed and 12 examined seed-to-whole brain). Other studies used data-driven approaches: three studies used Independent Components Analysis (ICA), and two studies used Multivoxel Pattern Analysis (MVPA). Single studies used other methods: Graph Theory, Voxel Mirrored Homotropic Connectivity (VMHC), Support Vector Machine (SVM), fractional Amplitude of Low-Frequency Fluctuations (fALFF), local Functional Connectivity Density (lFCD), and Intrinsic Connectivity Contrast (ICC).

Table 2.2 overviews emerging differences in rsFC in the reviewed samples of cannabis users compared to controls. All studies compared groups for rsFC between pairings of regions, the exception being for three studies that compared connectivity for networks (Filbey et al. 2018; Houck et al. 2013; Thijssen et al. 2017). The summary below therefore summarises group differences in rsFC between region pairings.

Figure 2.3. *Overview of definitions of fMRI methods used to measure rsFC*

rsFC Analysis	**Description**
Hypothesis driven approach:	
Seed-Based Connectivity	Measures rsFC as the average time-course between a priori selected ROIs (i.e., "seed", a cluster of voxels) and that of other regions either across the whole brain (seed-to-whole-brain), or within selected ROIs (seed-to-seed). It yields a map from cross-correlations coefficients between each seed voxel and all other voxels (Goebel et al. 1998).
Data driven approaches:	
ICA	A black-box data driven method that groups all voxels into different resting-state brain network based on their temporal and special information (Bartels and Zeki 2004).
SVM	A machine learning based class of algorithm that measures which combination of rsFC features in a data set most effectively differentiate between two categories e.g., cannabis vs controls (Huettel et al. 2014).
MVPA	A machine learning tool. It can be applied to rsFC to measure which patterns of rsFC best discriminate two conditions (e.g., cannabis vs control). MVPA comprises key steps (Cheng et al. 2014; Norman et al. 2006). *Step 1: 'feature selection'* It identifies, selects and constructs features (i.e., vectors) based on the BOLD amplitude within voxels (or regions; Huettel et al. 2014). *Step 2:* '*classifier training'* It is run on half of the dataset (i.e. 'training set'). The vectors of this training set are entered into a pattern classification function, which identifies which patterns of rsFC best discriminate two conditions (e.g., cannabis vs control). *Step3: 'evaluation'* It measures the degree to which the patterns classified in the 'training dataset' are generalised to the second half of the dataset i.e., 'testing set' (Huettel et al. 2014).
Graph Theory	Examines the following properties of complex networks: (i) a set of regions-of-interest (ROI) or voxels ('nodes'); (ii) the rsFC between the ROIs or the voxels (i.e., 'connections', 'edges'; Bullmore and Sporns 2009; van den Heuvel et al. 2008).
VMHC	Measures interhemispheric rsFC (i.e., 'functional homotopy') between each voxel in one hemisphere, and its counterpart in the other hemisphere (Wei et al. 2018; Zuo et al. 2010).
fALFF	Identifies brain areas with abnormal local functioning; by determining across all voxels in the brain, their relative contribution of low frequency fluctuations within a specific frequency band to the whole detectable frequency range (Chen et al. 2015; Zou et al. 2008).
lFCD	Identifies which functional hubs (i.e., networks with dense local clustering) are highly connected, by measuring the correlation between the BOLD time series of each voxel and all other voxels in the brain (Tomasi and Volkow 2010). lFCD does not indicate which specific regions are comprised within the hubs.
ICC	Measures clusters of different regions based on their rsFC profile, by accounting for the number of connections between one voxel and the rest of the brain, and the strength of these connections (Martuzzi et al. 2011; Walpola et al. 2017).

fALFF = fractional Amplitude of Low-Frequency Fluctuations, ICA = Independent Components Analysis, ICC = Intrinsic Connectivity Contrast, lFCD = local Functional Connectivity Density, MVPA = Multivoxel Pattern Analysis, SVM = Support Vector Machine, VMHC = Voxel Mirrored Homotropic Connectivity

Table 2.2. *Overview of rsFC analysis methods and examined regions, results from group differences in rsFC and from correlations between rsFC and behavioural data*

Author (year)	**rsFC analysis method**	**Examined brain area**	**Cannabis users vs Controls**		**Associations: rsFC and behavioural data in cannabis users**
Houck et al. (2013)	ICA (1 IC)	fronto-temporal network [frontal (mid.), hippocampus, occipital (sup.)]	-	-	***Pos. corr.*** cannabis use (MUS) & frontal (mid.) ***Neg. corr.*** cannabis use (MUS) & temporal (mid.)*
Orr et al. (2013)	fALFF	frontal (sup., inf.*), temporal (inf.)*, cerebellum (inf. semilunar lobe)*, parietal (sup.)	CB > CON	**Pos conn. (CB) / neg conn. (CON):** frontal (inf.) – parietal (sup.) **Pos conn. (CB) / neg conn. (CON):** frontal (inf.) – frontal (sup.)	-
				Pos. conn. temporal (inf.) – parietal (sup.)	
			CB < CON	**Pos. conn.** frontal (inf.) – temporal (inf.)	
	VMHC	temporal coherence between each voxel & its counterpart in opposite hemisphere	CB < CON	**Pos. conn.** left-right cerebellum (pyramis) **Pos. conn.** left-right sup. frontal gyri	***NS corr.*** onset age, dosage (joints past wk/mo/life) & VMHC
			CB > CON	**Pos. conn.** left-right supramarginal gyri	
Behan et al. (2014)	Seed-to-seed (22 ROIs)	frontal (mid., inf., sup., med.), ACC, lentiform nucleus, insular, temporal (mid.), parietal lobule, PCC, tuber (inf.), culmen	CB > CON	**Pos. conn.** parietal (inf.) – cerebellum (tuber)	***Pos. corr.*** dosage (joints past wk/mo) & parietal-cerebellar network
			CB = CON	frontal (inf.) – parietal (inf.) – cerebellum (tuber) [network]	
Cheng et al. (2014)	MVPA & SVM (11 clusters)	frontal (mid., inf., sup.), precentral, cingulate, fusiform, PCC, cerebellum	CB > CON	**Pos. conn.** frontal (mid., sup.) – precentral & cingulate **Pos. conn.** frontal (inf.) – fusiform gyrus	***Pos. corr.*** onset age, impulsivity (BIS motor/attention) & SVM mean accuracy ***NS corr.*** schizotypal personality characteristics (SPQ & PAS) & SVM mean accuracy
Filbey et al. (2014)	Seed-to-whole brain	OFC	CB > CON	**Pos. conn.** OFC – temporal gyrus	***Neg. corr.*** onset age, negative consequences of cannabis use (MPS) & OFC-temporal gyrus
Pujol et al. (2014)	Seed-to-whole brain	insular, hippocampus, PCC	CB > CON	**Pos. conn.** PCC – PCC (ventral) **Pos. conn.** insular (ant.) – insular (ant.), supramarginal	***Pos. corr.*** dosage **(**joints/year) & PCC*, insular ***Pos. corr.*** verbal recall (RALVT) & hippocampus – para-hippocampus, PCC (dorsal)/precuneus ***Neg. corr***. verbal recall (RALVT) & PCC (ventral)

			CB < CON	**Pos. conn.** PCC – PCC (dorsal)/precuneus **Pos. conn.** insular (ant.) – ACC, brainstem (sup.) **Pos. conn.** hippocampus – R hippocampus	***Neg. corr***. anxiety (STAI-Y) & insular
			CB > CON	**Neg. conn.** insular – frontal (med.), angular PCC (ventral) **Neg. conn.** PCC – frontal (inf.), insular, operculum, putamen, parietal (inf.)	
			CB < CON	**Neg. conn.** insular – visual area, sup. parietal **Neg. conn.** PCC – visual area	
Lopez-Larson et al. (2015)	Seed-to-whole brain	OFC	CB < CON	**Pos. conn.** OFC – parietal (sup.)*	***Pos. corr.*** dosage (lifetime events) & OFC – frontal, parietal, cingulate(mid., post.)/precuneus, cerebellum ***Pos. corr.*** onset age & OFC – occipital, cerebellum ***Pos. corr.*** impulsivity (BIS motor) & OFC – parietal, precunus, central (pre., post.), frontal (mid., inf.), temporal (sup.), SMA, cingulum (mid.), occipital ***Neg. corr.*** onset age & OFC – frontal (sup.), precentral, SMA ***NS corr.*** impulsivity (BIS attention/non-planning)
			CB > CON	**Pos. conn.** OFC – frontal (med., mid., sup.), ACC/MCC, precentral	
Wetherill et al. (2015)	Seed-to-whole brain	PCC	CB < CON	**Pos. conn.** PCC – parahippocampus	***Pos. corr.*** duration & PCC – insular (ant.)
			CB > CON	**Pos. conn.** PCC – insular (ant.)	
Blanco-Hinojo et al. (2017)	Seed-to-whole brain	ACC, caudate (dorsal, ventral), putamen (dorsal, ventral), fusiform	CB < CON	**Pos. conn.** ACC/frontal (mid.) – caudate/putamen & basal ganglia	***Pos. corr.*** arousal (IAPS) & caudate–mPFC, PCC, angular; ACC – basal ganglia ***Pos. corr.*** THC urine & putamen – fusiform
			CB < CON	**Neg. conn.** fusiform – caudate/putamen & basal ganglia	***Neg. corr.*** arousal (IAPS) & caudate – sensorimotor**;** fusiform – basal ganglia ***Neg. corr.*** duration & caudate – ACC/frontal (med.)
Camchong et al. (2017) *TIME 2*	Seed-to-whole brain	ACC (dorsal, caudal, peri, rostral, sub-genual)	CB < CON	**Pos. conn.** ACC (caudal) – dlPFC, frontal (sup.), OFC *Corrected for TIME 1*	***Neg. corr.*** days of cannabis use (during 18-months post time 1) & ACC (caudal) – OFC (*at baseline*)
Thijssen et al. (2017)	ICA (15 ICs)	frontoparietal, DMN, salience, ECN, primary visual, visual (med.), visual (lateral), sensorimotor, dorsal attention, auditory, precuneus,	CB = CON	-	***Pos. corr.*** duration & fronto-parietal – sensorimotor ***Neg. corr.*** duration & ECN – auditory, sensorimotor, attention (dorsal); DMN–fronto-parietal; salience–visual (med.); precuneus –visual (primary) auditory

Filbey et al. (2018)	ICA (12 ICs)	frontal (sup.), frontoparietal, DMN (ant., post.), salience, visual (higher), basal ganglia, insular, temporal (inf., sup.), lingual, cuneus/precuneus, attention (dorsal)	CB < CON	**Pos. conn.** salience network, PCC network	***NS corr.*** CUD symptoms & ICs ***Pos. corr.*** CUD symptoms & fronto-parietal network*
Manza et al. (2018)	Seed-to-whole brain	NAc, thalamus, brainstem substantia nigra & ventral tegmental area	CB = CON	-	***Neg. corr.*** onset age & subcortical lFCD ***Pos. corr.*** alienation (MPQ) & subcortical lFCD
	lFCD	NAc, thalamus, brainstem, caudate, putamen, amygdala, hippocampus, midbrain, pallidum	CB > CON	NAc, substantia nigra & ventral tegmental area, brainstem, thalamus	
Subramaniam et al. (2018)	Seed-to-whole brain	OFC	-	-	***Pos. corr***. depression (HAM-D) & OFC –parietal (inf.), angular ***Neg corr.*** anxiety (HAM-A) & OFC – occipital (mid.), temporal (mid.) ***Neg corr.*** anxiety (HAM-A) & OFC – occipital (mid.) ***NS corr.*** depression scores & R OFC
Zhou et al. (2018)	Seed-to-whole brain	NAc, caudate	CB = CON	-	***NS corr.*** onset age, lifetime dose (grams), abstinence days
	Seed-to-seed	dmPFC, ACC (rostral), NAc, caudate	CB < CON	**Neg. conn.** Nac – dmPFC	
			CB < CON	**Pos. conn.** caudate – dmPFC **Pos. conn.** caudate – ACC (rostral)	
			CB > CON	**Pos. conn.** NAc – ACC (rostral)	
	ICC & MVPA	NAc, caudate	CB ≠ CON	Altered global connectivity patterns	
Zimmerman et al. (2018)	Seed-to-seed	mOFC, cingulate, striatum (dorsal), insular (ant.), amygdala, hippocampus	CB > CON	**Pos. conn.** mOFC – striatum (dorsal)	***NS corr.*** duration, lifetime dose (grams)
Demiral et al. (2019)	Seed-to-whole brain	thalamic nuclei (frontal, motor, sensory, occipital, premotor, parietal, temporal)	CB > CON	**Pos. conn.** thalamic (motor) – pars triangularis, supramarginal/broca **Pos. conn.** thalamic (sensory) – parietal **Pos. conn.** thalamic (temporal) – parieto-temporal	-

			CB < CON	**Pos. conn.** thalamic (parietal) – parietal **Pos. conn.** thalamic (motor) – fusiform/lingual	
Kim et al. (2019)	Graph Theory	-	CB < CON	**Pos. conn.** hippocampus – frontal (sup., mid.), parietal (inf.), temporal (mid., inf.), mOFC **Pos. conn.** caudate – frontal (sup., mid.), temporal, parietal (inf., mid.), OFC, fusiform	-
			CB > CON	**Pos. conn.** hippocampus – insular, occipital (med.), temporal (sup.), ACC (caudal) **Pos. conn.** caudate – precentral, frontal (inf.), occipital	
Newman et al. (2019)	Seed-to-whole brain	ACC (dorsal)	CB = CON	-	***Pos regression:*** ACC (dorsal) glutamate x monthly CB use interaction & ACC (dorsal) – NAc ***NS regression:*** ACC (dorsal) glutamate x monthly CB use interaction & ACC (dorsal) – hippocampus
Shollenbarger et al. (2019)	Seed-to-seed	vmPFC, ACC (rostral, caudal), insular, amygdala	CB > CON	**Pos. conn.** left-right ACC (rostral) **Pos. conn.** ACC (rostral) – insular*, amygdala*	***Pos. corr.*** depression (BDI) & left-right ACC (rostral)
Sweigert et al. (2019)	Seed-to-whole brain	Cerebellum (crus I & II, lobules IX & VIIb)	CB > CON	**Pos. conn.** cerebellum (crus I) – temporal (inf.) **Pos. conn.** cerebellum (lobule IX) – precentral	***Pos. corr.*** craving (MCQ) & cerebellum (crus I) – parahippocampal (pos.); cerebellum (lobule VIIb) – frontal pole, caudate, insular
			CB < CON	**Pos. conn.** cerebellum (crus II) – occipital pole, temporal (ant., sup.), brainstem, frontal (mid.), lingual, central (post.), central opercula, insular **Pos. conn.** cerebellum (lobule VIIb) – insular, occipital pole **Pos. conn.** cerebellum (lobule IX) – cerebellum (lobule VI)	***Pos. corr.*** CUDIT & cerebellum (lobule IX) –supramarginal (post., ant.), angular, precentral, lingual ***Neg. corr.*** CUDIT & cerebellum (crus I)–cerebellum(lobule VI), brainstem ***NS corr.*** CUDIT & cerebellum (crura I, II, lobule VIIb) ***NS corr.*** craving (MCQ) & cerebellum (crura I, II; lobules VIIb, IX)

* = reduced threshold/did not survive multiple corrections/trend; ant. = anterior; ACC = anterior cingulate cortex; BDI = Beck Depression Inventory; BIS = Barratt Impulsivity Scale; CB = cannabis; CON = control; conn. = connectivity; corr. = correlation; CUD = cannabis use disorder; CUDIT = cannabis use disorder identification test; dlPFC = dorsolateral prefrontal cortex; dmPFC = dorsomedial prefrontal cortex; DMN = default mode network; ECN = executive control network; fALFF = fractional amplitude of low-frequency fluctuations; HAM-A = Hamilton Anxiety Rating Scale; HAM-D = Hamilton Depression Rating Scale; IAPS = International Affective Picture System; IC = independent component; ICA = independent component analysis; ICC = intrinsic connectivity contrast; inf. = inferior; lFCD = local functional connectivity density; MCC = middle cingulate cortex; MCQ = marijuana craving questionnaire;

med. = medial; mid. = middle; mo = month; mOFC = medial orbitofrontal cortex; mPFC = medial prefrontal cortex; MPS = marijuana-related problems scale; MPQ = Multidimensional Personality Questionnaire; MUS = Marijuana Use Scale; MVPA = multi-voxel pattern analysis; NAc = nucleus accumbens; Neg. = negative; NS = non-significant; OFC = orbitofrontal cortex; PAS = Perceptual Aberration Scale; PCC = posterior cingulate cortex; Pos. = positive; post. = posterior; R = right; RAVLT = Rey Auditory-Verbal Learning Test; ROI = region of interest; rsFC = functional connectivity; SMA = supplementary motor area; SPQ = schizotypal personality questionnaire; STAI-Y = State-Trait Anxiety Inventory – Y form; sup. = superior; SVM = support vector machine; vmPFC = ventromedial prefrontal cortex; VMHC = Voxel Mirrored Homotopic Connectivity; wk = week

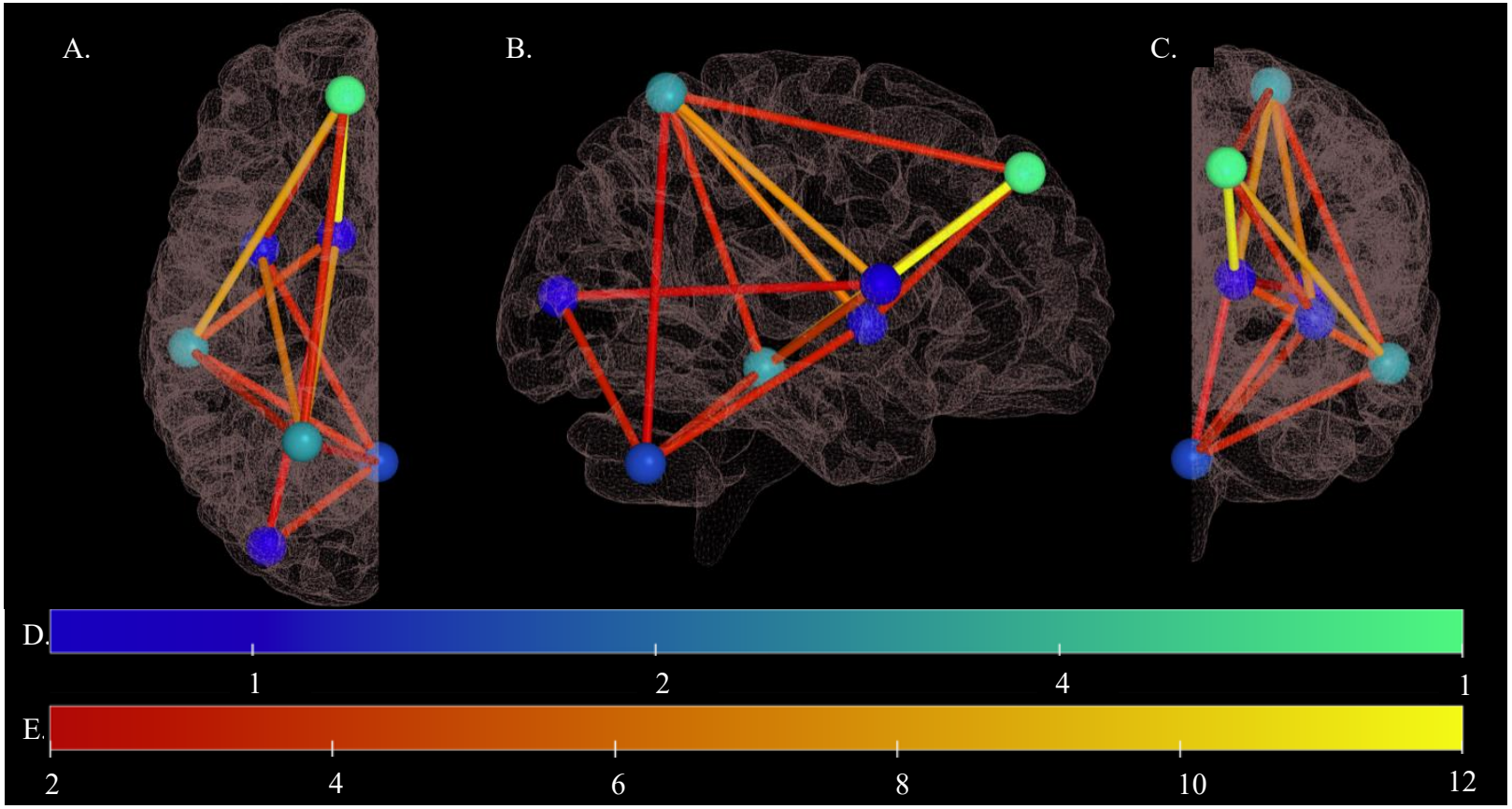
2.4.3.2 Brain Regions of Interest

Fifteen studies investigated rsFC from 22 different regions of interest (ROIs; i.e., 73 seeds were placed on these ROIs) using seed-based connectivity analysis. The most consistently studied ROI was the anterior cingulate cortex (ACC; *n=7* studies), followed by the orbitofrontal cortex (OFC) and the insular (*n=4* studies each), and by the nucleus accumbens (NAc) and the posterior cingulate cortex (PCC; *n=3* studies each). Other regions were used as seeds in *2* studies (i.e., prefrontal cortex [PFC], hippocampus, amygdala, caudate, thalamus, cerebellum), and in single studies (i.e., frontal gyrus [superior, middle, inferior and medial], fusiform, middle temporal and inferior parietal gyri, striatum [putamen, caudate], lentiform nucleus, brainstem, and midbrain).

2.4.4 Group Differences Between Region Pairings

Of the 19 studies reporting rsFC differences between region pairings, *15* reported group differences in rsFC between 91 region pairings, and *4* studies found no group difference (Manza et al. 2018; Newman et al. 2019; Subramaniam et al. 2018; Thijssen et al. 2017). Group differences in rsFC were most commonly reported between frontal regions and frontal (14 pairings), striatal (12 pairings), and temporal regions (8 pairings); see Figure 2.4.

Figure 2.4. *Group differences between region pairings, number of pairings displayed using left hemisphere*



A. Axial view; B. Sagittal view; C. Coronal view; D. No. of studies reporting inter-region pairings; E. No. of studies reporting between region pairings

Inter-region pairings: frontal-to-frontal = 14; parietal-to-parietal = 4; temporal-to-temporal = 4; cerebellum-to-cerebellum = 2; insular-to-insular = 1.

Between-region pairings: frontal-to-striatal = 12; frontal-to-temporal = 8; parietal-to-striatal = 7; parietal-to-insular = 6; temporal-to-striatal = 4; frontal-to-parietal = 3; parietal-to-temporal = 3; temporal-to-cerebellum = 3; occipital-to-cerebellum = 3; insular-to-cerebellum = 3; frontal-to-insular = 3; parietal-to-cerebellum = 2; occipital-to-striatal = 2; frontal-to-cerebellum = 1; temporal-to-occipital = 1; parietal-to-occipital = 1; occipital-to-insular = 1; temporal-to-insular = 1.

Note: between-region pairings reported in <2 studies are not represented in figure

2.4.4.1 Positive and Negative rsFC

Positive rsFC difference in cannabis users compared to controls was the most consistently reported finding by *all 15* of the studies reporting group differences (76 pairings, 84% of the total reviewed pairings). *Three* of the 15 studies also reported *negative rsFC* in cannabis users compared to controls, (13 pairings, 14% of the total reviewed pairings). A single study reported both *positive rsFC* in cannabis users and *negative rsFC* in controls (2 pairings, 2% of the total reviewed pairings).

2.4.4.2 Higher and Lower rsFC

Of 15 studies that found group differences, *13* studies reported that cannabis users versus controls had lower *rsFC* (in 44 of the region pairings, making up 48%). *Ten* studies found higher *rsFC* (in 47 of the region pairings, making up 52%).

2.4.5 Group Differences in rsFC

2.4.5.1 Positive rsFC

Cannabis users compared to controls had lower *positive rsFC* in 41 region pairings, across 10 out of 15 studies. Lower *positive rsFC* in cannabis users was most consistently reported for the following pairings: 7 frontal-striatal (*n*=3 studies), 4 frontal-to-frontal pairings, 3 parietal-striatal pairings, 3 temporal-temporal pairings (each *n*=2 studies), and 3 fronto-temporal, cerebellar-insular, and cerebellar-occipital (each single studies). Lower *positive rsFC* in cannabis users was reported in 2 parietal-temporal and cerebello-cerebellar areas (each *n=2* studies). Additional pairings were reported once-to-twice in single studies.

All 15 studies but *2* reported greater *positive rsFC* in cannabis users than controls, in 35 distinct region pairings. The locations were reported most consistently in the following pairings: 9 frontal-to-frontal pairings (*n=4* studies), 5 fronto-temporal pairings (*n=5* studies), 4 frontostriatal pairings (*n=3* studies), 3 parietal-striatal pairing (*single* study), 2 insular-parietal pairings (*n=2* studies). Additional pairings were reported once-to-twice in single studies.

2.4.5.2 Negative rsFC or “Anti-Correlations”

Single studies reported lower *negative rsFC* in cannabis users in 2 temporal-striatal and other single region pairings. Single studies reported higher *negative rsFC* in cannabis users between 3 parietal-insular and other single region pairings.

2.4.5.3 Group Differences

A single study (Orr et al., 2013), reported greater *rsFC* in cannabis users than controls between the inferior frontal gyrus and superior frontal/parietal gyri: these pairings were *positive* in cannabis users and *negative* in controls.

2.4.6 Associations between rsFC and Cannabis Users Parameters

A total of 18 studies ran 76 correlations between varying rsFC region pairings and the level of varying measures of cannabis exposure, cannabis use related problems, mental health symptoms, and cognitive performance (see Table 2.2 for each study’s findings). Most reported correlations were significant (62 out of 76). The significant correlations were most consistently reported in key rsFC regions pairings: frontal-to-frontal, fronto-parietal and fronto-temporal pairings were reported by *3* studies each (for a total of 26 correlations); while the frontal-striatal and parietal-cerebellum pairings were found by *2* studies each (yielding to 10 correlations). The results from the correlational analyses are further overviewed below based on the examined behavioural domain.

2.4.6.1 Cannabis Use Levels

Fourteen studies ran 37 correlations between distinct rsFC pairings and varying measures of cannabis use levels. The result from these correlations was largely significant. Specifically, 16 correlations were significantly positive (n=7 studies), 14 were significantly negative (n=7 studies), and 7 were non-significant (n=3 studies). Significant correlations implicated distinct single region pairings (e.g., fronto-parietal and frontal-to-frontal) and inconsistent measures of cannabis use level (e.g., lifetime cannabis events, number of joints in past week and month).

2.4.6.2 Problematic Cannabis Use

Three studies ran 13 correlations between distinct rsFC pairings and level of cannabis use levels. The results were mostly significant, as there were 6 significantly positive correlations, 3

significantly negative correlations, and 4 non-significant correlations (*n*=2 each). The emerging significant correlations involved varying single region pairings (e.g., parieto-to-cerebellar, fronto-to-cerebellar) and distinct measures of cannabis use related problems (e.g., scores on Marijuana Problem Scale, scores on CUDIT).

2.4.7 Associations between rsFC and Clinical and Cognitive Outcomes

2.4.7.1 Mental Health Symptoms

Five studies ran 11 correlations between rsFC and mental health symptom scores. Of these, most were significant. Specifically, there were 4 significantly positive correlations (*n*=3 studies), 4 significantly negative correlations (*n=2* studies), and 3 non-significant correlations (*n*=2 studies) reported. The significant correlations involved varying single region pairings (e.g., frontal-to-frontal, fronto-parietal) and distinct measures of mental health symptom related problems (i.e., depression, anxiety, alienation, schizotypal traits).

2.4.7.2 Cognitive Performance

Three studies ran 15 correlations between rsFC and cognitive performance (i.e., verbal recall, impulsivity). All correlations were significantly positive, with 2 exceptions (e.g., a negative correlation and a non-significant correlation). The significant correlations involved varying single region pairings (e.g., frontal-to-frontal, fronto-parietal) and distinct measures of cognitive performance (e.g., impulsivity which was also reported to be non-significant and verbal recall which was also negatively correlated).

2.5 Discussion

To our knowledge, this is the first systematic review of the evidence to date from rsFC fMRI studies comparing regular cannabis users and controls. The most consistent finding was that cannabis users compared to controls had greater *positive rsFC* between fronto-frontal, fronto-temporal, and frontostriatal pairings; followed by lower *positive rsFC* in partially overlapping pairings (frontal-striatal). Similar pairings (fronto-frontal, frontal-temporal, frontal-striatal) were preliminarily

associated (positive and negative) with varying measures of cannabis exposure, and with clinical (mental health symptoms) and cognitive (verbal recall and impulsivity) outcomes.

The most consistently reported finding in ~40% of the studies was that cannabis users had higher *positive rsFC* than controls, between fronto-frontal, fronto-temporal, and frontostriatal pairings. The location of the reviewed rsFC fMRI findings is partially overlapping with studies comparing cannabis users vs controls during cognitive task-based fMRI (e.g. alterations to temporal, frontal, and striatal activation; Blest-Hopley et al. 2018; Yanes et al. 2018), and structural MRI (e.g., lower volume of prefrontal [OFC], striatal [NAc] and temporal [hippocampus] regions; Lorenzetti et al. 2019). Together, these findings suggest that regular cannabis use is associated with neural alterations, particularly in frontal circuitry important for reward processing, and may therefore underlie typically observed cognitive changes. More research however is needed to determine if these alterations pre-date or follow onset of cannabis use.

In the current study, rsFC alterations between frontal region pairings were consistently associated with greater cannabis use levels in 50% of studies that ran correlations, including greater frequency, dosage, and/or level of cannabinoids in urine. Region pairings which were most consistently reported included fronto-frontal, frontostriatal and fronto-temporal regions. A variety of mechanisms may underlie greater *positive rsFC* in selected brain pathways in cannabis users compared to controls. First, repeated exposure to tetrahydrocannabinol (THC), the main psychoactive compound of cannabis with addiction liability (Zehra et al. 2018), may lead to aberrant function via endo-cannabinoid mediation in excitatory-inhibitory balance within the brain (Fratta and Fattore 2013). THC binds to cannabinoid type-1 (CB_1) receptors which are highly dense in frontal, striatal, and temporal regions and downregulated following repeated cannabis use (Burns et al. 2007).

Indeed, acute intoxication with THC has been shown to increase the rsFC between frontal and parietal regions, frontal and cerebellar regions, temporal and other temporal regions, and frontal and temporal regions (Bossong et al. 2019; Klumpers et al. 2012; Rabinak et al. 2018). Thus, the reported rsFC alterations might result from the residual-on-chronic effects of repeated exposure to THC on the central nervous system. rsFC alterations in cannabis users may normalise with abstinence, as indicated by preliminary evidence showing normalisation of rsFC after 28 days of controlled abstinence

(Blanco-Hinojo et al. 2017; Pujol et al. 2014), and of CB_1 receptors downregulation (Ceccarini et al. 2015; Hirvonen et al. 2012). Future research would benefit from examining longitudinal changes in rsFC networks in regular cannabis users following prolonged periods of abstinence, to inform mechanisms of brain recovery after repeated exposure to cannabis.

Frontal, temporal and striatal areas are an integral part of dopaminergic brain pathways that prominent neuroscientific theories implicate in addiction and in underlying altered reward processing, stress/craving, and disinhibition (Bloomfield et al. 2019; Bunge and Wright 2007; Martz et al. 2016; Nestor et al. 2010). Indeed, chronic THC exposure directly perturbs the dopamine system (Bloomfield et al. 2016). As frontal, temporal and striatal regions are innervated with dopamine projections, repeated THC exposure in cannabis users may alter dopaminergic transmission in these areas; and thus, alters their spontaneous BOLD fluctuations (Meck 2006; Paus 2001; Volkow and Fowler 2000), and the cognitive functions these are ascribed to e.g., disinhibition (Volkow et al., 2010).

2.5.1 Limitations of the Literature to Date and Directions for Future Work

The literature to date has several methodological limitations that need to be acknowledged and addressed by future work. Primarily, the design of the studies was cross-sectional and it remains unclear if rsFC alterations predate, predict or follow the onset of cannabis use and related problems, or whether rsFC alterations in cannabis users vary over time as cannabis exposure and related problems change or dissipate with abstinence (Blanco-Hinojo et al. 2017; Pujol et al. 2014), or exacerbate with continued use (Camchong et al. 2017). Further evidence is required to corroborate these notions. The use of already collected longitudinal neuroimaging consortia with available data on cannabis use and related problems data may prove useful to this end (e.g., The IMAGEN Dataset [https://imagen-europe.com/resources/imagen-dataset/], Imaging Data in Emerging Adults with Addiction [IDEAA; https://www.uwmbrainlab.com/research/ideaa-grant] Consortium, Connectome Coordination Facility [https://www.humanconnectome.org/])

Second, the direct integration of the results was hindered by the use of heterogeneous methodologies. For example, distinct brain regions were used as ROIs (e.g., two-third of the pairings examined, focused on 22 different combination of regions); which warrants the concurrent use of

whole-brain unbiased and hypothesis driven approaches to enable the comparability of results between studies and their consistency across analyses. Additionally, different cannabis use metrics and cut-offs to determine specific cannabis exposure/abstinence levels (e.g., cannabis dependence as defined by DSM-IV enforced in only one-third of studies, minimum use requirements vary from at least weekly use to at least twice daily use) hindered the interpretation of whether specific cannabis user groups show distinct alterations (e.g., were alterations driven by more frequent users, users with greater use duration, users of higher potency cannabis etc.) and the role of abstinence duration in driving the findings to date (i.e., abstinence varied from less than 24 hours to between 1 and 6 months). Few studies run correlations between rsFC and behavioural variables, and the correlation terms varied widely (due to inconsistent region pairings and metrics used to assess behavioural variables i.e. severity of CUD, cannabis-related problems, cannabis cravings, abstinence, urine metabolites, executive function, memory, depression and anxiety, schizotypal personality traits, and subjective arousal were each examined by only one-to-two studies) undermined the understanding of whether more vulnerable cannabis users show distinct or more marked neural alterations.

Furthermore, no study included in this review was pre-registered. In a move towards scientific best practices to minimise biases and increase the transparency of the scientific process and reproducibility of scientific results (Gonzales and Cunningham 2015), pre-registration to open access platforms is warranted for future work. Finally, the reviewed samples had a widely varying age range (from 16-to-42 years) with a mean of 23 years of age. As nonlinear neuromaturation trajectories are ongoing until age 24 (Sawyer et al. 2018), their role in the findings to date cannot be determined. Future studies are required to measure and account for variables related to neurodevelopment (e.g., age, pubertal stage, sex hormones).

2.5.2 Limitations of the Review

We did not include grey literature (e.g., conference abstracts, posters, dissertations, etc.). Thus, the results from this review may be influenced by publication biases (i.e., positive findings are more likely to be published and have a higher chance of being reported) and consequently over-represent positive findings (Dwan et al. 2013).

2.5.3 Conclusions

The evidence to date shows that regular cannabis exposure is consistently associated with alteration of spontaneous changes of rsFC in the absence of overt task performance. Regular cannabis users may affect the rsFC of selected pathways, as greater and lower *positive* rsFC was consistently reported between selected region pairings (fronto-frontal, fronto-temporal, and frontostriatal). Greater chronicity of use may drive such rsFC alterations in cannabis users, based on emerging correlational evidence with cannabis use level. The findings have implications for interpreting results from task-based fMRI studies of cannabis users which may additionally tax overlapping networks. Future longitudinal rsFC fMRI studies are required to determine the clinical relevance of the findings and their link to the chronicity of use, mental health, and cognitive abilities.

2.6 Supplementary Information

2.6.1 Inclusion and Exclusion Criteria

2.6.1.1 Cannabis Use Levels in Cannabis Groups

The inclusion criteria for the cannabis use group were heterogeneous between studies and referred to cannabis dependence, cannabis use levels, cannabinoids measured from specimens, and abstinence (see *Table S2.1* for details).

First, a diagnosis of *cannabis dependence* was required by 11 studies as determined by distinct tools: the DSM-IV (*n = 7* studies; American Psychiatric Association, 2013), the Kiddie Schedule for Affective Disorders and Schizophrenia (*n = 1* study; Kaufman et al., 1997), the Alcohol, Smoking, and Substance Involvement Screening Test (*n = 1* study; Humeniuk et al., 2008), DSM-III based Marijuana Use Scale score cut-off (*n = 1* study; Stephens, Roffman, & Curtin, 2000); and an unspecified tool (*n = 1* study).

Second, minimum *cannabis use levels* were required by 10 studies, which used various cut-offs: 'current cannabis use (n=1)', 'at least weekly use either for ≥3 months (n=1), ≥1 month (n=1) or unspecified duration (n=1)', 'use ≥4 times/week for >6 months (n=2)', 'use ≥100 times over previous year (n=2)', and 'use ≥14 times/week for >2 years (n=2)'.

Third, concurrently measured cannabinoids from specimens (i.e., THC-COOH in urine) and inclusion/exclusion criteria for cannabis dependence/use levels were required by 9 studies, of which 6 studies included presence of THC in urine and 3 studies excluded presence of THC in urine.

Last, *abstinence* from cannabis prior to testing was required by 12 studies using heterogeneous cut offs: ≥12 to 72 hours (*n = 6* studies); use up until the 'day prior'/'night prior'/'prior' (*n = 4* studies); ≥28 days abstinence (n=2).

2.6.1.2 Cannabis Use Levels in Control Group

Permitted levels of cannabis use in the control group varied between studies, outlined in *Table S2.1*. Cannabis use level was included as a screening criterion for controls only in 17 out of 21 studies, using distinct cut offs. Studies were heterogenous in applied

criteria, see *Table S2.1* for details. Eight studies additionally reported specific levels of cannabis use in controls ranging from a median lifetime use of 2 grams to previous cannabis abuse.

2.6.2 Recruitment Strategies

Samples were recruited from various outlets (outlined in *Table S2.1*): most commonly from the general community (n=12), followed by substance use treatment programs (n=5). Single studies selected samples from other outlets: a maximum-security facility, a justice program and distinct consortia (i.e., the Imaging Data in Emerging Adults with Addiction and the S500 release of the Washington University-University of Minnesota Consortium of the Human Connectome Project).

2.6.3 Handedness

Handedness was measured in half of the studies (11 out of 21 studies) and varied widely between samples (outlined in *Table S2.1*), which were either right-handed (8 studies), or mixed between right- and left-handed and ambidextrous (3 studies; only 1 of which matched handedness across groups).

2.6.4 MRI Scanner Strength and Brand

fMRI data was acquired using MRI scanners of distinct strengths and brands (outlined in *Table S2.2*). 3T Siemens scanners were most commonly used, in 14 studies. Five studies used 3T Philips. Two studies used 1.5T GE. Single studies used a 3T GE, a 4T Varian/Siemens, and a 1.5T Siemens.

2.6.5 MRI Protocol

MRI protocols were largely heterogeneous across studies (outlined in *Table S2.2*). Two studies (Filbey, Gohel, Prashad, & Biswal, 2018; Shollenbarger et al., 2019) reported multiple MRI protocols, varying by collection site. This resulted in a total of 24 protocols,

applied to the included samples. *Echo time* and *repetition time* was reported in all protocols, and varied from 20-to-50ms, and 720-to-2,580ms respectively. Eighteen protocols included the reporting of *flip* or *pulse angles*, varying from 52-to-90 degrees. *Field of view* was reported in 18 protocols, in millimetres (8 protocols; ranging from 208-to240mm), millimetres squared (8 protocols; ranging from 220 x 220mm^2 to 240 x 240mm^2), and millimetres cubed (2 protocols; 220 x 136 x 220mm^3 and 240 x 240 x 156mm^3). *Matrix size* was reported in all but one protocol. The majority of protocols included a matrix size of 64 x 64 (n=18), the remaining 5 protocols reported unique matrix sizes (i.e., 128 x 128, 64 x 64 x 64, 64 x 64 x 34, 104 x 90, 80 x 78). *Voxel size* was reported in 21 protocols varying from 1.7 x 1.7 x 3.8mm^3 to 3.8 x 3.8 x 4.6mm^3. *Number of slices* was reported in all studies except 1 and ranged from 22 to 72. Twelve protocols utilized axial slices and 1 utilized coronal slices; 11 did not specify slice plane. *Slice thickness* was reported in all protocols and varied between 2-to-5mm. The *gap between slices* was reported in 13 protocols. Of these, 8 left no gap, and 5 reported gaps between 0.4-to-1.5mm. *Number of images* or volumes was reported in 17 protocols and varied between 150 and 1,000. *Scan duration* was reported in 15 protocols and varied between 300 seconds and 873 seconds. Seventeen studies specified if participants were instructed to keep their *eyes open or closed* during the resting-state acquisition (12 open, 5 closed). Six of the studies that stipulated participant's eyes remain open also reported use of a fixation cross and 2 utilized an eye tracker. Finally, 1 study questioned participants following the scan to ascertain wakefulness and 6 studies reported instructing their participants to aim to think of nothing during the resting-state acquisition.

2.6.6 Risk of Bias

The quality of the study was largely consistently high, as presented in *Table S2.3*. All studies had a clearly stated *research question*, a specifically defined *study population*, *independent variable and outcome variable*, an adequate *participation rate* of eligible participants, a uniformly applied *inclusion and exclusion criteria*, and minimal *loss to*

follow-up after baseline (where applicable). All studies allowed a sufficient *timeframe* and measured the independent variable prior to the outcome variable. All but 1 study (Zhou et al., 2018) recruited participants during the same time period.

Only 3 studies (Behan et al., 2014; Camchong, Lim, & Kumra, 2017; Zhou et al., 2018) provided a *sample size justification*. Only 3 studies assessed the *independent variable more than once* over time (Blanco-Hinojo et al., 2017; Camchong et al., 2017; Pujol et al., 2014). The quality check highlighted inconsistencies in the reporting of *confounding variables*, whereby 8 studies reported age, gender, tobacco use, alcohol use, and IQ level (Camchong et al., 2017; Filbey et al., 2014; Filbey et al., 2018; Manza, Tomasi, & Volkow, 2018; Orr et al., 2013; Shollenbarger et al., 2019; Thijssen et al., 2017; Zimmermann et al., 2018). No studies *blinded* the researchers to the group status of the participants.

2.6.7 Number of Studies Measuring Metrics of Cannabis Use

Cannabis use levels were inconsistently measured in the reviewed literature (as detailed in Table 2.1 of the main text). *Age of cannabis use onset* and *cannabis use duration were* measured in 12 and 17 studies respectively. Two studies did not report either (Manza et al., 2018; Shollenbarger et al., 2019), therefore *age of cannabis use onset* was computed based on *duration* for 2 studies, and vice versa for 7 studies. *Frequency of cannabis use* was measured by 12 studies either in cannabis consumption days (*n = 7* studies) or number of smoking events (n = 5 studies) per month.

Cannabis dosage was measured by 10 studies using different metrics that referred to inconsistent periods of time: number of joints in past week (*n = 3* studies), number of joints per typical week (*n = 3* studies), grams per typical week (n=2), grams in past month, grams in lifetime, and milligrams of THC and of CBD (single studies).

The duration of *abstinence* from cannabis prior to scan, was measured in 7 studies. This was reported using different metrics: average number of days (*n = 5* studies), ranging from less than 1 day to 167 days, with an average of 35 days. Single studies reported the mode range of days, and total range of days.

Table S2.1. *Overview of cannabis-related inclusion and exclusion criteria for cannabis use levels and abstinence, cannabis use in control group, recruitment strategies, and handedness*

Author (yr)	**Screening of Cannabis Use Level**					**Recruitment Strategy**	**Handedness**
	Cannabis Group			**Control Group**		**Total Sample**	
	Inclusion Criteria	Exclusion criteria		Exclusion Criteria	Reported use		
		THC Urinalysis	Abstinence prior to testing	Cannabis use level			
Houck et al. (2013)	Score of 21-37 on Marijuana Use Scale	-	-	-	1-20 Score, Marijuana Use Scale	Juvenile justice program	-
Orr et al. (2013)	Cannabis dependence (DSM-IV)	No THC	'Night' before	-	Lifetime average 13 joints (in 4 control ppts); Month prior to testing average 3 joints (in 3 control ppts)	Drug treatment centre + community advertisement	Right
Behan et al. (2014)	Cannabis dependence (DSM-IV)	No THC	'Night' before	-	Lifetime average 13 joints (in 4 control ppts); Month prior to testing average 3 joints (in 3 control ppts)	Drug treatment centre + community advertisement	Right
Cheng et al. (2014)	Use ≥1x per week during the past month	-	12 hours	Exclude any use	0	Community advertisement	*Right (n=24); Left (n=1)
Filbey et al. (2014)	Use ≥4 x per week over past 6 months	No THC	-	Exclude regular use; positive urine screen	0	Community advertisement	-
Pujol et al. (2014)	Use onset < 16 yrs; smoking ≥14 x per week for ≥2 years prior to testing	No THC	12 hours	Exclude >14 lifetime uses; use in past month; positive urine screen	-	Community advertisement	Right
Lopez-Larson et al. (2015)	≥100 lifetime smoking events in year prior to testing	No THC	-	Exclude >15 lifetime uses	0	Community advertisement	-
Wetherill et al. (2015)	Cannabis dependence (untreated at time of testing)	No THC	-	Exclude any use	0	Community advertisement	-

Blanco-Hinojo et al. (2017)	Use onset before 16; smoking ≥14 x per week for ≥2 years prior to testing	No THC	12 hours	Exclude >14 lifetime cannabis uses; use in past month; positive urine screen	-	Community advertisement	Right
Camchong et al. (2017)	Cannabis dependence (DSM-IV); >50 exposures	Yes THC	-	Exclude >5 lifetime uses	Average 12 days of use of previous 18 months	Drug treatment centre + community advertisement	Right & Left (matched between groups)
Thijssen et al. (2017)	Cannabis dependence (KSADS)	-	-	Excluded current dependence	Previous cannabis abuse (in 29 control ppts)	Maximum security facility	*Right (n=120); Left (n=14); Ambidextrous (n=3); Missing (n=30)
Filbey et al. (2018)	Use ≥4 x per week over past 6 months	No THC	72 hours	Exclude regular or current use	0	Community advertisement	-
Manza et al. (2018)	Cannabis dependence (DSM-IV)	-	-	-	_	Consortium – Connectome	-
Subramaniam et al. (2018)	≥100 lifetime smoking events in year prior to testing	No THC	-	Exclude >15 lifetime uses	0	Community advertisement	-
Zhou et al. (2018)	Cannabis dependence (DSM-IV) at any stage during 18-months prior to testing	Yes THC	28 days	Exclude >14g lifetime use	Median lifetime use 2g	Drug treatment centre + community advertisement	Right
Zimmerman et al. (2018)	Cannabis dependence (DSM-IV)	Yes THC	28 days	Exclude >9g lifetime use	-	Drug treatment centre + community advertisement	Right
Demiral et al. (2019)	Cannabis dependence or abuse (DSM-IV)	-	-	Exclude history of abuse or dependence	0	Community advertisement	-
Kim et al. (2019)	Use ≥1x per week	-	Any 'Prior' use	Exclude >12 lifetime uses; use in past 3 months; positive urine screen	Average 4 lifetime events (in 6 control ppts)	Community advertisement	-
Newman et al. (2019)	Current cannabis use (unspecified amount)	-	'Day' before	Exclude use in past month; previous cannabis dependence; positive urine screen	Average 0.55 lifetime instances (in 5 control ppts)	Community advertisement	-

Shollenbarger et al. (2019)	Use ≥1x per week over past 3 months; duration of use >1 year	-	12 hours	Exclude regular use; use in past month; previous CUD	Not specified	Consortium – IDEAA	Right
Sweigert et al. (2019)	Moderate-to-high risk cannabis dependence (ASSIST); Weekly-to-daily cannabis use	-	48 hours	Exclude any use	0	Community advertisement	Right

*regular cannabis use and control groups not matched for handedness; ASSIST = Alcohol, Smoking, and Substance Involvement Screening Test; DSM-IV = Diagnostic and Statistical Manual on Mental Disorders, version four; IDEAA = Imaging Data in Emerging Adults with Addiction; KSADS = Kiddie Schedule for Affective Disorders and Schizophrenia

Table S2.2. *Overview of rsFC fMRI protocol*

Author (yr)	MRI magnet strength & brand	TE (*ms*)	TR (*ms*)	Flip / pulse angle	FOV	Matrix	Voxel size	No. of slices (plane)	Slice thickness (*mm*)	Slice gap (*mm*)	No. of images/ volumes	Scan duration (*seconds*)	Eyes	Other (e.g., fixation cross, eye monitoring, wakefulness)
Houck et al. (2013)	3T Siemens	29	2,000	-	224 mm	64 × 64	3.5 × 3.5 × 3.5 mm^3	33 (axial)	3.5	-	165	300	Open	Fixation cross. Think of nothing.
Orr et al. (2013)	3T Philips	30	2,000	-	224 mm	64 × 64	3.5 × 3.5 × 3.5 mm^3	39 (axial)	3.5	0.4	-	420	Open	
Behan et al. (2014)	3T Philips	30	2,000	-	224 mm	64 × 64	3.5 × 3.5 × 3.5 mm^3	39 (axial)	3.5	0.4	-	420	Open	
Cheng et al. (2014)	3T Siemens	30	2,500	70°	220 mm	128 × 128	1.7 × 1.7 × 3.8 mm^3	35 (axial)	3.8	-	175	-	Open	
Filbey et al. (2014)	3T Siemens	29	2,000	60°	240 × 240 mm^2	64 × 64	3.8 × 3.8 × 4.6 mm^3	32 (axial)	4.6	0	158	330	-	
Pujol et al. (2014)	1.5T GE	50	2,000	90°	240 mm	64 × 64	3.8 × 3.8 × 4 mm^3	22 (axial)	4	1.5	180	360	Closed	
Lopez-Larson et al. (2015)	3T Siemens	28	2,000	-	-	64 × 64	-	40 (-)	3	-	240	480	Open	Think of nothing.
Wetherill et al. (2015)	3T Siemens	24	2,000	-	220 × 220 mm^2	64 × 64 × 64	3.4 × 3.4 × 4 mm^3	- (-)	4	0	-	-	Open	Eye-tracker
Blanco-Hinojo et al. (2017)	1.5T GE	50	2,000	90°	240 mm	64 × 64	3.8 × 3.8 × 4 mm^3	22 (axial)	4	1.5	180	360	Closed	
Camchong et al. (2017)	3T Siemens	30	2,000	90°	-	64 × 64 × 34	3.4 × 3.4 × 4.0 mm^3	34 (axial)	4	0	180	360	Closed	Participants asked post scan if they remained awake.
Thijssen et al. (2017)	1.5T Siemens	39	2,000	90°	240 × 240 mm^2	64 × 64	3.8 × 3.8 × 5 mm^3	30 (-)	5	-	-	-	Open	Fixation cross. Eye tracker.
Filbey et al. (2018) *Ppt group:* 1	3T Siemens	29	2,000	60°	240 × 240 mm^2	64 × 64	3.8 × 3.8 × 4.6 mm^3	32 (axial)	4.6	0	158	300	-	

Filbey et al. (2018) *Ppt group:* 2	3T Philips	29	2,000	75°	220 × 136 × 220mm^3	64 × 64	3.8 × 3.8 × 3.9 mm^3	39 (axial)	3.9	-	150	312	-	
Manza et al. (2018)	3T Siemens	33	720	52°	208 mm	104 × 90	2 × 2 × 2 mm^3	72 (-)	2	0	-	873	Open	Fixation cross. Think of nothing.
Subramaniam et al. (2018)	3T Siemens	28	2,000	-	-	64 × 64	-	40 (-)	3	-	240	480	Open	Think of nothing.
Zhou et al. (2018)	3T Siemens	30	2,580	80°	224 × 224 mm^2	64 × 64	3.5 × 3.5 × 3.5 mm^3	47 (-)	3.5	0	180	-	Closed	Think of nothing.
Zimmerman et al. (2018)	3T Siemens	30	2,580	80°	224 × 224 mm^2	64 × 64	3.5 × 3.5 × 3.5 mm^3	47 (-)	3.5	0	180	-	Closed	Think of nothing.
Demiral et al. (2019)	4T Varian/ Siemens	20	1,600	90°	-	64 × 64	-	33 (coronal)	4	1	191	-	-	
Kim et al. (2019)	3T Siemens	28	813	60°	220 × 220 mm^2	64 × 64	3.4 × 3.4 × 3.4 mm^3	42 (axial)	3.4	-	1000	840	Open	Fixation cross.
Newman et al. (2019)	3T Siemens	28	813	60°	220 × 220 mm^2	64 × 64	3.4 × 3.4 × 3.4 mm^3	42 (axial)	3.4	-	1000	840	Open	Fixation cross.
Shollenbarger et al. (2019) *SITE 1:* UWM	3T GE	25	2,000	77°	240 mm	64 × 64	3.8 × 3.8 × 3.7 mm^3	40 (-)	3.7	-	240	-	-	
Shollenbarger et al. (2019) *SITE 2:* McLean	3T Siemens	30	2,500	82°	-	-	3.5 × 3.5 × 2.5 mm^3	41 (-)	2.5	-	-	-	-	
Shollenbarger et al. (2019) *SITE 3:* UTD	3T Philips	29	2,000	75°	-	64 × 64	3.4 × 3.4 × 3.5 mm^3	39 (-)	3.5	-	-	-	-	
Sweigert et al. (2019)	3T Philips	24	2,000	79°	240 × 240 × 156mm^3	80 × 78	3 × 3 × 4 mm^3	39 (-)	4	0	200	424	Open	Fixation cross.

FOV = Field of View; McLean = McLean Hospital/Harvard University; TE = Echo Time; TR = Repetition Time; UTD = University of Texas—Dallas; UWM = University of Wisconsin-Milwaukee

Table S2.3. *Overview of the Risk of Bias using the NIH National Heart, Lung, and Blood Institute – Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies Tool*

Author (yr)	**1**	**2**	**3**	**4a**	**4b**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Houck et al. (2013)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ; tobacco)
Orr et al. (2013)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Behan et al. (2014)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ; alcohol)
Cheng et al. (2014)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (tobacco)
Filbey et al. (2014)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Pujol et al. (2014)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	Yes	Yes	No	Yes	No (IQ; alcohol; tobacco)
Lopez-Larson et al. (2015)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ)
Wetherill et al. (2015)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ)
Blanco-Hinojo et al. (2017)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	Yes	Yes	No	Yes	No (IQ; alcohol; tobacco)
Camchong et al. (2017)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n/a	Yes	Yes	Yes	No	Yes	Yes
Thijssen et al. (2017)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Filbey et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Manza et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Subramaniam et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ; alcohol; tobacco)
Zhou et al. (2018)	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ)
Zimmerman et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Demiral et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ; alcohol)

Kim et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (tobacco)
Newman et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ)
Shollenbarger et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	Yes
Sweigert et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	n/a	Yes	No	Yes	No	n/a	No (IQ; tobacco)

1. Was the research question or objective in this paper clearly stated?; 2. Was the study population clearly specified and defined?; 3. Was the participation rate of eligible persons at least 50%?; 4a. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? 4b. Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?; 5. Was a sample size justification, power description, or variance and effect estimates provided?; 6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?; 7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?; 8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?; 9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?; 10. Was the exposure(s) assessed more than once over time?; 11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?; 12. Were the outcome assessors blinded to the exposure status of participants?; 13. Was loss to follow-up after baseline 20% or less?; 14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Note: Item 8. was deemed ‘not applicable’ as measuring cannabis use as a dichotomous variable (i.e., regular cannabis user versus control) was a key inclusion criteria.

Note: Item 14. Key potential confounding variables applied were Age, Gender, IQ, Tobacco use, Alcohol use – please see Figure 2.2 (main text) for more information.

Note: Optimal outcome = no shading; suboptimal outcome = dark grey shading; not applicable = light grey shading

NIH = National Institutes of Health

CHAPTER 3:

General Methods

Chapter Guide

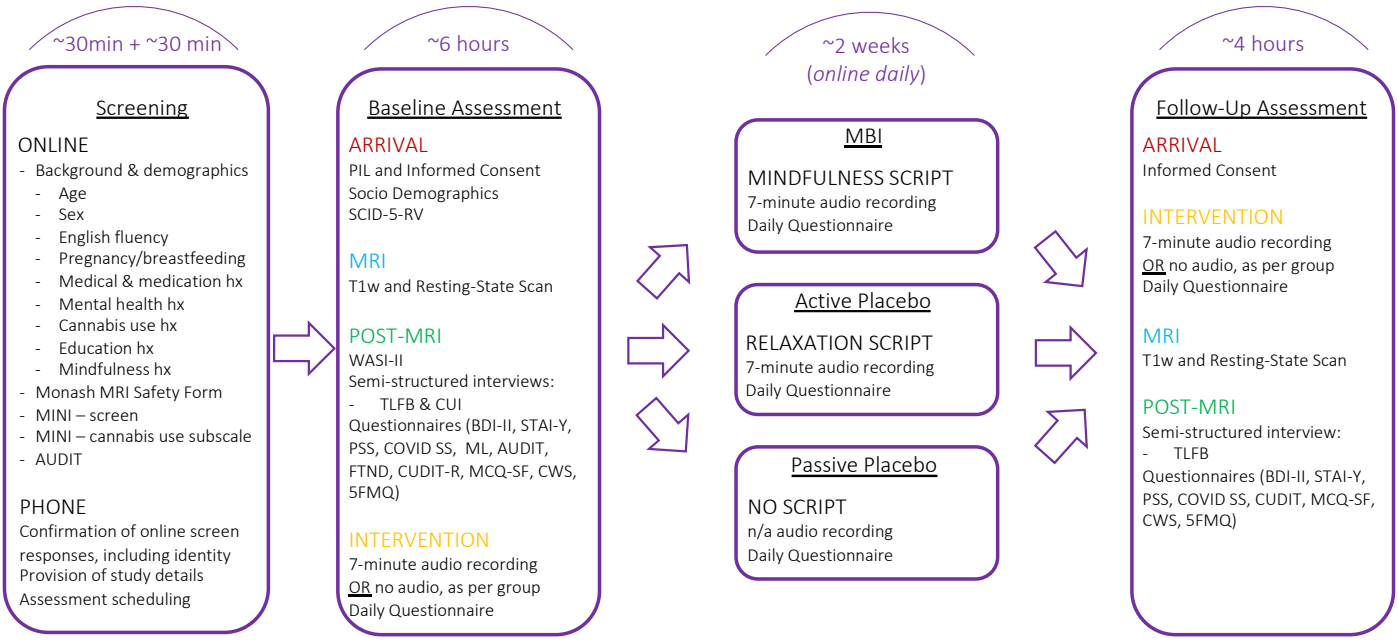
This chapter outlines the methodology of the two empirical experiments of this thesis: *'Study 2:* Investigating Resting-State Functional Connectivity Differences between people with a Moderate-to-Severe Cannabis User Disorder and Controls: An fMRI study (*Chapter 4*) and *'Study 3:* How does a Brief Mindfulness Intervention Reduce Resting-State Functional Connectivity Changes in Cannabis Use Disorder? A Double-Blind, Active and Passive Placebo-Controlled fMRI study (*Chapter 5*). The chapter will include specific information regarding the samples (i.e., how it was impacted by the COVID-19 pandemic, inclusion and exclusion criteria, target sample sizes, and recruitment procedures), which comprise people who use cannabis near daily and meet the criteria for moderate-to-severe Cannabis Use Disorder (CUD) and controls. Also, the chapter will detail the testing protocol: ethical procedures, study design, testing measures, data collected, study procedure, and data analyses planned for the two empirical experiments (*Chapter 4: Study 2 and Chapter 5: Study 3*). Of note, this chapter will provide specific details about the studies' methodologies, some of which were omitted from the specific methods sections for brevity and to adhere to publication writing standards.

As will become apparent across this chapter, the procedures described represent the combined efforts of a team of people including staff and student researchers. Within this team, my role in the data collection sessions was as a *blinded* tester. In this role, I collected the data of approximately one third of the total sample, specifically for a total of 41 participants, across 62 face-to-face testing sessions – cumulating to ~310 hours of testing time. I additionally reviewed online screens of approximately 250 prospective participants and completed ~150 15-to-60-minute screening phone calls; trained junior RAs and student researchers in cognitive, MRI, and clinical assessments; as well as overseeing participants' booking, scheduling, sending of session reminders, and pre-session covid screening of approximately half of the total sample.

3.1 Scope of the Experiments

The empirical experiments of this thesis are nested within a larger study: 'Mapping short-term brain changes in cannabis users: An fMRI study'. The larger study was pre-registered with ISRCTN (*Appendix 4*; found at https://doi.org/10.1186/ISRCTN76056942; registration ID: ISRCTN76056942). Selected measures were extracted from the larger study to address the aims and hypotheses of this thesis, therefore only the relevant components will be detailed within this General Methods chapter. Figure 3.1 briefly overview the study procedure with relevant measures, to be detailed within this chapter. Many of the included measures described were utilised on multiple occasions (i.e., during screening and at both empirical experiments), this will be noted throughout, and each measure only be described.

Figure 3.1. *Protocol testing flowchart with assessment tools measures and intervention components*



5FMQ = Five-Facet Mindfulness Questionnaire; AUDIT = Alcohol Use Disorder Identification Test; BDI-II = Beck Depression Inventory – II; COVID SS = COVID Stress Scale; CUDIT-R = Cannabis Use Disorder Identification Test – Revised; CUI = Cannabis Use Interview; CWS = Cannabis Withdrawal Scale; FTND = Fagerström Test Of Nicotine Dependence; MCQ-SF = Marijuana Craving Questionnaire – Short Form; MINI = The MINI International Neuropsychiatric Interview 7.0.2; ML = Marijuana Ladder; MRI = Magnetic Resonance Imaging; PIL = Participant Information Letter; PSS = Perceived Stress Scale; SCID-5-RV = Structured Clinical Interview of DSM-5 – Research Version; STAI-Y = State Trait Anxiety Inventory – Y form; TLFB = Timeline Follow Back; WASI-II = Wechsler Abbreviated Scale Of Intelligence – Second Edition

3.2 Ethics and Funding

The study was approved by the Australian Catholic University Human Research and Ethics Committee as meeting the requirements of the National Statement on Ethical Conduct in Human Research (HREC number 2019-71H; see *Appendix 5*). The study was supported by an Australian Government Research Training Program Scholarship and via an ACU internal competitive scheme. The Chief Investigator (Associate Professor Valentina Lorenzetti) was supported by an Al and Val Rosenstrauss Senior Research Fellowship (2022-2026) and by a NHMRC Investigator Grant (2023-2027).

3.3 Study Design

3.3.1 Study 2: (Chapter 4)

For the first empirical experiment, titled 'Investigating Resting-State Functional Connectivity Differences between people with a Moderate-to-Severe Cannabis User Disorder and Controls: An fMRI study' a cross sectional, between subject (people with Cannabis Use Disorder [CUD] vs controls), case control study design was used.

3.3.2 Study 3: (Chapter 5)

For the second empirical study, titled 'How does a Brief Mindfulness Intervention Reduce Resting-State Functional Connectivity Changes in Cannabis Use Disorder? A Double-Blind, Active and Passive Placebo-Controlled fMRI study', we used a double-blind, repeated-measure study with baseline and follow-up testing within ~2-weeks. Participants were allocated to one of three interventions using a pseudo-randomised order (stratified by age and sex). The study used a mindfulness-based intervention (MBI) as a primary intervention, and two placebo conditions: active-placebo (i.e., relaxation intervention) and passive-placebo (i.e., no intervention). It used a between-subject (i.e., MBI vs active placebo vs passive placebo) and within-subject design (baseline vs follow-up).

The researchers who were responsible for all data collection were *blinded* to the intervention condition of the participants, except for the delivery of the intervention which was done by separate

researchers *unblinded* to the intervention type. For details about the intervention procedures, please see section below 3.6.2.1 *Overview of Face-to-Face Session Procedures*. Additionally, participants were blinded to their intervention condition. Double-blinding was utilised to minimize biases on the data and on the intervention, given known expectancy effects of both testers and participants (Colagiuri, 2010). An active placebo condition was utilised to inform on intervention specific effects, whilst a passive placebo condition was utilised to parse apart the effects of engaging with the testing protocol.

3.4 Participants

3.4.1 A Word on the Impact of COVID-19

At the conception and commencement of this study, the recruitment target was a sample size of 120 people, comprising 90 people with a CUD, and 30 controls for cross-sectional comparison for Study 2. The 90 people with a CUD were to then be split into three equal groups of 30 people for examining intervention-group-by-time effects within Study 3. Data collection commenced in October 2019 and concluded in August 2022. Subsequently, several COVID-19-related hiatuses were encountered by the research team. Due to COVID-19-related disruptions and associated limitations on interaction with participants via data collection and on travel to testing sites, the target sample for the CUD group was reduced from 90 to 60 subjects, and to maintain power the target sample for controls was raised from 30 to 40 participants. The sample sizes achieved for both groups were ultimately larger than the COVID-adjusted targets (*Study 2:* 65 people with a CUD vs 42 controls).

In accordance with lockdown regulations specific to Melbourne, Australia, COVID-19, data collection was suspended between March and November 2020, as well as during the brief February 2021 lockdown (12/02/2021 to the 17/02/2021). Across the later three 2021 lockdowns (28/05/2021 – 10/06/2021, 15/07/2021 – 27/07/2021, and 5/08/2021 – 21/10/2021) the Victorian government issued permission for essential research to continue with data collection, via a statement released by acting Premier James Merlino MP on 27/05/2021. Data collection was therefore permitted to continue during this period on the grounds that it contained an intervention component that would benefit the wellbeing and mitigate health risks for members of the community who participated in the study

(Study 3). Furthermore, participation was permitted on the grounds that it would be prohibitive to restart, meaning prospective participants who had *already completed screening* were permitted to attend for their baseline session (and/or for their follow-up session if in the CUD group) due to the time sensitive nature of the inclusion and exclusion criteria. These reasons enabled the research team to maintain a somewhat consistent testing rate during 2021 of ~1.5 sessions/week. Meanwhile, we continued to screen prospective participants recruited via online platforms, and placement of recruitment flyers in the community immediately following the lifting of COVID-19 restrictions on people's travel distance and essential reasons for leaving the homes.

3.4.2 Sample Inclusion and Exclusion Criteria

3.4.2.1 Inclusion Criteria

Inclusion criteria for all participants were: (1) age between 18 years and 55 years; (2) having normal-to-corrected vision; (3) fluency in English; and (4) ability to attend testing sessions in person.

For people with a CUD only: (1) use of cannabis on a daily or almost daily basis for ≥12 months; (2) attempt to quit or to reduce cannabis use at least once within the past 24 months; and (3) have a diagnosis of moderate-to-severe CUD, confirmed by the endorsement of ≥ 4 CUD symptoms from the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5; American Psychiatric Association [APA], 2013), measured using the Structured Clinical Interview of DSM-5 – research version (SCID-5-RV; First et al., 2015). See *Chapter 1: Thesis Introduction and Overview, Figure 1.9, page 20* for DSM-5 CUD diagnostic criteria.

3.4.2.2 Exclusion Criteria

Exclusion criteria for all participants were: (1) Magnetic Resonance Imaging (MRI) contraindications, measured using the Monash Biomedical Imaging MRI Screening and Information Form; (2) unwillingness to refrain from any illicit substance and/or alcohol use in the 12 hours before testing (confirmed upon arrival at session); (3) current use of prescription medication that affects the central nervous system (CNS) except anti-depressants and anxiolytics, due to elevated depression/anxiety levels in CUD; (4) history of any diagnosed psychiatric disorders, with the exception for depression and anxiety disorders due to the high comorbidity with CUD, or current

suicidal ideation, as confirmed using The MINI International Neuropsychiatric Interview 7.0.2 (MINI; Lecrubier et al., 1998; Lecrubier et al., 1997; Sheehan et al., 2015; Sheehan et al., 1998); (5) history of any neurological disorders or major medical conditions (e.g., epilepsy, stroke, migraine, etc.); (6) history of acquired or traumatic brain injury or loss of consciousness > 5 minutes; (7) full scale intelligence quotient (FSIQ) estimate score < 70, confirmed using the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II; Wechsler, 2011); (8) current pregnancy and/or breastfeeding; (9) history of significant and regular mindfulness practice, defined as regular engagement in mindfulness practices over any extended period of time; or (10) significant use or dependence on alcohol confirmed by a score > 19 on the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2018).

For people with a CUD, exclusion criteria were: (1) significant exposure to substances other than cannabis or tobacco, as per >50 occasions of use over a 2-year period in the past 10 years; and (2) or use of any illicit drug other than cannabis in the four weeks prior to testing.

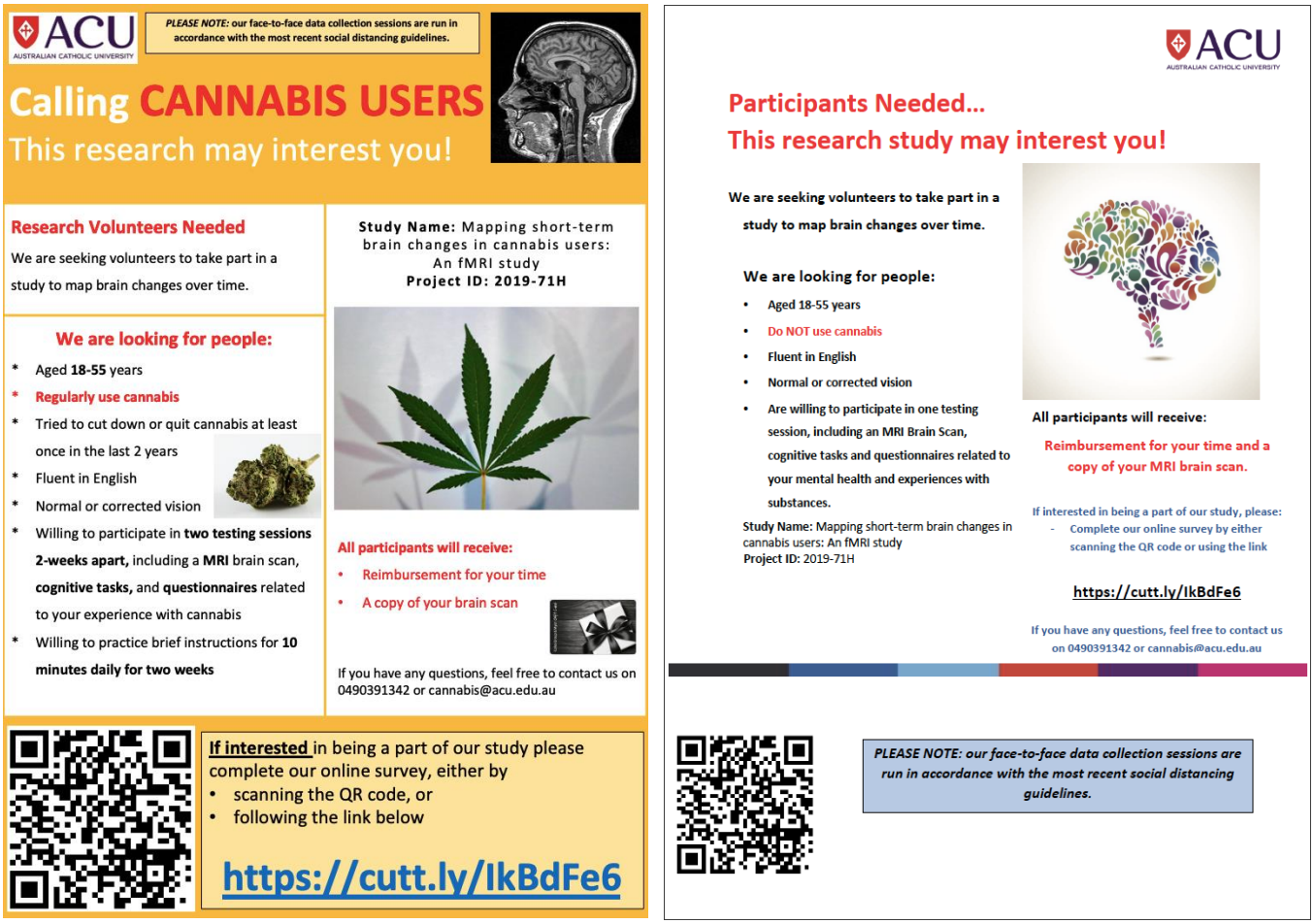
For controls only, exclusion criteria were: (1) significant exposure to substances other than tobacco, as per >50 occasions of use over a 2-year period in the past 10; (2) use of any illicit drug in the four weeks prior to testing; (3) use of cannabis at any stage in the 12 months prior to testing; or (4) >50 lifetime uses of cannabis.

3.4.3 Recruitment

Participants were recruited from the Greater Melbourne Metropolitan area, via online advertisements on various platforms (e.g., Google, Gumtree, Facebook, Instagram, Reddit, Tik Tok, Beat Online Magazine) and via printed flyers placed in public locations with high-foot traffic (see Figure 3.2). The flyers described key inclusion/exclusion criteria and directed interested members of the community to an online screening questionnaire via a link or QR code on the printed flyers. The online flyers provided a weblink to direct people to the online screening questionnaire (https://cutt.ly/lkBdFe6). The flyers also provided the study dedicated email address (cannabis@acu.edu.au) and phone number (+61 490 391 342), to enable members of the community

interested in participating to address any queries they might have prior to commencing screening questionnaires.

Figure 3.2. *Example of recruitment flyers for people with a CUD (A.) and controls (B.)*

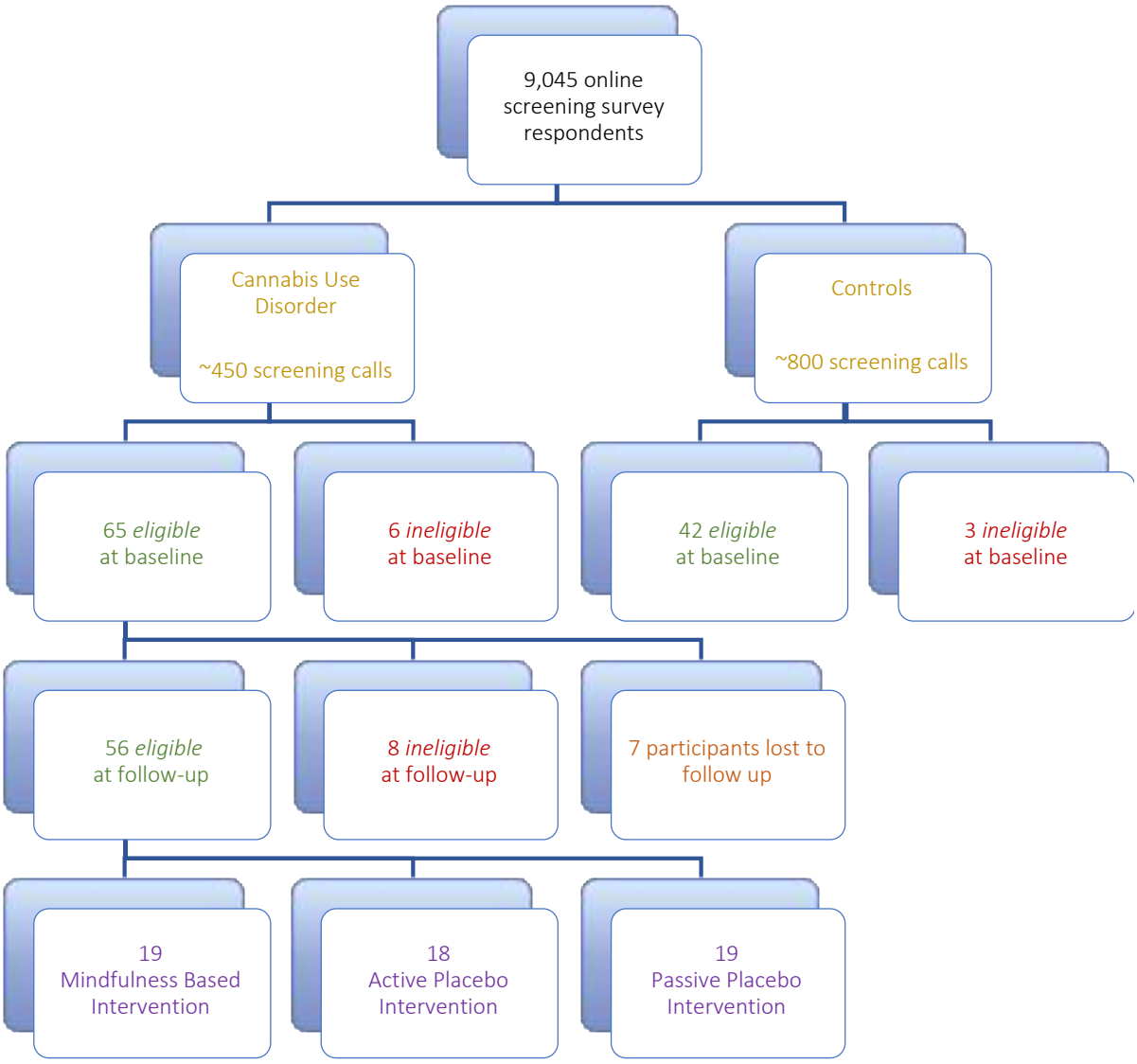


A. **B.**

3.4.4 The Sample: From Initial Enquiry to Data Analysis

For an overview of participant numbers across distinct stages of the recruitment process, please refer to Figure 3.3. Throughout the recruitment period (July 2019 to August 2022), there were 9,045 respondents to a ~30-minute online screening questionnaire to determine eligibility of community members against the study' inclusion and exclusion criteria. The testing measures used for the online screening are detailed below in section *3.5.1 Online Screening* and the procedures for the online screening are outlined in *3.6.1 Screening and Scheduling*.

Figure 3.3. *Overview of participant numbers through stages of the recruitment and testing process*



Of the survey respondents, ~800 people were deemed potentially eligible for the control group and were contacted for phone screening against the inclusion/exclusion criteria, using measures detailed in section *3.5.2 Phone Screening* and procedures as per *3.6.1 Screening and Scheduling*. Of the people called, 45 controls were deemed eligible and attended face-to-face data collection.

Furthermore, ~450 people were found to be potentially eligible for the CUD group and underwent phone screening to determine suitability for inclusion to the study. Of the people contacted, 71 people with a CUD were deemed eligible and attended the baseline session, and 64 completed the follow-up session (7 lost to follow-up). People who were deemed ineligible at either

the baseline or follow-up session continued through the entire assessment process and received reimbursement accordingly. They were then retrospectively excluded following data collection due to violation of exclusion criteria verified during face-to-face data collection. Of the 8 people whose ineligibility emerged at follow-up, 6 were the same individuals who were deemed as ineligible during baseline testing, and 2 were individuals deemed to be ineligible during follow up. For information relating to face-to-face data collection sessions, the measures are overviewed in section *3.5.3 Face-to-face Session* and the procedures in *3.6.2 Data Collection.*

A total of 166 participants attended at least the baseline session, 9 participants were excluded from Study 2 following data acquisition. They comprised 6 people with a CUD (of which 2 were female) and 3 controls (including 2 females), as they endorsed exclusion criteria during face-to-face baseline data collection. Specifically, the 3 controls had previously used cannabis at a level exceeding the limits of the exclusion criteria. Instead, for the CUD group, people were excluded for violating distinct exclusion criteria: FSIQ = 61 (n = 1); use of a prescription medication that affects the CNS (n = 1); and presence of neurological disorder (n = 4).

The final sample for Study 2 included 65 people with a CUD (19 females) with a median age of 25 years (*range:* 18-56 years), and 42 controls (16 females) with a median age of 25 years (*range:* 18-55 years). To note, one female participant in the CUD group who was 55 at the time of online screening but 56 at the time of data collection, was retained in the sample. Please see Study 2 (*Chapter 4*) for the specific sample characteristics for these groups.

The 65 participants with a CUD were invited to complete a two-week intervention involving pseudo-random allocation to one of three interventions unbeknownst to them (i.e., MBI, active control, or passive control) and attend a follow-up data collection session. Of the individuals with CUD, 58 participants completed baseline testing, the ~2-week intervention, and follow-up testing. An additional 7 people were lost to follow-up, during MBI (n=4, including 2 female), active placebo intervention (n = 1 female), and passive placebo intervention (n = 2, of which 1 was female). The analyses for the intervention (Study 3) did not include the 7 people who were lost to follow-up. An additional 2 people were excluded following data collection, due to an fMRI technical issue (n = 1), and for violating the exclusion criteria relating to use of illicit drug other than cannabis in the four

weeks prior to testing (n = 1). The final sample for the intervention Study 3 included 56 people with a CUD (14 females), aged a median of 25 years (*range:* 18-51). Please see Study 3 (*Chapter 5*) for the specific sample characteristics of this group.

3.4.5 Power Analysis

Post hoc power analyses were completed for both Study 2 and Study 3. Regrettably, an *a priori* post hoc power analysis was not possible due to the multiple novel elements of the project. Post hoc power analyses were generated in a multistep process. First, effect sizes were calculated for each of the resting-state Functional Connectivity (rsFC) analyses (i.e., cross-sectional for study 2, and pairwise comparison of the change of rsFC pre-to-post each intervention) by the utilisation of a Cohen's *d* converter (https://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD7.php). The Cohen's *d* effect size was then imputed into G*Power (Faul et al., 2009; Faul et al., 2007), to determine respective power values. Consequently, the rsFC analyses included within both studies were deemed to be adequately powered, see below for the specific power analyses for each Study.

3.4.5.1 Cross-sectional fMRI Study 2

The effect size (Cohen's *d*) was calculated for both the largest and the smallest p-value from comparing the rsFC between groups (CUD vs controls), utilising the final sample size of each group (CUD = 65; controls = 42). As detailed in *Chapter 4: section 4.4.2.2 Seed-Based Functional Connectivity*, the largest rsFC p-value was .0097 with a Benjamini-Hochberg (B-H) corrected α value of .0190, which corresponded to a Cohen's *d* of 0.52. The rsFC p-value threshold was <.0001 with a B-H corrected α value of .0071, which corresponded to a Cohen's *d* of at least 0.80.

The two effect sizes were then entered into G*Power, along with the sample sizes and respective α value (B-H corrected), resulting in a 1-β error probability range of .70 to at least 0.94.

3.4.5.2 Intervention fMRI Study 3

The effect size (Cohen's *d*) was calculated for both the largest and the smallest p-value of the pairwise comparisons of the rsFC between intervention groups (i.e., MBI vs active placebo; MBI vs passive placebo; active placebo vs passive placebo), utilising the final sample size of each group

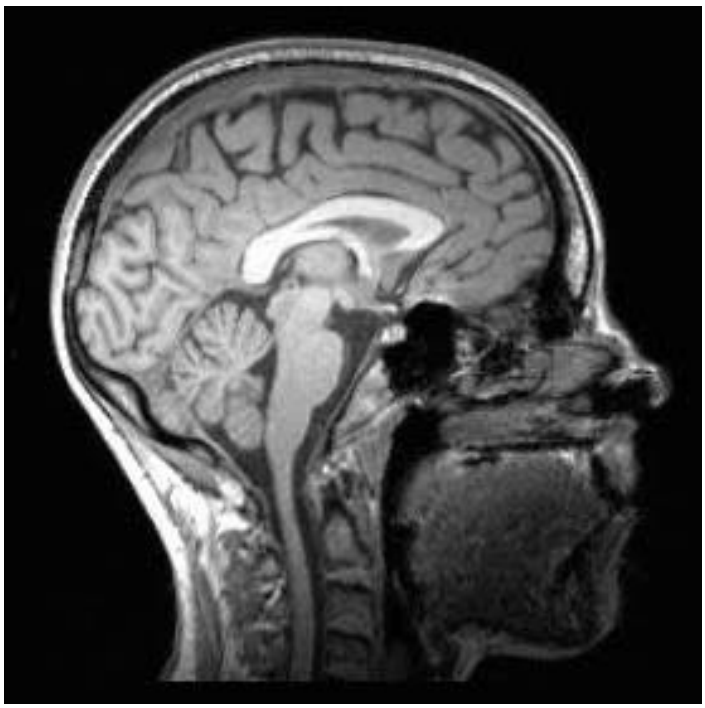
(MBI = 19, active placebo = 18, passive placebo = 19). As detailed in *Chapter 5: section 5.4.2.2 Post-Processing: Effects of Group-by-time on rsFC,* the largest rsFC p-value was .003 with a B-H corrected α value of .010 (i.e., MBI vs passive placebo), which corresponded to a Cohen's D of 1.04. The rsFC p-value threshold was <.001 with a B-H corrected α value of .002 (MBI vs active placebo), which corresponded to a Cohen's D of at least 1.16.

The two effect sizes were then entered into G*Power, along with the sample sizes and respective α value (B-H corrected), resulting in a 1-β error probability range of .69 to at least 0.76.

3.4.6 Informed Consent and Reimbursement

All participants provided informed consent prior to participation in the study, both online at the commencement of the online screening survey and in writing at the commencement of each face-to-face baseline and follow-up testing session. Please see *Appendix 6* for an electronic copy of the consent form for people with a CUD and controls. Participants were reimbursed for their time with a Coles Myer voucher (valued at AUD$150 for the CUD group and AUD$100 for controls). They also had the opportunity to obtain a 2D MR image of their brain, if interested – which entailed a 2D screenshot of a sagittal slice taken from the T1 scan of their own brain structure, see Figure 3.4.

Figure 3.4. *Example of a T1 weighted brain scan image, provided as a component of compensation*



3.5 Measures

All questionnaires used for participant screening and completed during face-to-face testing were administered via Qualtrics Software, version 2019-2022 (Qualtrics, Provo, UT).

3.5.1 Online Screening

3.5.1.1 Sociodemographic Information

Participants completed a standard sociodemographic questionnaire proforma, which contained items relating to participants' age, sex, and level of English fluency.

3.5.1.1.1 Edinburgh Handedness Inventory. Handedness was ascertained using the *Edinburgh Handedness Inventory – Short Form* (EHI-SF; Veale, 2014). This measure described four tasks (i.e., writing, throwing, teeth brushing, using a spoon). The participant was asked to rate their preferred hand (i.e., 'always right', 'usually right', 'both equally', 'usually left', 'always left'). Specifically, participants were asked to nominate their preferred hand for each task, and additionally indicate if their preference for their preferred side was so strong that they would not use the other side unless forced OR indicate if they did not have a preference between sides. The total scores indicated either left-handed, right-handed, or ambidextrous.

3.5.1.2 Health

All participants completed a standard health proforma, which contained questions relating to history of mental health related diagnoses, medication history, and status of pregnancy and/or breastfeeding.

3.5.1.2.1 MRI Safety Screen. The *Monash Biomedical Imaging MRI Screening and Information Form* was provided by the Monash Biomedical Imaging facility and is completed by all participants scanned at that site. It screened for contraindications for undergoing an MRI scan (i.e., currently pregnancy, metal in the body that cannot be removed, etc.). This screen can be viewed within *Appendix 7*, page 329.

3.5.1.2.2 MINI. *The MINI International Neuropsychiatric Interview* (MINI) 6.0.0 Screen contained 24 questions which screened for the 17 most common psychiatric disorders in the DSM-5,

including items determining suicidal ideation (Lecrubier et al., 1998). This screen can be viewed within *Appendix 7*, pages 325-328.

3.5.1.3 Substance Exposure and Related Problems

Participants completed a standard substance use history proforma, which contained questions relating to history of illicit or non-prescription substances. This included age of first use, frequency of use, duration of use, and date of most recent use, for all substances used by the participant (including cannabis). Participants were also asked about their history of attempting to quit or reduce their cannabis use, over the past 24-months.

3.5.1.3.1 MINI Cannabis Subscale. The *MINI 6.0.0 Substance Dependence/Abuse (Cannabis) Subscale* was administered to assess the presence and severity of a CUD based on the number of endorsed criteria (12 items; 1-3 = mild; 4-5 = moderate, 6+ = severe; Lecrubier et al., 1998). To note, this measure was used only as a screening tool for prospective participants, their DSM-5 CUD severity was confirmed via administration of the SCID-5-RV during face-to-face testing, see below section 3.4.3.1.3.

3.5.1.3.2 Alcohol Use Disorders Identification Test. The *Alcohol Use Disorders Identification Test* (AUDIT; Babor et al., 2018) is a 10-item screening tool developed by the World Health Organization (WHO) to assess alcohol consumption, drinking behaviour, and alcohol-related problems. The AUDIT provided diagnostic cut offs whereby scores of >19 indicated Alcohol Use Disorder.

3.5.1.4 Mindfulness

Participants responded to a standard set of questions indicating history of mindfulness practice, which included the frequency/duration of engagement in various types of mindfulness: Tai Chi, Meditation or Guided Meditation, Progressive Muscle Relaxation, Mindfulness, Yoga, Other.

3.5.2 Phone Screening

3.5.2.1 Physical health

During telephone screening, participants responded to a standard set of questions providing an indication of physical health. This included confirmation of normal (or corrected to normal) vision,

and history of any serious health condition which may impact results, including history of concussion and loss of consciousness, and neurological conditions.

3.5.2.2 Confirmation of Online Screen Responses

Responses provided during online screen were confirmed. Where necessary, online screen responses were probed to confirm details.

3.5.3 Face-to-Face Sessions

3.5.3.1 Baseline Face-to-Face Testing; To Address Study 2 & Study 3 Aims

A number of measures were administered only at the baseline session (i.e., were not monitored for change over time) and were required when addressing the aims of both Study 2 and Study 3.

3.5.3.1.1 Sociodemographic Information.

Participants completed a standard sociodemographic information questionnaire, which included questions relating to age, sex, and education history to determine total years of education.

3.5.3.1.2 Cognitive Testing.

The *Wechsler Abbreviated Scale of Intelligence* (WASI-II; Wechsler, 2011) is a standardized measure used to estimate participants' FSIQ. FSIQ estimates were derived from the administration of the Vocabulary and Matrix Reasoning subtests, which take approximately ~30 minutes total to administer.

3.5.3.1.3 Substance Exposure and Related Problems.

The SCID-5-RV (First et al., 2015) is a structured interview, which required participants to respond 'yes' or 'no' to 11-items (with additional probing if necessary) to determine the number of DSM-5 CUD items the participant endorsed. A score of 4-5 indicates moderate CUD, and a score of 6+ indicated severe CUD.

The *Cannabis Use Semi-Structured Interview* (CUI; Cuttler & Spradlin, 2017) was used to gather information on participants' cannabis use over their lifetime. This measure has been routinely utilised to map lifetime cannabis use in a research setting (Lorenzetti et al., 2015; Solowij et al., 2002;

Yücel et al., 2008). We extracted the age of *first* cannabis use, age of *regular* cannabis use (defined as onset of at least monthly use), and the duration of regular cannabis use.

The AUDIT was readministered in a face-to-face setting, see section 3.5.1.3.2 Online Screen above for details. The Fagerström Test for Nicotine Dependence (FTND; Fagerström et al., 2012) was administered to measure nicotine dependence. It is a standardised measure containing 6-items, which were scored as a mixture of either yes/no or Likert scale (0-3) responses. The possible score range was from 0 to 10. Scores ≥ 3 indicate presence of nicotine dependence.

3.5.3.2 Baseline Face-to-Face Testing; To Address Study 3 Aims

One measure was administered only at the baseline session (i.e., was not monitored for change over time); it was only required for analyses of the intervention Study 3.

3.5.3.2.1 Motivation to Change.

The *Marijuana Ladder* (Slavet et al., 2006) was administered to provide an indication of participant's motivation to stop cannabis use / readiness to change. Participants selected where they sat on a scale ranging from 0 (No thought about quitting, I cannot live without cannabis) to 9 (I have changed my cannabis use, but I still worry about slipping back, so I need to keep working on the changes I have made). Scores of 0-2 corresponded with the pre-contemplation stage, 3-5 with the contemplation stage, 6-7 with the preparation stage, 8 with the action stage, and 9 with the maintenance stage.

3.5.3.3 Baseline and Follow Up Face-to-Face Testing; To Address Study 2 & Study 3 Aims

The following measures were administered at the baseline testing session and again at the follow-up testing session (i.e., were monitored for change over time), and were required for the analyses of both Study 2 and Study 3. In order to monitor for change over time, the value of each variable *at baseline* was subtracted from the value of the corresponding variable *at follow-up.*

3.5.3.3.1 Substance Exposure and Related Problems.

The Timeline Follow Back (TLFB; Sobell & Sobell, 1992) is a semi-structured interview and it was used to gather information on recent (past 30 days or intervention period) substance use. Completion of the TLFB *at baseline* required participants to provide estimates of their use of substances (e.g., cannabis, alcohol, nicotine, and other) over the past 30 days, using a calendar-format

document to record substance use for every day in the month prior. Participants were encouraged to enter personal events into the calendar, and to check their social media and mobile phone history (i.e., recent posts to social media, recent photographs captured on camera roll) to act as a memory aid. The number of days used, and the number of units used/consumed per day (i.e., grams of cannabis, standard drinks of alcohol, number of cigarettes), over the past 30 days, were recorded for each relevant substance (i.e., cannabis, alcohol, and/or nicotine). Hours since last cannabis use was also recorded.

The TLFB was again administered *at follow-up* to gather information on cannabis use across the intervention period. Administration followed the same calendar 'memory-aid' procedure as utilised at baseline, however the TLFB duration was amended to reflect each participants specific intervention duration. The number of days cannabis was used on and the number of grams of cannabis consumed per day over the intervention period were recorded, as well as the hours since last cannabis use.

To directly compare cannabis use in the lead up to each session (i.e., pre-baseline compared to pre-follow-up), additional measures of 'cannabis days' and 'cannabis dose' were derived using the already collected data from the *TLFB* at baseline. For each participant, the total grams of cannabis consumed and the number of days on which cannabis was consumed on *pre-baseline* were calculated across a time interval which matched the participant's intervention duration. These variables are referred to as '*cannabis days – intervention duration'* and '*cannabis dose – intervention duration'*, to differentiate from 'number of days used, and the number of grams of cannabis consumed per day over the past 30 days' collected at baseline.

The *Cannabis Use Disorder Identification Test – Revised* (CUDIT-R; Adamson et al., 2010) is an 8-item, cannabis misuse screening tool. Items were rated on a 3- or 5-point Likert scale, with possible scores ranging from 0 to 32. Scores of 12 or more are indicative of severe CUD, the diagnostic cut off points have been shown to be consistent with the DSM-5 CUD severity cut offs, whereby score ≥ 13 on the CUDIT-R identify 'severe' CUD (SCID-5-RV ≥6). The CUDIT-R was utilised in brain-behaviour correlation analyses rather than the SCID-5-RV, given the increased variance within the CUDIT-R measure.

The *Beck Depression Index-II* (BDI-II; Beck et al., 1996) is a standardised measure of mood with diagnostic ranges, containing of 21- items. It was used to quantify participants' experiences of depression over the past fortnight. Each item was rated on a 4-point Likert scale, with higher scores representing greater severity of depression i.e., 0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression, 29–63: severe depression.

The *State-Trait Anxiety Index – Y Form*, 'state' sub-scale (STAI-Y; Spielberger et al., 1983) was used to measure state anxiety in the moments preceding the MRI acquisition. It contained 20-items, on a 4-point Likert scale. Higher scores indicated greater state anxiety.

The *Perceived Stress Scale* (PSS) – 10 items (Cohen et al., 1983) was used to quantify participant's perception of their stress over the past fortnight. It measured the degree to which situations in the participant's life were appraised as stressful. Items were designed to quantify how unpredictable, uncontrollable, and overloaded participants find their lives, as well as a number of direct queries about current levels of experienced stress. The 10 items were rated on a 5-point Likert scale. Scores ranging from 0-13 would be considered low perceived stress, 14-26 moderate perceived stress, and 27-40 high perceived stress.

The *COVID Stress Scale* (COVID SS; Taylor et al., 2020) was used to measure of COVID-related worries over the past week. It contained 36-items over 5 subscales (i.e., COVID danger and contamination, COVID socioeconomic consequences, COVID xenophobia, COVID traumatic stress, and COVID compulsive checking), summed to measure a specific 'COVID Stress Syndrome'.

3.5.3.4 Baseline and Follow Up Face-to-Face Testing; To Address Study 3 Aims

The following measures were administered at the baseline session and again at the follow-up session (i.e., were monitored for change over time), and were required only for the intervention Study 3 analyses. In order to monitor for change over time, the value of each variable *at baseline* was subtracted from the value of the corresponding variable *at follow-up.*

3.5.3.4.1 Substance Exposure and Related Problems.

The *Marijuana Craving Questionnaire – Short-Form* (MCQ-SF; Heishman et al., 2009) was administered to obtain an indication of the magnitude of cravings for cannabis experienced by the participant in the moment of questionnaire administration. It contained 12-items on a 7-point Likert

scale, with higher scores indicating greater cravings. The *Cannabis Withdrawal Scale* (Allsop et al., 2011) was administered to monitor participant's experiences of cannabis withdrawal over the past 24-hours. It contained 19-items, measured on a 10-point Likert scale (from not at all to extremely). Higher scores indicated greater impact of withdrawal symptoms on functioning.

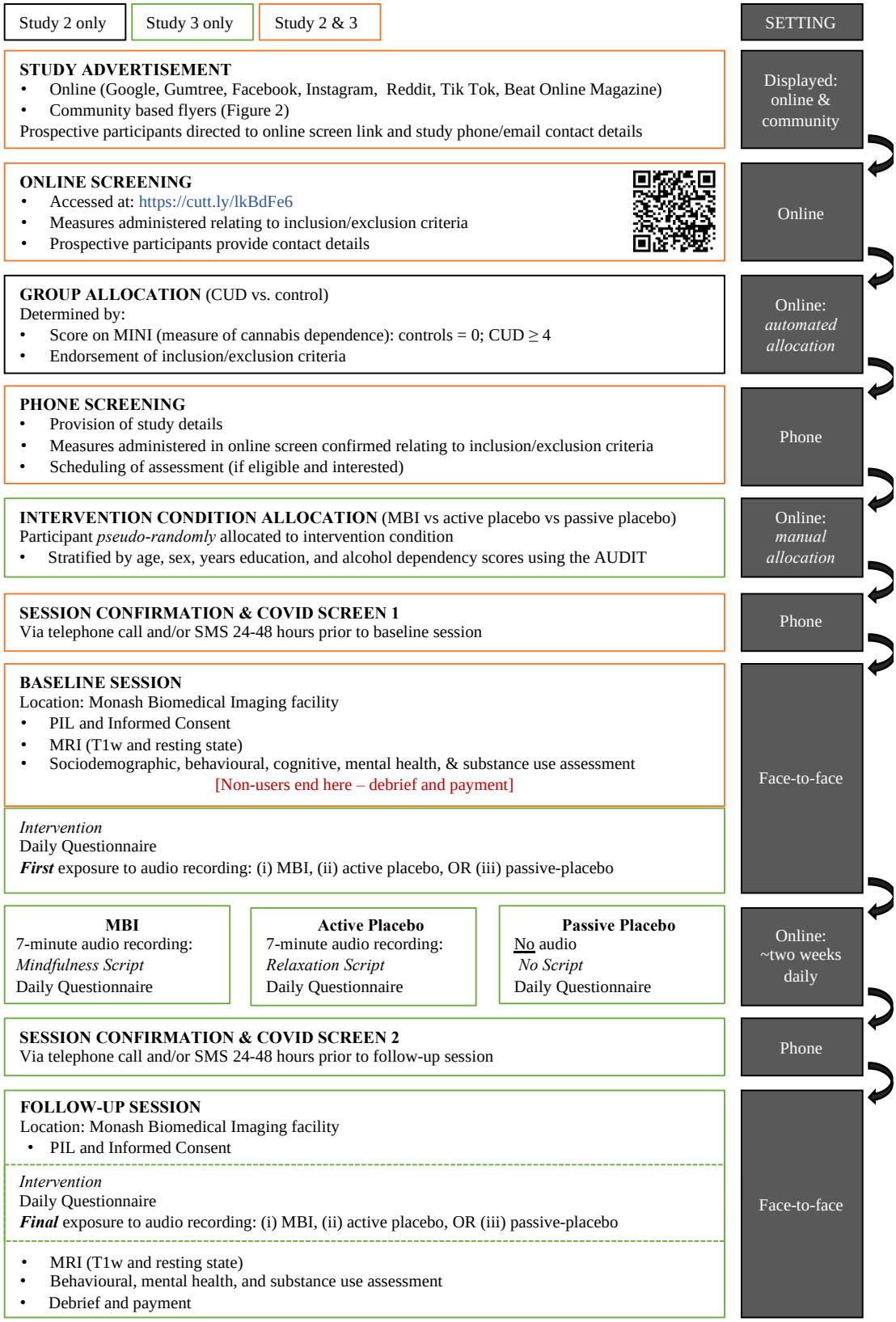
3.5.3.4.2 Mindfulness.

Participants completed the 5FMQ (Baer et al., 2008) to provide a measure of their perceived mindfulness and self-awareness. The five subscales (i.e., observation, description, aware actions, non-judgemental inner experience, and non-reactivity) were summed to provide a total score, whereby higher scores indicated greater mindfulness/self-awareness.

3.6 Procedure

All behavioural data collection and MRI acquisitions were conducted at the Monash Biomedical Imaging facility in Clayton, Victoria. Figure 3.5 overviews the testing procedure of the study, and the section below gives detailed information about each stage of the testing procedure.

Figure 3.5. *Overview of the testing procedure*



MINI = score on The MINI International Neuropsychiatric Interview 6.0.0 Substance Dependence / Abuse (Cannabis) Subscale; MBI = Mindfulness Based Intervention; MRI = Magnetic Resonance Imaging; PIL = Participant Information Letter; T1w = T1 weighted.

3.6.1 Screening and Scheduling

3.6.1.1 Online Screening: Determining Eligibility of Community Members Interested in the Study

Community members interested in the study were required to complete a ~30-minute online screening questionnaire (https://cutt.ly/lkBdFe6). The online screener consisted of validated questionnaires and additional items (detailed above 3.5.1 *Online Screen*), in order to collect data to ascertain if individuals met the study's inclusion and exclusion criteria e.g., age 18-to-55 years, history of substance use, medical health, mental health, and experience with mindfulness practices.

Student researchers carefully checked the responses from the online screener to determine if members of the community were either (i) ineligible for the study, or (ii) eligible for a *phone screening* call, in order to collect additional information to confirm eligibility or ineligibility.

3.6.1.2 Phone Screening: Confirming Participants' Eligibility

Phone calls placed to participants to complete telephone screening largely adhered to a script, please see *Appendix 7* for the Telephone Screen Script. Participants were provided with a study description including their specific involvement requirements, reimbursement, confidentiality, data storage, and risk and handling of incidental findings on MRI. The measures administered during the telephone screen are detailed in 3.5.2 *Phone Screen* above. The screening call was used to confirm responses to inclusion or exclusion criteria, as necessitated by their online screen. Medical history was confirmed with the participant and the MRI Safety screen (Monash Biomedical Imaging MRI Screening and Information Form, included within *Appendix 7*, page 329) was repeated (having already been administered during online screening). Any queries about participants' eligibility were resolved via discussion with the study Chief Investigator (VL) and research team members. In instances where there was a greater than four-week period between phone screening and scheduled session date, elements of the phone screen were repeated prior to the session to ensure eligibility was not violated (i.e., recent substance use).

3.6.1.3 Booking Protocol

Eligible participants were invited to participate, and a mutually convenient date and time was selected for the face-to-face session(s). Follow-up sessions were scheduled two weeks after the baseline session, with some variability to accommodate participant schedules. Using email

correspondence, participants were sent a booking confirmation email, with the consent form, participant information letter (PIL), and map attached (see *Appendices 6*, *8*, and *9*).

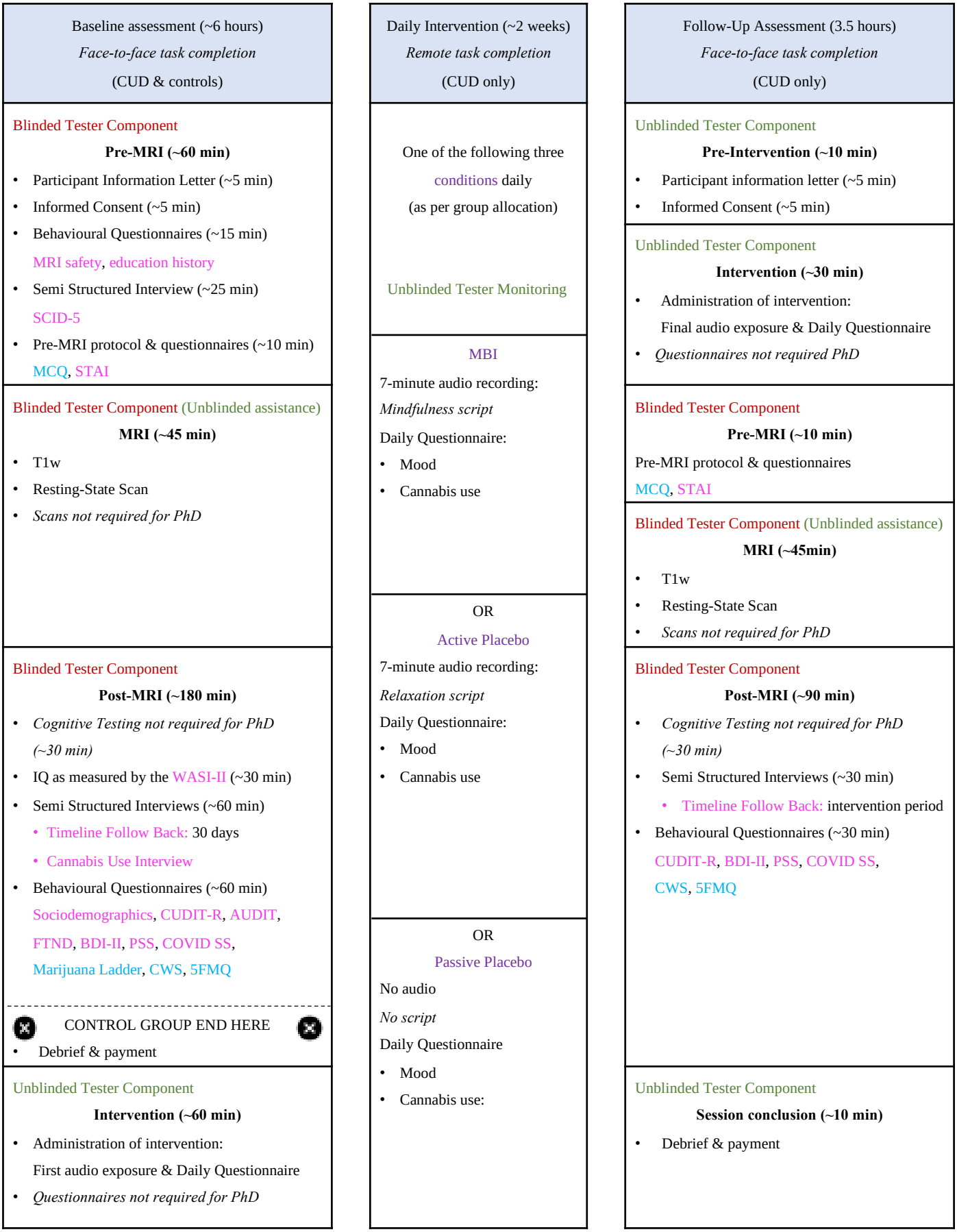
3.6.1.3.1 COVID-19 Screening. Approximately 48-hours prior to attendance of each session, a COVID-19 screening call was placed to participants to check for symptoms of COVID-19, or close contact exposures. If testing was scheduled 2+ weeks after the screening phone call, a COVID-19 screen was also completed about 2 weeks before testing. Participants who endorsed COVID-19 screen responses indicating risk of COVID-19 (i.e., symptomatic or recent COVID-19 exposure) had their sessions rescheduled. (*Note*: COVID-19 screening was introduced for all participants tested after November 2020). Participants received confirmation SMS messages 24 hours prior to their attendance at each session (which included a reminder to refrain from illicit substance and/or alcohol use in the 12 hours prior to testing).

3.6.1.3.2 Intervention Condition Allocation. Following booking confirmation, participants in the CUD group were pseudo-randomly allocated to an intervention condition (i.e., either MBI, active-placebo, or passive-placebo). This was facilitated by an *unblinded* study co-ordinator (AC or PH). Group allocation was stratified by age and sex.

3.6.2 Data Collection

This section provides an overview of the components of the data collection process, namely the two face-to-face data collection sessions (baseline and follow-up) and the brief intervention, see Figure 3.6.

Figure 3.6. *Overview of face-to-face sessions and intervention period*



Note: Data collected prior to this point ⊗ constitutes Study 2 (CUD vs controls), data collected across entire graphic constitutes Study 3 (pre-to-post intervention comparison). Measures written in pink were used for both Study 2 and Study 3 aims, measured written in blue were used for Study 3 aims only.
5FMQ = Five-Facet Mindfulness Questionnaire; AUDIT = Alcohol Use Disorder Identification Test; BDI-II= Beck Depression Inventory; COVID SS = COVID-19 Stress Scale; CUDIT-R = Cannabis Use Disorder Identification Test – Revised; CWS = Cannabis Withdrawal Scale; FTND = Fagerström Test for Nicotine Dependence; MCQ = Marijuana Craving Questionnaire; MRI = Magnetic Resonance Imaging; PSS = Perceived Stress Scale; SCID-5-RV = Structured Clinical Interview for DSM-5 Disorders; STAI = State-Trait Anxiety Inventory; T1w = T1 weighted; WASI-II = Wechsler Abbreviated Scale of Intelligence – Second Edition.

3.6.2.1 Overview of Face-to-Face Session Procedures

Both the CUD group and controls attended the baseline session, which lasted ~6 hours for people in the CUD group and ~5 hours for controls, as controls were not required to spend the final hour on the intervention. The CUD group also completed a ~2-week intervention whereby they were required to complete daily tasks; the specific requirements of the daily tasks were determined by the intervention condition of the participant. Participants within the CUD group also attended a follow-up session (~3.5 hours), approximately two weeks after the baseline session.

3.6.2.1.1 Tester Blinding Procedures. At each session, testing was run by both an *unblinded tester* and by a *blinded tester*. The *unblinded tester* was aware of the group allocation (i.e., MBI, active placebo, passive placebo) of each participant in the CUD group and administered their intervention-related components. The *unblinded tester* assisted with technical aspects of the MRI scanning relating to the administration of fMRI tasks, without interacting with the participant. The *unblinded tester* debriefed with and compensated the participants in the CUD group at the completion of the follow-up session. Finally, the *unblinded tester* monitored intervention compliance across the ~2-week intervention period.

The *blinded tester* was blinded to the intervention condition allocation of each participant in the CUD group. The *blinded tester* administered all remaining aspects of testing but importantly was not involved with intervention components. Specifically, the *blinded tester* oversaw initial informed

consent (follow-up informed consent was overseen by the *unblinded tester* as a part of the intervention administration), fMRI data acquisition, structured interviews relating to cannabis use, and cognitive testing. The *blinded tester* also oversaw questionnaires (delivered online via Qualtrics) covering the following domains: (i) demographic information, (ii) substance use and related problems, and (iii) mental health and wellbeing measures. The conclusion of the baseline session (debrief and reimbursement) for the controls was overseen by the *blinded* tester.

An overview is next provided of the baseline and follow-up session, detailing the order of events and the measures completed. The baseline and follow-up sessions followed a specific order, for which comprehensive checklists were utilised, please see *Appendix 10*. Specific information relating to the measures within this section is subsequently expanded below.

3.6.2.2 The Baseline Session

Upon arrival participants were provided with the informed consent form and the participant information letter (PIL; please see *Appendices 6 & 8*) in hardcopy format. The points on the consent form were discussed and participants were given the opportunity to ask questions prior to signing.

Participants completed the first phase of structured interviews and questionnaires (including the MRI Safety screen, education history, SCID-5-RV, MCQ, and STAI. Participants were then briefed on the MRI process, and completed the scan. Following the scan, participants completed the second phase of structured interviews and questionnaires, as well as cognitive testing (including the WASI-II, 30-day TLFB, CUI, sociodemographic questions, CUDIT-R, AUDIT, FTND, BDI-II, PSS, COVID SS, Marijuana Ladder, CWS, and 5FMQ.

Participants in the control group were then able to debrief and receive reimbursement. Participants in the CUD group were instead taken through the requirements of their daily ‘at-home’ questionnaire, and if applicable, listened to the first audio recording of their intervention (MBI and active-placebo).

3.6.2.3 The Follow-up Session

Participants in the CUD group returned following ~2-weeks for their follow up session. They commenced by again providing informed consent, before listening to the final audio recording of their intervention (if applicable) and responding to the final administration of their *daily questionnaire*.

Participants then completed the first phase of questionnaires (a repeat of the MCQ and STAI), before again completing the MRI scan, which following identical procedures as the baseline session. Following the scan, participants completed the second phase of structured interviews and questionnaires. They included the TLFB, covering the intervention period, and the CUDIT-R, BDI-II, PSS, COVID SS, CWS, and 5FMQ. Participants were then able to debrief and receive reimbursement.

3.6.3 Overview of the ~Two-Week Intervention

All participants in the CUD group completed a ~two-week intervention in one of three groups: MBI, active placebo, or passive placebo. The initial administration was completed at the final stage of the baseline session, participants then autonomously completed the intervention each day between their two sessions, and finally, participants again completed the intervention at the initial stage of the follow-up session. The completion of the intervention during each session was supervised by the *unblinded* tester.

Briefly, both the MBI and active placebo intervention conditions involved a combination of listening to a 7-minute audio track once per day and completion of a *daily questionnaire*; the passive placebo group completed the same *daily questionnaire*, without an audio track. The audio track and *daily questionnaire* were accessed remotely via a Qualtrics link, participants used their own personal device (typically a smart phone).

3.6.3.1 Monitoring Intervention Compliance

Daily intervention compliance was monitored by an *unblinded* tester, who was able to determine remotely if the participant had opened the Qualtrics link each day. SMS reminders were sent to participants who missed one day and again after two days. After missing three consecutive days, a phone call to participants was made to ascertain if they were experiencing any issues in completing or accessing the task(s) and assistance provided if required.

3.6.3.2 Daily Questionnaire

A *daily questionnaire* (~3-minutes) was used to collect information on behavioural variables across the intervention period to aid data interpretation, and was completed by participants in all three intervention conditions. All cannabis users were asked to provide daily estimates of their cannabis use (number of occasions and quantity), and instances of dangerous use (i.e., "have you been able to suspend your cannabis use to be 'safer' or to aid performance?"). All cannabis users were also asked each day to rate the intensity of their cannabis cravings and urges, their ability to "step back and be aware of cravings/urges without being taken over by them", their mental state, their levels of relaxation-tension and nervousness/stress, and judgement of thoughts as "good or bad". For participants in the MBI and active placebo group, their *daily questionnaire* also contained items on intervention compliance (e.g., "Since the last time you completed this questionnaire, have you listened to the audio track?"; "When you felt the urges or craving to smoke cannabis, have you practiced the strategy you have been listening to on the audio track?").

3.6.3.3 Audio Instructions for the MBI and Active Placebo Relaxation Intervention

The MBI group listened to a 7-minute mindfulness track and the active placebo group listened to a time-matched relaxation track. Both the MBI and the active placebo tracks were previously validated in an alcohol use intervention (Kamboj et al., 2017) and adapted for cannabis. The scripts were read and recorded by an experienced voice artist/actor, who has previously recorded guided meditations for the prominent digit meditation program, Smiling Mind (www.smilingmind.com.au). Please see *Appendix 11* for a copy of the complete MBI and active placebo scripts.

3.6.3.3.1 MBI, Mindfulness Script. The MBI group were told that "noticing, paying attention to, and accepting" their thoughts and physical sensations, could increase their ability to experience cannabis cravings without acting on them. It was emphasized that the aim was not to simply relax, but to be alert and attentive. Participants were guided through "open monitoring" of experience and particularly through "aware[ness] of feelings and bodily sensations" and experiencing "craving in a different way", in order to highlight 'craving' as a temporary event in the body.

3.6.3.3.2 Active Placebo, Relaxation Script. The active placebo group was told that cannabis craving intensity can be reduced by "softening the muscles…and calming and unwinding the

mind…releasing tension in your body" and that relaxation enables transformation of sensations into more calming, less unpleasant experiences. Participants were instructed to pay attention to their breath, to facilitate the release of tension. It was emphasized that this is a way of managing craving or urges to smoke cannabis.

3.6.3.3.3 Passive Placebo, No Script. Participants in the passive placebo group did not listen to any scripted recordings. Instead, this group only completed a *daily questionnaire*, to minimize discernment of allocation to the passive placebo group.

3.7 Statistical Analysis

All details relating to the statistical analyses of the behavioural data and brain behaviour correlations are contained within the Methods section of the relevant chapter. Specifically, for the cross-sectional Study 2 the statistical analyses performed to examine descriptive variables, fMRI group comparisons and brain behaviour correlations are outlined in Chapter 4, section 4.3.4 Statistical Analyses and 4.3.5.8 Brain Behaviour Correlations. Further, for Study 3 the group comparisons and group-by-time-interactions effects on descriptive variables and fMRI data, and brain behaviour correlations, are outlined in Chapter 5, section 5.3.5 Statistical Analyses and 5.3.6.8 Brain Behaviour Correlations.

3.8 Neuroimaging Data Acquisition and Processing

The protocol outlined below was administered identically for the baseline and follow-up session. All participants were scanned using the same group of experienced radiographers at the Monash Biomedical Imaging facility in Clayton, Victoria. The total MRI scanning time included additional MRI tasks not relevant to this PhD and was approximately 45 minutes. Table 3.1 provides a full list of the scan sequences administered to all participants.

Table 3.1. *Total MRI data acquisition sequence, in order*

Scan	Duration (minutes)
Localiser Scan	~0.5
T1-weighted Anatomical Scan	~6
Resting-State Scan	~8
Cue Reactivity Task*****	~9
Gradient Field Mapping***** (completed during MIDT practice)	~0.2
Monetary Incentive Delay Task (MIDT)*****	~12

***** Scans not required for this PhD

3.8.1 MRI Task Setup

Prior to the MRI, participants were briefed on what to expect and remember during the scan. This included information regarding the noise in the scanner and the importance of remaining still during scans and not moving between MRI acquisition sequences to ensure high image quality. Participants removed metal items prior to scanner entry (i.e., belt buckle, jewellery). The communication process between the tester outside the MRI scanner and the participant inside the MRI scanner, was explained. We informed the participants about the presence of the 'emergency buzzer', should the participant wish to urgently communicate with the tester or to leave the scanner, and intercom use during scan to receive task instructions and communicate with tester. A vitamin D capsule was placed on participants' *right* forehead (i.e., a marker) to create a bright spot in the MRI image, enabling clear identification of left vs right hemisphere of the brain on the MR images.

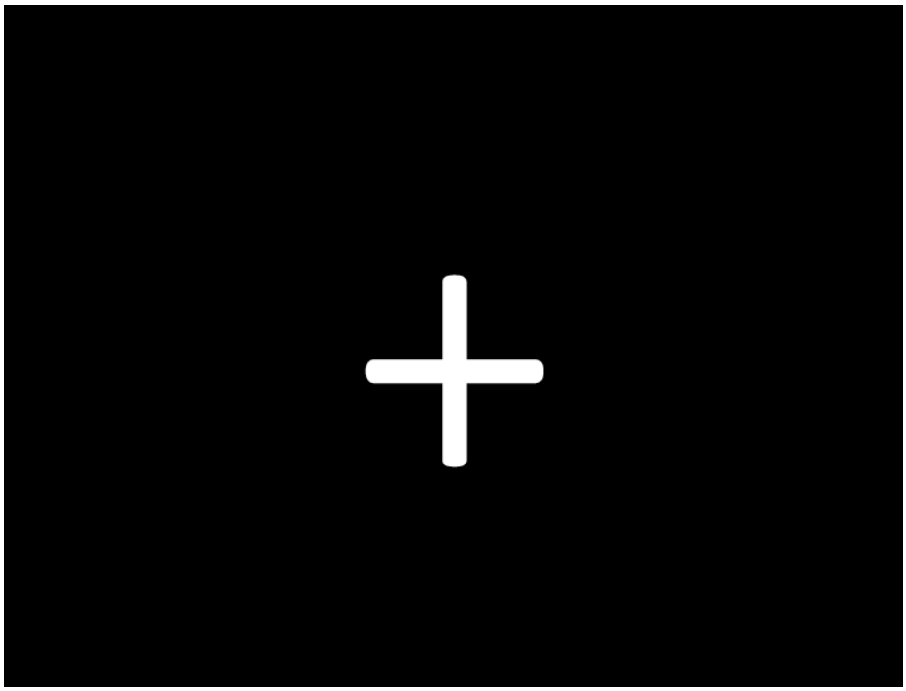
Prior to entering the scanner, participants were requested to stay awake; the tester checked in real-time that participants were awake and kept their eyes open throughout the scan, via an MRI-compatible camera placed inside the MRI scanner. Once the participant laid inside the scanner, a head-coil was placed on top of their head with a mirror attached, so they could view a screen outside the machine to visualise the images administered during the fMRI tasks, and also to ease anxiety of being in a narrow space.

To note, in the event that a participant dozed off or fell asleep during the resting-state scan, the MRI scanning was halted. The participant was gently woken up by the blinded tester by and removed from the scanner briefly until they reported feeling alert (they were given the opportunity to go outdoors, use the bathroom, have a stretch etc.). The participant then returned to the scanner to repeat the resting-state scan once they reported feeling fully vigilant. The localiser and T1w acquisition were also repeated.

3.8.2 MRI Instructions

Participants were instructed to keep still during the T1w scan; the radiographer notified the participant that the scan would run for approximately 6 minutes. Prior to the resting-state scan acquisition, whilst inside the MRI scanner, participants were instructed verbatim by the researcher and via written instructions: “The next scan will take about 10 minutes. Keep your eyes open, try not to think about anything in particular. Stay relaxed and try to keep your head still”. Through the resting-state scan, participants were shown a fixation cross (white cross on black background; see Figure 3.7).

Figure 3.7. *Image of the fixation cross, displayed on screen to be shown to participant throughout resting-state fMRI data acquisition, via an MRI compatible mirror*



3.8.3 MRI Data Acquisition Parameters

Participants were scanned on a Siemens Skyra 3 Tesla MRI scanner (Figure 3.8) using a 32-channel head coil. *Appendix 12* overviews in detail the parameters for acquiring the structural MRI data and the fMRI data. *T1-weighted* (T1w) scans were acquired using magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, with the following acquisition parameters: TE = 2.07ms, TR = 2300ms, flip angle = 9°, 192 sagittal slices without gap, field of view 256 x 256mm, yielding a 1 x 1 x 1mm resolution, for a total acquisition time of 5 minutes. *Resting-state* fMRI scans (189 volumes) using T2* weighted Echo Planar Imaging (EPI) were acquired over 8 minutes, using the following parameters: TR = 2500ms, TE = 30ms, flip angle = 90°, field of view = 192mm, matrix = 64, voxel size $3mm^3$, 44 slices without gap, and a total acquisition time of 480 seconds. Slice based acceleration Generalised Autocalibrating Partially Parallel Acquisitions (GRAPPA) = 2 was applied. The Siemens system includes an additional two 'dummy scans' at the beginning of the acquisition to allow the magnetization to stabilise to a steady state. These two scans were not collected for any data analysis.

Figure 3.8. *Siemens Skyra 3 Tesla MRI scanner located at Monash Biomedical Imaging facility, Clayton, Victoria, Australia*

Image retrieved from https://www.monash.edu/researchinfrastructure/mbi/facilities/human/3t-mri

3.8.4 MRI Data Handling

The raw MRI data acquired for each participant, were directly exported from the scanner to Monash Biomedical Imaging-XNAT (XNAT website, private server), where it was stored and backed up in Digital Imaging and Communications in Medicine (DICOM) format. MRI data in raw format (i.e., DICOM) were downloaded from the XNAT server and converted into Brain Imaging Data Structure (BIDS) format using dcm2niix (v1.0.20201102) for further analysis. All MRI data processing and analysis were performed on a cloud-based cluster-computational platform, MASSIVE (massive.org.au; Goscinski et al., 2014). The pre- and post-processing was conducted using CONN toolbox 20.b (www.nitrc.org/projects/conn, RRID:SCR_009550; Whitfield-Gabrieli & Nieto-Castanon, 2012), based on SPM12 on Matlab (2018a.r7487), which was pre-installed on MASSIVE.

3.8.5 MRI Data Pre-Processing

All raw MRI data were imported in BIDS format, then underwent a standard pre-processing using CONN toolbox 20.b, including 1) slice timing with interleaved slice order; 2) realignment and generation of motion parameters; 3) ARtifact-detection Tools (ART)-based outlier detection with intermediate settings (default 97th percentile in normative sample); 4) co-registered fMRI data with T1w images; 5) segmentations of T1w images; 6) normalising T1w images to Montreal Neurological Institute (MNI) space (standard space), and normalising fMRI to MNI space with the same parameters; 7) smoothing with 6mm kernel, and 8) temporal bandpass filtering (0.008-0.09). Default CONN toolbox 20.b pre-processing steps also include a combination of aCompCor, scrubbing, and motion correction. fMRI was then resampled at 2x2x2mm isotropic. Quality Assurance (QA) reports were generated and manually reviewed. Stringent criteria for detecting motion outliers, as outlined by Parkes et al. (2018), was followed, whereby limits of >0.25mm mean framewise displacement (mFD) and >5mm maximum framewise displacement were set; no participants violated these criteria.

3.9 Neuroimaging Data Analysis

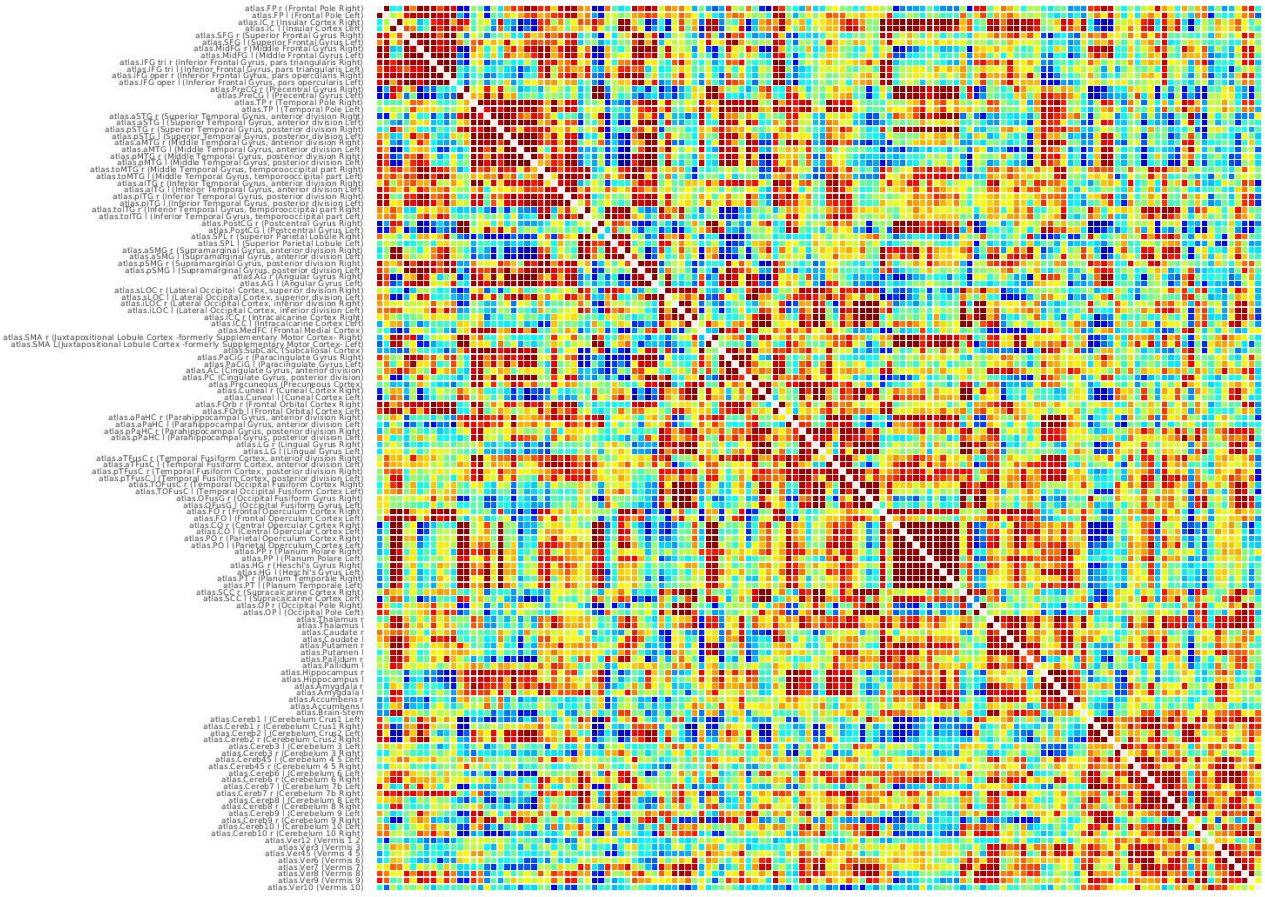
The methods and techniques of the neuroimaging data analysis are described below, specifically relating to each empirical experiment.

3.9.1 Study 2

3.9.1.1 Connectome Analysis

The connectome matrix (132 x 132) for each scan was generated using the default Harvard-Oxford parcellation (132 cortical and subcortical regions; Desikan et al., 2006) using CONN toolbox 20.b (conn/rois/atlas.nii), with method of bivariate correlation and haemodynamic response function (HRF) weightings. Figure 3.9 shows an example of a connectome matrix from a single subject within the sample, selected at random. These connectome matrices were fed into the graphic analysis using a General Linear Model (GLM) model using group as a factor (between subjects, two-group t-test), with the contrast CUD < controls. Covariates were age, sex, alcohol standard drinks/past 30 days and number of cigarettes/past 30 days, and BDI-II depression symptom scores. We corrected the results using the multiple comparison error method termed cluster-level false discovery rate (FDR), with Benjamini-Hochberg p-corrected < 0.05, and using a multi-voxel pattern analysis (MVPA) omnibus test (i.e., the current model versus the null model; Norman et al., 2006).

Figure 3.9. *Connectome matrix (132 x 132) of a single subject, selected at random from the sample*



3.9.1.2 Seed-Based Functional Connectivity

Brain regions selected as seeds were also referred to as regions of interest (ROIs). They were based on prior knowledge outlined in *Chapter 4*. Table 3.2 overviews the seeds examined and their coordinates in Montreal Neurological Institute (MNI) stereotaxic space. The location of the seeds was determined by the default Harvard-Oxford atlas.

Seed-based functional connectivity maps were generated using CONN toolbox 20.b. Briefly, a bivariate correlation coefficient was calculated between the timeseries of each seed and that of each other voxel in the whole brain, controlling for nuisance factors such as the six realignment parameters and six first order temporal derivatives, motion parameters, global signal, and signal from white matter, cerebral spinal fluid, and whole brain. The mean values within the seeds (i.e., mean rsFC values for the seed to cluster) were extracted using CONN toolbox 20.b for further brain behaviour correlation analyses.

Table 3.2. *Overview of seeds examined and coordinates in MNI stereotaxic space, for each Study*

Study 2	**Study 3**	**Seeds chosen as ROIs**	**Hemisphere**	**MNI Coordinates**
yes	yes	Nucleus Accumbens	Left	-11, 9, -7
			Right	11, 9, -7
yes	yes	Putamen	Left	-28, -3, 7
			Right	28, -3, 7
yes	yes	Pallidum	Left	-18, -7, 1
			Right	18, -7, 1
yes	-	Caudate	Left	-16, 2, 18
			Right	16, 2, 18
yes	yes	Hippocampus	Left	-27, -18, -16
			Right	27, -18, -16
yes	-	Amygdala	Left	-22, -4, -15
			Right	22, -4, -15
yes	-	Precentral Gyrus	Left	-38, -12, 55
			Right	38, -12, 55
yes	-	Anterior Cingulate Cortex	-	0, 33, -7

Note: MNI coordinates are taken from the rough centre of the mass, seeds are anatomic in shape, ROI = region of interest.

3.9.1.2.1 MRI Data Post-Processing. The group effect of the seed-based connectivity map was analysed using the same GLM model and contrasts as described above in section *3.9.1.1 Connectome Analysis*. The seed-based connectivity map was generated using the same GLM model and contrasts as described above in section *3.8.1.1 Connectome Analysis*. An FDR correction for multiple comparisons was applied. A Benjamini-Hochberg procedure was additionally applied to alpha values to decrease the FDR following the examination of multiple ROIs (Benjamini & Hochberg, 1995). The mean values within the ROIs (i.e., mean rsFC values for the seed to cluster) were extracted using CONN toolbox 20.b for further brain behaviour correlation analyses, for those seeds which survived the two multiple comparison corrections.

3.9.2 Study 3

3.9.2.1 Connectome Analysis

The connectome matrix (132 x 132; see example Figure 3.9 above) for each scan were generated using the default Harvard-Oxford parcellation on CONN toolbox 20.b, with method of bivariate correlation and HRF weightings. Then, these connectome matrices were fed into the graphic analysis using a longitudinal design. For the longitudinal design, the two timepoints were input as different sessions to set up the within subject contrast (follow-up – baseline). Intervention groups were set up as a three-level factor (i.e., MBI, active placebo, passive placebo). Three t-contrasts were selected between all combinations of two intervention types (i.e., MBI vs active placebo; MBI vs passive placebo; active placebo vs passive placebo). We applied a multiple comparison error correction, specifically a cluster-level FDR correction, with p-corrected < 0.05, using an MVPA omnibus test.

3.9.2.2 Seed-Based Functional Connectivity

ROIs were selected as seeds based on prior knowledge, as overviewed in Table 3.2 with their coordinates in MNI stereotaxic space. The seeds were determined by the default Harvard-Oxford atlas within CONN toolbox 20.b. Seed-based functional connectivity maps were generated using CONN toolbox 20.b. Briefly, a bivariate correlation coefficient was calculated between the timeseries of each seed and the that of each other voxel in the whole brain, controlling for nuisance factors such as

motions parameters, global signal, signal from white matter, and signal from cerebral spinal fluid. The seed-based rsFC maps for the four seeds were used for further post-processing.

3.9.2.2.1 MRI Data Post-Processing. Post-processing (statistical analysis) was conducted using CONN toolbox 20.b. The longitudinal design (i.e., follow-up – baseline) and contrasts (i.e., MBI vs active placebo; MBI vs passive placebo; active placebo vs passive placebo) was set up following the same parameters described above (section 3.9.2.1 *Connectome Analysis*). To control for multiple comparison errors, we applied a cluster level FDR correction (p-corrected < 0.05), with an initial default threshold ($p<0.001$). Next, for each of the four independent seeds, we applied a further Benjamini-Hochberg multiple comparison error correction, with a threshold of $p < 0.05$ (equivalent to p-FDR corrected < 0.001). Group-by-time interaction effects on the rsFC values between the seeds and the rest of the brain, were carried out for those seeds which survived the two multiple comparison corrections. The mean values within the ROIs (i.e., mean rsFC values for the seed to cluster) were extracted using CONN toolbox 20.b for further brain behaviour correlation analyses.

CHAPTER 4:

Study 2: The First Empirical Experiment

Chapter Guide

This chapter contains the first of the two empirical experiments included within this PhD, which result from the data collection detailed in the General Methods (*Chapter 3*). The ***primary aim*** of this first empirical experiment, which constitutes the current chapter, is to map the neurobiological differences between people with a moderate-to-severe Cannabis Use Disorder (CUD) who have tried to quit or reduce their cannabis use within the past two years, and controls. Neurobiology was measured via resting-state functional connectivity (rsFC), quantified using functional Magnetic Resonance Imaging (fMRI). The ***secondary aim*** of this empirical experiment is to explore how identified group differences in rsFC correlate with metrics of cannabis exposure and related problems i.e., Cannabis Use Disorders Identification Test – Revised (CUDIT-R) scores, age of *first* and of *regular* use onset, hours since last use, and frequency, duration, and dosage of cannabis use. Importantly, this paper aims to address important limitations identified in this field of research, as outlined in the published Systematic Literature Review (*Chapter 2*) and Thesis Introduction and Overview (*Chapter 1*).

This empirical experiment has been prepared with publication in mind. Therefore, sections of this study, in particular the Methods, are detailed succinctly where possible to adhere to word limits as per academic publishing standards. The examiners will be directed within this chapter where applicable back to the General Methods (*Chapter 3*) for a more comprehensive overview of methodologies, should they wish to revise specific details.

Title Page: For Planned Publication

Investigating Resting-State Functional Connectivity Differences between people with a Moderate-to-Severe Cannabis User Disorder and Controls: An fMRI study

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Conflict of interest: The authors report no potential conflicts of interest.

4.1 Abstract

Rationale: Cannabis Use Disorder (CUD) is experienced by 22 million people worldwide and can be associated with adverse psychosocial and health outcomes, particularly in those experiencing more severe forms of CUD. Such outcomes have been (partly) ascribed to neurobiological changes within the addiction neurocircuitry, corroborated by emerging functional neuroimaging (fMRI) evidence in regular cannabis users that measures brain function using resting-state functional connectivity (rsFC). However, no fMRI study to date has investigated rsFC in people with a diagnosis of CUD.

Objectives: We aimed to use fMRI and compare for the first time rsFC between people with a moderate-to-severe CUD who had tried to quit or reduce their use within the last two years, and controls. Regions which were previously shown to have altered rsFC in regular cannabis users, ascribed to neuroscientific theories of addiction and cognitive processes altered in cannabis users, and dense in cannabinoid receptors type 1 (CB_1R), were selected as regions-of-interest (ROIs). Secondarily, this study aimed to explore how identified rsFC differences would be associated with metrics of cannabis exposure and related problems (i.e., Cannabis Use Disorders Identification Test – Revised [CUDIT-R] scores, age of *first* and *regular* use onset, hours since last use, and frequency, duration, and dosage of use).

Methods: 107 participants (65 with a CUD, 42 controls) aged 18-to-56 years underwent comprehensive fMRI scanning, as well as substance use, mental health, and cognitive testing. We explored group differences by generating seed-based connectivity maps, controlling for age, sex, alcohol and nicotine dose, and depression scores in the GLM. Regions displaying altered rsFC were then correlated with metrics of cannabis exposure and related problems.

Results: CUD vs controls showed greater frontostriatal, occipito-striatal, and occipito-parietal-striatal rsFC, and lower occipito-hippocampal rsFC. Correlations were found between altered pallidum-occipital rsFC with frequency of cannabis use, altered pallidum-occipital/occipito-parietal rsFC with CUD symptom scores, and altered putamen-occipito-parietal rsFC with the age of *first* and of *regular* cannabis use onset.

Discussion: In the first study to utilize rsFC in people with a moderate-to-severe CUD vs controls, established rsFC alterations were largely consistent with hypotheses. Frontostriatal hyperconnectivity

supports the notion that CUD affects the addiction neurocircuitry akin to other SUDs, and such alterations may underlie (or predate) early onset, compulsive cannabis use and altered salience processing, characteristic of CUD. Given the growing rates of CUD and increasing access to high potency and addictive cannabis products, more research is required to confirm which people who use cannabis are most vulnerable to rsFC alterations and how to mitigate neurobehavioral problems established in those experiencing severe forms of CUD.

Keywords*:* functional magnetic resonance imaging, fMRI, resting-state functional connectivity, rsFC, seed-based connectivity, brain, cannabis, cannabis use disorder, CUD

4.2 Introduction

Cannabis Use Disorder (CUD) is experienced by 22 million people worldwide (Degenhardt et al., 2018). CUD is characterised by an inability to voluntarily cease consumption of cannabis, despite an underlying desire to do so, and/or in the face of related physical or psychological harms (American Psychiatric Association [APA], 2013; World Health Organisation [WHO], 2016). Such harms can include risk-taking behaviours (e.g., driving or operating machinery while intoxicated; Australian Institute of Health and Welfare, 2020), greater symptoms and prevalence of mental health disorders e.g., mood disorders (Gibbs et al., 2015; Gobbi et al., 2019; Lev-Ran et al., 2014; Twomey, 2017) and psychotic disorders (Curran et al., 2019; Kuepper et al., 2011; Large et al., 2011; Marconi et al., 2016; Rössler et al., 2012; Schoeler et al., 2016; Wright et al., 2021), as well as with reduced cognitive performance (e.g., executive function, attention, learning and memory, and psychomotor skills; Figueiredo et al., 2020; Grant et al., 2003; Lorenzetti et al., 2020; Lovell et al., 2020; Schreiner & Dunn, 2012). The psychosocial problems associated with CUD have been (partly) ascribed to neurobiological changes within the addiction neurocircuitry (Bloomfield et al., 2019; Koob & Volkow, 2010, 2016; Zehra et al., 2018).

Emerging evidence from functional neuroimaging (fMRI) research has shown that people who regularly use cannabis have altered brain function during rest, without performing any cognitively demanding tasks (Thomson et al., 2022 [*Chapter 2*]). Such function is measured via resting-state functional connectivity (rsFC), a proxy of brain integrity without cognitive confounds (Smitha et al., 2017), which measures correlations between the spontaneous fluctuations of the blood oxygen level dependent (BOLD) signal of two or more brain regions (Greicius et al., 2009; Smitha et al., 2017; van de Ven et al., 2004). Specifically, regular cannabis users vs controls, show greater rsFC in brain pathways within the addiction neurocircuitry (Koob & Volkow, 2010; Volkow et al., 2016), including fronto-frontal (e.g., anterior cingulate cortex [ACC], prefrontal gyrus [PFG], orbitofrontal gyrus [OFC]), fronto-temporal (e.g., OFC, ACC and hippocampus, amygdala), and frontostriatal region pairings (e.g., PFC, frontal pole, and nucleus accumbens [NAc], putamen, pallidum, caudate; Thomson et al., 2022 [*Chapter 2*]). Furthermore, rsFC alterations in cannabis users were shown to be

associated with varying measures of cannabis exposure (i.e., age of use onset and regular use duration; Thomson et al., 2022 [*Chapter 2*]).

The extant body of evidence on rsFC differences in regular cannabis users compared to controls is limited by methodological issues which preclude the mapping of which neurocircuitry is affected in cannabis users. First, no fMRI rsFC study to date has measured if participants endorsed a CUD using the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5; APA, 2013); while problems with cannabis use have been measured inconsistently (i.e., a third of studies measured cannabis dependence with diagnostic tools; Thomson et al., 2022 [*Chapter 2*]). Notably, problems with substance use are posited to be underscored by neuroadaptations in the pathways with reportedly different rsFC in cannabis users (Koob & Volkow, 2010; Volkow et al., 2016), and may drive neurobiological changes in cannabis users (Lorenzetti et al., 2016). Importantly, due to the unknown CUD status of participants, it is unclear if rsFC alterations shown in cannabis users thus far, are driven by a subgroup of cannabis users who endorse a CUD (Rossetti et al., 2021). Also, while it is known that more severe CUDs drive worse clinical and cognitive outcomes and costs to treatment services (Foster et al., 2018; van der Pol et al., 2013), there is a lack of neuroimaging studies on severe CUDs. Thus, the pathophysiology underlying severe forms of CUDs that represent the greatest burden of the disease in relation to cannabis use, remains unknown and neurobiological targets for treatment are yet to be identified.

Second, the literature to date has inconsistently controlled for a number of variables which could independently influence brain function (e.g., age and sex) and that are entrenched with CUD (e.g., high levels of alcohol and nicotine exposure, greater symptoms of depression./anxiety; Thomson et al., 2022 [*Chapter 2*]. Therefore, it is unclear if rsFC findings in cannabis users are (partly) driven by the demographic characteristics, alcohol/alcohol exposure or mental health comorbidities. Third, only few studies measured metrics of cannabis exposure and related problems, and explored their correlation with rsFC. Thus, whether cannabis exposure and related problems drive rsFC changes remain unconfirmed. Finally, the literature consists of small sample sizes (i.e., $n < 25$ in half of the examined samples; Thomson et al., 2022 [*Chapter 2*]), and may be therefore underpowered to detect subtle rsFC alterations in cannabis users.

This study sought to overcome the limitations of the literature to date in a general community sample of 107 people (35 female) aged 18-56 years. This sample was comprised of 65 people who met diagnosis of moderate-to-severe CUD and who had recently tried to cut down or quit cannabis, and 42 controls.

The ***primary aim*** was to compare groups by rsFC, accounting for age, sex, alcohol and nicotine exposure, and depression symptom scores. It was hypothesized that people with a CUD compared to controls would show different rsFC involving key regions of interest (ROIs). The selected ROIs were: striatal regions (i.e., NAc, putamen, pallidum, caudate), medial temporal regions (i.e., hippocampus, amygdala), and key cortical regions (i.e., ACC, and precentral gyrus). Importantly, the ROIs were selected on the basis of: (i) shown to have altered rsFC in regular cannabis users (Thomson et al., 2022 [*Chapter 2*]), (ii) being core components of the addiction neurocircuitry as per prominent neuroscientific theories of addiction (Koob & Volkow, 2010; Volkow et al., 2016), (iii) being implicated in cognitive processes altered in cannabis users (e.g. executive function, reward processing, inhibition, attention; Bloomfield et al., 2019); and (iv) being dense in cannabinoid receptors type 1 (CB_1R), to which delta-9-tetrahydrocannabinol (THC), the main psychoactive compound of cannabis (Radwan et al., 2017) binds to, to exert its effects (Glass et al., 1997; Herkenham et al., 1991; Julian et al., 2003; Zou & Kumar, 2018).

The ***secondary aim*** was to explore how rsFC differences identified in the CUD group vs controls would be associated with metrics of cannabis exposure and related problems (e.g., CUDIT-R scores, hours since last use, days/past 30 days, grams/past 30 days, age of *first* and of *regular* use onset, duration of regular use; Blanco-Hinojo et al., 2017; Filbey et al., 2014; Wetherill et al., 2015).

4.3 Method

This study was nested within a larger, pre-registered project (https://doi.org/10.1186/ISRCTN76056942; registration ID: ISRCTN76056942) and was approved by the Australian Catholic University Human Research and Ethics Committee (HREC:2019-71H).

4.3.1 Sample Inclusion and Exclusion Criteria

Unless otherwise specified, inclusion and exclusion criteria were confirmed by participant self-report and the use of a comprehensive online screening survey followed by a detailed screening over the phone.

4.3.1.1 Inclusion Criteria

Inclusion criteria for all participants were: (1) age between 18 years and 55 years; (2) having normal-to-corrected vision; (3) fluency in English; and (4) ability to attend testing sessions in person.

For people with a CUD only: (1) use of cannabis on a daily or almost daily basis for ≥12 months; (2) attempt to quit or to reduce cannabis use at least once within the past 24 months; and (3) have a diagnosis of moderate-to-severe CUD, confirmed by the endorsement of ≥ 4 CUD symptoms from the DSM-5 (APA, 2013), measured using the Structured Clinical Interview of DSM-5 – research version (SCID-5-RV; First et al., 2015). See *Chapter 1: Thesis Introduction and Overview, Figure 1.9, page 20* for DSM-5 CUD diagnostic criteria.

4.3.1.2 Exclusion Criteria

Exclusion criteria for all participants were: (1) MRI contraindications, measured using the Monash Biomedical Imaging Magnetic Resonance Imaging (MRI) Screening and Information Form; (2) unwillingness to refrain from any illicit substance and/or alcohol use in the 12 hours before testing (confirmed upon arrival at session); (3) current use of prescription medication that affects the central nervous system except anti-depressants and anxiolytics, due elevated depression/anxiety levels in CUD; (4) history of any diagnosed psychiatric disorders, with the exception for depression and anxiety disorders due to the high comorbidity with CUD, or current suicidal ideation, as confirmed using The MINI International Neuropsychiatric Interview 7.0.2 (MINI; Lecrubier et al., 1998; Lecrubier et al., 1997; Sheehan et al., 2015; Sheehan et al., 1998); (5) history of any neurological disorders or major medical conditions (e.g., epilepsy, stroke, migraine, etc.); (6) history of acquired or traumatic brain injury or loss of consciousness > 5 minutes; (7) full scale intelligence quotient (FSIQ) estimate score < 70, confirmed using the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II; Wechsler, 2011); (8) current pregnancy and/or breastfeeding; (9) history of significant and regular mindfulness practice, defined as regular engagement in mindfulness practices over any

extended period of time; or (10) significant dependence on alcohol, confirmed by a score > 19 on the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2018).

For people with a CUD only, exclusion criteria were: (1) significant exposure to substances other than cannabis or tobacco, as per >50 occasions of use over a 2-year period in the past 10 years; or (2) or use of any illicit drug other than cannabis in the four weeks prior to testing.

For controls only, exclusion criteria were: (1) significant exposure to substances other than tobacco, as per >50 occasions of use over a 2-year period in the past 10; (2) use of any illicit drug in the four weeks prior to testing; (3) use of cannabis at any stage in the 12 months prior to testing; or (4) >50 lifetime uses of cannabis.

4.3.2 Procedure

People were recruited from the Greater Melbourne Metropolitan area, via community-based flyers and online advertisements on various platforms (e.g., Google, Gumtree, Facebook, Instagram, Reddit, Tik Tok, Beat Online Magazine). Members of the community who were interested in the study were directed to (i) an online screening survey to determine their eligibility against inclusion and exclusion criteria (~30-minutes, 9,045 respondents), followed by (ii) a phone screener to confirm eligibility of potentially suitable participants (lasting ~10 minutes to ~1 hour; 450 prospective people with a CUD and 800 prospective controls were called). A detailed list of the tools used for the online and telephone screening is outlined in the study pre-registration (https://doi.org/10.1186/ISRCTN76056942; registration ID: ISRCTN76056942), and in *Chapter 3: General Methods, section 3.5.1 Online Screening* and *3.5.2 Phone Screening.* Eligible participants then attended face-to-face data collection at Monash Biomedical Imaging facility, Clayton, Victoria, Australia.

The testing session lasted approximately 5-6 hours (including the collection of measures beyond the scope of this study). Participants provided written informed consent and completed an assessment battery to profile socio-demographic data, substance use, mental health, and cognition, comprising questionnaires, face-to-face semi-structured interviews, standardised cognitive testing, as well as MRI scanning.

At the end of testing, participants were offered the opportunity to debrief and receive reimbursement in the form of a Coles Myer voucher to the value of AUD$100 for controls, and of AUD$150 for people with a CUD, as they underwent additional testing to address research questions beyond the scope of this study as described in *Chapter 3: General Methods* and *Chapter 5: Study 3*. All participants were also offered a picture from a single frame from their T1-weighted (T1w) scan.

All questionnaires used for participants' screening and face-to-face testing were administered via Qualtrics Software, version 2019-2022 (Qualtrics, Provo, UT).

4.3.3 Face-to-Face Assessment

4.3.3.1 Sociodemographic Data and Handedness

We measured participants' data on age, sex, and years of education using a standard demographic proforma. Handedness was ascertained using the *Edinburgh Handedness Inventory – Short Form* (EHI-SF; Veale, 2014).

4.3.3.2 Full Scale Intelligence Quotient

The *WASI-II* (Wechsler, 2011) was used to estimate participants' FSIQ, derived from the administration of the Vocabulary and Matrix Reasoning subtests.

4.3.3.3 Substance Exposure and Related Problems

The *SCID-5-RV* for DSM-5 (First et al., 2015) is a structured interview, which required participants to respond 'yes' or 'no' to 11-items (with additional probing if necessary) to determine the number of CUD symptoms the participants endorsed. A score of 4-5 indicates moderate CUD, and a score of 6+ indicated severe CUD.

The *Timeline Follow Back* (TLFB) methodology (Sobell & Sobell, 1992) is a structured interview, used to gather information on substance use over the past 30 days (e.g., cannabis, alcohol, nicotine, and other). We measured the number of days/past 30 days in which each substance was used (i.e., for cannabis, alcohol, nicotine) and the quantity of substance used/past 30 days (i.e., grams of cannabis, standard drinks of alcohol, number of cigarettes), as well as the number of hours since participants' last used cannabis.

The *Cannabis Use Semi-Structured Interview* (CUI; Cuttler & Spradlin, 2017) was used to gather information on participants' cannabis use over their lifetime. We extracted the age of *first* cannabis use, age of *regular* cannabis use (defined as onset of at least monthly use), and the duration of regular cannabis use.

Substance use and related problems were quantified for cannabis, alcohol, and nicotine, using the *Cannabis Use Disorders Identification Test – Revised (CUDIT-R;* Adamson et al., 2010), the *AUDIT* (Babor et al., 2018) and the *Fagerström Test for Nicotine Dependence (FTND;* Fagerström et al., 2012). Scores greater than 8 on the *CUDIT-R* indicate hazardous use and scores of 12 or more indicated a possible CUD. Scores greater than 19 on the AUDIT indicate Alcohol Use Disorder.

4.3.3.4 Mental Health

The *Beck Depression Inventory* – second edition (BDI-II; Beck et al., 1996), a standardised measure of mood with diagnostic ranges, was used to quantify participant's experiences of depression over the past fortnight. The *State-Trait Anxiety Index – Y* Form, 'state' sub-scale (STAI-Y; Spielberger et al., 1983) was used to measure state anxiety in the moments preceding the MRI acquisition. The *Perceived Stress Scale* (PSS) – 10 items (Cohen et al., 1983) was used to quantify participant's perception of their stress over the past fortnight. The *COVID Stress Scale* (Taylor et al., 2020) was used to measure of COVID-related worries over the past week. It contains 5 subscales (i.e., COVID danger and contamination, COVID socioeconomic consequences, COVID xenophobia, COVID traumatic stress, and COVID compulsive checking), summed to measure a specific 'COVID Stress Syndrome'.

4.3.4 Statistical Analyses

4.3.4.1 Sample Characteristics

We compared groups by sociodemographic data, IQ, substance use and related problems, and mental health symptom scores. We achieved this using Chi-squares for categorical variables (i.e., sex and handedness). We ran Mann-Whitney U for comparing groups for non-normally distributed data (i.e., age, years of education, depression scores, state anxiety scores, COVID stress scores, and alcohol/nicotine use and related problems). We performed independent samples t-tests for comparing

groups by normally distributed data (i.e., WASI-II FSIQ and perceived stress). All analyses were run using SPSS version 28.

4.3.5 Neuroimaging Data Acquisition and Processing

4.3.5.1 MRI Task Setup

Prior to entering the scanner participants were requested to stay awake; the tester checked in real-time that participants kept their eyes open throughout the scan, via an MRI-compatible camera placed inside the MRI scanner.

4.3.5.2 rsFC fMRI Task Instructions

Inside the MRI scanner, and prior to the resting-state scan acquisition, participants were instructed verbatim by the researcher and via written instructions: “The next scan will take about 10 minutes. Keep your eyes open, try not to think about anything in particular. Stay relaxed and try to keep your head still”. Through the resting-state scan, participants were shown a fixation cross (white cross on black background) via a mirror placed inside the MRI scanner.

4.3.5.3 MRI Data Acquisition Parameters

Participants were scanned using the same group of experienced radiographers at the Monash Biomedical Imaging facility in Clayton, Victoria. Participants were scanned on a Siemens Skyra 3 Tesla MRI scanner using a 32-channel head coil. T1-weighted (T1w) scans were acquired using the following acquisition parameters: TE = 2.07ms, TR = 2300ms, flip angle = 9°, 192 sagittal slices without gap, field of view 256 x 256mm, yielding a 1 x 1 x 1mm resolution, with a total acquisition time of 5 minutes. Resting-state scans (189 volumes) were acquired over 8 minutes, using the following parameters: TR = 2500ms, TE = 30ms, flip angle = 90°, field of view = 192mm, matrix = 64, voxel size 3 x 3 x 3mm^3, 44 slices without gap, and a total acquisition time of 480 seconds.

4.3.5.4 MRI Data Handling

All MRI data were directly exported from the scanner to Monash Biomedical Imaging-XNAT (XNAT website, private server), where it was stored and backed up in Digital Imaging and Communications in Medicine (DICOM) format. Raw format (i.e., DICOM) data were downloaded

from the XNAT server and converted into Brain Imaging Data Structure (BIDS) format using dcm2niix (v1.0.20201102) for further analysis. All imaging data processing and analysis were performed on a cloud-based cluster-computational platform, MASSIVE (massive.org.au; Goscinski et al., 2014). The pre- and post-processing was conducted using CONN toolbox 20.b (www.nitrc.org/projects/conn, RRID:SCR_009550; Whitfield-Gabrieli & Nieto-Castanon, 2012), based on SPM12 on Matlab (2018a.r7487), which was pre-installed on MASSIVE.

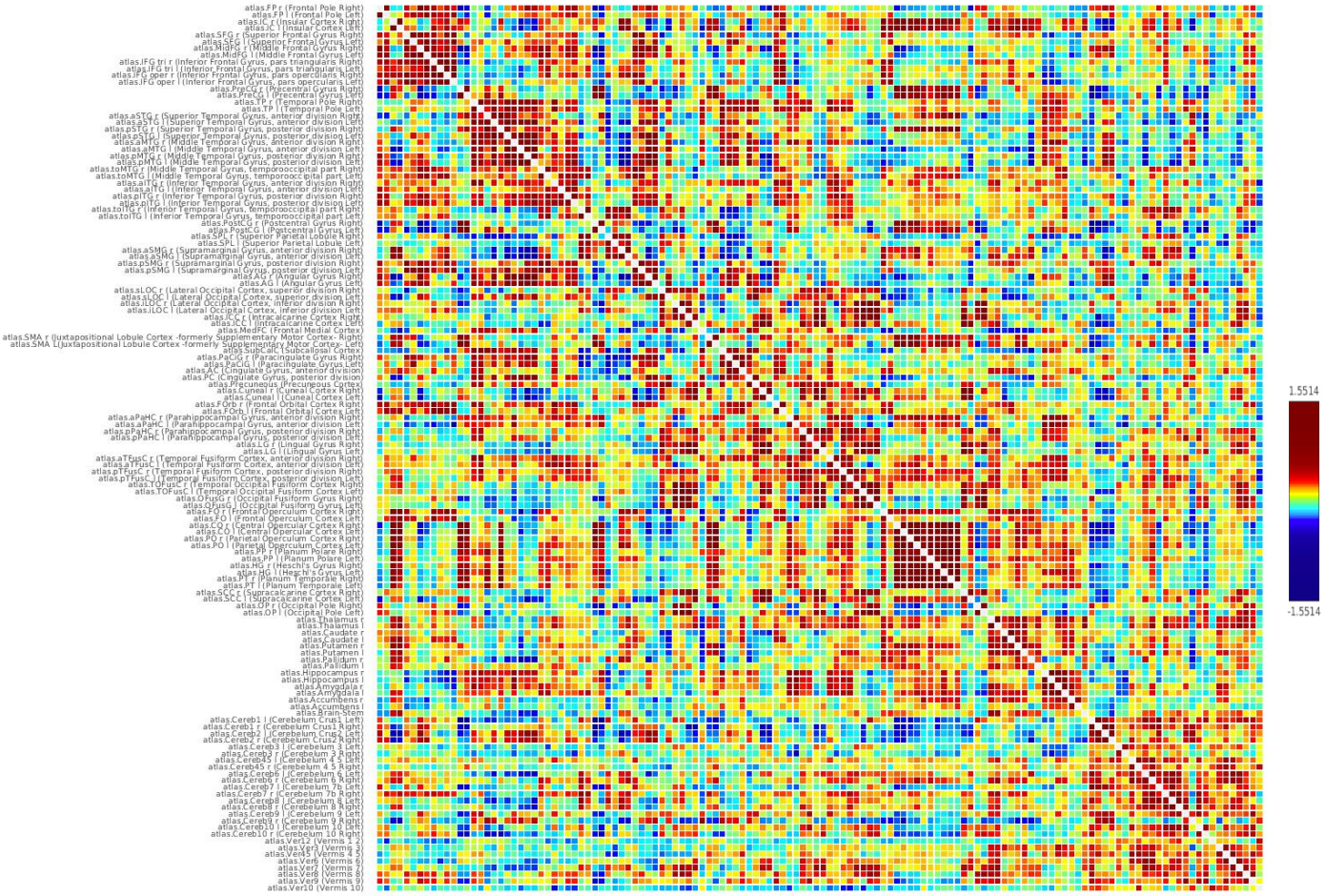
4.3.5.5 MRI Data Pre-Processing

All validated data were imported in BIDS format, then underwent a standard pre-processing using CONN toolbox 20.b, including: 1) slice timing with interleaved slice order; 2) realignment and generation of motion parameters; 3) ARtifact-detection Tools (ART)-based outlier detection with intermediate settings (default 97th percentile in normative sample) ; 4) co-registered fMRI data with T1w images; 5) segmentations of T1w images; 6) normalising T1w images to Montreal Neurological Institute (MNI) space (standard space) and normalising fMRI to MNI space with the same parameters; and 7) smoothing with 6mm kernel. fMRI was then resampled to 2x2x2mm isotropic voxels. Quality Assurance reports were generated and manually reviewed by authors HT and CS. Stringent criteria for detecting motion outliers, as outlined by Parkes et al. (2018), was followed, whereby limits of >0.25mm mean framewise displacement (mFD) and >5mm maximum framewise displacement were set; no participants violated these criteria.

4.3.5.6 Functional MRI Data Analysis

4.3.5.6.1 Connectome Analysis. The connectome matrix (132 x 132) for each scan were generated using the same Harvard-Oxford parcellation using CONN toolbox 20.b, with method of bivariate correlation and haemodynamic response function (HRF) weightings. Figure 4.1 shows an example of a connectome matrix from a single subject within the sample, selected at random. Then, these connectome matrices were fed into the graphic analysis using a General Linear Model (GLM) model using group as a factor, with the contrast CUD < controls. Covariates were age, sex, alcohol standard drinks/past 30 days and number of cigarettes/past 30 days, and BDI-II depression symptom scores. Multiple comparison error correction (cluster-level FDR correction, p-corrected < 0.05, multi-voxel pattern analysis (MVPA) omnibus test) was applied.

Figure 4.1. *Connectome matrix (132 x 132) of a single subject, selected at random from the sample*



4.3.5.6.2 Seed-Based Functional Connectivity. Brain regions selected as seeds (regions-of-interest; ROIs) based on prior knowledge, were determined by the default Harvard-Oxford atlas (132 cortical and subcortical regions; Desikan et al., 2006) within CONN toolbox 20.b (conn/rois/atlas.nii). Table 4.1 overviews the seeds examined and their coordinates in Montreal Neurological Institute (MNI) stereotaxic space.

Seed-based functional connectivity maps were generated using CONN toolbox 20.b. Briefly, a bivariate correlation coefficient was calculated between the timeseries of each ROI and the that of each other voxel in the brain, controlling for nuisance factors such as motions parameters, global signal, signal from white matter, and signal from cerebral spinal fluid. The seed-based rsFC maps for the four seeds were used for further post-processing.

Table 4.1. *Overview of seeds examined and coordinates in MNI stereotaxic space*

Seeds	**Hemisphere**	**MNI Coordinates**
Nucleus Accumbens	Left	-11, 9, -7
	Right	11, 9, -7
Putamen	Left	-28, -3, 7
	Right	28, -3, 7
Pallidum	Left	-18, -7, 1
	Right	18, -7, 1
Caudate	Left	-16, 2, 18
	Right	16, 2, 18
Hippocampus	Left	-27, -18, -16
	Right	27, -18, -16
Amygdala	Left	-22, -4, -15
	Right	22, -4, -15
Precentral Gyrus	Left	-38, -12, 55
	Right	38, -12, 55
Anterior Cingulate Cortex	-	0, 33, -7

Note: MNI coordinates are taken from the rough centre of the mass, seeds are anatomic in shape

4.3.5.7 MRI Data Post-Processing

A seed-based connectivity map was generated using the same GLM model and contrasts as described above in section 4.3.4.6.1. A False Discovery Rate (FDR) correction for multiple comparisons was applied. A Benjamini-Hochberg procedure was additionally applied to alpha values to decrease the false discovery rate following the examination of multiple ROIs (Benjamini & Hochberg, 1995).

4.3.5.8 Brain-Behaviour Correlations

We ran Spearman's rank-order correlations between the rsFC BOLD series values that differed significantly between people with a CUD and controls (i.e., beta values extracted via the CONN toolbox 20.b) and metrics of cannabis exposure and related problems. The metrics included: age of *first* cannabis use, age of *regular* cannabis use, duration of regular cannabis use, use days/past 30 days, total grams/past 30 days, CUDIT-R scores, and hours since last cannabis use. Correlations were run using SPSS (version 28).

4.4 Results

4.4.1 Sociodemographic, FSIQ, Substance Exposure and Related Problems, and Mental Health Characteristics

Sample characteristics are presented in Table 4.2. The sample included 107 people aged a median of 25 years, range 18-56 (35 female). Of these, 65 were people with a CUD (19 females) with a median age of 25 years (*range*: 18-56 years), and 42 controls (16 females) with a median age of 25 years (*range*: 18-55 years). This sample was determined to be of adequate size to ensure a high level of power for this study; please see *Chapter 3: General Methods, section 3.4.5* for details.

Table 4.2. *Sample characteristics and group differences for sociodemographic data, FSIQ, substance use and related problems, and mental health*

Characteristic	CUD	Controls	Group difference	
	mean (SD) or median [*range*]		Z_z / t_t / χ_x^{df}	p-value
Total *n* [Female]	65 [19]	42 [16]	0.91_x [1]	.340
Age, years	25 [*18-56*]	24.5 [18-55]	-0.21_z	.835
Education, years [+]	15 [*10-23*]	16 [*7-25*]	-0.80_z	.426
FSIQ ** [+++]	107 (*10*)	107 (13)	-0.80_t [60]	.426[a]
Handedness *n* [right] [++]	65 [61]	39 [36]	0.09_x [1]	.762
Depression *	9.5 [*1-46*]	3.5 [*0-37*]	-4.02_z	**<.001**
State Anxiety	31 [*20-60*]	29 [20-53]	-1.73_z	.084
Perceived Stress *	16 (*8*)	13 (*7*)	1.66_t [104]	.101
COVID Stress **** [++++]	5 [*0-88*]	8 [*0-43*]	-0.80_z	.428
Alcohol days/past 30 days *	3.5 [*0-29*]	2 [*0-25*]	-3.04_z	**.002**
Standard drinks/past 30 days *	13.5 [*0-207*]	3 [*0-81*]	-3.19_z	**.001**
AUDIT *	6 [*0-17*]	2 [*0-13*]	-4.837_z	**<.001**
Nicotine use days/past 30 days *	0 [*0-30*]	-	-	-
Cigarettes/past 30 days ***	0 [*0-600*]	-	-	-
FTND *	0 [*0-6*]	-	-	-
Cannabis Exposure and Related Problems				
DSM-5 CUD symptoms, n	7 [*4-11*]	-	-	-
CUDIT-R *	14.5 [*7-30*]	-	-	-
Days/past 30 days, n *	27.5 [*13-30*]	-	-	-
Grams/past 30 days, grams *	20.5 [*1-83*]	-	-	-
Age *first* use, years **	16.5 [*13-32*]	-	-	-
Age *regular* use, years *	18 [*14-32*]	-	-	-
Duration of use, years *	5 [*1-42*]	-	-	-
Abstinence, hours *	16.5 [*12-73*]	-	-	-

AUDIT = Alcohol Use Disorder Identification Test; CUD = Cannabis Use Disorder; CUDIT-r = Cannabis Use Disorder Identification Test-Revised; df = degrees of freedom; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, fifth edition; FTND = Fagerström Test for Nicotine Dependence; FSIQ = Full Scale Intelligence Quotient; *n* = sample size; *SD* = standard deviation; $_z$Mann-Whitney U, $_t$Independent samples t test, $_x$Chi-square; [a]Homogeneity of variance not assumed; *Note:* Medians and ranges which significantly differ are shaded yellow.

Sample size: cannabis users *n=64, **n=63, ***n=61, ****n=50; controls [+]n=40, [++]n=39, [+++]n=38, [++++]n=27

Groups were matched by sex and age. The CUD group and controls had non-significant differences for years of education, FSIQ scores, handedness, as well as for several mental health symptom scores: state anxiety, perceived stress and COVID stress.

The CUD group had significantly higher scores than controls, for depression symptoms and alcohol use metrics: alcohol use days/past 30 days, standard drinks/past 30 days, and AUDIT scores. There were 33 of 65 people with a CUD who did not use nicotine over the past 30-days; no controls reported past 30-day nicotine use. The remaining 32 of 65 people with a CUD reported using nicotine use within the past 30 days; this subset of participants scored a median of 1 on the FTND indicating 'very low' nicotine dependence.

4.4.1.1 Level of Cannabis Exposure and Related Problems

All people in the CUD group met the criteria for a moderate-to-severe CUD and most of them had a severe CUD; corroborated by a median number of CUD symptoms endorsed = 7 (*range*: 4-11) and a median score of 14.5 (*range*: 7-30) on the CUDIT-R. They used a median of 20 grams of cannabis in the past 30 days (*range*: 1-83), over a median of 27 days (*range*: 13-30). This approximately equated to 0.75 cannabis grams/day.

The median age of *first* cannabis use was 17 (*range*: 13-32), and for *regular* use was 18 (*range*: 14-32). All people with a CUD reported to have attempted to cut down or reduce their cannabis use over the past 2 years, used cannabis at least monthly for a median duration of 5 years (*range*: 1-40), and at least 4 days per week for 12 months (as per inclusion criteria). The CUD group were abstinent from cannabis for a median of 16 hours before testing (*range*: 12-73 hours).

4.4.2 Group Differences in Resting-State Functional Connectivity

4.4.2.1 Connectome Analysis

The connectome analysis did not yield any significant group differences in rsFC.

4.4.2.2 Seed-Based Functional Connectivity

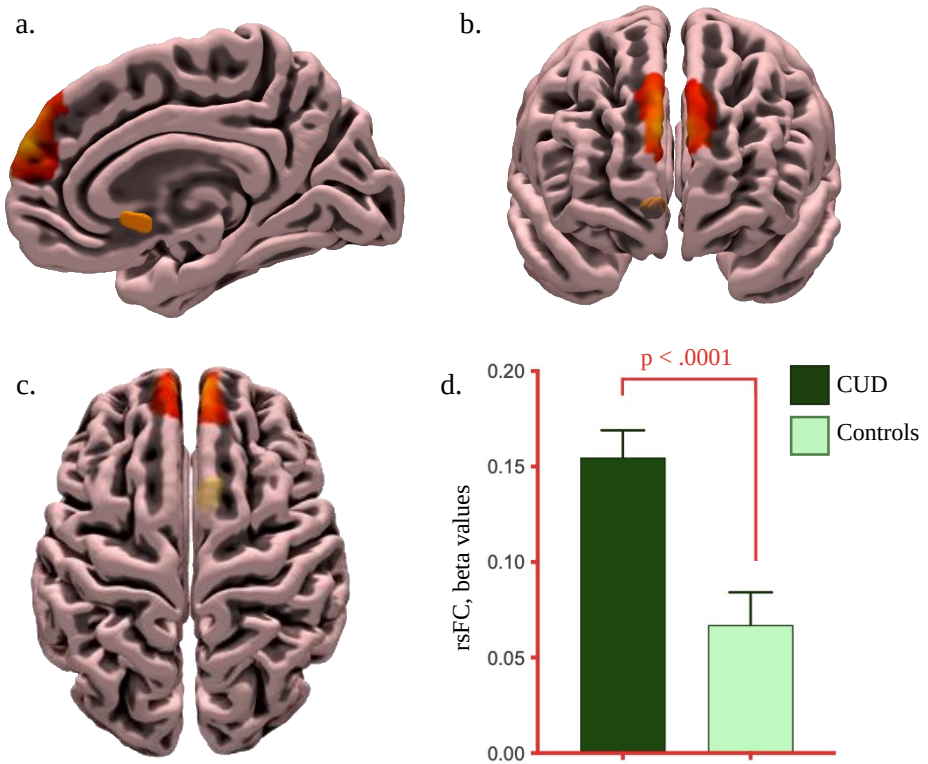
As shown in Table 4.3 and Figures 4.2-4.6, the CUD group compared to controls had different rsFC in – NAc (Figure 4.2), putamen (Figure 4.3), pallidum (Figures 4.4 & 4.5), and hippocampus (Figure 4.6) seeds – accounting for age, sex, past month standard drinks and cigarettes, and depression symptom scores, and using FDR and Benjamini-Hochberg corrections.

Table 4.3. *Overview of significant group differences (CUD < controls) in resting-state functional connectivity of a priori seeds, accounting for age, sex, past 30 days alcohol and nicotine dose, and depression scores, with Benjamini-Hochberg correction*

Figure	Seed	Cluster (*specific regions*)	Peak (x,y,z)	K	Size p-FDR	B-H adjusted α
Greater rsFC:						
Figure 4.2	*right* NAc	*bilateral* frontal pole & *left* SFG	2, 62, 26	318	<.0001	.0024
Figure 4.3	*left* Putamen	*right* superior lateral occipital cortex & superior parietal lobule	30, -56, 56	140	.0061	.0167
Figure 4.4 d.i	*left* Pallidum	*right* superior lateral occipital cortex & occipital pole	24, 80, 22	808	<.0001	.0071
d.ii		*right* intracalcarine cortex & lingual gyrus	4, -83, 4	178	.0008	.0095
d.iii		*left* intracalcarine cortex & lingual gyrus,	-20, -72, 00	164	.0009	.0119
d.iv		*right* superior lateral occipital cortex & superior parietal lobule	28, -56, 56	122	.0039	.0143
Figure 4.5	*right* Pallidum	*right* superior lateral occipital cortex & occipital pole	34, -72, 24	333	<.0001	.0048
Lower rsFC:						
Figure 4.6	*right* Hippocampus	*left* superior lateral occipital cortex & occipital pole	-16, -88, 18	122	.0097	.0190

B-H = Benjamini-Hochberg; FDR = false discovery rate; K = number of voxels; NAc = nucleus accumbens; SFG = superior frontal gyrus

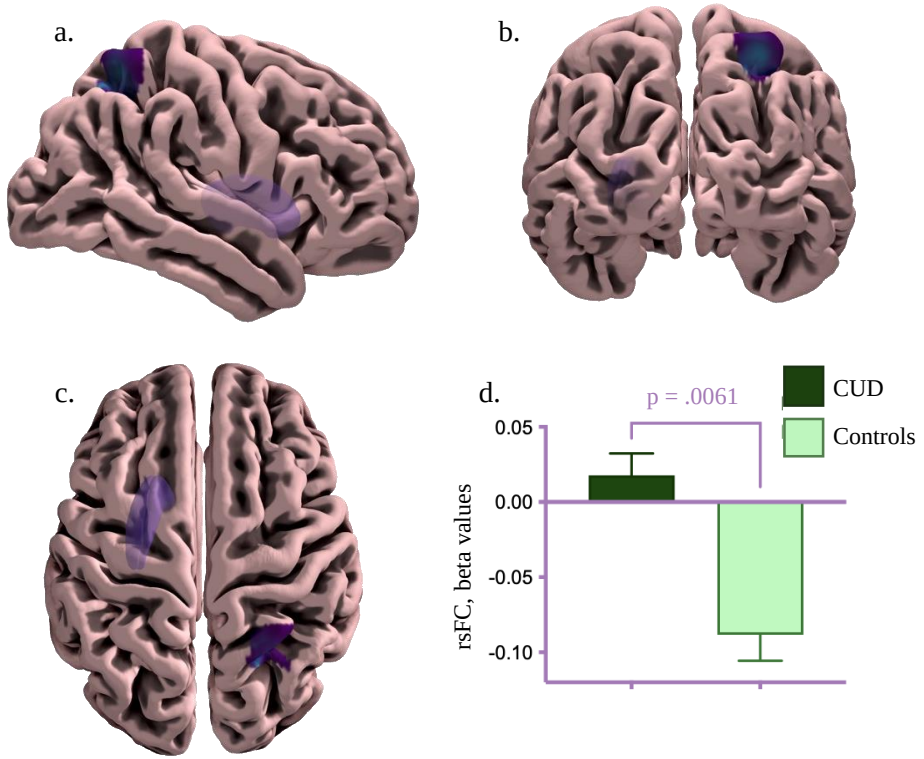
Figure 4.2. *Overview of greater resting-state functional connectivity between the right NAc seed and the bilateral frontal pole/left SFG cluster, in people with a Cannabis Use Disorder than controls*



a. interior sagittal view, right hemisphere
b. anterior view, bilateral hemispheres
c. superior view, bilateral hemispheres
d. histogram overview of beta values, in people with Cannabis Use Disorder and controls

CUD = Cannabis Use Disorder

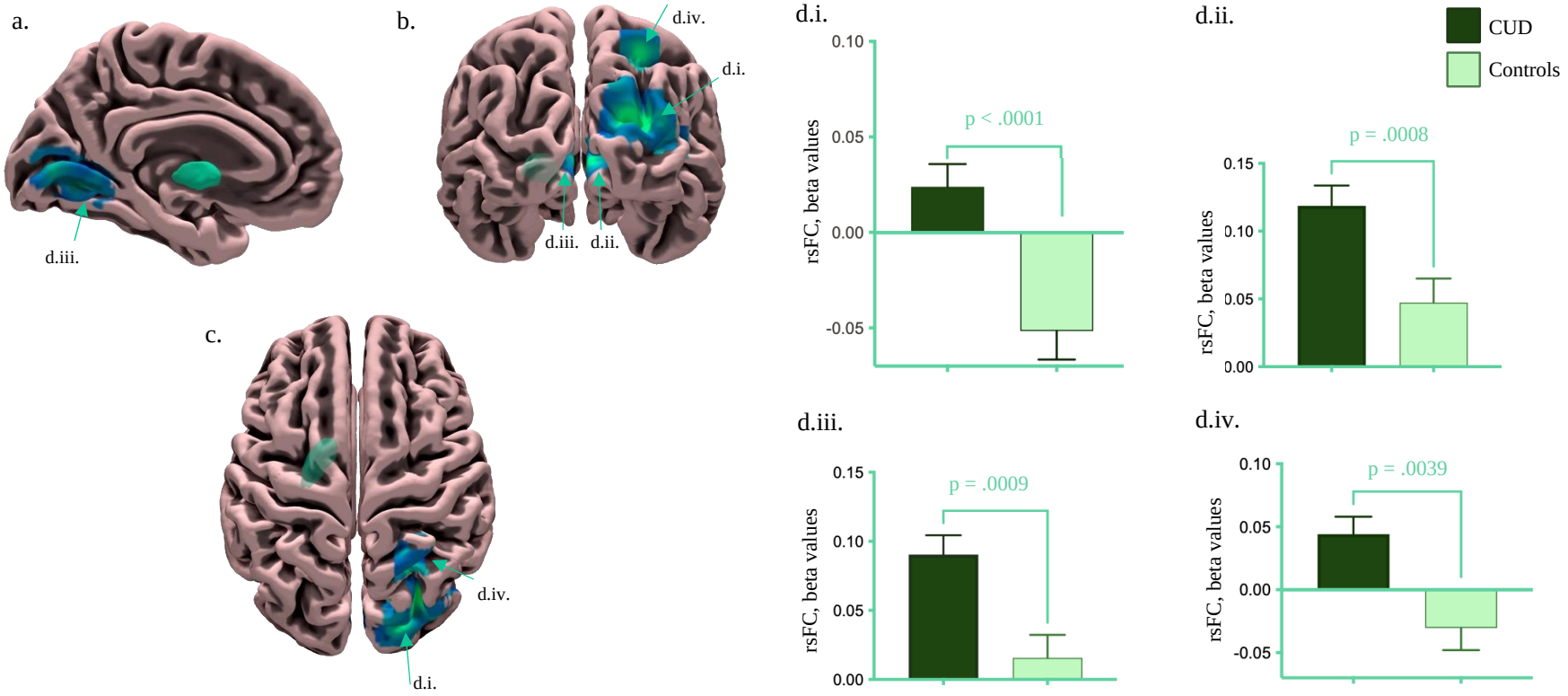
Figure 4.3. *Overview of greater resting-state functional connectivity between the left putamen and right superior lateral occipital cortex/superior cluster, in people with a Cannabis Use Disorder than controls*



a. exterior sagittal view, right hemisphere
b. posterior view, bilateral hemispheres
c. superior view, bilateral hemispheres
d. histogram overview of beta values, in people with Cannabis Use Disorder and controls

CUD = Cannabis Use Disorder

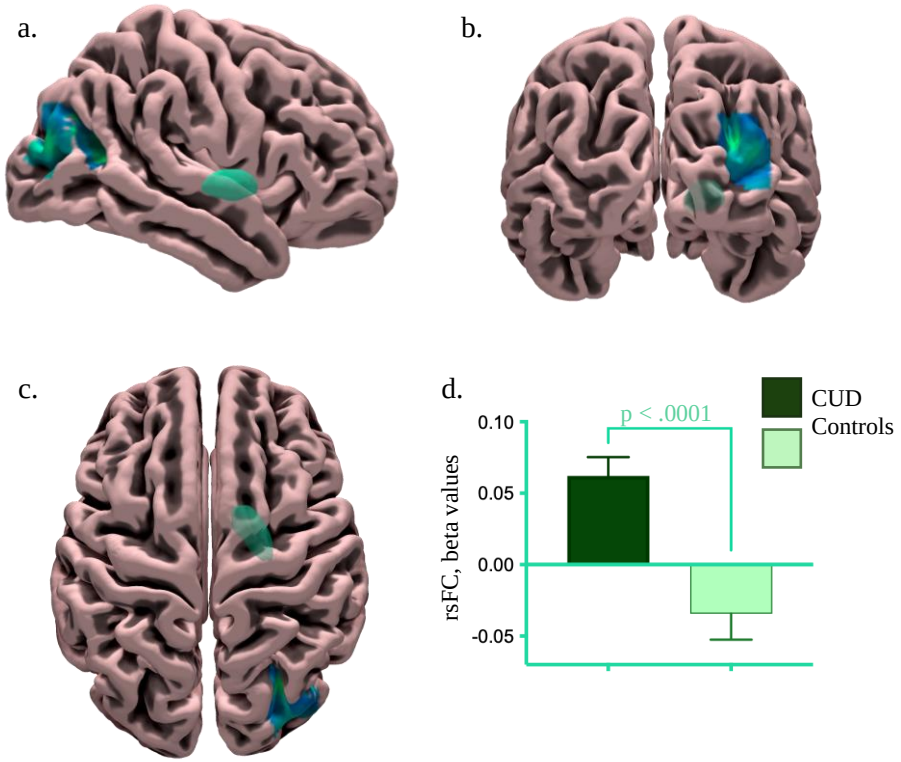
Figure 4.4. *Overview of lower resting-state functional connectivity between the left pallidum and occipital/occipito-parietal clusters in people with a Cannabis Use Disorder than controls*



a. exterior sagittal view, right hemisphere
b. posterior view, bilateral hemispheres
c. superior view, bilateral hemispheres
d. histogram overview of beta values, in people with Cannabis Use Disorder and controls:
- d.i. left pallidum and right superior lateral occipital cortex/occipital pole cluster;
- d.ii. right intracalcarine cortex/lingual gyrus cluster;
- d.iii. left intracalcarine cortex/lingual gyrus pairing; and
- d.iv. right superior lateral occipital cortex/superior parietal lobule cluster,

CUD = Cannabis Use Disorder

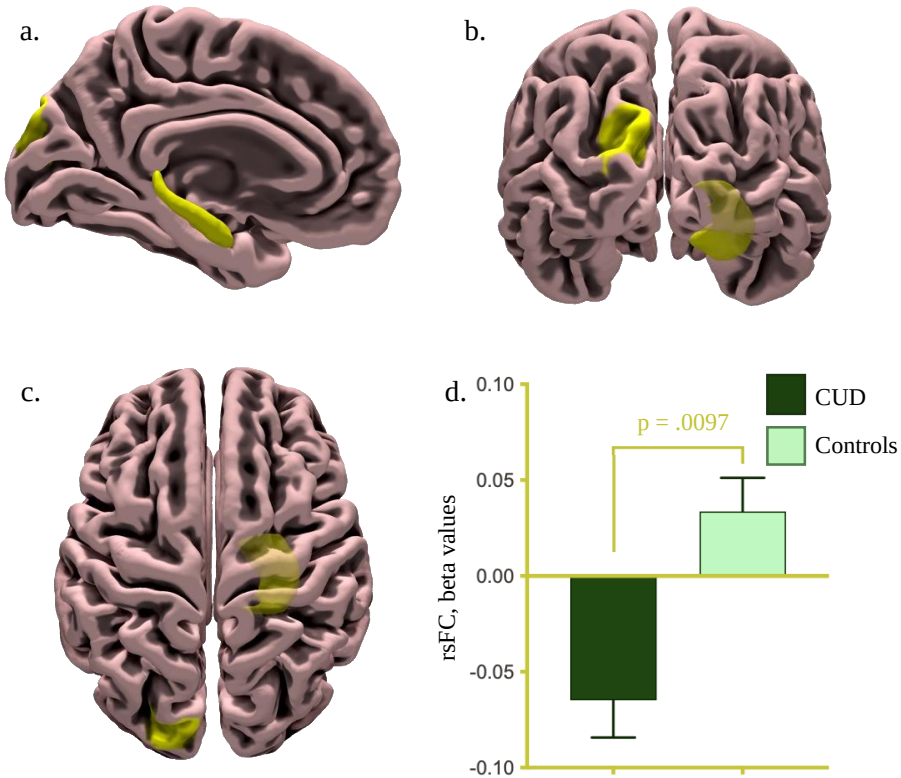
Figure 4.5. *Overview of greater resting-state functional connectivity between the right pallidum and right superior lateral occipital cortex/occipital pole cluster, in people with a Cannabis Use Disorder than controls*



a. exterior sagittal view, right hemisphere
b. posterior view, bilateral hemispheres
c. superior view, bilateral hemispheres
d. histogram overview of beta values, in people with Cannabis Use Disorder and controls

CUD = Cannabis Use Disorder

Figure 4.6. *Overview of lower resting-state functional connectivity between the right hippocampus – left superior lateral occipital cortex/occipital pole cluster, in people with a Cannabis Use Disorder than controls*



a. interior sagittal view, left hemisphere
b. posterior view, bilateral hemispheres
c. superior view, bilateral hemispheres
d. histogram overview of beta values, in people with Cannabis Use Disorder and controls

CUD = Cannabis Use Disorder

The CUD group had higher rsFC between the following region pairings: the right NAc and a frontal cluster (Figure 4.2), the left putamen and an occipito-parietal cluster (Figure 4.3), the left pallidum and three occipital clusters (Figure 4.4; d.i, d.ii, and d.iii) and one occipito-parietal cluster (Figure 4. 4; d.iv), and the right pallidum and an occipital cluster (Figure 4.5), than controls. The CUD group also had lower rsFC between the right hippocampus and an occipital cluster than controls (Figure 4.6). There was no group difference in rsFC using the other seeds (i.e., ACC, caudate, amygdala, and precentral gyrus).

4.4.3 Brain-Behaviour Correlations

Table 4.4 overviews significant correlations between rsFC pairs altered in people with a CUD than controls, and cannabis exposure and related problems. We found that greater putamen-occipito-parietal rsFC correlated with an earlier age of *first* cannabis use onset ($p < .05$) and an earlier age of *regular* cannabis use onset ($p < .05$). Furthermore, greater pallidum-occipital rsFC correlated with lower CUDIT-R scores, and less cannabis use days/past month. ($p < .001 – p < .05$). Finally, greater pallidum-occipito-parietal rsFC also correlated with lower CUDIT-R scores ($p < .001$).

Table 4.4. *Correlation between resting-state functional connectivity pairs showing group differences (rsFC beta values) and metrics of cannabis exposure and related problems, within the CUD group*

Seed region	Cluster region/s	Grams, past 30 days		Days used, past 30 days		CUDIT-R		Duration, regular use		Age, *first* use		Age, *regular* use		Abstinence hours	
		r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
right NAc	frontal	.02	.905	.07	.582	.12	.378	.07	.591	.04	.737	.12	.347	-.08	.520
left Putamen	Occipito-parietal	.03	.836	-.19	.143	-.19	.141	.12	.355	-.29	.025	-.29	**.022**	.22	.082
left Pallidum	occipital	.10	.429	-.17	.181	-.41	**.001**	.05	.678	-.17	.185	-.09	.495	.18	.178
	occipital	-.04	.786	-.27	**.037**	-.45	**<.001**	-.02	.853	-.16	.235	-.09	.475	.18	.163
	occipital	-.15	.246	-.35	**.006**	-.28	**.028**	-.13	.302	-.07	.621	.03	.844	.05	.706
	Occipito-parietal	-.02	.856	-.12	.364	-.40	**.001**	.18	.171	-.03	.802	-.10	.470	.22	.090
right Pallidum	occipital	-.06	.665	-.19	.160	-.17	.181	-.20	.133	.15	.269	.13	.309	.18	.155
right Hippocampus	occipital	.15	.253	.16	.231	<.01	.986	.17	.187	-.18	.166	-.13	.318	-.15	.239

CUDIT-R = Cannabis Use Disorder Identification Test-Revised; NAc = nucleus accumbens; r_s = Spearman's Rho

Note: significant *moderate* (r_s: 0.40 – 0.69) correlations are shaded orange, significant *weak* (r_s <0.39) correlations are shaded green

4.5 Discussion

To our knowledge, this is the first fMRI study examining the rsFC of people who meet criteria for a CUD, specifically a moderate-to-severe CUD with attempt to cut down or reduce use in the past 2 years. As hypothesized, people with a CUD vs controls showed significantly different (predominantly greater) rsFC, controlling for age, sex, past 30 days alcohol and nicotine dose, and depression scores. It has been suggested that increases in rsFC during a non-intoxicated state may reflect adaptive processes (i.e., allostatic/compensatory) which follow rsFC 'interruptions' commonly observed during acute cannabis intoxication (Ertl et al., 2023). In people with a CUD compared to controls, rsFC was greater between NAc-frontal regions, putamen-occipito-parietal regions (which correlated with an earlier age of *first* and of *regular* cannabis use), and pallidum-occipital/occipito-parietal regions (which correlated with CUDIT-R scores and days of cannabis use/past 30 days); rsFC was lower between hippocampus-occipital regions. Notably region pairings with altered rsFC are part of reward and incentive pathways implicated in prominent neuroscientific theories of addiction (Berridge & Robinson, 2016; Koob & Volkow, 2010; Volkow et al., 2016) and with known alterations in regular cannabis users (Thomson et al., 2022 [*Chapter 2*]). This is the first time altered rsFC has been reported between these specific region pairs. Associations between increased rsFC and earlier age of use onset were partly in keeping with past research which demonstrated increased rsFC between the bilateral OFC correlated with earlier age of use onset (Filbey et al., 2014).

We reported greater NAc-frontal rsFC in people with a CUD. The finding may reflect increased engagement of salience pathways underlying sensitivity to THC exposure (Berridge & Robinson, 2016). Indeed, THC affects dopamine synthesis within the NAc (Bossong et al., 2009; Pierce & Kumaresan, 2006), which might subsequently affect the function of NAc and interconnected frontal pathways implicated in salience processing. This may play a key role in cannabinoid reinforcement (Lupica et al., 2004; Tanda & Goldberg, 2003), whereby projections from the NAc to the PFC are thought to mediate experiences of 'wanting' and urges to use cannabis (Berridge & Robinson, 2016). In line with this notion, the NAc plays a key role in the predictive value of rewarding stimuli (Knutson & Gibbs, 2007), whilst frontal regions have been linked to a loss of control over substance use (George & Koob, 2010). Therefore, these regions may be implicated in more severe forms of

CUD, where a loss of control over cannabis use can be a key feature. Overall, our finding of greater frontostriatal connectivity in people with more severe forms of CUD are in keeping with the fMRI literature in regular cannabis users (for a review, see Thomson et al., [2022] [*Chapter 2*]). Specifically, the literature reports frontostriatal rsFC alterations in current cannabis users (Blanco-Hinojo et al., 2017) as well as abstinent cannabis users compared to controls (Zhou et al., 2018; Zimmermann et al., 2018) . Taken together, the findings suggest that frontostriatal rsFC alterations are associated with CUD, even though it is unclear if they predate or follow CUD (or both); further these rsFC alterations may persist following abstinence from cannabis. Finally, alterations to frontostriatal rsFC reported herein, have also been observed with exposure to other substances/other SUDs, including cocaine (Hu et al., 2015; Zhang & Li, 2018), nicotine (Hong et al., 2009; Hong et al., 2010), opioids (McConnell et al., 2020; Wang et al., 2021), stimulants (Wang et al., 2019), and polysubstance use (Motzkin et al., 2014). Therefore, CUD may affect the addiction neurocircuitry, in a similar fashion to other SUDs (Berridge & Robinson, 2016; Koob & Volkow, 2010; Volkow et al., 2016; Zehra et al., 2018).

We found greater striatal-occipital rsFC in the CUD group vs controls, comprising putamen-occipito-parietal, pallidum-occipital, and pallidum-occipito-parietal region pairings, in correlation with severity of cannabis dependence (CUDIT-R scores) and frequency of cannabis use. Interestingly, both the putamen and pallidum have been implicated in cognitive processes reportedly altered in regular cannabis users, e.g., habit formation (Grahn et al., 2008; Yin & Knowlton, 2006), the automation of behaviour (Everitt & Robbins, 2005) and compulsive substance use (Everitt & Robbins, 2005). Furthermore, occipital regions have been shown to underlie attentional control (Beffara et al., 2022; Gilbert & Li, 2013), particularly when processing and directing attention toward salient information (Kim et al., 2021). Previous task-based fMRI research (Sehl et al., 2021) has demonstrated that greater activation in striatal regions (including both the putamen and the pallidum) emerged in cannabis users with greater severity of cannabis-related problems, frequency of use and/or earlier age of onset. Task-based fMRI studies have also detected decreased activity in the putamen and in occipital regions, in long-term cannabis users (Blest-Hopley et al., 2018). Therefore, greater putamen/pallidum-occipital/occipito-parietal rsFC, may underlie habitual cannabis use and altered

salience processing observed in CUD. rsFC between these regions may reflect habituation of brain pathways to compulsive cannabis use that can characterise CUD (Everitt & Robbins, 2005), but this notion is not supported by the lack of their association with cannabis dosage and duration.

An alternative interpretation of changes in putamen-occipito-parietal rsFC is that they may reflect an effect of cannabis exposure on neurodevelopmental processes within these pathways, or a pre-existing vulnerability to commence cannabis use. Indeed, greater putamen-occipito-parietal rsFC significantly correlated both with an earlier age of *first* and of *regular* cannabis use onset; participants with a CUD were exposed to cannabis for the first time during youth: they *tried* cannabis for the first time at a median age of 16, and started consuming cannabis *regularly* at a median age of 18. The correlations are in keeping with previous work which has demonstrated a correlation between earlier age of use onset with increased rsFC (in frontal pairings; Filbey et al., 2014); and with evidence that adolescent cannabis users have demonstrated increased putamen activation during task-based fMRI (Blest-Hopley et al., 2018). Taken together, cannabis exposure during youth may change neurodevelopment (Blest-Hopley, Colizzi, et al., 2020), via altering endocannabinoid signalling that regulates brain maturation (Galve-Roperh et al., 2009). Consistently, cannabis use during adolescence may be associated with adverse neurobiological outcomes persisting into adulthood, as shown for cognition and mental health (e.g., risk of psychosis and heightened mental health symptom scores; Blest-Hopley, Colizzi, et al., 2020; Lawn, Mokrysz, et al., 2022; Lubman et al., 2015). It should be noted that emerging evidence examining rsFC in both adults and adolescents compared to age matched controls (Ertl et al., 2023), failed to demonstrate that adolescents are more vulnerable than adults to the putatively harmful impacts of chronic cannabis use on rsFC. It is possible however that this study was under-powered to detect significant findings. It is also possible that the discrepancy in results stems from differences in the characteristics of their sample, whereby Ertl et al., (2023) examined current adolescents, and adults who commenced using cannabis in *adulthood*, rather than adults with an earlier age of onset. Future research could compare rsFC alterations, as well as associated behavioural outcomes, between adolescent (i.e., aged 10-17) and adult (i.e., aged 18+) onset cannabis users, in order to explore the differing impacts of THC exposure across stages of development (Lawn, Fernandez-Vinson, et al., 2022; Lawn, Mokrysz, et al., 2022). Additionally,

longitudinal research monitoring neurobiological changes predating and following the onset of cannabis use and of CUD over time is required to increase understanding of the time course of rsFC alterations detected herein.

We found that the CUD group showed lower hippocampus-occipital rsFC than controls. The rsFC differences were not significantly correlated with metrics of cannabis exposure or related problems, and it remains unclear which variables may drive the group difference. Interestingly, the hippocampus is implicated in learning, memory and stress processes (Clark et al., 2019; Corkin, 2002; Howland & Wang, 2008; Maguire et al., 2016), the alteration of which has been documented in cannabis users (Lorenzetti et al., 2021; Scott, 2023; Scott et al., 2018). Meanwhile, occipital regions have been implicated in salience processing also shown to be different in cannabis users (Berridge & Robinson, 2016). Task-based fMRI research, has shown increased hippocampal activation for regular cannabis users during cue-reactivity (Goldman et al., 2013; Sehl et al., 2021), thought to reflect memory of prior substance use (Franklin et al., 2007). In contrast, hippocampal hypoactivation has been established during tasks of hippocampus-dependent associative memory in regular cannabis users compared to controls (Carey et al., 2015; Jager et al., 2007), associated with poorer memory performance. It has been theorised that hypoactivation of the hippocampus may result from CB_1R-mediated disruption of hippocampal plasticity (Blest-Hopley, Giampietro, et al., 2020), or to cannabis exposure-associated cerebrovascular or structural changes (Jager et al., 2007). In further support of this, reductions in hippocampal volume in regular cannabis users have been well established (Lorenzetti et al., 2019), potentially due to the neuroadaptations from chronic exposure to THC (Chan et al., 1998; Landfield et al., 1988). Therefore, different hippocampus-occipital rsFC may reflect altered BOLD signal of the hippocampus and interconnected occipital regions implicated in salience processing. To identify the specific neurobiological mechanisms underlying the rsFC changes identified in the sample examined in this experiment, multimodal neuroimaging research is required to explore if and how rsFC differences occur in parallel to structural alterations of the same pathways – such as volumes as examined by t1-weighted MRI, structural connectivity measured with diffusion-weighted imaging, and metabolites as examined by Magnetic resonance Spectroscopy.

4.5.1 Strengths of the Current Study

There were a number of strengths relating to the research aims, as well as to the study design and methodologies undertaken in the current study. First, to our knowledge, this is the first fMRI study to examine rsFC in current cannabis users with a diagnosis of moderate-to-severe CUD. Therefore the study findings extend upon: (i) previous knowledge on the neurobiology of people who use regularly cannabis with unknown status regarding problems with use (Thomson et al., 2022 [*Chapter 2*]); (ii) existing theories of addiction (Berridge & Robinson, 2016; Koob & Volkow, 2010; Volkow et al., 2016), which are largely based on substances other than cannabis and on outdated diagnostic systems, which have shown lack of consistency with the DSM-5 e.g., DSM-IV (Livne et al., 2021); and (iii) knowledge of neural underpinnings of moderate-to-severe CUD, therefore relating to the cannabis using population shown most to be impacted by negative outcomes associated with their use (Foster et al., 2018; van der Pol et al., 2013).

Second, we used robust methodologies to minimise the influence of confounding variables on rsFC. Specifically, the CUD and control groups did not significantly differ in the majority of measured sociodemographic and mental health-related variables (i.e., years of education, FSIQ, handedness, and scores of state anxiety, perceived stress, and COVID specific stress). Variables that did significantly differ between groups (i.e., past 30 days alcohol and nicotine exposure, and depression scores) or that exert a major influence on brain function (i.e., age and sex), were controlled for in all rsFC analyses and therefore, their influence on the results was minimised. Additionally, we asked participants to abstain from cannabis use for 12 hours prior to scanning; and participants' duration of abstinence from cannabis did not correlate with rsFC alterations in the CUD group. Therefore, it was unlikely that acute effects of cannabis (i.e., intoxication) confounded results. Finally, as per methodological standards of neuroimaging research in substance using populations, we thoroughly screened for significant use of other substances (other than alcohol and/or nicotine), comorbid diagnosis of psychiatric disorders (other than depression or anxiety), and central nervous system altering medications. Given the heterogenous rates of depression, anxiety, alcohol use, and nicotine use levels within the sample examined herein, and their elevated levels in the examined CUD group, the examined sample and therefore findings are likely representative of cannabis using

populations in the general community where these mental health and substance use variables are elevated (Onaemo et al., 2021; Stinson et al., 2006). Furthermore, given that we systematically accounted for confounders, our results may be specific to populations with more severe forms of CUD with comorbid depression and anxiety disorders.

4.5.2 Limitations and Future Directions

The results from this study should be considered in light of some methodological limitations, detailed in this section. First and foremost, the cross-sectional study design prevented establishing whether rsFC changes predated or followed cannabis use, or the development of moderate-to-severe CUD. Longitudinal neuroimaging studies are required to unpack the time course of rsFC in youth who will vs will not go on to develop a CUD. Second, although the sample size in the current study provided adequate power, it has been suggested that sample sizes of N≳2,000 may be required to secure reproducibility and identify stabilised behavioural phenotypes (Marek et al., 2020; Marek et al., 2022). It would therefore be ideal to aim to validate the current findings in larger, multi-site neuroimaging studies and neuroimaging consortia data sets that confer increased power to detect the effects detected in this study, to confirm generalisability of our findings. At present, such datasets are yet to exist, as current consortia are largely based on normative samples, where the prevalence of cannabis use tends to be low and even lower for cannabis-used related problems that are seldom assessed, or the datasets from multi-site studies – such as the ENIGMA Addiction Working Group - conducted using now outdated diagnostic systems (i.e., prior to DSM-5) and with poor characterisation of substances use. In future, these limitations could be addressed in multi-site consortia such as the ENIGMA Addiction may collate additional datasets comprising cannabis with CUD assessed with the DSM-5, or other initiatives such as the Adolescent Brain Cognitive Development (ABCD) study (https://abcdstudy.org/), for which data collection is currently underway. The ABCD study commenced in 2015, and follows ~10,000 adolescents aged 9-10 years, who complete cognitive and behavioural assessment, and fMRI every two years, for ten years. Therefore, the ABCD study may provide data useful to clarify with precision the rsFC correlates of CUD.

Meanwhile, robust neuroimaging studies with targeted samples with a CUD such as the current experiment, are required to pave the way to advance the understanding of the neurobiology of CUD.

Third, our study did not integrate measures of cannabinoids in the cannabis used by participants, or objective measures of cannabinoids or cannabinoid metabolites from specimens (e.g., blood plasma, urine). Various cannabinoids have previously been shown to affect rsFC in a distinct fashion (Lorenzetti et al., 2022), and sometimes in an opposite fashion (Wall et al., 2022; Wall et al., 2019). Specifically, acutely administered THC has been shown to disrupt key brain networks, whilst acute CBD administration restores disruption to other key networks. Future studies are required examining how rsFC is affected in people with a CUD during acute intoxication and while non-intoxicated. To note, within the current study, urine samples were collected from all participants, however due to time delays out of researcher's control for urinalysis, the results were unable to be included here in the thesis at this stage, but *results from urine toxicology analyses will be reported and examined in relation to rsFC in the manuscript that will be prepared for publication from this study*. Hence, the contribution of cannabinoids or of cannabis potency (i.e., the level of THC) to residual rsFC alterations reported in the CUD group examined in this experiment, remains to be elucidated.

Fourth, the cannabis use estimates included in this study relied exclusively on self-report measures. Self-reported substance use is subject to underreporting biases (Khalili et al., 2021), memory fallibility, or gaps in the knowledge of participants relating to their cannabis supply (i.e., participants may be unaware of the strength of their cannabis supply or the specific amount that they consume via any given method or over time periods). The use of the TLFB (Sobell & Sobell, 1992) enabled us to somewhat mitigate this shortcoming, as it utilized a memory aid to improve the validity and accuracy of the data collected (i.e., calendar with personally relevant events to the participant inputted to aid memory). Future research could implement methodologies to mitigate such biases, for example a 'roll a joint' paradigm (Casajuana et al., 2017; Hindocha et al., 2017), to extrapolate typical volume of cannabis use more accurately. Also Ecological Momentary Assessment (EMA) could be incorporated over a set period to provide a real-world estimate of cannabis use patterns. Ideally, self-

report measures (including the ecologically valid TLFB), could be integrated with a 'roll a joint' paradigm and EMA reporting to maximise accuracy in future research.

4.5.3 Clinical Implications

This research contributes to the understanding of how changes in rsFC are related to moderate-to-severe CUD, and how these changes may impact, or be impacted by, cannabis exposure and related behaviours; thus, there are a number of clinical implications of these results. First, region pairings shown in this study to display altered rsFC in the CUD group, could be utilized as neural treatment targets in intervention for people with a moderate-to-severe CUD aiming to reduce, or gain increased control over, their cannabis use. Specifically, this could be applied via implementation of interventions thought to target neurobiological mechanisms associated with substance use disorders i.e., Mindfulness Based Interventions (Kirlic et al., 2021; Witkiewitz et al., 2013), neuromodulation tools (e.g., neurofeedback) or others (Martz et al., 2020) Future research is warranted to fully understand how rsFC changes in people with a CUD can be mitigated. Second, the correlations established between rsFC alterations and age of *first* or *regular* use, could ultimately provide valuable information about brain development and maturation via identifying pathways vulnerable to adolescent cannabis use or, or pre-disposing adolescents to commence using cannabis. Third, patterns of rsFC alterations identified here may contribute to the mapping of biomarkers for CUD, or to the identification of at-risk populations. This would prove especially useful if rsFC alterations were indeed shown to predate the onset of CUD development, through longitudinal investigation.

4.5.4 Conclusions

In the first fMRI study to examine rsFC in selected ROIs people with a moderate-to-severe CUD vs controls, with a focus on ROIs implicated in neuroscientific theories of addiction (Koob & Volkow, 2010), high in CB1 receptors (Glass et al., 1997) and known to differ between cannabis users with unknown CUD status and controls (Thomson et al., 2022 [*Chapter 2*]). We demonstrated largely greater rsFC between the NAc, putamen, pallidum, and with clusters in frontal, occipital, and parietal regions, and lower hippocampus-occipito-parietal rsFC. The frontostriatal rsFC alterations associated

with CUD may result from neuroadaptations of dopaminergic pathways postulated to occur across SUDs, though longitudinal neuroimaging studies are required to confirm if they predated or follow CUD (or both). Further, greater striatal-occipital rsFC may underlie habitual cannabis use and altered salience processing observed in CUD, particularly in those who commence using cannabis earlier in youth. Finally, lower hippocampus-occipital rsFC was in line with task-based fMRI studies and may reflect CB_1R-mediated disruption of hippocampal plasticity, or to cannabis associated cerebrovascular or structural changes. Future multimodal longitudinal neuroimaging research in larger samples is required to elucidate the nature and mechanism of rsFC alterations in CUD in order to pave the way for preventative interventions in youth and adults with a CUD. Given the growing rates of CUD and increasing access to high potency and addictive cannabis products, more research is required to confirm which people who use cannabis are most vulnerable to rsFC alterations and how to mitigate neurobehavioral problems established in those experiencing severe forms of CUD.

CHAPTER 5:

Study 3: The Second Empirical Experiment

Chapter Guide

This chapter contains the second of the two empirical studies included within this PhD, which results from the data collection process detailed within the General Methods (*Chapter 3*) and from the sample assessed in the first empirical experiment where brain dysfunction between specific regions was shown (*Chapter 4*). This second empirical experiment has two key aims. The ***primary aim*** was to map how a brief Mindfulness Based Intervention (MBI) targeting cannabis cravings, compared to *active* and *passive placebo*-controlled conditions, mitigates brain dysfunction shown in people with a Cannabis Use Disorder (CUD) who tried to cut down/reduce use, which was documented in the first empirical experiment. This was achieved via utilisation of functional Magnetic Resonance Imaging (fMRI) tools to measure intervention-group-by-time effects on resting-state functional connectivity (rsFC) between *a priori* regions-of-interest (ROIs) shown to have different rsFC in CUD compared to controls. The ***secondary aim*** was to explore how group-by-time effects were associated with metrics of cannabis exposure, related problems, and mindfulness levels. To this end, correlations were run between change in rsFC over time (pre-to-post MBI or *active placebo* or *passive placebo*), with behavioural variables which also changed pre-to-post interventions i.e., change in cannabis dose and frequency, cannabis withdrawal, COVID specific stress, and mindfulness levels.

This empirical experiment has been prepared with publication in mind. Therefore, sections of this study, in particular the Methods, are detailed succinctly where possible to adhere to word limits as per academic publishing standards. The examiners will be directed within this chapter where applicable back to the General Methods (*Chapter 3*) for a more comprehensive overview of methodologies, should they wish to revise specific details.

The sample size of the participant group used in this study was ultimately smaller (N=56) than originally intended (N=90). As noted in the General Methods (*Chapter 3: 3.4.1 A Word on the Impact of COVID-19*), this sample size reduction was largely due to COVID-19 related disruptions throughout our data collection period.

Title Page: For Planned Publication

How does a Brief Mindfulness Intervention Reduce Resting-State Functional Connectivity Changes in Cannabis Use Disorder? A Double-Blind, Active and Passive Placebo-Controlled fMRI study

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5.1 Abstract

Rationale: Cannabis Use Disorder (CUD) is experienced by 22 million people worldwide. Worryingly, CUD can be associated with continued use despite attempts to quit, and the experience of significant adverse outcomes including craving and brain dysfunction while unintoxicated, 'at rest', and without performing cognitive tasks – measured with resting-state functional connectivity (rsFC) functional neuroimaging. Research on how to reduce altered brain function in CUD is lacking. Emerging evidence shows that mindfulness-based interventions (MBIs) can reduce cravings in cannabis users and brain dysfunction in substance use disorders (SUDs). Yet, no study has tested how MBIs affect brain dysfunction in CUD, and no fMRI studies in SUD – including CUD – used robust *active* and *passive placebo* conditions to isolate effects specific to MBI.

Objectives: We aimed to map how a brief MBI compared to *active* and *passive placebo* control interventions, mitigated rsFC in *a priori* regions of interest (ROIs) known to be altered in CUD, high in cannabinoid receptors, and implicated in neuroscientific theories of addiction. We explored if rsFC changes pre-to-post MBI, *active* or *passive placebo*, were associated with those in cannabis exposure, related problems, and mindfulness levels.

Methods: 56 people (14 females) with a CUD who tried to cut down or quit cannabis, underwent comprehensive fMRI, substance use, cognitive, and mental health assessment pre-to-post an MBI (n=19); *active placebo* control (i.e., relaxation, n=18); or *passive placebo* control (i.e., daily monitoring of cannabis, n=19). We examined group-by-time effects on rsFC. We then correlated region pairings that drove group-by-time effects, with changes pre-to-post intervention in cannabis grams, use days and withdrawal, COVID-specific-stress, and perceived mindfulness/self-awareness.

Results: There were group-by-time effects on rsFC observed for the putamen, pallidum, and the hippocampus seeds with various brain clusters. Putamen-frontal pole rsFC decreased pre-to-post MBI; it increased pre-to-post *active placebo*, in correlation with decreased cannabis grams, and it also increased pre-to-post *passive placebo* in correlation with decreased cannabis use days. Putamen-SFG rsFC decreased pre-to-post MBI and increased pre-to-post *active placebo*. Hippocampus-anterior cingulate rsFC increased pre-to-post MBI, in correlation with more cannabis use days, and decreased pre-to-post *passive placebo*. Pallidum-anterior superior temporal gyrus rsFC decreased pre-to-post

active placebo and increased pre-to-post *passive placebo*. Further, putamen-cerebellum/brainstem rsFC increased pre-to-post *active placebo* and decreased pre-to-post *passive placebo*.

Discussion: As hypothesised, brief MBI (vs *active* and *passive placebo*) altered rsFC in ROIs – putamen-frontal pole – of the addiction neurocircuitry, high in cannabinoid receptors, and known to be altered in this group individuals with a moderate-to-severe CUD. Thus, a brief MBI, relaxation intervention, and daily monitoring intervention could affect selected putamen-frontal pole/STG and hippocampus-ACC pathways dysfunctional in CUD, in differing directions; and additional pathways are selectively affected specifically by relaxation and by daily monitoring of cannabis use (e.g., putamen-cerebellum/brainstem and pallidum-aSTG).Future research with larger samples of individuals with a CUD, who endorse motivation to change use and adhere to intervention requirements, is required to further expand on how brief MBI mitigate brain dysfunction in CUD.

Keywords*:* functional magnetic resonance imaging, fMRI, resting-state functional connectivity, rsFC, seed-based connectivity, brain, cannabis, cannabis use disorder, CUD, substance use intervention, mindfulness

5.2 Introduction

Cannabis Use Disorder (CUD) is endorsed by approximately 22 million people worldwide (Degenhardt et al., 2018). Rates of 'disordered' cannabis use have increased, from 10% of regular users endorsing *cannabis dependency* in the early 90s (Anthony et al., 1997), to 18-26% of regular users now estimated to meet criteria of CUD (Leung et al., 2020). Concurrently, the demand for effective treatments for CUDs have also risen (Manthey et al., 2021; United Nations Office on Drugs and Crime [UNODC], 2019; World Health Organisation [WHO], 2016). Specifically, based on 22 countries with available data, treatment entries per 100,000 adults went from went from 27 entries in 2010, to 35 entries in 2019, representing a statistically significant increase in 10 countries (Manthey et al., 2021). A number of psychosocial treatments have been shown to reduce cannabis use and related problems, however this treatment effect is seldom maintained over time (Gates et al., 2016), and pharmacotherapies have proven to be ineffective (Bahji et al., 2021; Kondo et al., 2020; Nielsen et al., 2019). Furthermore, the limited effectiveness of treatments can be compounded by the fact that only 8% of adults with a CUD receive a CUD-specific treatment (Wu et al., 2017). These statistics underscore the urgent need to find interventions that can target the core pathophysiology of CUD and the adverse outcomes.

Worryingly, the adverse outcomes of CUD and heavy cannabis use constitute a significant burden of the disease on treatment services, with AUD$714million spent annually in Australia on healthcare costs (Whetton et al., 2020). Adverse outcomes of CUD are associated with a number of negative psychosocial and health outcomes (Foster et al., 2018; Hall & Degenhardt, 2014; van der Pol et al., 2013; Volkow et al., 2014). They can include: mental ill health - such as mood disorders, anxiety disorders (Gibbs et al., 2015; Gobbi et al., 2019; Leadbeater et al., 2019; Lev-Ran et al., 2014; Twomey, 2017) and psychotic disorders (Kuepper et al., 2011; Leadbeater et al., 2019; Rössler et al., 2012; Wright et al., 2021), reduced cognitive abilities (Figueiredo et al., 2020; Grant et al., 2003; Lovell et al., 2020; Schreiner & Dunn, 2012), and neurobiological alterations within the addiction neurocircuitry (Thomson et al., 2022 [*Chapter 2*]).

Importantly, neurobiological alterations have been observed while people who use cannabis are non-intoxicated using 'resting-state functional connectivity' (rsFC); a functional Magnetic

Resonance Imaging modality collected while participants are not performing any cognitively demanding tasks (e.g., 'at rest', watching a fixation cross while letting one's mind wander; Thomson et al., 2022 [*Chapter 2*]; Thomson et al., *in preparation* [Chapter 4]). Importantly, neurobiological alterations in people who suffer from addiction have been (partly) ascribed to the adverse psychosocial outcomes they experience; and implicated in prominent theories of addiction (Koob & Volkow, 2010; Volkow et al., 2016; Zehra et al., 2018). However, it remains unclear how brain dysfunction in CUD can be reduced. Indeed, interventions aimed a targeting aberrant neurobiology in CUD are largely unexplored (Verdejo-Garcia et al., 2019).

In recent years, mindfulness-based interventions (MBI) have been developed to address substance use disorders (SUDs). MBIs foster "awareness through paying attention on purpose, in the present moment, and non-judgmentally, to the unfolding of experiences moment by moment" (Kabat-Zinn, 1991). MBIs can include treatments targeting aspects of SUDs, with a range of durations, from single sessions that target cravings (e.g., urge surfing), to one or a few weeks (Kamboj et al., 2017; Serfaty et al., 2018), and a few months (Korecki et al., 2020). Emerging evidence shows that MBIs can reduce SUDs and related problems. For example, the literature to date including meta-analyses show that MBIs can reduce substance cravings and frequency of use, while reducing mental ill health (i.e., depression, stress; Cavicchioli et al., 2018; Chiesa & Serretti, 2014; Korecki et al., 2020; Li et al., 2017; Ramadas et al., 2021). Preliminary evidence has also shown that MBI can reduce the frequency of cannabis use, for up to 3 months post intervention in regular cannabis users (de Dios et al., 2012). Thus, MBIs have shown promise for reducing substance use and related problems in SUD populations.

Emerging fMRI evidence also show that MBIs can target brain dysfunction in SUD (Garland et al., 2014; Lorenzetti et al., *under review*). For instance, MBIs have been shown to increase rsFC between cortical regions underlying cognitive control, implicated within the addiction neurocircuitry (e.g., anterior cingulate cortex [ACC]-orbitofrontal cortex [OFC]) in people with nicotine dependence, as a function of smoking reduction pre-to-post MBI (Froeliger et al., 2017). Further, MBIs have been shown to reduce rsFC between cortical regions shown to be dysfunctional in CUD, that are implicated in inhibitory control (e.g., superior frontal gyrus [SFG]) and in interoception (e.g.,

default mode network [DMN]) in people with opioid dependence, in correlation with increased mindfulness (Fahmy et al., 2019). Overall, this early evidence suggests that MBIs can reduce brain dysfunction in SUD, and such changes may underlie substance use reduction and increase in mindfulness levels.

While emerging evidence shows that MBIs may target brain dysfunction in SUDs, methodological limitations of the literature to date preclude the understanding of the mechanisms of MBIs targeting SUDs including CUDs. First, the evidence relies on a few studies of limited SUDs with distinct psychopharmacological signatures (e.g., nicotine and opioids), therefore the findings cannot be generalised across distinct SUDs and the neurobiology of MBIs in CUD is unknown. Second, the extant fMRI studies of MBI comprised small sample sizes (e.g., 13-28; Fahmy et al., 2019; Froeliger et al., 2017), and therefore may have been underpowered to detect subtle changes in rsFC pre-to-post MBI. Third, the evidence lacks control groups (e.g., either *active*- or *passive-placebo* or both), which prevents the disentangling of MBI-specific effects from expectancy and general treatment effects. Fourth, the requirements for attendance of the MBIs might not have made it feasible to engage people with SUDs from the general community who can have limited cognitive resources to organise highly structured interventions over time, or people with work commitments or parental responsibilities. For example, one study required the attendance of 16 sessions over a month and targeted inpatients (Fahmy et al., 2019), and another study required 10 weekly 2-hour face-to-face sessions (Froeliger et al., 2017). Such interventions, while effective, might not be feasible for people with a CUD, who seek self-reliance and informal treatment settings (van der Pol et al., 2013). These limitations highlight a need for research examining rsFC in individuals with a CUD pre-to-post MBIs compared to control conditions, that are feasible to deliver to map the underlying neurobiological changes and their link to behavioural changes (e.g., lower dosage, lower stress, greater mindfulness).

The ***primary aim*** of this study is to examine for the first time how a brief MBI previously shown to reduce alcohol consumption (Kamboj et al., 2017) reduced brain dysfunction – measured with rsFC fMRI – in people with a current moderate-to-severe CUD, who had tried to cut down or quit their use in the previous 2 years. We used a robust double-blind, *active* and *passive placebo*-controlled intervention. In detail, the 56 participants were allocated in a pseudo-randomised order

stratified on age and sex, to one of three interventions: (i) a brief MBI adapted to target cannabis cravings (n = 19), (ii) an *active placebo* (relaxation) intervention adapted to cannabis cravings and matched to the MBI by number of words, complexity, and all components but mindfulness (n = 18), (iii) a *passive placebo* control group with daily monitoring of cannabis use, a component which was also embedded in both MBI and *active placebo* (n = 19).

We hypothesised that MBI vs *active* and *passive placebo*, would change rsFC between *a priori* brain regions of the addiction neurocircuitry (Koob & Volkow, 2010), high in cannabinoid receptors type 1 (CB_1R; Glass et al., 1997) and with demonstrated altered rsFC in this sample of people with CUD vs controls (Thomson et al., *in preparation* [Chapter 4]). The regions-of-interest (ROIs) were: the nucleus accumbens (NAc), the hippocampus, the pallidum, and the putamen.

We ***secondarily aimed*** to explore if changes in rsFC pre-to-post MBI were associated with changes in metrics of cannabis exposure (e.g., grams, use days) and related problems (e.g., withdrawal), as well as psychological measures (e.g., COVID-related-stress, and mindfulness levels).

5.3 Method

This study was nested within a larger, pre-registered study (https://doi.org/10.1186/ISRCTN76056942; registration ID: ISRCTN76056942). The Australian Catholic University Human Research and Ethics Committee approved the study protocols (HREC number 2019-71H).

5.3.1 Sample Inclusion and Exclusion Criteria

Unless otherwise specified, inclusion and exclusion criteria were confirmed by participant self-report and the use of a comprehensive online screening survey followed by a detailed screening over the phone.

5.3.1.1 Inclusion Criteria

Inclusion criteria were: (1) age between 18 years and 55 years; (2) normal-to-corrected vision; (3) fluency in English; (4) ability to attend sessions; (5) use of cannabis on a daily or almost daily basis for ≥12 months; (6) attempt to quit or reduce their cannabis use at least once within the

past 24 months; and (7) diagnosis of moderate-to-severe CUD, confirmed by the endorsement of ≥ 4 CUD symptoms from the DSM-5 (American Psychiatric Association [APA], 2013), measured using the Structured Clinical Interview of DSM-5 – research version (SCID-5-RV; First et al., 2015). See *Chapter 1: Thesis Introduction and Overview, Figure 1.0, page 20* for DSM-5 CUD diagnostic criteria.

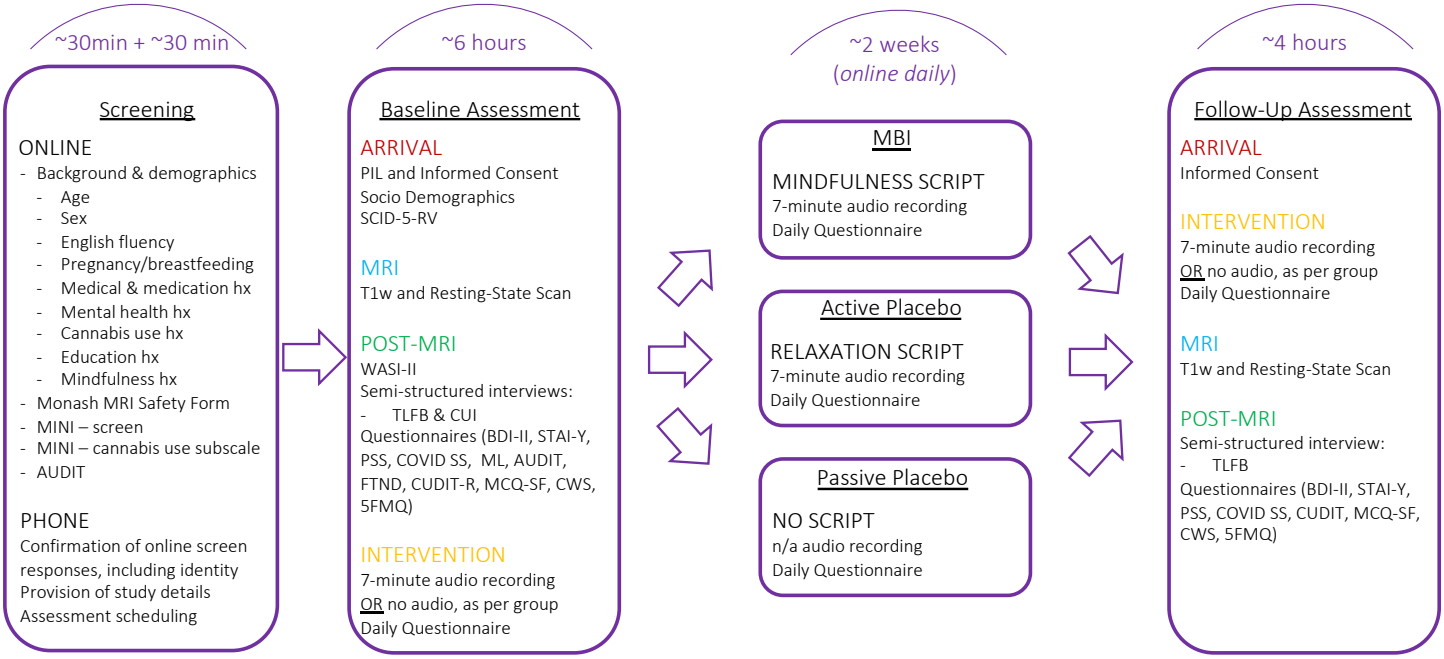
5.3.1.2 Exclusion Criteria

Exclusion criteria were: (1) Magnetic Resonance Imaging (MRI) contraindications, measured using the Monash Biomedical Imaging MRI Screening and Information Form; (2) unwillingness to refrain from any illicit substance and/or alcohol use in the 12 hours before testing (confirmed upon arrival at session); (3) current use of prescription medication that affects the central nervous system (CNS) except anti-depressants and anxiolytics, due elevated depression/anxiety levels in CUD; (4) history of any diagnosed psychiatric disorders, with the exception of depression and anxiety disorders due to the high comorbidity with CUD, or current suicidal ideation, as confirmed using The MINI International Neuropsychiatric Interview 7.0.2 (MINI; Lecrubier et al., 1998; Lecrubier et al., 1997; Sheehan et al., 2015; Sheehan et al., 1998); (5) history of any neurological disorders or major medical conditions (e.g., epilepsy, stroke, migraine, etc.); (6) history of acquired or traumatic brain injury or loss of consciousness > 5 minutes; (7) full scale intelligence quotient (FSIQ) estimate score < 70, confirmed using the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II; Wechsler, 2011); (8) current pregnancy and/or breastfeeding; (9) history of significant and regular mindfulness practice, defined as regular engagement in mindfulness practices over any extended period of time; (10) significant dependence on alcohol, confirmed by a score > 19 on the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2018); (11) significant exposure to substances other than cannabis or tobacco, as per >50 occasions of use over a 2-year period in the past 10 years; or (12) or use of any illicit drug other than cannabis in the four weeks prior to testing.

5.3.2 Procedure

Please refer to Figure 5.1 for a brief overview of the participant flow through data collection, as well as the measures administered at each stage, and intervention components.

Figure 5.1. *Protocol testing flowchart with assessment tools measures and intervention components*



5FMQ = Five-Facet Mindfulness Questionnaire; AUDIT = Alcohol Use Disorder Identification Test; BDI-II = Beck Depression Inventory – II; COVID SS = COVID Stress Scale; CUDIT-R = Cannabis Use Disorder Identification Test – Revised; CUI = Cannabis Use Interview; CWS = Cannabis Withdrawal Scale; FTND = Fagerström Test Of Nicotine Dependence; MCQ-SF = Marijuana Craving Questionnaire – Short Form; MINI = The MINI International Neuropsychiatric Interview 7.0.2; ML = Marijuana Ladder; MRI = Magnetic Resonance Imaging; PIL = Participant Information Letter; PSS = Perceived Stress Scale; SCID-5-RV = Structured Clinical Interview of DSM-5 – Research Version; STAI-Y = State Trait Anxiety Inventory – Y form; TLFB = Timeline Follow Back; WASI-II = Wechsler Abbreviated Scale Of Intelligence – Second Edition

5.3.1.2 Recruitment

All participants were recruited from the Greater Melbourne Metropolitan area, using flyers displayed in public locations and online (e.g., Google, Gumtree, Facebook, Instagram, Reddit, Tik Tok, Beat Online Magazine). The flyers displayed a summary of key inclusion and exclusion criteria.

5.3.2.2 Screening

Members of the community who were interested in the study were directed to (i) an online screening survey to determine their eligibility against inclusion and exclusion criteria (~30-minutes,

9,045 respondents), followed by (ii) a phone screener to confirm eligibility of potentially suitable participants (lasting ~10 minutes to ~1 hour; 450 prospective people with a CUD and 800 prospective controls were called). A detailed list of the tools used for the online and telephone screening is outlined in the study pre-registration (https://doi.org/10.1186/ISRCTN76056942; registration ID: ISRCTN76056942), and in *Chapter 3: General Methods, section 3.5.1 Online Screening* and *3.5.2 Phone Screening.*

5.3.2.3 Intervention Group Allocation

Following confirmation of eligibility, the study-coordinator pseudo-randomly allocated participants to an intervention group (MBI OR *active placebo* OR *passive placebo*), stratified by age and sex. Years of education, alcohol dependency scores (using the AUDIT; Babor et al., 2018) and cannabis dosage/frequency at baseline across intervention groups were also monitored while recruiting.

5.3.2.4 Blinding Procedure

The study was supported by an ***unblinded*** study coordinator (i.e., responsible for pseudo-randomization process of participants to intervention groups and consequently aware of specific participant allocations), ***unblinded*** testers (i.e., responsible for intervention administration, compliance monitoring, and data collection relating to the intervention), and ***blinded*** testers (i.e., responsible for all remaining data collection including neuroimaging acquisition, cognitive testing, semi-structured interviews, and questionnaire administration). Participants were blind to their respective intervention allocation and to the study aims.

5.3.2.5 Overview of Structure for Data Collection

Face-to-face data collection sessions at baseline and at follow-up ~2 weeks later were completed at Monash Biomedical Imaging facility, Clayton, Victoria, Australia.

5.3.2.5.1 Baseline. Participants first attended a baseline session (*see Chapter 3: General Methods for detailed procedure*), at which data was largely collected by a *blinded* tester. The testing session lasted approximately 5-6 hours (including the collection of measures beyond the scope of this study). Participants provided written informed consent and completed an assessment battery to profile socio-demographic data, substance use, mental health, and cognition, comprising questionnaires, face-

to-face semi-structured interviews, standardised cognitive testing, as well as MRI scanning. Additionally, at the conclusion of the baseline session, an *unblinded* tester guided participants through their respective intervention component (~45-minutes), including the initial administration of the intervention audio instructions if applicable (i.e., for the MBI or *active placebo* group).

5.3.2.5.2 Intervention. Participants then completed the specific daily tasks (audio track containing intervention instructions, if applicable, and brief questions to monitor cannabis use and cravings) required for their respective intervention (5-15 minutes per day) over a 2-week period. See section 5.3.4 below for details. Some duration variability between baseline and follow-up was permitted to accommodate participant scheduling requirements.

5.3.2.5.3 Follow-up. The follow-up session lasted ~4-hours. It started with the administration of the intervention including audio instructions (as applicable) with an *unblinded* tester upon arrival. Participants then completed the follow-up session battery with a *blinded* tester, which largely replicated the baseline session. At the completion of the follow-up session, participants were given the opportunity to debrief and received reimbursement in the form of a Coles Myer voucher, to the value of AUD$150. All participants were also offered a picture from a single frame from their T1-weighted (T1w) scan.

5.3.3 Face-To-Face Assessment Tools

All questionnaires used for participants' screening and face-to-face testing were administered via Qualtrics Software, version 2019-2022 (Qualtrics, Provo, UT).

5.3.3.1 Overview of Tools Administered at Baseline Face-to-Face Testing Only

5.3.3.1.1 Sociodemographic Data and Handedness. We measured participants' data on age, sex, and years of education using a standard demographic proforma. Handedness was ascertained using the Edinburgh Handedness Inventory – Short Form (EHI-SF; Veale, 2014).

5.3.3.1.2 Full Scale Intelligence Quotient Testing. The WASI-II (Wechsler, 2011) was used to estimate participants' FSIQ, derived from the administration of the Vocabulary and Matrix Reasoning subtests.

5.3.3.1.3 Substance Exposure and Related Problems. The *SCID-5-RV* (First et al., 2015) is a structured interview, which required participants to respond 'yes' or 'no' to 11-items (with additional probing if necessary) to determine the number of DSM-5 CUD items the participant endorsed. A score of 4-5 indicated moderate CUD, and a score of 6+ indicated severe CUD.

The *Timeline Follow Back (TLFB;* Sobell & Sobell, 1992) is a structured interview, used to gather information on substance use over the past 30 days (e.g., cannabis, alcohol, nicotine, and other). We measured the number of days/past 30 days in which each substance was used (i.e., for cannabis, alcohol, nicotine) and the quantity of substance used/past 30 days (i.e., grams of cannabis, standard drinks of alcohol, number of cigarettes), as well as the number of hours since participants' last used cannabis.

The *Cannabis Use Semi-Structured Interview (CUI;* Cuttler & Spradlin, 2017) was used to gather information on participants' cannabis use over their lifetime. We extracted the age of *first* cannabis use, age of *regular* cannabis use (defined as onset of at least monthly use), and the duration of regular cannabis use.

The *Marijuana Ladder* (Slavet et al., 2006) was administered to provide an indication of participant's motivation to stop cannabis use / readiness to change. Participants selected where they sat on a scale ranging from 0 (No thought about quitting, I cannot live without cannabis) to 9 (I have changed my cannabis use, but I still worry about slipping back, so I need to keep working on the changes I have made). Scores of 0-2 correspond with the pre-contemplation stage, 3-5 with the contemplation stage, 6-7 with the preparation stage, 8 with the action stage, and 9 with the maintenance stage.

Substance use-related problems were quantified for alcohol and nicotine, via the *AUDIT* (Babor et al., 2018) and the *Fagerström Test for Nicotine Dependence (FTND)*, respectively (Fagerström et al., 2012).

5.3.3.2 Overview of Tools Administered at Both Baseline and Follow-Up Face-to-Face Testing

5.3.3.2.1 Substance Exposure and Related Problems. The *TLFB* (Sobell & Sobell, 1992) was administered at follow-up following the same procedure as described above (*section 5.3.4.1.3.3),* to determine the number of days cannabis was used on and the number of grams of cannabis consumed per day, across the participants' intervention period, as well as hours since last cannabis use. This therefore meant that the period of time recorded varied across participants, according to their respective intervention duration (i.e., 14-day target with some variability to accommodate participant schedules).

To directly compare cannabis use in the lead up to each session (i.e., pre-baseline compared to pre-follow-up), additional measures of 'cannabis days' and 'cannabis dose' were derived using the already collected data from the *TLFB* at baseline. For each participant, the total grams of cannabis consumed and the number of days on which cannabis was consumed on *pre-baseline* were calculated across a time interval which matched the participant's intervention duration. These variables are referred to as '*cannabis days – intervention duration'* and '*cannabis dose – intervention duration'*, to differentiate from 'number of days used, and the number of grams of cannabis consumed per day over the past 30 days' collected at baseline.

CUD-related problems were quantified using the *Cannabis Use Disorders Identification Test – Revised (CUDIT-R;* Adamson et al., 2010). Scores greater than 8 indicated hazardous use and scores of 12 or more indicated a possible CUD.

The *Marijuana Craving Questionnaire – Short-Form (MCQ-SF;* Heishman et al., 2009) was administered to obtain an indication of the magnitude of cravings for cannabis experienced by the participant in the moment of questionnaire administration. It contained 12-items, with higher scores indicating greater cravings. The *Cannabis Withdrawal Scale* (Allsop et al., 2011) was administered to monitor participant's experiences of cannabis withdrawal over the past 24-hours. It contained 19-items, with higher scores indicating greater withdrawal.

The *Beck Depression Inventory – second edition (BDI-II;* Beck et al., 1996), a standardised measure of mood with diagnostic ranges, was used to quantify participant's experiences of depression over the past fortnight. The *State-Trait Anxiety Index – Y Form, (STAI-Y)* 'state' sub-scale

(Spielberger et al., 1983) is a commonly used measure of state anxiety. It provided a measure of feelings of anxiety in the moments preceding the MRI acquisition. The *Perceived Stress Scale – 10 items (PSS;* Cohen et al., 1983) is a widely used measure to quantify participant's perception of their stress over the past fortnight. It contained 5 subscales (i.e., COVID danger and contamination, COVID socioeconomic consequences, COVID xenophobia, COVID traumatic stress, and COVID compulsive checking), summed to measure a specific 'COVID Stress Syndrome'.

Participants completed the *5FMQ* (Baer et al., 2008) to provide a measure of their perceived mindfulness and self-awareness. The five subscales (observation, description, aware actions, non-judgemental inner experience, and non-reactivity) were summed to provide a total score, whereby higher scores indicate greater mindfulness/self-awareness.

5.3.4 Intervention

The intervention daily online tasks (e.g., brief questionnaire to monitor cannabis use and cravings, and audio track where applicable) were carried out by participants over a ~2-week between the baseline and the follow-up face-to-face testing session. Additionally, the initial and final daily online tasks were completed under *unblinded* supervision at the completion of the baseline and commencement of the follow-up session. For the *passive placebo* group, this consisted of completing a *daily questionnaire* only; for the MBI and *active placebo* group this included the *daily questionnaire* in addition to listening to 7-minutes of audio instructions. The *daily questionnaire* (and audio instructions if applicable) were accessed online via Qualtrics link.

5.3.4.1 Monitoring Intervention Compliance

Daily compliance of completion of the daily tasks (*daily questionnaire* and audio instructions if applicable) was monitored by an *unblinded* tester, who was able to determine remotely if the participant had opened their Qualtrics link each day. If participants missed one day or two days, an SMS reminder was sent. If participants missed a third consecutive day, a phone call was placed to participants was made to ascertain if they were experiencing any issues in completing or accessing the task(s) and assistance provided if required. A binary measure of intervention compliance was created by determining whether or not the participant had accessed the task each day.

5.3.4.2 Audio Instructions for MBI and Active Placebo Interventions

The two sets of audio instructions were matched for time and cadence. The scripts were near identical but for the alteration of key words to tailor the interventions.

5.3.4.2.1 MBI Script. The MBI group were told that "noticing, paying attention to, and accepting" their thoughts and physical sensations, could increase their ability to experience cannabis cravings without acting on them. It was emphasized that the aim was not to simply relax, but to be alert and attentive. Participants were guided through "open monitoring" of experience and particularly through "aware[ness] of feelings and bodily sensations" and experiencing "craving in a different way". To reduce expectancy effects relating to the increasing popularity and public discussion of complementary medicine approaches, no mention of the term "mindfulness" (or "relaxation") was made in any experimental or recruitment material.

5.3.4.2.2 Active Placebo, Relaxation Script. The *active placebo* group was told that cannabis craving intensity can be reduced by "softening the muscles…and calming and unwinding the mind…releasing tension in your body" and that relaxation enables transformation of sensations into more calming, less unpleasant experiences. Participants were instructed to pay attention to their breath, to facilitate the release of tension. It was emphasized that this is a way of managing craving or urges to smoke cannabis.

5.3.4.2.3 Passive Placebo, No Script. Participants in the *passive placebo* group did not listen to any scripted recordings. Instead, this group only completed a *daily questionnaire*, to minimize discernment of allocation to the *passive placebo* group.

5.3.4.3 Daily Questionnaire, In All Intervention Groups

All participants completed a *daily questionnaire* (~3 minutes), that collected information on cannabis exposure and related variables across the intervention period to aid data interpretation. All cannabis users were asked to provide daily estimates of their cannabis use (number of occasions and quantity), and instances of dangerous use (i.e., "have you been able to suspend your cannabis use to be 'safer' or to aid performance?"). All cannabis users were also asked each day to rate the intensity of their cannabis cravings and urges, their ability to "step back and be aware of cravings/urges without being taken over by them", their mental state, their levels of relaxation-tension and

nervousness/stress, and judgement of thoughts as "good or bad". For participants in the MBI and *active placebo* group, their *daily questionnaire* also contained items on intervention compliance (e.g., "Since the last time you completed this questionnaire, have you listened to the audio track?"; "When you felt the urges or craving to smoke cannabis, have you practiced the strategy you have been listening to on the audio track?").

5.3.5 Statistical Analyses

5.3.5.1 Behavioural Data: Group Differences at Baseline

We used a series of Chi-squares to compare sex and handedness between the intervention groups at baseline (i.e., MBI vs *active placebo* vs *passive placebo*). We performed a series of Kruskal-Wallis tests to compare groups for non-normally distributed scalar variables, i.e., age, years of education, all substance use variables, and intervention duration. Finally, we ran a series of ANOVAs for normally distributed scalar data, i.e., WASI-II FSIQ, motivation to change, and intervention compliance measured as the proportion of days out of the total intervention on which the participant accessed their respective daily task.

5.3.5.2 Behavioural Data: Effect of Group, Effect of Time, and Effect of Group-by-Time

Effects of group, effects of time and effects of group-by-time were analysed using a linear mixed-effect model. Sample characteristics examined included *cannabis days – intervention duration*, *cannabis dose – intervention duration*, CUD-related problems (CUDIT-R), withdrawal (CWS), and craving (MCQ-SF), abstinence from cannabis pre-session (hours), mental health symptom scores (i.e., depression [BDI], state anxiety [STAI-Y], perceived stress [PSS], and COVID-specific stress [COVID Stress Scale]), and measure of mindfulness (5FMQ). The timepoint (baseline and follow-up) and the intervention group were entered into the model as fixed factors, with the participant entered as a random factor to account for natural heterogeneity in the responses of different individuals.

5.3.5.3 Sample Characteristics: Post Hoc Pairwise Comparison

Post hoc pairwise comparisons were completed on variables at baseline and follow-up which showed either a significant (state anxiety) or a trend (perceived stress) effect of group in the linear

mixed-effects model. Mann Whitney U pairwise comparisons for non-normally distributed data were used.

5.3.6 Neuroimaging

5.3.6.1 fMRI Resting-State Task Setup

Prior to entering the scanner participants were requested to stay awake; the tester checked in real-time that participants kept their eyes open throughout the scan, via an MRI-compatible camera placed inside the MRI scanner.

5.3.6.2 fMRI Resting-State Task Instructions

Inside the scanner, prior to the resting-state scan acquisition, participants were instructed verbatim "the next scan will take about 10 minutes. Keep your eyes open, try not to think about anything in particular. Stay relaxed and try to keep your head still". Through the resting-state scan, participants were shown a fixation cross (white cross on black background) via a mirror placed inside the MRI scanner.

5.3.6.3 MRI Acquisition Parameters

Participants were scanned using the same group of experienced radiographers at Monash Biomedical Imaging at baseline and follow-up. Participants were scanned on a Siemens Skyra 3 Tesla MRI scanner using a 32-channel head coil. T1-weighted (T1w) scans were acquired using the following acquisition parameters: TE = 2.07ms, TR = 2300ms, flip angle = 9°, 192 sagittal slices without gap, field of view 256 x 256mm, yielding a 1 x 1 x 1mm resolution, with a total acquisition time of 5 minutes. Resting-state scans (189 volumes) were acquired over 8 minutes, using the following parameters: TR = 2500ms, TE = 30ms, flip angle = 90°, field of view = 192mm, matrix = 64, voxel size 3 x 3 x 3mm^3, 44 slices without gap, and a total acquisition time of 480 seconds.

5.3.6.4 MRI Data Handling

All MRI data were directly exported from the scanner to Monash Biomedical Imaging-XNAT (XNAT website, private server), where it was stored and backed up in Digital Imaging and Communications in Medicine (DICOM) format. Raw format (i.e., DICOM) data were downloaded

from the XNAT server and converted into Brain Imaging Data Structure (BIDS) format using dcm2niix (v1.0.20201102) for further analysis. All imaging data processing and analysis were performed on a cloud-based cluster-computational platform, MASSIVE (massive.org.au; Goscinski et al., 2014). The pre- and post-processing was conducted using CONN toolbox 20.b (www.nitrc.org/projects/conn, RRID:SCR_009550; Whitfield-Gabrieli & Nieto-Castanon, 2012), based on SPM12 on Matlab (2018a.r7487), which was pre-installed on MASSIVE.

5.3.6.5 MRI Data Pre-Processing

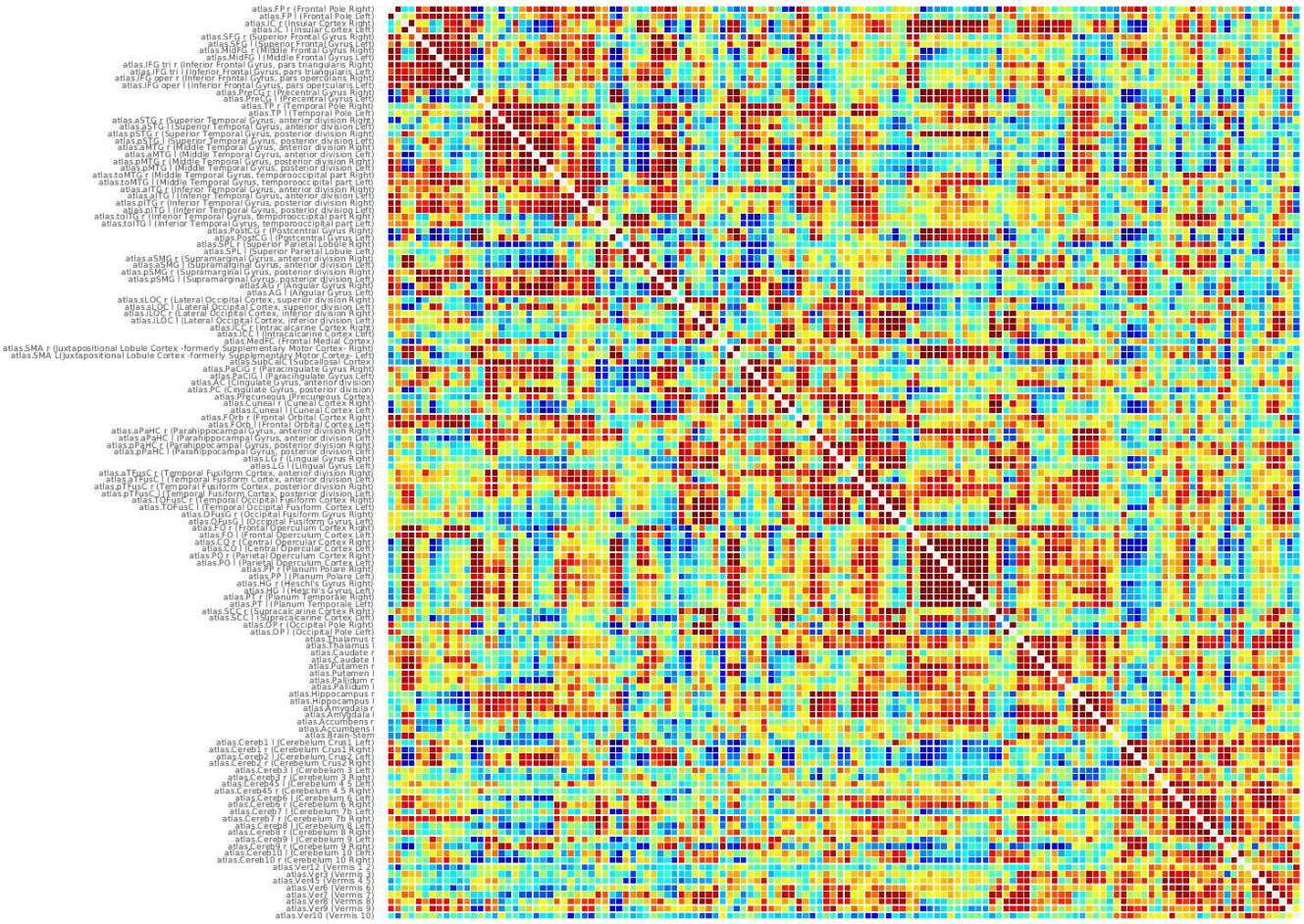
All validated data were imported in BIDS format, then underwent a standard pre-processing pipeline using CONN toolbox 20.b, including 1) slice timing with interleaved slice order, 2) realignment and generation of motion parameters, 3) ARtifact-detection Tools (ART)-based outlier detection with intermediate settings (default 97th percentile in normative sample), 4) co-registered fMRI data with T1w images, 5) segmentations of T1w images, 6) normalising T1w images to Montreal Neurological Institute (MNI) space (standard space), and normalising fMRI to MNI space with the same parameters, and 7) smoothing with 6mm kernel. fMRI was then resampled at 2x2x2mm isotropic. Quality Assurance (QA) reports were generated and manually reviewed by authors HT and CS. Stringent criteria for detecting motion outliers, as outlined by Parkes et al. (2018), was followed, whereby limits of >0.25mm mean framewise displacement (mFD) and >5mm maximum framewise displacement were set; no participants violated these criteria.

5.3.6.6 functional MRI Data Analysis of Intervention-Group-by-Time Effects

5.3.6.6.1 Connectome Analysis. The connectome matrix (132 x 132) for each scan were generated using the default Harvard-Oxford atlas (132 cortical and subcortical regions; Desikan et al., 2006) within the CONN toolbox 20.b (conn/rois/atlas.nii), with method of bivariate correlation and haemodynamic response function (HRF) weightings. See Figure 5.2 for an example of a connectome matrix from a single subject within the sample, selected at random. Then, these connectome matrices were fed into the graphic analysis using a General Liner Model (GLM) using a group-by-time design: the two timepoints were input as different sessions to set up the within subject contrast (follow-up – baseline). The intervention groups were set up as a three-level factor (i.e., MBI, *active placebo*, *passive placebo*). Three t-contrasts were selected between all combinations of two intervention types

(MBI vs *active placebo*; MBI vs *passive placebo*; *active placebo* vs *passive placebo*). We used multiple comparison error correction, specifically using a cluster-level FDR correction, p-corrected < 0.05, multi-voxel pattern analysis (MVPA) omnibus test.

Figure 5.2. *Connectome matrix (132 x 132) of a single subject, selected at random from the sample*



5.3.6.6.2 Seed-Based Functional Connectivity Analysis. Brain regions selected as seeds (ROIs) based on prior knowledge, were determined by the default Harvard-Oxford atlas within the CONN toolbox 20.b. See Table 5.1 for an overview of the seeds examined and their coordinates in Montreal Neurological Institute (MNI) stereotaxic space. Seed-based functional connectivity maps were generated using CONN toolbox 20.b. Briefly, a bivariate correlation coefficient was calculated between the timeseries of each ROI and the that of each other voxel in the brain, controlling for

nuisance factors such as motions parameters, global signal, signal from white matter, and signal from cerebral spinal fluid. The seed-based rsFC maps for the four seeds were used for post-processing.

Table 5.1. *Overview of seeds examined and coordinates in MNI stereotaxic space*

Seeds	**Hemisphere**	**MNI Coordinates**
Putamen	Left	-28, -3, 7
	Right	28, -3, 7
Nucleus Accumbens	Left	-11, 9, -7
	Right	11, 9, -7
Pallidum	Left	-18, -7, 1
	Right	18, -7, 1
Hippocampus	Left	-27, -18, -16
	Right	27, -18, -16

Note: MNI coordinates are taken from the rough centre of the mass, seeds are anatomic in shape

5.3.6.7 MRI Data Post-Processing

Post-processing statistical analyses of fMRI data were conducted using CONN toolbox 20.b. For the group-by-time design, the same GLM with t-contrasts were used, as applied for the Connectome Analysis. Briefly, a group-by-time design: the two timepoints were input as different sessions to set up the within subject contrast (follow-up – baseline). The intervention groups were set up as a three-level factor (i.e., MBI, *active placebo*, *passive placebo*). Three t-contrasts were selected between all combinations of two intervention types (MBI vs *active placebo*; MBI vs *passive placebo*; *active placebo* vs *passive placebo*. To control for multiple comparison errors across voxels, we applied a cluster level False Discovery Rate (FDR) correction (p-corrected < 0.05), with an initial default threshold ($p<0.001$). Next, among the four independent seeds, we applied a further conservative Benjamini-Hochberg multiple comparison error correction, with a threshold of $p < 0.05$ (equivalent to p-FDR corrected < 0.001). rsFC group-by-time interaction effects were defined for those seeds which survived the two multiple comparison corrections. We extracted the mean values within the ROIs (i.e., mean rsFC beta values for the seed to cluster) using CONN toolbox 20.b for further brain behaviour correlation analyses.

5.3.6.8 Brain Behaviour Correlations

We ran Pearson's correlations between the BOLD series values that displayed a group-by-time interaction effect (i.e., rsFC beta values extracted via the CONN toolbox 20.2) and metrics of cannabis exposure, related problems, and mindfulness levels, which significantly changed over time, within each intervention group. For rsFC pairings showing a group-by-time interaction effect a 'change in rsFC' was calculated by subtracting the mean rsFC beta value within the ROI for each participant at baseline from their value at follow-up. Similarly, for metrics of cannabis exposure, related problems, and mindfulness levels which changed over time in the linear-mixed-effects model, a 'change' measure was calculated by subtracting the value at baseline from the value at follow-up (i.e., change in: *cannabis days – intervention duration, cannabis dose – intervention duration*, cannabis withdrawal, COVID-specific stress, and 5FMQ mindfulness). Correlations were run using SPSS (version 28).

5.4 Results

5.4.1 Sample Characteristics

5.4.1.1 Baseline

The total sample included 56 cannabis users (14 females), aged a median of 25 years (*range:* 18-51). Of these, 19 participants (5 female) were allocated to the MBI, 18 participants (4 female) to the *active placebo* group, and 19 participants (5 female) to the *passive placebo* group. This sample was determined to be of adequate size to ensure a high level of power for this study; please see *Chapter 3: General Methods, section 3.4.5* for details.

Table 5.2 overviews the characteristics of all three groups at baseline. Groups were matched by age and sex. Additionally, at baseline the groups were not significantly different for handedness, FSIQ, years of education and substance exposure and related problems ($p > .05$). The intervention compliance (percentage of days completed out of total intervention per participant) was significantly different between MBI and *passive placebo*, with MBI having lower compliance than the *passive placebo* group.

Table 5.2. *Sample Characteristics measured at baseline only*

Variable		MBI	Active placebo	Passive Placebo	Group Differences	
		Mean (*SD*) or median [*range*]			χ^{χ} / H^{H} / F^{F}	*p*
Total *n* [female]		19 [5]	18 [4]	19 [5]	0.11 $^{\chi}$	.947
Age		26 [*20-44*]	25.5 [*18-51*]	23 [*18-36*]	1.32 ^{H}	.517
Handedness *n* [right]		19 [18]	18 [17]	19 [17]	0.47 $^{\chi}$	.789
FSIQ		110 (*11*) $^{n=18}$	106 (*8*)	105 (*9*) $^{n=18}$	1.36 ^{F}	.266
Education, years		13.5 [*11-21*]	15.5 [*13-19*]	16 [*12-23*]	5.56 ^{H}	.062
Alcohol	days, past 30 days	4 [*0-22*]	3 [*0-12*]	5 [*1-29*]	4.60 ^{H}	.101
	standard drinks, past 30 days	16 [*0-207*]	5 [*0-119*]	23 [*3-201*]	5.02 ^{H}	.081
	AUDIT	6 [*0-17*]	5 [*1-12*]	7 [*2-17*]	4.20 ^{H}	.123
Nicotine	days, past 30 days	0 [*0-30*]	0 [*0-30*]	0 [*0-30*]	1.73 ^{H}	.421
	cigarettes, past 30 days	0 [*0-262*] $^{n=18}$	0 [*0-200*]	0.5 [0-580] $^{n=17}$	0.63 ^{H}	.728
	FTND	0 [*0-6*]	0 [*0-5*]	0 [*0-5*]	0.70 ^{H}	.705
Cannabis	age at *first* use, years	17 [*13-32*]	15 [*14-21*]	16.5 [*14-21*]	1.85 ^{H}	.396
	age at *regular* use, years	18 [*15-32*]	18 [*15-26*]	19 [*14-26*]	2.18 ^{H}	.336
	duration regular use, years	5 [*1-29*]	7 [*3-35*]	4.5 [*1-16*]	3.15 ^{H}	.207
	use days, past 30 days	28 [*13-30*]	27 [*14-30*]	26 [*14-29*]	1.96 ^{H}	.375
	grams, past 30 days	24 [*3-76*]	21 [*5-81*]	18 [*1-59*]	0.76 ^{H}	.684
	motivation to change	5 (*2*)	5 (*2*)	6 (*2*)	0.30 ^{F}	.750
	N CUD symptoms	7 [*4-9*]	7 [*4-11*]	8 [*4-11*]	0.01 ^{H}	.997
Intervention	duration, days	15 [*11-33*]	15 [*14-29*]	15 [*10-32*]	2.95 ^{H}	.228
	% completed	60 (*19*)	68 (*23*)	83 (*15*)	6.70 ^{F}	**.003**

AUDIT = Alcohol Use Disorder Identification Test; ^{F} = ANOVA; FTND = Fagerström Test Of Nicotine Dependence; FSIQ = full scale intelligence quotient; ^{H} = Kruskal Wallis; *n* = group size; *SD* = standard deviation; $^{\chi}$ = Chi-square. *Note:* Means and standard deviations which significantly differ are shaded pink.

5.4.1.2 Sample Characteristics: Effect of Group, Time, and Group-by-Time

Table 5.3 overviews sample characteristics at baseline and follow-up, as well as the effects of group, effects of time, and effects of group-by-time. There was an effect of intervention group on anxiety and stress scores. Post-hoc pair-wise comparisons revealed that group effects were driven by significantly higher anxiety at follow-up in both the MBI and *active placebo* groups than the *passive placebo* group; perceived stress was trend-level greater at follow-up in MBI than *passive placebo*. There were significant effects of time on *cannabis days – intervention duration*, *cannabis dose – intervention duration*, COVID-specific stress scores, and 5FMQ mindfulness, which decreased from pre-to-post intervention in all groups, and on cannabis withdrawal, which increased pre-to-post intervention in all groups. There was no group-by-time effect on any variables.

Table 5.3. *Sample characteristics measured at baseline and at follow-up*

Variable			MBI	Active placebo	Passive Placebo	Group		Time		Group-by-Time	
			Median [*range*]			*F* (*df*)	*p*	*F* (*df*)	*p*	*F* (*df*)	*p*
Cannabis	use days (*intervention duration*)	BL	15 [*5-23*]	15 [*9-21*] n=16	13 [*0-18*] n=17	1.35 (*2*)	.268	7.87 (*1*)	**.007**	0.29 (*2*)	.751
		FU	14 [2-22]	13 [*6-26*]	13 [*1-15*]						
	dose, grams (*intervention duration*)	BL	6 [*2-59*] n=18	16 [*2-74*] n=16	12 [*0-21*] n=16	1.64 (*2*)	.204	8.51 (*1*)	**.005**	0.41 (*2*)	.665
		FU	10 [*2-65*]	7 [*1-75*]	6 [*1-20*]						
	CUDIT-R	BL	14 [*8-26*]	15 [*12-30*]	16 [*7-27*]	0.71 (*2*)	.496	2.54 (*1*)	.117	0.49 (*2*)	.614
		FU	14 [*5-21*]	16 [*10-29*]	15 [*4-29*]						
	abstinence, hours	BL	16 [*13-44*]	17 [*12-65*]	16 [*12-44*]	0.58 (*2*)	.566	1.70 (*1*)	.198	0.76 (*2*)	.472
		FU	15 [*12-127*]	16 [*1-91*]	18 [*12-761*]						
	cravings, MCQ-SF	BL	27 [*18-75*]	40 [*17-63*]	31 [*17-70*]	1.21 (*2*)	.305	0.245 (*1*)	0.623	.038 (*2*)	.687
		FU	35 [*15-75*]	37 [*15-57*]	29 [*13-68*]						
	withdrawal, CWS	BL	26 [*0-118*]	28 [*8-91*]	14 [*1-98*]	1.83 (2)	.171	5.22 (*1*)	**.026**	0.126 (*2*)	.882
		FU	43 [*6-91*]	31 [*8-118*]	21 [*3-101*]						
Depression, BDI-II		BL	10 [*1-27*]	10 [*2-46*]	6 [*2-25*]	2.15 (*2*)	.127	0.55 (*1*)	.462	0.47 (*2*)	.630
		FU	9 [*0-39*]	10 [*2-45*]	5 [*0-33*]						
State Anxiety, STAI-Y		BL	32 [*20-60*]	32 [*21-54*]	26 [*21-45*]	3.35 (*2*)	**.043**	1.81 (*1*)	.184	0.61 (*2*)	.546
		FU	34 [*21-80*]	34 [*23-56*]	25 [*20-49*]						
Perceived Stress, PSS		BL	16 [*1-33*]	15 [*5-33*]	12 [*4-32*]	2.65 (*2*)	**.080**	2.60 (*1*)	.113	0.83 (*2*)	.441
		FU	19 [*5-36*]	17 [*9-36*]	11 [*6-33*]						
COVID Stress Scale		BL	3 [*0-88*] n=17	8 [*1-45*] n=12	3 [*0-21*] n=15	0.32 (*2*)	.728	8.65 (*1*)	**.005**	0.30 (*2*)	.739
		FU	2 [*0-73*]	3 [*0-53*]	4 [*0-16*]						
Mindfulness, 5FMQ		BL	135 [*104-173*]	124 [*94-171*]	134 [*70-161*]	0.43 (*2*)	.656	4.10 (*1*)	**.048**	0.32 (*2*)	.731
		FU	131 [*104-165*]	129 [*93-161*]	131 [*103-141*]						

FMQ = Five Facet Mindfulness Questionnaire; BDI-II = Beck Depression Inventory; CUDIT-R = Cannabis Use Disorder Identification Test-Revised; CWS = Cannabis Withdrawal Scale; *df* = degrees of freedom; MCQ-SF = Marijuana Craving Questionnaire – Short Form; PSS = Perceived Stress Scale; STAI-Y = State Trait Anxiety Inventory – Y Form; TLFB = Timeline Follow Back
Note: significant effects of time are shaded orange; significant effects of intervention group are shaded green (trend effect light green); medians and ranges which significantly differ at follow up only are shaded pink.

5.4.2 Group-by-Time Effects on Resting-State Functional Connectivity

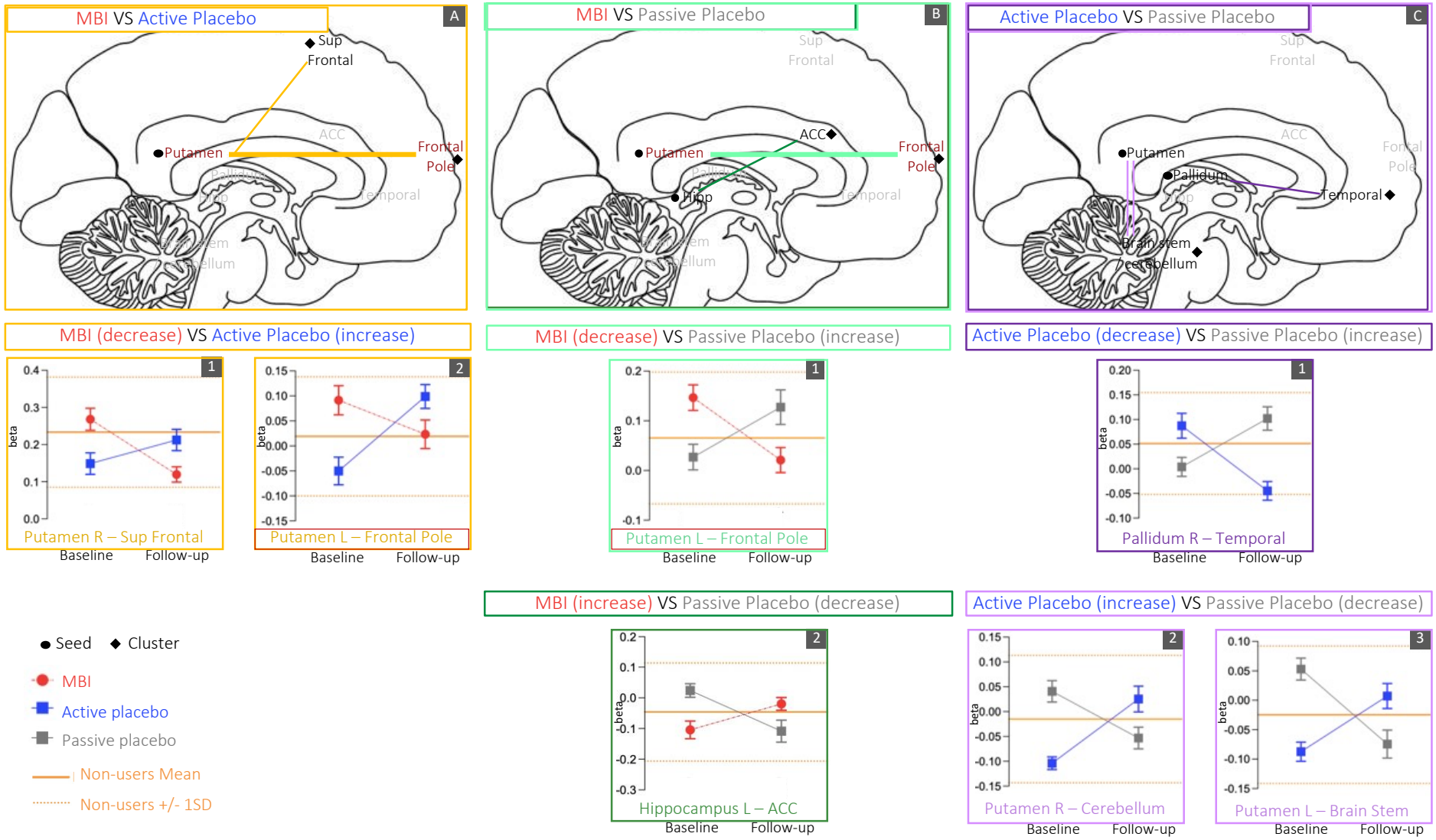
5.4.2.1 Group-by-Time Effects on the Connectome Analysis

There were no significant effects of group, time, or group-by-time on connectome analysis.

5.4.2.2 Group-by-time Effects on Seed-Based Resting-State Functional Connectivity

Figure 5.3 and Table 5.4 show effects of group-by-time on rsFC, using FDR-corrected p-value plus conservative Benjamini-Hochberg correction to α values. There were group-by-time effects on rsFC observed for the putamen, pallidum, and the hippocampus seeds with various brain clusters (expanded below). The NAc seed did not yield significant intervention-group-by-time interactions. Visual plots were used to identify the nature of the interaction effects, which are outlined below as a function of MBI vs *active placebo*, MBI vs *passive placebo*, and *passive placebo* vs *active placebo*.

Figure 5.3. *rsFC group-by-time interactions, with accompanying plots. Regions labelled in dark red text/box indicate pairing observed in two contrasts*



ACC = anterior cingulate cortex; aSTG = anterior superior temporal gyrus; cerebellum = cerebellum (vermis); hipp = hippocampus; L = left; MBI = Mindfulness Based Intervention; R = right; SD = standard deviation; Sup = superior; VS = versus

Note: Means and standard deviations (*orange lines in plots*) come from a non-using-control comparison sample, utilised in *Chapter 4: Study 2*.

Table 5.4. *Seed-based functional connectivity maps*

	Seed	Cluster	Cluster Peak (x,y,z)	K (voxels)	Size p-FDR	B-H adjusted α
A1	Putamen R	SFG	04, 14, 56	120	**.001**	.005
A2	Putamen L	Frontal Pole	02, 66, 12	105	**.003**	.010
B1	Putamen L	Frontal Pole	46, 52, 08	155	**<.001**	<.001
B2	Hippocampus L	ACC	12, 18, 34	124	**<.001**	.002
C1	Pallidum R	aSTG	-56, -08, -04	112	**.001**	.001
C2	Putamen L	Brainstem	04, -46, -28	111	**.002**	.004
C3	Putamen R	Cerebellum Vermis	00, -48, -28	107	**.002**	.003

ACC = Anterior Cingulate Cortex; aSTG = Anterior Superior Temporal Gyrus; B-H = Benjamini-Hochberg; FDR = False Discovery Rate; L = Left; K = Cluster Size; R = Right; SFG = Superior Frontal Gyrus

Note: A1 – C3 refer to pairing labels within Figure 5.3

5.4.2.2.1 Changes in rsFC Pre-To-Post MBI vs Pre-To-Post Active Placebo. Figure 5.3A shows that putamen-SFG and putamen-frontal pole rsFC decreased pre-to-post *MBI* and increased pre-to-post *active placebo*.

5.4.2.2.2 Changes in rsFC Pre-To-Post MBI vs Pre-To-Post Passive Placebo. Figure 5.3B shows that putamen-frontal pole rsFC decreased pre-to-post *MBI* and increased pre-to-post *passive placebo*. In contrast, hippocampus-ACC rsFC increased pre-to-post *MBI* and decreased pre-to-post *passive placebo*.

5.4.2.2.3 Changes in rsFC Pre-To-Post Active Placebo vs Pre-To-Post Passive Placebo. Figure 5.3C shows that pallidum-anterior superior temporal gyrus (aSTG) rsFC decreased pre-to-post *active placebo* and increased pre-to-post *passive placebo*. Further, putamen-cerebellum (vermis) and putamen-brainstem rsFC increased pre-to-post *active placebo* and decreased pre-to-post *passive placebo*.

5.4.2.3 Brain Behaviour Correlations

Correlations between the change in rsFC and the change in cannabis exposure and related variables (i.e., behavioural variables which displayed a main effect of time) are displayed in Tables 5.5, 5.6, and 5.7.

5.4.2.3.1 MBI Group. Increased hippocampus-ACC rsFC pre-to-post *MBI* significantly correlated with more cannabis use days pre-to-post *MBI* (Table 5.5).

5.4.2.3.2 Active Placebo Group. Increased putamen-frontal pole rsFC pre-to-post *active placebo* correlated with decreased cannabis grams pre-to-post *active placebo* (Table 5.6).

5.4.2.3.3 Passive Placebo Group. Increased putamen-frontal pole rsFC pre-to-post *passive placebo* correlated with less cannabis use days pre-to-post *passive placebo* (Table 5.7).

Table 5.5. *Overview of correlations between change in rsFC and changes in cannabis use, withdrawal, perceived stress, and mindfulness, within the MBI group*

Contrast	ΔrsFC		Δcannabis, grams (n=18)		Δcannabis, days		Δwithdrawal, CWS		ΔCOVID stress (n=17)		Δ5FMQ	
	Seed	*Cluster*	r	p	r	p	r	p	r	p	r	p
MBI vs AP $_{A1}$	*Putamen R*	*SFG*	-.145	.565	.073	.768	.238	.326	.006	.983	-.227	.349
MBI vs AP $_{A2}$	*Putamen L*	*Frontal Pole*	-.196	.435	-.229	.345	-.075	.760	.076	.772	-.192	.430
MBI vs PP $_{B1}$	*Putamen L*	*Frontal Pole*	-.090	.721	.059	.811	.386	.103	-.369	.145	.031	.898
MBI vs PP $_{B2}$	*Hippocampus L*	*ACC*	-.131	.604	.629	**.004**	.358	.132	.439	.078	.007	.977

Δ = Change Pre-To-Post Intervention; 5FMQ = Five Facet Mindfulness Questionnaire; ACC = Anterior Cingulate Cortex; AP = Active Placebo; CWS = Cannabis Withdrawal Scale; L = Left; MBI = Mindfulness Based Intervention; PP = Passive Placebo; R = Right; rsFC = Resting-State Functional Connectivity; SFG = Superior Frontal Gyrus

Note: significant strong positive correlations are shaded red; A1 – B2 refer to pairing labels within Figure 5.3.

Table 5.6. *Overview of correlations between change in rsFC and changes in cannabis use, withdrawal, perceived stress, and mindfulness, within the active placebo group*

Contrast	ΔrsFC		Δcannabis, grams (n=16)		Δcannabis, days (n=16)		Δwithdrawal, CWS		ΔCOVID stress (n=17)		Δ5FMQ	
	Seed	*Cluster*	r	p	r	p	r	p	r	p	r	p
MBI vs AP $_{A1}$	*Putamen R*	*SFG*	.065	.810	.098	.718	.002	.994	.121	.707	-.153	.546
MBI vs AP $_{A2}$	*Putamen L*	*Frontal Pole*	-.573	**.020**	-.477	.061	.178	.479	-.363	.246	-.240	.338
AP vs PP $_{C1}$	*Pallidum R*	*aSTG*	.405	.120	.260	.331	-.100	.693	-.187	.561	-.026	.919
AP vs PP $_{C2}$	*Putamen R*	*Cerebellum*	-.053	.847	.270	.313	.176	.485	-.252	.429	-.152	.547
AP vs PP $_{C3}$	*Putamen L*	*Brainstem*	.189	.484	.249	.353	.030	.906	-.388	.213	-.147	.560

Δ = Change Pre-To-Post Intervention; 5FMQ = Five Facet Mindfulness Questionnaire; aSTG = Anterior Superior Temporal Gyrus; CWS = Cannabis Withdrawal Scale; AP = Active Placebo; L = Left; MBI = Mindfulness Based Intervention; PP = Passive Placebo Group; R = Right; rsFC = Resting-State Functional Connectivity

Note: significant strong negative correlations are shaded blue; A1 – C3 refer to pairing labels within Figure 5.3.

Table 5.7. *Overview of correlations between change in rsFC and changes in cannabis use, withdrawal, perceived stress, and mindfulness, within the passive placebo group*

Contrast	Δ rsFC		n=16 Δcannabis, grams		n=17 Δcannabis, days		Δwithdrawal, CWS		n=17 ΔCOVID stress		Δ5FMQ	
	Seed	*Cluster*	r	p	r	p	r	p	r	p	r	p
MBI vs PP $_{B1}$	*Putamen L*	*Frontal Pole*	-.050	.855	-.517	**.034**	.321	.181	-.001	.998	.096	.696
MBI vs PP $_{B2}$	*Hippocampus L*	*ACC*	.193	.474	.105	.688	-.118	.629	-.388	.153	-.263	.277
AP vs PP $_{C1}$	*Pallidum R*	*aSTG*	.189	.483	-.164	.530	.016	.949	.111	.695	.413	.079
AP vs PP $_{C2}$	*Putamen R*	*Cerebellum*	-.020	.940	.294	.252	.272	.260	.100	.723	-.340	.155
AP vs PP $_{C3}$	*Putamen L*	*Brainstem*	-.028	.917	.201	.438	.377	.112	-.043	.878	-.285	.237

Δ = Change Pre-To-Post Intervention; 5FMQ = Five Facet Mindfulness Questionnaire; ACC = Anterior Cingulate Cortex; AP = Active Placebo; aSTG = Anterior Superior Temporal Gyrus; CWS = Cannabis Withdrawal Scale; L = Left; MBI = Mindfulness Based Intervention; PP = Passive Placebo Group; R = Right; rsFC = Resting-State Functional Connectivity

Note: significant strong negative correlations are shaded blue; B1 – C3 refer to pairing labels within Figure 5.3.

5.5 Discussion

In the first fMRI study to examine how a brief MBI changes brain dysfunction in CUD, we reported significant rsFC changes in three ROIs: the putamen, pallidum, and hippocampus. Putamen-frontal pole rsFC decreased pre-to-post MBI; it increased pre-to-post *active placebo*, in correlation with decreased cannabis grams, and it also increased pre-to-post *passive placebo* in correlation with decreased cannabis use days. Putamen-SFG rsFC decreased pre-to-post MBI and increased pre-to-post *active placebo*. Hippocampus-ACC rsFC increased pre-to-post MBI, in correlation with more cannabis use days, and decreased pre-to-post *passive placebo*. Pallidum-anterior superior temporal gyrus rsFC decreased pre-to-post *active placebo* and increased pre-to-post *passive placebo*. Further, putamen-cerebellum/brainstem rsFC increased pre-to-post *active placebo* and decreased pre-to-post *passive placebo*. The findings provide tentative evidence that MBI, relaxation intervention, and daily monitoring intervention could affect selected putamen-frontal pole/STG and hippocampus-ACC pathways dysfunctional in CUD, in differing directions; and additional pathways are selectively

affected specifically by relaxation and by daily monitoring of cannabis use (e.g., putamen-cerebellum/brainstem and pallidum-aSTG).

We found that putamen-frontal pole rsFC decreased pre-to-post MBI. Notably, this effect emerged in contrast to both *active* and *passive placebo,* therefore suggesting MBI-specific decreases within this pathway. Both regions have been implicated in cognitive processes implicated in addiction and CUD. The putamen has been implicated in habit formation and compulsive substance use (Ersche et al., 2021; Yin & Knowlton, 2006), while the frontal pole has been ascribed to cognitive control (Goldstein & Volkow, 2002; Hanlon et al., 2018; Orr et al., 2015). Of note, MBI has been associated with changes in the function of the putamen in cigarette smokers (Froeliger et al., 2017) and in normative samples (Santarnecchi et al., 2021). Additionally, increased putamen volume has been established in individuals experienced with MBI, compared to non-mindfulness practicing controls (Chiesa & Serretti, 2010). Thus, the putamen may be a promising target for MBI in SUDs including CUD, possibly via attenuating reward prediction signals to salient stimuli such as cannabis-related stimuli (Kirk & Montague, 2015). Also, the frontal pole has been suggested as a target for the treatment of SUDs (Clinical TMS Society, 2023). Prefrontal regions are thought to be involved in a feedback loop with the putamen to regulate behaviour. Consequently, putamen-frontal pole rsFC changes pre-to-post MBI may underlie a transition from compulsive to regulated substance use via increasing cognitive control over people's own cannabis use, via engaging inhibitory PFC pathways over striatal pathways implicated in habit formation such as reduced cannabis dosage (Koob & Volkow, 2010). However, there were no correlations between the decrease in putamen-frontal pole rsFC pre-to-post MBI with reduced cannabis use and the behavioural relevance of the brain functional changes is to be elucidated.

We reported decrease in putamen-SFG rsFC pre-to-post MBI (vs *active placebo*). Importantly, frontostriatal dysfunction has been consistently associated with SUDs, including CUD (Bloomfield et al., 2019; Thomson et al., *in preparation* [Chapter 4]), opioid use disorder (Ma et al., 2010), nicotine use disorder (Hong et al., 2009), and alcohol use disorder (Sullivan & Pfefferbaum, 2019). Frontostriatal alterations have been thought to contribute to impaired reward processing demonstrated in CUD (Bloomfield et al., 2019). Hence, frontostriatal alterations (putamen-frontal

pole/SFG) shown pre-to-post MBI support the notion that MBI may 'reverse, repair, or compensate for' neuroadaptive changes associated with addiction neurocircuitry (Kirlic et al., 2021; Witkiewitz et al., 2013) and contribute to restoration of natural reward processes among individuals with an SUD (Garland et al., 2014). Future work is required to establish the precise mechanism whereby frontostriatal dysfunction is targeted by MBI.

Interestingly, putamen-frontal pole rsFC changed pre-to-post *active* and *passive placebo*, in an opposite direction than the MBI. Putamen-frontal pole rsFC increased pre-to-post *active* (relaxation) and *passive* (daily monitoring) *placebo*, and the change in rsFC correlated with decreased cannabis dosage and frequency pre-to-post intervention, respectively. The findings suggested that changes observed in *active* and *passive placebo* groups are linked to changes in cannabis exposure. It remains unclear if decreased cannabis exposure caused rsFC changes, or of if brain changes in these interventions drove reduced cannabis exposure. To our knowledge, this is the first time rsFC alterations have been demonstrated pre-to-post relaxation-based intervention (*active placebo*) and daily monitoring of substance use (*passive placebo*) in any SUD. This finding suggests that these interventions may influence the addiction neurocircuitry in CUD in their own right, as well as changes to cannabis exposure. While relaxation and daily monitoring of substance use may represent viable alternative intervention options, the specific mechanisms driving rsFC changes in these interventions are yet to be clarified. Importantly, future findings reporting changes of putamen-frontal pole rsFC should be interpreted with caution as this pathway might be targeted by multiple interventions entailing separate cognitive processes and mechanisms.

We found increased hippocampus-ACC rsFC pre-to-post MBI (vs *active placebo*), as a function of increased cannabis use days pre-to-post MBI. Interestingly, ACC-cortical connectivity was previously reported to increase pre-to-post MBI in cigarette smokers, in correlation with decreased cigarette smoking (Froeliger et al., 2017). Therefore, MBI may affect how ACC connects with cortical and sub-cortical regions. Interestingly, the ACC plays a key role in reward processing (Rolls, 2019), and emotion-regulation (Ichikawa et al., 2011; Posner et al., 2007). Following MBI, the ACC showed altered activity during reward processing and cue reactivity, linked to improved self-control and reduced craving (Froeliger et al., 2017; Westbrook et al., 2013). The hippocampus is

known to be critical to learning, memory and stress (Clark et al., 2019; Corkin, 2002; Maguire et al., 2016). Further, alterations to hippocampus-ACC rsFC have been established in SUDs (i.e., cocaine dependence), and are thought underlie altered reward processing in SUD (Gu et al., 2010). Additionally, functional connectivity strengthening between the hippocampus and ventromedial PFC (vmPFC; on which the ACC sits) has previously been linked to successful inhibition of a conditioned fear response (Milad et al., 2007). Both the ACC and hippocampus regions have also been implicated in mindfulness practice (Chiesa & Serretti, 2010; Lu et al., 2014; Tang et al., 2015), further demonstrating their potential to be targeted by MBI in people with SUD. It has been theorised that MBI may act on a network of regions, including the hippocampus and ACC, to enhance fear extinction (Tang et al., 2015). It may be therefore possible, that strengthening of rsFC between these same regions (hippocampus and ACC), may facilitate the extinguishing of habitual substance use, however future research is required to investigate this further.

We found that *active placebo* intervention increased putamen-brainstem/cerebellum rsFC (vs *passive placebo*). It has been theorised that processes of relaxation establish parasympathetic dominance (Luberto et al., 2020), which includes reduced heart rate and respiration rate (Luberto et al., 2020). Of interest, the putamen is known to underlie habit formation (Balleine & O'Doherty, 2010; Yin et al., 2004), while the brainstem underlies respiratory processes (Dutschmann & Dick, 2012; Holstege, 2014) and the cerebellum is involved with craving for substances including cannabis (Moreno-Rius, 2019; Moreno-Rius & Miquel, 2017). Thus, increased putamen-brainstem/cerebellum rsFC pre-to-post *active placebo* relaxation intervention may reflect a coupling between putamen-related habitual cannabis use in CUD, with relaxation-related breath changes implicated by the brainstem, as well as experiences of craving mediated by the cerebellum. Further fMRI research is required to confirm how relaxation-based interventions affect brain function.

Finally, we found that the *active placebo (*relaxation) intervention reduced pallidum-aSTG rsFC (vs *passive placebo*). Previously, rsFC alterations between striatal-temporal regions have been implicated in low mood (Ma et al., 2012). Notably, the pallidum is implicated in inhibitory control (Aron, 2007) and the direction of attention (Klaassen et al., 2021); whilst the aSTG is known to contribute to increased severity mood-related symptomology (Kang et al., 2022). Relaxation-based

techniques have proven useful in reducing symptoms of depression and anxiety (Klainin-Yobas et al., 2015), which have shown to be highly comorbid in populations who experience CUD (Onaemo et al., 2021). Thus, the relaxation intervention might have targeted neural pathways implicated in low mood and elevated anxiety within this sample, which can affect neurobiology independently of interaction with CUD (Kaiser et al., 2016; Northoff, 2020; Rabany et al., 2017). Future work is required to confirm how relaxation-based interventions can change brain dysfunction in people with a CUD, with entrenched mood disorders.

5.5.1 Limitations and Future Directions

The results of this study should be interpreted considering the following limitations. First, the included sample size (N=56) was much smaller than recently published recommendations examining power to detect group differences in single site rsFC fMRI studies (Marek et al., 2020; Marek et al., 2022). It was reported that to secure reproducibility and identify stabilised behavioural phenotypes, sample sizes of N≳2,000 would be advisable (Marek et al., 2020; Marek et al., 2022). It was however acknowledged that comparatively 'small' neuroimaging samples may remain necessary when examining clinical conditions or in longitudinal studies, and those authors suggested that measurement reliability and effect sizes may still be adequate (Marek et al., 2022). It should however be acknowledged that sample size recommendations provided by Marek et al., 2022 have come under some recent scrutiny (Makowski et al., 2023; Spisak et al., 2023). It has been posited that brain-behaviour correlations are adequately replicable in much smaller samples, consisting of as low as 20 (Spisak et a., 2023) or 42 (Makowski et al., 2023) participants. Indeed, the current study was adequately powered to detect group differences, as evidenced by both significant findings and power analysis (see *Chapter 4: General Methods, 3.4.5 Power Analysis*). Our sample size was larger than similar published studies (N=13; Froeliger et al., 2017; N=28; Fahmy et al., 2019) and median neuroimaging sample sizes more broadly (median N=25; Poldrack et al., 2017; Szucs & Ioannidis, 2020). The sample size was also largely consistently with research suggesting that sample size be dictated by scan length. For example, it has been posited that 40 participants would be required for a 14-minute scan and 100 participants for a 7-minute scan (Termenon et al., 2016). To note, sample

sizes within the current study were hindered by the targeting of a difficult to recruit population, in conjunction with coinciding COVID-19 restrictions spanning the data collection period. Future research should recruit larger sample sizes to reproduce and confirm these effects, or use more powerful MRI scanners (i.e., 7-Tesla), which can reliably detect signal in smaller samples (Willems & Henke, 2021).

A second limitation of this study was participants' low level of motivation to change reported by participants, who largely endorsed being in the 'contemplation' stage, or in preliminary stages of the 'preparation' stage. This meant that most participants ultimately reported being *not yet ready*, or *almost ready* to change their use. Motivation/readiness to change predicts successful behavioural change in substance using populations (Myers et al., 2016), and may outweigh treatment adherence as a facilitator of change (Collins et al., 2012). Therefore, heterogeneous level of motivation to change might have somehow affected neurobiological changes over time. To note however, it was a study eligibility requirement that participants had actively attempted to quit or reduce their cannabis use *within the past 24-months,* and participants significantly reduced their cannabis dose and frequency, in relation to brain functional changes. This may indicate that the intervention process might have been sufficient to detect neurobehavioral changes. Future research should replicate the findings in a sample with a strong intention to implement change (i.e., a score of ≥7 on the Marijuana Ladder; Slavet et al., 2006), to determine if detected changes may become more robust.

A third limitation of the study was the level of intervention compliance in the MBI and *active* placebo intervention groups (intervention was completed on ~66% of days, on average across these groups) and was significantly lower for the MBI than the *passive placebo* group (83% completion on average). Reduced intervention compliance could be driven by reduced motivation to change (Brocato & Wagner, 2008; Clair et al., 2011; Hiller et al., 2002; Longshore & Teruya, 2006; McMurran & Ward, 2010). Considering the exclusion criterion that participants have little-to-no lifetime experience with any mindfulness-related practices, it is feasible that the resultant eligible population may have an inadvertent aversion to mindfulness-related practices. Inclusion of mindfulness averse participants may partially account for an unexpected reduction in 5FMQ mindfulness observed over time across groups (i.e., participants may disengage with strategies) and may also further contribute to low

compliance. Furthermore, poor adherence to treatment/intervention has been shown to limit treatment effectiveness (Hansen et al., 2002; Harris et al., 2010; McMurran & Ward, 2010). Therefore, low intervention compliance may have undermined the detection of addition rsFC changes, which are subtle in nature. With that in mind however, rsFC alterations established within this study, may reflect a 'real world' effect of intervention related changes, as 'perfect' compliance with intervention for SUDs is unlikely to be achieved in day-to-day life (Herbeck et al., 2005). Future research could consider an additional layer of reimbursement that is contingent upon high intervention compliance. Additional research is warranted to measure how rsFC changes pre-to-post MBI in mindfulness-naïve participants vs mindfulness-experienced participants, and measure level of mindfulness experience in their samples, to untangle how baseline levels of mindfulness affect rsFC changes when MBIs are administered in CUD.

A final limitation of the study was the use of a *daily questionnaire* across all intervention groups, which required self-report of cannabis use daily. This meant that self-monitoring of cannabis dosage was required in the *passive placebo* group to ensure that any effect pre-to-post MBI and active placebo relaxation were due to the interventions themselves, not the self-monitoring component. However, self-monitoring alone has previously been shown to decrease substance use (Gass et al., 2021). Therefore, self-monitoring in the passive placebo group may have acted as an 'active intervention' and might have contributed to the rsFC changes in the passive placebo condition. Future research that uses daily monitoring of substance use as passive placebo, should consider the introduction of a 'waitlist control' to unpack the effects of daily monitoring. Yet in our study, all 3 conditions included daily monitoring of cannabis use, and the effects of MBI accounted for those due to daily monitoring.

5.5.2 Strengths of the Current Study

A number of strengths emerged in the first study to investigate the neurobiological correlates of a brief, cost effective, and remotely delivered MBI in a population with moderate-to-severe CUD. First, the study followed a robust double-blinding design. The double-blinding process removed expectancy effects of treatment outcomes on rsFC from both the participants and the researchers

conducting data collection and analyses. Second, the pseudo-randomised allocation to one of three interventions ensured that the participants within each group did not significantly differ on key demographic variables (i.e., age, sex), known to influence neurobiology. Furthermore, the groups did not differ on other additional variables (e.g., demographics, cannabis, alcohol, and nicotine use variables, mental health symptom scores, or subjective mindfulness). Therefore, the influence of confounders on rsFC changes might have been minimised. Third, the inclusion of both an *active placebo* control group and *passive placebo* control group enabled us to detect MBI-specific changes from general treatment effects or the effects of participation in a study.

5.5.3 Clinical Implications

This study reported intervention-group-by-time interactions on rsFC, which may indicate that over time brief MBI may ultimately affect behavioural change. Foremost, hippocampus-ACC rsFC changes pre-to-post MBI were a function of change to number of cannabis use days. Furthermore, evidence has suggested that there may exist a temporal ordering of top-down and bottom-up brain changes following MBI, i.e., emergence of change in regions associated with cognitive control (top down) and cravings (bottom up) may occur in a sequence (Witkiewitz et al., 2013). Therefore, neurobiological changes may continue to evolve and further reductions in associated behaviours i.e., substance use and/or symptoms of cravings, may emerge following the onset of rsFC alterations; and were thus not yet detectable immediately post-MBI. Future research should include a long-term (i.e., 1-, 3- & 6-months post intervention) follow-up to monitor for continued or sustained behavioural change (i.e., continued reduction/abstinence from substance).

Additionally, decreased frontostriatal rsFC pre-to-post MBI, as well as increased hippocampus-frontal rsFC pre-to-post MBI – correlated with reduced cannabis frequency – adds support to the notion that MBI may be an effective tool for the targeting of neuroadaptive changes associated with addiction (Kirlic et al., 2021; Witkiewitz et al., 2013). Meanwhile, rsFC pre-to-post *active* and *passive placebo* also correlated with a reduction in dosage/frequency. Thus, the utility of relaxation and daily monitoring interventions to mitigate brain dysfunction and cannabis exposure in CUD should be further investigated.

5.5.4 Conclusion

In conclusion, the findings from this study confirmed our hypothesis that a brief MBI (vs *active* and *passive placebo*) changed rsFC in ROIs of the addiction neurocircuitry, high in CB_1R, and known to be altered in this group individuals with a moderate-to-severe CUD. Specifically, MBI increased rsFC in frontostriatal pathways implicated in habituation and inhibitory control (e.g., putamen-frontal pole/SFG), and in association with changes in cannabis exposure (e.g., frequency of use). Also, *active placebo* relaxation intervention affected rsFC in partly overlapping neural pathways implicated in respiratory control and craving (i.e., putamen-brainstem/cerebellum) and may be an effective intervention to mitigate brain dysfunction, in correlation with cannabis dosage. Meanwhile, *passive placebo* including monitoring of daily cannabis use also affected rsFC in frontostriatal pathways. Together, the findings suggest that MBI, relaxation intervention, and daily monitoring intervention affect distinct putamen-frontal pole/STG and hippocampus-ACC pathways dysfunctional in CUD, in differing directions. Additional pathways are selectively affected specifically by relaxation and by daily monitoring of cannabis use. Future research with larger samples of individuals with a CUD, who endorse high motivation to change use, is required to further expand on rsFC changes over time. Overall, findings suggest that MBI may be a promising intervention for individuals with CUD, by targeting altered neurocircuitry associated with addiction. Observed changes provide insight into the neural mechanisms underlying MBI, as well as relaxation- and self-monitoring-based intervention, in CUD.

CHAPTER 6:

General Discussion

Chapter Guide

The overarching aims of this thesis were threefold. The ***first aim*** was to systematically review the fMRI literature on the neural correlates of regular cannabis use, using resting-state functional connectivity (rsFC; *Chapter 2: Systematic Literature Review*). The review was published in the journal *Psychopharmacology* in 2022. The ***second aim*** was to identify if people with a Cannabis Use Disorder (*CUD*) showed different rsFC than controls, via utilising a purposefully collected novel dataset (*Chapter 4: Study 2*). The ***third aim*** was to explore how altered rsFC in CUD participants from Study 2, was mitigated using a ~two-week Mindfulness Based Intervention (MBI; compared to *active* and *passive placebo* control interventions) with an *active* and *passive placebo* controlled design (*Chapter 5: Study 3*).

The following chapter will review the findings of the three separate studies, then detail the contributions that the thesis has made to the understanding of neural correlates of (i) regular cannabis use, (ii) CUD, and (iii) brief MBI in CUD. Strengths of the research and clinical implications of the findings will be discussed. Limitations and future directions will be outlined before a concluding statement on the thesis.

Taken together, the findings from all three studies within this thesis make a significant contribution to the understanding of neural mechanisms involved with CUD, and the development of potential neurobiological targets for the treatment of CUD.

6.1 Summary of Aims and Main Findings

6.1.1 Study 1: Systematic Literature Review

6.1.1.1 Study Aims

The ***primary aim*** of the systematic literature review (SLR) was to summarise the findings to date on resting-state functional connectivity (rsFC) differences between non-intoxicated people who regularly use cannabis and controls. 'Regular cannabis use' was defined as per the inclusion/exclusion criteria of the reviewed studies. The targeting of 'regular' cannabis users who were non-intoxicated at the time of rsFC functional Magnetic Resonance Imaging (fMRI) acquisition, was to measure the 'residual' chronic effects of cannabis on the brain.

The ***secondary aim*** of the SLR was to systematically synthesise the evidence to date on the associations between rsFC in people who regularly use cannabis and cannabis use levels (e.g., duration, dosage), cognitive performance (e.g., executive function), and mental health symptoms (e.g., depression). The summarised findings helped to shed light on whether specific subgroups of people who regularly use cannabis are more vulnerable to greater alterations of rsFC.

6.1.1.2 Key Study Findings

To our knowledge, this was the first SLR to be published, on the evidence to date from rsFC fMRI studies comparing people who regularly use cannabis and controls. The reviewed literature comprised of 21 studies, published between 2013 and 2019. The most consistent finding was that people who regularly use cannabis compared to controls had greater positive rsFC between frontostriatal, fronto-frontal, and frontotemporal pairings; followed by lower positive rsFC in partially overlapping pairings (frontostriatal). Similar pairings (frontostriatal, fronto-frontal, frontotemporal) were preliminarily associated with varying measures of cannabis exposure, and with mental health symptom scores, and cognitive outcomes (verbal recall and impulsivity).

Higher positive rsFC in people who regularly use cannabis compared to controls, was reported in ~40% of the studies between frontostriatal, fronto-frontal, and frontotemporal pairings. Interestingly, the location of the reviewed rsFC group differences was partially overlapping with that reported by studies comparing people who regularly use cannabis and controls during cognitive task-based fMRI (e.g., temporal, frontal, and striatal activation; Blest-Hopley et al., 2018; Yanes et al.,

2018); and also examined with structural MRI (e.g., lower volume of the orbitofrontal cortex, and hippocampus regions; Lorenzetti et al., 2019). Together, the findings from the literature to date show that regular cannabis use is associated with rsFC alterations within regions of the addiction neurocircuitry e.g., PFC, striatum, amygdala, and basal ganglia.

6.1.2 Study 2: Comparing rsFC in People with a Cannabis Use Disorder vs Controls

6.1.2.1 Study Aims

The second study of the thesis was designed to address the limitations uncovered across the SLR (*Chapter 2: Study 1*), such as small sample sizes, the lack of assessment of whether cannabis users endorsed a Cannabis Use Disorder (CUD) and lack of accounting for confounders.

The ***primary aim*** was to compare rsFC for the first time between 65 people with a diagnosis of moderate-to-severe CUD and who had recently tried to cut down or quit cannabis, and 42 controls, accounting for age, sex, and variables that differed between the two groups (i.e., alcohol and nicotine exposure, and depression symptom scores).

The ***secondary aim*** was to explore how rsFC differences identified between people with a CUD and controls would be associated with metrics of cannabis exposure and related problems. The metrics included: severity of CUD, age of first and regular use onset, hours since last use, and the duration of use in years, frequency (number of days on which cannabis was used in the past 30 days), and cannabis dosage (total number of grams used across the past 30 days).

6.1.2.2 Key Study Findings

To our knowledge, this was the first study that examined rsFC in individuals who met criteria for a CUD. As hypothesised, significant alterations in rsFC in the CUD group compared to controls were observed, controlling for age, sex, past 30 days alcohol and nicotine dose, and depression scores in the GLM. While the SLR uncovered rsFC alterations in widespread brain pathways, Study 2 implicated only frontostriatal and occipito-striatal pathways. Specifically, in people with a CUD compared to controls, rsFC was greater between NAc-frontal regions, putamen-occipito-parietal regions, and pallidum-occipital/occipito-parietal regions. Greater putamen-occipito-parietal rsFC correlated with an earlier age of *first* and of *regular* cannabis use, and greater pallidum-

occipital/occipito-parietal rsFC correlated with severity of CUD and days of cannabis use/past 30 days. Further, rsFC was lower between hippocampus-occipital regions.

6.1.3 Study 3: Pre-to-Post Brief Intervention

6.1.3.1 Study Aims

The third study was designed to use a brief Mindfulness-Based Intervention (MBI) to mitigate altered rsFC shown in Study 2, in individuals with a moderate-to-severe CUD, who have attempted to quit or reduce their cannabis use within the past 24-months.

The ***primary aim*** of study 3 was to examine for the first time how a brief MBI previously shown to reduce alcohol consumption (Kamboj et al., 2017) affected rsFC in people with a moderate-to-severe CUD. A robust double-blind, *active* and *passive placebo*-controlled design was utilised. In detail, the 56 participants were allocated in a pseudo-randomised order stratified on age and sex, to one of three interventions: (i) a brief MBI adapted to target cannabis cravings (n = 19), (ii) an *active placebo* (relaxation) intervention adapted to cannabis cravings and matched to the MBI by number of words, complexity, and all components but mindfulness (n = 18), (iii) a *passive placebo* control group with daily monitoring of cannabis use, a component which was also embedded in both MBI and *active placebo* (n = 19).

The ***secondary aim*** was to explore if changes in rsFC pre-to-post MBI were associated with changes in cannabis exposure, related variables, and psychological measures, pre-to-post MBI.

6.1.3.2 Key Study Findings

To the best of our knowledge, this was the first fMRI study to examine how a brief MBI mitigates altered rsFC in CUD. We reported significant rsFC changes in three ROIs that showed alterations in the CUD sample as shown in Study 2: the putamen, pallidum, and hippocampus. **Pre-to-post MBI**, putamen-frontal pole rsFC decreased (vs *active* and *passive placebo*); putamen- superior frontal gyrus (SFG) rsFC decreased (vs *active placebo*); and hippocampus-ACC rsFC increased (vs *passive placebo*), which correlated with increased frequency of cannabis use. **Pre-to-post *active placebo*** (vs MBI), participants showed increased putamen-frontal pole rsFC, in correlation with lower cannabis grams; and increased putamen-SFG rsFC. Also pre-to-post *active placebo*, pallidum-aSTG

rsFC decreased, and putamen-cerebellum/brainstem rsFC increased (vs *passive placebo*). Third, **pre-to-post *passive placebo***, putamen-frontal pole rsFC increased (vs MBI) which correlated with decreased cannabis use days; putamen-SFG rsFC and putamen-cerebellum/brainstem rsFC both decreased (vs MBI and vs *active placebo* respectively) while pallidum-aSTG rsFC increased (vs *active placebo*).

6.2 Strengths of the Research

6.2.1 Novelty

6.2.1.1 Novelty of Study 1: Systematic Literature Review

This SLR was the first to collate the growing body of evidence to date which has examined the rsFC of people who regularly use cannabis in comparison to controls. Previously published reviews examining residual effects of cannabis use using fMRI have either included task-based fMRI research only (Blest-Hopley et al., 2018), or included rsFC but within a much broader scope (Bloomfield et al., 2019). For instance, Bloomfield et al. (2019) reported on structural, functional, and pharmacological neuroimaging modalities, focusing on adolescence and adults, as well as acute intoxication effects, and subsequently included only 4 of the 21 papers integrated in Study 1.

Study 1 provided the first comprehensive synthesis of the literature and revealed emerging patterns of rsFC alterations in regular cannabis users in pathways that had not yet become evident in individual studies alone or narrative reviews. Similarly, the literature shows preliminary trends for associations between changes in rsFC and level of cannabis use, mood, and cognitive performance. This SLR therefore advanced our understanding of the impact of regular cannabis use on rsFC, and provided insight necessary when developing a framework for the two thesis experiments. Specifically, the SLR identified methodological limitations of the literature, which enabled the identification of broad and specific future directions for the field, and which were addressed by the first experiment of the thesis. Importantly, no study yet had included a measure of whether their cannabis using participants met the diagnostic criteria for a CUD, as per the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5; American Psychiatric Association [APA], 2013). Two thirds of studies within the SLR did not utilise any DSM edition diagnostic criteria relating to cannabis use.

The remaining one third of the studies reported participants' degree of 'cannabis dependence', as measured by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). A notable change between the two editions, was the inclusion of a criterion relating to experiences of 'withdrawal' within the DSM-5. Of relevance, withdrawal is associated with increased functional impairment in SUDs (Katz et al., 2014). Furthermore, the DSM-5 introduced a dimensional approach (i.e., degrees of severity) rather than a categorical approach employed by the DSM-IV (i.e., dependence present; yes or no). Thus, the fourth and fifth edition of the DSM lack full agreement (Livne et al., 2021), and that the evidence in the published literature on cannabis dependence does not translate to the new diagnostic criteria for a CUD.

6.2.1.2 Novelty of Study 2: Cannabis Use Disorder vs Controls

A key novelty of Study 2 was the inclusion of people with a CUD. In addition, we included people who tried to cut down or quit their cannabis use and were on the more severe end of the CUD spectrum. This was also novel and important because more severe forms of CUD are associated with more severe negative psychosocial outcomes. Many of the negative outcomes associated with regular cannabis use (e.g., increased antisocial behaviour, use of other illicit substances, legal trouble, unemployment, and mood disorders [depression and anxiety]) have been shown to be significantly worse for people who regularly use who *do* meet diagnostic criteria for CUD than for people who regularly use who *do not* (Foster et al., 2018; van der Pol et al., 2013). It is thought that cannabis users who experience a CUD may drive the neurobiological changes reported in cannabis using samples where problematic cannabis use is unknown (Lorenzetti et al., 2016). In mapping the neural correlates of CUD using rsFC, this study advanced the understanding of the specific neurobiological outcomes experienced by individuals who are most impacted by cannabis use. Therefore, this study paves the way to identify neurobiological targets for the treatment of more severe forms of CUD. Additionally, this study has extended upon the SLR and confirms that alterations occur in selected pathways in regular cannabis users with unknown CUD levels (i.e., frontostriatal, occipito-striatal, and occipito-hippocampal). Perhaps, alteration of such pathways in regular cannabis users were driven by people with a more severe CUD, and other pathways reportedly altered in regular cannabis users in the SLR might reflect the effect of moderators or confounders.

6.2.1.3 Novelty of Study 3: Pre-to-Post Brief Intervention

This was the first fMRI study to date which explored how a brief MBI that targets cannabis cravings, impacts rsFC in individuals with a CUD, with a focus on ROIs (i) implicated in the addiction neurocircuitry, (ii) high in CB_1R and (iii) with known alterations in this sample compared to controls. Further, the study explored how rsFC changes pre-to-post MBI correlate with those in cannabis use behaviour, mindfulness and stress. Pre-to-post MBI, rsFC decreased between the putamen and the frontal pole/SFG; and increased between the hippocampus and the ACC. Further, ACC-hippocampus rsFC correlated with increased frequency of cannabis use pre-to-post MBI. Thus, a brief MBI may target altered rsFC in CUD in regions of the addiction neurocircuitry, adding to similar results in samples who use substances other than cannabis (Garland et al., 2014; Lorenzetti et al., *under review*). This thesis shows for the first time that MBI can change rsFC in CUD, and extends upon the literature to date that examines substances other than cannabis. Specifically, changes in frontostriatal rsFC were noted in people with a CUD examined herein, but also in opiate users and nicotine users examined in previous work. Thus, selected pathways may be targeted by MBI across different SUDs.

To our knowledge, this was also the first study to explore neural correlates of relaxation-based (*active placebo* condition) and daily-monitoring-based (*passive placebo* condition) intervention in people with a moderate-to-severe CUD. The rsFC changes observed pre-to-post *active* and *passive placebo*, also correlated with a reduction in cannabis dosage/frequency. Thus, the mechanisms underlying rsFC changes pre-to-post relaxation- and daily monitoring-based interventions should be further investigated using multimodal MRI studies and psychophysiological measures (e.g., respiration) that may confound or confirm the specificity of the changes.

6.2.2 Accounting for Confounds

The study designs applied both in Study 2 and Study 3 were robust to account for the influence of many confounding variables on rsFC changes.

6.2.2.1 Confounds Accounted For, Within Study 2: Cannabis Use Disorder vs Controls

A number of methodologies were implemented in the first experiment to account for confounds. First, participants were subject to a thorough inclusion and exclusion criteria in order to screen for confounders including: significant use of other substances (other than alcohol and/or nicotine); comorbid diagnosis of psychiatric disorders other than depression or anxiety which are known to be highly comorbid with CUD (Onaemo et al., 2021); and exposure to medications that affect the central nervous system. The high level of depression and anxiety symptoms, alcohol use, and nicotine use within the CUD sample examined herein, was noted in previous samples of people who regularly use cannabis compared to controls (Chye et al., 2017; Koenders et al., 2017; Lorenzetti et al., 2021; Rossetti et al., 2021). Therefore, the sample of people with a CUD examined herein, may have represented cannabis using populations with entrenched nicotine/alcohol use and mental health problems. Furthermore, the characteristics of the cannabis using group examined in Study 2 were monitored throughout the recruitment phase, in order to concurrently target controls which best matched our clinical sample. This enabled to obtain non-significant group differences for several variables, therefore further mitigating the contributions from additional confounding variables i.e., age, sex, and years of education. Similarly, the two groups were non-significantly different on full scale intelligence quotient (FSIQ) estimate, handedness, and scores on measures of anxiety, stress, and COVID-specific stress. Variables that we were significantly different between groups (i.e., depression scores, and alcohol and nicotine use) were controlled for in all analyses of rsFC data. Therefore, given that the experiments systematically accounted for the influence of confounding variables, our results may represent a ‘true’ effect of CUD with entrenched depression/anxiety comorbidity, and heterogenous rates of alcohol and nicotine use.

6.2.2.2 Confounds Accounted For, Within Study 3: Pre-to-Post Brief Intervention

This study was the first of its kind to investigate the neurobiological correlates of a brief, cost effective, and remotely delivered MBI in a population with moderate-to-severe CUD. The thoroughly screened group of cannabis users with a CUD involved in Study 2, also formed the sample for Study 3. Inclusion and exclusion selection criteria applied which specifically related to Study 3 was

exclusion of participants with past mindfulness-related experience. This was to minimise the confounding influence of pre-existing mindfulness levels on rsFC changes pre-to-post MBI.

The study used a robust double-blinding design, that required managing significant logistical challenges through the project. The double-blinding process was used to mitigate expectancy effects on rsFC outcomes from both researchers collecting data from participants and analysing the data, and from included participants. Second, the pseudo-randomised allocation to one of three interventions ensured that the participants within each group did not significantly differ on stratified variables known to influence neurobiology (i.e., age, sex). Furthermore, the groups did not differ on other measured variables, including demographics, cannabis, alcohol and nicotine use, mental health symptom scores, or subjective mindfulness level. Thus, study 3 examined the impact of intervention condition on rsFC, while minimising the influence of confounders. Third, the inclusion of both an *active placebo* control group and *passive placebo* control group enabled us to detect MBI-specific changes, whilst mitigating possible confounding variables i.e., we were able to parse apart effects which are MBI specific vs effects of intervention engagement vs placebo effects.

6.2.3 Statistical Power

A strength of both Study 2 and Study 3 was the adequate degree of statistical power afforded by size of the two samples. As demonstrated in the General Methods (*Chapter 3: 3.4.5 Power Analysis*) both studies were sufficiently powered to detected between- and within-group differences. This was further evidenced by the survival of the rsFC alterations reported in both studies against the employed strict thresholding criteria - including False Discovery Rate and Benjamini-Hochberg correction. It should still be acknowledged that the sample sizes lacked the power of multi-site neuroimaging studies and consortia data sets and were consequently less likely to secure reproducibility and identify stabilised behavioural phenotypes (Marek et al., 2020; Marek et al., 2022). It has been acknowledged however, that comparatively ‘small’ neuroimaging samples may remain necessary when examining clinical conditions or in longitudinal studies, and measurement reliability and effect sizes may still be adequate (Marek et al., 2022). Furthermore, it has more recently been posited that brain-behaviour correlations are adequately replicable in samples of as few

as 20 (Spisak et a., 2023) or 42 (Makowski et al., 2023) participants. Indeed, our sample across both studies were largely consistent with research suggesting that sample size be dictated by scan length (i.e., 40 participants for a 14-minute scan and 100 participants for a 7-minute scan; Termenon et al., 2016). Our sample was also larger than median neuroimaging sample sizes more broadly (median N=25; Poldrack et al., 2017; Szucs & Ioannidis, 2020), and larger than similar published studies (Fahmy et al., 2019; Froeliger et al., 2017; Thomson et al., 2022 [*Chapter 2*]) and therefore represented an advancement in this field.

6.2.4 Risk of Bias

An additional strength of this thesis was the implementation of 'Risk of Bias' measures across the three included studies. This enabled identification of potential sources of bias, both from the field of literature within the SLR, and within our own research. The National Institute of Health (NIH), National Heart, Lung and Blood Institute's 'Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies' (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools) was utilised for all studies within the SLR. The purpose of the risk of bias within the SLR was to provide a background upon which to interpret the included study findings, i.e., no studies within the SLR produced misleading and/or false results.

The same NIH Quality Assessment Tool was also utilised for Study 2, whilst the Cochrane Collaboration's Tool for Assessing Risk of Bias in Randomised Trials was utilised for Study 3 (Higgins et al., 2011), both detailed below.

6.2.4.1 Risk of Bias Assessment in Study 2: Cannabis Use Disorder vs Controls

The NIH Quality Assessment Tool provided 14 criteria on which to score cross-sectional research. Of the 14 criteria, 2 were deemed not applicable due to their relevance only to longitudinal studies. Of the remaining 12 criteria, only one point was lost due to the lack of blinding of the testers to the participants 'CUD' or 'control' status. Due to the volume of data collection relating specifically to cannabis use, this was unavoidable. The remaining criteria were adequately passed, resulting in a 'Good' quality rating for Study 2 (11 scored out of 12).

6.2.4.2 Risk of Bias Assessment in Study 3: Pre-to-Post Brief Intervention

The Cochran Collaboration's Tool for Assessing Risk of Bias in Randomised Trials provided 6 domains, on which the risk of bias could be scored as either low, unclear, or high. In all 6 domains, the evaluated risk was considered to be 'Low'. This was due to several items: (i) the clear reporting of the pseudorandomisation-with-stratification process resulting in well matched samples (mitigating risk of confounding variable; Sella et al., 2021); (ii) the inclusion of and effective use of the *active* and *passive placebo* conditions; (iii) the high retention rate of participants; and (iv) the clear detailing of methods and results. In addition, the 'gold standard' double-blinding procedure further contributed to the 'Low' risk rating (Evans et al., 2021). Findings from Study 3, specifically the group-by-time-interaction effects observed on rsFC for all three intervention conditions, highlighted the importance of highly controlled experimental designs in order to parse apart variables contributing to the results.

6.2.5 Pre-Scan Abstinence

A final strength of the two empirical experiments within this thesis (Study 2 and 3) was the requirement for participants to abstain from cannabis for at least 12 hours prior to the baseline and follow-up session. This this time period enabled us to measure the 'residual' effects of chronic cannabis use on rsFC, without the confounding contribution from acute intoxication which typically peak within 10 minutes and taper off within 2-3 hours (when smoked; Grotenhermen, 2003).

The abstinence prior to each session across Study 2 and Study 3 was a median of 16 hours. It has been demonstrated that following the cessation of regular/heavy cannabis use, cannabis withdrawal can occur following 24-to-48 hours, and can last between 1-to-2 weeks (Budney et al., 2003; Davis et al., 2016). Therefore, participants in the examined sample were scanned *after* acute intoxication effects of cannabis had worn off, but *before* symptoms of withdrawal had commenced. Therefore, the influence of withdrawal on brain and behaviour was mitigated. Indeed, symptoms of cannabis withdrawal can include irritability and anxiety, as well as physiological symptoms such as decreased appetite and sleep disturbance (Curran et al., 2016; Gates et al., 2016).

6.3 Key Implications Resulting from the Three Studies

6.3.1 Implications for Prominent Neuroscientific Theories of Addiction

The findings from this thesis significantly contribute to the broader field of research examining the neurobiology of SUDs, in so far as uncovered rsFC alterations support the validation of prominent neuroscientific theories of addiction. Specifically, this relates to both the 'three-stage' model first proposed by Koob and Volkow (2010), and the 'incentive-sensitization' theory proposed by Berridge and Robinson (2016). Additionally, the findings herein further support that these theories may partly apply to CUD, suggesting partially overlapping neurocircuitry with other SUDs. Finally, the findings support the notion that a brief psychological intervention has the potential to act upon neurocircuitry thought to underlie these theories.

6.3.1.1 Findings in relation to the 'Three-Stage' Model of Addiction by Koob and Volkow (2010)

The neuroscientific theory of addiction developed by Koob and Volkow (2010) postulates that drug addiction is a "chronically relapsing disorder", driven by neural changes underlying three stages: (i) the binge/intoxication stage (with associated changes to the mesocorticolimbic dopaminergic reward pathway, and includes alterations to the basal ganglia, pallidum, putamen, and NAc), (ii) the withdrawal/negative affect stage (with associated changes to the extended amygdala, also including the NAc), and (iii) the preoccupation/anticipation stage (with associated changes in the PFC; Koob & Volkow, 2016; Volkow, Koob, et al., 2016; Volkow et al., 2019). The findings from this thesis suggest that the neurobiological alterations reported to underlie each respective stage may pervasively impact addiction related neural circuitry, detectable during 'resting-state' i.e., when the participant is not currently engaged in any of the three stages.

Primarily, the findings on frontostriatal *hyperconnectivity* from both the SLR (Study 1) and the first empirical experiment (Study 2) suggest that CUD is associated with hyperactivity of the mesocorticolimbic dopaminergic reward pathway (e.g., increased NAc-frontal pole rsFC), which underlies the binge/intoxication stage (Zehra et al., 2018). To note, the mesocorticolimbic dopamine pathway is comprised of the PFC, the NAc and the ventral tegmental area (Volkow & Morales, 2015; Wise, 1996). The experiments also identified altered rsFC in CUD vs controls, in other regions also implicated within distinct stages of addiction, including preoccupation/anticipation (i.e., frontal pole),

and withdrawal/negative affect (i.e., pallidum and putamen). The mechanisms underlying increased NAc-frontal pole rsFC are unclear. Perhaps, chronically repeated neurobiological alterations of these pathways during cannabis intoxication, may result over time into compensatory neuroadaptations that persist through resting-state. In alterative, rsFC alterations of this pathway may exist pre-dating the onset of cannabis use or CUD (or both). Of interest, findings from the second empirical experiment (i.e., increased frontostriatal rsFC pre-to-post brief MBI) suggest that a brief MBI to restore alterations within the neurocircuitry of binge/intoxication.

Future research should examine rsFC in CUD in relation to the three stages of addiction – binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation – as per Koob and Volkow's (2010) 'three-stage' addiction model.

6.3.1.2 Findings in relation to 'Incentive-Sensitization' Theory by Berridge and Robinson (2016)

The 'incentive-sensitization' neuroscientific theory of addiction proposed by Berridge & Robinson (2016) postulates that addiction is driven by attribution of excessive motivational value to substances (i.e., greater incentive salience). It is theorised that repeated exposure to a substance activates the mesocorticolimbic dopamine system, involved in reward processing. Over time, an individual would assign greater value to a substance which is activating the dopamine system, whilst disregarding other, 'naturally' rewarding, stimuli.

Greater NAc-frontal pole rsFC observed in people with a CUD may therefore reflect increased engagement of the mesocorticolimbic salience pathway, underlying sensitivity to THC exposure. THC has been shown to affect dopamine synthesis within the NAc (Bossong et al., 2009; Pierce & Kumaresan, 2006), which might subsequently affect the function of NAc and interconnected frontal pathways implicated in salience processing. This may play a key role in cannabinoid reinforcement (Lupica et al., 2004; Tanda & Goldberg, 2003), whereby projections from the NAc to the PFC are thought to mediate experiences of 'wanting' and urges to use cannabis (Berridge & Robinson, 2016). In line with this notion, the NAc plays a key role in the predictive value of rewarding stimuli (Knutson & Gibbs, 2007), whilst frontal regions have been linked to a loss of control over substance use (George & Koob, 2010).

Further research is required to elucidate the role of occipital regions in the neurobiology of CUD. Perhaps, occipital-striatal changes in CUD reflect habituation of reward and attentional pathways to reflect higher incentive salience towards rewarding stimuli people are regularly exposed to such as cannabis in people with a CUD. Occipital regions are not traditionally implicated in either the 'incentive salience' model (Berridge & Robinson, 2016) or the 'three-stage' model (Koob & Volkow, 2016; Volkow, Koob, et al., 2016; Volkow et al., 2019), and may therefore represent alterations to neurocircuitry unique to individuals with a CUD.

6.3.2 Implications for Neuroscientific Theories of Mindfulness

As proposed by Garland et al. (2014), mindfulness practice and MBIs may regulate the brain's reward system by promoting greater awareness of internal experiences. Furthermore, MBIs may disrupt neural 'habit loops' underlying SUDs (Brewer et al., 2013), and replace habitual reactions with adaptive responses (Houlihan & Brewer, 2016). It has been suggested that MBIs may promote self-control/decision making to mitigate risk of substance relapse, by acting upon the PFC. MBIs also may enable conscious awareness of substance seeking thoughts and behaviours, via enhanced connectivity of a cortico-thalamic loop (including the PFC, ACC, and thalamus; Garland et al., 2014). Specifically, MBI is postulated to increase awareness of changes to body state and associated drive to use substances, and to disrupt 'unregulated craving', via affecting the function of the hippocampus, which underlies associative memory processes, and of the thalamus, which acts to relay information between striatal and cortical structures, (Garland et al., 2014).

The findings from the current thesis partly support the above notions, in that MBI changed rsFC in (partly) overlapping regions i.e., the putamen-frontal pole/SFG, and the hippocampus-ACC as a function of change to cannabis use pre-to-post MBI. Thus, MBI may affect rsFC alterations in SUD, in pathways implicated in cognitive and inhibitory control (Fahmy et al., 2019; Froeliger et al., 2017). Overall, MBI may contribute to the 'reversal, reparation, or compensation for' altered addition neurocircuitry (Kirlic et al., 2021; Witkiewitz et al., 2013), though future research is necessary to confirm this notion.

6.3.3 Contribution to Advancing Interventions Targeting rsFC in CUD

6.3.3.1 Contribution of Examining a Mindfulness-Based Intervention

As covered in the section above, the research comprising Study 3 (*Chapter 5*) adds to the growing field of research supporting the utility of MBIs in targeting functional brain pathways implicated in addiction and reward processing (Kirlic et al., 2021; Lorenzetti et al., *under review*; Witkiewitz et al., 2013), and extends the theory that MBIs may restore natural reward processes among individuals with an SUD (Garland et al., 2014). Brief MBIs may facilitate an increase in control over cannabis use, by acting on a frontostriatal pairing thought to underlie the transition between compulsive and regulated use/abstinence (Koob & Volkow, 2010). Furthermore, brief MBIs may enhance inhibitory control over- or extinguish habitual substance use, by strengthening rsFC between regions previously shown to underlie inhibition, self- and emotion- regulation, and reward processing (i.e., the hippocampus and ACC; Gu et al., 2010; Ichikawa et al., 2011; Milad et al., 2007; Posner et al., 2007; Rolls, 2019). Whilst there were no behavioural changes reported specific to the MBI, behavioural change may require a longer period of time than the ~two weeks used in this study, to be detected via rsFC fMRI.

As mentioned above, these findings having meaningful implications for those who provide health care support for people with a CUD. Of note, the brief MBI utilised here was delivered online and would be cost effective to administer, straight forward to roll out, adaptable to the routine of individuals who are using it, and not requiring of perfect adherence to be effective. The implementation of this brief MBI or similar, should be considered for individuals with a CUD who are treatment seeking.

6.3.3.2 Contribution of Examining a Relaxation-Based Intervention

To date, there are no known published interventions which specifically examine neurobiological underpinnings of relaxation-based strategies for SUDs. Group-by-time interaction effects showed that the brief *active placebo* intervention (consisting of relaxation strategies) affected frontostriatal, hindbrain-striatal (i.e., putamen-brainstem and putamen-cerebellum), and temporo-striatal pathways also implicated in SUDs. Frontostriatal rsFC increased pre-to-post *active placebo* relaxation intervention, as a function of reduced cannabis dose over the intervention period. It is not

yet understood what mechanisms might be driving this change. Hindbrain-striatal rsFC changes were thought to be linked to the relaxation strategies, as implicated brain regions (brainstem and cerebellum) are involved with respiration and cravings (Dutschmann & Dick, 2012; Holstege, 2014; Moreno-Rius, 2019; Moreno-Rius & Miquel, 2017). Future work is required to confirm how relaxation-based interventions can change brain dysfunction in people with a CUD.

6.3.3.3 Contribution of Examining Interventions Including Self-Monitoring of Substance Use

Participants with a CUD across all three conditions completed a daily practice of 'self-monitoring' which included daily reporting of cannabis use over the intervention period. This was employed (i) to ensure that changes observed within the MBI and *active placebo* condition were not attributable to the daily monitoring component, (ii) to reduce the likelihood that individuals within the *passive placebo* group would be able to discern their 'control group' status, and (iii) to collect data relating to cannabis use patterns of participants across the intervention. This is the first study that examined neurobiological correlates of self-monitoring-based intervention for SUD. Individuals within the *passive placebo* condition demonstrated a correlation between increased frontostriatal rsFC with decreased frequency of cannabis use. Thus, the process of self-monitoring substance use alone may have acted as an 'active intervention' and may additionally target CUD-associated neural pathways in a way that is unique, as opposed to when it is used in combination with MBI or *active placebo*. At present, there is no known neuroimaging research which examines brain changes associated with daily monitoring in the context of SUD, and therefore future research is required to expand this finding.

The presence of self-monitoring across all three conditions may therefore contribute to the observed reduction in cannabis use across participants overall, via the process of 'assessment reactivity'. Assessment reactivity refers to the process of behavioural change that occurs due to self-monitoring (Nelson & Hayes, 1981). It is thought that daily monitoring may impact substance use due to increased participant awareness of use (Moos, 2008). Daily monitoring has been shown to be effective in the reduction of substance use (primarily alcohol and nicotine use; Gass et al., 2021), and indeed in the reduction of cannabis use (Buu et al., 2020; Isaacs et al., 2021). The results of the current study therefore provide further support for the utility of self-monitoring (a low-cost and

widely accessible intervention), both to support behavioural change and target neural alterations associated with CUD. In order to mitigate the effects of assessment-reactivity, future research could consider the introduction of a 'waitlist control' group.

6.3.4 Contribution to Addressing Gaps in the Existing Field, and A Call for Evidence

Across the completion of this thesis, there were two major limitations uncovered in the existing field of literature, which were addressed during completion of this thesis: (i) a lack of replication of extant studies, and (ii) a lack of research implementing diagnostic criteria from the current version of the DSM (the DSM-5). Hence, a call for evidence to further address these limitations is provided here, with justification.

6.3.4.1 Replication of Findings Between Studies of rsFC in People Who Use Cannabis

Limitations uncovered across the course of the SLR related to the highly heterogenous nature of the rsFC methodologies applied, which made the integration of rsFC alterations challenging and which precluded the completion of a meta-analysis. The current thesis aimed to further the work within the field of rsFC research in people who regularly use cannabis, by selecting commonly implicated brain regions as seeds in *a priori* hypothesis based analysis. A possible contributing factor to the application of heterogenous rsFC methodologies may be the phenomenon of 'publication bias'. Publication bias is a pitfall of academia, whereby significant findings are more likely to be published than null findings (Dickersin, 1997). Thus, *a priori* planned methodologies may be altered after researchers uncover they result in a null finding (Miyakawa, 2020), and this may result into reduced replication of studies methodologies/results and high heterogeneity in methods used and results.

In order to facilitate the replication of findings in studies examining rsFC in people who regularly use cannabis, the following recommendations are made. *First,* it is recommended that a coordinated approach is undertaken by researchers in the field, whereby a portion of the literature is dedicated to the formulation of *a priori* driven hypotheses intended to expand upon previously established findings by replicating similar methodological approaches e.g., seed-to-whole brain analyses which select previously implicated brain regions as seeds. *Second,* it is advised that future research report planned fMRI analyses in advance, i.e., pre-registration, which encourages

transparency and re-producibility in research, whilst reducing publication bias (van 't Veer & Giner-Sorolla, 2016). *Third*, research should subsequently report any attempted rsFC methodologies regardless of significance within their manuscripts so that the reader can observe when a replication attempt has been unsuccessful (see *Chapter 4: section 4.4.2.1 Connectome Analysis* and *Chapter 5: section 5.4.2.1 Group-by-Time Effects on the Connectome Analysis* for an example). *Fourth*, raw data should be made available alongside published manuscripts to increase transparency (Gorgolewski & Poldrack, 2016; Miyakawa, 2020; Poldrack & Gorgolewski, 2014). The provision of raw data may additionally further facilitate the development of multi-site and consortia data sets in fMRI research (Breeze et al., 2012; Poldrack & Gorgolewski, 2014; Poline et al., 2012). We plan to share the data from this study once the main analyses on rsFC have been tested.

6.3.4.2 Updated CUD Criteria; Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

Study 2 is the first rsFC study to apply the up-to-date diagnostic criteria for CUD, as published within the DSM-5 (APA, 2013). Future work is required to replicate the findings to corroborate their robustness. The studies herein extend upon previous research examining people who use regularly cannabis with unknown status regarding problems with use (Thomson et al., 2022 [*Chapter 2*]) and further the knowledge of neural underpinnings of moderate-to-severe CUD, which therefore relates to cannabis using population shown most to be impacted by negative outcomes associated with their use (Foster et al., 2018; van der Pol et al., 2013). In order to improve the generalisability of findings across populations with CUD, and to explore moderators which may exacerbate rsFC alterations in CUD, future research is required (please see below for details outlining directions for future research, in *section 6.4.1.2 Lack of Power to Examine Sub-Groups of People with CUD*).

6.3.5 Contribution to Raising Public Awareness Towards Possible Consequences of Cannabis Use

The findings of this thesis contribute to the extensive body of research demonstrating adverse outcomes associated with cannabis use (Foster et al., 2018; Hall & Degenhardt, 2009; Lev-Ran et al., 2014; Solowij & Battisti, 2008; van der Pol et al., 2013; Volkow et al., 2014; Volkow, Swanson, et al., 2016). Building upon established risks of cannabis use, findings from Study 1 (SLR) and Study 2

(first empirical experiment), demonstrate that cannabis acts on brain circuits involved in the reward system, similarly to other drugs of abuse (Zehra et al., 2018). It is advised that public awareness be raised via population-based mass media campaign, previously demonstrated to reach a target audience and raise awareness of links between substance use and associated negative outcomes (Dixon et al., 2015). This could provide the public with a balanced narrative i.e., that risks exist alongside medicinal benefits (Hall, 2020). A population-based mass media campaign could be targeted at increasing knowledge regarding (i) the commonalities between cannabis and other substances established within this thesis (i.e., frontostriatal alterations), and (i) negative psychosocial health outcomes of cannabis use more broadly. This increased awareness may enable people to be better informed when making decisions. As the legal status of cannabis continues to evolve in modern society, it will be essential for policy makers to be aware of the associated risks of use, to mitigate the risk that people using cannabis develop a CUD, and to support those who do. To this end, both policy makers directing health support funding, and those who provide support to people with a CUD, should be made aware of potential interventions which are able to target the neurobiological alterations demonstrated in individuals with a CUD (detailed in the next section).

6.4 Limitations and Future Directions

The results of this thesis must be interpreted alongside a number of limitations outlined below, alongside suggestions for future research which could address them.

6.4.1 Limitations of Study 2: CUD vs Controls

6.4.1.1 Limitations of A Cross Sectional Study Design

The results discussed in Study 2 relied upon a cross-sectional study design. The use of a cross-sectional study design prevented the ability to make conclusions regarding the direction or existence of causal relationships between moderate-to-severe CUD and rsFC changes. Additionally, we were unable to determine if rsFC changes predated or followed the development of a CUD. The implication of this being that it remains unclear if the identified rsFC changes are the result of exposure to cannabis use or are pre-existing and predispose an individual to the development of a

CUD. Longitudinal fMRI studies are required to track rsFC changes predating and following the onset of CUD and monitor how they change with the continuation of CUD and changes of cannabis use levels.

6.4.1.2 Lack of Power to Examine Sub-Groups of People with CUD

Although the sample size of Study 2 was adequately powered to detect between group differences (CUD vs controls), the sample size did not provide adequate power to further divide the CUD group into various subgroups, to explore the impact of moderating variables such as sex. Furthermore, CUD is known to have comorbidities (expanded below), which increases difficulty in pinpointing specific factors influencing rsFC alterations. Future research, utilising a larger sample of people with a CUD, is required to explore which moderators exacerbate rsFC alterations in CUD. Possible outstanding moderators of rsFC alterations in CUD include:

- Sex: Neurobiological alterations have been shown to differ as a function of sex, in populations who use cannabis (Calakos et al., 2017; Hammond et al., 2022; Rosseti et al., 2021) as well as in SUDs more broadly (Cornish & Prasad, 2021), and which should therefore be further explored in CUD.
- Psychiatric Disorders: Individuals with comorbid substances use disorder and psychiatric disorders have been shown to display unique neurobiological alterations to individuals who endorse substance use disorders without psychiatric comorbidity (Balhara et al., 2017). Given the high rates of comorbid CUD with psychiatric illness (Hasin & Walsh, 2020), future research is required exploring how such comorbidities may interact with established rsFC alterations.
- Age of Use Onset: As established within this study, increased rsFC between the putamen and occipito-parietal regions was observed as a function of age of *first* and *regular* cannabis use. Furthermore, evidence has shown that exposure to cannabis during key neurodevelopmental periods such as adolescence may result in changes to corticolimbic circuitry (Meyer et al., 2018). Distinct patterns of neurobiological alterations have been observed when examining both adults and adolescents who use cannabis (Blest-Hopley et al., 2018), and hence, how the neurobiology of ‘adult onset’ versus ‘adolescent onset’ cannabis use differs, should be further explored.

6.4.1.3 Reduced Generalisability of CUD Sample to the Population Who Use Cannabis

An additional limitation related to the enforcement of strict exclusion criteria during the recruitment period. This may have inadvertently generated a sample who are less representative of people with a CUD or people who regularly use cannabis. Key exclusion criteria which may have reduced the generalizability of the findings were: (i) history of diagnosed psychiatric disorder, (ii) history of mindfulness practice, and (iii) current suicidal ideation. Regular cannabis use is known to be highly comorbid with psychosis and schizophrenia (Hasin & Walsh, 2020; Hunt et al., 2018), and with suicidal ideation (Borges et al., 2016; van Ours et al., 2013). Furthermore, we observed that history of mindfulness practice was one of the most often violated exclusion criteria throughout recruitment. Thus, the subsequent exclusion of these participants may have reduced the generalizability of the findings. Despite this limitation, the sample utilised in the current thesis was largely consistent with samples utilised in the field of research. Future research should incorporate in their samples, cannabis use participants who endorse psychiatric disorders, suicidality, and history of mindfulness practice to increase generalizability and understand how these variables may influence rsFC changes.

It was beyond the scope of this thesis to examine neural correlates of regular cannabis use, in individuals who *do not* meet moderate-to-severe CUD diagnostic criteria. Therefore, the impact 'cumulative THC exposure' versus 'behavioural changes that accompany CUD' remains hitherto unexplored. Given the commonality of identified rsFC differences between individuals with a CUD and individuals with other SUDs who do not ingest cannabis, it seems likely that there may be a common factor beyond the effect of various substances on dopamine pathways (i.e., an SUD-related behavioural change) eliciting disturbance to frontostriatal pathways. Although it has been established that negative behavioural outcomes associated with regular cannabis use are significantly worse for regular users who *do* meet diagnostic criteria for CUD than for regular users who *do not* (Foster et al., 2018; van der Pol et al., 2013), how these two groups differ neurobiologically remains unclear. To understand this, future research utilising rsFC could compare two groups, who are differing on CUD status but matched for key variables including cannabis use duration, frequency, and dose.

6.4.1.4 Inability to Determine Cannabinoid Contributions to rsFC Alterations

A key limitation of the study was the lack of inclusion of an objective measure of cannabinoids in users' typical cannabis supply (THC vs cannabidiol [CBD] vs other phytocannabinoids). Thus, the relative contributions from varying cannabis strains/strengths to the observed rsFC changes is unclear. Recent work examining rsFC following acute administration of THC and CBD has demonstrated that acute THC and CBD have differing (Lorenzetti et al., 2022), and sometimes opposing effects on rsFC (Wall et al., 2022; Wall et al., 2019). The collection of cannabis samples for determining cannabinoid profiles of participants' typical cannabis supply was beyond the scope of this project. Future research should look to extend this work further to residual rsFC, to aid in the interpretation of the uncovered rsFC differences.

The current study was also limited by the lack of biological metrics of cannabinoid exposure including cannabis metabolites from participants' specimens (e.g., levels of 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH). Following ingestion, THC is initially metabolized into a psychoactive metabolite (11-hydroxy-tetrahydrocannabinol; 11-OH-THC), and shortly thereafter (within hours) into the non-psychoactive metabolite THC-COOH (Huestis et al., 1992). THC-COOH is detectable in urine for up to a month following abstinence in people who regularly use cannabis (Bergamaschi et al., 2013), and it provides a measure of recent cannabis use. Levels of THC-COOH have previously been shown to correlate with degree of cannabis dependency (DSM-IV; Curran et al., 2019). To note, during data collection process for the larger project within which this PhD sits, urine samples were taken from participants in order to monitor their THC-COOH levels, but analyses on this data could not be completed on time for its inclusion here. The future planned publications resulting from this thesis (Study 2 and 3) will incorporate the urinalysis results in a later manuscript revision – both as a correlation within rsFC outcomes in Study 2, and to check if intervention conditions are matched (and if not then entered as a covariate) in Study 3.

6.4.2 Limitations of Study 3: Pre-to-Post Brief Intervention

6.4.2.1 Low Sample Size

The sample collected for Study 3 was ultimately smaller than originally planned, due to COVID-19 related disruption during the recruitment and data collection phase, as detailed within the General Methods (*Chapter 3: 3.4.1 A Word on the Impact of COVID-19*). Therefore Study 3 possessed less statistical power than originally intended, which may have reduced our ability to detect neurobiological changes which are subtle in nature. Nevertheless, the current study was adequately powered to detect group-by-time interactions. Additionally, our sample size was larger than similar published studies (N = 13; Froeliger et al., 2017; N = 28; Fahmy et al., 2019). Regardless, future research should recruit larger sample sizes to reproduce and confirm these effects, or use more powerful MRI scanners (i.e., 7-Tesla [7T]), which can reliably detect signal in smaller samples (Willems & Henke, 2021).

6.4.3 Functional Magnetic Resonance Imaging Methodologies

The application of *a priori* hypothesis driven approach for seed selection (i.e., within the seed-to-whole-brain analysis), limited the ability of this study to discover results which may be unexpected. To this end, data driven approaches, including machine learning approaches and multi-variance analysis, or whole brain analysis including independent components analysis (ICA) and connectome (graph theory) analysis may be used to examine rsFC in CUD. There is an array of data driven approaches available for the analysis of rsFC. For a review of these approaches, please see *Chapter 2: Systematic Literature Review*, Figure 2.3 (page 51)

To note however, the rigorous process for the *a priori* hypothesis driven seed selection within this study ensured the seed selection was justified and relevant to the research (i.e., seeds are selected based upon established findings), this method also provided an opportunity to replicate past findings and answer specific research questions (Poldrack et al., 2011).

6.4.3.1 A Novel rsFC Analysis Approach: Dynamic Causal Modelling

The traditional rsFC analyses utilised within this study, which relied on the non-causal establishment of relationships between spatially distinct brain regions, were limited in the ability to enable the inference of how the data were caused or generated, or the understanding of an underlying level of neuronal interaction (Frassle et al., 2021). Furthermore, traditional rsFC analyses are not

directional and do not capture asymmetries in reciprocal connections (Frassle et al., 2021). One such method that has been developed, which may give the ability to infer causal relationships between brain region connectivity utilising fMRI data, is Dynamic Causal Modelling (DCM; David et al., 2008; Friston, 2009). DMC provides a measure of 'Effective Connectivity' (EC) which measures the causal effect that one region's activity has on another (Friston, 1994). At present, there exists only a small field of research which has utilised DCM in people who regularly use cannabis (Ma et al., 2021) and people with a CUD (Ma et al., 2020). In order to expand upon the knowledge gained from the present thesis and deepen the understanding of the relationship between brain regions displaying altered rsFC, future research could incorporate DCM; ideally utilising a sample of people with CUD and seed selection based on the findings of the research within this thesis.

6.5 Summary and Conclusions

Together, the findings from this thesis make a significant contribution to the understanding of neural mechanisms of CUD by demonstrating for the first time, increased rsFC between frontostriatal regions and occipito-parietal/occipital-striatal regions, and decreased rsFC between occipito-parietal-hippocampal regions. Additionally, this research contributed to the development of potential treatment targets for CUD, by demonstrating decreased frontostriatal and increased fronto-hippocampal rsFC pre-to-post brief MBI.

This thesis involved the completion of three studies. The initial study was the first systematic integration of the existing fMRI studies which have examined rsFC in people who regularly use cannabis. The results of the SLR, along with prominent neuroscientific theory of addiction and the location of CB_1R, were used to inform the neural targets of Study 2. Subsequently, the results of Study 2 were used to inform the neural targets of Study 3. The aims and the key findings of Study 2 and Study 3 (the two empirical experiments) were:

6.5.1 Aims of Study 2: Cannabis Use Disorders vs Controls

- Aim 1: To compare rsFC for the first time between people with a diagnosis of moderate-to-severe CUD and who had recently tried to cut down or quit cannabis and non-cannabis-using

controls, whilst accounting for age, sex, and variables that differed between the two groups (i.e., alcohol and nicotine exposure, and depression symptom scores).

- Aim 2: To explore how rsFC differences identified in the CUD group vs controls would be associated with cannabis use exposure and related behaviours.
- Key Findings: In people with a CUD compared to controls, rsFC was greater between NAc-frontal regions, putamen-occipito-parietal regions (which correlated with an earlier age of *first* and of *regular* cannabis use), and pallidum-occipital/occipito-parietal regions (which correlated with severity of CUD and days of cannabis use/past 30 days); rsFC was lower between hippocampus-occipital regions.

6.5.2 Aims of Study 3: Pre-to-Post Brief Intervention

- Aims 1: To examine for the first time how a brief MBI reduced brain dysfunction – measured with rsFC fMRI – in people with a current moderate-to-severe CUD, who had tried to cut down or quit their use in the previous 2 years, compared to *active* and *passive placebo* control intervention conditions.
- Aim 2: To explore if changes in rsFC pre-to-post MBI were associated with changes in cannabis exposure (e.g., grams, use days) and cannabis-use related problems (e.g., withdrawal), as well as psychological measures (e.g., COVID-related-stress, and mindfulness levels).
- Key Findings: Pre-to-post MBI, putamen-frontal pole rsFC decreased (vs *active* and *passive placebo*); putamen-SFG rsFC decreased (vs *active placebo*); and hippocampus-ACC rsFC increased (vs *passive placebo*), which correlated with increased frequency of cannabis use. Pre-to-post *active placebo* (vs MBI), participants showed increased putamen-frontal pole rsFC, in correlation with lower cannabis grams; and increased putamen-SFG rsFC. Also pre-to-post *active placebo*, pallidum-aSTG rsFC decreased, and putamen-cerebellum/brainstem rsFC increased (vs *passive placebo*). Third, pre-to-post *passive placebo*, putamen-frontal pole rsFC increased (vs MBI) which correlated with decreased cannabis use days; putamen-SFG rsFC and putamen-

cerebellum/brainstem rsFC both decreased (vs MBI and vs *active placebo* respectively) while pallidum-aSTG rsFC increased (vs *active placebo*).

6.5.3 Concluding Statements

In conclusion, this thesis provides significant advances in the understanding of the neurobiology of CUD. Specifically, the research within this thesis uncovered rsFC alterations in people who regularly use cannabis (Study 1), and then (partly) replicated these findings in overlapping and novel altered rsFC pairings, in a sample of people with CUD compared to controls (Study 2). The group differences in CUD implicated pathways previously reported in other SUDs as per prominent neuroscientific theories of addiction. Thus, CUD may affect key brain circuits involved in the reward system similar to other SUDs. Importantly, this thesis also demonstrated that brief MBI may target identified rsFC alterations and additional novel rsFC pairings. It was thought that brief MBI may target altered neurocircuitry known to be established in CUD and thought to underlie addiction-related cognitive processes (i.e., frontostriatal rsFC). Furthermore, MBI may facilitate extinguishing of habitual substance use, via strengthening of key regions previously shown to underlie cognitive inhibition (i.e., hippocampus-ACC).

Taking into consideration the novelty of the research, future research is warranted to replicate the findings established herein and to expand the thesis findings in a larger sample size, in vulnerable CUD populations with psychiatric comorbidities, and including a measure of both cannabis metabolites (i.e., THC-COOH) and cannabinoid profiles of user supplies. Furthermore, future research may further explore the neurobiological correlates brief interventions (with a focus on mindfulness-based, but also including relaxation-based, and self-monitoring-based), in samples who are motivated to change their cannabis use, and adherent to the intervention protocols. Finally, future research could look to expand upon findings by collaborating in multi-site neuroimaging data collection and utilising novel rsFC data analyses approaches, such as DCM, or more powerful MRI scanners (i.e., 7T).

Whilst the neural correlates of both CUD and brief MBI as treatment for CUD require further exploration, this thesis makes an important contribution to a growing foundation of knowledge. The

findings herein will serve to inform future neuroimaging research, both in understanding potential neurobiological vulnerabilities of CUD, and informing the identification of neurobiological targets for the treatment of CUD in vulnerable people.

~ FIN ~

References

Abdullaev, Y., Posner, M. I., Nunnally, R., & Dishion, T. J. (2010). Functional MRI evidence for inefficient attentional control in adolescent chronic cannabis abuse. *Behavioural Brain Research, 215*(1), 45-57. https://doi.org/10.1016/j.bbr.2010.06.023

Adamson, S. J., Kay-Lambkin, F. J., Baker, A. L., Lewin, T. J., Thornton, L., Kelly, B. J., & Sellman, J. D. (2010). An improved brief measure of cannabis misuse: The Cannabis Use Disorders Identification Test-Revised (CUDIT-R). *Drug and Alcohol Dependence, 110*(1-2), 137-143. https://doi.org/10.1016/j.drugalcdep.2010.02.017

Adinoff, B., Gu, H., Merrick, C., McHugh, M., Jeon-Slaughter, H., Lu, H., Yang, Y., & Stein, E. A. (2015). Basal hippocampal activity and its functional connectivity predicts cocaine relapse. *Biological Psychiatry, 78*(7), 496-504. https://doi.org/10.1016/j.biopsych.2014.12.027

Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, W., Al-Hosaini, K., Back, M., Barnes, N. M., Bathgate, R., . . . Ye, R. D. (2021). The concise guide to pharmacology 2021/22: G protein-coupled receptors. *British Journal of Psychopharmacology, 178*(Suppl 1), S27-S156. https://doi.org/10.1111/bph.15538

Allsop, D. J., Norberg, M. M., Copeland, J., Fu, S., & Budney, A. J. (2011). The Cannabis Withdrawal Scale development: Patterns and predictors of cannabis withdrawal and distress. *Drug and Alcohol Dependence, 119*(1-2), 123-129. https://doi.org/10.1016/j.drugalcdep.2011.06.003

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). https://doi.org/https://doi.org/10.1176/appi.books.9780890425596

Anderson, L. C. (1980). Leaf variation among cannabis species from a controlled garden. *Botanical Museum Leaflets, Harvard University, 28*(1), 61-69. http://www.jstor.org/stable/41762825

Anthony, J. C., Warner, L. A., & Kessler, R. C. (1994). Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: Basic findings from the National

Comorbidity Survey. *Experimental and Clinical Psychopharmacology, 2*(3), 244–268. https://doi.org/10.1037/1064-1297.2.3.244

Aron, A. R. (2007). The neural basis of inhibition in cognitive control. *Neuroscientist, 13(3),* 214-228. https://doi.org/10.1177/1073858407299288

Ashtari, M., Avants, B., Cyckowski, L., Cervellione, K. L., Roofeh, D., Cook, P., Gee, J., Sevy, S., & Kumra, S. (2011). Medial temporal structures and memory functions in adolescents with heavy cannabis use. *Journal of Psychiatric Research, 45*(8), 1055-1066. https://doi.org/10.1016/j.jpsychires.2011.01.004

Australian Institute of Health and Welfare. (2019). *National Drug Strategy Household Survey 2016: Detailed findings.* Canberra: AIHW https://doi:10.25816/e42p-a447

Australian Institute of Health and Welfare. (2020). *National Drug Strategy Household Survey 2019: Detailed findings.* Canberra: AIHW

Babor, T., Higgins-Biddle, J., Saunders, J., & Monteiro, M. (2018). AUDIT: The alcohol use disorders identification test: Guidelines for use in primary health care. Geneva: World Health Organization. *BMC Public Health, 18*(1), 735.

Badhwar, A., Tam, A., Dansereau, C., Orban, P., Hoffstaedter, F., & Bellec, P. (2017). Resting-state network dysfunction in Alzheimer's disease: A systematic review and meta-analysis. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring (Amst), 8,* 73-85. https://doi.org/10.1016/j.dadm.2017.03.007

Baer, R. A., Smith, G. T., Lykins, E., Button, D., Krietemeyer, J., Sauer, S., Walsh, E., Duggan, D., & Williams, J. M. (2008). Construct validity of the five facet mindfulness questionnaire in meditating and nonmeditating samples. *Assessment, 15*(3), 329-342. https://doi.org/10.1177/1073191107313003

Bahji, A., Meyyappan, A. C., Hawken, E. R., & Tibbo, P. G. (2021). Pharmacotherapies for cannabis use disorder: A systematic review and network meta-analysis. *International Journal of Drug Policy, 97,* 103295. https://doi.org/https://doi.org/10.1016/j.drugpo.2021.103295

Balhara, Y. P., Kuppili, P. P., & Gupta, R. (2017). Neurobiology of comorbid substance use disorders and psychiatric disorders: Current state of evidence. *Journal of Addictions Nursing*, *28*(1), 11-26. https://doi.org/10.1097/JAN.0000000000000155

Balleine, B. W., & O'Doherty, J. P. (2010). Human and rodent homologies in action control: Corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology*, *35*(1), 48-69. https://doi.org/10.1038/npp.2009.131

Bandettini, P. A., Wong, E. C., Hinks, R. S., Tikofsky, R. S., & Hyde, J. S. (1992). Time course EPI of human brain function during task activation. *Magnetic Resonance in Medicine*, *25*(2), 390-397. https://doi.org/10.1002/mrm.1910250220

Barcaccia, G., Palumbo, F., Scariolo, F., Vannozzi, A., Borin, M., & Bona, S. (2020). Potentials and challenges of genomics for breeding cannabis cultivars. *Frontiers in Plant Science*, *11*, 573299. https://doi.org/10.3389/fpls.2020.573299

Bartels A, Zeki S (2004). The chronoarchitecture of the human brain: Natural viewing conditions reveal a time-based anatomy of the brain. *NeuroImage*, *22*, 419-433 https://doi.org/10.1016/j.neuroimage.2004.01.007

Batalla, A., Bhattacharyya, S., Yucel, M., Fusar-Poli, P., Crippa, J. A., Nogue, S., Torrens, M., Pujol, J., Farre, M., & Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: A systematic review of adolescent and adult findings. *PLoS One*, *8*(2), e55821. https://doi.org/10.1371/journal.pone.0055821

Beck, A. T., Steer, R. A., & Brown, G. (1996). Beck depression inventory–II. *Psychological Assessment.*

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., Spuentrup, E., & Daumann, J. (2010a). Altered parahippocampal functioning in cannabis users is related to the frequency of use. *Psychopharmacology (Berl)*, *209*(4), 361-374. https://doi.org/10.1007/s00213-010-1805-z

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., Spuentrup, E., & Daumann, J. (2010b). The impact of early-onset cannabis use on functional brain correlates of working memory. *Progress in*

Neuro-Psychopharmacology and Biological Psychiatry, 34(6), 837-845. https://doi.org/10.1016/j.pnpbp.2010.03.032

Beffara, B., Hadj-Bouziane, F., Hamed, S. B., Boehler, C. N., Chelazzi, L., Santandrea, E., & Macaluso, E. (2022). Dynamic causal interactions between occipital and parietal cortex explain how endogenous spatial attention and stimulus-driven salience jointly shape the distribution of processing priorities in 2D visual space. *Neuroimage, 255*, 119206. https://doi.org/10.1016/j.neuroimage.2022.119206

Behan, B., Connolly, C. G., Datwani, S., Doucet, M., Ivanovic, J., Morioka, R., Stone, A., Watts, R., Smyth, B., & Garavan, H. (2014). Response inhibition and elevated parietal-cerebellar correlations in chronic adolescent cannabis users. *Neuropharmacology 84,* 131-137 https://doi:10.1016/j.neuropharm.2013.05.027

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological), 57*(1), 289-300.

Bergamaschi, M. M., Karschner, E. L., Goodwin, R. S., Scheidweiler, K. B., Hirvonen, J., Queiroz, R. H., & Huestis, M. A. (2013). Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. *Clinical Chemistry, 59*(3), 519-526. https://doi.org/10.1373/clinchem.2012.195503

Berlingeri, M., Losasso, D., Girolo, A., Cozzolino, E., Masullo, T., Scotto, M., Sberna, M., Bottini, G., & Paulesu, E. (2017). Resting state brain connectivity patterns before eventual relapse into cocaine abuse. *Behavioural Brain Research, 327,* 121-132. https://doi.org/10.1016/j.bbr.2017.01.002

Berridge, K. C., & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist, 71*(8), 670-679. https://doi.org/10.1037/amp0000059

Binkowska, A. A., Ruban, A., Gogolewska, M., Sawicz, P., Rychlewski, L., & Brzezicka, A. (2022). Who is at risk of developing cannabis dependence? Findings from an extensive online study on cannabis users. *Journal of Addictions Nursing, 33*(1), 37-44. https://doi.org/10.1097/jan.0000000000000448

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine, 34*(4), 537-541. https://doi.org/10.1002/mrm.1910340409

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

Blest-Hopley G, Giampietro V, Bhattacharyya S (2018). Residual effects of cannabis use in adolescent and adult brains: A meta-analysis of fMRI studies. *Neuroscience & Biobehavioral Reviews, 88*, 26-41 https://doi.org/10.1016/j.neubiorev.2018.03.008

Blest-Hopley, G., Colizzi, M., Giampietro, V., & Bhattacharyya, S. (2020). Is the adolescent brain at greater vulnerability to the effects of cannabis? A narrative review of the evidence. *Frontiers in Psychiatry, 11*, 859. https://doi.org/10.3389/fpsyt.2020.00859

Blest-Hopley, G., Giampietro, V., & Bhattacharyya, S. (2020). A systematic review of human neuroimaging evidence of memory-related functional alterations associated with cannabis use complemented with preclinical and human evidence of memory performance alterations. *Brain Science, 10*(2). https://doi.org/10.3390/brainsci10020102

Bloomfield, M. A. P., Hindocha, C., Green, S. F., Wall, M. B., Lees, R., Petrilli, K., Costello, H., Ogunbiyi, M. O., Bossong, M. G., & Freeman, T. P. (2019). The neuropsychopharmacology of cannabis: A review of human imaging studies. *Pharmacology & Therapeutics, 195*, 132-161. https://doi.org/10.1016/j.pharmthera.2018.10.006

Bloomfield, M. A., Ashok, A. H., Volkow, N. D., & Howes, O. D. (2016). The effects of Δ-9-tetrahydrocannabinol on the dopamine system. *Nature, 539*(7629), 369-377. https://doi.org/10.1038/nature20153

Bloomfield, M. A., Morgan, C. J., Egerton, A., Kapur, S., Curran, H. V., & Howes, O. D. (2014). Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biological Psychiatry, 75*(6), 470-478. https://doi.org/10.1016/j.biopsych.2013.05.027

Bodor, A. L., Katona, I., Nyiri, G., Mackie, K., Ledent, C., Hajos, N., & Freund, T. F. (2005). Endocannabinoid signalling in rat somatosensory cortex: Laminar differences and involvement of specific interneuron types. *Journal of Neuroscience, 25*(29), 6845-6856. https://doi.org/10.1523/JNEUROSCI.0442-05.2005

Boggs, D. L., Nguyen, J. D., Morgenson, D., Taffe, M. A., & Ranganathan, M. (2018). Clinical and preclinical evidence for functional interactions of cannabidiol and delta-9-tetrahydrocannabinol. *Neuropsychopharmacology*, *43*(1), 142-154. https://doi.org/10.1038/npp.2017.209

Bonini, S. A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., & Mastinu, A. (2018). Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *Journal of Ethnopharmacology*, *227*, 300-315. https://doi.org/10.1016/j.jep.2018.09.004

Borges, G., Bagge, C. L., & Orozco, R. (2016). A literature review and meta-analyses of cannabis use and suicidality. *Journal of Affective Disorders*, *195*, 63-74. https://doi.org/10.1016/j.jad.2016.02.007

Bossong, M. G., van Hell, H. H., Schubart, C. D., van Saane, W., Iseger, T. A., Jager, G., van Osch, M. J. P., Jansma, J. M., Kahn, R. S., Boks, M. P., & Ramsey, N. F. (2019). Acute effects of delta 9-tetrahydrocannabinol (THC) on resting state brain function and their modulation by COMT genotype. *European Neuropsychopharmacology, 29*, 766-776 https://doi:10.1016/j.euroneuro.2019.03.010

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., van Gerven, J. M., Ramsey, N. F., Lammertsma, A. A., & Kahn, R. S. (2009). Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology, 34*(3), 759-766. https://doi.org/10.1038/npp.2008.138

Borges, G., Bagge, C. L., & Orozco, R. (2016). A literature review and meta-analyses of cannabis use and suicidality. *Journal of Affective Disorders*, *195*, 63-74. https://doi.org/10.1016/j.jad.2016.02.007

Boyd, J. E., Lanius, R. A., & McKinnon, M. C. (2018). Mindfulness-based treatments for posttraumatic stress disorder: A review of the treatment literature and neurobiological evidence. *Journal of Psychiatry Neuroscience, 43*(1), 7-25. https://doi.org/10.1503/jpn.170021

Braganza, D. L., Fitzpatrick, L. E., Nguyen, M. L., & Crowe, S. F. (2022). Interictal cognitive deficits in migraine sufferers: A meta-analysis. *Neuropsychology Review, 32*(4), 736–757. https://doi.org/10.1007/s11065-021-09516-1

Breeze, J. L., Poline, J. B., & Kennedy, D. N. (2012). Data sharing and publishing in the field of neuroimaging. *Gigascience, 1*(1), 9. https://doi.org/10.1186/2047-217X-1-9

Brenan, M. (2020). Support for legal marijuana inches up to new high of 68%. *Age, 18*(29), 79.

Brewer, J. A., Elwafi, H. M., & Davis, J. H. (2013). Craving to quit: Psychological models and neurobiological mechanisms of mindfulness training as treatment for addictions. *Psychology of Addiction Behaviour, 27*(2), 366-379. https://doi.org/10.1037/a0028490

Brocato, J., & Wagner, E. F. (2008). Predictors of retention in an alternative-to-prison substance abuse treatment program. *Criminal Justice and Behavior, 35*(1), 99-119.

Budney, A. J., Moore, B. A., Vandrey, R. G., & Hughes, J. R. (2003). The time course and significance of cannabis withdrawal. *Journal of Abnormal Psychology, 112*(3), 393-402. https://doi.org/10.1037/0021-843X.112.3.393

Bullmore, E., & Sporns, O. (2009). Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nature Reviews Neuroscience, 10*, 186-198 https://doi:10.1038/nrn2575

Bunge, S. A., & Wright, S. B. (2007). Neurodevelopmental changes in working memory and cognitive control. *Current Opinion in Neurobiology, 17*, 243-250 https://doi.org/10.1016/j.conb.2007.02.005

Burns, H. D., Van Laere, K., Sanabria-Bohórquez, S., Hamill, T. G., Bormans, G., Eng, W. S., Gibson, R., Ryan, C., Connolly, B., Patel, S., Krause, S., Vanko, A., Van Hecken, A., Dupont, P., De Lepeleire, I., Rothenberg, P., Stoch, S. A., Cote, J., Hagmann, W. K., Jewell,

J. P., … Hargreaves, R. J. (2007). [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proceedings of the National Academy of Sciences of the United States of America, 104*(23), 9800-9805 https://doi:10.1073/pnas.0703472104

Buu, A., Yang, S., Li, R., Zimmerman, M. A., Cunningham, R. M., & Walton, M. A. (2020). Examining measurement reactivity in daily diary data on substance use: Results from a randomized experiment. *Addictive Behaviours, 102*, 106198. https://doi.org/10.1016/j.addbeh.2019.106198

Calakos, K. C., Bhatt, S., Foster, D. W., & Cosgrove, K. P. (2017). Mechanisms underlying sex differences in cannabis use. *Current Addiction Reports, 4*(4), 439-453. https://doi.org/10.1007/s40429-017-0174-7

Camchong, J., Lim, K. O., & Kumra, S. (2017). Adverse effects of cannabis on adolescent brain development: A longitudinal study. *Cerebral Cortex 27*, 1922-1930 https://doi:10.1093/cercor/bhw015

Campanella, F., Crescentini, C., Urgesi, C., & Fabbro, F. (2014). Mindfulness-oriented meditation improves self-related character scales in healthy individuals. *Comprehensive Psychiatry, 55*(5), 1269-1278. https://doi.org/10.1016/j.comppsych.2014.03.009

Campos, D., Modrego-Alarcon, M., Lopez-Del-Hoyo, Y., Gonzalez-Panzano, M., Van Gordon, W., Shonin, E., Navarro-Gil, M., & Garcia-Campayo, J. (2019). Exploring the role of meditation and dispositional mindfulness on social cognition domains: A controlled study. *Frontiers in Psychology, 10*, 809. https://doi.org/10.3389/fpsyg.2019.00809

Carey, S. E., Nestor, L., Jones, J., Garavan, H., & Hester, R. (2015). Impaired learning from errors in cannabis users: Dorsal anterior cingulate cortex and hippocampus hypoactivity. *Drug and Alcohol Dependence, 155*, 175-182. https://doi.org/10.1016/j.drugalcdep.2015.07.671

Carliner, H., Brown, Q. L., Sarvet, A. L., & Hasin, D. S. (2017). Cannabis use, attitudes, and legal status in the U.S.: A review. *Preventative Medicine, 104*, 13-23. https://doi.org/10.1016/j.ypmed.2017.07.008

Casajuana, C., Lopez-Pelayo, H., Mercedes Balcells, M., Miquel, L., Teixido, L., Colom, J., & Gual, A. (2017). Working on a Standard Joint Unit: A pilot test. Estableciendo la unidad de porro estandar: Estudio piloto. *Adicciones, 29*(4), 227-232. https://doi.org/10.20882/adicciones.721

Cavicchioli, M., Movalli, M., & Maffei, C. (2018). The clinical efficacy of mindfulness-based treatments for alcohol and drugs use disorders: A meta-analytic review of randomized and nonrandomized controlled trials. *European Addiction Research, 24*(3), 137-162. https://doi.org/10.1159/000490762

Ceccarini, J., Kuepper, R., Kemels, D., van Os, J., Henquet, C., & Van Laere, K. (2015). [18F]MK-9470 PET measurement of cannabinoid CB1 receptor availability in chronic cannabis users. *Addiction Biology, 20*(2), 357-367. https://doi.org/10.1111/adb.12116

Chan, G. C., Hinds, T. R., Impey, S., & Storm, D. R. (1998). Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *Journal of Neuroscience, 18*(14), 5322-5332. https://doi.org/10.1523/JNEUROSCI.18-14-05322.1998

Chandra, S., Lata, H., Khan, I. A., & ElSohly, M. A. (2017). Cannabis sativa L.: Botany and horticulture. In Chandra, S., Lata, H., & ElSohly, M. A. (Eds.), *Cannabis sativa L. - Botany and Biotechnology* (pp. 79-100). Springer International Publishing. https://doi.org/10.1007/978-3-319-54564-6_3

Chang, L., Yakupov, R., Cloak, C., & Ernst, T. (2006). Marijuana use is associated with a reorganized visual-attention network and cerebellar hypoactivation. *Brain, 129*(Pt 5), 1096-1112. https://doi.org/10.1093/brain/awl064

Chen. C.-Y., O'Brien, M. S., & Anthony, J. C. (2005). Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000–2001. *Drug and Alcohol Dependence 79*, 11-22 https://doi.org/10.1016/j.drugalcdep.2004.11.014

Chen, Y. C., Xia, W., Luo, B., Muthaiah, V. P., Xiong, Z., Zhang, J., Wang, J., Salvi, R., & Teng, G. J. (2015). Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus. *Frontiers in Neural Circuits, 9*, 1-13 doi:10.3389/fncir.2015.00067

Cheng, H., Skosnik, P. D., Pruce, B. J., Brumbaugh, M. S., Vollmer, J. M., Fridberg, D. J., O'Donnell, B. F., Hetrick, W. P., & Newman, S. D. (2014). Resting state functional magnetic resonance imaging reveals distinct brain activity in heavy cannabis users - A multi-voxel pattern analysis. *Journal of Psychopharmacology, 28,* 1030-1040 https://doi:10.1177/0269881114550354

Chiesa, A., & Serretti, A. (2009). Mindfulness-based stress reduction for stress management in healthy people: A review and meta-analysis. *Journal of Alternative Complementary Medicine, 15*(5), 593-600. https://doi.org/10.1089/acm.2008.0495

Chiesa, A., & Serretti, A. (2010). A systematic review of neurobiological and clinical features of mindfulness meditations. *Psychological Medicine, 40*(8), 1239-1252. https://doi.org/10.1017/S0033291709991747

Chiesa, A., & Serretti, A. (2014). Are mindfulness-based interventions effective for substance use disorders? A systematic review of the evidence. *Substance Use Misuse, 49*(5), 492-512. https://doi.org/10.3109/10826084.2013.770027

Chiesa, A., Calati, R., & Serretti, A. (2011). Does mindfulness training improve cognitive abilities? A systematic review of neuropsychological findings. *Clinical Psychology Review, 31*(3), 449-464. https://doi.org/10.1016/j.cpr.2010.11.003

Chye, Y., Solowij, N., Suo, C., Batalla, A., Cousijn, J., Goudriaan, A. E., Martin-Santos, R., Whittle, S., Lorenzetti, V., & Yucel, M. (2017). Orbitofrontal and caudate volumes in cannabis users: a multi-site mega-analysis comparing dependent versus non-dependent users. *Psychopharmacology (Berl), 234*(13), 1985-1995. https://doi.org/10.1007/s00213-017-4606-9

Chye, Y., Suo, C., Yucel, M., den Ouden, L., Solowij, N., & Lorenzetti, V. (2017). Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users. *Psychopharmacology (Berl), 234*(14), 2149-2157. https://doi.org/10.1007/s00213-017-4620-y

Clair, M., Stein, L. A. R., Martin, R., Barnett, N. P., Colby, S. M., Monti, P. M., Golembeske, C., Jr., & Lebeau, R. (2011). Motivation to change alcohol use and treatment engagement in

incarcerated youth. *Addictive Behaviors, 36*(6), 674-680. https://doi.org/10.1016/j.addbeh.2011.01.007

Clark, I. A., Hotchin, V., Monk, A., Pizzamiglio, G., Liefgreen, A., & Maguire, E. A. (2019). Identifying the cognitive processes underpinning hippocampal-dependent tasks. *Journal of Experimental Psychology General, 148*(11), 1861-1881. https://doi.org/10.1037/xge0000582

Classen, A. (2001). The great herbal of Leonhart Fuchs. De historia stirpium commentarii insignes, 1542, Frederick G. Meyer, Emily Emmart Trueblood, & John L. Heller. *German Studies Review, 24*(3), 595-597. https://doi.org/10.2307/1433419

Clinical TMS Society. (2023) The frontal pole: An emerging TMS treatment target for drug and alcohol use disorders. Retrieved from https://www.clinicaltmssociety.org/events/frontal-pole-emerging-tms-treatment-target-drug-and-alcohol-use-disorders

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385-396.

Colagiuri, B. (2010). Participant expectancies in double-blind randomized placebo-controlled trials: Potential limitations to trial validity. *Clinical Trials 7*(3), 246-255. https://doi.org/10.1177/1740774510367916

Coleman, J. R., Madularu, D., Ortiz, R. J., Athanassiou, M., Knudsen, A., Alkislar, I., Cai, X., Kulkarni, P. P., Cushing, B. S., & Ferris, C. F. (2022). Changes in brain structure and function following chronic exposure to inhaled vaporised cannabis during periadolescence in female and male mice: A multimodal MRI study. *Addiction Biology, 27*(3), e13169. https://doi.org/10.1111/adb.13169

Colizzi, M., Fazio, L., Ferranti, L., Porcelli, A., Masellis, R., Marvulli, D., Bonvino, A., Ursini, G., Blasi, G., & Bertolino, A. (2015). Functional genetic variation of the cannabinoid receptor 1 and cannabis use interact on prefrontal connectivity and related working memory behavior. *Neuropsychopharmacology, 40*(3), 640-649. https://doi.org/10.1038/npp.2014.213

Collins Dictionaries. (2020). *Collins Latin Essential Dictionary* HarperCollins.

Collins, S. E., Malone, D. K., & Larimer, M. E. (2012). Motivation to change and treatment attendance as predictors of alcohol-use outcomes among project-based Housing First residents. *Addictive Behaviors, 37*(8), 931-939. https://doi.org/10.1016/j.addbeh.2012.03.029

Corkin, S. (2002). What's new with the amnesic patient H.M.? *Nature Reviews Neuroscience, 3*(2), 153-160. https://doi.org/10.1038/nrn726

Cornish, J. L., & Prasad, A. A. (2021). Sex differences in substance use disorders: a neurobiological perspective. *Frontiers in Global Women's Health, 2*, 778514. https://doi.org/10.3389/fgwh.2021.778514

Cousijn, J., Wiers, R. W., Ridderinkhof, K. R., van den Brink, W., Veltman, D. J., Porrino, L. J., & Goudriaan, A. E. (2013). Individual differences in decision making and reward processing predict changes in cannabis use: A prospective functional magnetic resonance imaging study. *Addiction Biology, 18*(6), 1013-1023. https://doi.org/10.1111/j.1369-1600.2012.00498.x

Curran, H. V., Freeman, T. P., Mokrysz, C., Lewis, D. A., Morgan, C. J., & Parsons, L. H. (2016). Keep off the grass? Cannabis, cognition and addiction. *Nature Reviews Neuroscience, 17*(5), 293-306. https://doi.org/10.1038/nrn.2016.28

Curran, H. V., Hindocha, C., Morgan, C. J. A., Shaban, N., Das, R. K., & Freeman, T. P. (2019). Which biological and self-report measures of cannabis use predict cannabis dependency and acute psychotic-like effects? *Psychological Medicine, 49*(9), 1574-1580. https://doi.org/10.1017/s003329171800226x

Cuttler, C., & Spradlin, A. (2017). Measuring cannabis consumption: Psychometric properties of the Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory (DFAQ-CU). *PLoS One, 12*(5), e0178194. https://doi.org/10.1371/journal.pone.0178194

D'Souza, D. C., Cortes-Briones, J. A., Ranganathan, M., Thurnauer, H., Creatura, G., Surti, T., Planeta, B., Neumeister, A., Pittman, B., Normandin, M., Kapinos, M., Ropchan, J., Huang, Y., Carson, R. E., & Skosnik, P. D. (2016). Rapid changes in CB1 receptor availability in

cannabis dependent males after abstinence from cannabis. *Biological Psychiatry Cognitive Neuroscience Neuroimaging, 1*(1), 60-67. https://doi.org/10.1016/j.bpsc.2015.09.008

David, O., Guillemain, I., Saillet, S., Reyt, S., Deransart, C., Segebarth, C., & Depaulis, A. (2008). Identifying neural drivers with functional MRI: An electrophysiological validation. *PLoS Biology, 6*(12), 2683-2697. https://doi.org/10.1371/journal.pbio.0060315

Davis, J. P., Smith, D. C., Morphew, J. W., Lei, X., & Zhang, S. (2016). Cannabis withdrawal, posttreatment abstinence, and days to first cannabis use among emerging adults in substance use treatment: A prospective study. *Journal of Drug Issues, 46*(1), 64-83. https://doi.org/10.1177/0022042615616431

de Dios, M. A., Herman, D. S., Britton, W. B., Hagerty, C. E., Anderson, B. J., & Stein, M. D. (2012). Motivational and mindfulness intervention for young adult female marijuana users. *Journal of Substance Abuse Treatment, 42*(1), 56-64. https://doi.org/10.1016/j.jsat.2011.08.001

De Petrocellis, L., Cascio, M. G., & Di Marzo, V. (2004). The endocannabinoid system: A general view and latest additions. *British Journal of Psychopharmacology, 141*(5), 765-774. https://doi.org/10.1038/sj.bjp.0705666

Degenhardt, L., Alcohol, G. B. D., & Drug Use, C. (2018). The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Psychiatry, 5*(12), 987-1012. https://doi.org/10.1016/S2215-0366(18)30337-7

Demirakca, T., Sartorius, A., Ende, G., Meyer, N., Welzel, H., Skopp, G., Mann, K., & Hermann, D. (2011). Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. *Drug and Alcohol Dependence, 114*(2-3), 242-245. https://doi.org/10.1016/j.drugalcdep.2010.09.020

Demiral, Ş. B., Tomasi, D., Wiers, C. E., Manza, P., Shokri-Kojori, E., Studentsova, Y., Wang, G. J., & Volkow, N. D. (2019). Methylphenidate's effects on thalamic metabolism and functional connectivity in cannabis abusers and healthy controls. *Neuropsychopharmacology : official*

publication of the American College of Neuropsychopharmacology, 44(8), 1389-1397. https://doi:10.1038/s41386-018-0287-2

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage, 31*(3), 968-980. https://doi.org/10.1016/j.neuroimage.2006.01.021

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

Devinsky, O., Cilio, M. R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W. G., Martinez-Orgado, J., Robson, P. J., Rohrback, B. G., Thiele, E., Whalley, B., & Friedman, D. (2014). Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia, 55*(6), 791-802. https://doi.org/10.1111/epi.12631

Di Marzo, V., & Piscitelli, F. (2015). The endocannabinoid system and its modulation by phytocannabinoids. *Neurotherapeutics, 12*(4), 692-698. https://doi.org/10.1007/s13311-015-0374-6

Dickersin, K. (1997). How important is publication bias? A synthesis of available data. *AIDS Education and Prevention, 9*(1 Suppl), 15-21.

Dixon, H. G., Pratt, I. S., Scully, M. L., Miller, J. R., Patterson, C., Hood, R., & Slevin, T. J. (2015). Using a mass media campaign to raise women's awareness of the link between alcohol and cancer: Cross-sectional pre-intervention and post-intervention evaluation surveys. *BMJ Open, 5*(3), e006511. https://doi.org/10.1136/bmjopen-2014-006511

Downer, E., Boland, B., Fogarty, M., & Campbell, V. (2001). Delta 9-tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB1 receptor. *Neuroreport, 12*(18), 3973-3978. https://doi.org/10.1097/00001756-200112210-00024

Drug Enforcement Administration. (2018). *Controlled Substances Act.* https://www.dea.gov/druginfo/csa.shtml. [28.02. 2017].

Dutschmann, M., & Dick, T. E. (2012). Pontine mechanisms of respiratory control. *Comprehensive Physiology, 2*(4), 2443-2469. https://doi.org/10.1002/cphy.c100015

Dwan. K., Gamble. C., Williamson. P. R., & Kirkham, J. J. (2013). Systematic review of the empirical evidence of study publication bias and outcome reporting bias — An updated review. *PLOS ONE 8*, e66844 https://doi:10.1371/journal.pone.0066844

ElSohly, M. A., Radwan, M. M., Gul, W., Chandra, S., & Galal, A. (2017). Phytochemistry of Cannabis sativa L. *Progress in the Chemistry of Organic Natural Products, 103*, 1-36. https://doi.org/10.1007/978-3-319-45541-9_1

Emboden, W. A. (1974). Cannabis: A polytypic genus. *Economic Botany, 28*(3), 304-310. http://www.jstor.org/stable/4253520

Enzi, B., Lissek, S., Edel, M. A., Tegenthoff, M., Nicolas, V., Scherbaum, N., Juckel, G., & Roser, P. (2015). Alterations of monetary reward and punishment processing in chronic cannabis users: An fMRI study. *PLoS One, 10*(3), e0119150. https://doi.org/10.1371/journal.pone.0119150

Ersche, K. D., Lim, T. V., Murley, A. G., Rua, C., Vaghi, M. M., White, T. L., Williams, G. B., & Robbins, T. W. (2021). Reduced glutamate turnover in the putamen is linked with automatic habits in human cocaine addiction. *Biological Psychiatry, 89*(10), 970-979. https://doi.org/10.1016/j.biopsych.2020.12.009

Ersche, K. D., Meng, C., Ziauddeen, H., Stochl, J., Williams, G. B., Bullmore, E. T., & Robbins, T. W. (2020). Brain networks underlying vulnerability and resilience to drug addiction. *Proceedings of the National Academy of Sciences, 117*(26), 15253. https://doi.org/10.1073/pnas.2002509117

Ertl, N., Lawn, W., Mokrysz, C., Freeman, T. P., Alnagger, N., Borissova, A., Fernandez-Vinson, N., Lees, R., Ofori, S., Petrilli, K., Trinci, K., Viding, E., Curran, H. V., & Wall, M. B. (2023). Associations between regular cannabis use and brain resting-state functional connectivity in adolescents and adults. *Journal of Psychopharmacology 37*(9), 904–919. https://doi.org/10.1177/02698811231189441

European Monitoring Centre for Drugs and Drug Addiction [EMCDDA]. (2015). *European Drug Report 2015: Trends and Developments.* http://www.emcdda.europa.eu/edr2015/

Evans, K., Colloca, L., Pecina, M., & Katz, N. (2021). What can be done to control the placebo response in clinical trials? A narrative review. *Contemporary Clinical Trials, 107*, 106503. https://doi.org/10.1016/j.cct.2021.106503

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience, 8*(11), 1481-1489. https://doi.org/10.1038/nn1579

Fagerström, K., Russ, C., Yu, C. R., Yunis, C., & Foulds, J. (2012). The Fagerström Test for Nicotine Dependence as a predictor of smoking abstinence: A pooled analysis of varenicline clinical trial data. *Nicotine & Tobacco Research, 14*(12), 1467-1473. https://doi.org/10.1093/ntr/nts018

Fahmy, R., Wasfi, M., Mamdouh, R., Moussa, K., Wahba, A., Schmitgen, M. M., Kubera, K. M., Wolf, N. D., Sambataro, F., & Wolf, R. C. (2019). Mindfulness-based therapy modulates default-mode network connectivity in patients with opioid dependence. *European Neuropsychopharmacology, 29*(5), 662-671. https://doi.org/https://doi.org/10.1016/j.euroneuro.2019.03.002

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41*(4), 1149-1160. https://doi.org/10.3758/BRM.41.4.1149

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*(2), 175-191. https://doi.org/10.3758/bf03193146

Ferland, J. N., & Hurd, Y. L. (2020). Deconstructing the neurobiology of cannabis use disorder. *Nature Neuroscience, 23*(5), 600-610. https://doi.org/10.1038/s41593-020-0611-0

Ferreira, L. K., & Busatto, G. F. (2013). Resting-state functional connectivity in normal brain aging. *Neuroscience Biobehavior Reviews, 37*(3), 384-400. https://doi.org/10.1016/j.neubiorev.2013.01.017

Figueiredo, P. R., Tolomeo, S., Steele, J. D., & Baldacchino, A. (2020). Neurocognitive consequences of chronic cannabis use: A systematic review and meta-analysis. *Neuroscience Biobehavior Reviews, 108,* 358-369. https://doi.org/10.1016/j.neubiorev.2019.10.014

Filbey, F. M., Gohel, S., Prashad, S., & Biswal, B. B. (2018). Differential associations of combined vs isolated cannabis and nicotine on brain resting state networks. *Brain Structure and Function, 223,* 3317-3326 https://doi:10.1007/s00429-018-1690-5

Filbey, F. M., Aslan, S., Calhoun, V. D., Spence, J. S., Damaraju, E., Caprihan, A., & Segall, J. (2014). Long-term effects of marijuana use on the brain. *Proceedings Of The National Academy Of Sciences Of The United States Of America, 111*(47), 16913-16918. https://doi.org/10.1073/pnas.1415297111

Filbey, F. M., McQueeny, T., Kadamangudi, S., Bice, C., & Ketcherside, A. (2015). Combined effects of marijuana and nicotine on memory performance and hippocampal volume. *Behavioural Brain Research, 293,* 46-53. https://doi.org/10.1016/j.bbr.2015.07.029

First, M. B., Williams, J. B., Karg, R. S., & Spitzer, R. L. (2015). Structured clinical interview for DSM-5 – Research version (SCID-5-RV). *Arlington, VA:* American Psychiatric Association (1–94).

Foster, K. T., Arterberry, B. J., Iacono, W. G., McGue, M., & Hicks, B. M. (2018). Psychosocial functioning among regular cannabis users with and without cannabis use disorder. *Psychological Medicine, 48*(11), 1853-1861. https://doi.org/10.1017/s0033291717003361

Fox, M. D., & Greicius, M. (2010). Clinical applications of resting state functional connectivity. *Frontiers in Systems Neuroscience, 4,* 19. https://doi.org/10.3389/fnsys.2010.00019

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews Neuroscience, 8*(9), 700-711. https://doi.org/10.1038/nrn2201

Franklin, T. R., Wang, Z., Wang, J., Sciortino, N., Harper, D., Li, Y., Ehrman, R., Kampman, K., O'Brien, C. P., Detre, J. A., & Childress, A. R. (2007). Limbic activation to cigarette smoking cues independent of nicotine withdrawal: A perfusion fMRI study. *Neuropsychopharmacology, 32*(11), 2301-2309. https://doi.org/10.1038/sj.npp.1301371

Frassle, S., Harrison, S. J., Heinzle, J., Clementz, B. A., Tamminga, C. A., Sweeney, J. A., Gershon, E. S., Keshavan, M. S., Pearlson, G. D., Powers, A., & Stephan, K. E. (2021). Regression dynamic causal modeling for resting-state fMRI. *Human Brain Mapping, 42*(7), 2159-2180. https://doi.org/10.1002/hbm.25357

Fratta, W., & Fattore, L. (2013). Molecular mechanisms of cannabinoid addiction. *Current Opinion in Neurobiology, 23*, 487-492 https://doi.org/10.1016/j.conb.2013.02.002

Freckelton, I. (2021). Children as voices and images for medicinal cannabis law reform. *Monash Bioethics Review, 39*(Suppl 1), 4-25. https://doi.org/10.1007/s40592-021-00139-z

Friston, K. (2009). Causal modelling and brain connectivity in functional magnetic resonance imaging. *PLoS Biology, 7*(2), e33. https://doi.org/10.1371/journal.pbio.1000033

Friston, K. J. (1994). Functional and effective connectivity in neuroimaging: A synthesis. *Human Brain Mapping, 2*(1-2), 56-78. https://doi.org/10.1002/hbm.460020107

Froeliger, B., Mathew, A. R., McConnell, P. A., Eichberg, C., Saladin, M. E., Carpenter, M. J., & Garland, E. L. (2017). Restructuring reward mechanisms in nicotine addiction: A pilot fMRI study of mindfulness-oriented recovery enhancement for cigarette smokers. *Evidence-Based Complementary and Alternative Medicine, 2017*, 7018014. https://doi.org/10.1155/2017/7018014

Gallant, S. N. (2016). Mindfulness meditation practice and executive functioning: Breaking down the benefit. *Conscious Cognition, 40*, 116-130. https://doi.org/10.1016/j.concog.2016.01.005

Galve-Roperh, I., Palazuelos, J., Aguado, T., & Guzman, M. (2009). The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders. *European Archives of Psychiatry and Clinical Neuroscience, 259*(7), 371-382. https://doi.org/10.1007/s00406-009-0028-y

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

Gardner, E. L. (2005). Endocannabinoid signalling system and brain reward: Emphasis on dopamine. *Pharmacology, Biochemistry and Behavior*, *81*(2), 263-284. https://doi.org/10.1016/j.pbb.2005.01.032

Garland, E. L., Froeliger, B., & Howard, M. O. (2014). Mindfulness training targets neurocognitive mechanisms of addiction at the attention-appraisal-emotion interface. *Frontiers in Psychiatry*, *4*, 173. https://doi.org/10.3389/fpsyt.2013.00173

Garrison, K. A., Pal, P., O'Malley, S. S., Pittman, B. P., Gueorguieva, R., Rojiani, R., Scheinost, D., Dallery, J., & Brewer, J. A. (2020). Craving to quit: A Randomized Controlled Trial of smartphone app-based mindfulness training for smoking cessation. *Nicotine & Tobacco Research*, *22*(3), 324-331. https://doi.org/10.1093/ntr/nty126

Gass, J. C., Funderburk, J. S., Shepardson, R., Kosiba, J. D., Rodriguez, L., & Maisto, S. A. (2021). The use and impact of self-monitoring on substance use outcomes: A descriptive systematic review. *Substance Abuse*, *42*(4), 512-526. https://doi.org/10.1080/08897077.2021.1874595

Gates, P., Albertella, L., & Copeland, J. (2016). Cannabis withdrawal and sleep: A systematic review of human studies. *Substance Abuse*, *37*(1), 255-269. https://doi.org/10.1080/08897077.2015.1023484

Gates, P. J., Sabioni, P., Copeland, J., Le Foll, B., & Gowing, L. (2016). Psychosocial interventions for cannabis use disorder. *Cochrane Database of Systematic Reviews*, (5). https://doi.org/10.1002/14651858.CD005336.pub4

George, O., & Koob, G. F. (2010). Individual differences in prefrontal cortex function and the transition from drug use to drug dependence. *Neuroscience Biobehavior Reviews, 35*(2), 232-247. https://doi.org/10.1016/j.neubiorev.2010.05.002

Gerdeman, G., & Lovinger, D. M. (2001). CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *Journal of Neurophysiology, 85*(1), 468-471. https://doi.org/10.1152/jn.2001.85.1.468

Gibbs, M., Winsper, C., Marwaha, S., Gilbert, E., Broome, M., & Singh, S. P. (2015). Cannabis use and mania symptoms: A systematic review and meta-analysis. *Journal of Affective Disorders, 171,* 39-47. https://doi.org/10.1016/j.jad.2014.09.016

Gilbert, C. D., & Li, W. (2013). Top-down influences on visual processing. *Nature Reviews Neuroscience, 14*(5), 350-363. https://doi.org/10.1038/nrn3476

Glass, M., Dragunow, M., & Faull, R. L. (1997). Cannabinoid receptors in the human brain: A detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience, 77*(2), 299-318. https://doi.org/10.1016/s0306-4522(96)00428-9

Gleeson, P. (2019). The challenge of medicinal cannabis to the political legitimacy of therapeutic goods regulation in Australia. *Melb. UL Rev.,* 43, 558.

Gobbi, G., Atkin, T., Zytynski, T., Wang, S., Askari, S., Boruff, J., Ware, M., Marmorstein, N., Cipriani, A., Dendukuri, N., & Mayo, N. (2019). association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: A systematic review and meta-analysis. *JAMA Psychiatry, 76*(4), 426-434. https://doi.org/10.1001/jamapsychiatry.2018.4500

Goebel, R., Linden, D. E. J., Lanfermann, H., Zanella, F. E., & Singer, W. (1998). Functional imaging of mirror and inverse reading reveals separate coactivated networks for oculomotion and spatial transformations. *NeuroReport 9,* 713-719

Goldberg, S. B., Pace, B., Griskaitis, M., Willutzki, R., Skoetz, N., Thoenes, S., Zgierska, A. E., & Rösner, S. (2021). Mindfulness-based interventions for substance use disorders. *Cochrane Database of Systematic Reviews* (10). https://doi.org/10.1002/14651858.CD011723.pub2

Goldberg, S. B., Riordan, K. M., Sun, S., & Davidson, R. J. (2022). The empirical status of mindfulness-based interventions: A systematic review of 44 meta-analyses of randomized controlled trials. *Perspectives on Psychological Science, 17*(1), 108-130. https://doi.org/10.1177/1745691620968771

Goldman, M., Szucs-Reed, R. P., Jagannathan, K., Ehrman, R. N., Wang, Z., Li, Y., Suh, J. J., Kampman, K., O'Brien, C. P., Childress, A. R., & Franklin, T. R. (2013). Reward-related brain response and craving correlates of marijuana cue exposure: A preliminary study in treatment-seeking marijuana-dependent subjects. *Journal of Addiction Medicine, 7*(1), 8-16. https://doi.org/10.1097/ADM.0b013e318273863a

Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: Neuroimaging evidence for the involvement of the frontal cortex. *American Journal of Psychiatry*, *159*(10), 1642-1652. https://doi.org/10.1176/appi.ajp.159.10.1642

Gonzales. J. E., & Cunningham, C. A. (2015). The promise of pre-registration in psychological research. *Psychological Science Agenda.* http://www.apa.org/science/about/psa/2015/08/pre-registration.aspx.

Gorgolewski, K. J., & Poldrack, R. A. (2016). A practical guide for improving transparency and reproducibility in neuroimaging research. *PLoS Biol*, *14*(7), e1002506. https://doi.org/10.1371/journal.pbio.1002506

Goscinski, W. J., McIntosh, P., Felzmann, U., Maksimenko, A., Hall, C. J., Gureyev, T., Thompson, D., Janke, A., Galloway, G., Killeen, N. E., Raniga, P., Kaluza, O., Ng, A., Poudel, G., Barnes, D. G., Nguyen, T., Bonnington, P., & Egan, G. F. (2014). The multi-modal Australian ScienceS Imaging and Visualization Environment (MASSIVE) high performance computing infrastructure: Applications in neuroscience and neuroinformatics research. *Frontiers Neuroinformatics, 8*, 30. https://doi.org/10.3389/fninf.2014.00030

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus. *Progress in Neurobiology, 86*(3), 141-155. https://doi.org/10.1016/j.pneurobio.2008.09.004

Grant, I., Gonzalez, R., Carey, C. L., Natarajan, L., & Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: A meta-analytic study. *Journal of the International Neuropsychology Society*, *9*(5), 679-689. https://doi.org/10.1017/s1355617703950016

Grant, S., Colaiaco, B., Motala, A., Shanman, R., Booth, M., Sorbero, M., & Hempel, S. (2017). Mindfulness-based relapse prevention for substance use disorders: A systematic review and meta-analysis. *Journal of Addiction Medicine*, *11*(5), 386-396. https://doi.org/10.1097/ADM.0000000000000338

Greicius, M. D., Kiviniemi, V., Tervonen, O., Vainionpaa, V., Alahuhta, S., Reiss, A. L., & Menon, V. (2008). Persistent default-mode network connectivity during light sedation. *Human Brain Mapping*, *29*(7), 839-847. https://doi.org/10.1002/hbm.20537

Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral Cortex*, *19*(1), 72-78. https://doi.org/10.1093/cercor/bhn059

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

Gruber, S. A., & Yurgelun-Todd, D. A. (2005). Neuroimaging of marijuana smokers during inhibitory processing: A pilot investigation. *Cognitive Brain Research*, *23*(1), 107-118. https://doi.org/10.1016/j.cogbrainres.2005.02.016

Gu, H., Salmeron, B. J., Ross, T. J., Geng, X., Zhan, W., Stein, E. A., & Yang, Y. (2010). Mesocorticolimbic circuits are impaired in chronic cocaine users as demonstrated by resting-state functional connectivity. *Neuroimage*, *53*(2), 593-601. https://doi.org/10.1016/j.neuroimage.2010.06.066

Hall, W. (2020). The costs and benefits of cannabis control policies. *Dialogues in Clinical Neuroscience*, *22*(3), 281-287. https://doi.org/10.31887/DCNS.2020.22.3/whall

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*(9698), 1383-1391. https://doi.org/10.1016/s0140-6736(09)61037-0

Hall, W., & Degenhardt, L. (2014). The adverse health effects of chronic cannabis use. *Drug Test and Analysis, 6*(1-2), 39-45. https://doi.org/10.1002/dta.1506

Hammond, C. J., Allick, A., Park, G., Rizwan, B., Kim, K., Lebo, R., Nanavati, J., Parvaz, M. A., & Ivanov, I. (2022). A meta-analysis of fMRI studies of youth cannabis use: Alterations in executive control, social cognition/emotion processing, and reward processing in cannabis using youth. *Brain Science, 12*(10). https://doi.org/10.3390/brainsci12101281

Hanlon, C. A., Dowdle, L. T., Gibson, N. B., Li, X., Hamilton, S., Canterberry, M., & Hoffman, M. (2018). Cortical substrates of cue-reactivity in multiple substance dependent populations: transdiagnostic relevance of the medial prefrontal cortex. *Translational Psychiatry, 8*(1), 186. https://doi.org/10.1038/s41398-018-0220-9

Hansen, N. B., Lambert, M. J., & Forman, E. M. (2002). The psychotherapy dose-response effect and its implications for treatment delivery services. *Clinical Psychology: Science and Practice, 9*(3), 329. https://doi.org/10.1093/clipsy.9.3.329

Harris, A. H., Humphreys, K., Bowe, T., Tiet, Q., & Finney, J. W. (2010). Does meeting the HEDIS substance abuse treatment engagement criterion predict patient outcomes? *The Journal of Behavioral Health Services & Research, 37*(1), 25-39. https://doi.org/10.1007/s11414-008-9142-2

Hasin, D. S., Kerridge, B. T., Saha, T. D., Huang, B., Pickering, R., Smith, S. M., Jung, J., Zhang, H., & Grant, B. F. (2016). Prevalence and correlates of DSM-5 Cannabis Use Disorder, 2012-2013: Findings from the National Epidemiologic Survey on Alcohol and Related Conditions-III. *American Journal of Psychiatry, 173*(6), 588-599. https://doi.org/10.1176/appi.ajp.2015.15070907

Hasin, D. S., Saha, T. D., Kerridge, B. T., Goldstein, R. B., Chou, S. P., Zhang, H., Jung, J., Pickering, R. P., Ruan, W. J., Smith, S. M., Huang, B., & Grant, B. F. (2015). Prevalence of Marijuana Use Disorders in the United States between 2001-2002 and 2012-2013. *JAMA Psychiatry, 72*(12), 1235-1242. https://doi.org/10.1001/jamapsychiatry.2015.1858

Hasin, D., & Walsh, C. (2020). Cannabis use, cannabis use disorder, and comorbid psychiatric illness: A narrative review. *Journal of Clinical Medicine, 10*(1). https://doi.org/10.3390/jcm10010015

Heishman, S. J., Evans, R. J., Singleton, E. G., Levin, K. H., Copersino, M. L., & Gorelick, D. A. (2009). Reliability and validity of a short form of the Marijuana Craving Questionnaire. *Drug and Alcohol Dependence, 102*(1-3), 35-40. https://doi.org/10.1016/j.drugalcdep.2008.12.010

Herbeck, D. M., Fitek, D. J., Svikis, D. S., Montoya, I. D., Marcus, S. C., & West, J. C. (2005). Treatment compliance in patients with comorbid psychiatric and substance use disorders. *American Journal on Addictions, 14*(3), 195-207. https://doi.org/10.1080/10550490590949488

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

Hester, R., Nestor, L., & Garavan, H. (2009). Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. *Neuropsychopharmacology, 34*(11), 2450-2458. https://doi.org/10.1038/npp.2009.67

Higgins, J. P., Altman, D. G., Gotzsche, P. C., Juni, P., Moher, D., Oxman, A. D., Savovic, J., Schulz, K. F., Weeks, L., Sterne, J. A., Cochrane Bias Methods, G., & Cochrane Statistical Methods, G. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ, 343*, d5928. https://doi.org/10.1136/bmj.d5928

Hill, S. Y., Sharma, V., & Jones, B. L. (2016). Lifetime use of cannabis from longitudinal assessments, cannabinoid receptor (CNR1) variation, and reduced volume of the right anterior cingulate. *Psychiatry Research Neuroimaging, 255*, 24-34. https://doi.org/10.1016/j.pscychresns.2016.05.009

Hiller, M. L., Knight, K., Leukefeld, C., & Simpson, D. D. (2002). Motivation as a predictor of therapeutic engagement in mandated residential substance abuse treatment. *Criminal Justice and Behavior, 29*(1), 56-75.

Hillig, K. W. (2005). Genetic evidence for speciation in Cannabis (Cannabaceae). *Genetic Resources and Crop Evolution, 52*(2), 161-180. https://doi.org/10.1007/s10722-003-4452-y

Hindocha, C., Freeman, T. P., & Curran, H. V. (2017). Anatomy of a joint: Comparing self-reported and actual dose of cannabis and tobacco in a joint, and how these are influenced by controlled acute administration. *Cannabis Cannabinoid Research, 2*(1), 217-223. https://doi.org/10.1089/can.2017.0024

Hirvonen, J., Goodwin, R. S., Li, C. T., Terry, G. E., Zoghbi, S. S., Morse, C., Pike, V. W., Volkow, N. D., Huestis, M. A., & Innis, R. B. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Molecular Psychiatry, 17*(6), 642-649. https://doi.org/10.1038/mp.2011.82

Hofmann, S. G., Sawyer, A. T., Witt, A. A., & Oh, D. (2010). The effect of mindfulness-based therapy on anxiety and depression: A meta-analytic review. *Journal of Consulting and Clinical Psychology, 78*(2), 169183. https://doi.org/10.1037/a0018555

Holstege G. (2014). The periaqueductal gray controls brainstem emotional motor systems including respiration. *Progress in Brain Research, 209*, 379–405. https://doi.org/10.1016/B978-0-444-63274-6.00020-5

Hölzel, B. K., Lazar, S. W., Gard, T., Schuman-Olivier, Z., Vago, D. R., & Ott, U. (2011). How does mindfulness meditation work? Proposing mechanisms of action from a conceptual and neural perspective. *Perspectives on Psychological Science, 6*(6), 537-559. https://doi.org/10.1177/1745691611419671

Hong, L. E., Gu, H., Yang, Y., Ross, T. J., Salmeron, B. J., Buchholz, B., Thaker, G. K., & Stein, E. A. (2009). Association of nicotine addiction and nicotine's actions with separate cingulate cortex functional circuits. *Archives of General Psychiatry, 66*(4), 431-441. https://doi.org/10.1001/archgenpsychiatry.2009.2

Hong, L. E., Hodgkinson, C. A., Yang, Y., Sampath, H., Ross, T. J., Buchholz, B., Salmeron, B. J., Srivastava, V., Thaker, G. K., Goldman, D., & Stein, E. A. (2010). A genetically modulated, intrinsic cingulate circuit supports human nicotine addiction. *Proceedings of the National Academy of Sciences USA, 107*(30), 13509-13514. https://doi.org/10.1073/pnas.1004745107

Houck, J. M., Bryan, A. D., & Ewing, S. W. F. (2013). Functional connectivity and cannabis use in high-risk adolescents. *The American Journal of Drug and Alcohol Abuse, 39*(6), 414-423. https://doi.org/10.3109/00952990.2013.837914

Houlihan, S. D., & Brewer, J. A. (2016). The emerging science of mindfulness as a treatment for addiction. In: Shonin, E., Gordon, W., Griffiths, M. (eds). *Mindfulness and Buddhist-Derived Approaches in Mental Health And Addiction*, *Advances in Mental Health and Addiction.* Springer, Cham. https://doi.org/10.1007/978-3-319-22255-4_9

Howell, A. J., Digdon, N. L., Buro, K., & Sheptycki, A. R. (2008). Relations among mindfulness, well-being, and sleep. *Personality and Individual Differences, 45*(8), 773-777. https://doi.org/10.1016/j.paid.2008.08.005

Howland, J. G., & Wang, Y. T. (2008). Chapter 8: Synaptic plasticity in learning and memory: Stress effects in the hippocampus. In W. S. Sossin, J.-C. Lacaille, V. F. Castellucci, & S. Belleville (Eds.), *Progress in Brain Research, 169*, 145-158). Elsevier. https://doi.org/https://doi.org/10.1016/S0079-6123(07)00008-8

Hu, Y., Salmeron, B. J., Gu, H., Stein, E. A., & Yang, Y. (2015). Impaired functional connectivity within and between frontostriatal circuits and its association with compulsive drug use and trait impulsivity in cocaine addiction. *JAMA Psychiatry, 72*(6), 584-592. https://doi.org/10.1001/jamapsychiatry.2015.1

Huestis, M. A., Henningfield, J. E., & Cone, E. J. (1992). Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. *Journal of Analytical Toxicology, 16*(5), 276-282. https://doi.org/10.1093/jat/16.5.276

Huettel, S. A., Song, A. W., & McCarthy, G. (2014). Functional magnetic resonance imaging. 3rd edn. Sinauer Associates, Inc, Massachusetts

Humeniuk, R., Ali, R., Babor, T. F., Farrell, M., Formigoni, M. L., Jittiwutikarn, J., & Simon, S. (2008). Validation of the alcohol, smoking and substance involvement screening test (ASSIST). *Addiction, 103,* 1039-1047. https://doi.org/10.1111/j.1360-0443.2007.02114.x

Hunt, G. E., Large, M. M., Cleary, M., Lai, H. M. X., & Saunders, J. B. (2018). Prevalence of comorbid substance use in schizophrenia spectrum disorders in community and clinical settings, 1990-2017: Systematic review and meta-analysis. *Drug and Alcohol Dependence, 191,* 234-258. https://doi.org/10.1016/j.drugalcdep.2018.07.011

Ichikawa, N., Siegle, G. J., Jones, N. P., Kamishima, K., Thompson, W. K., Gross, J. J., & Ohira, H. (2011). Feeling bad about screwing up: Emotion regulation and action monitoring in the anterior cingulate cortex. *Cognitive, Affective, & Behavioral Neuroscience, 11*(3), 354-371. https://doi.org/10.3758/s13415-011-0028-z

Isaacs, J., MacKinnon, S., Joyce, K., & Stewart, S. (2021). Reactivity to daily self-monitoring of cannabis use in biological females. *Cannabis, 4*(2), 17-30. http://doi.org/10.26828/cannabis/2021.02.002

Jager, G., Block, R. I., Luijten, M., & Ramsey, N. F. (2010). Cannabis use and memory brain function in adolescent boys: A cross-sectional multicenter functional magnetic resonance imaging study. *Journal of American Academy of Child and Adolescent Psychiatry, 49*(6), 561-572, 572.e561-563. https://doi.org/10.1016/j.jaac.2010.02.001

Jager, G., Block, R. I., Luijten, M., & Ramsey, N. F. (2013). Tentative evidence for striatal hyperactivity in adolescent cannabis-using boys: A cross-sectional multicenter fMRI study. *Journal of Psychoactive Drugs, 45*(2), 156-167. https://doi.org/10.1080/02791072.2013.785837

Jager, G., Van Hell, H. H., De Win, M. M., Kahn, R. S., Van Den Brink, W., Van Ree, J. M., & Ramsey, N. F. (2007). Effects of frequent cannabis use on hippocampal activity during an associative memory task. *European Neuropsychopharmacology, 17*(4), 289-297. https://doi.org/10.1016/j.euroneuro.2006.10.003

Janes, A. C., Datko, M., Roy, A., Barton, B., Druker, S., Neal, C., Ohashi, K., Benoit, H., van Lutterveld, R., & Brewer, J. A. (2019). Quitting starts in the brain: A randomized controlled trial of app-based mindfulness shows decreases in neural responses to smoking cues that predict reductions in smoking. *Neuropsychopharmacology, 44*(9), 1631-1638. https://doi.org/10.1038/s41386-019-0403-y

Janischevsky, D. (1924). Forma Konopli na sornykh mestakh vyugo-vostochnoi rosii. Uchenye. Zapiski, Saratov Gosud Univ, 2, 2-3.

Jiang, H. E., Li, X., Zhao, Y. X., Ferguson, D. K., Hueber, F., Bera, S., Wang, Y. F., Zhao, L. C., Liu, C. J., & Li, C. S. (2006). A new insight into Cannabis sativa (Cannabaceae) utilization from 2500-year-old Yanghai Tombs, Xinjiang, China. *Journal of Ethnopharmacology, 108*(3), 414-422. https://doi.org/10.1016/j.jep.2006.05.034

Julian, M. D., Martin, A. B., Cuellar, B., Rodriguez De Fonseca, F., Navarro, M., Moratalla, R., & Garcia-Segura, L. M. (2003). Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. *Neuroscience, 119*(1), 309-318. https://doi.org/10.1016/s0306-4522(03)00070-8

Kabat-Zinn, J. (1991). *Full catastrophe living: Using the wisdom of your body and mind to face stress, pain, and illness.* Delta.

Kabat-Zinn, J. (2003). Mindfulness-based stress reduction (MBSR). *Constructivism in the Human Sciences, 8*(2), 73. https://www.proquest.com/scholarly-journals/mindfulness-based-stress-reduction-mbsr/docview/204582884/se-2

Kaiser, R. H., Whitfield-Gabrieli, S., Dillon, D. G., Goer, F., Beltzer, M., Minkel, J., Smoski, M., Dichter, G., & Pizzagalli, D. A. (2016). dynamic resting-state functional connectivity in major depression. *Neuropsychopharmacology, 41*(7), 1822-1830. https://doi.org/10.1038/npp.2015.352

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience, 10*(8), 561-572. https://doi.org/10.1038/nrn2515

Kamboj, S. K., Irez, D., Serfaty, S., Thomas, E., Das, R. K., & Freeman, T. P. (2017). Ultra-brief mindfulness training reduces alcohol consumption in at-risk drinkers: A randomized double-

blind active-controlled experiment. *International Journal of Neuropsychopharmacology, 20*(11), 936-947. https://doi.org/10.1093/ijnp/pyx064

Kanayama, G., Rogowska, J., Pope, H. G., Gruber, S. A., & Yurgelun-Todd, D. A. (2004). Spatial working memory in heavy cannabis users: A functional magnetic resonance imaging study. *Psychopharmacology (Berl), 176*(3-4), 239-247. https://doi.org/10.1007/s00213-004-1885-8

Kang, L., Wang, W., Zhang, N., Nie, Z., Gong, Q., Yao, L., Tu, N., Feng, H., Zong, X., Bai, H., Wang, G., Bu, L., Wang, F., & Liu, Z. (2022). Superior temporal gyrus and cerebellar loops predict nonsuicidal self-injury in major depressive disorder patients by multimodal neuroimaging. *Translational Psychiatry, 12*(1), 474. https://doi.org/10.1038/s41398-022-02235-y

Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E. S., Mackie, K., & Freund, T. F. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *Journal of Neuroscience, 19*(11), 4544-4558. https://www.ncbi.nlm.nih.gov/pubmed/10341254

Katz, G., Lobel, T., Tetelbaum, A., & Raskin, S. (2014). Cannabis withdrawal - a new diagnostic category in DSM-5. *Israel Journal of Psychiatry Related Sciences, 51*(4), 270-275. https://www.ncbi.nlm.nih.gov/pubmed/25841223

Katz, D., & Toner, B. (2013). A systematic review of gender differences in the effectiveness of mindfulness-based treatments for substance use disorders. *Mindfulness, 4*(4), 318-331. https://doi.org/10.1007/s12671-012-0132-3

Kaufman, J., Birmaher, B., Brent, D., Rao, U. M. A., Flynn, C., Moreci, P., & Ryan., N. (1997). Schedule for affective disorders and schizophrenia for school-age children: Present and lifetime version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*, 980-988. doi:https://doi.org/10.1097/00004583-199707000-00021

Khalili, P., Nadimi, A. E., Baradaran, H. R., Janani, L., Rahimi-Movaghar, A., Rajabi, Z., Rahmani, A., Hojati, Z., Khalagi, K., & Motevalian, S. A. (2021). Validity of self-reported substance

use: research setting versus primary health care setting. *Substance Abuse Treatment, Prevention, and Policy, 16*(1), 66. https://doi.org/10.1186/s13011-021-00398-3

Kilpatrick, L. A., Suyenobu, B. Y., Smith, S. R., Bueller, J. A., Goodman, T., Creswell, J. D., Tillisch, K., Mayer, E. A., & Naliboff, B. D. (2011). Impact of Mindfulness-Based Stress Reduction training on intrinsic brain connectivity. *Neuroimage, 56*(1), 290-298. https://doi.org/10.1016/j.neuroimage.2011.02.034

Kim, D. J., Schnakenberg Martin, A. M., Shin, Y. W., Jo, H. J., Cheng, H., Newman, S. D., Sporns, O., Hetrick, W. P., Calkins, E., & O'Donnell, B. F. (2019). Aberrant structural-functional coupling in adult cannabis users. *Human Brain Mapping, 40*(1), 252–261. https://doi.org/10.1002/hbm.24369

Kim, H., Nanavaty, N., Ahmed, H., Mathur, V. A., & Anderson, B. A. (2021). Motivational salience guides attention to valuable and threatening stimuli: Evidence from behavior and functional magnetic resonance imaging. *Journal Cognitive Neuroscience, 33*(12), 2440-2460. https://doi.org/10.1162/jocn_a_01769

Kirk, U., & Montague, P. R. (2015). Mindfulness meditation modulates reward prediction errors in a passive conditioning task. *Frontiers in Psychology, 6*, 90. https://doi.org/10.3389/fpsyg.2015.00090

Kirlic, N., Cohen, Z., & Stewart, J. L. (2021). Neurocircuitry of Mindfulness-Based Interventions for substance use prevention and recovery. *Current Addiction Reports, 8*(4), 520-529. https://doi.org/10.1007/s40429-021-00396-2

Klaassen, A. L., Heiniger, A., Vaca Sánchez, P., Harvey, M. A., & Rainer, G. (2021). Ventral pallidum regulates the default mode network, controlling transitions between internally and externally guided behavior. *Proceedings of the National Academy of Sciences USA, 118*(36). https://doi.org/10.1073/pnas.2103642118

Klainin-Yobas, P., Oo, W. N., Suzanne Yew, P. Y., & Lau, Y. (2015). Effects of relaxation interventions on depression and anxiety among older adults: A systematic review. *Aging Mental Health, 19*(12), 1043-1055. https://doi.org/10.1080/13607863.2014.997191

Klugah-Brown, B., Di, X., Zweerings, J., Mathiak, K., Becker, B., & Biswal, B. (2020). Common and separable neural alterations in substance use disorders: A coordinate-based meta-analyses of functional neuroimaging studies in humans. *Human Brain Mapping, 41*(16), 4459-4477. https://doi.org/10.1002/hbm.25085

Klumpers, L. E., Cole, D. M., Khalili-Mahani, N., Soeter, R. P,, te Beek, E. T., Rombouts, S. A. R. B., & van Gerven, J. M. A. (2012). Manipulating brain connectivity with delta9-tetrahydrocannabinol: A pharmacological resting state fMRI study. *NeuroImage 63*, 1701-1711 http://dx.doi.org/10.1016/j.neuroimage.2012.07.051

Knutson, B., & Gibbs, S. E. (2007). Linking nucleus accumbens dopamine and blood oxygenation. *Psychopharmacology (Berl)*, 191(3), 813-822. https://doi.org/10.1007/s00213-006-0686-7

Kober, H., Brewer, J. A., Height, K. L., & Sinha, R. (2017). Neural stress reactivity relates to smoking outcomes and differentiates between mindfulness and cognitive-behavioral treatments. *Neuroimage, 151,* 4-13. https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.09.042

Koenders, L., Lorenzetti, V., de Haan, L., Suo, C., Vingerhoets, W., van den Brink, W., Wiers, R. W., Meijer, C. J., Machielsen, M., Goudriaan, A. E., Veltman, D. J., Yucel, M., & Cousijn, J. (2017). Longitudinal study of hippocampal volumes in heavy cannabis users. *J Psychopharmacology, 31*(8), 1027-1034. https://doi.org/10.1177/0269881117718380

Kondo, K. K., Morasco, B. J., Nugent, S. M., Ayers, C. K., O'Neil, M. E., Freeman, M., & Kansagara, D. (2020). Pharmacotherapy for the treatment of cannabis use disorder: a systematic review. *Annals of Internal Medicine, 172*(6), 398-412. https://doi.org/10.7326/m19-1105

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology, 35*(1), 217-238. https://doi.org/10.1038/npp.2009.110

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: A neurocircuitry analysis. *Lancet Psychiatry, 3*(8), 760-773. https://doi.org/10.1016/S2215-0366(16)00104-8

Korecki, J. R., Schwebel, F. J., Votaw, V. R., & Witkiewitz, K. (2020). Mindfulness-based programs for substance use disorders: A systematic review of manualized treatments. *Substance Abuse Treatment, Prevention, and Policy, 15*(1), 51. https://doi.org/10.1186/s13011-020-00293-3

Kragel, E. A., Sweitzer, M. M., & Davis, J. M. (2019). The effect of brief mindfulness training on brain reactivity to food cues during nicotine withdrawal: A pilot functional imaging study. *Mindfulness, 10*(11), 2272-2276. https://doi.org/10.1007/s12671-019-01201-y

Kral, T. R. A., Imhoff-Smith, T., Dean, D. C., Grupe, D., Adluru, N., Patsenko, E., Mumford, J. A., Goldman, R., Rosenkranz, M. A., & Davidson, R. J. (2019). Mindfulness-Based Stress Reduction-related changes in posterior cingulate resting brain connectivity. *Social Cognitive and Affective Neuroscience, 14*(7), 777-787. https://doi.org/10.1093/scan/nsz050

Kuepper, R., van Os, J., Lieb, R., Wittchen, H. U., Hofler, M., & Henquet, C. (2011). Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. *BMJ, 342*, d738. https://doi.org/10.1136/bmj.d738

Kuhn, S., & Gallinat, J. (2013). Resting-state brain activity in schizophrenia and major depression: A quantitative meta-analysis. *Schizophrenia Bulletin, 39*(2), 358-365. https://doi.org/10.1093/schbul/sbr151

Kwak, S., Lee, T. Y., Jung, W. H., Hur, J. W., Bae, D., Hwang, W. J., Cho, K. I. K., Lim, K. O., Kim, S. Y., Park, H. Y., & Kwon, J. S. (2019). the immediate and sustained positive effects of meditation on resilience are mediated by changes in the resting brain. *Frontiers in Human Neuroscience, 13*, 101. https://doi.org/10.3389/fnhum.2019.00101

Lamarck, J. d. (1786). Encyclopedie Methodique Botanique, Vol. 2. Chez Pankoucke, Paris.

Landfield, P. W., Cadwallader, L. B., & Vinsant, S. (1988). Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: Possible mediation by glucocorticoid systems. *Brain Research, 443*(1-2), 47-62. https://doi.org/10.1016/0006-8993(88)91597-1

Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and earlier onset of psychosis: A systematic meta-analysis. *Archives of General Psychiatry, 68*(6), 555-561. https://doi.org/10.1001/archgenpsychiatry.2011.5

Lawn, W., Fernandez-Vinson, N., Mokrysz, C., Hogg, G., Lees, R., Trinci, K., Petrilli, K., Borissova, A., Ofori, S., Waters, S., Michor, P., Wall, M. B., Freeman, T. P., & Curran, H. V. (2022). The CannTeen study: Verbal episodic memory, spatial working memory, and response

inhibition in adolescent and adult cannabis users and age-matched controls. *Psychopharmacology (Berl), 239*(5), 1629-1641. https://doi.org/10.1007/s00213-022-06143-3

Lawn, W., Mokrysz, C., Lees, R., Trinci, K., Petrilli, K., Skumlien, M., Borissova, A., Ofori, S., Bird, C., Jones, G., Bloomfield, M. A., Das, R. K., Wall, M. B., Freeman, T. P., & Curran, H. V. (2022). The CannTeen Study: Cannabis use disorder, depression, anxiety, and psychotic-like symptoms in adolescent and adult cannabis users and age-matched controls. *Journal of Psychopharmacology, 36*(12), 1350-1361. https://doi.org/10.1177/02698811221108956

Leadbeater, B. J., Ames, M. E., & Linden-Carmichael, A. N. (2019). Age-varying effects of cannabis use frequency and disorder on symptoms of psychosis, depression and anxiety in adolescents and adults. *Addiction, 114*(2), 278-293. https://doi.org/10.1111/add.14459

Lecrubier, Y., Sheehan, D. V., Weiller, E., Amorim, P., Bonora, I., Sheehan, K. H., Janavs, J., & Dunbar, G. C. (1997). The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. *European Psychiatry, 12*(5), 224-231.

Lecrubier, Y., Sheehan, D., Hergueta, T., & Weiller, E. (1998). The MINI international neuropsychiatric interview. *European Psychiatry, 13*(1004), 198s-198s.

Lee, M. A. (2012). The discovery of the endocannabinoid system. *The Prop,* 215.

Lees, R., Hines, L. A., D'Souza, D. C., Stothart, G., Di Forti, M., Hoch, E., & Freeman, T. P. (2021). Psychosocial and pharmacological treatments for cannabis use disorder and mental health comorbidities: A narrative review. *Psychological Medicine, 51*(3), 353-364. https://doi.org/10.1017/S0033291720005449

Leung, J., Chan, G. C. K., Hides, L., & Hall, W. D. (2020). What is the prevalence and risk of cannabis use disorders among people who use cannabis? A systematic review and meta-analysis. *Addictive Behaviors, 109*, 106479. https://doi.org/10.1016/j.addbeh.2020.106479

Lev-Ran, S., Roerecke, M., Le Foll, B., George, T. P., McKenzie, K., & Rehm, J. (2014). The association between cannabis use and depression: A systematic review and meta-analysis of

longitudinal studies. *Psychological Medicine, 44*(4), 797-810. https://doi.org/10.1017/s0033291713001438

Li, S., Hu, N., Zhang, W., Tao, B., Dai, J., Gong, Y., Tan, Y., Cai, D., & Lui, S. (2019). Dysconnectivity of multiple brain networks in schizophrenia: A meta-analysis of resting-state functional connectivity. *Frontiers in Psychiatry, 10*, 482. https://doi.org/10.3389/fpsyt.2019.00482

Li, W., Howard, M. O., Garland, E. L., McGovern, P., & Lazar, M. (2017). Mindfulness treatment for substance misuse: A systematic review and meta-analysis. *Journal of Substance Abuse Treatment, 75*, 62-96. https://doi.org/10.1016/j.jsat.2017.01.008

Livne, O., Shmulewitz, D., Stohl, M., Mannes, Z., Aharonovich, E., & Hasin, D. (2021). Agreement between DSM-5 and DSM-IV measures of substance use disorders in a sample of adult substance users. *Drug and Alcohol Dependence*, *227*, 108958. https://doi.org/10.1016/j.drugalcdep.2021.108958

Longshore, D., & Teruya, C. (2006). Treatment motivation in drug users: A theory-based analysis. *Drug and Alcohol Dependence, 81*(2), 179-188. https://doi.org/10.1016/j.drugalcdep.2005.06.011

Lopez-Larson, M. P., Rogowska, J., & Yurgelun-Todd, D. (2015). Aberrant orbitofrontal connectivity in marijuana smoking adolescents. *Developmental Cognitive Neuroscience 16*, 54-62 https://doi:10.1016/j.dcn.2015.08.002

Lopez-Larson, M. P., Rogowska, J., & Yurgelun-Todd, D. (2015). Aberrant orbitofrontal connectivity in marijuana smoking adolescents. *Developmental Cognitive Neuroscience, 16*, 54-62. https://doi.org/10.1016/j.dcn.2015.08.002

Lorenzetti, V., & Cousijn, J. (2016). Chapter 72 - Cannabis Use Disorders and Brain Morphology. In V. R. Preedy (Ed.), *Neuropathology of Drug Addictions and Substance Misuse* (pp. 773-785). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-800213-1.00072-9

Lorenzetti, V., Chye, Y., Silva, P., Solowij, N., & Roberts, C. A. (2019). Does regular cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural

neuroimaging studies. *European Archives of Psychiatry and Clinical Neuroscience, 269*(1), 59-71. https://doi.org/10.1007/s00406-019-00979-1

Lorenzetti, V., Cousijn, J., Solowij, N., Garavan, H., Suo, C., Yücel, M., & Verdejo-García, A. (2016). The Neurobiology of Cannabis Use Disorders: A Call for Evidence. *Frontiers in Behavioral Neuroscience, 10,* 86. https://doi.org/10.3389/fnbeh.2016.00086

Lorenzetti, V., Gaillard, A., Beyer, E., Kowalczyk, M., Kamboj, S., Manning, V., & Gleeson, J. (*under review*) Do mindfulness-based interventions changes brain function in people with substance dependence? A systematic review of the fMRI evidence.

Lorenzetti, V., Gaillard, A., Thomson, D., Englund, A., & Freeman, T. P. (2022). Effects of cannabinoids on resting state functional brain connectivity: A systematic review. *Neuroscience Biobehavior Reviews, 145*, 105014. https://doi.org/10.1016/j.neubiorev.2022.105014

Lorenzetti, V., Hoch, E., & Hall, W. (2020). Adolescent cannabis use, cognition, brain health and educational outcomes: A review of the evidence. *European Neuropsychopharmacology, 36*, 169-180. https://doi.org/10.1016/j.euroneuro.2020.03.012

Lorenzetti, V., Lubman, D. I., Whittle, S., Solowij, N., & Yücel, M. (2010). Structural MRI findings in long-term cannabis users: What do we know? *Substance Use Misuse, 45*(11), 1787-1808. https://doi.org/10.3109/10826084.2010.482443

Lorenzetti, V., Solowij, N., & Yucel, M. (2016). The role of cannabinoids in neuroanatomic alterations in cannabis users. *Biological Psychiatry, 79*(7), e17-31. https://doi.org/10.1016/j.biopsych.2015.11.013

Lorenzetti, V., Solowij, N., Whittle, S., Fornito, A., Lubman, D. I., Pantelis, C., & Yücel, M. (2015). Gross morphological brain changes with chronic, heavy cannabis use. *British Journal of Psychiatry, 206*(1), 77-78. https://doi.org/10.1192/bjp.bp.114.151407

Lorenzetti, V., Takagi, M., van Dalen, Y., Yucel, M., & Solowij, N. (2021). Investigating the residual effects of chronic cannabis use and abstinence on verbal and visuospatial learning. *Frontiers in Psychiatry, 12,* 663701. https://doi.org/10.3389/fpsyt.2021.663701

Lovell, M. E., Akhurst, J., Padgett, C., Garry, M. I., & Matthews, A. (2020). Cognitive outcomes associated with long-term, regular, recreational cannabis use in adults: A meta-analysis. *Experimental and Clinical Psychopharmacology, 28*(4), 471-494. https://doi.org/10.1037/pha0000326

Lu, H. C., & Mackie, K. (2021). Review of the endocannabinoid system. *Biological Psychiatry Cognitive Neuroscience Neuroimaging, 6*(6), 607-615. https://doi.org/10.1016/j.bpsc.2020.07.016

Lu, H., Song, Y., Xu, M., Wang, X., Li, X., & Liu, J. (2014). The brain structure correlates of individual differences in trait mindfulness: A voxel-based morphometry study. *Neuroscience, 272,* 21-28. https://doi.org/10.1016/j.neuroscience.2014.04.051

Luberto, C. M., Hall, D. L., Park, E. R., Haramati, A., & Cotton, S. (2020). A perspective on the similarities and differences between mindfulness and relaxation. *Global Advances in Integrative Medicine and Health, 9*, 2164956120905597. https://doi.org/10.1177/2164956120905597

Lubman, D. I., Cheetham, A., & Yucel, M. (2015). Cannabis and adolescent brain development. *Pharmacology & Therapeutics, 148*, 1-16. https://doi.org/10.1016/j.pharmthera.2014.11.009

Luciana, M. (2020). Risks versus consequences of adolescent and young adult substance use: A focus on executive control. *Current Addiction Report, 7*(4), 453-463. https://doi.org/10.1007/s40429-020-00341-9

Lupica, C. R., Riegel, A. C., & Hoffman, A. F. (2004). Marijuana and cannabinoid regulation of brain reward circuits. *British Journal of Psychopharmacology, 143*(2), 227-234. https://doi.org/10.1038/sj.bjp.0705931

Lutz, A., Jha, A. P., Dunne, J. D., & Saron, C. D. (2015). Investigating the phenomenological matrix of mindfulness-related practices from a neurocognitive perspective. *American Psychologist, 70*(7), 632-658. https://doi.org/10.1037/a0039585

Lv, H., Wang, Z., Tong, E., Williams, L. M., Zaharchuk, G., Zeineh, M., Goldstein-Piekarski, A. N., Ball, T. M., Liao, C., & Wintermark, M. (2018). Resting-state functional MRI: Everything

that nonexperts have always wanted to know. *AJNR American Journal of Neuroradiology, 39*(8), 1390-1399. https://doi.org/10.3174/ajnr.A5527

Ma, C., Ding, J., Li, J., Guo, W., Long, Z., Liu, F., Gao, Q., Zeng, L., Zhao, J., & Chen, H. (2012). Resting-state functional connectivity bias of middle temporal gyrus and caudate with altered gray matter volume in major depression. *PLoS One, 7*(9), e45263. https://doi.org/10.1371/journal.pone.0045263

Ma, N., Liu, Y., Li, N., Wang, C. X., Zhang, H., Jiang, X. F., Xu, H. S., Fu, X. M., Hu, X., & Zhang, D. R. (2010). Addiction related alteration in resting-state brain connectivity. *Neuroimage, 49*(1), 738-744. https://doi.org/10.1016/j.neuroimage.2009.08.037

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol, 168*, 299-325. https://doi.org/10.1007/3-540-26573-2_10

MacPhail, S. L., Bedoya-Pérez, M. A., Cohen, R., Kotsirilos, V., McGregor, I. S., & Cairns, E. A. (2022). Medicinal cannabis prescribing in Australia: An analysis of trends over the first five years. *Frontiers in Pharmacology, 13*, 885655. https://doi.org/10.3389/fphar.2022.885655

Maguire, E. A., Intraub, H., & Mullally, S. L. (2016). Scenes, spaces, and memory traces: What does the hippocampus do? *Neuroscientist, 22*(5), 432-439. https://doi.org/10.1177/1073858415600389

Makowski, C., Brown, T. T., Zhao, W., Hagler, D. J., Parekh, P., Garavan, H., Nichols, T. E., Jernigan, T. L., & Dale, A. M. (2023). Reports of the death of brain-behavior associations have been greatly exaggerated. *bioRxiv* https://doi.org/10.1101/2023.06.16.545340

Malinowski, P. (2013). Neural mechanisms of attentional control in mindfulness meditation. *Frontiers in Neuroscience, 7*, 8. https://doi.org/10.3389/fnins.2013.00008

Mannheim, M., & Lowrey, T. (2020, Jan 31). Canberrans can now grow and smoke cannabis, though some questions remain unanswered *The News Daily* https://thenewdaily.com.au/news/national/2020/01/31/canberra-cannabis-questions-remain/

Manthey, J., Freeman, T. P., Kilian, C., Lopez-Pelayo, H., & Rehm, J. (2021). Public health monitoring of cannabis use in Europe: Prevalence of use, cannabis potency, and treatment

rates. *The Lancet Regional Health - Europe, 10,* 100227. https://doi.org/10.1016/j.lanepe.2021.100227

Manza, P., Tomasi, D., & Volkow, N. D. (2018). Subcortical local functional hyperconnectivity in cannabis dependence. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 3*, 285-293 https://doi:10.1016/j.bpsc.2017.11.004

Marconi, A., Di Forti, M., Lewis, C. M., Murray, R. M., & Vassos, E. (2016). Meta-analysis of the association between the level of cannabis use and risk of psychosis. *Schizophrenia Bulletin, 42*(5), 1262-1269. https://doi.org/10.1093/schbul/sbw003

Marek, S., Tervo-Clemmens, B., Calabro, F. J., Montez, D. F., Kay, B. P., Hatoum, A. S., ... & Dosenbach, N. U. (2020). Towards reproducible brain-wide association studies. *BioRxiv.* 2020-08. https://doi.org/10.1101/2020.08.21.257758

Marek, S., Tervo-Clemmens, B., Calabro, F. J., Montez, D. F., Kay, B. P., Hatoum, A. S., Donohue, M. R., Foran, W., Miller, R. L., Hendrickson, T. J., Malone, S. M., Kandala, S., Feczko, E., Miranda-Dominguez, O., Graham, A. M., Earl, E. A., Perrone, A. J., Cordova, M., Doyle, O., . . . Dosenbach, N. U. F. (2022). Reproducible brain-wide association studies require thousands of individuals. *Nature, 603*(7902), 654-660. https://doi.org/10.1038/s41586-022-04492-9

Marel, C., Sunderland, M., Mills, K. L., Slade, T., Teesson, M., & Chapman, C. (2019). Conditional probabilities of substance use disorders and associated risk factors: Progression from first use to use disorder on alcohol, cannabis, stimulants, sedatives and opioids. *Drug and Alcohol Dependence, 194,* 136-142. https://doi.org/10.1016/j.drugalcdep.2018.10.010

Martuzzi, R., Ramani, R., Qiu, M., Shen, X., Papademetris, X., & Constable, R. T. (2011). A whole-brain voxel based measure of intrinsic connectivity contrast reveals local changes in tissue connectivity with anesthetic without a priori assumptions on thresholds or regions of interest. *NeuroImage 58,* 1044-1050. https://doi.org/10.1016/j.neuroimage.2011.06.075

Martz, M. E., Hart, T., Heitzeg, M. M., & Peltier, S. J. (2020). Neuromodulation of brain activation associated with addiction: A review of real-time fMRI neurofeedback studies. *Neuroimage Clin, 27,* 102350. https://doi.org/10.1016/j.nicl.2020.102350

Martz, M. E., Trucco, E. M., Cope, L. M., Hardee, J. E., Jester, J. M., Zucker, R. A., & Heitzeg, M. M. (2016). Association of marijuana use with blunted nucleus accumbens response to reward anticipation. *JAMA Psychiatry, 73*(8), 838-844. https://doi.org/10.1001/jamapsychiatry.2016.1161

Matochik, J. A., Eldreth, D. A., Cadet, J. L., & Bolla, K. I. (2005). Altered brain tissue composition in heavy marijuana users. *Drug and Alcohol Dependence, 77*(1), 23-30. https://doi.org/10.1016/j.drugalcdep.2004.06.011

McConnell, P. A., Garland, E. L., Zubieta, J. K., Newman-Norlund, R., Powers, S., & Froeliger, B. (2020). Impaired frontostriatal functional connectivity among chronic opioid using pain patients is associated with dysregulated affect. *Addiction Biology, 25*(2), e12743. https://doi.org/10.1111/adb.12743

McHugh, M. J., Demers, C. H., Braud, J., Briggs, R., Adinoff, B., & Stein, E. A. (2013). Striatal-insula circuits in cocaine addiction: implications for impulsivity and relapse risk. *American Journal of Drug and Alcohol Abuse, 39*(6), 424-432. https://doi.org/10.3109/00952990.2013.847446

McKenna, G. J. (2014). The current status of medical marijuana in the United States. *Hawai'i Journal of Medicine and Public Health, 73*(4), 105-108.

McMurran, M., & Ward, T. (2010). Treatment readiness, treatment engagement and behaviour change. *Criminal Behaviour and Mental Health, 20*(2), 75-85. https://doi.org/10.1002/cbm.762

McPartland, J. M. (2018). Cannabis systematics at the levels of family, genus, and species. *Cannabis Cannabinoid Research, 3*(1), 203-212. https://doi.org/10.1089/can.2018.0039

McPartland, J. M., Hegman, W., & Long, T. (2019) *Cannabis* in Asia: Its center of origin and early cultivation, based on synthesis of subfossil pollen and archaeobotanical studies. *Vegetation History and Archaeobotany, 28,* 691-702. https://doi.org/10.1007/s00334-019-00731-8

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

Mechoulam, R., & Shvo, Y. (1963). Hashish. I. The structure of cannabidiol. *Tetrahedron, 19*(12), 2073-2078. https://doi.org/10.1016/0040-4020(63)85022-x

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

Meck, W. H. (2006). Neuroanatomical localization of an internal clock: A functional link between mesolimbic, nigrostriatal, and mesocortical dopaminergic systems. *Brain Research, 1109*(1), 93-107. https://doi.org/https://doi.org/10.1016/j.brainres.2006.06.031

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

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry, 62*(5), 446-454. https://doi.org/10.1016/j.biopsych.2006.10.011

Miyakawa, T. (2020). No raw data, no science: Another possible source of the reproducibility crisis. *Molecular Brain, 13*(1), 24. https://doi.org/10.1186/s13041-020-0552-2

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., The Prisma Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLOS Medicine 6*:e1000097 https://doi:10.1371/journal.pmed.1000097

Moos, R. H. (2008). Context and mechanisms of reactivity to assessment and treatment. *Addiction, 103*(2), 249-250. https://doi.org/10.1111/j.1360-0443.2007.02123.x

Morein-Zamir, S., & Robbins, T. W. (2015). Frontostriatal circuits in response-inhibition: Relevance to addiction. *Brain Research, 1628,* 117-129. https://doi.org/https://doi.org/10.1016/j.brainres.2014.09.012

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

Moreno-Rius, J. (2019). The cerebellum, THC, and cannabis addiction: Findings from animal and human studies. *Cerebellum, 18*(3), 593-604. https://doi.org/10.1007/s12311-018-0993-7

Moreno-Rius, J., & Miquel, M. (2017). The cerebellum in drug craving. *Drug and Alcohol Dependence, 173*, 151-158. https://doi.org/10.1016/j.drugalcdep.2016.12.028

Morris, L. S., Kundu, P., Dowell, N., Mechelmans, D. J., Favre, P., Irvine, M. A., Robbins, T. W., Daw, N., Bullmore, E. T., Harrison, N. A., & Voon, V. (2016). Fronto-striatal organization: Defining functional and microstructural substrates of behavioural flexibility. *Cortex, 74*, 118-133. https://doi.org/https://doi.org/10.1016/j.cortex.2015.11.004

Motzkin, J. C., Baskin-Sommers, A., Newman, J. P., Kiehl, K. A., & Koenigs, M. (2014). Neural correlates of substance abuse: Reduced functional connectivity between areas underlying reward and cognitive control. *Human Brain Mapping, 35*(9), 4282-4292. https://doi.org/10.1002/hbm.22474

Myers, B., van der Westhuizen, C., Naledi, T., Stein, D. J., & Sorsdahl, K. (2016). Readiness to change is a predictor of reduced substance use involvement: Findings from a randomized controlled trial of patients attending South African emergency departments. *BMC Psychiatry, 16*, 35. https://doi.org/10.1186/s12888-016-0742-8

Nelson, R. O., & Hayes, S. C. (1981). Theoretical explanations for reactivity in self-monitoring. *Behavior Modification, 5*(1), 3-14.

Nestor, L., Hester, R., & Garavan, H. (2010). Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage, 49*(1), 1133-1143. https://doi.org/10.1016/j.neuroimage.2009.07.022

Nestor, L., Roberts, G., Garavan, H., & Hester, R. (2008). Deficits in learning and memory: Parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. *Neuroimage, 40*(3), 1328-1339. https://doi.org/10.1016/j.neuroimage.2007.12.059

Newman, S. D., Cheng, H., Kim, D. J., Schnakenberg-Martin, A., Dydak, U., Dharmadhikari, S., Hetrick, W., & O'Donnell, B. (2020). An investigation of the relationship between glutamate and resting state connectivity in chronic cannabis users. *Brain imaging and behavior*, *14*(5), 2062–2071. https://doi.org/10.1007/s11682-019-00165-w

Nielsen, S., Gowing, L., Sabioni, P., & Le Foll, B. (2019). Pharmacotherapies for cannabis dependence. *Cochrane Database of Systematic Reviews* (1). https://doi.org/10.1002/14651858.CD008940.pub3

Norman, K. A,, Polyn, S. M., Detre, G. J., & Haxby, J. V. (2006). Beyond mind-reading: Multi-voxel pattern analysis of fMRI data. *Trends in Cognitive Sciences. 10* 424-430 https://doi.org/10.1016/j.tics.2006.07.005

Northoff, G. (2020). Anxiety disorders and the brain's resting state networks: From altered spatiotemporal synchronization to psychopathological symptoms. *Advances in Experimental Medicine and Biology, 1191*, 71-90. https://doi.org/10.1007/978-981-32-9705-0_5

Ogawa, S., Lee, T. M., Kay, A. R., & Tank, D. W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Sciences USA, 87*(24), 9868-9872. https://doi.org/10.1073/pnas.87.24.9868

Onaemo, V. N., Fawehinmi, T. O., & D'Arcy, C. (2021). Comorbid cannabis use disorder with major depression and generalized anxiety disorder: A systematic review with meta-analysis of nationally representative epidemiological surveys. *Journal of Affective Disorders, 281*, 467-475. https://doi.org/10.1016/j.jad.2020.12.043

Orr, C., Morioka, R., Behan, B., Datwani, S., Doucet, M., Ivanovic, J., Kelly, C., Weierstall, K., Watts, R., Smyth, B., & Garavan, H. (2013). Altered resting-state connectivity in adolescent cannabis users. *The American Journal of Drug and Alcohol Abuse, 39*(6), 372–381. https://doi.org/10.3109/00952990.2013.848213

Orr, J. M., Smolker, H. R., & Banich, M. T. (2015). Organization of the human frontal pole revealed by large-scale DTI-based connectivity: Implications for control of behavior. *PLoS One, 10*(5), e0124797. https://doi.org/10.1371/journal.pone.0124797

Pacher, P., Bátkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev, 58*(3), 389-462. https://doi.org/10.1124/pr.58.3.2

Pain, S. (2015). A potted history. *Nature, 525*(7570), S10-11. https://doi.org/10.1038/525S10a

Parkes, L., Fulcher, B., Yucel, M., & Fornito, A. (2018). An evaluation of the efficacy, reliability, and sensitivity of motion correction strategies for resting-state functional MRI. *Neuroimage, 171*, 415-436. https://doi.org/10.1016/j.neuroimage.2017.12.073

Pate, D. W. (1999). The phytochemistry of Cannabis: Its ecological and evolutionary implications. *Advances in Hemp Research.* Food Product Press, New York, 21-42.

Paus, T. (2001). Primate anterior cingulate cortex: Where motor control, drive and cognition interface. *Nature Reviews Neuroscience, 2*(6), 417-424. https://doi.org/10.1038/35077500

Pellati, F., Borgonetti, V., Brighenti, V., Biagi, M., Benvenuti, S., & Corsi, L. (2018). Cannabis sativa L. and nonpsychoactive cannabinoids: Their chemistry and role against oxidative stress, inflammation, and cancer. *BioMed Research International, 2018*, 1691428. https://doi.org/10.1155/2018/1691428

Pertwee, R. G. (2008). Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addiction Biology, 13*(2), 147-159. https://doi.org/10.1111/j.1369-1600.2008.00108.x

Philippi. C. L., Floyd, T., & Dahl, G. (2020). Resting-state functional magnetic resonance imaging and neuropsychiatric conditions. In: Paul RH, Salminen LE, Heaps J, Cohen LM (ed). *The Wiley Encyclopedia of Health Psychology.* 131-140. https://doi.org/10.1002/9781119057840.ch16

Philippi, C. L., Reyna, L., Nedderman, L., Chan, P., Samboju, V., Chang, K., Phanuphak, N., Ratnaratorn, N., Hellmuth, J., Benjapornpong, K., Dumrongpisutikul, N., Pothisri, M., Robb, M. L., Ananworanich, J., Spudich, S., Valcour, V., Paul, R., Search, R. V., & teams,

R. S. s. (2020). Resting-state neural signatures of depressive symptoms in acute HIV. *Journal of NeuroVirology, 26*(2), 226-240. https://doi.org/10.1007/s13365-020-00826-3

Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience Biobehavior Reviews, 30*(2), 215-238. https://doi.org/10.1016/j.neubiorev.2005.04.016

Piet, J., & Hougaard, E. (2011). The effect of mindfulness-based cognitive therapy for prevention of relapse in recurrent major depressive disorder: A systematic review and meta-analysis. *Clinical Psychology Review, 31*(6), 1032-1040. https://doi.org/10.1016/j.cpr.2011.05.002

Piluzza, G., Delogu, G., Cabras, A., Marceddu, S., & Bullitta, S. (2013). Differentiation between fiber and drug types of hemp (Cannabis sativa L.) from a collection of wild and domesticated accessions. *Genetic Resources and Crop Evolution, 60*(8), 2331-2342. https://doi.org/10.1007/s10722-013-0001-5

Pisanti, S., & Bifulco, M. (2017). Modern history of medical cannabis: from widespread use to prohibitionism and back. *Trends in Pharmacological Sciences, 38*(3), 195-198. https://doi.org/10.1016/j.tips.2016.12.002

Pisanti, S., & Bifulco, M. (2019). Medical cannabis: A plurimillennial history of an evergreen. *Journal of Cellular Physiology, 234*(6), 8342-8351. https://doi.org/10.1002/jcp.27725

Poissant, H., Mendrek, A., Talbot, N., Khoury, B., & Nolan, J. (2019). behavioral and cognitive impacts of mindfulness-based interventions on adults with attention-deficit hyperactivity disorder: A systematic review. *Behavioral Neurology, 2019*, 5682050. https://doi.org/10.1155/2019/5682050

Poldrack, R. A., Baker, C. I., Durnez, J., Gorgolewski, K. J., Matthews, P. M., Munafo, M. R., Nichols, T. E., Poline, J. B., Vul, E., & Yarkoni, T. (2017). Scanning the horizon: Towards transparent and reproducible neuroimaging research. *Nature Reviews Neuroscience, 18*(2), 115-126. https://doi.org/10.1038/nrn.2016.167

Poldrack, R. A., & Gorgolewski, K. J. (2014). Making big data open: data sharing in neuroimaging. *Nature Neuroscience, 17*(11), 1510-1517. https://doi.org/10.1038/nn.3818

Poldrack, R. A., Mumford, J. A., & Nichols, T. E. (2011). *Handbook of Functional MRI Data Analysis*. Cambridge University Press.

Poline, J. B., Breeze, J. L., Ghosh, S., Gorgolewski, K., Halchenko, Y. O., Hanke, M., Haselgrove, C., Helmer, K. G., Keator, D. B., Marcus, D. S., Poldrack, R. A., Schwartz, Y., Ashburner, J., & Kennedy, D. N. (2012). Data sharing in neuroimaging research. *Frontiers in Neuroinformatics, 6*, 9. https://doi.org/10.3389/fninf.2012.00009

Posner, M. I., Rothbart, M. K., Sheese, B. E., & Tang, Y. (2007). The anterior cingulate gyrus and the mechanism of self-regulation. *Cognitive, Affective, & Behavioral Neuroscience, 7*(4), 391-395. https://doi.org/10.3758/cabn.7.4.391

Poulton, R., Moffitt, T. E., Harrington, H., Milne, B. J., & Caspi, A. (2001). Persistence and perceived consequences of cannabis use and dependence among young adults: Implications for policy. *N Z Medical Journal, 114*(1145), 544-547.

Priddy, S. E., Howard, M. O., Hanley, A. W., Riquino, M. R., Friberg-Felsted, K., & Garland, E. L. (2018). Mindfulness meditation in the treatment of substance use disorders and preventing future relapse: Neurocognitive mechanisms and clinical implications. *Substance Abuse and Rehabilitation, 9,* 103-114. https://doi.org/10.2147/sar.S145201

Pujol, J., Blanco-Hinojo, L., Batalla, A., López-Solà, M., Harrison, B. J., Soriano-Mas, C., Crippa, J. A., Fagundo, A. B., Deus, J., de la Torre, R., Nogué, S., Farré, M., Torrens, M., & Martín-Santos, R. (2014). Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. *Journal of Psychiatric Research, 51,* 68–78. https://doi.org/10.1016/j.jpsychires.2013.12.008

Rabany, L., Diefenbach, G. J., Bragdon, L. B., Pittman, B. P., Zertuche, L., Tolin, D. F., Goethe, J. W., & Assaf, M. (2017). Resting-state functional connectivity in generalized anxiety disorder and social anxiety disorder: Evidence for a dimensional approach. *Brain Connect, 7*(5), 289-298. https://doi.org/10.1089/brain.2017.0497

Rabinak, C. A., Peters, C., Marusak, H. A., Ghosh, S., & Phan, K. L. (2018). Effects of acute Delta 9-tetrahydrocannabinol on next-day extinction recall is mediated by post-extinction resting-

state brain dynamics. *Neuropharmacology, 143*, 289-298 https://doi:10.1016/j.neuropharm.2018.10.002

Radwan, M. M., Chandra, S., Gul, S., & ElSohly, M. A. (2021). Cannabinoids, phenolics, terpenes and alkaloids of cannabis. *Molecules, 26*(9). https://doi.org/10.3390/molecules26092774

Radwan, M. M., Wanas, A. S., Chandra, S., & ElSohly, M. A. (2017). Natural Cannabinoids of Cannabis and Methods of Analysis. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa L. - Botany and Biotechnology* (pp. 161-182). Springer International Publishing. https://doi.org/10.1007/978-3-319-54564-6_7

Ramadas, E., Lima, M. P., Caetano, T., Lopes, J., & Dixe, M. D. A. (2021). Effectiveness of mindfulness-based relapse prevention in individuals with substance use disorders: A systematic review. *Behavioral Sciences, 11*(10). https://doi.org/10.3390/bs11100133

Rapp, R. C., Xu, J., Carr, C. A., Lane, D. T., Wang, J., & Carlson, R. (2006). Treatment barriers identified by substance abusers assessed at a centralized intake unit. *Journal of Substance Abuse Treatment*, 30(3), 227-235. https://doi.org/10.1016/j.jsat.2006.01.002

Reagan, N. (1986). Just say no. *School Safety*, 3, 4-5.

Ren, G., Zhang, X., Li, Y., Ridout, K., Serrano-Serrano, M. L., Yang, Y., Liu, A., Ravikanth, G., Nawaz, M. A., Mumtaz, A. S., Salamin, N., & Fumagalli, L. (2021). Large-scale whole-genome resequencing unravels the domestication history of Cannabis sativa. *Science Advances, 7*(29). https://doi.org/10.1126/sciadv.abg2286

Riba, J., Valle, M., Sampedro, F., Rodríguez-Pujadas, A., Martínez-Horta, S., Kulisevsky, J., & Rodríguez-Fornells, A. (2015). Telling true from false: Cannabis users show increased susceptibility to false memories. *Molecular Psychiatry*, *20*(6), 772-777. https://doi.org/10.1038/mp.2015.36

Rolls, E. T. (2019). The cingulate cortex and limbic systems for emotion, action, and memory. *Brain Structure and Function, 224*(9), 3001-3018. https://doi.org/10.1007/s00429-019-01945-2

Rosenbaum, D. M., Rasmussen, S. G., & Kobilka, B. K. (2009). The structure and function of G-protein-coupled receptors. *Nature, 459*(7245), 356-363. https://doi.org/10.1038/nature08144

Rossetti, M. G., Mackey, S., Patalay, P., Allen, N. B., Batalla, A., Bellani, M., Chye, Y., Conrod, P., Cousijn, J., Garavan, H., Goudriaan, A. E., Hester, R., Martin-Santos, R., Solowij, N., Suo, C., Thompson, P. M., Yucel, M., Brambilla, P., & Lorenzetti, V. (2021). Sex and dependence related neuroanatomical differences in regular cannabis users: findings from the ENIGMA Addiction Working Group. *Translational Psychiatry, 11*(1), 272. https://doi.org/10.1038/s41398-021-01382-y

Rössler, W., Hengartner, M. P., Angst, J., & Ajdacic-Gross, V. (2012). Linking substance use with symptoms of subclinical psychosis in a community cohort over 30 years. *Addiction, 107*(6), 1174-1184. https://doi.org/10.1111/j.1360-0443.2011.03760.x

Russo, E. B., Jiang, H. E., Li, X., Sutton, A., Carboni, A., del Bianco, F., Mandolino, G., Potter, D. J., Zhao, Y. X., Bera, S., Zhang, Y. B., Lu, E. G., Ferguson, D. K., Hueber, F., Zhao, L. C., Liu, C. J., Wang, Y. F., & Li, C. S. (2008). Phytochemical and genetic analyses of ancient cannabis from Central Asia. *Journal of Experimental Botonny, 59*(15), 4171-4182. https://doi.org/10.1093/jxb/ern260

Sadaka, A. H., Canuel, J., Febo, M., Johnson, C. T., Bradshaw, H. B., Ortiz, R., Ciumo, F., Kulkarni, P., Gitcho, M. A., & Ferris, C. F. (2023). Effects of inhaled cannabis high in Δ9-THC or CBD on the aging brain: A translational MRI and behavioral study. *Frontiers in Aging Neuroscience, 15,* 1055433. https://doi.org/10.3389/fnagi.2023.1055433

Sancho, M., De Gracia, M., Rodriguez, R. C., Mallorqui-Bague, N., Sanchez-Gonzalez, J., Trujols, J., Sanchez, I., Jimenez-Murcia, S., & Menchon, J. M. (2018). Mindfulness-Based Interventions for the treatment of substance and behavioral addictions: A systematic review. *Frontiers in Psychiatry, 9,* 95. https://doi.org/10.3389/fpsyt.2018.00095

Sanelli, P. C., Schaefer, P. W., & Loevner, L. A. (Eds.). (2016). *Neuroimaging: The essentials.* Wolters Kluwer.

Santarnecchi, E., Egiziano, E., D'Arista, S., Gardi, C., Romanella, S. M., Mencarelli, L., Rossi, S., Reda, M., & Rossi, A. (2021). Mindfulness-based stress reduction training modulates striatal and cerebellar connectivity. *Journal of Neuroscience Research, 99*(5), 1236-1252. https://doi.org/10.1002/jnr.24798

Sarvet, A. L., Wall, M. M., Keyes, K. M., Cerdá, M., Schulenberg, J. E., O'Malley, P. M., Johnston, L. D., & Hasin, D. S. (2018). Recent rapid decrease in adolescents' perception that marijuana is harmful, but no concurrent increase in use. *Drug and Alcohol Dependence, 186,* 68-74. https://doi.org/10.1016/j.drugalcdep.2017.12.041

Sawyer, S. M., Azzopardi, P. S., Wickremarathne, D., & Patton, G. C. (2018). The age of adolescence. *The Lancet Child & Adolescent Health, 2,* 223-228 https://doi.org/10.1016/S2352-4642(18)30022-1

Schoeler, T., Monk, A., Sami, M. B., Klamerus, E., Foglia, E., Brown, R., Camuri, G., Altamura, A. C., Murray, R., & Bhattacharyya, S. (2016). Continued versus discontinued cannabis use in patients with psychosis: A systematic review and meta-analysis. *Lancet Psychiatry, 3*(3), 215-225. https://doi.org/10.1016/S2215-0366(15)00363-6

Schrantee, A., Ferguson, B., Stoffers, D., Booij, J., Rombouts, S., & Reneman, L. (2016). Effects of dexamphetamine-induced dopamine release on resting-state network connectivity in recreational amphetamine users and healthy controls. *Brain Imaging and Behavior, 10*(2), 548-558. https://doi.org/10.1007/s11682-015-9419-z

Schreiner, A. M., & Dunn, M. E. (2012). Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: A meta-analysis. *Experimental and Clinical Psychopharmacology, 20*(5), 420-429. https://doi.org/10.1037/a0029117

Schultes, R. E., Klein, W. M., Plowman, T., & Lockwood, T. E. (1975). Cannabis: An example of taxonomic neglect. *Cannabis andCculture,* 21-38. https://doi.org/10.1515/9783110812060

Scott, J. C. (2023). Impact of Adolescent Cannabis Use on Neurocognitive and Brain Development. *Child and Adolescent Psychiatric Clinics of North America, 32* (1), 21-42. https://doi.org/10.1016/j.chc.2022.06.002

Scott, J. C., Slomiak, S. T., Jones, J. D., Rosen, A. F. G., Moore, T. M., & Gur, R. C. (2018). Association of cannabis with cognitive functioning in adolescents and young adults: A systematic review and meta-analysis. *JAMA Psychiatry, 75*(6), 585-595. https://doi.org/10.1001/jamapsychiatry.2018.0335

Sehl, H., Terrett, G., Greenwood, L. M., Kowalczyk, M., Thomson, H., Poudel, G., Manning, V., & Lorenzetti, V. (2021). Patterns of brain function associated with cannabis cue-reactivity in regular cannabis users: A systematic review of fMRI studies. *Psychopharmacology (Berl), 238*(10), 2709-2728. https://doi.org/10.1007/s00213-021-05973-x

Sella, F., Raz, G., & Cohen Kadosh, R. (2021). When randomisation is not good enough: Matching groups in intervention studies. *Psychonomic Bulletin & Review, 28*, 2085-2093.

Serfaty, S., Gale, G., Beadman, M., Froeliger, B., & Kamboj, S. K. (2018). Mindfulness, acceptance and defusion strategies in smokers: A systematic review of laboratory studies. *Mindfulness (NY), 9*(1), 44-58. https://doi.org/10.1007/s12671-017-0767-1

Sezer, I., Pizzagalli, D. A., & Sacchet, M. D. (2022). Resting-state fMRI functional connectivity and mindfulness in clinical and non-clinical contexts: A review and synthesis. *Neuroscience Biobehavior Reviews, 135*, 104583. https://doi.org/10.1016/j.neubiorev.2022.104583

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry, 59*(20), 22-33.

Sheehan, D., Janavs, J., Baker, R., Sheehan, K., Knapp, E., & Sheehan, M. (2015). Mini international neuropsychiatric interview–version 7.0.0 DSM-5. 2014.

Shollenbarger, S., Thomas, A. M., Wade, N. E., Gruber, S. A., Tapert, S. F., Filbey, F. M., & Lisdahl, K. M. (2019). Intrinsic frontolimbic connectivity and mood symptoms in young adult cannabis users. *Frontiers in Public Health, 7*, 1-12 https://doi:10.3389/fpubh.2019.00311

Slavet, J. D., Stein, L. A., Colby, S. M., Barnett, N. P., Monti, P. M., Golembeske, C., Jr., & Lebeau-Craven, R. (2006). The Marijuana Ladder: Measuring motivation to change marijuana use in incarcerated adolescents. *Drug and Alcohol Dependence*, *83*(1), 42-48. https://doi.org/10.1016/j.drugalcdep.2005.10.007

Small, E., & Beckstead, H. D. (1973). Cannabinoid phenotypes in Cannabis sativa. *Nature, 245*(5421), 147-148. https://doi.org/10.1038/245147a0

Small, E., & Cronquist, A. (1976). A practical and natural taxonomy for cannabis. *Taxon, 25*(4), 405-435. https://doi.org/10.2307/1220524

Small, E., & Marcus, D. (2002). Hemp: A new crop with new uses for North America. *Trends in New Crops and New Uses, 24*(5), 284-326.

Smith, B., Metzker, K., Waite, R., & Gerrity, P. (2015). Short-form mindfulness-based stress reduction reduces anxiety and improves health-related quality of life in an inner-city population. *Holist Nursing Practice, 29*(2), 70-77. https://doi.org/10.1097/hnp.0000000000000075

Smitha, K. A., Akhil Raja, K., Arun, K. M., Rajesh, P. G., Thomas, B., Kapilamoorthy, T. R., & Kesavadas, C. (2017). Resting state fMRI: A review on methods in resting state connectivity analysis and resting state networks. *Neuroradiology Journal, 30*(4), 305-317. https://doi.org/10.1177/1971400917697342

Sneider, J. T., Gruber, S. A., Rogowska, J., Silveri, M. M., & Yurgelun-Todd, D. A. (2013). A preliminary study of functional brain activation among marijuana users during performance of a virtual water maze task. *Journal of Addiction, 2013*, 461029. https://doi.org/10.1155/2013/461029

Sobell, L. C., & Sobell, M. B. (1992). Timeline Follow-Back. In R. Z. Litten & J. P. Allen (Eds.), *Measuring Alcohol Consumption: Psychosocial and Biochemical Methods* (pp. 41-72). Humana Press. https://doi.org/10.1007/978-1-4612-0357-5_3

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: A review. *Curr Drug Abuse Rev, 1*(1), 81-98. https://doi.org/10.2174/1874473710801010081

Solowij, N., Stephens, R. S., Roffman, R. A., Babor, T., Kadden, R., Miller, M., Christiansen, K., McRee, B., Vendetti, J., & Marijuana Treatment Project Research, G. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA, 287*(9), 1123-1131. https://doi.org/10.1001/jama.287.9.1123

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P., & Jacobs, G. (1983). Manual for the state-trait anxiety scale. *Consulting Psychologists.*

Spisak, T., Bingel, U., & Wager, T. D. (2023). Multivariate BWAS can be replicable with moderate sample sizes. *Nature, 615*(7951), E4–E7. https://doi.org/10.1038/s41586-023-05745-x

Stephens, R. S., Roffman, R. A., & Curtin, L. (2000). Comparison of extended versus brief treatments for marijuana use. *Journal of consulting and clinical psychology, 68*, 898.

Stinson, F. S., Ruan, W. J., Pickering, R., & Grant, B. F. (2006). Cannabis use disorders in the USA: Prevalence, correlates and co-morbidity. *Psychological Medicine, 36*(10), 1447-1460. https://doi.org/10.1017/S0033291706008361

Subramaniam, P., Rogowska, J., DiMuzio, J., Lopez-Larson, M., McGlade, E., & Yurgelun-Todd, D. (2018). Orbitofrontal connectivity is associated with depression and anxiety in marijuana-using adolescents. *Journal of Affective Disorders, 239*, 234-241. https://doi.org/https://doi.org/10.1016/j.jad.2018.07.002

Substance Abuse and Mental Health Services Administration. (2014). Results from the 2013 National Survey on Drug Use: Summary of National Findings. *NSDUH Series H-48, HHS Publication No. (SMA) 14-4863*, 1-143.

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

Sullivan, E. V., & Pfefferbaum, A. (2019). Brain-behavior relations and effects of aging and common comorbidities in alcohol use disorder: A review. *Neuropsychology, 33*(6), 760-780. https://doi.org/10.1037/neu0000557

Sweigert, J., Pagulayan, K., Greco, G., Blake, M., Larimer, M., & Kleinhans, N. M. (2019). A multimodal investigation of cerebellar integrity associated with high-risk cannabis use. *Addiction Biology 25*(6), e12839. https://doi.org/10.1111/adb.12839

Szasz, T. (2003). *Ceremonial chemistry: The ritual persecution of drugs, addicts, and pushers.* Syracuse University Press.

Sznitman, S. R., & Bretteville-Jensen, A. L. (2015). Public opinion and medical cannabis policies: Examining the role of underlying beliefs and national medical cannabis policies. *Harm Reduction Journal,* 12, 46. https://doi.org/10.1186/s12954-015-0082-x

Szucs, D., & Ioannidis, J. P. (2020). Sample size evolution in neuroimaging research: An evaluation of highly-cited studies (1990-2012) and of latest practices (2017-2018) in high-impact journals. *Neuroimage, 221,* 117164. https://doi.org/10.1016/j.neuroimage.2020.117164

Taebi, A., Becker, B., Klugah-Brown, B., Roecher, E., Biswal, B., Zweerings, J., & Mathiak, K. (2022). Shared network-level functional alterations across substance use disorders: A multi-level kernel density meta-analysis of resting-state functional connectivity studies. *Addiction Biology, 27*(4), e13200. https://doi.org/10.1111/adb.13200

Tan, L. B., Lo, B. C., & Macrae, C. N. (2014). Brief mindfulness meditation improves mental state attribution and empathizing. *PLoS One, 9*(10), e110510. https://doi.org/10.1371/journal.pone.0110510

Tanda, G., & Goldberg, S. R. (2003). Cannabinoids: Reward, dependence, and underlying neurochemical mechanisms: A review of recent preclinical data. *Psychopharmacology (Berl), 169*(2), 115-134. https://doi.org/10.1007/s00213-003-1485-z

Tang, Y. Y., Hölzel, B. K., & Posner, M. I. (2015). The neuroscience of mindfulness meditation. *Nature Reviews Neuroscience, 16*(4), 213-225. https://doi.org/10.1038/nrn3916

Tang, Y.-Y., Tang, R., & Posner, M. I. (2013). Brief meditation training induces smoking reduction. *Proceedings of the National Academy of Sciences, 110*(34), 13971-13975. https://doi.org/doi:10.1073/pnas.1311887110

Taylor, S., Landry, C. A., Paluszek, M. M., Fergus, T. A., McKay, D., & Asmundson, G. J. G. (2020). Development and initial validation of the COVID Stress Scales. *Journal of Anxiety Disorders, 72,* 102232. https://doi.org/10.1016/j.janxdis.2020.102232

Termenon, M., Jaillard, A., Delon-Martin, C., & Achard, S. (2016). Reliability of graph analysis of resting state fMRI using test-retest dataset from the Human Connectome Project. *Neuroimage, 142,* 172-187. https://doi.org/10.1016/j.neuroimage.2016.05.062

Tervo-Clemmens, B., Simmonds, D., Calabro, F. J., Day, N. L., Richardson, G. A., & Luna, B. (2018). Adolescent cannabis use and brain systems supporting adult working memory encoding, maintenance, and retrieval. *Neuroimage, 169,* 496-509. https://doi.org/10.1016/j.neuroimage.2017.12.041

Thijssen, S., Rashid, B., Gopal, S., Nyalakanti, P., Calhoun, V. D., & Kiehl, K. A. (2017). Regular cannabis and alcohol use is associated with resting-state time course power spectra in incarcerated adolescents. *Drug & Alcohol Dependence, 17,* 492-500 https://doi:10.1016/j.drugalcdep.2017.05.045

Thomson, H., Labuschagne, I., Greenwood, L. M., Robinson, E., Sehl, H., Suo, C., & Lorenzetti, V. (2022). Is resting-state functional connectivity altered in regular cannabis users? A systematic review of the literature. *Psychopharmacology (Berl), 239*(5), 1191-1209. https://doi.org/10.1007/s00213-021-05938-0

Thomson, H., Suo, C., Labuschagne, I., & Lorenzetti, V. (*in preparation*). Investigating resting-state functional connectivity differences between people with a moderate-to-severe cannabis use disorder and controls: An fMRI study.

Tolomeo, S., & Yu, R. (2022). Brain network dysfunctions in addiction: a meta-analysis of resting-state functional connectivity. *Translational Psychiatry, 12*(1), 41. https://doi.org/10.1038/s41398-022-01792-6

Tomasi, D., & Volkow, N. D. (2010). Functional connectivity density mapping. *Proceedings of the National Academy of Sciences, 107,* 9885 https://doi:10.1073/pnas.1001414107

Twomey, C. D. (2017). Association of cannabis use with the development of elevated anxiety symptoms in the general population: A meta-analysis. *Journal of Epidemiological Community Health, 71*(8), 811-816. https://doi.org/10.1136/jech-2016-208145

United Nations Office on Drugs and Crime [UNODC]. (2019). *World Drug Report* (United Nations publication, Sales No. E.19.XI.8), Issue. https://www.unodc.org/unodc/en/data-and-analysis/wdr2021.html

United Nations Office on Drugs and Crime [UNODC]. (2021). *World Drug Report* (United Nations publication, Sales No. E.20.XI.6), Issue. https://www.unodc.org/unodc/en/data-and-analysis/wdr2021.html

United Nations Office on Drugs and Crime, UNODC (2020). *World Drug Report*. (United Nations publication, Sales No. E.20.XI.6). https://wdr.unodc.org/wdr2020/

Vaghi, M. M., Vértes, P. E., Kitzbichler, M. G., Apergis-Schoute, A. M., van der Flier, F. E., Fineberg, N. A., Sule, A., Zaman, R., Voon, V., Kundu, P., Bullmore, E. T., & Robbins, T. W. (2017). Specific frontostriatal circuits for impaired cognitive flexibility and goal-directed planning in obsessive-compulsive disorder: Evidence from resting-state functional connectivity. *Biological Psychiatry, 81*(8), 708-717. https://doi.org/10.1016/j.biopsych.2016.08.009

van 't Veer, A. E., & Giner-Sorolla, R. (2016). Pre-registration in social psychology: A discussion and suggested template. *Journal of Experimental Social Psychology, 67*, 2-12. https://doi.org/https://doi.org/10.1016/j.jesp.2016.03.004

van de Ven, V. G., Formisano, E., Prvulovic, D., Roeder, C. H., & Linden, D. E. (2004). Functional connectivity as revealed by spatial independent component analysis of fMRI measurements during rest. *Human Brain Mapping, 22*(3), 165-178. https://doi.org/10.1002/hbm.20022

van den Heuvel, M. P., Stam, C. J., Boersma, M., & Hulshoff Pol, H. E. (2008). Small-world and scale-free organization of voxel-based resting-state functional connectivity in the human brain. *NeuroImage, 43*, 528-539 https://doi.org/10.1016/j.neuroimage.2008.08.010

van den Heuvel, M. P., Mandl, R. C., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Human Brain Mapping, 30*(10), 3127-3141. https://doi.org/10.1002/hbm.20737

van der Pol, P., Liebregts, N., de Graaf, R., Korf, D. J., van den Brink, W., & van Laar, M. (2013). Facilitators and barriers in treatment seeking for cannabis dependence. *Drug and Alcohol Dependence, 133*(2), 776-780. https://doi.org/10.1016/j.drugalcdep.2013.08.011

van der Pol, P., Liebregts, N., de Graaf, R., Ten Have, M., Korf, D. J., van den Brink, W., & van Laar, M. (2013). Mental health differences between frequent cannabis users with and without dependence and the general population. *Addiction, 108*(8), 1459-1469. https://doi.org/10.1111/add.12196

van Hell, H. H., Vink, M., Ossewaarde, L., Jager, G., Kahn, R. S., & Ramsey, N. F. (2010). Chronic effects of cannabis use on the human reward system: An fMRI study. *European Neuropsychopharmacology, 20*(3), 153-163. https://doi.org/10.1016/j.euroneuro.2009.11.010

van Ours, J. C., Williams, J., Fergusson, D., & Horwood, L. J. (2013). Cannabis use and suicidal ideation. *J Health Econ, 32*(3), 524-537. https://doi.org/10.1016/j.jhealeco.2013.02.002

Vargas, C., Lopez-Jaramillo, C., & Vieta, E. (2013). A systematic literature review of resting state network-functional MRI in bipolar disorder. *Journal of Affective Disorders*, *150*(3), 727-735. https://doi.org/10.1016/j.jad.2013.05.083

Veale, J. F. (2014). Edinburgh Handedness Inventory - Short Form: A revised version based on confirmatory factor analysis. *Laterality*, *19*(2), 164-177. https://doi.org/10.1080/1357650X.2013.783045

Verdejo-Garcia, A., Lorenzetti, V., Manning, V., Piercy, H., Bruno, R., Hester, R., Pennington, D., Tolomeo, S., Arunogiri, S., Bates, M. E., Bowden-Jones, H., Campanella, S., Daughters, S. B., Kouimtsidis, C., Lubman, D. I., Meyerhoff, D. J., Ralph, A., Rezapour, T., Tavakoli, H., . . . Ekhtiari, H. (2019). A roadmap for integrating neuroscience into addiction treatment: A consensus of the neuroscience interest group of the international society of addiction medicine. *Frontiers in Psychiatry, 10*, 877. https://doi.org/10.3389/fpsyt.2019.00877

Volkow, N. D., & Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. *Cerebral Cortex, 10*(3), 318-325. https://doi.org/10.1093/cercor/10.3.318

Volkow, N. D., & Morales, M. (2015). The brain on drugs: From reward to addiction. *Cell, 162*(4), 712-725. https://doi.org/10.1016/j.cell.2015.07.046

Volkow, N. D., Baler, R. D., Compton, W. M., Weiss, S. R. B. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine, 370*, 2219-2227 https://doi:10.1056/NEJMra1402309

Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine, 370*(23), 2219-2227. https://doi.org/10.1056/NEJMra1402309

Volkow, N. D., Koob, G. F., & McLellan, A. T. (2016). Neurobiologic Advances from the Brain Disease Model of Addiction. *New England Journal of Medicine, 374*(4), 363-371. https://doi.org/10.1056/NEJMra1511480

Volkow, N. D., Michaelides, M., & Baler, R. (2019). The neuroscience of drug reward and addiction. *Physiol Rev, 99*(4), 2115-2140. https://doi.org/10.1152/physrev.00014.2018

Volkow, N. D., Swanson, J. M., Evins, A. E., DeLisi, L. E., Meier, M. H., Gonzalez, R., Bloomfield, M. A., Curran, H. V., & Baler, R. (2016). Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: A review. *JAMA Psychiatry, 73*(3), 292-297. https://doi.org/10.1001/jamapsychiatry.2015.3278

Wall, M. B., Freeman, T. P., Hindocha, C., Demetriou, L., Ertl, N., Freeman, A. M., Jones, A. P., Lawn, W., Pope, R., Mokrysz, C., Solomons, D., Statton, B., Walker, H. R., Yamamori, Y., Yang, Z., Yim, J. L., Nutt, D. J., Howes, O. D., Curran, H. V., & Bloomfield, M. A. (2022). Individual and combined effects of cannabidiol and Delta(9)-tetrahydrocannabinol on striato-cortical connectivity in the human brain. *Journal of Psychopharmacology, 36*(6), 732-744. https://doi.org/10.1177/02698811221092506

Wall, M. B., Pope, R., Freeman, T. P., Kowalczyk, O. S., Demetriou, L., Mokrysz, C., Hindocha, C., Lawn, W., Bloomfield, M. A., Freeman, A. M., Feilding, A., Nutt, D., & Curran, H. V. (2019). Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity. *Journal of Psychopharmacology, 33*(7), 822-830. https://doi.org/10.1177/0269881119841568

Walpola, I. C., Nest, T., Roseman, L., Erritzoe, D., Feilding, A., Nutt, D. J., & Carhart-Harris, R. L. (2017). Altered insula connectivity under MDMA. *Neuropsychopharmacology, 42*, 2152-2162 https://doi:10.1038/npp.2017.35

Wanden-Berghe, R. G., Sanz-Valero, J., & Wanden-Berghe, C. (2011). The application of mindfulness to eating disorders treatment: A systematic review. *Eat Disorders. The Journal of Tretment & Prevention, 19*(1), 34-48. https://doi.org/10.1080/10640266.2011.533604

Wang, L., Hermens, D. F., Hickie, I. B., & Lagopoulos, J. (2012). A systematic review of resting-state functional-MRI studies in major depression. *Journal of Affective Disorders, 142*(1-3), 6-12. https://doi.org/10.1016/j.jad.2012.04.013

Wang, S., Zhang, M., Liu, S., Xu, Y., Shao, Z., Chen, L., Li, J., Yang, W., Liu, J., & Yuan, K. (2021). Impulsivity in heroin-dependent individuals: structural and functional abnormalities within frontostriatal circuits. *Brain Imaging and Behavior, 15*(5), 2454-2463. https://doi.org/10.1007/s11682-020-00445-w

Wang, Y., Yan, K. J., Fan, C. X., Luo, X. N., & Zhou, Y. (2019). Altered functional connectivity of the nucleus accumbens subdivisions in amphetamine-type stimulant abusers: A resting-state fMRI study. *BMC Neuroscience, 20*(1), 66. https://doi.org/10.1186/s12868-019-0548-y

Watts, G. (2006). Cannabis confusions. *BMJ, 332*(7534), 175-176. https://doi.org/10.1136/bmj.332.7534.175

Wechsler, D. (2011). *Wechsler Abbreviated Scale of Intelligence – Second Edition* (WASI-II). San Antonio, TX: Pearson.

Wei, J., Wei, S., Yang, R., Yang, L., Yin, Q., Li, H., Qin, Y., Lei, Y., Qin, C., Tang, J., Luo, S., & Guo, W. (2018). Voxel-mirrored homotopic connectivity of resting-state functional magnetic resonance imaging in blepharospasm. *Frontiers in Psychology, 9*, 1620. https://doi.org/10.3389/fpsyg.2018.01620

Westbrook, C., Creswell, J. D., Tabibnia, G., Julson, E., Kober, H., & Tindle, H. A. (2011). Mindful attention reduces neural and self-reported cue-induced craving in smokers. *Social Cognitive and Affective Neuroscience, 8*(1), 73-84. https://doi.org/10.1093/scan/nsr076

Wetherill, R. R., Fang, Z., Jagannathan, K., Childress, A. R., Rao, H., & Franklin, T. R. (2015). Cannabis, cigarettes, and their co-occurring use: Disentangling differences in default mode network functional connectivity. *Drug And Alcohol Dependence, 153*, 116-123. https://doi.org/10.1016/j.drugalcdep.2015.05.046

Whetton, S., Tait, R. J., Chrzanowska, A., Donnelly, N., McEntee, A., Mukhtar, S. A., Zahra, E., Campbell, G., Degenhardt, L., Dey, T., Halim, S. A., Hall, W., Makate, M., Norman, R., Peacock, A., Roche, A., & Allsop, S. (2020). *Quantifying the Social Costs of Cannabis Use to Australia in 2015/16.* National Drug Research Institute. https://ndri.curtin.edu.au/publications-resources/publication-search#search

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity*, *2*(3), 125-141. https://doi.org/10.1089/brain.2012.0073

Wilkinson, S. T., Yarnell, S., Radhakrishnan, R., Ball, S. A., & D'Souza, D. C. (2016). Marijuana legalization: Impact on physicians and public health. *Annual Review of Medicine, 67*, 453-466. https://doi.org/10.1146/annurev-med-050214-013454

Willems, T., & Henke, K. (2021). Imaging human engrams using 7 Tesla magnetic resonance imaging. *Hippocampus, 31*(12), 1257-1270. https://doi.org/10.1002/hipo.23391

Wise, R. A. (1996). Neurobiology of addiction. *Current Opinion Neurobiology*, *6*(2), 243-251. https://doi.org/10.1016/s0959-4388(96)80079-1

Witkiewitz, K., Lustyk, M. K. B., & Bowen, S. (2013). Retraining the addicted brain: A review of hypothesized neurobiological mechanisms of mindfulness-based relapse prevention. *Psychology of Addiction Behaviour, 27*(2), 351-365. https://doi.org/10.1037/a0029258

Wolters, A. F., van de Weijer, S. C. F., Leentjens, A. F. G., Duits, A. A., Jacobs, H. I. L., & Kuijf, M. L. (2019). "Resting-state fMRI in Parkinson's disease patients with cognitive impairment: A meta-analysis": Answer to Wang and colleagues. *Parkinsonism Related Disorders, 66*, 253-254. https://doi.org/10.1016/j.parkreldis.2019.07.014

World Health Organisation [WHO]. (2016). *The health and social effects of nonmedical cannabis use.* World Health Organization. https://apps.who.int/iris/handle/10665/251056

World Health Organisation [WHO]. (2018). *ICD-11 International classification of diseases for mortality and morbidity statistics.* https://icd.who.int/browse11/l-m/en

Wright, A. C., Cather, C., Farabaugh, A., Terechina, O., Pedrelli, P., Nyer, M., Fava, M., & Holt, D. J. (2021). Relationship between cannabis use and psychotic experiences in college students. *Schizophrenia Research, 231,* 198-204. https://doi.org/10.1016/j.schres.2021.04.004

Wu, J. (2019). Cannabis, cannabinoid receptors, and endocannabinoid system: yesterday, today, and tomorrow. *Acta Pharmacologica Sinica, 40*(3), 297-299. https://doi.org/10.1038/s41401-019-0210-3

Wu, L. T., Zhu, H., Mannelli, P., & Swartz, M. S. (2017). Prevalence and correlates of treatment utilization among adults with cannabis use disorder in the United States. *Drug and Alcohol Dependence, 177,* 153-162. https://doi.org/10.1016/j.drugalcdep.2017.03.037

Yanes, J. A., Riedel, M. C., Ray, K. L., Kirkland, A. E., Bird, R. T., Boeving, E. R., Reid, M. A., Gonzalez, R., Robinson, J. L., Laird, A. R., & Sutherland, M. T. (2018). Neuroimaging meta-analysis of cannabis use studies reveals convergent functional alterations in brain regions supporting cognitive control and reward processing. *Journal of Psychopharmacology, 32*(3), 283-295. https://doi.org/10.1177/0269881117744995

Yang, C. C., Barros-Loscertales, A., Pinazo, D., Ventura-Campos, N., Borchardt, V., Bustamante, J. C., Rodriguez-Pujadas, A., Fuentes-Claramonte, P., Balaguer, R., Avila, C., & Walter, M. (2016). State and training effects of mindfulness meditation on brain networks reflect neuronal mechanisms of its antidepressant effect. *Neural Plasticity, 2016,* 9504642. https://doi.org/10.1155/2016/9504642

Yin, H. H., & Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience, 7*(6), 464-476. https://doi.org/10.1038/nrn1919

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European Journal of Neuroscience, 19*(1), 181-189. https://doi.org/10.1111/j.1460-9568.2004.03095.x

Yip, S. W., DeVito, E. E., Kober, H., Worhunsky, P. D., Carroll, K. M., & Potenza, M. N. (2014). Pretreatment measures of brain structure and reward-processing brain function in cannabis

dependence: An exploratory study of relationships with abstinence during behavioral treatment. *Drug and Alcohol Dependence, 140*, 33-41. https://doi.org/10.1016/j.drugalcdep.2014.03.031

Yücel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., & Lubman, D. I. (2008). Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry, 65*(6), 694-701. https://doi.org/10.1001/archpsyc.65.6.694

Zakiniaeiz, Y., Scheinost, D., Seo, D., Sinha, R., & Constable, R. T. (2017). Cingulate cortex functional connectivity predicts future relapse in alcohol dependent individuals. *NeuroImage Clinical, 13*, 181-187. https://doi.org/10.1016/j.nicl.2016.10.019

Zehra, A., Burns, J., Liu, C. K., Manza, P., Wiers, C. E., Volkow, N. D., & Wang, G. J. (2018). Cannabis addiction and the brain: A review. *Journal of Neuroimmune Pharmacology, 13*(4), 438-452. https://doi.org/10.1007/s11481-018-9782-9

Zgierska, A., Rabago, D., Chawla, N., Kushner, K., Koehler, R., & Marlatt, A. (2009). Mindfulness meditation for substance use disorders: A systematic review. *Substance Abuse, 30*(4), 266-294. https://doi.org/10.1080/08897070903250019

Zhang, D., Lee, E. K. P., Mak, E. C. W., Ho, C. Y., & Wong, S. Y. S. (2021). Mindfulness-based interventions: An overall review. *British Medical Buletinl, 138*(1), 41-57. https://doi.org/10.1093/bmb/ldab005

Zhang, R., & Volkow, N. D. (2019). Brain default-mode network dysfunction in addiction. *Neuroimage, 200*, 313-331. https://doi.org/10.1016/j.neuroimage.2019.06.036

Zhang, S., & Li, C. R. (2018). Ventral striatal dysfunction in cocaine dependence: Difference mapping for subregional resting state functional connectivity. *Translational Psychiatry, 8*(1), 119. https://doi.org/10.1038/s41398-018-0164-0

Zhou, F., Zimmermann, K., Xin, F., Scheele, D., Dau, W., Banger, M., Weber, B., Hurlemann, R., Kendrick, K. M., & Becker, B. (2018). Shifted balance of dorsal versus ventral striatal communication with frontal reward and regulatory regions in cannabis-dependent males. *Human Brain Mapping, 39*(12), 5062-5073. https://doi.org/10.1002/hbm.24345

Zilverstand, A., Huang, A. S., Alia-Klein, N., & Goldstein, R. Z. (2018). Neuroimaging impaired response inhibition and salience attribution in human drug addiction: A systematic review. *Neuron, 98*(5), 886-903. https://doi.org/10.1016/j.neuron.2018.03.048

Zimmermann, K., Yao, S., Heinz, M., Zhou, F., Dau, W., Banger, M., Weber, B., Hurlemann, R., & Becker, B. (2018). Altered orbitofrontal activity and dorsal striatal connectivity during emotion processing in dependent marijuana users after 28 days of abstinence. *Psychopharmacology (Berl), 235*(3), 849-859. https://doi.org/10.1007/s00213-017-4803-6

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., Wang, Y. F., & Zang, Y. F. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: Fractional ALFF. *Journal of Neuroscience Methods, 172*(1), 137–141. https://doi:10.1016/j.jneumeth.2008.04.012

Zou, S., & Kumar, U. (2018). cannabinoid receptors and the endocannabinoid system: Signalling and function in the central nervous system. *International Journal of Molecular Sciences, 19*(3). https://doi.org/10.3390/ijms19030833

Zuardi, A. W. (2006). History of cannabis as a medicine: A review. *Brazilian Journal of Psychiatry, 28*(2), 153-157. https://doi.org/10.1590/s1516-44462006000200015

Zuo, X. N., Kelly, C., Di Martino, A., Mennes, M., Margulies, D. S., Bangaru, S., Grzadzinski, R., Evans, A. C., Zang, Y. F., Castellanos, F. X., & Milham, M. P. (2010). Growing together and growing apart: regional and sex differences in the lifespan developmental trajectories of functional homotopy. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience, 30*(45), 15034–15043. https://doi.org/10.1523/JNEUROSCI.2612-10.2010

Appendices

Appendix 1. Permission to Reprint Diagnostic Criteria for Cannabis Use Disorder

AMERICAN PSYCHIATRIC ASSOCIATION PUBLISHING

800 Maine Avenue, S.W. Suite 900
Washington, DC 20024
Phone (202) 559-3900

22 February 2023

Hannah Thomson, Australian Catholic University
115 Victoria Parade
Fitzroy, Victoria
Australia 3065
Permission Request #PL19528

hannah.thomson@myacu.edu.au

Dear Hannah,

I am responding to your request to reproduce the items listed below in Investigating resting state functional connectivity differences in cannabis use disorder and exploring their mitigation through brief mindfulness based intervention, presented by Australian Catholic University:

From Diagnostic and Statistical Manual of Mental Disorders, DSM-5, fifth edition, diagnostic criteria for Cannabis Use Disorder

One-time and non-exclusive permission is granted under the following conditions:

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Sincerely,

Nicole Wilder
Permissions Coordinator

Appendix 2. PROSPERO Registration Document

2020 CRD420220181355

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

1. * Review title.

Give the working title of the review, for example the one used for obtaining funding. Ideally the title should state succinctly the interventions or exposures being reviewed and the associated health or social problems. Where appropriate, the title should use the PI(E)COS structure to contain information on the Participants, Intervention (or Exposure) and Comparison groups, the Outcomes to be measured and Study designs to be included.

A systematic review of resting-state functional connectivity differences between cannabis users compared to controls

2. Original language title.

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3. * Anticipated or actual start date.

Give the date when the systematic review commenced, or is expected to commence.

11/03/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

18/12/2020

5. * Stage of review at time of this submission.

Indicate the stage of progress of the review by ticking the relevant Started and Completed boxes. Additional information may be added in the free text box provided.
Please note: Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. Should evidence of incorrect status and/or completion date being supplied at the time of submission come to light, the content of the PROSPERO record will be removed leaving only the title and named contact details and a statement that inaccuracies in the stage of the review date had been identified.
This field should be updated when any amendments are made to a published record and on completion and publication of the review. If this field was pre-populated from the initial screening questions then you are not able to edit it until the record is published.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here (e.g. Funded proposal, protocol not yet finalised).

6. * Named contact.

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

Hannah Thomson

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Hannah

7. * Named contact email.

Give the electronic mail address of the named contact.

hannah.thomson@myacu.edu.au

8. Named contact address

Give the full postal address for the named contact.

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Australian Catholic University

Organisation web address:

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country are now mandatory fields for each person.**

Miss Hannah Thomson. Australian Catholic University
Dr Valentina Lorenzetti. Australian Catholic University
Dr Izelle Labuschagne. Australian Catholic University
Ms Alexandra Gorelik. Australian Catholic University Ms Hannah Sehl. Australian Catholic University

12. * Funding sources/sponsors.

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Include any unique identification numbers assigned to the review by the individuals or bodies listed. Not applicable

Grant number(s)

13. * Conflicts of interest.

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country are now mandatory fields for each person.**

15. * Review question.

State the question(s) to be addressed by the review, clearly and precisely. Review questions may be specific or broad. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS where relevant.

Cannabis is currently the most commonly used and arguably the most frequently debated illicit drug in Australia and globally, with about 188 million people worldwide reporting use (United Nations Office on Drugs and Crime, 2019). Heavy cannabis use is associated with adverse psychosocial and mental health outcomes (APA, 2013). Emerging evidence shows that cannabis users versus controls have different restingstate functional connectivity, particularly between prefrontal and striatal pathways implicated in inhibitory control, craving and reward processing, all of which are altered in addiction (Weinstein, Livny & Weizman, 2016). No study to date however has systematically synthesised the literature of differences between restingstate functional connectivity in cannabis users compared to controls, and the association between functional connectivity differences, and behavioural outcomes (e.g. cannabis use, psychopathology symptom scores, and cognitive performance) and cannabinoid levels from toxicology analysis (e.g. THC, CBD). This systematic

review aims to synthesise the current body of literature examining resting-state functional connectivity in cannabis users compared to controls, to determine whether different patterns of functional connectivity exist between groups (i.e. strength, direction, and location). Furthermore, we aim to explore whether there is an association between differences in functional connectivity and cannabis use (e.g. frequency of use, severity of craving), psychopathology symptom scores (e.g. depression, anxiety and psychosis), cognitive performance (e.g. attentional bias, impulsivity and working memory) and cannabinoid levels from toxicology analysis (e.g. THC, CBD).

Note: If the resultant studies uncovered during the systematic review permit, data will be extracted for meta-analysis.

16. * Searches.

State the sources that will be searched. Give the search dates, and any restrictions (e.g. language or publication period). Do NOT enter the full search strategy (it may be provided as a link or attachment.) The following electronic databases will be searched: CINAHL, MEDLINE, PubMed, PsycINFO, Scopus, Web of Science, Embase, and Cochranes. The reference lists of eligible studies will also be searched. Search terms include: (cannabis OR marijuana OR hashish OR THC OR tetrahydrocannabinol) AND (“resting state” OR “resting-state” OR “at rest” OR resting) AND (“connect*” OR “funct*”) AND ("Magnetic Resonance Imaging" OR MRI OR "functional Magnetic Resonance Imaging" OR fMRI OR BOLD OR "Blood Oxygen Level Dependent") Databases searches were conducted on 11/03/2020. Duplicates will be removed.

17. URL to search strategy.

Give a link to a published pdf/word document detailing either the search strategy or an example of a search strategy for a specific database if available (including the keywords that will be used in the search strategies), or upload your search strategy.Do NOT provide links to your search results.

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

This systematic review will cover two domains: (1) to identify resting-state functional connectivity differences in cannabis users compared to controls, and (2) to explore for cannabis users, whether there is an association between altered functional connectivity and levels of cannabis use (e.g. frequency of use, severity of craving), psychopathology symptom scores (e.g. depression, anxiety and psychosis), cognitive performance (e.g. attentional bias, impulsivity and working memory), and cannabinoid levels from toxicology analysis (e.g. THC, CBD).

19. * Participants/population.

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

(1)**The targeted** be regular cannabis users (as defined in each study), **participants will:**

(2) of any age.**Participants will be excluded if they:**(1) meet diagnostic criteria for substance use disorders

(2)other than Cannabis Use Disorder and/or Tobacco Us meet diagnostic criteria for severe mental health, neurological or neurodevelopmental disorders (e.g. Dis rder,

Attention Deficit Hyperactive Disorder, Schizophrenia, Post Traumatic Stress Disorder, Multiple Sclerosis).

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the nature of the interventions or the exposures to be reviewed. None

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria. controls

22. * Types of study to be included.

Give details of the types of study (study designs) eligible for inclusion in the review. If there are no restrictions on the types of study design eligible for inclusion, or certain study types are excluded, this should be stated. The preferred format includes details of both inclusion and exclusion criteria.

Studies will be included if they meet the following eligibility criteria:

(1) use human participants, include regular cannabis using participants (as defined in each study) compared to controls,

(2) measure functional connectivity using resting-state fMRI,

(3) are written in English, and

(4) are peer reviewed.

Studies will be excluded that:

(1) measure brain integrity using imaging techniques other than fMRI (e.g. EEG, CT, PET, SPECT, structural neuroimaging),

(2) are non-peer reviewed, non-published, or non-empirical studies (e.g. dissertations, corrigendums, editorials, single case-reports, book chapters, conference abstracts only),

(3) are reviews or meta-analyses of the literature, and

(4) examine a primary drug of use other than cannabis (e.g. cocaine, methamphetamines).

23. Context.

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

Identification of differences in resting-state functional connectivity between cannabis users and controls.

* Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Differences between groups (cannabis users versus controls) in resting-state functional connectivity (strength, location and direction).

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state ‘None’ or ‘Not applicable’ as appropriate to the review

In cannabis users, associations between altered functional connectivity and levels of cannabis use (e.g. frequency of use, severity of craving), psychopathology symptom scores (e.g. depression, anxiety and psychosis), cognitive performance (e.g. attentional bias, impulsivity and working memory), and cannabinoid levels from toxicology analysis (e.g. THC, CBD).

* Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

The direction (e.g., positive or negative) and the strength of the associations (e.g., Pearson's R, Spearman's rho, regression coefficient) between altered functional connectivity and levels of cannabis use (e.g. frequency of use, severity of craving), psychopathology symptom scores (e.g. depression, anxiety and psychosis), cognitive performance (e.g. attentional bias, impulsivity and working memory), and cannabinoid levels from toxicology analysis (e.g. THC, CBD) within the cannabis using group.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Study selection will follow PRISMA guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). To determine which studies will be included, two reviewers (blinded to each other's decisions) will screen the titles and abstracts and then the resultant full-text articles that have not been excluded. Any discrepancies during the review process will be resolved through discussion between the two reviewers under the supervision of a senior staff member. Inter-rater reliability will be calculated.
The data which will be extracted from the selected papers is outlined below, grouped by the categories; study characteristics, participant characteristics, MRI related variables, and results.

Study characteristics - First author.

- Year of publication.
- Recruitment strategy.

Participant characteristics

- Sample size
- Age
- Sex
- Handedness
- Cannabis use (e.g., dosage, duration, age of onset, frequency/occasions, abstinence duration)
- Cannabis use disorder/dependence (e.g., tool used, presence/absence, level)
- Cannabinoid level (e.g., THC, CBD) and specimen (e.g., urine, saliva, hair, breath)
- Other substance use (e.g., tobacco, alcohol)
- Psychopathology levels (e.g., symptom scores for depression, anxiety, psychosis)
- Recruitment location and strategy
- Treatment status

MRI related variables:

- Scanner strength and brand

- Head coil (number of channels)
- fMRI data acquisition protocol
- fMRI task characteristics (e.g. duration, eyes open or closed)
- Functional connectivity analysis method (e.g., whole brain, ROI-based, seed-based, details of relevant regions)
- Thresholding

Results:

- Group differences in patterns of brain function (location, direction)
- Brain-behaviour associations within cannabis users (location, direction)

One individual will extract data (by recording it in an excel spreadsheet) and a second individual (trained member of the research team) will quality check the extracted data. Any disagreements between individual judgements will be resolved via discussion. In cases of missing data, study investigators will be contacted for unreported data or additional details.

Meta-analysis: Strength (e.g., Beta/t), location (e.g., ROI/seed based, voxel coordinates, nodes and edges, low frequency spontaneous fluctuations), and direction.

27. * Risk of bias (quality) assessment.

Describe the method of assessing risk of bias or quality assessment. State which characteristics of the studies will be assessed and any formal risk of bias tools that will be used.

In observation of PRISMA guidelines, we will use the Cochrane Collaboration's tool for examining study bias.

This tool assesses bias (high risk, low risk, or unclear risk) across seven domains which will be conducted where applicable. Assessment will be done at both study and outcome levels. One individual will assess risk of bias and a second individual (trained member of the research team) will review outcomes. Any disagreements between individual judgements will be resolved via discussion.

28. * Strategy for data synthesis.

Provide details of the planned synthesis including a rationale for the methods selected. This **must not be generic text** but should be **specific to your review** and describe how the proposed analysis will be applied to your data.

As part of the systematic review, a summary of the findings from all included studies will be presented in tables and/or figures. Data extracted and measures of effect (as listed above) will be reported for the main and secondary outcomes.

Results will be meta-analysed if there is enough power (i.e., a sufficient number of studies) using relevant toolboxes e.g. the Matlab toolbox; multilevel kernel density analysis (MKDA) toolbox (http://wagerlab.colorado.edu).

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach. Subject to data availability, sub-analyses will be performed on associations between resting state functional connectivity and key variables (e.g., sex, age, cannabis use levels, hours of abstinence).

30. * Type and method of review.

Select the type of review and the review method from the lists below. Select the health area(s) of interest for your review.

Type of review

Cost effectiveness
No

Diagnostic
No

Epidemiologic
No

Individual patient data (IPD) meta-analysis
No

Intervention
No

Meta-analysis
Yes

Methodology
No

Narrative synthesis
No

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews

No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of review

Alcohol/substance misuse/abuse
Yes

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

Child health
No

Complementary therapies

No

COVID-19
No

Crime and justice
No

Dental
No

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest

No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing
No

Obstetrics and gynaecology
No

Oral health
No

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
No

Rehabilitation No Respiratory disorders
No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological

No

Wounds, injuries and accidents
No

Violence and abuse
No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error. English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Australia

33. Other registration details.

Give the name of any organisation where the systematic review title or protocol is registered (such as with The Campbell Collaboration, or The Joanna Briggs Institute) together with any unique identification number assigned. (N.B. Registration details for Cochrane protocols will be automatically entered). If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

Give the citation and link for the published protocol, if there is one

Give the link to the published protocol.

Alternatively, upload your published protocol to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

Do you intend to publish the review on completion?

Yes

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords will help users find the review in the Register (the words do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

cannabis; marijuana; resting state; functional connectivity; mri; fmri; magnetic resonance imaging; functional magnetic resonance imaging

37. Details of any existing review of the same topic by the same authors.

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38. * Current review status.

Review status should be updated when the review is completed and when it is published. For newregistrations the review must be Ongoing. Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information the review team feel is relevant to the registration of the review.

40. Details of final report/publication(s) or preprints if available.

This field should be left empty until details of the completed review are available OR you have a link to a preprint.

Give the link to the published review.

Appendix 3. Authorship Statement of Contribution by Others

The following statement of contribution is made regarding Chapter 2: Systematic Literature Review (Study 1) of this thesis, which was published as:

Thomson, H., Labuschagne, I., Greenwood, L. M., Robinson, E., Sehl, H., Suo, C., & Lorenzetti, V. (2022). Is resting-state functional connectivity altered in regular cannabis users? A systematic review of the literature. *Psychopharmacology (Berl)*, *239*(5), 1191-1209. https://doi.org/10.1007/s00213-021-05938-0

First author: Hannah Thomson
I acknowledge that my contribution to the above paper is 70%.
Extent of contribution: H.T. was involved in the conceptualisation of the work, performed literature searches, article screening, data extraction and checking, synthesised the data for reporting, wrote manuscript drafts, and finalised the manuscript for publication.

Signature: 
Date: 27/02/2023

Second author: Izelle Labuschagne
I acknowledge that my contribution to the above paper is 5%.
Extent of contribution: I.L. provided feedback on manuscript drafts.

Signature: 
Date: 18/02/2023

Third author: Lisa Greenwood
I acknowledge that my contribution to the above paper is 4%.
Extent of contribution: L.G. provided feedback on manuscript drafts.

Signature: 
Date: 19/02/2023

Fourth author: Emily Robinson
I acknowledge that my contribution to the above paper is 3%.
Extent of contribution: E.R. contributed to the synthesis of data and its subsequent visual representation in a figure.

Signature: 
Date: 19/02/2023

Fifth author: Hannah Sehl
I acknowledge that my contribution to the above paper is 3%.
Extent of contribution: H.S. performed re-screening of search results to ensure accuracy of included/excluded articles.

Signature: 
Date: 21/02/2023

Sixth author: Chao Suo
I acknowledge that my contribution to the above paper is 5%.
Extent of contribution: C.S. provided feedback on manuscript drafts, assisted with the synthesis of rsFC results and its subsequent visual representation in a figure.

Signature: 
Date: 17/02/2023

Last author: Valentina Lorenzetti
I acknowledge that my contribution to the above paper is 10%.
Extent of contribution: V.L. contributed significantly to the conceptualisation of the work, discussion of ideas and revisions, data analysis and provided comments and edits on all manuscript drafts.

Signature: 
Date: 12/02/2023

Appendix 4. ISRCTN Registration

Study Information

Submission date: 28/04/2020

Registration date: 12/05/2020

Last edited: 26/04/2022

ISRCTN

Mapping short-term brain changes in cannabis users: An fMRI study

HYPOTHESES

Brain function will be assessed during rest, and during fMRI tasks including (i) a cue reactivity fMRI task that involves exposure to cannabis pictures and carefully matched neutral pictures (Cousijn, Goudriaan, Ridderinkhof, van den Brink, Veltman, & Wiers, 2013), (ii) a monetary incentive delay fMRI task (van Hell, Vink, Ossewaarde, Jager, Kahn & Ramsey, 2010), and (iii) an avoidance learning fMRI task (Kim, Shimojo, & O'Doherty, 2006).

It is hypothesized that:

1. People with a moderate-to-severe cannabis use disorder (CUD) compared to non-cannabis using controls, will show altered structure (e.g. volumes and thickness) and function (e.g. activity and connectivity) within brain pathways ascribed to addiction-relevant cognitive processes, including:
   1.1 reward processing (e.g. striatum, orbitofrontal cortex),
   1.2 stress/negative affect (e.g. amygdala),
   1.3 cognitive control (e.g. parietal cortex, dorsolateral prefrontal cortex, cerebellum),
   1.4 learning and memory (e.g. hippocampus), and
   1.5 interoception (e.g. insula).
2. Brain function will change in brain pathways regions implicated in:
   2.1 reward processing, cognitive control and interoception, pre-to-post a brief ~2-week mindfulness-based intervention, which targets cannabis craving compared to no intervention, as shown in early work examining normative samples (Fox et al., 2016; Reese, Zielinski, & Veilleux, 2015).
   2.2 stress and interoception, pre-to-post a brief, ~2-week active placebo-controlled relaxation intervention, compared to no intervention, as shown by emerging work investigating normative samples (Sevinc et al., 2018).
3. We will explore the association between changes in measures of brain integrity and level of cannabis use severity, psychopathology symptom scores (e.g. depression, anxiety and psychotic-like experiences) and cognitive performance (e.g. attentional bias, impulsivity and working memory).

DOUBLE-BLIND PROCEDURE

The study includes "blinded" and "unblinded" testers, with distinct roles described below.

1. Selected researchers will administer face-to-face clinical and cognitive assessment, and MRI to the participant, without knowing which intervention condition cannabis users have been allocated to. These researchers will be referred to as "blinded" testers.
2. Selected researchers will be unblinded to each CUD participant's allocation to the three intervention conditions. These will be referred to as "unblinded" testers.

Unblinded testers will not administer any testing other than the intervention. Specifically, "unblinded" testers will:

1.1 allocate CUD participants to one of the three distinct intervention conditions in a pseudo-randomised fashion. This is to ensure group matching for age and sex across all three intervention conditions and the non-using control group, and for the number of CUD symptoms at baseline across the three intervention conditions.
1.2 administer the intervention at baseline and follow up face-to-face assessments.
1.3 administer scales immediately before and after the intervention at baseline and follow up face-to-face assessments, to monitor its effectiveness.
1.4 give participants information and material relevant to the online practice of the intervention.
1.5 monitor the participant's completion of the online daily intervention for the 2-week intervention period (e.g. VAS scales and/or audio tracks).
1.6 communicate with the participants about any issues during the intervention period.
1.7 debrief the participants on the intervention.

INTERVENTION

1. INTERVENTION CONDITIONS

There will be three intervention conditions, all of which will be accessible via online weblinks in Qualtrics:

1.1 A 2-week mindfulness-based intervention, consisting of a guided mindfulness audio track and VAS scales (e.g. stress, anxiety, substance use levels on the day of completion; see OUTCOMES section 2 below for detailed explanation of measures).
1.2 A 2-week active placebo-controlled relaxation-based intervention, consisting of a guided relaxation audio track and VAS scales (e.g. stress, anxiety, substance use levels on the day of completion).
1.3 A 2-week passive placebo no intervention consisting of VAS scales only (e.g. stress, anxiety, substance use levels on the day of completion).

Note: Non-cannabis using controls will not be administered an intervention. This group will undergo only the baseline face-to-face assessment, which will be identical to that of cannabis users.

2. ADMINISTRATION OF THE INTERVENTION

The allocated intervention (i.e. VAS scales and/or audio tracks) will be administered in three different phases outlined below.

2.1 PHASE I (BASELINE FACE-TO-FACE ASSESSMENT)

The first delivery of the intervention will occur at the end of the baseline face-to-face assessment. An unblinded tester will run this component of the assessment, which will include:

2.1.1 VAS and Toronto Scale (administered pre- and post-intervention), and the Credibility/Manipulation Check (administered post-intervention). See OUTCOMES section 2 below for detailed explanation of measures.
2.1.2 Audio track with the content of the intervention. The unblinded tester will start the track (i.e. press play) so the participant will hear the audio track via headphones connected to a laptop.
2.1.3 The first audio track will encapsulate 4 parts:
2.1.3.1. Part 1: A 30-second introduction. This explains the aim of the intervention. This part is identical for both the mindfulness and the relaxation intervention conditions;
2.1.3.2. Part 2: A 3-minute explanation of the psychological strategy that they will be asked to practice;

2.1.3.3. Part 3: A 4-minute preliminary experiential practice;
2.1.3.4. Part 4: The 7-minute "main" track that encapsulates the intervention that the participant will be asked to practice daily (either mindfulness or relaxation). The word 'mindfulness' will not be mentioned in either intervention to minimise expectancy effects.

2.1.4 During the first delivery of the intervention at baseline face-to-face testing, assessment of credibility and expectance will be run using The Credibility/Expectancy Questionnaire (CEQ; Devilly & Borkovec, 2000). These are described in detail in the section 'Secondary Outcome Measures – Mindfulness and Interventions Measures'.

2.1.5 At the conclusion of the first delivery of the intervention, an unblinded tester will:

2.1.5.1 SMS the participant with the online web-link to access the intervention in order to complete it at home

2.1.5.2 give the participant a USB stick with back-up files necessary to practice the intervention (i.e. VAS scales in a word document, and/or MP4 audio tracks), to facilitate compliance of people with limited access to online data.

2.2 PHASE 2 (ONLINE, OFF-SITE DAILY INTERVENTION)

The participant will be required to practice the intervention (using the online link or the USB files) daily offsite for ~2-weeks, between the baseline and the follow up face-to-face testing.

The allocated intervention will consist of the VAS scales (the sole component in the "no intervention condition"), followed by 7-minute long audio tracks (i.e. described in bullet-point 2.1.4 above) for either the mindfulness or relaxation intervention condition.

An unblinded tester will measure compliance via monitoring the participant's daily completion of the intervention, through the study's online Qualtrics server.

2.3 PHASE 3 (FOLLOW UP FACE-TO-FACE ASSESSMENT)

The final delivery of the intervention will occur at the start of the follow up face-to-face assessment (immediately after informed consent). This is in order to boost the ~2-week intervention effect on the outcomes of interest at follow up. An unblinded tester will run this component of the assessment, which will include:

2.3.1 VAS and Toronto Scale (administered pre- and post-intervention). See OUTCOMES section 2 below for detailed explanation of measures

2.3.2 Audio track with the content of the intervention. The unblinded tester will start the track (i.e. press play) so the participant will hear the audio track via headphones connected to a laptop. The intervention will be the 7-minute track as used across the previous 2-weeks and at baseline (see 2.1.3.4).

Audio-tracks containing the interventions will be made available to all participants after the completion of the study.

3. INTERVENTION SCRIPTS

3.1 The scripts used for the mindfulness and relaxation intervention conditions have the following characteristics:

3.1.1 They do not contain the word 'mindfulness', to mitigate expectancy effects

3.1.2 They rely on already establish scripts used for delivering a similar intervention in hazardous drinkers, which was published by Co-Investigators Prof Sunjeev Kamboj and Dr Tom Freeman (PMID: 29016995).

3.1.3 They are delivered on high-quality audio tracks, which were read and recorded by Tamblyn Lord, who is a qualified mindfulness instructor with >20 years of experience, is the voice of the Smiling Mind application, and is a career voice artist/actor.

3.1.4 They are matched by the following parameters: length (15 minutes for the first delivery at baseline, and 7-minutes for subsequent deliveries during the intervention and at follow up), number of smoking- and craving-related words, language complexity (Flesch-Kincaid grade level 8), key words relating to craving and cannabis, sequence of components and readability scores.

3.1.5 They are matched by number of words for the mindfulness intervention i.e. 1,779 words. These include 946 words for the baseline assessment audio track and 833 words for subsequent at home intervention and follow up assessment audio tracks.

3.1.6 They are matched by number of words for the relaxation intervention: 1,783 words. These include 949 words for the baseline assessment audio tracks and 834 words for subsequent at home intervention and follow up assessment audio tracks.

3.2 Example phrases used for the interventions:

3.2.1 Relaxation script: During the explanation of the intervention, the participant is instructed that craving intensity can be reduced by "softening the muscles...and calming and unwinding the mind…releasing tension in your body" and that relaxation enables transformation of sensations into more calming, less unpleasant experiences. It is also emphasized that this is a way of gaining control over craving.

3.2.2 Mindfulness script: By contrast, instructions for the mindfulness script did not include any mention of reduced "craving or of controlling, transforming, or regulating internal experience. It was clarified that the aim was not to simply relax, but to be alert and attentive. The emphasis was on "open monitoring" of experience and particularly on "aware[ness] of feelings and bodily sensations" and to "experience craving in a different way." The participant was told that by noticing bodily sensations they could "experience them as temporary events in the body," helping the participant to "tolerate [bodily sensations] without acting on them." To minimize expectancy effects relating to the increasing popularity and public discussion of complementary medicine approaches, there was no mention of the term "mindfulness" (or "relaxation") in any experimental or recruitment material.

OUTCOMES

The study outcomes have been grouped as (1) primary outcome measures, and (2) secondary outcome measures. These are described below.

1. Primary outcome measures

Structural and functional brain outcomes will be measured using Magnetic Resonance Imaging (MRI) at baseline and follow up.

1.1 Brain structure will be measured by assessing the volumes and thickness of the hypothesised brain regions of interest (see HYPOTHESES section 1.1-1.5 above for details).

1.2 Brain function will be measured while performing a number of fMRI tasks outlined below:

1.2.1 A Cue reactivity fMRI task (10 minutes) will be run to examine brain function when the participant views cannabis-related pictures versus matched neutral pictures.
There are two versions of this task, which are identical in procedure but contain different pictures (matched for picture complexity, object size, colours, and brightness) in order to minimise the confounding impact of memory and recognition on cue reactivity. The two task versions are delivered in counter balanced order at baseline and follow up assessment, via pseudorandomised procedure.

1.2.2 A Monetary Incentive Delay fMRI task (15 minutes) will be run to investigate brain function while:
1.2.2.1 anticipation (vs receipt) of monetary outcomes;

1.2.2.2 anticipation of monetary outcomes (vs neutral outcomes);
1.2.2.3 receipt (vs anticipation) of monetary outcomes;
1.2.2.4 receipt of neutral outcomes (vs monetary outcomes)

1.2.3 An Avoidance Learning fMRI task (15 minutes) will be run to measure brain function while:
1.2.3.1 anticipating rewards and losses,
1.2.3.2 learning to avoid losses and obtain rewards.

1.2.4 A resting state fMRI task (10 minutes) will be run to investigate functional connectivity during rest (eyes open, while looking at a fixation cross).

2. Secondary outcome measures

Measures on substance use and related problems, mood and personality, mindfulness and wellbeing (e.g. sleep, physical activity) will be used as descriptive variables, covariates, or moderators to interpret the study results. These are grouped in pattern of administration and key domains below.

2.1 REPEATED MEASURES OF CRAVING, ANXIETY AND OTHER PSYCHOLOGICAL STATES THROUGHOUT THE FACE-TO-FACE BASELINE AND FOLLOW UP ASSESSMENTS.
These measures are delivered online via Qualtrics

2.1.1 Changes to cannabis craving, relaxation, tension, and mindful attention level:

2.1.1.1 The Visual Analogue Scale (VAS) will be used to measure on a 1-to-10 point scale current levels of cannabis craving, relaxation, tension, and mindful attention.
The number of VAS administrations will vary according to which group the participant is allocation to. Cannabis users allocated to the mindfulness or relaxation intervention group will complete five administrations of the VAS (I-V outlined below), cannabis users allocated to the no-intervention group will complete four administrations of the VAS (I-IV outlined below), and non-using controls will complete three administrations of the VAS (I-III outlined below).
(I) immediately pre-MRI scan,
(II) during the MRI scan, immediately before the cue reactivity fMRI task (see 1.2.1 above),
(III) during the MRI scan, immediately after the cue post cue reactivity fMRI task,
(IV) immediately before the delivery of the audio intervention,
(V) immediately after the delivery of the audio intervention.

2.1.1.2 A single item from the VAS will be used to measure on a 1-to-10 point scale the participant's current level of cannabis craving.
This will be administered twice:
(I) immediately pre-attentional bias dot probe task (see 2.4.1.1 below)
(II) immediately post-attentional bias dot probe task

2.1.2 Changes to state anxiety and cannabis craving symptom scores pre-to-post the MRI scan:

2.1.2.1 The Marijuana Craving Questionnaire (MCQ; Heishman et al., 2009). It has 45-items rated on a seven-point Likert-type scale ranging from "strongly disagree" to "strongly agree."
The items relate to four distinct constructs: (1) compulsivity e.g. inability to control marijuana use; (2) emotionality, e.g. use of marijuana in anticipation of relief from withdrawal or negative mood; (3) expectancy, e.g. anticipation of positive outcomes from using marijuana; and (4) purposefulness, e.g. intention and planning to use marijuana for positive outcomes.

2.1.2.2 The State Anxiety Subscale of the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). It has 20 items rated on a 4-point scale (e.g., from 1 = “Almost Never” to 4 = “Almost Always”).

2.1.3 Changes to state mindfulness levels before and after the mindfulness and relaxation audio interventions:
NOTE: Not completed by cannabis users allocated to the no intervention group or non-using controls.

2.1.3.1 The Toronto Mindfulness Scale (TMS; Lau et al., 2006). It has 42-items rated on a 5-point scale Likert scale from 0 = “Not At All” to 4 = “Very Much”. It measures "state-like" experiences during meditation.

2.1.3.2 State Mindfulness Scale (SMS; Tanay & Bernstein, 2013). It has 23-items rated on a 5-point Likert scale ranging from 1 = “Not At All” to 5 = “Very Well”. It measures state mindfulness of both mind and body.

2.2 SUBSTANCE USE AND RELATED PROBLEMS

2.2.1 Semi-structured interviews (online and printed), administered at baseline only:

2.2.1.1 The Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV). The SCID-5-RV (First, Williams, Karg, & Spitzer, 2015) is an 11-item semi-structured interview that measures cannabis dependence according to specific DSM-5 criteria for CUD. This will be used to confirm a diagnosis of moderate-to-severe CUD in cannabis users.

2.2.1.2 Cannabis Use Interview (CUI) measures lifetime cannabis exposure. The CUI is adapted from the Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory (Cuttler & Spradlin, 2017). It has been previously utilised for the testing of cannabis users in research settings (Solowij et al., 2011).

2.2.2 Self-report online questionnaires (exception of the TLFB, completed face-to-face), administered at baseline and follow up:

2.2.2.1 The Timeline Follow Back (TLFB; Sobell & Sobell, 1992). The TLFB is administered in a paper- calendar-based format. It is a researcher administered semi structured interview, to gather retrospective estimates of number of days of substance use and quantity of use over the previous 30 days (at baseline testing) or ~ 2-weeks (at follow up testing). We will additionally collect information about the type, amount and strength of the cannabis use.

2.2.2.2 The Cannabis Withdrawal Scale (CWS; Allsop, Norberg, Copeland, Fu, & Budney, 2011). It has 19-items rated on a 10-point scale from ‘Not at all’ to ‘Extremely’. The CWS is used in clinical and research settings to measure how cannabis withdrawal symptoms affect daily activities.

2.2.2.3 The Cannabis Use Identification Test-Revised (CUDIT-R; Adamson et al., 2010). It has 8-items rated on a 5-point Likert scale. It is a screening tool as it has diagnostic cut-offs for the DSM-5 CUD severity, validated with clinical and normative samples.

2.2.2.4 The Obsessive Compulsive Drug Use Scale – Cannabis (OCDUS; Dekker et al., 2012). It has 12-items rated on a 5-point Likert scale. It measures compulsive cannabis use.

2.2.2.5 Fagerström Test for Nicotine Dependence (FTND; Fagerstrom, Russ, Yu, Yunis, & Foulds, 2012). It has 8-items rated on yes/no and Liker scales. It measures the severity of physical dependence to nicotine related to cigarette smoking.

2.2.2.6 One item on cannabis use to sleep i.e. “In the past two weeks have you used cannabis to help you sleep?”.

2.2.3 Self-report online questionnaires, administered at baseline only:

2.2.3.1 The Marijuana Motives Questionnaire (MMQ; Lee, Neighbors, Hendershot, & Grossbard, 2009). It assesses motivation of marijuana use and related consequences. It has 25-items rated on a 4-point Likert scale from 'Never/Almost never' to 'Almost always/Always'.

2.2.3.2 The Alcohol Use Disorders Identification Test (AUDIT; Babor, Higgins-Biddle, Saunders, & Monteiro, 2001). It has 10-items. The AUDIT is screening tool developed by the World Health Organization. It assesses alcohol use and the level of hazardous drinking.

2.2.3.3 The Credibility/Expectancy Questionnaire (CEQ; Devilly & Borkovec, 2000). It has 6-items rated on a Likert scale. It measures: the momentary belief that the received therapy will help to reduce anxiety; what the participant thinks will happen and what the participant feels will happens a result of the intervention.

2.3 MINDFULNESS AND INTERVENTION-RELATED MEASURES

These include self-report online questionnaires, administered both at baseline and follow up assessment:

2.3.1 The Five Facet Mindfulness Questionnaire (5FMQ; Baer et al., 2008). This scale has 39-items, rated on a 5-point Likert scale. Items relate to 5 factors: (1) observing (2) describing (3) acting with awareness (4) non-judging of inner experience (5) non-reactivity to inner experience.

2.3.2 Motivation to Stop Scale (MSS; Kotz, Brown, & West 2013). It has 1-item, which is rated on a 7-point Likert scale, which reflects desire and intention to stop substance use.

2.3.3 The Credibility/Manipulation Check (CMC; Kamboj et al., 2017). It has 9 intervention specific items, which assess the participant's compliance to the intervention and comprehension of the intervention.

2.3.4 Debrief / task feedback.
It consists of 19 open and closed questions regarding the participants experience completing the daily tasks and if applicable, audio tracks.
NOTE: This is completed at follow up only.

2.4 COGNITIVE PERFORMANCE MEASURES

2.4.1 Cognitive performance will be assessed via computerised cognitive tasks (administered at baseline and follow up):

2.4.1.1 A 'dot probe' task (Morgan et al., 2010), will be used to measure attentional bias towards cannabis-related pictures and pictures matched for composition. There are two identical versions of this task, delivered in counter balanced order at baseline and follow up assessment, via pseudorandomised procedure. The two task versions are identical in procedure, but contain different pictures (matched for picture complexity, object size, colours, and brightness) to minimise the confounding impact of memory and recognition on attentional bias.

2.4.1.2 A '2, 3, & 4-N-back task' (Jaeggi et al., 2010), will be run to assess working memory. Participants are shown a sequence of visual stimulus on a computer and must respond each time the current stimulus is identical to the one presented 'n' positions back in the sequence

2.4.1.3 A 'Go/No-Go task' (Fillmore, Rush, & Hays, 2006), will be run to test response inhibition. Participants are shown cues on a computer; the cues provide preliminary information regarding the type of target (i.e. go or stop) that is likely to follow. The cues have a high probability of signalling the correct target, to which the participant must response. The response time and accuracy of the participant is measured.

2.4.2 IQ, will be assessed at baseline only

2.4.2.1 The Wechsler Abbreviated Scales of Intelligence, 2nd edition (WASI-II; Wechsler, 2011) is a short form standardised measure of intellectual ability. It provides an estimate of

full-scale IQ using the two-subtest administration consisting of the Vocabulary and Matrix Reasoning subtests.

2.5 MENTAL HEALTH AND WELLBEING MEASURES

2.5.1 Self-report online questionnaires, administered at baseline only:

2.5.1.1 The 36 Item Short Form Survey Instrument (SF-36; Ware, Sherbourne, & Davies, 1992). Items are rated on yes/no and Likert scale responses. It is a set of generic, coherent, and easily administered items measuring quality-of-life. It is widely utilised by managed care organizations and by Medicare for routine monitoring and assessment of care outcomes in adult patients.

2.5.1.2 Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002). It has 42 items rating the frequency (rated on a 4-point scale: Never, Sometimes, Often, Nearly Always) and distress (rated on a 4-point scale: Not distressed, A bit distressed, Quite distressed, Very distressed) of positive and negative psychotic symptoms.

2.5.2 Self-report online questionnaires, administered at baseline and follow up:

2.5.2.1 The Emotion Regulation Questionnaire (ERQ; Gross & John, 2003). It has 10-items rated on a 7-point Likert-type scale ranging from 1 (strongly disagree) to 7 (strongly agree). It measures the tendency to regulate emotions via Cognitive Reappraisal and Expressive Suppression.

2.5.2.2 Beck's Depression Inventory – 2nd edition (BDI-II; Beck et al., 1996). It has 21-items rated on a 4-point Likert scale. It measures the severity of depression and its total score has diagnostic cut-offs, i.e. 0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression, 29–63: severe depression.

2.5.2.3 The Confidence Ladder (CL; Slavet et al., 2006). This visual scale measures motivation/readiness to change. It has 11 rungs and 5 statements represent stages of change, rated on a scale from 0 (least motivated) to 10 (most motivated).

2.5.2.4 The Apathy Evaluation Scale (AES; Marin, Biedrzycki, & Firinciogullari, 1991). It has 18 items rated on a 3-point Likert scale ranging from 1 (not at all) to 3 (somewhat a lot). It provides global measure of apathy.

2.5.2.5 The Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). It has 10 items rated on a 5-point Likert scale ranging from 0 (never) to 4 (very often). It measures how unpredictable, uncontrollable, stressful and overloaded respondents find their lives.

2.5.2.6 International Physical Activity Questionnaire (short form) (IPAQ; Craig et al., 2003). It has 9 items measuring the frequency and duration of vigorous activity, moderate activity, walking, and sitting over the previous seven days.

2.5.2.7 Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency – Short Form (S-UPPS-P; Cyders, Littlefield, Coffey, & Karyadi, 2014). It has 20 items, rated on a 4-point Likert scale ranging from (1) agree strongly to (4) disagree strongly. It measures 5 distinct domains of impulsivity i.e., Negative Urgency, (lack of) Premeditation, (lack of) Perseverance, Sensation Seeking, and Positive Urgency). Two more second order factors can be extracted i.e. Emotion Based Rash Action (Positive & Negative Urgency) and Deficits in Conscientiousness (Premeditation and Perseverance).

PARTICIPANT ELIGIBILITY

Target number of participants

We aim to recruit N = 120 participants, including: n = 90 moderate-to-severe cannabis users who have tried to cut down or quit in the past 24 months and n= 30 non-cannabis using controls.

1. Participant inclusion criteria

1.1 Inclusion criteria for all participants are:
- 1.1.1 Aged 18 to 55 years
- 1.1.2 Normal-to-corrected vision
- 1.1.3 Fluent in English
- 1.1.4 Meeting safety criteria for MRI scan

1.2 Inclusion criteria for cannabis users are:
- 1.2.1 Daily/almost daily (>3 days per week) cannabis use for >12 months
- 1.2.2 CUD 4+ DSM-5 symptoms
- 1.2.3 Tried to quit/reduce cannabis use at least once within the past 24 months

2. Participant exclusion criteria

2.1 Exclusion criteria for all participants are:
- 2.1.1 Any illicit substance and alcohol use for 12 hours before assessment (confirmed by self- report)
- 2.1.2 Currently using prescription medication that affect the central nervous system
- 2.1.3 Current or past diagnosed psychiatric disorders
- 2.1.4 Any current severe psychiatric diagnosis, excepting diagnoses of depression or anxiety
- 2.1.5 History of any neurological disorders
- 2.1.6 History of acquired or traumatic brain injury
- 2.1.7 Currently pregnant
- 2.1.8 Suicidality

2.2 Exclusion criteria for cannabis users are:
- 2.2.1 Significant use or dependence on alcohol and any illicit substances other than cannabis
- 2.2.2 Illicit drug use past 4 weeks (other than cannabis)

2.3 Exclusion criteria for non-cannabis using controls are:
- 2.3.1 Significant use or dependence on alcohol and any illicit substances
- 2.3.2 Illicit drug use past 4 weeks

3. Selection process

Study advertisement (printed and online flyers) will direct all people interested in participating in the study to an online screening survey. All potential participants will undergo a selection process to determine their eligibility against our study inclusion and exclusion criteria.

This includes a ~ 25-minute online screening survey (detailed in section 3.1), which will be followed up by a phone call to determine study inclusion (explained in section 3.2) and if possible schedule session (described in section 3.3).

3.1 Online screening survey

3.1.1 Socio-demographic, medical and handedness data
- 3.1.1.1 Demographic data (e.g. age, date of birth, English fluency, sex, education, income)
- 3.1.1.2 Pregnancy/breastfeeding status (yes/no)
- 3.1.1.3 Previous experience with psychological strategies such as Mindfulness, Tai Chi, Meditation, Progressive Muscle Relaxation, Mindfulness, Yoga, other
- 3.1.1.4 Lifetime prescription medication (yes/no, type and details)
- 3.1.1.5 Lifetime personal diagnoses of mental health related problem or psychopathology (yes/no, type and details)
- 3.1.1.6 Lifetime diagnoses of mental health disorders in family members (yes/no, type and details)
- 3.1.1.7 Previously seen psychologist/psychiatrist/counsellor or other related therapy type (yes/no, type and details)
- 3.1.1.8 MRI safety Screening Questionnaire (provided by the testing facility Monash Biomedical Imaging Centre) & information regarding the MRI scanning process

3.1.1.9 Edinburgh Handedness Inventory – Short Form (EHI-SF; Veale, 2014). It comprises four tasks (writing, throwing, teeth brushing, using a spoon) and asks the participant to rate their preferred hand (i.e., 'always right', 'usually right', 'both equally', 'usually left', 'always left') for carrying out each task.

3.1.2 Substance Use data:

3.1.2.1 Cannabis Use Identification Test – Revised (CUDIT-R; Adamson et al., 2010). It has 8-items rated on a 5-point Likert scale. It is a screening tool as it has diagnostic cut-offs for the DSM-5 CUD severity, validated with clinical and normative samples.

3.1.2.2 Severity of Dependence Scale (SDS; Gossop et al., 1995). It is a 5-item measure of cannabis dependency.

3.1.2.3 Alcohol Use Identification Test (AUDIT; Babor, Higgins-Biddle, Saunders, & Monteiro, 2001). It has 10-items. This screening tool has been developed by the World Health Organization to assess hazardous drinking.

3.1.3.4 Substance Use History (SUH; adapted from Sobell, Kwan, & Soball, 1995). It is adapted from the Drug History Questionnaire, and contains up to 96 questions depending on the number of substances endorsed.

3.1.3 Mental health data:

3.1.3.1 Mini International Neuropsychiatric Interview 6.0.0 Screen (Lecrubier Sheehan, Hergueta, & Weiller, 1998). It is a standardised measure which includes 24 questions to screen for the 17 most common psychiatric disorders based on DSM-5 criteria. Twelve questions assess the presence of CUD and its severity based on how many criteria apply (1-3 = mild; 4-5 = moderate; 6-11 = severe).

3.1.3.2 Depression, Anxiety, and Stress Scale (DASS; Lovibond & Lovibond 1995). It is a 21-item questionnaire that measures depression, anxiety, and stress. Responses are given via a 5-point Likert scale ranging from 0 (did not apply to me at all) to 4 (applied to me very much, or most of the time).

3.1.3.3 Motivation to Stop Scale (MSS; Kotz, Brown, & West 2013). It has 1 item, which is rated on a 7-point Likert scale, which reflects desire and intention to stop substance use.

3.2 Eligibility of selected participants will be confirmed via a phone call. Any queries about participants' eligibility will be resolved via a discussion with the study CI and the research team.

3.3 All eligible participants will be contacted by phone to schedule an assessment time.

PLAIN ENGLISH SUMMARY

Background and Aims

Cannabis is currently the most commonly used and arguably the most frequently debated illicit drug globally, with about 188 million people worldwide reporting use. A significant portion of cannabis users smoke daily-to-weekly and endorse Cannabis Use Disorder (CUD; as defined by the Diagnostic and Statistical Manual, fifth edition).

Heavy cannabis use is associated with adverse psychosocial and mental health outcomes. This includes cannabis dependence, reduced performance at work, school and some cognitive tasks, engaging in risk-taking behaviour (e.g. smoking while driving), and higher symptoms of mood, anxiety, and psychotic disorders. Worryingly, only ~36% of those experiencing problems with cannabis use seek treatment, and many of those who receive treatment for CUD fail to reduce their use or to quit. Emerging evidence suggests that mindfulness-based strategies that target core features of CUD – such as the experience of craving and withdrawal – may mitigate brain, mental health and cognitive harms associated with CUD.

The first aim of this multimodal MRI study is to map how brain, cognitive performance and mental health differs between people with a CUD (moderate-to-severe) compared to non-using controls. The second aim of this study is to examine how brain, cognitive performance and mental health harms in people with a CUD are mitigated pre-to-post a brief 2-week mindfulness intervention, versus a 2-week active placebo-controlled relaxation intervention and a 2-week no intervention period. The intervention has been successfully tested in hazardous drinkers by Co-Investigators Professor Kamboj and Dr Freeman (please see PMID: 29016995).

Finally, this study will explore how brain alterations in CUD are associated with the level of cannabis use (e.g. dosage, duration of use), psychopathology symptom scores (e.g. depression, anxiety and psychosis), and cognitive performance (e.g. attentional bias, impulsivity and working memory).

Hypotheses

It is hypothesized that:

4. People with a moderate-to-severe cannabis use disorder (CUD) compared to non-cannabis using controls, will show altered structure (e.g. volumes and thickness) and function (e.g. activity and connectivity) within brain pathways ascribed to addiction-relevant cognitive processes, including:
   1.1 reward processing (e.g. striatum, orbitofrontal cortex),
   1.2 stress/negative affect (e.g. amygdala),
   1.3 cognitive control (e.g. parietal cortex, dorsolateral prefrontal cortex, cerebellum),
   1.4 learning and memory (e.g. hippocampus), and
   1.5 interoception (e.g. insula).
5. Brain function will change in brain pathways regions implicated in:
   2.3 reward processing, cognitive control and interoception, pre-to-post a brief ~2-week mindfulness-based intervention, which targets cannabis craving compared to no intervention, as shown in early work examining normative samples (Fox et al., 2016; Reese, Zielinski, & Veilleux, 2015).
   2.4 stress and interoception, pre-to-post a brief, ~2-week active placebo-controlled relaxation intervention, compared to no intervention, as shown by emerging work investigating normative samples (Sevinc et al., 2018).
6. We will explore the association between changes in measures of brain integrity and level of cannabis use severity, psychopathology symptom scores (e.g. depression, anxiety and psychotic-like experiences) and cognitive performance (e.g. attentional bias, impulsivity and working memory).

Research design

A pseudorandomised, double-blind, placebo-controlled design will be used. Ninety frequent cannabis users will be assessed at baseline and 2-week follow up and will be divided into three groups to be allocated to either a 2-week daily mindfulness intervention and brief questionnaires (n = 30), a 2-week daily active placebo controlled relaxation and brief questionnaires (n = 30) and 2-week no intervention period with daily brief questionnaires (n=30). Thirty non-cannabis using controls will be assessed at baseline only for comparative purposes.

Who can participate?

We will recruit 120 participants aged 18-to-55 years from the general community, including 90 frequent cannabis users and 30 non-using controls.

What does the study involve?

Participation includes:

- an online screening questionnaire (~25 minutes) in order to confirm eligibility,
- a phone conversation to further confirm the participant's eligibility and details and schedule assessments,
- two near-identical face-to-face 4-to-5-hour assessments at baseline and ~2-week follow up, comprising psychological questionnaires, computer tasks and a 1-hour MRI scan,
- between baseline and follow up, the participant completes a daily intervention (or no intervention depending on group allocation) and brief questionnaire.
- non-cannabis using control participants will complete the baseline assessment only (no intervention).

What are the possible benefits and risks of participating?

Possible benefits from participating include a potential reduction in cravings for cannabis use and improved mood. The research is considered to be low risk.

Where is the study run from?

Assessments will be run at the Monash Biomedical Imaging facility (MBI). The participant will complete the intervention online, at a location convenient for them.

When is the study starting and how long is it expected to run for?

The approximate start date for the trial is November 2019, data collection is expected to conclude December 2020. The approximate duration of the trial will be 13 months.

Who is funding the study?

The Healthy Brain and Mind Research Centre, Neuroscience of Addiction and Mental health group, within the Australian Catholic University.

Who is the main contact?

Dr Valentina Lorenzetti (Valentina.Lorenzetti@gmail.com)

OVERALL STRUCTURE OF THE ASSESSMENT AND INTERVENTION PROTOCOL

1. THREE MAIN PHASES

The testing protocol comprises three main phases:

- I.1 Face-to-face baseline assessment, ~4 hours (here on referred to as 'baseline assessment')
- I.2 ~2-week daily off-site, online intervention, ~10-15 minutes daily
- I.3 Face-to-face follow up assessment, ~3 hours (~2-weeks post baseline) (here on referred to as 'follow up assessment')

2. ROLES OF BLINDED AND UNBLINDED TESTERS

Both blinded and unblinded testers will be present at the start of the two (baseline and follow up) assessments and will drive distinct part of the assessment. Specifically:

- 2.1 A blinded tester will run all experimental procedures and assessments of socio-demographic variables, substance use, mental health and cognitive performance.
- 2.2 An unblinded tester will administer all *information* specifically pertaining to the intervention (the intervention itself and pre-to-post intervention related scales).
- 2.3 An unblinded tester will be responsible for debrief at baseline and at follow up with queries on intervention and obtaining consent at follow up.
- 2.4 An unblinded tester will be responsible for daily monitoring of the online tasks/intervention (e.g. VAS scales and/or audio tracks) and SMS reminders if these are missed, as well as communicating with the participant about any issues during the intervention period.
- 2.5 During the MRI scan:
  - 2.5.1 A blinded tester will interact with the participant and read scripts relating to the delivery of the assessment
  - 2.5.2 An unblinded tester will support the running of the technical aspects of the MRI that do not require direct interaction with the participant (e.g. open and save relevant fMRI task files and logs, to ensure timely completion of the MRI).

3. OVERVIEW OF BASELINE FACE-TO-FACE ASSESSMENT

- 3.1. First, at the start of the baseline assessment, a blinded tester will ask the participant to review and clarify all study details explained in the Participant Information Letter and to provide written informed consent to participate in the study.
- 3.2. Second, a blinded tester will ask the participant to provide a urine sample to confirm the presence and absence of THC metabolites in cannabis users and non-users, respectively, and the absence of any other drug metabolites.
- 3.3. Then, a blinded tester will administer to the participant a battery of validated cognitive tasks (to assess IQ, attentional bias, working memory, disinhibition), semi-structured interviews and self-report questionnaires (relating to mindfulness, substance use, and mental health); as well as an MRI scan to measure brain structure and function.
- 3.4. Finally, an unblinded tester will administer the intervention (i.e. press play on the intervention audio track and/or provision of VAS scales and debrief the participant). Non-cannabis using controls will be reimbursed and debriefed for their participation at this stage.

4. OVERVIEW OF THE ~2-WEEK OFF-SITE INTERVENTION PERIOD

4.1 Online delivery of the daily tasks

The ~2-week intervention will be run off-site, during the period between baseline and follow up assessment. The participant will be able to practice the intervention tasks via either an online link or via relevant files on the USB, both of which will be provided at the end of baseline testing by an unblinded tester.

4.2 Content of the daily tasks

Daily tasks will be given to the three CUD groups and will differ based on the intervention condition:

4.2.1 Those allocated to any intervention condition, will complete:

4.2.1.1 a 1-point VAS scale to indicate the levels of: craving for cannabis, relaxation, tension, and mindful attention.

4.2.1.2 a short questionnaire to indicate compliance, risk behaviour, mood, cravings, and cannabis use level.

4.2.2 Those allocated to the mindfulness and relaxation groups, will:

4.2.2.1 listen to the 7-minute audio track with the allocated intervention

4.2.2.2 complete a short questionnaire to indicate if they practiced the psychological strategy explained during the audio track, when they experience cannabis craving in moments other than during the audio track.

4.3 Monitoring of participants' compliance to daily tasks

An unblinded tester will monitor the participant's completion of daily tasks through Qualtrics and send reminders if the participant does not complete the tasks. Reminders will be provided as follows:

4.3.1 A SMS reminder, after the participant does not complete their tasks for *one* day

4.3.2 A SMS reminder, after the participant does not complete their tasks for *two* days

4.3.3 Phone call the participant to confirm if they are experiencing any issues to do the daily tasks, if the participant does not complete their tasks for > *two consecutive* days.

4.3.4 daily (either SMS or phone) reminders from an unblinded tester if the participant remains non-compliant.

Regardless of the level of compliance, the follow up assessment will take place. The amount of intervention completed (e.g. total number of days or total number of minutes practiced) may be used as predictors of the outcomes of interest.

5. OVERVIEW OF THE FOLLOW UP FACE-TO-FACE ASSESSMENT

The follow up assessment takes place ~2-weeks after the baseline assessment. These assessments are identical, with some exceptions. Specifically, at follow up:

5.1. The intervention is administered at the start of the assessment after participant' written informed consent is provided. This is to boost the effect that the 2-week intervention might have on the outcomes of interest.

5.2. The debrief includes additional questions about their experience of the intervention (e.g. if the participant found it useful and when they practiced it).

5.3. "Trait" variables already assessed at baseline will be not be measured, as these are unlikely to change over time (e.g. socio-demographic data, menstrual cycle details for females, CAPE, CUI, AUDIT, MMQ, CUD module of the SCID-5-RV, and SF-36).

5.4. The WASI testing of IQ will not be administered, as this is already measured at baseline.

5.5. Measures that are irrelevant are not administered (i.e. the planning session for the two-week intervention period).

Appendix 5. Ethics Approval

From: Res Ethics <Res.Ethics@acu.edu.au>

Subject: 2019-71H Ethics application approved!

Date: 9 June 2019 at 8:27:12 pm GMT-7

To: Valentina Lorenzetti <Valentina.Lorenzetti@acu.edu.au>

Cc: Bernardo Jarrin <Bernardo.Jarrin@acu.edu.au>, Res Ethics <Res.Ethics@acu.edu.au>

Dear Applicant,

Chief Investigator: Dr Valentina Lorenzetti

Co-Investigators: Dr Izelle Labuschagne, Prof Valerie Curran, Ms Hannah Sehl, Assoc. Prof. Gill Terrett, Professor Peter Rendell, Dr Tom Freeman

Ethics Register Number: 2019-71H

Project Title: Mapping short term brain changes in cannabis use: An fMRI study

Date Approved: 10/06/2019

End Date: 30/04/2022

This is to certify that the above application has been reviewed by the Australian Catholic University Human Research Ethics Committee (ACU HREC). The application has been approved for the period given above.

Continued approval of this research project is contingent upon the submission of an annual progress report which is due on/before each anniversary of the project approval. A final report is due upon completion of the project. A report proforma can be downloaded from the ACU Research Ethics website.

Researchers are responsible for ensuring that all conditions of approval are adhered to and that any modifications to the protocol, including changes to personnel, are approved prior to implementation. In addition, the ACU HREC must be notified of any reportable matters including, but not limited to, incidents, complaints and unexpected issues.

Researchers are also responsible for ensuring that they adhere to the requirements of the National Statement on Ethical Conduct in Human Research, the Australian Code for the Responsible Conduct of Research and the University's Research Code of Conduct.

Any queries relating to this application should be directed to the Ethics Secretariat (res.ethics@acu.edu.au). Please quote your ethics approval number in all communications with us.

If you require a formal approval certificate in addition to this email, please respond via reply email and one will be issued.

We wish you every success with your research.

Kind regards,

Kylie Pashley
on behalf of ACU HREC Chair, Assoc Prof. Michael Baker
Senior Research Ethics Officer | Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
T: +61 2 9739 2646 E:
res.ethics@acu.edu.au

Appendix 6. Participant Consent Form

People with a CUD

CONSENT FORM

TITLE OF PROJECT: **Mapping short-term brain changes in cannabis users: An fMRI study**

APPLICATION NUMBER: 2019-71H

(NAME OF) PRINCIPAL INVESTIGATOR (or SUPERVISOR): Senior Lecturer Valentina Lorenzetti

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Peter Rendell

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Associate Professor Gill Terrett

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Dr Izelle Labuschagne

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Valerie Helen Curran

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Dr Tom Freeman

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Sunjeev Kamboj

(NAME OF) MASTER/PhD RESEARCH STUDENT: Miss Hannah Sehl, Miss Hannah Thomson, Miss Marianna Gabriela Quinones Valera

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I asked, have been answered to my satisfaction.

I agree to participate in the activities as outlined in the information letter. The study involves participating in two 4.5 - 5.5 hour assessment sessions at the Monash Biomedical Imaging facility,

two weeks apart. Activities include providing urine, questionnaires on mental health, wellbeing and substance use, two MRI scans, and brief daily activities for 2 weeks.

I understand that

- I will be allocated to one of three research conditions and neither I nor the researcher will know which group I have been allocated too.
- Each assessment session involves: questions about my background, past and present use of any drugs; brief computer tasks and short questionnaires about my history and current general physical health, mental health and cognitive function, brief tasks in a MRI scanner, and providing urine samples.
- Every day during the two-weeks between the testing sessions, I will be required to complete a brief 3-4 minute daily online questionnaire about cannabis use and measures of wellbeing. I may also be asked to listen to a brief 7-minute audio recording each day.
- I can withdraw from participating in the study at any time without any adverse consequences for the relationship with the study investigators.
- I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.
- I have been informed that my responses to the questionnaires will be initially stored online on servers managed by Qualtrics, subsequently stored on internal servers at Australian Catholic University and will be destroyed ten years after the publication of the findings relative to this study.
- I agree to participate in this activity realizing that information gathered will remain confidential and secure except when it is required by law, and or failure to disclose the information would place myself or others at risk.

I realise that I can withdraw my consent to participate in the study at any time (without adverse consequences). Unless otherwise requested by me, data collected prior to withdrawing, will be

included in the group dataset for aggregated data analysis. If I withdraw after the completion of data analysis, my data will be retained within the dataset.

I freely agree for my data to be used in future studies that are an extension of or closely related to the present project.	*Please tick:*	Yes		No	
I give permission to be contacted again for future studies.	*Please tick:*	Yes		No	
Would you like to hear about the outcomes of this study?	*Please tick:*	Yes		No	
If you have ticked YES to either of the above please provide your contact details below: Email:					
Phone:					
Date of birth:					
Handedness (left, right, ambidextrous):					

NAME OF PARTICIPANT: ……

SIGNATURE: .....DATE: .....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR): DATE: ……...

SIGNATURE OF STUDENT RESEARCHER: ……DATE: .....…

Controls

CONSENT FORM

TITLE OF PROJECT: **Mapping short-term brain changes in cannabis users: An fMRI study**

APPLICATION NUMBER: 2019-71H

(NAME OF) PRINCIPAL INVESTIGATOR (or SUPERVISOR): Senior Lecturer Valentina Lorenzetti

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Peter Rendell

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Associate Professor Gill Terrett

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Dr Izelle Labuschagne

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Valerie Helen Curran

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Dr Tom Freeman

(NAME OF) CO-INVESTIGATOR (or SUPERVISOR): Professor Sunjeev Kamboj

(NAME OF) MASTER/PhD RESEARCH STUDENT: Miss Hannah Sehl, Miss Hannah Thomson, Miss Marianna Gabriela Quinones Valera

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I asked, have been answered to my satisfaction.

I agree to participate in the activities as outlined in the information letter. The study involves participating in one 4 – 4.5 hour assessment session at the Monash Biomedical Imaging facility. Activities include providing urine, questionnaires on mental health, wellbeing and substance use, and an MRI scan.

I understand that

- The assessment session involves: questions about my background, past and present use of any drugs; brief computer tasks and short questionnaires about my history and current general physical health, mental health and cognitive function, brief tasks in a MRI scanner, and providing urine samples.
- I can withdraw from participating in the study at any time without any adverse consequences for the relationship with the study investigators.
- I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.
- I have been informed that my responses to the questionnaires will be initially stored online on servers managed by Qualtrics, subsequently stored on internal servers at Australian Catholic University and will be destroyed ten years after the publication of the findings relative to this study.
- I agree to participate in this activity realizing that information gathered will remain confidential and secure except when it is required by law, and or failure to disclose the information would place myself or others at risk.

I realise that I can withdraw my consent to participate in the study at any time (without adverse consequences). Unless otherwise requested by me, data collected prior to withdrawing, will be included in the group dataset for aggregated data analysis. If I withdraw after the completion of data analysis, my data will be retained within the dataset.

I freely agree for my data to be used in future studies that are an extension of or closely related to the present project.	*Please tick:*	Yes		No	
I give permission to be contacted again for future studies.	*Please tick:*	Yes		No	
Would you like to hear about the outcomes of this study?	*Please tick:*	Yes		No	
If you have ticked YES to either of the above please provide your contact details below: Email:					
Phone:					
Date of birth:					
Handedness (left, right, ambidextrous):					

NAME OF PARTICIPANT: ……

SIGNATURE: .....DATE: .....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR): DATE: ……...

SIGNATURE OF STUDENT RESEARCHER: ……DATE: .....…

Appendix 7. Telephone Screen Script

Group ☑ Cannabis
☐ Control

Screening Number _____
Initials _____
(person making screening decision)

Date ___ /___ /___ (online screening decision)

☐ **Male**
Age _____ ☐ **Female**

☐ Booked

NOTE: COVID-related changes highlighted in green

Telephone Screen Script

Brain-Cann Cannabis Group | Project ID: 2019-71H

1st Call	Date	Initials	1st SMS	Date	Initials
☐	_____	_____	☐	_____	_____

2nd Call	Date	Initials	2nd SMS	Date	Initials
☐	_____	_____	☐	_____	_____

Notes on red flags:

.....

.....

.....

.....

.....

⚠ **Participant quit smoking? Ask** 1) When did you stop smoking? (be as specific as possible, e.g. three months ago, in February 2020, etc.) .....

2) How frequently were you smoking for before quitting? (e.g. 3 days/week) .....

3) For how long? (e.g. two years): .....

☐ *See additional notes on back page*

COVID-19
Please check for COVID-related updates and questions before continuing

General Tips

NOTE: For participant's "Yes" answers – respond with "that's great" or similar that's natural to you.

Be familiar with the entire script & study procedures, so that if you're asked a question you can easily respond or know where to find the information (or that you'll need to find out and call them back). Based on required questions, estimate the time for the call (Page 6) and advise participant.

PRE-FILL pages 7 onwards with available info, so that you can confirm details with the participant (e.g., confirm correct/best contact information) and have relevant screening questions/probes for more information.

Have the MRI lab calendar open and ready to use; skim over it in advance and be familiar with roughly what's available/roughly how far in advance you need to book when initiating conversation about session times.

Page ⑦ has space for an estimate of how long it will take to conduct additional screening (e.g., where there were responses to the MINI that might impact eligibility).

Advise participant - Any information you give will be kept strictly confidential and will be destroyed upon completion of the study or if you decide not to participate.

Other notes:

MRI Safety - MRIs don't involve radiation & are very safe. They just use a strong magnetic field to take pictures, and are quite noisy (e.g., it is normal to hear knocking/banging sounds etc.). However, people with some kinds of implant or metal in their body, such as a pacemaker, can't have a scan because of that magnetic field. So, we have to ask specific questions to check, and a radiographer will also check these questions on the day of a scan.

The ***MBI address*** is 770 Blackburn Rd, Clayton. MBI is also accessible by PT, primarily via **bus, route 703** (note that the stop may appear as "Telstra Labs" on the bus); Clayton train station is ~45min walk (Cranbourne/Pakenham lines*; Westall station also*), but there are multiple connecting buses from there and also from Huntingdale station, that go to the Monash Uni campus. The Monash Uni campus bus loop is ~15min walk or take the 703 from there (or Clayton station; or Syndal station on the Glen Waverley line).

PARKING: There is FREE parking available on site, however we require the car registration number (for participants and testers) prior to each assessment date.
Note to Researchers: **Car registration numbers must be collected from each participant at time of booking and logged in the BrainCann participant booking form**, along with blinded and unblinded tester car registrations. This information along with duration of parking will be populated and emailed to MBI prior to each session, for entry into online parking system.

Study contact details are: cannabis@acu.edu.au; [redacted].

INTRODUCTION

Hello, this is *[insert your name];* I'm calling from Australian Catholic University, about your interest in participating in a research study. Am I speaking with …*[insert their name]?; [CONFIRM CORRECT PERSON].* Thank you for registering your interest in our project and completing the online survey. The purpose of this call is to provide further information about the study and if you are interested in taking part, to ask a few more questions, similar to those you completed online, to make sure this study is right for you.

Would you like to know more about the study?

*IF **NO*** Is there something that concerns you about the project, or would you like to know a little more information before you make up your mind?

*If **Yes** Explore the participant's concerns and clarify any information*

*If still **No*** Thank the participant for their interest in the study and time

*IF **YES*** This information will take a few minutes, is now an okay time? *[If applicable:]* Or would you like us to call you back at a different time or on a different number?

*If **NO** Contact participant at mutually convenient time/date.*

*If **YES** Continue with script:*

STUDY DESCRIPTION

In this study, we are looking at how the brain and behaviour may be affected in those using cannabis over time when we use strategies that may help to manage cannabis cravings. Participation involves taking part in two assessments of approximately 2.5 hours each and practicing some short activities at home on the days in between them. You would be randomly assigned to one of three groups (1 intervention and 2 control groups). Neither you nor the researcher doing the testing knows which group you are allocated to, although all participants will be offered the intervention task at the end of the study. The appointments would be booked at a mutually convenient date and time for you and the researcher and take place at Monash Biomedical Imaging on Blackburn Road, Clayton.

Is this somewhere you will be able to travel to?

*IF **YES*** *Continue; Respond* "that's great" *or similar.*

IF UNSURE *Refer to travel info; can offer to discuss and confirm Y/N later.*

*IF **NO*** *Thank them for their time & refer to for INELIGIBLE procedure, page 21.*

You will be asked to refrain from using any drugs and alcohol during the 12 hours before each appointment. This will be confirmed with a urine sample at the start of each session.

Is that something that you will be able to do for us? *[OR] Will you be able to refrain from using any drugs and alcohol during the 12 hours before the assessment sessions?*

*IF **YES*** *Continue; Respond* "that's great" *or similar.*

*IF **NO*** *Thank them for their time & refer to INELIGIBLE procedure, page 21.*

We will need to call you 5 or more days before the research session and then send you a text message 12-48 hours before your booking, to run through a quick covid-19 check. You will also be required to have your temperature taken upon entry into the MRI scanning room, this is keeping with hospital policies regarding MRI scan procedures during the covid pandemic. Both the researchers and yourself will need to wear a mask during the sessions. These requirements are part of Victorian Government and MBI's standard covid-related health and safety regulations.

Is that all ok with you?

*IF **YES*** *Continue; Respond* "that's great" *or similar.*

*IF **NO*** *Thank them for their time & refer to INELIGIBLE procedure, page 21.*

I'll now tell you a bit more about what's involved. During each session, you will be asked to complete questionnaires about your mood, reactions to cannabis-related and other pictures, substance use, and do activities like short computer tasks. You will also undergo a MRI scan during each session that will take pictures of your brain. MRI scans do not involve radiation and are very safe.

Did the participant endorse items of concern regarding MRI safety in their online screen?

If YES: Turn to MBI MRI safety questionnaire (page 17). Probe participant about relevant endorsed items and administer the MBI MRI safety questionnaire. If participant remains eligible after probing and safety questionnaire completion, return to this point in the script and continue.

If NO: Continue from here with the script, ensuring to administer the MBI MRI safety questionnaire when it arises in the script (page 17).

Once the assessments are complete, you will have the opportunity to ask questions and debrief. As compensation for your time, you will receive a $150 Coles-Myer Voucher at the completion of your second assessment session. If you are interested, a high-resolution picture of your brain can also be provided to you at the end of the study.

In addition, every day for two-weeks between the assessments you will be asked to do one or both of the following tasks: 1) answer a 3-minute online questionnaire about your mood and substance use; 2) listen to a 7-minute set of audio instructions. You may find the audio-instructions helpful for your wellbeing, interesting and enjoyable. Your participation will help us gain a better understanding of how some instructions can help the way people deal with their daily experiences and which brain pathways are involved in this.

Is 2 weeks of short tasks something you can commit to?

*IF **YES*** *Continue; Respond* "that's great" *or similar:*

*IF **NO*** *Thank them for their time & refer to INELIGIBLE procedure, page 21.*

To maintain confidentiality, your data from this study will be stored electronically using a code so that your information cannot be personally identified.

Do you have any questions or concerns about that? *[OR: Would you like to know more about that before we go on?]*

*IF **YES*** ➔ *[extra info:]* - Electronic data will be stored securely on both online and internal ACU servers. Hardcopy data will also be stored with restricted access at ACU's Melbourne Campus. Only researchers directly involved in the study will have access to the data. Identifying personal information will also be stored separately from participant data files. Only results of overall group data will be reported, and may be published in academically reviewed journals and presented at research conferences. No individual data will be reported or published. Also, data will be destroyed ten years after the publication of the findings related to this study.

*IF **NO*** ➔ *Continue:*

💬 Some questionnaires include sections asking about the use of substances that are unlawful. We collect this information to help describe participant groups overall, rather than individuals. All efforts are made to ensure the confidentiality of participant information; we cannot guarantee, though, that a third party could not use some legal process to gain access to the data (for instance a subpoena or search warrant). This would be unlikely.

💬 Participation in this study is completely voluntary. You can withdraw at any stage, even after you've signed the consent form. If you withdraw after we've started collecting your data, we may still use that existing data in the group analysis unless you ask for us not to. However, if you withdraw after the data has been analysed, your deidentified data will still be included.

💬 All MRI scans collected will be analysed for research rather than diagnostic purposes. While there are no known risks from MRI scans of the brain used in this study, there are occasionally cases where an atypical or significant finding might be made. For instance, this could be a cyst with no adverse impact, or something with possible clinical implications. If researchers become aware of a significant finding during the course of the study, you <u>will</u> be notified. Although this is unlikely, this could have consequences such as affecting your ability to work in certain professions, or to obtain health or life insurance. Please consider what knowing about something like this would mean for you. If you don't want to know, it is suggested that you do not participate.

Does this study still sound like something that you would be interested in?

*IF **YES*** → *Respond* "that's great" *or similar and continue with script*

*IF **NO*** ⊘ *Thank them for their time & refer to INELIGIBLE procedure, page 21.*

STUDY ELIGIBILITY

Now I need to ask you a few questions to ensure that you are eligible for the study. This will take about ……. minutes. *[insert estimate prior to call based on required Qs.]*

Are you in a quiet place where you can talk and answer honestly at the moment?

*IF **YES*** → *Continue with script:*

*IF **NO*** *Offer to call back, & record details in contact information file.*

Advise participant - Any information you give will be kept strictly confidential and will be destroyed upon completion of the study or if you decide not to participate.

First, I need to confirm some of the personal information you entered during the online survey. It is important that you provide accurate information, and [as I've said,] any information that you provide will be treated as confidential. If you do not wish to answer questions about your substance use or psychiatric history, you may withdraw at any time.

DEMOGRAPHICS

1. What is your age? and DOB
2. Which sex are you, male or female? **Male / Female**
3. To confirm, you are able to travel to the Monash Biomedical Imaging centre in Clayton, on two separate occasions, approximately 2 weeks apart? **Yes / No**
4. Are you able and willing to take part in a two-week online intervention that takes approximately 5-10min a day? **Yes / No**
   a. Do you have a device with an internet connection that you will be able to use for the daily activity, like an iPad, laptop, or mobile phone? [What sort of device?] _____
   b. Is it ok for a research team member to potentially contact you via telephone during this time? **Yes / No**

5. What is your current occupation? Full-time / Part-time / Unemployed

---

6. What is the highest level of education you have completed, or are undertaking?

---

7. Did you complete any studies overseas? If so, what, for how long, did you complete this study? *[Gather information briefly for matching purposes]*

---

8. How did you hear about our research?

---

9. What suburb/area do you currently live in?

---

10. Will you be driving to MBI and require car parking?

    Yes No

11. If yes, so that we can book you a parking spot, can please advise your Car registration number?

OTHER SPECIFIC EXCLUSION CRITERIA (IF APPLICABLE)

Other experiences

In the online survey you completed, you indicated that:

You'd used some sort of psychological strategy/strategies; please tell me more about:

Insert questions about psychological strategy practice:

We ask that you don't take up and new psychological strategy/strategies such as tai chi, yoga, meditation, mindfulness, progressive muscle relaxation, etc; in the time between now and the end of your assessment/s.

You'd participated in other research studies; please tell me more about that: *Insert questions about previous study participation:*

If calling >4 weeks after online screen:

Substance Use

⚠ Have you used any illicit substance in the past month? **Yes No** *If applicable:*

What?

When?

How much?

How often?

Have you ever tried (drug)? If Yes continue If No go onto the next drug	How old were you when you first used (drug)?	How old were you when you first used the (drug) on a regular basis? * *Note that regular use is considered to be weekly use*	Over your lifetime... What's the most frequently you have ever used? 5. Daily or almost daily 4. Weekly 3. 2-3 times a month 2. Monthly 1. Less than monthly	Over the past 12 months... How often do you usually use the drug? 5. Daily or almost daily 4. Weekly 3. 2-3 times a month 2. Monthly 1. Less than monthly 0. None in the past 12 months	Over the past 3 months... How often do you usually use the drug? 5. Daily or almost daily 4. Weekly 3. 2-3 times a month 2. Monthly 1. Less than monthly 0. None in the past 3 months	How much a day do you use usually? *Estimate units*	How long has it been since you last used? Write date OR No. of days, weeks, months, years ago
Tobacco ❑ Yes ❑ No							
Alcohol ❑ Yes ❑ No						*Standard units*	
Cannabis (eg. marijuana, hash) ❑ Yes ❑ No						____ g/oz *Joints/Cones/Bongs* *Leaf/Hydro/Buds/Hash*	
Amphetamines (eg. speed, ice) ❑ Yes ❑ No IV use ever? ❑ Yes ❑ No						____ *g* ____ *Hits* *Oral Snorts IV*	
Cocaine (eg. coke) ❑ Yes ❑ No IV use ever? ❑ Yes ❑ No						____ *g* ____ *Hits* *Oral Snorts IV*	
Hallucinogens (eg. LSD, mushrooms) ❑ Yes ❑ No						*Tabs/Pills*	
Ecstasy ❑ Yes ❑ No						*Tabs/Pills*	

Duration/ other questions
……
……
……
……
……
……
……
……
……
……

Have you ever tried (drug)? If Yes continue If No go onto the next drug	How old were you when you first used (drug)?	How old were you when you first used the (drug) on a regular basis? * *Note that regular use is considered to be weekly use*	Over your lifetime ... What's the most frequently you have ever used? **5.** Daily or almost daily **4.** Weekly **3.** 2-3 times a month **2.** Monthly **1.** Less than monthly	Over the past 12 months ... How often do you usually use the drug? **5.** Daily or almost daily **4.** Weekly **3.** 2-3 times a month **2.** Monthly **1.** Less than monthly **0.** None in the past 3 months	Over the past 3 months ... How often do you usually use the drug? **5.** Daily or almost daily **4.** Weekly **3.** 2-3 times a month **2.** Monthly **1.** Less than monthly **0.** None in the past 3 months	How much a day do you use usually? *Estimate units*	How long has it been since you last used? Write date OR No. of days, weeks, months, years ago	Duration/ other questions
Inhalants (eg. petrol, paint, glue) ❑ Yes ❑ No						*Sniffs/Cans/Bags*		
Opiates (eg. heroin, codeine) ❑ Yes ❑ No IV use ever? ❑ Yes ❑ No						___*g* ___*Hits* *Licit Illicit* *Oral Smokes IV*		
Benzos/Sedatives (non-prescribed use) ❑ Yes ❑ No IV use ever? ❑ Yes ❑ No						___*mg* ___*Tabs/Pills* *Oral IV* Prescribed? *Y N*		
Other _____ ❑ Yes ❑ No								

MEDICAL

I now need to ask you some medical questions.

1. Have you ever had a serious head injury that resulted in trauma to the brain, or required surgery, prolonged hospitalisation, or rehabilitation, and may have involved prolonged unconsciousness or concussion? *[If person can't recall head injuries, prompt by asking whether they have ever had concussion or been unconscious]*. **Yes / No** *If applicable:* Please tell me more about that: _____

_____

2. Have you ever had any of the following? For confidentiality, please do not tell me or elaborate on the particular diagnosis, simply provide a yes or no answer after the list is completed. Fits, convulsions, epileptic seizures; stroke, brain tumour, meningitis, encephalitis, multiple sclerosis; Positive for HIV. Again, only state 'yes' or 'no', please do not elaborate. **Yes / No**

3. Have you ever had any other serious illness not mentioned in the previous question but that you suspect might affect our research question in any way? **Yes / No**

*IF **YES*** What condition? How long ago was this?

_____

4. The next question follows the current ACU policies around COVID-19, which states that everyone those attending this research study must be fully vaccinated due to close proximities during testing. Are you fully vaccinated? **Yes / No**

*IF **YES*** You do not have to send through your proof-of-vaccination, however, a member of the research team needs to see this upon arrival at your first testing session. Would you be willing to present this certificate at your first testing session? This can be when you check into the venue. **Yes / No**

*IF **NO**, explain to participant that we need to site the certificate in order for them to participate*

*IF **NO*** Do you intend to get vaccinated? **Yes / No**

*IF **YES***

Date first dose: Click or tap to enter a date.

Date second dose: Click or tap to enter a date.

*IF **NO***

Inform participant that you will not be able to book them due to the current ACU policies for this study, but that you can continue screening them to keep them in our records in case there are any changes.

VISION

1. Have you ever had your vision assessed?

   YES *[continue with question 2]* NO ➔ *[skip to next section]*

2. Do you require glasses or contact lenses?

   YES *[continue with question 3]* NO ➔ *[skip to next section]*

3. Will you be able to wear contact lenses to the assessment sessions?

   YES *[continue with next section]*

   NO *[Marginal eligibility, but continue screening:]* I'll have to check later whether we can access a special set of glasses for you to use during the MRI. Do you know the script for your glasses, or how to find this out? *Record if known:*

Left	*Right*

FEMALE PARTICIPANTS ONLY

1. Are you currently breastfeeding?

   *IF **NO*** ✓ *Continue [question 2]*

   *IF **YES*** ⊘ *Thank them for their time & refer to INELIGIBLE procedure, page* 21.

2. To the best of your knowledge, are you currently pregnant?

   *IF **YES*** ⊘ *Thank them for their time & refer to INELIGIBLE procedure, page* 21.

   *IF **NO*** ✓ Are you thinking of or trying to get pregnant?

   *If **Yes*** ⊘ *Thank them for their time & refer to INELIGIBLE procedure, page* 21.

   *If **No*** ✓ *Continue with script*

MINI questions *(tick the items you need to probe)*

I now need to ask some more questions about your survey responses.

[For each relevant item, prompt and probe by asking "You indicated/reported that…" and insert specifics from the relevant question (which are included in grey text for reference). E.g.:

"You indicated that you'd been depressed or down, nearly every day, for two weeks. Can you tell me more about that?"]

*Answered Yes to **MINI_1**: Have you been depressed or down, or felt sad, empty or hopeless most of the day, **nearly every day**, for the past two weeks?*

_____

_____

_____

*Answered Yes to **MINI_2**: In the past two weeks, were you much less interested in most things or much less able to enjoy the things you used to enjoy **most of the time**?*

_____

_____

_____

*Answered Yes to **MINI_3**: In the past month did you think that you would be better off dead or wish you were dead?*

_____

_____

_____

*Answered Yes to **MINI_4**: In the past month have you thought about killing yourself, or wanted to be dead, or planned to kill yourself, or done anything that you hoped would cause your death?*

_____

_____

_____

*Answered Yes to **MINI_5**: Have you **ever** had a period of time when you were feeling 'up' or 'high' or 'hyper' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.)*

_____

_____

_____

*Answered Yes to **MINI_6**: Have you ever been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family?*

OR

Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified?

_____

_____

_____

*Answered Yes to **MINI_7**: Have you, on more than one occasion, had spells or attacks when you suddenly felt anxious, very frightened, uncomfortable or uneasy, even in situations where most people would not feel that way? Did the spells surge to a peak, within 10 minutes of starting?*

_____

_____

_____

*Answered Yes to **MINI_8**: Did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner?*

_____

_____

*Answered Yes to **MINI_9**: Do you feel anxious or uneasy in places or situations where help might not be available, or escape might be difficult: like being in a crowd or enclosed space, standing in a line (queue), when you are away from home or alone at home, or when crossing a bridge, traveling in a bus, train or car?*

_____

_____

_____

*Answered Yes to **MINI_10**: In the past **month** did you have persistent fear and significant anxiety of being watched, being the focus of attention, or of being humiliated or embarrassed or rejected? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.*

_____

_____

_____

*Answered Yes to **MINI_11**: In the past **month** have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing?*

*(e.g., the idea that you were dirty, contaminated **or** had germs, or fear of contaminating others, **or** fear of harming someone even though you didn't want to, **or** fearing you would act on some impulse, **or** fear or superstitions that you would be responsible for things going wrong, **or** obsessions with sexual thoughts, images or impulses, or religious obsessions.)*

_____

_____

_____

*Answered Yes to **MINI_12**: In the past **month**, did you feel driven to do something repeatedly in response to a rigid rule or obsession, like washing or cleaning excessively, counting or checking things over and over, or repeating or arranging things, or other superstitious rituals?*

_____

_____

_____

*Answered Yes to **MINI_13**: Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury or sexual violence to you or someone else?*

Examples of traumatic events include: Serious accidents, sexual or physical assault, a terrorist attack, being held hostage, kidnapping, fire, discovering a body, war, natural disaster, witnessing the violent or sudden death or someone close to you, or a life-threatening illness.

_____

_____

_____

*Answered Yes to **MINI_14**: During the past month, have you re-experienced the event in an unwanted distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?*

_____

_____

_____

*Answered Yes to **MINI_17**: Have you ever believed that people were spying on you or that someone was plotting against you or trying to hurt you?*

_____

_____

_____

*Answered Yes to **MINI_18**: Have you ever heard things other people couldn't hear such as voices?*

_____

_____

_____

*Answered Yes to **MINI_19**: Have you ever had visions when you were awake, or have you ever seen things other people couldn't see?*

_____

_____

_____

*Answered a BMI <18 to **MINI_20-21**:*

_____

_____

_____

*Answered Yes to **MINI_22-23**: In the past 3 months, did you have eating binges or times when you ate a very large amount of food within a 2-hour period? In the last **3 months**, during these binges, did you feel that your eating was out of control?*

_____

_____

*Answered Yes to **MINI_24**: Were you **excessively** anxious or worried about several routine things over the past 6 months?*

_____

_____

_____

(TESTER: this is <u>compulsory to administer</u> to confirm eligibility and that the person is safe to be tested. Administer all items and keep a close eye on those flagged during phone screen)

If applicable: Before we continue, I just want to confirm some information about your …… that you noted in the online survey and other similar items.

MBI MRI SAFETY QUESTIONNAIRE

Have you ever had any eye injury caused by metal? .....NO / YES
If YES:
Did you see a doctor at the time? ....NO / YES
Did they remove the foreign body? ....NO / YES
Did they tell you that they got it all out? ....NO / YES
Was this the last injury involving metal? ....NO / YES

Are you pregnant, suspect you may be pregnant or breastfeeding?.....NO / YES

Do You Have (Or Have You Ever Had):

A Cardiac Pacemaker/stent/defibrillator/wire.....NO / YES
Any heart operation or valve replacement..... .....NO / YES
Any Brain operation ……NO / YES
Abdominal Aneurysm repair or IVC filter.....NO / YES
Brain Aneurysm Clips.....NO / YES
Deep Brain Stimulator.....NO / YES
Brain Shunt Tube ,.....NO / YES
If YES, is it programmable .....NO / YES
Any Ear operations /cochlear or stapes implants.....NO / YES
Implanted drug infusion devices.....NO / YES
Neuro or Bone growth stimulator.....NO / YES
Shrapnel, bullet, gunshot.....NO / YES
Any stents, vascular, oesophageal or biliary .....NO / YES
Any Surgical clips/wire sutures/screws/mesh/prosthesis.....NO / YES
Joint Replacement or Prosthesis.....NO / YES

Do You Have:

Ocular prosthesis (eye implants).....NO / YES
A Swan-Ganz Catheter .....NO / YES
Skin patches .....NO / YES
Intrauterine device (IUD).....NO / YES
A penile prosthesis .....NO / YES
Any other implant, or breast tissue expander .....NO / YES
Tattooes eyelids or tattoos.....NO / YES
Hearing Aid .....NO / YES
Removable dentures.....NO / YES
Any Piercings or braces that CANNOT be removed.....NO/ YES
Hair Extensions ……NO / YES

Have You: **What? / When?**

Had an operation or procedure within the last 8 weeks NO / YES .....
Had a history of seizures or epilepsy .....NO / YES

IF YES to any items, check with Richard if the person is eligible for an MRI scan 9905 0100

[Specific questions from Richard/MBI:]

*IF **Ok**, or eligibility **TBC*** 💬 *Return to page 5 and continue with script **OR** continue from this point, as required.*

*IF **NOT** eligible* ✎ *Refer to INELIGIBLE procedure script, page 21.*

Inclusion /Exclusion Check List:

- ☐ Aged 18-55 years
- ☐ Normal-to-corrected vision
- ☐ Fluent in English
- ☐ Meet safety criteria for MRI
- ☐ Informed of 12-hour abstinence from illicit substance use and alcohol for ax
- ☐ No current medication that affects the CNS
- ☐ No history of diagnosed psychiatric conditions
- ☐ No neurological disorders
- ☐ No history of ABI
- ☐ Current CUD with 4+ DSM-V symptoms
- ☐ Current daily/almost daily CB use for > 12-months
- ☐ Tried to quit/reduce CB use at least once within the past 24 months
- ☐ No significant use or dependence on alcohol or illicit substances (except CB)
- ☐ No illicit drug use in past 4-weeks (except CB)

ELIGIBLE PARTICIPANTS

Thank you for your time in answering those questions. We would like to invite you to participate in the study. *[Or similar – affirm interest and be enthusiastic!]*

If necessary, remind: Both sessions will be held at Monash Biomedical Imaging in Clayton and will involve further questions about any current or previous drug use and your general physical and mental health. We will also ask you to complete a few questionnaires and some computer tests.

Inform ppt: when describing assessment length – especially if their assessment runs across a main mealtime such as lunch or dinner – that they should eat before they come. Let them know that we do have some light snacks, but they are welcome to bring food with them

Would you like to make a time now to participate in the study?

*IF **NO*** Is there a good time for me to call you back to make an appointment? *Record details in Contact Info file*.

*IF **YES*** We need to find times for two sessions, two weeks apart. Are there particular days or times during the week that suit you?

[CHECK LAB CALENDAR and discuss potential dates/times accordingly]

……

……

Can we book you in for ……?

Two weeks later is …… *[session 1 date+14 days; CHECK LAB CALENDAR]*. Can we book you in for the second session on …….. *[date]* at ……*[time]*?

Can we please give you a call you the day before, to remind you about the appointment?

[confirm best number & record in contact info file]

We would also like to send you a copy of our Participant Information Letter, so you can read about the study in full, as well as a map showing you the location of the appointment. Can I please confirm the best email address to send this to you?

*IF **YES*** Thank you. We will send these out to you in the **email** today *[or as soon as possible]* so you should receive these shortly.

*IF **NO*** Is there something that concerns you about the study or would you like to know a little more information?

> *If **Yes** Explore the participant's concerns and clarify any information. If helpful, can offer to have a senior team member call them back.*
>
> *If still **No** Thank the participant for their interest and the time for the call.*

Wrap Up

Thank you for your interest in the project and the time you've taken to speak with me. Is there anything about the study that you'd like to talk through further?

We look forward to meeting you *[in a few weeks/as applicable].* In the meantime, please feel free to get in contact with the team if you have any questions when reading the study information. *[Confirm study contact info if needed.]* Thanks again. Bye!

MARGINALLY ELIGIBLE PARTICIPANTS

Thank you for your time in answering those questions. *[Could insert: "I need to confirm some details with my supervisor/the study leader" or similar.]* We will confirm with you in the next …… if the study is right for you. Are there particular days or times that suit you for us to call you back?

Can remind if indicated: Any information will be confidential and will be destroyed if you do not participate.

Record details in contact info section, page ⑦

Wrap Up

Thank you for your interest in the project and the time you've taken to speak with me. We'll look forward to speaking with you *[in a few weeks/as applicable].* In the meantime, please feel free to get in contact with the team if you have any questions about the project. *[Confirm study contact info if needed.]* Thanks again. Bye!

INELIGIBLE PARTICIPANTS

Explain that unfortunately the study has very strict inclusion criteria (do NOT give specific reason for participant being ineligible unless it is MRI safety). Thank participant and ask whether they would like their name and contact detail recorded for any future studies. [confirm this and record details if appropriate on the next page]

Below are example explanations for ineligibility:

E.g. Thank-you for your time but unfortunately, due to the requirements of the study, we already have enough participants with your characteristics (and/or information that you provided suggests that procedures we use in the study, such as the MRI, may compromise your safety if you were to participate in this study). This means that this study is not appropriate for you at this time. I'd like to thank you for your time and for taking an interest in our study.

E.g. Unfortunately, we have enough participants with your characteristics this time. This does not mean that you won't be able to take part in other studies at the university later on. What this does mean is that due to the specific requirements of the present study and the number of people that we need, we have enough people with your characteristics. If you are interested in participating in future studies by the same research team, we are able to add your name to a participant database and we can notify you about future studies, which you may be eligible to participate.

Consent future studies(N/A)

NB: Consent for future studies is now captured via the online survey screener and via the consent form.

Thanks again. Bye!

NOTES

Appendix 8. Participant Information Letter

People with a CUD

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: Mapping short-term brain changes in cannabis users: An fMRI study

APPLICATION NUMBER: 2019-71H HREC

PRINCIPAL INVESTIGATOR: Dr Valentina Lorenzetti

CO-INVESTIGATOR: Professor Peter Rendell

CO-INVESTIGATOR: Associate Professor Gill Terrett

CO-INVESTIGATOR: Dr Izelle Labuschagne

CO-INVESTIGATOR: Professor Valerie Helen Curran

CO-INVESTIGATOR: Dr Tom Freeman

CO-INVESTIGATOR: Professor Sunjeev Kamboj

STUDENT RESEARCHER: Ms Hannah Sehl, Ms Hannah Thomson, Ms Marianna Gabriela Quinones Valera

STUDENT'S DEGREE: Research Higher Degree, Masters of Psychology (Clinical), Doctor of Philosophy

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?

There are over 200 million cannabis users globally. Some scientific findings suggest that using cannabis regularly may affect our behaviour, how we think and our brain. This study aims to test how our brain and behaviour changes in cannabis users over time and when we use strategies that may help manage cannabis craving.

Who is undertaking the project?

This project is being led by Dr Valentina Lorenzetti, an expert in neurocognitive mechanisms of addiction and lead of the Neuroscience of Addiction and Mental Health Program. Co-Investigators include Prof Rendell, Prof Terrett and Dr Labuschagne - members of the Healthy Brian and Mind Research Centre and international experts in Memory, Addiction, and Neuroimaging. Prof Valerie Helen Curran, Prof Sunjeev Kamboj and Dr Tom Freeman – world class experts in substance use at the Clinical Psychopharmacology Unit, University College London who have led similar studies.

Are there any risks associated with participating in this project?

Participants will not be asked to take any illicit substances. Participants with concerns about their health and/or regarding substance use should contact their general practitioner or drug use hot line such as the 24 hour Direct Line 1800 888 236, mental health help lines such as Lifeline 13 11 26 Web: www.lifeline.org.au/ and Beyond Blue 1300 22 4636 www.beyondblue.org.au; ACU students can contact the university's counselling services Tel: (03) 9953 3006 | Fax: 03 9953 3195 | Email: melbournepsychologyclinic@acu.edu.au | Web: www.acu.edu.au/psychologyclinic; and for those who require a psychological referral, Dr Barbara Jones of ACU Melbourne can be contacted. In the event of a crime, there is a chance that a court will demand access to the data on illicit substance use. By checking the corresponding box in the consent form, participants declare their awareness and consent to this risk. It is possible that incidental findings are detected during the brain scan. Knowing about an incidental finding may affect your ability to work in certain professions, obtain life or health insurance and other aspects of daily living. Please take the time to consider carefully what it would mean to you if we told you about an incidental finding in your brain that might, or might not, affect you in later life. If you do not want to know, then it is better not to take part.

What will I be asked to do?

- You will be asked to refrain from using any drugs and alcohol during the **12 hours before each assessment session.** Abstinence will be confirmed with a urine sample at the start of each session.

- You will be randomly allocated to one of 3 groups (1 intervention and 2 control groups). Neither you nor the researcher doing the testing knows which group you are allocated to. (NB: All participants will be offered the intervention task at the end of the study).
- Participation involves taking part in **two assessments (4.5 - 5.5 hours each)** at Monash Biomedical Imaging (Address: 762-772 Blackburn Rd, Clayton VIC 3168). The second session is usually shorter than the first one, and you can take breaks as needed during the appointments. Some light refreshments are provided (e.g., tea, coffee, small snacks), or you are welcome to bring your own food.
- The assessments will occur at a mutually convenient dates/times for you and the researcher.
- **Every day** for **two-weeks between assessments,** you will be asked to do one or both of the following tasks:

  i) answer a 3-minute online questionnaire about your mood and substance use; ii) listen to a 7-minute audio recording.

At both assessment sessions, you will be asked to:

- Provide a urine sample to confirm your regular and recent substance usage.
- Complete questionnaires about your mood, reactions to cannabis-related and other stimuli (pictures), substance use, questions about COVID-related and other stressful events, discuss your availability to complete the daily tasks you are assigned, and to perform short computer tasks.
- Undergo a 1-hour MRI scan that will take pictures of your brain so we can map how the brain changes over a brief period of time. Eye-tracking will be used during the scan to check that you have your eyes open to attend to the tasks.
- Debrief with the researcher to address any questions you have.

How much time will the project take?

Participation involves taking part in **two assessments** at the Monash Biomedical Imaging at Monash University (Clayton campus), two weeks apart. Both **assessments** will take up to5.5 hours. We will

also ask you to practice the instructions in the audio-recording for two weeks between the assessments, every day, for about 10 minutes, and provide some information on your mood / substance use via an online link. As compensation for your time, you will receive a $150 Coles/Myer Voucher at the completion of the second assessment.

What are the benefits of the research project?

We will provide you with a high-resolution image of your brain at the end of the study. You may find the audio-instructions helpful for your wellbeing, interesting and enjoyable. However, this is not certain. Your participation will help us gain a better understanding of how some instructions can help the way people deal with their daily experiences and which brain pathways are involved in this.

Can I withdraw from the study?

Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate and have signed the consent form you can still withdraw from the study at any time without adverse consequences. Unless otherwise requested by you, data collected prior to you withdrawing, will be included in the group dataset for aggregated data analysis. If you withdraw after the completion of data analysis your data will be retained within the dataset.

Will anyone else know the results of the project?

To maintain confidentiality your data from this study will be stored electronically using a numeric code so that your information cannot be personally identified. Electronic data will be stored online on servers managed by Qualtrics, subsequently stored on internal servers at Australian Catholic University and will be destroyed ten years after the publication of the findings relative to this study. Only researchers directly involved in the study will have access to the data. Some questionnaires include questions regarding use of substances some of which are unlawful. This information is collected for the purposes of describing sample characteristics. Given illicit substance use is unlawful, the researchers cannot guarantee that a third party could not use some legal process to gain access to the data (i.e., subpoena or search warrant). All hardcopy and electronic data will be securely stored with restricted access at the ACU, Melbourne Campus and consent forms will be stored separately from data files. Only results of group (aggregated) data will be reported and may be

published in refereed psychological or medical journals and presented at research conferences. No individual data will be reported or published.

Will I be able to find out the results of the project?

If you are interested in finding out the results of the study, please tick the relevant box on your consent form. You will then receive a summary of the outcomes and an image of your brain at the end of the study.

Who do I contact if I have questions about the project?

If you have any questions or concerns regarding this project, before or after participating, please contact the study researcher, via email: cannabis@acu.edu.au or telephone our dedicated research line, 0490391342. If leaving a voice message, please provide your name, telephone number and/or email address and a convenient time to return your call. Alternatively, you can contact the Principal Supervisor, Senior Lecturer Dr Valentina Lorenzetti via email valentina.lorenzetti@acu.edu.au at the Australian Catholic University, to discuss your participation or the project in general.

What if I have a complaint or any concerns?

The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (review number 2019-71H HREC). If you have any complaints or concerns about the conduct of the project, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics

c/o Office of the Deputy Vice Chancellor (Research) Australian Catholic University

North Sydney Campus PO Box 968

NORTH SYDNEY, NSW 2059

Ph.: 02 9739 2519

Fax: 02 9739 2870

Email: resethics.manager@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?

If you are willing to participate please sign the attached informed consent form. You should sign both copies of the consent form and retain one copy for your records and then contact me on our dedicated research phone on 0490391342 or email me at Cannabis@acu.edu.au to book a session. You will need to bring the researcher's copy of the signed consent form to the session before we can start. Your support for the research project will be most appreciated.

Yours sincerely,

The research team,

Master Research Students & Principal Investigators

Neuroscience of Addiction and Mental Health

Healthy Brain and Mind Research Centre

School of Behavioural & Health Sciences

Faculty of Health Sciences

Australian Catholic University

115 Victoria Pde, Fitzroy, VIC, 3065

Controls

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: Mapping short-term brain changes in cannabis users: An fMRI study

APPLICATION NUMBER: 2019-71H HREC

PRINCIPAL INVESTIGATOR: Dr Valentina Lorenzetti

CO-INVESTIGATOR: Professor Peter Rendell

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STUDENT RESEARCHER: Ms Hannah Sehl, Ms Hannah Thomson, Ms Marianna Gabriela Quinones Valera

STUDENT'S DEGREE: Research Higher Degree, Masters of Psychology (Clinical), Doctor of Philosophy

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?

There are over 200 million cannabis users globally. Some scientific findings suggest that using cannabis regularly may affect our behaviour, how we think and our brain. This study aims to test how our brain and behaviour changes in cannabis users over time and when we use strategies that may help manage cannabis craving.

Who is undertaking the project?

This project is being led by Dr Valentina Lorenzetti, an expert in neurocognitive mechanisms of addiction and lead of the Neuroscience of Addiction and Mental Health Program. Co-Investigators include Prof Rendell, Prof Terrett and Dr Labuschagne - members of the Healthy Brian and Mind Research Centre and international experts in Memory, Addiction, and Neuroimaging. Prof Valerie Helen Curran, Prof Sunjeev Kamboj and Dr Tom Freeman – world class experts in substance use at the Clinical Psychopharmacology Unit, University College London who have led similar studies.

Are there any risks associated with participating in this project?

Participants will not be asked to take any illicit substances. Participants with concerns about their health and/or regarding substance use should contact their general practitioner or drug use hot line such as the 24 hour Direct Line 1800 888 236, mental health help lines such as Lifeline 13 11 26 Web: www.lifeline.org.au/ and Beyond Blue 1300 22 4636 www.beyondblue.org.au; ACU students can contact the university's counselling services Tel: (03) 9953 3006 | Fax: 03 9953 3195 | Email:

melbournepsychologyclinic@acu.edu.au | Web: www.acu.edu.au/psychologyclinic; and for those who require a psychological referral, Dr Barbara Jones of ACU Melbourne can be contacted. In the event of a crime, there is a chance that a court will demand access to the data on illicit substance use. By checking the corresponding box in the consent form, participants declare their awareness and consent to this risk. It is possible that incidental findings are detected during the brain scan. Knowing about an incidental finding may affect your ability to work in certain professions, obtain life or health insurance and other aspects of daily living. Please take the time to consider carefully what it would mean to you if we told you about an incidental finding in your brain that might, or might not, affect you in later life. If you do not want to know, then it is better not to take part.

What will I be asked to do?

- You will be asked to refrain from using any drugs and alcohol during the **12 hours before the assessment session.** Abstinence will be confirmed with a urine sample at the start of the session.
- Participation involves taking part in **one assessment (4 - 4.5 hours)** at Monash Biomedical Imaging (Address: 762-772 Blackburn Rd, Clayton VIC 3168), and you

can take breaks as needed during the appointment. Some light refreshments are provided (e.g., tea, coffee, small snacks), or you are welcome to bring your own food.

- The assessment will occur at a mutually convenient date/time for you and the researcher.

At the assessment session, you will be asked to:

- Provide a urine sample to confirm your regular and recent substance usage.
- Complete questionnaires about your mood, reactions to cannabis-related and other stimuli (pictures), substance use, questions about COVID-related and other stressful events, and to perform short computer tasks.
- Undergo a 1-hour MRI scan that will take pictures of your brain so we can map how the brain changes over a brief period of time. Eye-tracking will be used during the scan to check that you have your eyes open to attend to the tasks.
- Debrief with the researcher to address any questions you have.

How much time will the project take?

Participation involves taking part in **one assessment** at the Monash Biomedical Imaging at Monash University (Clayton campus). The **assessment** will take up to 4.5 hours. As compensation for your time, you will receive a $100 Coles/Myer Voucher at the completion of the assessment.

What are the benefits of the research project?

We will provide you with a high-resolution image of your brain at the end of the study. You may find the audio-instructions helpful for your wellbeing, interesting and enjoyable. However, this is not certain. Your participation will help us gain a better understanding of how some instructions can help the way people deal with their daily experiences and which brain pathways are involved in this.

Can I withdraw from the study?

Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate and have signed the consent form you can still withdraw from the study at any time without adverse consequences. Unless otherwise requested by you, data collected prior to you

withdrawing, will be included in the group dataset for aggregated data analysis. If you withdraw after the completion of data analysis your data will be retained within the dataset.

Will anyone else know the results of the project?

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If you have any questions or concerns regarding this project, before or after participating, please contact the study researcher, via email: cannabis@acu.edu.au or telephone our dedicated research line, 0490391342. If leaving a voice message, please provide your name, telephone number and/or email address and a convenient time to return your call. Alternatively, you can contact the Principal Supervisor, Senior Lecturer Dr Valentina Lorenzetti via email valentina.lorenzetti@acu.edu.au at the Australian Catholic University, to discuss your participation or the project in general.

What if I have a complaint or any concerns?

The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (review number 2019-71H HREC). If you have any complaints or concerns about the conduct of the project, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics c/o Office of the

Deputy Vice Chancellor (Research)

Australian Catholic University

North Sydney Campus

PO Box 968

NORTH SYDNEY, NSW 2059

Ph.: 02 9739 2519

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I want to participate! How do I sign up?

If you are willing to participate please sign the attached informed consent form. You should sign both copies of the consent form and retain one copy for your records and then contact me on our dedicated research phone on 0490391342 or email me at Cannabis@acu.edu.au to book a session. You will need to bring the researcher's copy of the signed consent form to the session before we can start. Your support for the research project will be most appreciated.

Yours sincerely,

The research team

Master Research Student & Principal Investigators

Neuroscience of Addiction and Mental Health

Healthy Brain and Mind Research

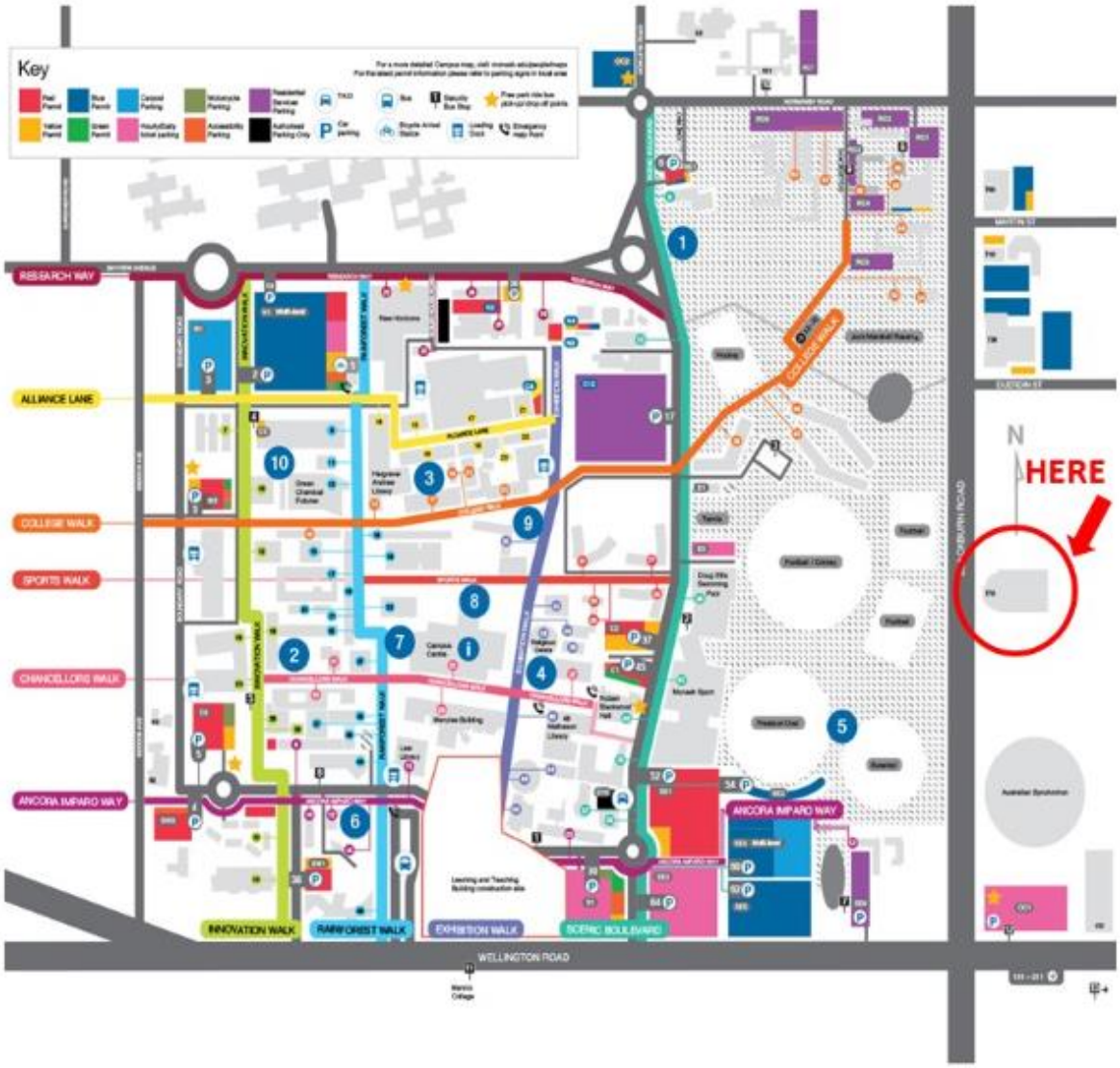
School of Behavioural & Health Sciences

Faculty of Health Sciences

Australian Catholic University

115 Victoria Pde, Fitzroy, VIC, 3065

Appendix 9. Map to Locate Scanning Facility



Appendix 10. Session Checklist

Baseline Session Checklist

Main Baseline Assessment Checklist (blinded)

Appt date: ……..
Group:
Appt time: …….. Cue Reactivity fMRI: **A** / **B**
Next Appt: …….. Attentional Bias: **a** / **b**

Wash hands & sanitize workstations, testing rooms, and laptops used by participants and research staff/students. Observe social distancing protocols & avoid close contact with team members.

Maximal face-to-face time (total)
Blinded tester & participant: 120min
Unblinded tester & participant: 60min

Examiner-led tasks Computer-led tasks

Item	Time	Task	Y/N	Administration	Time Face-to-face	Page
Introduction					**10min**	
0.1	5min	Consent & Covid Tracing Record		Face-to-face	5min	4
0.2	5min	MRI Screening form			5min	4
Toxicology - ADHERE TO MBI PROTOCOL FOR URINE SAMPLE COLLECTION					**2min**	
1.1	5-10min	Urine Sample ***drop off before 4:30pm***		Face-to-face	2min	5
Pre-MRI Tasks					**~12-17min**	
Baseline Survey – *Use Qualtrics Baseline Survey Link*						
2.1	3-5min	Demographics		Show Link	1min	7
2.2	10-15min	SCID		Face-to-face	10-15min	7
2.3	2-4min	Education		2nd Room		9
Pre-scan: *Use MRI Sandwich Qualtrics Survey Link*				Show Link	**1min**	
Error! Reference source not found.	3-5min	MCQ (1/2)		2nd Room	-	10
Error! Reference source not found.		STAI-Y (1/2)				10
Error! Reference source not found.		VAS (1/5)				10
MRI preparation & stretch					**1min**	

Error! Reference source not found.	5min	MRI preparation & stretch		Face-to-face	1min	11
Enter MRI*: See MRI Script*						
Post-MRI Tasks					**1hr 31min**	
Post-scan: *Use MRI Sandwich Qualtrics Survey Link (Cont'd)* Show Link					**1min**	
Error! Reference source not found.	3-4min	MCQ (2/2)		2nd Room	-	12
Error! Reference source not found.		STAI-Y (2/2)				12
Post-MRI – Part A: Cognition					**1hr**	
4.1	20-30min	WASI (IQ): *Use external materials*		Face-to-face	20-30min	13
4.2.1	10min	Pre-task craving question Score:___			10min	14
4.2.2		Attentional Bias Dot Probe				14
4.2.3		Post-task craving question Score:___				14
4.5	10min	N-back			10min	15
4.6	10min	Go/No-Go			10min	15
*****Planned break*** ENCOURAGE PPTs TO GO OUTSIDE AND SANTISE UPON REENTERING MBI**					**1min**	
	~ 5-10min	Show tea/coffee & note Break time: ____		Face-to-face	-	16
Post-MRI – Part B: TLFB/CUQ				**Set-up virtual screenshare**	**1min**	
5.1.1	15-20min	Timeline Follow Back – Part 1		2nd Room	-	16
5.1.2	10-15min	Timeline Follow Back – Part 2			-	16
5.1.3	10-15min	CUQ (Cannabis Hx)			-	16
Post-MRI – Part C: Questionnaires				Show Link	**1min**	
Baseline Survey Cont'd - *Use Qualtrics Baseline Survey Link*						
5.2	20-35min	AUDIT (Alcohol)		2nd Room	-	17
5.3		FNTD (Nicotine)				17
5.4		CUDIT (Cannabis)				17
5.5		OCDUS-CAN				17
5.6		CWS (Withdrawal)				17
5.7		MMQ (Marijuana Motives)				17
5.8		Sleep				17
5.9		CL (Change)				18
5.10		5F (Thoughts)				18

5.11		ERQ (Emotional Regulation)				18
5.12		PSS (Stress)				18
5.13		BDI-II (Depression)				18
5.14		AES (Apathy)				18
5.15		SF-36 (Health)				19
5.16		SUPPS-P (Impulsivity)				19
5.17		CAPE (Psychotic symptoms)				19
5.18		IPAQ (Physical activity)				19
5.19		COVID stress scale (COVID)				19
5.20		MTSS (Cannabis use)				19
5.21		SMS (Mindfulness)				19
5.22		COROTRAS (COVID)				20
Post-MRI – Part D: Planning session					**10min**	
6.1	5min	2-week Daily Plan: *Use external materials*		Face-to-face	5min	20
6.2	5min	Psych Strategy Check			5min	20
Post-MRI – Part E: Picture rating				Show Links	**1min**	
7.1	5-10min	Cue Reactivity		2nd Room	-	20
7.2	5-10min	Attentional Bias Dot Probe				21
Handover to Un-blind examiner (if cannabis)						
	Intervention *(IF CANNABIS)*				**40-50min**	
8.1	40-50min	Intervention Sandwich		Face-to-face	-	21
	Debrief *(IF CONTROL)*				**5-10min**	
9.1		Debrief		Face-to-face	2-5min	21
9.2		Reimbursement *(IF CONTROL)*			3-5min	21
	Post-Ax Tasks					
						21

Notes:

Follow-up Session Checklist

Main Follow-Up Assessment Checklist (Blind)

Appt date: ……..
Group:
Appt time: …….. Cue Reactivity fMRI: **A** / **B**

Attentional Bias: **a** / **b**

Wash hands & sanitize workstations, testing rooms, and laptops used by participants and research staff/students. Observe social distancing protocols & avoid close contact with team members.

Maximal face-to-face time (total) **Blinded tester & participant: 110min** **Unblinded tester & participant: 52min** **Blinded tester & unblinded tester: 62min**

Examiner-led tasks Computer-led tasks

Task	Time	Task	Y/N	Administration	Time Face-to-face	Page
		Handover to Blind examiner				
Pre-MRI Tasks						
Pre-scan: *Use Qualtrics MRI Sandwich survey*				Show Link	**1min**	
1.1.1	3-5min	MCQ (1/2)		2nd Room	-	3
1.1.2		STAI-Y (1/2)				3
1.1.3		VAS (3/5)				3
MRI preparation & stretch					**1min**	
1.2		MRI preparation & stretch		Face-to-face	1min	4
Enter MRI: *See MRI script*						
Post-MRI Tasks					**1hr**	
Post-scan: *Use MRI Sandwich Qualtrics Survey Link (Cont'd)* Show Link					**1min**	
1.5.1	3-4min	MCQ (2/2)		2nd Room	-	5
1.5.2		STAI-Y (2/2)				5
Post-MRI – Part A: Cognition					**30min**	
2.1.0	10min	Pre-task craving question *Score:*_____		Face-to-face	10min	6
2.1		Attentional Bias Task				6
2.1.1		Pre-task craving question *Score:*_____				6
2.2	10min	N-back			10min	6
2.3	10min	Go/No-Go			10min	6
*****Planned Break*** ENCOURAGE PPTs TO GO OUTSIDE AND SANTISE UPON REENTERING MBI**					**1min**	
	5-10min	Show tea/coffee & note Break time: _____		Face-to-face	-	7
Post-MRI – Part B: TLFB					**15min**	
3.1.1	10min	Timeline Follow Back – Part 1		Face-to-face	10min	7

3.1.2	5min	Timeline Follow Back – Part 2			5min	7
Post-MRI – Part C: Questionnaires				Show Link	**1min**	
Follow-up Survey - *Use Qualtrics Follow-up Survey Link*						
3.2	15-25min	FNTD – (Nicotine)		2nd Room	-	8
3.3		CUDIT (Cannabis)				8
3.4		OCDUS-CAN				8
3.5		CWS (Withdrawal)				8
3.6		Sleep				8
3.7		CL (Change)				8
3.8		5F (Thoughts)				8
3.9		ERQ (Emotional Regulation)				9
3.10		PSS (Stress)				9
3.11		BDI-II (Depression)				9
3.13		SUPPS-P (Impulsivity)				9
Error! Reference source not found.		IPAQ (Physical activity)				10
3.15		COVID stress scale (COVID)				10
3.16		MTSS (Cannabis use)				10
3.17		SMS (Mindfulness)				10
3.18		COROTRAS (COVID)				10
Post-MRI – Part D: Picture rating				Show Links	**1min**	
4.1	5min	Cue Reactivity		2nd Room	-	10
4.2	5min	Attentional Bias				11
Handover back to Un-blind examiner						

Notes:

Appendix 11. Audio Scripts for MBI and Active Placebo Groups

PART A

TRACK 1/3 (A & B) 3 MIN 40 SEC	
MBI (Mindfulness) **TRACK 1_A**	**Active Placebo (Relaxation)** **TRACK 1_B**
Introduction about 30 sec	
In this recording you will learn about a strategy for managing craving or urges to smoke cannabis. [2 sec] This strategy can be used whenever you experience a difficult feeling, but here we are thinking specifically about how to manage craving for cannabis. [2 sec] First there will be an explanation about what this strategy involves [1 sec] and then you'll have a chance to practice it briefly before the main task. [3 sec]	In this recording you will learn about a strategy for managing craving or urges to smoke cannabis. [2 sec] This strategy can be used whenever you experience a difficult feeling, but here we are thinking specifically about how to manage craving for cannabis. [2 sec] First there will be an explanation about what this strategy involves [1 sec] and then you'll have a chance to practice it briefly before the main task. [3 sec]
"An explanation of the strategy" [about 3 min]	
When we notice a strong desire for something, like a favourite food or drink or drug, especially if it's right in front of us, it is often the case that we will simply consume it without too much thought. This is a kind of automatic response. We do not notice how full or hungry we are but just respond to stimuli automatically. [2 sec] A similar thing can happen with cannabis, leading to over-consumption and occasionally, to more serious problems related to smoking cannabis. [2 sec] We may be responding automatically to external events, such as seeing someone smoking cannabis, or we may be responding automatically to internal negative feelings in our bodies. [3 sec]	When we notice a strong desire for something, like a favourite food or drink or drug, especially if it's right in front of us, it is often the case that we will simply consume it without too much thought. This is a kind of automatic response. We do not notice how full or hungry we are but just respond to stimuli automatically. [2 sec] A similar thing can happen with cannabis, leading to over-consumption and occasionally to more serious problems related to smoking cannabis. [2 sec] We may be responding automatically to external events such as seeing someone smoking cannabis, or we may be responding automatically to internal negative feelings in our bodies. [3 sec]
A craving or urge to smoke cannabis is generally experienced as a feeling in the body, that can be accompanied by thoughts like "I could really do with a smoke right now". Craving is often related to stress and negative feelings, like anxiety. Experiencing craving, stress and uncomfortable bodily sensations, can lead to automatic smoking. [4 sec] **Being in touch with and aware of your thoughts, feelings and bodily sensations** - can help you **experience cravings in a different way**. [4 sec] **Noticing your thoughts, and what sensations are currently being felt in your body can help you experience craving as a temporary event in the body.** [2 sec] **Paying attention to the exact experiences and processes, that are going through your body and mind, can help you tolerate** your cravings, without having to act on them. [2 sec]	A craving or urge to smoke cannabis is generally experienced as a feeling in the body, that can be accompanied by thoughts like "I could really do with a smoke right now". Craving is often related to stress and negative feelings, like anxiety. Experiencing craving, stress and uncomfortable bodily sensations, can lead to automatic smoking. [4 sec] **Softening the muscles in your body - and calming and unwinding your mind** - can help you **reduce your** craving. [4 sec] **Releasing tension in your body can help you reduce the intensity of your cravings by helping you experience them less intensely in the body.** [2 sec] **Easing-up and de-stressing the tense feelings in your body, and reaching a state of tranquillity, can help you to control** the intensity of your cravings, reducing the need to act on them. [2 sec]

Some people find that **noticing, paying attention to, and accepting** what's going on inside their minds and bodies, **without trying to change these experiences** – can help them **experience cravings, in a different way** – in a way that does not automatically lead to smoking. [3 sec]	Some people find that **calming** and unwinding what's going on inside their minds **and releasing and easing up the tension from their bodies** - can help them to **reduce their craving levels -** in a way that does not automatically lead to smoking. [3 sec]
The main benefits of **noticing and being aware of your thoughts and bodily sensations,** are believed to lie, in a greater ability to **understand** that unpleasant, thoughts and feelings **come and go, like clouds in the sky**. [2 sec] You begin to **realise that you do not have to get caught up in them – you can just allow** unpleasant thoughts and feelings**,** to **come, to stay for as long as they will**, and eventually begin to experience them in a different way. [1 sec]	The main benefits of **calming down, and de-stressing your mind and releasing the tension in your body,** are believed to lie in a greater ability to calm and **reduce** strong, unpleasant, **or unwanted** feelings**, sensations** and thoughts that arise. [2 sec] You begin to **develop the ability to deliberately release tension from your body and to calm down your mind, and find, that with practice, these** unpleasant feelings, sensations, and thoughts will gradually change**,** and **decrease**, and eventually**,** they may even disappear. [1 sec]
The key thing is **allowing** yourself to **fully experience** bodily reactions, and thoughts, **without trying to get rid of them**, and without automatically reacting to them. [1 sec] This can be achieved by the simple method, of **observing** your thoughts and feelings, **with curiosity**, **without analysing or judging them.** [3 sec] This leads to **greater acceptance** of difficult experiences and the ability to respond to them more purposefully.	The key thing is **transforming** your bodily reactions, emotions, and thoughts, **to more calming experiences so that they are less unpleasant**, so you do not have to automatically respond to them. [1 sec] This can be achieved by the simple method of **soothing** your thoughts, and **loosening up**, **any tension from your muscles.** [3 sec] This leads to difficult thoughts, feelings, and sensations **changing into less unpleasant ones.**
TRACK 2/3 (A & B) 3 MIN 59 SEC **Strategy practice**	
Mindfulness **TRACK_2A**	**Relaxation** **TRACK_2B**
[1 sec] Let's see how this approach might work in practice. Start by letting your eyes gently close or fix them on the floor in front of you. Take a moment and **notice the sensations of sitting on the chair. [1 sec] Maybe notice the parts of your body in contact with the chair**. [pause 3 sec] **Notice the sensations in those parts of** your body. [2 sec] **Notice the sensations in** your legs and in your feet, where they make contact with your shoes, and the floor [pause 5 seconds]. **Notice sensations** in other parts of your body. **[pause 5 sec]**	[1 sec] Let's see how this approach might work in practice. Start by letting your eyes gently close or fix them on the floor in front of you. Take a moment **to adopt a calm state of mind and a relaxed posture. [1 sec] Make sure you are sitting in a comfortable position in the chair and relax and unwind your mind**. [pause 3 sec] **Loosen up any stiffness that you feel** in your body. [2 sec] **Start by releasing tension from the muscles** in your legs and feet and then **ease and soften** other parts in your body [pause 5 seconds].
Now imagine that you have cannabis with you: your favourite kind of cannabis. Imagine that your favourite kind of cannabis is in front of you. Concentrate fully on this image, get caught up in it, bring it to life as if it's right in front of you, and give it your full attention.	*Now imagine that you have cannabis with you: your favourite kind of cannabis. Imagine that your favourite kind of cannabis is in front of you. Concentrate fully on this image, get caught up in it, bring it to life as if it's right in front of you, and give it your full attention.*

Imagine holding the cannabis; it's as if it's really there. Imagine the smell. Now imagine preparing it so you can smoke it. And now imagine getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it, and exhale. Immerse yourself in this experience and the different sensations [3 sec].	*Imagine holding the cannabis; it's as if it's really there. Imagine the smell. Now imagine preparing it so you can smoke it. And now imagine getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it, and exhale. Immerse yourself in this experience and the different sensations [3 sec].*
As you keep this image in mind you, may notice some craving or urges to smoke. [2 sec] As you **notice** these feelings, focus your **attention inward,** on those feelings. Allow your **attention to scan the sensations throughout your body.** [3 sec] **Notice** where in your body you experience the craving, or any difficult feeling, and **what the sensations are like**. **Notice** fully each area in your body where you experience the urge and simply tell yourself **what you are experiencing**. For example, you might say, "**I feel my craving**, **in my abdomen"**, **or**, **"I feel my craving**, **in my chest".** **Focus** on the area in your body where you are experiencing the craving most strongly. **Notice the exact sensations in that area. [1 sec] How does it feel? Is it hot, cold, tingly, or numb? Perhaps there is another word to describe the feeling, that you are noticing? [1 sec] Are your muscles tense or relaxed? [1 sec] How large an area of your body is involved? [1 sec]** **Notice the craving sensations, stay with them, and describe them to yourself**. [pause 5 sec] **Notice how the sensations change in your body: how they change in shape or location, or intensity. [1 sec] Do not struggle against the feelings; allow yourself to experience them and follow the way they shift and change.** [3 sec]	As you keep this image in mind you, may start feeling craving and urges to smoke. [2 sec] As you **have** these feelings, focus on **calming your body**. Allow your **body to feel more and more loose and at ease.** [3 sec] As you experience craving, or any difficult feeling in your body, **just loosen and untense your muscle**s and allow yourself to relax fully. When you experience an urge, simply tell yourself **to relax and think relaxing thoughts**. For example, you might say, **"I am managing my craving**, **by relaxing my muscles"**, **or**, **"I am managing my craving**, **by calming my mind".** Try to **relax** the area in your body where you are experiencing the craving most strongly. **Start by taking a few slow deep breaths….. [1 sec] Slowly breathe in through your nostrils and breathe out from your mouth. [1 sec] As you breathe out, release any tension that you may be experiencing. [1 sec] Allow the muscles to feel more and more loose and relaxed in other parts of your body. [1 sec]** **Calm each area where you experience craving,** [pause 5 sec]. **Continue to take slow and deep breaths… As you breathe out unwinding your mind, and releasing any further tension, felt in your body. [1 sec] Allow any feelings, to change to more calming and less unpleasant ones.** [3 sec]
The purpose of this exercise is **not to make the craving go away**, **but to experience craving**, **in a different way**, **and learn that these feelings can be accepted**, **and tolerated**, **rather than acted upon**. [**16 sec silence till the end]**	The purpose of this exercise **is to reduce the craving**, **and change the unpleasant experience of the craving**, **into a less unpleasant one, through releasing tension in the muscles**, **and calming and unwinding the mind**. [**16 sec silence till the end]**

TRACK 3/3 (A & B) 7 MIN 20 SEC **Main task/main strategy practice**	
Mindfulness **TRACK_3A**	**Relaxation** **TRACK_3B**
Now we are going to practice the strategy again with a bit more detail and depth. While doing this exercise, your attention will probably wander from time to time. In fact, this is	Now we are going to practice the strategy again with a bit more detail and depth. While doing this exercise, your attention will probably wander from time to time. In fact, this is

quite normal, and it may happen repeatedly **but try not to get caught up in these different, unrelated thoughts**. pause]. **Each time you notice your mind wandering; take a second to notice this and bring yourself back to the present experience of thoughts, feelings, and sensations** [pause 5 seconds].	quite normal, and it may happen repeatedly, **but try not to get too distracted and continue to calm the mind** [pause]. **Just allow your body to continue to be relaxed by softening any tension and by letting your mind to continue to unwind and slow down** [pause 5 seconds].
To start, let your eyes gently close, or fix them on a point in front of you. Try to sit in a way that ensures that you are **awake and alert**. The idea is not necessarily to **become relaxed.** The main idea is to be **awake and attentive** to fully notice and focus on what you experience in your body and mind. This will enable you to **learn how to experience craving without reacting to it**.	To start, let your eyes gently close, or fix them on a point in front of you. Try to sit in a way that ensures that you are **comfortable and tranquil**. The main idea is to learn how to deliberately **become relaxed, calm, and at ease**. This will enable you to fully **release tension from your body and unwind your mind, so that you can change how you experience cravings and reduce the intensity of them**.
As before, take a moment now to **notice the sensation of sitting in the chair** [pause]. Start **to notice where each part of your body touches the chair and feel your feet on the ground** [pause 5 seconds].	As before, take a moment now to **adopt a calm state of mind** [pause]. **Make sure you sit in a comfortable position in the chair and relax any tension that you feel in your body** [pause 5 seconds].
Now take a slow and deep breath and **direct your attention to focus on the physical sensations of your breath** [pause 5 seconds]. You don't need to do anything special with your breathing. Simply **notice the rise and fall of your chest or abdomen as you breathe in through your nose and gently breathe out.** [pause 5 seconds]. As you breathe in **notice** the cool air coming into your nostrils [pause], and the warm air as you breathe out.	Now take a slow and deep breath. **As you breathe in, naturally allow your belly to rise, and to fall, as you breathe out, making sure that it feels comfortable** [pause 5 seconds]. B**reathe in through your nose and gently breathe out.** [pause 5 seconds.] **Feel relaxed and calm through your body and mind.** B**reathe in through your nose and gently breathe out.** **Feel calm** as you breathe in [pause], and feel any tension leave as you breathe out.
Now again, imagine that you have your favourite kind of cannabis with you. Imagine holding the cannabis; as if it's really there. Imagine the smell.. Now imagine preparing it so you can smoke it. And now imagine that it is ready to smoke, and that now you are getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it, [1 sec] and exhale it. Immerse yourself in this experience and the different sensations [3 sec].	*Now again, imagine that you have your favourite kind of cannabis with you. Imagine holding the cannabis; as if it's really there. Imagine the smell.. Now imagine preparing it so you can smoke it. And now imagine that it is ready to smoke, and that now you are getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it [1 sec] and exhale it. Immerse yourself in this experience and the different sensations [3 sec].*
Become aware of whatever you are experiencing in this moment as you imagine this scene, even if it is difficult or unpleasant. In fact, it is important especially in such moments **to be open hearted and non-reactive as you notice and observe the sensations and thoughts the best you can** [pause]. **Let go of the tendency that we all have to want things to be different from how they are right now and allow things to be exactly as you find them** [5 seconds pause].	As you imagine this scene **you may** experience difficult or unpleasant thoughts or sensations. **Try to wind down your mind and release any tension from your body completely.** In fact, it is important especially in such moments **to ease any stiffness in your muscles and calm any thoughts that may be distressing in your mind** [pause]. **If you feel tension, try and release it and make yourself feel more at ease and relaxed, in order to**

	allow things to be less unpleasant. [5 seconds pause].
Returning to the experience of smoking your favourite kind of cannabis - and the different sensations - you may start to feel some craving or urges to smoke. As you **notice** these feelings, focus **your attention inward on those feelings**. Allow your **attention to scan the sensations throughout your body.** **Notice** where in your body you experience the craving or any difficult feelings and **what the sensations are like**. **Notice** fully each area where you experience the urge and simply tell yourself **what you are experiencing**. For example, you might say to yourself **"I feel my craving in my abdomen" or "I feel my craving in my chest".** **Focus** on one area where you are experiencing the craving most vividly. **Notice the exact sensations in that area. How does it feel? Is it hot, cold, tingly, or numb? Perhaps there is another word to describe the feeling you are noticing? Are your muscles tense or relaxed? How large an area of your body is involved?** **Notice the sensations, stay with them and describe them to yourself.** [pause] **Notice also how the sensations change in your body: how they change in shape or location or intensity. Do not struggle against the feelings; allow them and follow the way they shift and change.** **Become aware of any thoughts about craving you might be having. Describe them to yourself [pause]. Do not try to suppress the thoughts; allow them and notice how they come and go.**	Returning to the experience of smoking your favourite kind of cannabis - and the different sensations - you may start to feel some craving and urges to smoke. As you **have** these feelings, focus **on softening your body.** Allow your **body to feel more and more loose and at ease.** As you experience craving, or any difficult feeling in your body, **just loosen and untense your muscles and allow yourself to relax fully.** When you experience an urge, simply tell yourself **to relax and think relaxing thoughts**. For example, you might say, **"I am managing my craving**, **by calming my muscles"**, **or**, **"I am managing my craving**, **by thinking relaxing thoughts".** **Relax** the area where you are experiencing the craving most vividly. **Take a few slow and deep breaths…. As you breathe out, release any tension that you may experience. Allow your muscles to feel more and more loose and floppy in all the parts of your body, paying particular attention to the tenser areas.** **Calm each area where you experience tension and difficult feelings. Allow any unpleasant thoughts to be calmed down** [pause]. **Continue to take slow and deep breaths… As you breathe out continue to unwind your mind and release any further tension felt in your body. Allow any thoughts and feelings to change to more calming and less unpleasant ones [pause]. Allow yourself to soften and feel relaxed. Continue to take slow deep breaths… With each exhale feel calm and relaxed.**
Repeat by **focusing** on each part of your body that experiences the craving. **Pay attention to and describe to yourself the changes that occur in the sensations**. **Notice how the urges come and go.** Remember, the purpose of this exercise is **not to make the craving go away but to experience it in a different way and learn that these thoughts and feelings can be accepted and tolerated rather than acted upon** [30 secs].	Repeat **releasing the tension** from each part of your body that experiences craving. **Calm down your entire body and let the muscles loosen up gradually**. **Take a few more deep breaths in order to reduce the tension.** Remember, the purpose of this exercise **is to reduce the craving and change the feelings of craving into less unpleasant ones, through releasing tension all through the muscles in the body and calming the mind** [30 secs].
And now bring your attention back to the room, gently open your eyes if they were closed. **Notice what you can see, notice what you can hear** [pause]. ***Remember that if or when you experience craving or urges to smoke cannabis, you can refrain from it by using the strategies you have been taught.***	And now bring your attention back to the room, open your eyes if they were closed. **You can stretch and move the different parts of your body** [pause]. ***Remember that if or when you experience craving or urges to smoke cannabis, you can refrain from it by using the strategies you have been taught.***

Notice and observe your thoughts, feelings, and any physical reactions non-judgmentally as they arise. Allow them to be there, notice how they come and go like clouds in the sky.	***Use slow, deep breaths and release any tension in your body as it arises. Allow all your muscles to relax and allow your mind to feel calm and at ease.***

PART B – SCRIPT TO RECORD FOR TAKE HOME AUDIO TRACK

HOME PRACTICE AUDIO	
Main task/main strategy practice [exactly as TRACK 3 7 MIN 20 SEC]	
Mindfulness [exactly as TRACK_3A except highlighted intro]	**Relaxation [exactly as TRACK_3B except highlighted intro]**
Welcome back, we are going to practice the strategy again. While doing this exercise, your attention will probably wander from time to time. In fact, this is quite normal, and it may happen repeatedly **but try not to get caught up in these different, unrelated thoughts**. pause]. **Each time you notice your mind wandering; take a second to notice this and bring yourself back to the present experience of thoughts, feelings, and sensations** [pause 5 seconds].	Welcome back, we are going to practice the strategy again. While doing this exercise, your attention will probably wander from time to time. In fact, this is quite normal, and it may happen repeatedly, **but try not to get too distracted and continue to calm the mind** [pause]. **Just allow your body to continue to be relaxed by softening any tension and by letting your mind to continue to unwind and slow down** [pause 5 seconds].
To start, let your eyes gently close, or fix them on a point in front of you. Try to sit in a way that ensures that you are **awake and alert**. The idea is not necessarily to **become relaxed.** The main idea is to be **awake and attentive** to fully notice and focus on what you experience in your body and mind. This will enable you to **learn how to experience craving without reacting to it**.	To start, let your eyes gently close, or fix them on a point in front of you. Try to sit in a way that ensures that you are **comfortable and tranquil**. The main idea is to learn how to deliberately **become relaxed, calm, and at ease**. This will enable you to fully **release tension from your body and unwind your mind, so that you can change how you experience cravings and reduce the intensity of them**.
As before, take a moment now to **notice the sensation of sitting in the chair** [pause]. Start **to notice where each part of your body touches the chair and feel your feet on the ground** [pause 5 seconds].	As before, take a moment now to **adopt a calm state of mind** [pause]. **Make sure you sit in a comfortable position in the chair and relax any tension that you feel in your body** [pause 5 seconds].
Now take a slow and deep breath and **direct your attention to focus on the physical sensations of your breath** [pause 5 seconds]. You don't need to do anything special with your breathing. Simply **notice the rise and fall of your chest or abdomen as you breathe in through your nose and gently breathe out.** [pause 5 seconds]. As you breathe in **notice** the cool air coming into your nostrils [pause], and the warm air as you breathe out.	Now take a slow and deep breath. **As you breathe in, naturally allow your belly to rise, and to fall, as you breathe out, making sure that it feels comfortable** [pause 5 seconds]. B**reathe in through your nose and gently breathe out.** [pause 5 seconds.] **Feel relaxed and calm through your body and mind.** B**reathe in through your nose and gently breathe out.** **Feel calm** as you breathe in [pause], and feel any tension leave as you breathe out.
Now again, imagine that you have your favourite kind of cannabis with you. Imagine holding the cannabis; as if it's really there. Imagine the smell..	*Now again, imagine that you have your favourite kind of cannabis with you. Imagine holding the cannabis; as if it's really there. Imagine the smell..*

Now imagine preparing it so you can smoke it. And now imagine that it is ready to smoke, and that now you are getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it, [1 sec] and exhale it. Immerse yourself in this experience and the different sensations [3 sec].	*Now imagine preparing it so you can smoke it. And now imagine that it is ready to smoke, and that now you are getting ready to smoke it. Bring it to your lips, and breathe it in [1 sec], inhaling deeply. Sense how it feels to smoke it, feeling it in your chest [pause] and the taste in your mouth. Inhale it [1 sec] and exhale it. Immerse yourself in this experience and the different sensations [3 sec].*
Become aware of whatever you are experiencing in this moment as you imagine this scene, even if it is difficult or unpleasant. In fact, it is important especially in such moments **to be open hearted and non-reactive as you notice and observe the sensations and thoughts the best you can** [pause]. **Let go of the tendency that we all have to want things to be different from how they are right now and allow things to be exactly as you find them** [5 seconds pause].	As you imagine this scene **you may** experience difficult or unpleasant thoughts or sensations. **Try to wind down your mind and release any tension from your body completely.** In fact, it is important especially in such moments **to ease any stiffness in your muscles and calm any thoughts that may be distressing in your mind** [pause]. **If you feel tension, try and release it and make yourself feel more at ease and relaxed, in order to allow things to be less unpleasant.** [5 seconds pause].
Returning to the experience of smoking your favourite kind of cannabis - and the different sensations - you may start to feel some craving or urges to smoke. As you **notice** these feelings, focus **your attention inward on those feelings**. Allow your **attention to scan the sensations throughout your body.** **Notice** where in your body you experience the craving or any difficult feelings and **what the sensations are like**. **Notice** fully each area where you experience the urge and simply tell yourself **what you are experiencing**. For example, you might say to yourself **"I feel my craving in my abdomen" or "I feel my craving in my chest".** **Focus** on one area where you are experiencing the craving most vividly. **Notice the exact sensations in that area. How does it feel? Is it hot, cold, tingly, or numb? Perhaps there is another word to describe the feeling you are noticing? Are your muscles tense or relaxed? How large an area of your body is involved?** **Notice the sensations, stay with them and describe them to yourself.** [pause] **Notice also how the sensations change in your body: how they change in shape or location or intensity. Do not struggle against the feelings; allow them and follow the way they shift and change.** **Become aware of any thoughts about craving you might be having. Describe them to yourself [pause]. Do not try to suppress the thoughts; allow them and notice how they come and go.**	Returning to the experience of smoking your favourite kind of cannabis - and the different sensations - you may start to feel some craving and urges to smoke. As you **have** these feelings, focus **on softening your body.** Allow your **body to feel more and more loose and at ease.** As you experience craving, or any difficult feeling in your body, **just loosen and untense your muscles and allow yourself to relax fully.** When you experience an urge, simply tell yourself **to relax and think relaxing thoughts**. For example, you might say, **"I am managing my craving**, **by calming my muscles"**, **or**, **"I am managing my craving**, **by thinking relaxing thoughts".** **Relax** the area where you are experiencing the craving most vividly. **Take a few slow and deep breaths….As you breathe out, release any tension that you may experience. Allow your muscles to feel more and more loose and floppy in all the parts of your body, paying particular attention to the tenser areas.** **Calm each area where you experience tension and difficult feelings. Allow any unpleasant thoughts to be calmed down** [pause]. **Continue to take slow and deep breaths… As you breathe out continue to unwind your mind and release any further tension felt in your body. Allow any thoughts and feelings to change to more calming and less unpleasant ones [pause]. Allow yourself to soften and feel relaxed. Continue to take slow**

	deep breaths… With each exhale feel calm and relaxed.
Repeat by **focusing** on each part of your body that experiences the craving. **Pay attention to and describe to yourself the changes that occur in the sensations**. **Notice how the urges come and go.** Remember, the purpose of this exercise is **not to make the craving go away but to experience it in a different way and learn that these thoughts and feelings can be accepted and tolerated rather than acted upon** [pause 30 secs].	Repeat **releasing the tension** from each part of your body that experiences craving. **Calm down your entire body and let the muscles loosen up gradually**. **Take a few more deep breaths in order to reduce the tension.** Remember, the purpose of this exercise **is to reduce the craving and change the feelings of craving into less unpleasant ones, through releasing tension all through the muscles in the body and calming the mind** [pause 30 secs].
And now bring your attention back to the room, gently open your eyes if they were closed. **Notice what you can see, notice what you can hear** [pause]. ***Remember that if or when you experience craving or urges to smoke cannabis, you can refrain from it by using the strategies you have been taught.*** ***Notice and observe your thoughts, feelings, and any physical reactions non-judgmentally as they arise. Allow them to be there, notice how they come and go like clouds in the sky.***	And now bring your attention back to the room, open your eyes if they were closed. **You can stretch and move the different parts of your body** [pause]. ***Remember that if or when you experience craving or urges to smoke cannabis, you can refrain from it by using the strategies you have been taught.*** ***Use slow, deep breaths and release any tension in your body as it arises. Allow all your muscles to relax and allow your mind to feel calm and at ease.***

Appendix 12. MRI Data Acquisition Parameters

SIEMENS MAGNETOM Skyra

\\USER\Valentina\new_protocol_use_this\Brain_cann\t1_mprage_sag_p2_iso_1_ADNI

TA: 5:12 PM: FIX Voxel size: 1.0×1.0×1.0 mmPAT: 2 Rel. SNR: 1.00 : tfl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	50 %
Position	R1.2 P13.1 F17.2 mm
Orientation	S > C2.3 > T0.3
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	16.7 %
Slices per slab	192
FoV read	256 mm
FoV phase	93.8 %
Slice thickness	1.00 mm
TR	2300.0 ms
TE	2.07 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast - Common

TR	2300.0 ms
TE	2.07 ms
Magn. preparation	Non-sel. IR
TI	900 ms
Flip angle	9 deg
Fat suppr.	None
Water suppr.	None

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution - Common

FoV read	256 mm
FoV phase	93.8 %
Slice thickness	1.00 mm
Base resolution	256
Phase resolution	100 %
Slice resolution	100 %
Phase partial Fourier	Off
Slice partial Fourier	Off
Interpolation	Off

Resolution - iPAT

PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	32
Accel. factor 3D	1
Reference scan mode	Integrated

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	50 %
Position	R1.2 P13.1 F17.2 mm
Orientation	S > C2.3 > T0.3
Phase enc. dir.	A >> P
Slice oversampling	16.7 %
Slices per slab	192
FoV read	256 mm
FoV phase	93.8 %
Slice thickness	1.00 mm
TR	2300.0 ms
Multi-slice mode	Single shot
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
Position	R1.2 P13.1 F17.2 mm
Orientation	S > C2.3 > T0.3
Phase enc. dir.	A >> P
AutoAlign	---
Initial Position	R1.2 P13.1 F17.2
R	1.2 mm
P	13.1 mm
F	17.2 mm
Initial Rotation	0.00 deg
Initial Orientation	S > C
S > C	2.3
> T	0.3

Geometry - Navigator

Geometry - Tim Planning Suite

Set-n-Go Protocol	Off
Table position	H
Table position	0 mm
Inline Composing	Off

System - Miscellaneous

Positioning mode	FIX

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System - Miscellaneous

Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
AutoAlign	---
Coil Select Mode	Off - AutoCoilSelect

System - Adjustments

B0 Shim mode	Tune up
B1 Shim mode	TrueForm
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	263 mm
R >> L	350 mm
F >> H	350 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm
Excitation	Non-sel.

System - Tx/Rx

Frequency 1H	123.251913 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2300.0 ms
Concatenations	1

Physio - Cardiac

Magn. preparation	Non-sel. IR
TI	900 ms
Fat suppr.	None
Dark blood	Off
FoV read	256 mm
FoV phase	93.8 %
Phase resolution	100 %

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

Subtract	Off
Measurements	1

Inline - Common

StdDev	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Composing

Inline Composing	Off
Distortion Corr.	Off

Inline - MapIt

Save original images	On
MapIt	None
Flip angle	9 deg
Measurements	1
TR	2300.0 ms
TE	2.07 ms

Sequence - Part 1

Introduction	On
Dimension	3D
Elliptical scanning	Off
Reordering	Linear
Asymmetric echo	Allowed
Flow comp.	No
Multi-slice mode	Single shot
Echo spacing	6.3 ms
Bandwidth	230 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Normal
Excitation	Non-sel.
RF spoiling	On
Incr. Gradient spoiling	Off
Turbo factor	224

Sequence - Assistant

Mode	Off

SIEMENS MAGNETOM Skyra

\\USER\Valentina\new_protocol_use_this\Brain_cann\A-P_Resting_ep2d_p2_3mm
TA: 8:02 PM: FIX Voxel size: 3.0×3.0×3.0 mmPAT: 2 Rel. SNR: 1.00 : epfid

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	On
Start measurements	Single measurement

Routine

Slice group	1
Slices	44
Dist. factor	0 %
Position	R0.6 P18.9 F11.5 mm
Orientation	T > C-16.2 > S1.3
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	2500 ms
TE	30.0 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast - Common

TR	2500 ms
TE	30.0 ms
MTC	Off
Flip angle	90 deg
Fat suppr.	Fat sat.

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	189
Delay in TR	0 ms
Multiple series	Off

Resolution - Common

FoV read	192 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
Base resolution	64
Phase resolution	100 %
Phase partial Fourier	Off
Interpolation	Off

Resolution - iPAT

Accel. mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24

Resolution - iPAT

Reference scan mode	EPI/separate

Resolution - Filter Image

Distortion Corr.	Off
Prescan Normalize	On

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
Hamming	Off

Geometry - Common

Slice group	1
Slices	44
Dist. factor	0 %
Position	R0.6 P18.9 F11.5 mm
Orientation	T > C-16.2 > S1.3
Phase enc. dir.	A >> P
FoV read	192 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	2500 ms
Multi-slice mode	Interleaved
Series	Interleaved
Concatenations	1

Geometry - AutoAlign

Slice group	1
Position	R0.6 P18.9 F11.5 mm
Orientation	T > C-16.2 > S1.3
Phase enc. dir.	A >> P
AutoAlign	---
Initial Position	R0.6 P18.9 F11.5
R	0.6 mm
P	18.9 mm
F	11.5 mm
Initial Rotation	0.00 deg
Initial Orientation	T > C
T > C	-16.2
> S	1.3

Geometry - Saturation

Fat suppr.	Fat sat.
Special sat.	None

Geometry - Tim Planning Suite

Set-n-Go Protocol	Off
Table position	H
Table position	0 mm
Inline Composing	Off

System - Miscellaneous

Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Sum of Squares

SIEMENS MAGNETOM Skyra

System - Miscellaneous

Matrix Optimization	Off
AutoAlign	---
Coil Select Mode	Off - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
B1 Shim mode	TrueForm
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	R0.6 P18.9 F11.5 mm
Orientation	T > C-16.2 > S1.3
Rotation	0.00 deg
A >> P	192 mm
R >> L	192 mm
F >> H	132 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm

System - Tx/Rx

Frequency 1H	123.251913 MHz
Correction factor	1
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2500 ms
Concatenations	1

BOLD

GLM Statistics	Off
Dynamic t-maps	Off
Ignore meas. at start	0
Ignore after transition	0
Model transition states	Off
Temp. highpass filter	Off
Threshold	4.00
Paradigm size	20
Meas[1]	Baseline
Meas[2]	Baseline
Meas[3]	Baseline
Meas[4]	Baseline
Meas[5]	Baseline
Meas[6]	Baseline
Meas[7]	Baseline
Meas[8]	Baseline
Meas[9]	Baseline
Meas[10]	Baseline
Meas[11]	Active
Meas[12]	Active
Meas[13]	Active
Meas[14]	Active
Meas[15]	Active
Meas[16]	Active
Meas[17]	Active

BOLD

Meas[18]	Active
Meas[19]	Active
Meas[20]	Active
Motion correction	Off
Spatial filter	Off
Measurements	189
Delay in TR	0 ms
Multiple series	Off

Sequence - Part 1

Introduction	On
Multi-slice mode	Interleaved
Free echo spacing	Off
Echo spacing	0.65 ms
Bandwidth	1776 Hz/Px

Sequence - Part 2

EPI factor	64
RF pulse type	Normal
Gradient mode	Fast