Mapping brain function associated with cue-reactivity and changes pre-to-post a mindfulness-based intervention in cannabis use disorder

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Mapping Brain Function Associated with Cue-Reactivity and Changes Pre-to-Post a Mindfulness-Based Intervention in Cannabis Use Disorder

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Declaration of Authorship and Sources

This thesis contains no material that has been extracted in whole or in 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: 

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"You can't stop the waves,
but you can learn to surf"
-Jon Kabat-Zinn[1]

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[1]Kabat-Zinn, J. (1994). Wherever you go, there you are: Mindfulness meditation in everyday life: New York: Hyperion

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Abstract

Globally, cannabis is used by ~210 million people and 10-to-30% endorse symptoms consistent with a cannabis use disorder (CUD), which constitutes a substantial social burden including health and treatment services. CUD is characterised by a loss of control over cannabis consumption despite significant adverse outcomes including strong cravings when exposed to cannabis cues. Such outcomes have been (partly) ascribed to altered brain function in addiction related pathways.

Preliminary functional magnetic resonance imaging (fMRI) evidence in cannabis users, show different brain activity when exposed to cannabis (vs neutral) cues, in prefrontal, striatal and parietal regions. However, no study has examined cannabis users with a DSM-5 diagnosis of CUD, or tested if psychological interventions targeting cravings (e.g., mindfulness-based interventions [MBI]) reduce neural cue-reactivity in CUD. This thesis comprises three studies aimed to examine brain activity during cannabis cue-reactivity in cannabis users and CUD, and whether such activity can be reduced with a MBI.

Study 1 was a systematic review of the fMRI literature on brain function during cue-reactivity in cannabis users. It synthesised findings on brain function during fMRI cue-reactivity tasks (cannabis vs neutral stimuli) in regular cannabis users, and their association with behavioural variables (e.g., craving). Eighteen studies showed that cannabis users had greater activity in prefrontal, striatal, and parietal regions, some of which (orbitofrontal cortex [OFC]) correlated with and greater subjective craving. The literature was limited by the lack of assessment of CUD using the DSM-5 and the inclusion of a non-using control group.

Study 2 aimed to examine differences in brain activity during a cue-reactivity task (cannabis vs neutral images), i) between 49 adults with moderate-to-severe CUD and 30 controls; and ii) their association with craving, cannabis exposure and mental health. CUD vs

controls had greater activity in the lingual gyrus (*FWE*-corrected $p < .05$, $k > 10$), and in the MFG, medial OFC, and cerebellum (uncorrected, $p < .001$, $k > 10$). Greater MFG activity correlated with more past month cannabis grams.

Overall, the findings from this thesis provide novel information on the current understanding of the neural correlates of cannabis cue-reactivity in CUD. The results of the first two studies suggest that CUD has a (partly) overlapping neurobiology with that of other SUDs as per prominent neuroscientific theories of addiction. Different brain function during cannabis cue-reactivity may reflect alterations in reward processing, including salience evaluation and attention pathways resulting from regular exposure to cannabis/related cues; or predating CUD. As such, interventions that target these regions may be effective at reducing cue-reactivity/craving in CUD.

Study 3 was a double-blind fMRI experiment. It aimed to investigate for the first time if a brief MBI compared to both an active relaxation and passive no intervention placebo controls, reduces neural cue-reactivity in the regions of interest (ROIs) functionally different in Study 2 (i.e., MFG, OFC, lingual gyrus and cerebellum), in the same sample with CUD ($N = 40$). It also explored if changes in brain activity pre-to-post MBI were associated with changes in behaviour. It was hypothesised that the greater activity in the ROIs would significantly decrease pre-to-post the MBI only. A significant decrease in the activity of the OFC was observed pre-to-post all three interventions, as well as in subjective craving and arousal rating of cannabis images. No correlations emerged.

Overall, the findings from the research in this thesis demonstrates that cannabis cue-reactivity in CUD is associated with different activity in selected brain pathway implicated in salience and reward processing; and the activity of some of these regions (e.g. OFC) can be reduced during a brief engagement with monitoring of daily cannabis use, cravings and mood. More research in larger samples is required to identify with precision the neurobiology

of cannabis cue-reactivity in CUD and to reduce these with novel interventions. Such new knowledge is necessary to alleviate the harmful impacts of the increasing prevalence of CUD to both the individual and to society, particularly when cannabis products and related cues are increasingly accessible and visible to vulnerable members of the community.

Research Outputs

Published Peer Review Paper as Chapter of the Thesis

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Table of Contents

List of Tables

List of Figures

List of Commonly Used Abbreviations

ACC	Anterior cingulate cortex
ApBM	Approach Bias Modification
AUDIT	Alcohol use disorder identification test
ANOVA	Analysis of variance
BOLD	Blood oxygen level dependent
CBD	Cannabidiol
CBT	Cognitive behavioural therapy
CBM	Cognitive bias modification
CUD	Cannabis use disorder
CUDIT	Cannabis use disorder identification test
DMN	Default mode network
DSM	Diagnostic and statistical manual of mental disorders
DQ	Daily questionnaire
fMRI	Functional magnetic resonance imaging
FWE	Family wise error
FTND	Fagerstrom test for nicotine dependence
IAPS	International Affective Picture System
MBCT	Mindfulness-based cognitive therapy
MBI	Mindfulness-based intervention
MBRP	Mindfulness-based relapse prevention
MBSR	Mindfulness-based stress reduction
MCQ	Marijuana Craving Questionnaire
MFG	Middle frontal gyrus
ML	Marijuana Ladder

MORE	Mindfulness-oriented recovery enhancement
NAcc	Nucleus accumbens
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
PCC	Posterior cingulate cortex
ROI	Region of interest
SCID	Structured clinical interview for DSM
SUD	Substance use disorder
THC	ΔTetrahydrocannabinol
VAS	Visual analogue scale
VTA	Ventral tegmental area

Chapter 1: Thesis Introduction and Overview

1.1 Chapter Guide

This thesis aims to examine the neurobiological mechanisms associated with regular cannabis use, in particular CUD and how a novel intervention targets such mechanisms. As such, this chapter will first provide an overview of the prevalence and diagnostic criteria of CUD. Second, the chemical compounds underlying the psychopharmacological effects and addictive liability of cannabis will be summarised, followed by an address of the implications of the global trends in changes in the legal status of cannabis products. Next, an overview of a prominent neuroscientific theory of addiction (Volkow and colleagues, *Physiological Reviews*, 2019), with a focus on cue-elicited craving (i.e., cue-reactivity) will be summarised to provide a background relating to the neurobiology associated with the development and maintenance of CUD. A review of the current neuroimaging evidence examining the neurobiological correlates of cue-reactivity is provided and the limitations of the current literature outlined. Current pharmacological and psychological treatments for CUD will be briefly summarised, as well as mindfulness-based interventions (MBIs). A summary of the neuroscientific theories of MBIs for addiction will provide context for the use of MBIs as treatment for CUD, followed by a review of how MBIs effect cue-reactivity in substance use disorders and the current limitations in the literature. This chapter will conclude by presenting the overall objective and aims of this thesis, with a brief outline of the thesis chapters.

1.2 Cannabis Use Disorder

1.2.1 Prevalence and Diagnostic Criteria

Worldwide cannabis is used by ~209 million people (1). A significant increase in daily or near-daily cannabis use has been reported over the past few years (i.e., since 2018), with ~10% of cannabis users consuming cannabis daily (2). Prevalence of cannabis use and demographic characteristics of cannabis users varies widely across countries. However, cannabis use incidence is consistently highest for young adults (aged 15-24) and men across all age groups, with the highest prevalence of cannabis users reported in North America, Australia, New Zealand, and West Africa (see United Nations Office on Drugs and Crime World Drug Report, Booklet 3 2021-2022 for comprehensive statistics; 1),

Whilst conventionally being considered a "soft option" drug (3) cannabis is the substance with the highest dependence rates with ~10-30% of users developing a cannabis use disorder (CUD; 1, 4, 5). CUD has been defined by the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) as a chronically relapsing substance use disorder (SUD; 6). A diagnosis of CUD can be made using the DSM-5 based on people endorsing two or more of eleven criteria. In the DSM-5 the severity of a CUD is defined on a range from mild, to moderate, to severe depending on the number of established criteria that apply (2-3, 4-5, 6-11 respectively; 6). Approximately 47% of individuals with a CUD have a moderate-to-severe CUD (5, 6).

DSM-5 CUD criteria include: a loss of control in limiting cannabis intake and a substantial amount of time using and procuring cannabis or recovering from intoxication. CUD criteria also includes a compulsion to find and consume cannabis despite adverse outcomes, such as interpersonal conflict related to use, interference with activities of daily living and recreation and impacts on physical and mental well-being respectively. CUD is also associated with tolerance to the desired (e.g., psychoactive) effects of cannabis. This

entails requiring greater quantities of cannabis to achieve the rewarding effects of cannabis over time (6). CUD is also associated with withdrawal symptoms such as sleep disruption, irritability, and/or mood disturbance when cannabis is inaccessible (6). CUD can be characterised by continued use despite repeated attempts to cut down or quit (6). Further, the experience of cravings – intense desire/preoccupation to use cannabis - is a key criterion of CUD that can trigger continued cannabis use despite the experience of harms (6, 7).

The relationship between craving and continued substance use despite experiencing harms/desire to quit is considered integral to the maintenance of SUDs including CUD (6, 8, 9). This is reflected in "craving" being added as a criterion for all SUDs in the latest edition of the DSM (i.e., 5th Edition; 6). See Figure 1.1 for DSM-5 criteria of CUD.

Figure 1.1

The DSM-5 Diagnostic Criteria of CUD

Diagnostic Criteria for Cannabis Use Disorder

A. 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 fulfil 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. A 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 (refer to DSM-5 for further details).
    b. Cannabis (or a closely related substance) is taken to relieve or avoid withdrawal symptoms.

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.

1.2.2 Patterns of Cannabis Use

The most common method of cannabis consumption (non-medical use) is smoking (79%), followed by edibles (53%) and vaping (31%; (1). Common reasons people report using cannabis is to manage stress or to relax (10, 11), which is consistent with studies associating its use for mental health management (e.g., depression, anxiety, social anxiety/social discomfort, post-traumatic stress disorder, sleep disturbance, and chronic pain; (12-16). Among adolescents, socially conforming, experimenting, and enjoyment were most common motivators for cannabis use (10, 12, 17, 18).

1.2.3 Cannabis Composition and Pharmacological Effects in Humans

The cannabis plant is comprised of over 400 chemical compounds, including cannabinoids (e.g., cannabidiol; CBD, Δtetrahydrocannabinol; THC) that can produce pharmacological effects on the human body (19). The psychoactive experience of cannabis intoxication commonly reported as “euphoria”, “stoned” or “high” is primarily caused by THC (19, 20). As such, THC is the cannabinoid responsible for the addictive potential of cannabis due to its associated effects on brain dopaminergic function (21, 22) similar to other drugs of abuse (23, 24).

In human laboratory studies, THC has been shown to cause dose-dependent increases in intoxication, anxiety and symptoms of psychosis, as well as cognitive impairment (25, 26). CBD, which is not intoxicating at typical doses, has been shown to be the protective component of the cannabis plant by moderating the effects of THC (27, 28). Experimental studies have shown CBD to reduce the acute effects of THC on reward (29) and emotion processing (30), the intensity of psychotic symptoms and attentional bias to drug-cues (31).

Cannabis plants producing higher levels of THC:CBD ratios are the most common source for both recreational and medicinal cannabis products (32). Variations in the cannabis

genus as well as methods of consumption (e.g., smoked, vaped, ingested) produce varied and often unpredictable concentrations of THC (33). This is of concern, as to date, there are no clear safety guidelines regulating the cannabis industry in regard to THC levels in cannabis products (4).

1.2.4 Legal Status of Cannabis Use

The legal status of cannabis use and related products, for both medical and recreational purposes varies internationally, as well as at national and regional levels (34). The observed increase in cannabis use in the past few years is associated with global legislation trends expanding the decriminalisation, depenalisation and legalisation of its use (1, 34). Indeed, associations between increased past-month cannabis use and decreased perception of risk or harm from occasional or regular cannabis use have been reported in high school students in the United States, Europe, Latin America and the Caribbean (1). The decreasing perception of harm is related to the propagation of the potential health benefits (e.g., media coverage claiming medical benefits of cannabis, particularly products containing CBD) and changes in legal status minimising the perception of risk associated with non-medical use (1, 34-36).

1.2.5 Legalisation and Implications on Cannabis Composition

The most recent report by the United Nations Office on Drugs and Crime (2022; 1) has observed an association between cannabis legalisation and the availability of cannabis products with higher levels of THC. Prior to 1990 THC was less than 2%, rising to 4% in the 1990s, to 17-28% in 2017 and some cannabis products such as oil and edibles that have THC concentrations as high as 70% or more (1, 37-39). Despite the increase in the potency of cannabis products over the past two decades, there has been a significant decline in the

percentage of adolescents who consider regular cannabis use to be harmful (1). This is concerning as high-potency cannabis use has been associated with increased likelihood of developing mood disturbances (e.g., anxiety and psychotic disorders) and a CUD (40-43). This is five times more likely in people who use on a daily basis, with cannabis potency of THC ≥ 10% (42, 43).

1.2.6 Adverse Neurobiological Impacts Associated with Cannabis Use

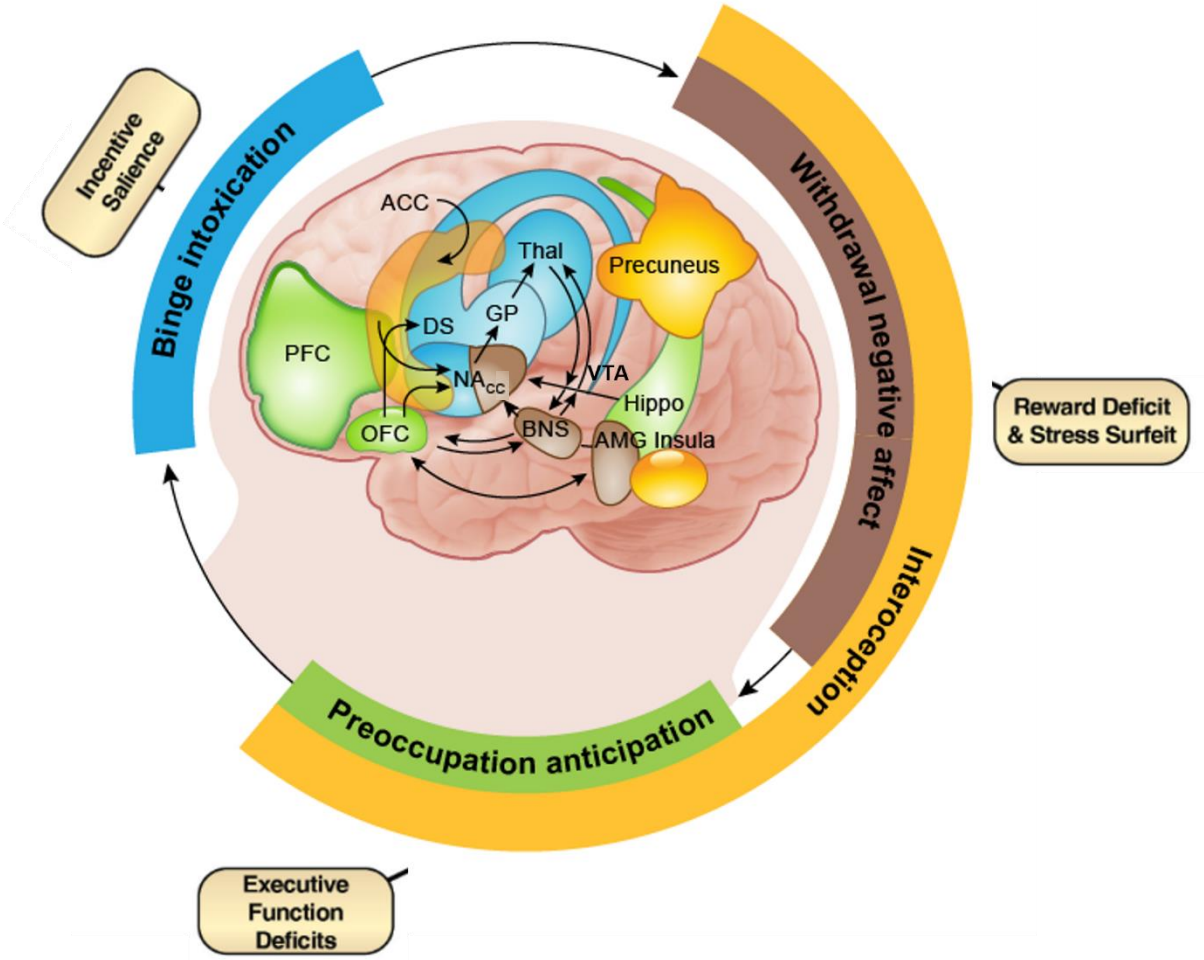
Despite the perception of harm related to cannabis use decreasing (36), regular cannabis use is evidenced to be associated with alterations in brain reward pathways (i.e., basal ganglia, anterior cingulate and prefrontal cortex; (24, 44, 45). Altered brain function and metabolism has been associated with both acute and long-term THC exposure (46-48).

1.3. Neuroscientific Theory of Addiction

One of the most prominent and influential models of addiction is the 3-stage model of neuroadaptation initially proposed by Koob and Volkow (2010; 49). This neuroscientific theory of addiction postulates that SUD is a recurring cycle comprising three stages (1) *binge and intoxication (2) withdrawal and negative affect and (3) preoccupation and anticipation* occurring in key brain pathways. This model was recently revised and outlined in Figure 1.2 and described below (53).

Figure 1.2

The Three Stage Neuroscientific Model of Addiction



Note. The three stages of addiction model adapted from "The neuroscience of drug reward and addiction" by N. Volkow, M. Michaelides and R. Baler, 2019, *Physiological reviews*, *99*(4), pg. 2115 (Abstract). Copyright 2019 by The American Physiological Society. Blue regions indicate neuroadaptations associated with *binge and intoxication.* Brown regions indicate neuroadaptations attributed to *withdrawal and negative affect.* Green regions indicate neuroadaptations implicated in *preoccupation and anticipation.* Yellow regions indicate neuroadaptations associated with *interoception* processes. The psychological processes indicated in the gold boxes are ascribed to neuroadaptations in these respective regions. *Abbreviations*: NAcc – nucleus accumbens, DS – dorsal striatum, GP – globus

pallidus, Thal – Thalamus, OFC – orbitofrontal cortex, PFC – prefrontal cortex, ACC – anterior cingulate cortex, Hippo – hippocampus, AMG – amygdala, BNS – bed-nucleus of the stria terminalis.

(i) *Binge and intoxication stage*. This stage refers to when a person experiences the rewarding effects from intake of an intoxicating substance. This stage is ascribed to brain regions implicated in the experience of reward whilst intoxicated (i.e., basal ganglia including the nucleus accumbens/ventral striatum, ventral tegmental area; (50, 51, 53). Repeated intoxication is ascribed to brain regions implicated in tolerance, which is when a person requires a higher dosage of the substance to achieve the same experience of reward as that from the initial dosage, and habit formation (i.e., thalamus, dorsal striatum; 52).

(ii) *Withdrawal and negative affect stage.* This stage refers to when a person experiences altered physiological (e.g., increased arousal, muscle pain, vomiting) and/or psychological (e.g., negative emotional states) symptoms. These follow abrupt discontinuation of the substance. This stage has been ascribed to brain regions implicated in stress and emotion regulation (i.e., amygdala), as well as reward processing (i.e., NAcc/ventral striatum; 52, 53).

(iii) *Preoccupation and anticipation stage and Interoception.* This is the stage at which an individual is preoccupied with, and anticipates the use of substances, either when such access is not immediately available or after periods of abstinence. This stage has been ascribed to brain regions implicated in motivation and attention (i.e., prefrontal cortex; PFC), cognitive control and

reward evaluation (i.e., anterior cingulate cortex; ACC, occipitofrontal cortex; OFC), response inhibition (i.e., medial prefrontal cortex; mPFC), conditioned learning (i.e., hippocampus), and interoception/awareness (i.e., insula, precuneus, posterior parietal cortex; PCC; 52, 53).

1.3.1 Neuroscientific Theory of Addiction and Substance-Related Cue-Reactivity

Prominent theoretical models of addiction posit that cue-elicited craving (i.e., strong desire/urge/preoccupation to use a drug after being exposed to a related cue) may be a powerful form of craving and a key factor contributing to compulsive substance-use/relapse (50, 53, 54). The theory of drug reward developed by Volkow and colleagues, *Physiological Reviews*, (2019; 53) postulates that with repeated substance use, neuroadaptations occur within brain regions involved in motivation and reward (i.e., limbic and prefrontal regions) as people become sensitised to drugs and their related stimuli (55, 56). As a result of this neural sensitisation, the rewarding effect of intoxication (i.e., dopamine increase) is paired with substance-related stimuli (50).

Such stimuli can include drug paraphernalia (i.e., drug-taking apparatus, drug-related images), sensory cues (i.e., associated smells), environmental contexts (i.e., routine location of use, social situations) and strong emotional states (i.e., feeling stressed, happy, depressed; (8). This paired association between the rewarding effects of intoxication and substance related-cues gives such cues increased incentive salience (i.e., value, motivation, desire) and the capacity to elicit a release of dopamine and subsequent substance-taking anticipation and preoccupation (i.e., craving; 57-59).

The nature of the incentivisation of substance-related cues is also postulated to be cyclical (53). That is, repeated substance use (i.e., binge and intoxication) alters neural activity, this subsequently increases the incentive salience of substance-related cues that

triggers preoccupation with/anticipation of substance use further altering brain function (60, 61).

Similarly, the withdrawal symptoms that are resultant of reduced or discontinued substance use and negative affect (e.g., stress, depression, anxiety) are also posited to increase the incentive salience of substance-related cues (52). In turn, experience of withdrawal symptoms may also prompt preoccupation and craving and a cycle of further neuroadaptation (62, 63).

Exposure to substance related cues has been shown to be a key factor in triggering substance use behaviour, relapse and in maintaining compulsive patterns of substance use (7, 57-59). For instance, exposure to a cue associated with cannabis (e.g., a bong, skunk smell, lighter) can elicit craving even in the absence of cannabis. This response (including behavioural, physiological, psychological and neural) is often referred to as "cue-reactivity" (8).

1.3.2 Cue-Reactivity in SUD including CUD: Behavioural Evidence

In laboratory studies, cue-reactivity paradigms involve the systematic exposure of stimuli related to the individual's primary substance of use (often compared to neutral or naturally rewarding stimuli; (64, 65). Using cue-reactivity paradigms, researchers have repeatedly demonstrated the ability of substance-related cues to elicit psychophysiological response patterns related to motivational processes involved in substance use (64). These include changes in subjective reports of craving, stress, and withdrawal symptoms, as well as, heart rate variability, galvanised skin response (i.e., skin conductance and temperature), event-related potentials (Pz P300) and attentional bias; (7, 66-68). In cannabis users, a meta-analyses comprised of 12 studies demonstrated that cannabis cues compared to control conditions elicit moderate changes in psychophysiological responses (i.e., heart rate, skin

conductance, event-related potentials, subjective craving; 66). However, the findings do not clearly inform on the underlying mechanisms driving cue-reactivity.

Alterations in brain function associated with substance use (including cannabis) are posited to contribute to the development and severity of cue-reactivity and subsequently a long-lasting vulnerability to relapse despite efforts and/or desire to reduce or quit (7, 53, 55, 56). Importantly, meta-analysis of 237 studies representing 21216 participants with a confirmed SUD, found an association between biological indices of cue-reactivity (e.g., heart rate, neural activity) and subjective craving with substance use/relapse. Specifically, every 1-unit increase in cue and craving indices was associated with a more than double the odds of future substance use or relapse (7).

1.3.3 Overview of Functional Magnetic Resonance Imaging for Measuring Neural Cue-Reactivity

Neurobiological theories of addiction including cue-reactivity can now be tested in humans via imaging technology that can provide pictures of the brain and its functioning (69). *Functional magnetic resonance imaging* (fMRI) is a non-invasive and safe technique for measuring and mapping brain activity (69). The use of this imaging technique can enable the examination of the neurobiological mechanisms of cue-reactivity in cannabis users. The use of fMRI methods allows for the investigation of related research questions by creating an image of anatomical reference (i.e. structural MRI; 70) with magnetic resonance signals to map changes in blood oxygenation level dependent (BOLD), which is considered a proxy for neuronal function (71, 72).

1.3.3.1 fMRI for Task-Based Measures of Neural Cue-Reactivity

The application of fMRI in studies designed to examine task-based activations, which measure brain function during task engagement (e.g., cue-reactivity), seeks to measure differences in neural responses as the stimulus (e.g., visual images) is manipulated during the scan (69). Activation maps are produced by a comparison of the signals induced during task-based conditions (e.g., cannabis vs neutral stimuli). Task-based activations can be detected by using either a block design – the alternate presentation of experimental and control conditions or an event-related design – the jittered (i.e., varied timing of an inter-stimulus interval) and relatively brief presentation of conditions with longer presentation of the control condition (Glover et al., 2011). Whilst block design is considered better for detecting activation, a jittered event-related design is considered more effective when a more specific classification of the amplitude or the timing of the BOLD response is required (73, 74). Jittering the timing between the condition events of the task can detect transient variations in BOLD responses providing greater temporal frequency in the overall time series, and allows for individual responses to condition events to be analysed (75). An event-related design may also be used to avoid expectation effects or maintain attention (74), and is not as sensitive to motion (76).

The use of fMRI methods and employment of an event-related cue-reactivity task design can facilitate the investigation of brain function associated with cue-reactivity by safely measuring differences in brain-specific regions in response to cannabis vs neutral cues. The identification of patterns of brain function associated with cue-reactivity in cannabis users can aid in the identification of neural vulnerabilities that may underscore CUD.

1.3.4 Cue-Reactivity in SUDs: Functional Neurobiological Evidence

The use of fMRI tools has enabled the study of the neurocorrelates of substance related cue-reactivity. A meta-analyses of imaging studies (i.e., predominantly fMRI)

employing a cue-reactivity task has demonstrated that activation of brain regions ascribed to prominent models of addiction (i.e., striatum, PFC, parietal cortex) are associated with cue-reactivity across multiple substances (i.e., alcohol *n* = 44, nicotine *n* = 33, cocaine *n* = 22, heroin *n* = 11, cannabis *n* = 5; 77). Thus, this evidence suggests that there may be key neuroadaptations underlying cue-reactivity that may drive craving and relapse in SUD. Indeed, meta-analytic research investigating cue-reactivity in nicotine, alcohol and cocaine users found evidence for associations between cue-induced brain activity (i.e., ACC, ventral striatum) and subjective craving (60). However, due to methodological heterogeneity in the meta-analyses conducted by Noori and colleagues, 2016, it was not possible to determine if neural activity during cue-exposure is associated with subjective experiences of craving (77). Further, due to the limited inclusion of studies examining cannabis cue-reactivity specifically (*n* = 5), it is unclear if the findings are generalisable to CUD (78).

1.3.5 Cue-Reactivity in CUD: Functional Neurobiological Evidence

Individual functional neuroimaging studies examining cannabis cue-reactivity do suggest some overlap with patterns of cue-induced brain function and regions implicated in addiction theory (i.e., basal ganglia, amygdala, PFC, parietal cortex; (79-86). However, there are inconsistent results, with different patterns of cue-elicited brain activity being reported across studies. Similarly, associations between cannabis cue-induced brain function and measures of subjective craving were also inconsistent, with the most consistent correlation between the OFC and craving reported in four studies (80, 84, 85, 87).

1.3.6 Limitations of fMRI Evidence of Cue-Reactivity in CUD

Overall, the inconsistent findings may be due to several methodological limitations. First, CUD severity was heterogeneous across study samples. This may be driving the

variation in the findings, as the severity of CUD samples may be associated with more marked or additional alterations (82, 86, 88). No study to date has used the most recent diagnostic classification system to measure CUD severity (i.e., DSM-5). This is a problem because previous versions of the DSM classify CUD using different criteria (i.e., craving) and severity metrics, which means current research data cannot be linked to current diagnostic classification. Further, the generalisability of the existing literature is limited by small sample sizes and low power due to very few studies with a non-using control group, and has yet to be systematically reviewed and meta-analysed. There is a need for further research to map in detail the neural mechanisms of cannabis cue-reactivity and associations with craving and the more severe forms of CUD. The harmful impacts of CUD to both the individual and to society, highlight the importance of identifying the neurobiological mechanisms of cue-reactivity in cannabis users that could inform treatment targets.

1.4 Treatments for CUD

1.4.1 Pharmacological Approaches

To date, no pharmacological medications have been approved by the Food and Drug Administration for the treatment of CUD due to lack of efficacy (89). Current pharmacological treatment approaches for cannabis dependence target aspects of the three stages of addiction as outlined by Volkow and colleagues, *Physiological Reviews*, (2019; 53). Targeted treatments for the binge/intoxication stage include the use of cannabinoid receptor antagonists to block the "high" effects of smoked cannabis, however, ~10% of users experience anxiety and depression following its use. Additionally, cannabis abstinence is required prior to administration of antagonists resulting in poor compliance rates (90). There are currently no partial agonists for cannabis (91).

Treatments targeting the withdrawal stage that have been the most successful are cannabinoid receptor agonists, however these drugs have abuse potential and are recommended to be administered in conjunction with psychosocial therapeutic approaches for relapse prevention (92, 93). Medications used to stabilise mood (e.g., anti-depressants) have been studied to address withdrawal symptoms of insomnia and anxiety, however they have not demonstrated effectiveness for continuous abstinence at a group level (93).

Treatment for the preoccupation/anticipation stage focusses on reducing craving (24). *N*-acetylcysteine is under current investigation as an anti-craving agent for CUDs, however the results have been inconclusive (94, 95).

1.4.2 Psychological and Behavioural Interventions

Leading psychological treatment for CUD consist of interventions aimed at craving management via cognitive restructuring and distress tolerance (Cognitive Behvioural Therapy [CBT]) and promoting motivation to change (Motivational Enhancement Therapy [MET]; (96). A Cochrane review (2016) reported that CBT and MET may provide some effectiveness at reducing the frequency and quantity of cannabis use, but have limited effects on sustained abstinence (97).

Cue-exposure therapy has shown preliminary evidence for increased ability to tolerate cravings in cannabis users, however the impact on cannabis use and abstinence has yet to be tested (98).

1.4.2.1 Mindfulness-Based Interventions

A meta-analyses of Mindfulness-Based Interventions (MBIs) for SUDs (including one cannabis study) found that MBIs were more effective than treatment as usual (including CBT) in reducing substance use, craving, and stress, as well as improving cognitive control

(99). The development of psychological interventions for SUDs are based on addiction theory which posits that maladaptive drug-taking behaviours are preceded by cognitive and affective states (i.e., craving, arousal; (100, 101). Therefore, a reduction in the intensity of these states may reduce impulsive/reactionary substance use and promote behaviour change (102). Treatment of SUDs including CUD often employ relaxation techniques for emotion regulation to reduce the intensity of arousal states associated with craving and stress (103, 104).

In contrast, MBIs do not aim to eliminate or alter craving or associated arousal including thoughts, feelings, and sensations. Instead, MBIs for SUDs address craving by training individuals to become familiar with their experience of craving in the mind and body and observe the transient nature of it (i.e., noticing how the craving changes and dissipates over time; 100). This is often termed "urge surfing" and uses the analogy of a wave rising to a peak and eventually crashing and disappearing (105).

1.4.2.1.1 What is Mindfulness? Traditionally considered a path for spiritual enlightenment, mindfulness is defined as non-judgemental awareness of one's own psychological processes from moment-to-moment (106). This includes the capacity to be aware of automatic tendencies (e.g., phone checking, turning on the T.V., self-evaluation) and remain non-reactive to distressing thoughts and emotions (e.g., not giving into road rage or engaging in self-harm). It also includes the ability to recognise emotional states and observe internal and external experiences without fixating on or attaching meaning to them (106). The practice of mindfulness is understood as a two-component process: i) *Focussed attention* – which uses an object (often one's own breath) or an analogy of an impermanent entity (e.g., waves in the ocean, clouds in the sky) to repeatedly return to when inevitable distractions of thoughts, feelings, and/or sensations occur. ii) *Open monitoring* – which promotes a general awareness of experiences including cognitive, emotional, and physical

without attaching any judgment label (e.g., good/bad, right/wrong, happy/sad) to them (107). Repeated practice (i.e., daily) of these two techniques is evidenced to produce robust neuroplastic changes in the brain (functional and structural) which are hypothesised to increase the ability to be mindful in daily life even when not engaging in mindfulness practice (i.e., trait mindfulness; 108, 109).

1.4.2.1.2 Types of MBIs for SUD. In the context of addiction, MBIs have been adapted (i.e., structure and format) from the first generation of MBIs developed for depression and anxiety by Jon Kabat-Zinn (i.e., Mindfulness-Based Stress Reduction, Mindfulness-Based Cognitive Therapy; 106). These include interventions such as Mindfulness-Based Relapse Prevention (MBRP; 110), Mindfulness-Orientated Recovery Enhancement (MORE; 111) and Mindfulness Training for Smoking (112). MBIs for addiction are often delivered in a group therapy context and run across 8-12 weeks. At each weekly session, a clinician guides participants through various mindfulness practices including both formal (i.e., meditation, mindful breathing) and informal (i.e., body-scans). In each subsequent week, these mindfulness techniques are debriefed, and new psychoeducational content is presented. In the time between sessions, participant are assigned therapeutic homework including mindfulness practices and self-monitoring exercises to increase awareness of craving and emotional states. Variation between MBIs for addiction tend to be in the delivery and debrief style (open vs. directive approach), the types of mindfulness practices taught, psychoeducation content, and the amount of homework and length of mindfulness practice sessions (100, 110-112).

MBIs designed for SUDs are tailored to target craving, autonomic arousal and affective states (100). Exercises designed to simulate drug-craving are employed to teach participants how to deconstruct their craving into its constituent sensory, affective, and cognitive components (i.e., noticing how the craving changes and dissipates over time – aka

'urge surfing' (105). With practice of these exercises, participants learn how to deliberately and adaptively respond to their urges instead of automatically reacting in maladaptive ways (100). The cognitive training implicated in focussed attention and open monitoring techniques taught in MBIs are posited to strengthen cognitive processes central to self-regulation of substance-dependent behaviour (i.e., attention, awareness, evaluation, and inhibition; (100). Over time, these practices are theorised to strengthen the prefrontal regions (i.e., OFC, ACC, MFG) conditioned by substance cue-elicited craving implicated in the preoccupation and anticipation stage of addiction (113). As prefrontal control is regained, individuals desiring to reduce or quit using are able to respond to their craving deliberately and adaptively instead of automatically and maladaptively (100).

1.4.3 Emerging Neurobehavioural Evidence of MBIs for SUDs

1.4.3.1 Behavioural Evidence in SUD Populations

There is emerging evidence supporting the hypothesis that MBIs may be an effective treatment for SUDs (99, 114, 115). For example, meta-analytic research including 42 studies has demonstrated that MBIs are more effective in reducing weekly substance use compared to controls (e.g., treatment as usual, relaxation, CBT, no intervention; 99). Further, the MBIs included in the meta-analysis were also shown to be more effective in reducing cravings, withdrawal symptoms, and stress, as well as increasing cognitive control (i.e., attention). However, high attrition rates associated with the length and intensity of standard MBIs for SUD populations limit interpretation that MBIs are clinically useful (99, 101).

In response to high attrition rates, brief MBIs – as short as 1-day have demonstrated a reduction in substance use (110, 116, 117). Specifically, a reduction in weekly alcohol consumption in at-risk drinkers (116) and number of cigarettes smoked per day by dependent smokers (110) at 7-day follow-up post engagement in a 10-minute mindfulness audio or

script compared to active-control (i.e., relaxation or effective techniques previously used) respectively. However, in these two studies, subjective craving and negative affect did not differ from pre-to-post MBI when exposed to related cues. Whilst compliance in the daily mindfulness-based task was not monitored, > 90% of participants were retained at 7-day follow-up.

Of particular relevance to CUD, a MBI comprising of two clinician guided 45-minute sessions 2-weeks apart in female cannabis users reported a significant reduction in cannabis dosage compared to an assessment-only control group (117). Engagement of participants with at least 1-session was 100% with 73% attending the 2nd session, and ~80% engaged in at least one follow-up assessment (1, 2, 3-month). Compliance was monitored (daily practice via a self-report record) but associations with cannabis use reduction were not explored, limiting the knowledge-base on the relationship between length of MBI and reduction in cannabis use.

Taken together, the findings suggest that individuals can be trained easily and quickly in MBIs with preliminary evidence for brief MBIs effectively reducing craving and substance use, which may circumvent the high attrition typically reported with longer MBIs (99). However, this evidence does not inform on the underlying mechanisms of MBIs for treating SUDs. With emerging evidence showing differences in neural function during cannabis cue-reactivity (80, 82, 85, 86), it is unclear if this can be mitigated.

1.4.3.2 Neurobiological Evidence of MBIs using fMRI in SUDs

To date, there are only seven known studies using fMRI that have examined the neural mechanisms of change in SUDs associated with MBIs. Of these, six are in cigarette smokers (118-123) and one in opiate users (124). Interestingly, there was reduction from pre-to-post MBI in differences in substance user's functional brain patterns associated with

emotion regulation (i.e., amygdala, hippocampus, insula; (118, 122), reward processing (i.e., striatum, prefrontal and parietal cortex (121-124), attention and awareness (i.e., precuneus/default mode network; 120, 124). Changes in brain activity (i.e., striatum, amygdala, insula, hippocampus, prefrontal and parietal cortex) during emotion processing was correlated with changes in cigarette dosage (118, 122). Pre-to-post MBI, changes in striatal activity was correlated with changes in subjective craving and PFC activity was correlated with positive affect (122). Reductions in subjective craving and cigarette dosage were also reported from pre-to-post MBI (118, 120, 122). Overall, emerging evidence shows that MBIs may attenuate differences in neural function during cue-reactivity and behavioural measures associated with SUDs. However, it is unclear if the findings generalise to altered brain function associated with cannabis cue-reactivity.

1.4.4 Can MBIs Mitigate Altered Brain Function Associated with Cue-Reactivity in CUD?

In the context of SUDs there is very limited neuroimaging research investigating cue-reactivity targeted MBIs, with three studies to date. The three studies have investigated the neural mechanisms of MBIs for cue-reactivity in cigarette smokers. Two studies using a whole brain analysis approach, observed that post-MBI there was a reduction in cue-elicited activations in prefrontal regions including the MFG and ACC-OFC connectivity (122, 123). A reduction in weekly cigarettes consumed and levels of self-reported stress was also reported (123), with a relationship between changes in dosage and affect with changes in ACC-OFC connectivity (122). A study also reported pre-to-post changes in cue-induced activations via ROI analyses approach in the PCC and a correlation between PCC changes and a reduction in cigarette dosage (121). Taken together, the preliminary findings suggest

that MBIs reduce neural activation associated with cue-reactivity, however this notion remains to be tested in CUD.

1.4.5 Limitations of Current Neuroimaging Literature on MBIs for SUD

While the emerging evidence is informative and suggests that MBIs may have clinical utility for CUD, there are some methodological limitations. First, MBI's employed for SUDs include varying levels of complexity, structure (i.e., length in weeks, session duration), format (i.e., individual vs face-to-face, guided vs self-directed) and number of components (e.g., required engagement and homework; for review see Li et al., 2017; 99 and Cavicchioli et al., 2018; 125). Reviews of the dissemination of evidenced-based treatments (including MBIs) from controlled settings to clinical care suggest that MBIs delivered in easily accessible (e.g., self-administered) and flexible (e.g., brief) formats may prove more effective (e.g., uptake, retention (126, 127). Mindfulness is considered a form of cognitive training which requires consistent practice – much like physical training – to produce sustained results (e.g., changes in cognitive function; (128). However, there have been promising effects of brief MBIs (e.g., ~10-minutes/day) over 1-week in at-risk drinkers (116) and a single session in smokers (123). Therefore, it is important to determine if a brief MBI is effective in changing the neural correlates of cannabis cue-reactivity in CUD, with potential for improved clinical significance in treatment uptake and retention.

Second, in laboratory studies of MBIs control conditions are varied, with no known study including both an active and passive control; therefore limiting the ability to parse apart intervention specific changes and control for placebo effects (129). Further, very few studies have employed imaging techniques to elucidate brain-behaviour relationships. The lack of imaging data limits our understanding of the underlying mechanisms of change needed to inform the development of more relevant and target-specific treatments.

These aforementioned limitations demonstrate the need for a double-blind randomised-control trial employing fMRI techniques to compare a condition matched MBI vs treatment as usual (i.e., relaxation) vs no intervention for people with mild-to-moderate CUD. Such a design will enable the highest level of experimental control to determine efficacy and unveil underlying neural mechanisms.

1.5 Overall Objectives and Summary of the Thesis

The overall objective of this thesis is to better understand the neurobiological mechanisms implicated in CUD to identify potential neurobiological treatment targets and to test how interventions can reduce brain changes in individuals with moderate-to-severe CUD. To achieve this three studies will be conducted. First, functional brain patterns will be mapped in detail via a systematic review of the fMRI literature to date on cannabis cue-reactivity in regular cannabis users. Second, brain activity in cannabis users will be compared to controls during an fMRI cue-reactivity task to confirm if the findings of the systematic review hold in cannabis users with a moderate-to-severe CUD assessed using the DSM-5 for the first time. Brain activity will be measured during a cannabis cue-reactivity task (cannabis vs neutral stimuli) via functional magnetic resonance imaging (fMRI), which map brain function in-vivo and non-invasively.

The current thesis also aims to inform on whether differences in brain function in CUD can be reduced using a brief MBI aimed at mitigating craving. The MBI-related changes will be measured by comparing outcomes to those obtained with an active control intervention and passive control. Overall, the research aims addressed by this thesis have implications for confirming if brain differences in CUD affect the same pathways implicated in Volkow and colleagues, (2019; 53) three-stage addiction theory and identifying potential neurobiological targets for CUD treatment relevant to cue-reactivity. Further, investigation of

MBI-related changes in cue-elicited brain function, will inform on the development of new interventions for people from the general community with a moderate-to-severe CUD who have tried to cut down or quit.

1.6 Overview of Study Aims

Study 1 (Systematic Review): "Patterns of Brain Function Associated with Cannabis Cue-Reactivity in Regular Cannabis Users: A Systematic Review of fMRI Studies"

Aim 1: The first aim of this systematic literature review is to synthesise the fMRI evidence to date on the neurocorrelates of cannabis cue-reactivity in cannabis users using fMRI, while participants are exposed to cannabis and neutral pictures.

Aim 2: The second aim is to systematically summarise the evidence on the correlations between brain function during cannabis cue reactivity fMRI tasks, and level of cannabis use/misuse (e.g., craving, duration, dosage, dependence) and psychopathology symptom scores (e.g., depression).

Aim 3: An additional aim is to overview the methodological quality of the reviewed literature in order to inform on the methodological standards in this area of research.

Study 2 (Experiment 1): "Mapping the Functional Neural Correlates of Cue-Reactivity in Moderate-to-Severe Cannabis Use Disorder"

Aim 1: To identify differences in brain function during cue-reactivity (watching cannabis vs neutral images) in people with a DSM-5 diagnosis of moderate-to-severe CUD who tried to cut down or quit, compared to non-using controls, examined with fMRI.

*Aim 2: T*o explore within the CUD group, the associations between brain activity during cue-reactivity (cannabis vs neutral) and behavioural measures. They include: subjective craving, withdrawal symptoms, and cannabis exposure levels e.g. dosage (grams),

frequency (days), duration (years of regular use), age of onset and abstinence duration (time since last use), and arousal rating of cannabis images.

Study 3 (Experiment 2): "Does a Brief Mindfulness-Based Intervention Reduce Cue-Reactivity Related Brain Function in Cannabis Use Disorder? An Active and Passive Placebo-Controlled Pseudo-Randomised fMRI Study"

Aim 1: The aim of this pseudo-randomised fMRI experiment is to investigate for the first time in adults with moderate-to-severe CUD, how a brief MBI targeting cannabis craving compares to active and passive placebo control interventions in terms of reduced activation during a cannabis cue-reactivity task. The active control (relaxation) will inform on MBI-specific effects on brain function, and the passive control, will parse a-part potential placebo effects.

The aim of this double-blind pseudo-randomised fMRI experiment is to investigate for the first time whether functional brain differences during cannabis cue-reactivity can be mitigated following a brief 2-week MBI in adults with moderate-to-severe CUD. This will be assessed using an active placebo-controlled protocol previously validated in at-risk alcohol users; (116). CUD participants will be allocated to one of three groups: i) MBI, ii) relaxation, iii) no intervention. The active control (relaxation) will inform on MBI-specific effects on brain function, and the passive control, will parse a part potential placebo effects.

Aim 2: The secondary aim is to examine if changes in brain activity pre-to-post-intervention is associated with changes in behaviour. Exploratory analyses will be conducted to determine if intervention-related changes in brain function during cue-reactivity correlates with changes in subjective craving, cannabis dosage, mental health symptoms, mindfulness, and arousal ratings of cannabis stimuli.

1.7 Outline of the Thesis Structure

This thesis comprises six chapters. The current chapter (Chapter 1) provides a general overview of the thesis including the background, rationale and aims. Chapter 2 (Study 1) provides a systematic review of patterns of brain function associated with cannabis cue-reactivity in regular cannabis users examined with fMRI, including brain-behaviour correlations. Chapter 3 overviews the design and methodology of the empirical studies, including sampling procedure and proposed statistical data analysis. Chapter 4 (Study 2) outlines the first experiment. It examines patterns of brain function during a fMRI cue-reactivity task in adults with moderate-to-severe CUD who tried to cut down or quit compared to non-using controls, and correlations between cue-induced brain function and behavioural measures (e.g., subjective craving). Chapter 5 (Study 3) outlines the second experiment. It investigates whether altered brain function during a fMRI cue-reactivity task in the CUD group, is reduced pre-to-post a brief 2-week MBI with daily monitoring of cannabis use, compared to an active control intervention (i.e., relaxation) and a passive control intervention (i.e., no intervention). Chapter 6 outlines the General Discussion. It summarises and discusses the findings presented throughout the thesis and their implications for addiction theory, further research and clinical practice.

Chapter 2: Patterns of Brain Function Associated with Cannabis Cue-Reactivity In Regular Cannabis Users: A Systematic Review of fMRI Studies

Study 1

2.1 Chapter Guide

This chapter present Study 1, which is the first comprehensive systematic review of the literature to date investigating the functional neural correlates of cannabis cue-reactivity. The first aim of this systematic review was to synthesise the evidence to date on the functional neural correlates of cannabis cue-reactivity in regular cannabis users examined using fMRI tasks which entail participants' exposure to cannabis *vs* neutral stimuli (henceforth CAN vs NEU). The secondary aim of this review is to summarise the evidence on the associations between cue-elicited brain function in cannabis users and the level of various behavioural variables including subjective cannabis craving, cannabis exposure (e.g., duration, dosage, frequency), cannabis use related problems and exposure to substances other than cannabis. An additional aim is to overview the methodologies used to measure cannabis cue-reactivity using fMRI in regular cannabis users in order to inform on the methodological standards in this area of research.

Database searches were re-run prior to submission. There are no known additional studies that meet the inclusion criteria. As such, the findings of the review reflect the current literature (*N* = 18 studies). This review has been published in *Psychopharmacology* and has been presented in this chapter without any alterations to the content.

Citation:

Sehl, H., Terrett, G., Greenwood, L. M., Kowalczyk, M., Thomson, H., Poudel, G., . . . 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. doi:10.1007/s00213-021-05973-x

2.2 Abstract

Rationale: Regular cannabis use (i.e., ≥ monthly) is highly prevalent, with past year use reported by ~200 million people globally. Greater reactivity to cannabis cues is a key feature of regular cannabis use and has been ascribed to greater cannabis exposure and craving, but the underlying neurobiological mechanisms are yet to be systematically integrated.

Objectives: We aim to systematically summarise the findings from fMRI studies which examined brain function in cannabis users while exposed to cannabis vs neutral stimuli during a cue-reactivity fMRI task.

Methods: A systematic search of PsychInfo, PubMed, and Scopus databases was pre-registered in PROSPERO (CRD42020171750) and conducted following PRISMA guidelines. Eighteen studies met inclusion/exclusion criteria – samples comprised 918 participants (340 female) aged 16-38-years. Of these, 603 were regular cannabis users, 315 were controls.

Results: Overall, the literature consistently reported greater brain activity in cannabis users while exposed to cannabis vs neutral stimuli in three key brain areas: the striatum, the prefrontal (anterior cingulate, middle frontal), and the parietal cortex (posterior cingulate/precuneus) and additional brain regions (e.g., hippocampus, amygdala, thalamus, occipital cortex). Preliminary correlations emerged between the function of similar regions (striatum, orbitofrontal cortex, amygdala) and cannabis-related cravings but not dosage/frequency.

Conclusions: Overall, the evidence to date shows greater brain function during cannabis cue-reactivity in regular cannabis users which may be associated with greater cannabis craving. The inconsistent methodological standards highlight a need for more robust standardised assessments of cannabis use and related mental health problems, to profile and mitigate altered neurobiology underlying cue-reactivity in cannabis users.

Keywords: *cannabis, craving, functional magnetic resonance imaging, fMRI, cue-reactivity, neuroimaging, brain*

2.3 Introduction

Cannabis is the most widely used substance globally, with ~192 million users in the past year (1). A significant and increasing minority of ~10% of users consume cannabis on a regular basis (1). This is concerning as regular cannabis use (i.e., at least once a month; 130) is associated with a range of psychosocial outcomes including severe cannabis use disorders (CUD) and mental health disorders (6, 5), lower IQ, education and cognitive performance (e.g., working memory; 131). Cannabis use related problems are reported to incur a substantial financial burden globally from a range of issues e.g., traffic accidents, hospital/treatment services, psychological disorders, work absenteeism (1). For these reasons, it is critical to understand the pathophysiological mechanisms of regular cannabis use in order to develop effective intervention strategies to prevent these issues and/or mitigate their effects. From a neurobiological perspective, we are yet to fully understand the key processes and brain regions that are associated with regular cannabis use. In spite of this, the implementation of Magnetic Resonance Imaging (MRI) has caused increasingly advanced efforts to identify the pathophysiology of regular cannabis use.

A core feature of regular cannabis use is greater reactivity to cannabis cues vs neutral cues (henceforth called “cue-reactivity”; 8). Greater cannabis cue-reactivity has been robustly demonstrated using self-report (e.g., higher valence, arousal and craving rating) and various psychophysiological indices (e.g., higher heart rate, blood pressure, skin temperature, and P300 amplitude (66). Greater cannabis cue-reactivity in regular cannabis users has been posited to develop as a result of repeated cannabis consumption; whereby cannabis related cues (e.g., paraphernalia, smell, contexts) progressively acquire a rewarding value in that they signal, predate/anticipate the experience of the reward (i.e., pleasure, feeling high) that will come from the consumption of cannabis (8). Thus, reactivity to cannabis related cues has been posited to underlie symptoms consistent with a CUD: increased motivation for using

cannabis, habitual/repeated cannabis use and in some also the experience of cravings for cannabis (i.e., strong desires, urges and preoccupation to use), loss of control of cannabis use and relapse following attempts to reduce or quit (55, 56). Notably, cannabis and related products have become increasingly available and advertised (either in a licit or illicit fashion) in outlets online and in communities globally due to trends towards the decriminalisation of recreational and medical cannabis. Therefore, investigating how exposure to cannabis related cues affect the brain, and how brain alterations in relation to cannabis cue-exposure relate to cannabis craving and chronicity of use, is timely to inform users and their relatives in the general community, clinical practitioners and policy makers (132).

Animal studies and meta-analysis of drug cue-reactivity studies (e.g., alcohol, nicotine, cocaine), show that greater reactivity to substance related cues in regular substance users is ascribed to sensitisation of brain pathways implicated in reward processing with repeated exposure to substances. These include striatal areas implicated in reward processing, limbic regions mediating stress, and prefrontal cortex (PFC) areas implicated in motivation and disinhibition (24, 51, 77). Specifically, such reward brain pathways would be activated with cannabis consumption in occasional users; however with repeated cannabis use the activation of these pathways would occur also in response to exposure to cannabis related cues that signal/predate cannabis use, thereby triggering repeated/automatic cannabis use behaviour, motivation for using, and in some, also craving and relapse when attempting to cut down or quit (55, 56).

However, the neurobiology of reactivity to cannabis cues in regular cannabis users are yet to be fully mapped. The evidence from functional magnetic resonance imaging (fMRI) studies that have mapped brain function with high-resolution, in-vivo, non-invasively during exposure to cannabis cues in regular cannabis users has yet to be synthetised (44, 45). A careful profiling of the neurobiological correlates of cannabis cue-reactivity is required to

further neurobiological theories of addiction (i.e., anticipation/motivation stage) as these are largely based on evidence on substances other than cannabis (24, 51). A synthesis of the evidence on the neurobiology of cannabis cue-reactivity will also create a knowledge-base that can be used to inform the development of neurobiological targets for treatment that aim to mitigate reactivity to cannabis cues, consequent automated use, and in some, craving and relapse.

The first aim of this systematic review is to synthesise the evidence to date on the brain functional correlates of cannabis cue-reactivity in regular cannabis users examined using fMRI tasks which entail participants' exposure to cannabis *vs* neutral stimuli (henceforth CAN vs NEU). The secondary aim of this review is to summarise the evidence on the associations between brain function in cannabis users (while exposed to CAN vs NEU stimuli) and the level of various variables including subjective cannabis craving, cannabis exposure (e.g., duration, dosage, frequency), cannabis use related problems and exposure to substances other than cannabis. An additional aim is to overview the methodologies used to measure cannabis cue-reactivity using fMRI in regular cannabis users in order to inform on the methodological standards in this area of research.

2.4 Method

2.4.1 Search Strategy

This review was pre-registered via PROSPERO (ID: CRD42020171750; See Appendix B). A systematic search of the literature to date (5th November, 2020) was reported in-line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (133), full checklist in Online Resource 1. Searches were completed using PsychInfo, PubMed, and Scopus databases. Search terms included: ("cannabis use disorder" OR cannabis OR marijuana) AND (fMRI OR "functional magnetic resonance

imaging" OR MRI OR "magnetic resonance imaging" OR "brain activity" OR "brain function" OR connectivity OR "neural activity") AND ("cue-reactivity" OR "cue-salience" OR craving OR reward OR sensitization). All terms were searched within title, abstract and keywords. No time limits were placed on the search.

2.4.2 Inclusion and Exclusion Criteria

Inclusion criteria were: i) the manuscript was written in English; ii) the sample included human participants; iii) the mean age of the sample ranged between 14 and 65 years; iv) the sample comprised people who regularly use cannabis (i.e., at least once a month; 130) or meeting criteria for a cannabis use disorder/dependence); v) fMRI was used as a technique to measure brain function; vi) a cue-reactivity fMRI task was used to measure brain function; vii) brain function was measured via contrasting presentation of CAN vs NEU stimuli; and viii) the manuscript was published in peer reviewed journal.

Exclusion criteria were: i) the sample was defined as endorsing a diagnosis of any major mental health disorder (e.g., depression, schizophrenia), or neurological disorders (e.g., epilepsy); ii) the sample had regular/disordered/dependent use of substances other than cannabis, alcohol or tobacco (as defined by each study); iii) brain integrity was measured using neuroimaging techniques other than fMRI (e.g., structural MRI, diffusion weighted imaging, electroencephalography, positron emission tomography); and iv) the study was not an experiment (e.g., single case report, case studies, review or meta-analysis); and v) the manuscript was not published in a peer reviewed journal (e.g., conference abstract, book chapter, dissertation).

Figure 2.1 outlines the PRISMA flowchart which summarises the systematic study selection process for inclusion in this review. Screening of all records' titles, abstracts, and full-texts (after duplicates were removed) against the inclusion and exclusion criteria was

done independently by two student researchers (H.S., H.T.). The resulting article selection were then disclosed, and any discrepancies were resolved via discussion with a senior researcher (V.L.). As a result of this process, 18 manuscripts were identified as eligible for this review.

2.4.3 Data Extraction

The following data was extracted from all studies: i) study characteristics (e.g., first author, year of publication, and recruitment strategy); ii) sample socio-demographic characteristics (e.g., sample size, age, sex); iii) level of cannabis use and related problems in the cannabis group (e.g., dosage, duration, age of onset, frequency/occasions, abstinence duration); iv) method used to analyse fMRI data (e.g., whole brain, ROI), v) brain functional differences comparing exposure to CAN vs NEU stimuli a) within cannabis users, b) between cannabis users compared to controls (i.e., additional brain functional differences between cannabis subgroups were extracted), and vi) correlations between brain function (while exposed to CAN vs NEU stimuli) and behavioural variables (e.g., subjective craving, level of cannabis use).

Supplementary materials (including Supplementary Tables 2.7.1-2.7.4) available in Online Resource 1, overviews data that was additionally extracted: i) inclusion/exclusion criteria and assessment methods at study level (e.g., co-morbid psychopathology, concurrent substance use, medical conditions); ii) group inclusion/exclusion criteria and assessment methods at study level (e.g., severity and/or diagnosis of cannabis use disorder/dependence, treatment status and abstinence duration in the cannabis group, level of alcohol and tobacco use); iii) biological measures of cannabinoids from various specimens; iv) details of the cannabis cue-reactivity fMRI task (e.g., craving rating, stimuli type, presentation protocol); v)

technical characteristics of imaging data acquisition (e.g., fMRI acquisition parameters, MRI scanner strength and manufacturer, number of head coil' channels).

2.4.4 Additional Data Handling

We extracted data from cross-sectional comparisons of brain function within cannabis users (CAN vs NEU) and between cannabis users and controls. The design of two studies was prospective with assessment of cannabis users and controls at baseline (82) and 3 years later (88). From these datasets, results on the primary outcome variable (i.e., brain function comparing cannabis vs control groups) were extracted during baseline administered of the cue-reactivity fMRI task (82), and associations with cannabis use patterns and related problems 3 years later (88).

2.4.5 Risk of Bias

Results from the quality assessment showed consistency in the quality of studies included in this review; see Supplementary Table 2.7.5. All 18 studies stated the research question/s clearly, with the study population/s specifically defined and selected from similar populations and time periods. Inclusion/exclusion criteria was applied uniformly to all participants across all included studies. The independent and outcome variables were prespecified and implemented consistently across all studies, with sufficient time so that one could expect to see an association between exposure and outcome if it existed. No study provided a sample size justification, power description, or effect estimates. Similarly, no study blinded the researchers to the group status of the participants. Only eight of the 18 studies controlled for potential confounding variables in their statistical analysis, which were also inconsistent. Only four studies respectively controlled for cannabis problems/dependence severity, cannabis use patterns, and age. Five studies controlled for alcohol, and three

controlled for cigarettes, two for IQ/education years, and one for lifetime use of other psychotropic substances.

2.5 Results

A total of 18 studies were included in this review (80-88, 134-142).

2.5.1 Overview of Groups Compared

We extracted brain function during exposure to CAN vs NEU stimuli, in the following groups: i) within cannabis users in 12 out of 18 studies (80-85, 87 137-140, 143), ii) between cannabis users and non-using controls in eight studies (80, 82, 85, 86, 134, 135, 140, 142). We additionally extracted brain function during exposure to CAN vs NEU stimuli between distinct cannabis subgroups, which was reported in 1-to-2 studies: dependent vs non-dependent users (2 studies; 86, 138); high vs low problem cannabis use (2 studies; 82, 88); early vs late cannabis use onset (1 study; 82, 141, frequent vs sporadic; n = 1); cannabis use only vs cannabis *and* tobacco use (142); and male vs female cannabis users (1 study; 87).

2.5.2 Overview of Sample Socio-Demographic and Cannabis Use Characteristics

Table 2.1 overviews the socio-demographic characteristics of the reviewed samples. The reviewed samples comprised a total of 918 participants (340 female), of which 603 were cannabis users and 315 were non-cannabis using controls (i.e. henceforth controls). The sample size ranged from 12 to 144 participants aged between 16 and 38 years. Males were slightly represented in ten studies, and the ratio of males and females was even in the remainder eight studies. Participants' recruitment source was described in all studies but one (140). Most samples were recruited from the general community (15 studies, using flyers,

newspapers, internet, media) and two samples were recruited from other sources, e.g. juvenile justice programs (143), drug counselling services (86).

2.5.3 Overview of Cannabis use Levels

Table 2.1 overviews the levels of cannabis use in the cannabis groups. The age of onset of cannabis use ranged from 12 to 20 years, with a mean age of onset of 16 years across studies. The duration of cannabis use varied widely from 2 to 19 years, with a mean duration of cannabis use of 8 years across studies. Most studies reported how often cannabis was currently used in either weekly consumption days (11 studies) or occasions (3 studies). The level of cannabis use was from 5 days to everyday of the week, and the number of weekly cannabis use occasions ranged from 5 to 21. Cannabis dosage was measured in 13 of the 18 studies, using heterogeneous metrics and over different period of times. These ranged from two to 28 grams a week (11 studies) and 8-to-84 joints a week (2 studies). Only two studies quantified THC metabolites in urine, which corroborated presence of cannabis use. The duration of abstinence from cannabis at the time of scan was reported only by four studies, and ranged widely from 14 hours to ~4 days.

2.5.4 Overview of fMRI Methods

Table 2.2 summarises the fMRI data analysis methods used. The most consistently used fMRI data analysis method was a region of interest approach (ROI; 7 studies), followed by a whole brain approach (5 studies), and by a seed-based connectivity approach (seed/network to whole brain; 3 studies). Some of these studies used multiple methods concurrently: ROI *and* whole brain (5 studies; 82, 84-86, 139, 142).

2.5.5 Summary of ROIs Examined Across the Studies

A total of 347 ROIs were examined across the 18 studies. The most examined ROI was the ventral striatum/nucleus accumbens (NAcc; 13 studies) followed by the amygdala (9 studies). These were followed by the orbitofrontal cortex (OFC), and the dorsal striatum (7 studies, respectively; the latter included the caudate; 2 studies, pallidum; 2 studies, and the putamen; 1 study), and then by the anterior cingulate cortex (ACC; 6 studies), and the insula and the hippocampus (5 studies, respectively). Single studies examined the thalamus and a variety of other regions, as well as networks (i.e., default mode, salience, central executive). Table 2.2 overviews results on differences in brain function between exposure to CAN vs NEU within cannabis users, between cannabis users and controls, and between various cannabis using subgroups.

2.5.6 Brain Functional Differences in Cannabis Users During Exposure to CAN vs NEU Stimuli

Greater brain activity was reported in 11 of 12 within cannabis using samples while exposed to CAN vs NEU stimuli. Single studies reported lower activity (i.e. in parietal and occipital cortices), and non-significantly different brain function while cannabis users were exposed to CAN vs NEU stimuli. Greater brain function in cannabis users while exposed to CAN vs NEU stimuli was located most consistently in the hippocampus/parahippocampus (8 studies) and the amygdala (6 studies), followed by the thalamus, the PFC (middle frontal gyrus, ACC), parietal regions (i.e. precuneus, posterior cingulate cortex (PCC), inferior gyrus), the occipital cortex (5 studies each respectively), and other PFC regions (OFC, inferior/superior frontal gyrus), striatum (ventral), insula, fusiform/inferior temporal gyri and cerebellum (4 studies). The activity of additional regions was reported to be greater in cannabis users while exposed to CAN vs NEU by three studies (for each region):

precentral/postcentral gyrus; temporal gyrus (inferior, middle, superior) and inferior occipital gyrus. Greater function (CAN vs NEU) in cannabis users was reported by ≤ 2 studies in other striatal, parietal and occipital areas. A single study reported higher functional connectivity during exposure to CAN vs NEU stimuli between the NAcc and the caudate head, ACC, and cerebellum.

2.5.7 Brain Functional Differences Between Cannabis Users and Controls During Exposure to CAN vs NEU Stimuli

Greater brain activity was found in cannabis users compared to controls while exposed to CAN vs NEU stimuli in most studies that compared these groups (i.e. 7 out of 8 studies). The location of greater activity was most consistently in the striatum (i.e., NAcc, caudate, 3 studies respectively) and parietal cortex (i.e., precuneus). Additional regions with greater activity were reported by two studies respectively, and included the PFC (ACC, middle frontal gyrus), and parietal regions (i.e., superior parietal cortex, PCC).

Non-significant differences in brain function between cannabis users and controls emerged in two studies. Single studies reported greater brain function in additional regions; and lower brain function in cannabis users compared to controls while exposed to CAN vs NEU stimuli: Lower activity in the striatum, amygdala and other areas (e.g., inferior frontal gyrus, ACC, amygdala), and lower functional connectivity between the striatum and the hippocampus/amygdala.

2.5.8 Brain Functional Differences Between Cannabis User Subgroups

A range of findings emerged from three or less studies that compared varying cannabis using subgroups. Greater function of striatal regions (i.e., caudate, putamen, pallidum, NAcc) was reported in more severely using cannabis subgroups: dependent vs non-dependent users, high vs low problem users, frequent users vs sporadic users, and early onset

vs late onset users. Whereas greater function in the ventral striatum was reported in non-dependent vs dependent and connectivity between NAcc – parietal/postcentral gyri late vs early onset cannabis users.

Greater and lower function of parietal regions was also reported in dependent cannabis users by two studies. These included: i) greater activity of the precuneus and the PCC in dependent vs non-dependent cannabis users, ii) within dependent cannabis users, greater/lower functional connectivity between parietal areas (postcentral gyrus, superior and inferior gyri) and other regions (ACC, NAcc); and greater functional connectivity between other parietal regions (precuneus) and the ACC. Greater function of PFC regions was reported in two studies comparing high vs low problem users (higher activity of the medial frontal gyrus, ACC, OFC) and within non-dependent cannabis users (greater functional connectivity between the OFC and the superior frontal, precentral and postcentral gyri). In a single study, greater amygdala connectivity with the PFC (middle, inferior), and temporal gyrus (superior) was reported in dependent cannabis users and greater hippocampus connectivity with the precuneus was reported within non-dependent cannabis users.

No difference in brain function was reported between other cannabis user subsamples with vs without tobacco use, or male vs female.

2.5.9 Overview of Associations Between Brain Function (CAN vs NEU stimuli) and Other Variables

Table 2.3 overviews results from studies (all but two) that examined the association between the level of brain function and that of various variables. The results are overviewed below grouped by the type of variable that was correlated with brain function in the following order: subjective craving, cannabis exposure, level of cannabis use related problems, use of substances other than cannabis.

2.5.10 Brain Function and Subjective Cannabis Craving

Overall, 13 studies examined the association between subjective cannabis craving and brain activity. Of these, correlations were run with brain function measured i) in specific ROIs (9 studies) or across the whole brain (4 studies); ii) as either activity (11 studies) or connectivity (2 studies).

2.5.10.1 Correlations with ROI

The nine studies that focused on ROIs ran a total of 51 correlations between brain function and subjective craving. The results were non-significant in about two third of these correlations (n = 38) and significant in opposite directions in the remainder correlations (positive in eight studies, and negative in three studies).

2.5.10.2 Correlations with Regions from Whole-Brain Approach

Of the four studies that used a whole brain approach, only two found significant correlations and reported 36 consistently positive correlations between a range of brain areas and subjective craving.

2.5.10.3 Direction and Location of Correlations with Subjective Craving

The direction of the significant correlations between subjective craving and brain function was mixed for some of the examined regions (i.e. both positive and negative correlations were reported). The most consistently reported region that was correlated with subjective craving was the dorsal striatum (i.e. putamen, pallidum, caudate; 6 studies), followed by the OFC (4 studies), the amygdala and the insula (3 studies, respectively). Other regions were reported to be significantly correlated with subjective craving by two studies

(i.e. ventral striatum), the inferior frontal gyrus, and pre/post central gyri, or single studies (i.e. PFC, parietal, temporal, limbic areas, and cerebellum).

The direction of correlations between subjective craving and striatal (dorsal) activity was mixed, with a total of four positive correlations across four subgroups: i) within cannabis users, ii) in male cannabis users, iii) early onset users, and iv) dependent users, and two negative correlations in two subgroups: i) within cannabis users and ii) non-dependent users. In the OFC three positive correlations were within cannabis users, and one negative correlation was reported in female users. In the ventral striatum, one positive correlation was within cannabis users and one negative correlation was reported in male users. Correlations reported in single studies were all positive, with the exception of a single negative correlation with the dlPFC in frequent cannabis users.

2.5.10.4 Correlations with Functional Connectivity

Two studies reported significant correlations between subjective craving and functional connectivity between two key regions (i.e. NAcc, amygdala) and the function/modularity of other regions/networks.

2.5.11 Brain Function and Levels of Cannabis Exposure

Six studies examined the association between brain function and the level of cannabis use (frequency/quantity) and found non-significant results. There were two exceptions to this: single studies found significant correlations between brain function in distinct regions (ventral tegmental area (VTA), lingual gyrus/cuneus) and cannabis dosage i.e. weekly cannabis gram consumption in cannabis + tobacco users and greater urinary THC/creatinine metabolites (e.g., THC-COOH ng/ml), respectively.

2.5.12 Brain Function and Level of Cannabis Use Related Problems

Three studies examined the association between brain function and level of cannabis use related problems. Two of these reported positive correlations between the activity of the PFC (mOFC, ACC) and the striatum/NAcc and greater Marijuana Problem Scale scores, and between the activity of the dorsal striatum/putamen and Cannabis Use Disorders Identification Test scores at baseline and three years later. There was no association between brain function and the severity of DSM-IV cannabis dependence symptoms.

2.5.13 Brain Function and Level of Use of Substances Other than Cannabis

Two studies examined associations between brain function and level of cigarette use in cannabis users and reported non-significant results (i.e., number of cigarettes/day, duration of use, Fagerstrom Test of Nicotine Dependence scores and cannabis users with vs without concurrent tobacco use. A single study found non-significant associations between brain function and Alcohol Use Disorders Identification Test scores.

2.6 Discussion

To our knowledge, this is the first systematic review of the literature to date on the functional neural correlates of cue-reactivity fMRI tasks while regular cannabis users are exposed to cannabis vs neutral stimuli (i.e., CAN vs NEU). The literature consistently reported greater brain activity in cannabis users in three key brain areas: the striatum, the PFC (ACC, middle frontal), and the parietal cortex (PCC/precuneus; relative to controls) and additional brain regions (e.g., hippocampus, amygdala, thalamus, occipital cortex) among cannabis users in studies without controls. Early evidence showed associations between greater brain function in similar brain regions (e.g., dorsal striatum, OFC, amygdala, insula) during cannabis cue-reactivity and higher subjective cannabis craving. The methodologies

used to assess cannabis users and cue-reactivity using fMRI tasks varied widely between studies. Overall, the evidence points to greater brain function during cannabis cue-reactivity in regular cannabis users and such greater brain function may drive stronger cannabis craving in response to exposure to cannabis related cues, and to a need for improved standardised assessment of the neurobiology of cue-reactivity in cannabis users.

The literature to date shows that reactivity to CAN vs NEU cues is consistently associated with greater brain function in addiction relevant pathways encompassing the striatum, the PFC, and parietal regions implicated in cognitive processes reportedly different between cannabis users vs controls (i.e., reward processing, motivation/disinhibition and cognitive control (44, 45). The results from the literature on cue-reactivity in regular cannabis users are consistent with other existing findings from samples of cannabis and other substance users. First, the location of these functional differences is consistent with that reported by meta-analyses of fMRI studies in cannabis users while performing a variety of cognitive tasks (e.g., attention, memory, inhibition, reward processing; 44, 45). Thus, altered brain function in cannabis users might occur across a variety of cognitive tasks including but not limited to cannabis cue-reactivity. Second, the location of greater activity in cue-reactivity tasks (i.e. striatum, PFC, parietal regions) overlapped with that reported in cannabis users during reward processing fMRI tasks other than cue-reactivity (e.g., gambling; Yanes, 2018) but not distinct during cognitive control and attention-related tasks (e.g., Go/No-Go, N-back; 45). Therefore, alteration of specific pathways might be ascribed with altered reward processing in regular cannabis users. Third, the location of the group differences reported in this review (e.g., dorsal striatum, ACC, middle frontal gyrus, PCC/precuneus, and temporal regions*)*, was consistent with that reported in meta-analyses of brain function measured with fMRI tasks of cue-reactivity predominantly to substances other than cannabis (Noori et al. 2018). Thus, reactivity to any substance related cues might recruit a common neurobiological

correlate across regular users of different substances (77). Interestingly, additional brain regions were implicated in both cannabis users and controls, during the cannabis cue-reactivity fMRI tasks (i.e., hippocampus, amygdala, thalamus, occipital cortex) in studies that did not include a control group. Functional activations of additional regions may be ascribed to salience processing, as images of illicit substances vs neutral stimuli, may be more salient in both substance using and normative samples. Future work on picture rating of illicit substances vs neutral using controls is needed to confirm this notion. In sum, the neurobiological correlates of reactivity to cannabis related cues in regular cannabis users may overlap with those implicated in i) reward processing in cannabis users; and ii) reactivity to distinct substances in regular users of substance other than cannabis, a notion that is consistent with neuroscientific theories of addiction (24, 51, 144).

Among correlations subjective craving was the most consistently examined and reported to be significant. Notably, the location of the region of which the activity correlated with craving, (partially) overlapped with that of areas with different activity between cannabis users and controls. Thus, altered function during cue-reactivity in these regions may drive higher self-reported subjective craving experienced as a result of cannabis cue-exposure. These regions included the dorsal striatum, OFC, and amygdala, and these regions are implicated in key aspects of cue-reactivity: habitual/compulsive use (24, 50, 51), reward evaluation/motivational drive (51, 145), and craving/stress levels respectively (24, 51).

Emerging evidence from correlational analyses suggest that greater cannabis dependence and problems related with use, earlier cannabis use onset, and comorbid tobacco use might be moderators of cue-reactivity related functional brain alterations in regular cannabis users (86, 141). In a prospective study, cue-reactivity in the dorsal striatum was associated with cannabis dependence severity at 3-year follow-up, and cannabis dependence severity and subjective craving were also positively correlated (88). The findings from this

review provides preliminary evidence, which is in-line with animal studies (50), and other substances of abuse (8), that the dorsal striatum may be a key brain region involved in cannabis dependence and cue-reactivity. Taken together, there is suggestion that the results may be driven by subgroups of cannabis users and explain some of the variance in the literature, which may include noise from inclusion of cannabis users with varying dependence severity (e.g., on the mild end of dependence) and cannabis use history (e.g., later age of onset). Future studies that include individuals with CUD on the more severe end and detailed reporting of cannabis use history are needed to examine this further.

Interestingly, the literature reported no association between brain function during cue-reactivity and measures of cannabis exposure (e.g., dosage, frequency). This is inconsistent with prominent neuroscientific theories of addiction which posit that neurobiological alterations in reward pathways occur with repeated substance exposure and related psychological correlates (e.g., tolerance, craving, withdrawal; 51, 52). This is also inconsistent with meta-analyses showing that greater cannabis dosage is associated with altered brain integrity (i.e., function and structure; 44, 146). It could be that exposure to cannabis is not consistently assessed across the reviewed studies and was examined by few studies, so this evidence might not be conclusive and needs to be corroborated by future work with sound assessment of cannabis exposure (e.g., detailed cannabis use history across the lifespan). Varying levels of cannabis exposure across the included samples prevents examination of this systematically as samples had cannabis users with different patterns of regular use (e.g., days/week) and varying level of exposure (grams/week). There may also be protective factors that preserve and/or moderate reward processing despite repeated cannabis exposure, such as, age of onset, duration of use, treatment exposure, socio-economic status (8). Further, assessing cannabis potency/cannabinoid content is needed as different compounds (i.e., THC and CBD) have opposite effects on brain function (the latter being

neuroprotective and former associated with psychotogenic effects), and these may conflate the results (147).

Importantly, the design of the reviewed evidence was cross-sectional. Indeed, our review aimed to cross-sectionally compare brain function between cannabis users and controls and the design of most studies to date was also cross-sectional. Thus, future longitudinal neuroimaging studies are warranted to investigate how the neurobiological correlates of cannabis cue-reactivity change over time. Specifically, a priority of future work to determine if i) functional alterations represent a neurobiological vulnerability that predates or predicts the onset of cannabis use and related problems, as greater sensitivity to reward has been implicated in increased risk of substance use and related problems (50); ii) change over time with variations in the level of cannabis exposure and related problems (e.g., exacerbate with the progression to more chronic/severe CUD, or mitigate with the transition to lighter forms of use), and iii) dissipate or persist with abstinence from cannabis use.

The reviewed literature is limited by the use of inconsistent methodologies to measure cannabis use and cue-reactivity, and this issue prevents the direct integration of the study findings. First, the measurement of cannabis use and use related problems occurred in limited studies and varied widely, and only a few studies ran correlations between brain function and cannabis use levels (e.g., distinct inclusion/exclusion criteria, use of different indices of exposure and over different periods of time, only two studies reported cannabinoids via toxicology analyses of biological specimens, four studies assessed duration of abstinence from cannabis and not in relation to brain function). Thus, an important area for future work is to use standardised measures of cannabis use. These include, detailed measurement of current and lifetime use via Timeline Follow back methodologies (148), which may clarify if frequency of use plays a role in cue-reactivity in cannabis users. Furthermore detailed measurement of cannabis dosage, type, strength and method of uses via integrating to-scale

visual aids to the TLFB could investigate if these parameters of use drives reactivity to specific cannabis related cues. For example, people who use cannabis via joints may experience greater reactivity when viewing images of joints, and this may direct clinicians to implement interventions to target reactivity to specific triggers of relapse. Additionally, a greater understanding of the role of craving and withdrawal on brain function in cannabis users would be achieved with reporting of abstinence duration at the time of data collection, and with running correlations between abstinence duration and brain functional indices during cue-reactivity. Finally, to determine whether specific subgroups of cannabis users show more marked neural alterations during cue-reactivity, it would be useful to perform a clinical assessment of cannabis use related problems that identifies the vulnerable of users (e.g., presence and/or severity of CUD and of psychopathologies). Vulnerable cannabis users might include those with a more severe CUD, or those using more potent and addictive cannabis varieties with high level of THC and low level of CBD with known distinct properties on brain function (147), or people who have been abstinent from cannabis for longer time periods prior to scan. Such new knowledge is required to understand how brain functional alterations relate to clinical and public health issues.

Second, a comparison control group of non-cannabis users was used in less than half of the studies, and more evidence is required to confirm the location and direction of the group differences. Third, a meta-analysis could not be run as only one study (86) met criteria for inclusion in a meta-analysis (i.e., reported all coordinates and utilised a whole brain approach; 149a). Future research is needed that employ methods and report details that allow for inclusion in meta-analysis to provide a systematic synthesis of findings to further our understanding of the neurobiology of reactivity to cannabis cues in regular cannabis users. Fourth, inconsistent methodologies were used to examine cue-reactivity, such as which stimuli were used as cues (e.g., modalities and matching of CAN and NEU), the fMRI cue-

reactivity task design (e.g., duration, presentation order), measurement of subjective craving (e.g., at different time points in relation to the fMRI task). Future research is required to use designs that allow for replicability of findings and their direct integration, and to use and share via open access platforms, cannabis stimuli with stronger ecological validity (e.g., favourite product, people's own cannabis, and internal cues such as specific emotional states) which could be subsequently used to target in cue-exposure therapy for the treatment of CUD.

Last, a major limitation of the literature is the lack of any analyses that explored associations between brain function during cue-reactivity and the severity of sub-clinical or diagnosed mental disorders that are commonly associated with cannabis use, dependence and greater reactivity to cannabis cues, and cannabis craving (e.g., depression, anxiety, psychotic symptoms; 149), or of well-being measures associated with cannabis use (e.g., increased contact with peers, greater relaxation; 150). Future work is warranted to embrace the systematic assessment of mental health and wellbeing in the cannabis using samples, so that the clinical significance of the literature findings can be appreciated.

2.6.1 Clinical Implications

In the context of CUDs there is very limited neuroimaging research investigating cue-reactivity targeted interventions. The findings from the literature can be used to inform the development of interventions designed to mitigate aberrant brain function associated with cue-reactivity in regular cannabis users. A reduction in brain cue-evoked activation in the amygdala and medial PFC (which are both implicated in cannabis cue-reactivity and subjective cannabis craving) has been reported after Cognitive Bias Modification (CBM) relative to a sham-training control condition with alcohol-dependent participants, with the reduction significantly correlated with reduced subjective craving scores (151). Whilst there

has only been one small pilot randomised controlled trial of Approach Bias Modification (ApBM) with cannabis users to date, those receiving the active intervention showed blunted cannabis cue-induced craving at the end of training compared to those in the sham-training controls, though greater reductions in cannabis use were only observed among male participants in the active-condition (152). Two pilot studies on MBIs showed a reduction in subjective craving and weekly cigarette dosage (mindfulness vs passive placebo) and in brain function during a cigarette cue-reactivity task pre-to-post intervention in cigarette smokers (i.e., ventral striatum, ACC, ventral and medial PFC; 123). These regions are also associated with cannabis cue-reactivity in cannabis users as per this review. In sum, interventions such as CBM, ApBM and mindfulness-based interventions may be effective in reducing cue-reactivity and craving in users of substances other than cannabis, and future work is required to test this notion in regular cannabis users.

2.6.2 Conclusions

Overall, the evidence points to greater brain function during cannabis cue-reactivity in regular cannabis users in specific brain pathways (striatal, PFC, and parietal regions, followed by the hippocampus, amygdala and other regions), which might reflect a common neurobiology of altered reward processing across cannabis and other substances. Preliminary findings also show that greater brain function within such pathways (striatum, OFC and amygdala) may drive greater cannabis subjective craving in response to cannabis related cue exposure, and may not be relevant to cannabis use itself (i.e., no correlation between dosage and brain function). Our review also highlights the need for greater standardised assessment of the neurobiology of cue-reactivity in cannabis users, cannabis use and use related problems, and (sub-clinical and diagnosed) mental health problems. Finally, longitudinal studies are required to profile how brain function during cannabis cue-reactivity changes over

time and in people as they develop greater severity of CUD, relapse or quit cannabis consumption. Overall, more robust fMRI evidence is required in order to fully determine the clinical relevance of altered brain function that cannabis users have in response to cannabis related cues.

Table 2.1

Sample Socio-Demographics Characteristics and Cannabis Use Levels

Author, year	Total N (female)			Age, *yrs* mean (SD)		Education, *yrs* mean (SD)		Cannabis use level				
	Cannabis Subgroups	Cannabis	Control	Cannabis	Control	Cannabis	Control	Age onset, *yrs*	Duration, *yrs*	Frequency, *day/occasions per week*	Weekly dosage, *grams/joints*	Abstinence duration (hrs)
Filbey, 2009	_	31 (3)	_	23.7 (7.3)	_	_	_	17	7	6 day/wk, 21 occ/wk	_	_
Charboneau, 2013	_	16 (11)	_	23.7 (3.9)	_	_	_	15	_	16 occ/wk	_	14
Cousijn, 2013	Frequent	31 (11)	21 (8)	21.3 (2.3)	22.1 (2.5)	_	_	_	3	5 day/wk	3 gram/wk, 10 joints/wk[c]	_
	Sporadic	20 (7)		22.1 (2.4)		_	_	_	_	_	10 joints/life[c]	_
	High Problem	16 (7)		21.4 (2.4)		_	_	-	3	5 day/wk	4 gram/wk, 12 joints/wk[c]	_
	Low Problem	15 (4)		21.1 (2.3)		_	_	_	2	5 day/wk	2 gram/wk, 12 joints/wk[c]	_
Goldman, 2013	_	12 (2)	_	37.6 (10.7)	_	13.0 (2.0)	_	_	19	7 day/wk[c]	21 gram/wk[c] 84 joints/wk[c]	_
Feldstein Ewing, 2013	_	43 (7)	_	16.1 (1.1)	_	_	_	12 [a]	_	4 day/wk[c]	_	_
Bitter, 2014	_	13 (5)	15 (9)	19.0 (1.0)	18 (2)	_	_	_	_	_	2 gram/wk[c] 8 joints/wk[c]	26
Filbey, 2014	Dependent	37 (9)	_	24.5 (6.9)	_	13.8 (3.2)	_	18	6	6 day/wk[c]	_	_
	Non-dependent	34 (9)		24.5 (8.1)		13.4 (2.2)		17	8	6 day/wk[c]		

Wetherill, 2014	_	20 (12)	_	29.1 (9.7)	_	12.4 (3.0)	_	_	11	7 day/wk[c]	14 gram/wk[c]	_
Wetherill, 2015	Female	17	_	30.0 (6.8)	_	13.0 (2.2)	_	_	11	6 day/wk	21 gram/wk[c]	_
	Male	27		29.3 (8.2)		13.0 (1.5)					28 gram/wk[c]	
Filbey, 2016	_	53 (20)	68 (35)	30.7 (7.5)	31.4 (10.2)	13.1 (3.1)	16.8 (2.8)	_	12	_	14 gram/wk[c] 2 THC/CR (ng/ml)[b]	_
de Sousa Fernandes Perna, 2017	_	21 (6)	20 (10)	22.5 (2.3)	22.5 (2.3)	_	_	_	_	5 occ/wk[c]	1.2 THC (µg/l) 0.4 THC-OH (µg/l) 16 THC-COOH (µg/l)	_
Vingerhoets, 2016	Baseline	12 (4)	_	20.9 (2.4)	_	13.8 (2.2)	_	_	_	5 day/wk	3 gram/wk	_
	Follow-up	11 (3)	_	24.1 (2.4)	_	16.7 (3.2)	_	_	_	5 day/wk	3 gram/wk	_
Wetherill, 2016	Early Onset	15 (8)	_	29.0 (2.0)	_	12.5 (0.4)	_	13	5	_	21 gram/wk[c]	_
	Late Onset	26 (7)		28.7 (1.4)		13.1 (0.3)		20			28 gram/wk[c]	
Karoly, 2019	_	40 (21)	_	18.8 (1.0)	_	_	_	_	_	5 day/wk[c]	_	_
Zhou, 2019	Dependent	18 (0)	44 (0)	22.9 (2.7)	23.2 (4.3)	15.3 (2.5)	15.6 (2.5)	15	5	_	6 gram/wk[c]	40
	Non-Dependent	20 (0)		21.5 (2.5)			14.4 (1.7)	16			4 gram/wk[c]	83
Kuhns, 2020	Cannabis + Tobacco	16 (6)	14 (8)	21.3 (2.0)	20.7 (2.5)	_	_	_	_	5 day/wk[c]	3 gram/wk[c]	_
	Cannabis only	18 (10)	18 (10)	20.7 (2.1)	21.6 (2.4)	_	_	_	_	4 day/wk[c]	3 gram/wk[c]	

Yoo, 2020	_	54 (23)	90 (45)	29.4 (7.9)	29.4 (10.2)	_	_	19	10	_	14 gram/wk[c] 2 THC/creatinine (ng/ml)	79.4
Kleinhans, 2020	_	25 (12)	25 (12)	26.2 (4.2)	26.2 (5.1)	_	_	17	4	2-4 day/wk[d]	3 gram/wk[c]	98.4

Note: Yrs = years, N = Number, SD. = standard deviation, wk = week, occ = occasions, Edu = Education, THC = Tetrahydrocannabinol, THC-OH = 11-Hydroxy-Δº-tetrahydrocannabinol, THC-COOH = 11-Nor-9-carboxy-Δº-tetrahydrocannabinol, , ADHD = attention deficit hyperactivity disorder. [a] Age of first use. [b] Measure during pre-assessment abstinence period. [c] Estimated averages calculated via past number of day per month divided into weeks and number of grams per day or lifetime into weeks to aid comparability across studies. [d] 80% of users

Table 2.2

Summary of fMRI Analysis Method and Brain Alteration During Cue-Reactivity Task (CAN > NEU) in Cannabis Users

Author, Year	fMRI data analysis approach	Regions of interest	Brain functional differences using contrast CAN > NEU		
			Within Cannabis users	Cannabis users vs Controls	Cannabis subgroups
Filbey, 2009	Whole brain	_	thalamus, amygdala, frontal (inferior, middle), lOFC, pre/postcentral, dACC, parietal (inferior), precuneus, temporal (superior, middle), fusiform, insula, occipital (middle, inferior), cerebellum, VTA	_	_
Charboneau, 2013	Whole brain	_	Amygdala, hippocampus, uncus, temporal (inferior, middle), fusiform, occipital (inferior middle), lingual, cerebellum	_	_
Cousijn, 2013[a]	Whole brain	_	PCC, precuneus, frontal (medial, superior), OFC, occipital/angular (lateral)		*High > Low-problem*: frontal (medial), temporal (pole) *Frequent+Tobacco = Frequent Non-tobacco*[b]
	ROI	amygdala, OFC, ACC, striatum/VTA		*CB > HC:* frontal (medial), temporal pole, VTA	*High > Low-problem*: striatum (NAcc, caudate, putamen), ACC, OFC
Feldstein Ewing, 2013	Whole brain	_	thalamus, striatum (caudate), parahippocampus, uncus, frontal (superior, middle, inferior), pre/postcentral, ACC, parietal (inferior, superior), temporal (inferior, superior), insula, cerebellum (tonsil), occipital (middle)	_	_
Goldman, 2013	ROI	amygdala, hippocampus, OFC, striatum (ventral), insula (ventral)	amygdala, hippocampus	_	_
Bitter, 2014	ROI	amygdala, thalamus, striatum (NAcc), vmPFC	*CAN = NEU*	*CB>HC:* striatum/NAcc	_
Filbey, 2014	seed-based	amygdala, hippocampus, OFC, ACC, striatum (Nacc, VTA), insula	striatum (NAcc) – striatum (caudate), ACC, cerebellum	_	*Dependent > Non-dependent:* amygdala –frontal (middle, inferior), temporal (superior); ACC – postcentral, parietal (superior, inferior), precuneus *Dependent < Non-dependent:* NAcc – postcentral, parietal (superior, inferior), OFC – frontal (superior), pre/postcentral; hippocampus – precuneus

Wetherill, 2014	ROI	amygdala, hippocampus, mOFC, periACC, striatum (ventral), insula	amygdala, striatum (ventral), insula	_	_
Wetherill, 2015	ROI	amygdala, hippocampus, mOFC, periACC, striatum (ventral), insula	hippocampus, amygdala, lOFC, ACC, striatum, insula	_	*Male = Female*
Filbey, 2016	Whole brain	_	parahippocampus, thalamus, frontal (medial, inferior), ACC, precuneus, cerebellum, cuneus, occipital (lateral) *CAN < NEU:* occipital (lateral), cuneus, precuneus	*CB > HC:* caudate, ACC, precuneus, PCC, subcallosal, cerebellum	_
de Sousa Fernandes Perna, 2017	ROI	striatum (putamen, caudate, pallidum)	pallidum	*CB = HC*	_
Wetherill, 2016	ROI	amygdala, hippocampus, mOFC, periACC, striatum (ventral), insula	_	_	*Early > Late onset:* striatum (dorsal) *Early < Late onset*: striatum (ventral)
Karoly, 2019	Whole brain	_	parahippocampus, thalamus, frontal (superior, medial), fusiform, PCC, cuneus, parietal (inferior), precuneus, temporal (inferior), occipital (middle)	_	_
Zhou, 2019	Whole brain	_	_	*CB > HC:* striatum (NAcc, ventral caudate), frontal (inferior, medial, superior), mPFC, ACC, parietal (inferior, superior), PCC, precuneus, temporal, fusiform, occipital	*Dependent > Non-dependent:* PCC, precuneus
	ROI	striatum (ventral, dorsal)	_	_	*Dependent > Non-dependent:* striatum (dorsal)
	seed-based	striatum (ventral, dorsal)	_	*CB Dependent > HC*: striatum (dorsal) – frontal (inferior), vACC *CB Dependent < HC:* striatum (ventral/dorsal) – hippocampus, amygdala	_
Kuhns, 2020	Whole brain	_	_	*HC+Tobacco > CB+ & - Tobacco*: frontal pole, inferior frontal	
	ROI	amygdala, OFC, ACC, striatum/VTA	_	*CB-Tobacco > HC-Tobacco:* amygdala	*CB +Tobacco = CB No Tobacco*

				HC+Tobacco > CB+Tobacco: striatum, amygdala, ACC	
Yoo, 2020	ROI	347 regions: DMN, CEN, SN, amygdala, NAcc	_	*CB > HC:* NAcc – Central Executive Network	
Kleinhans, 2020	ROI	striatum (NAcc, pallidum, VTA)	_	*CB = HC*	_
	Whole brain	_	*Cannabis > flower (picture & odor):* parahippocampus, amygdala, striatum (NAcc, putamen), frontal (superior, middle, medial, inferior, pole), OFC, para/subcallosal/ACC, juxtapositional, precentral, temporal (middle, inferior, superior), fusiform, temporal pole, intracalcarine, cuneal, angular, occipital (pole), crus, lingual *Cannabis > Flower (picture):* parahippocampus, amygdala, striatum (pallidum), OFC, lingual, temporal (inferior, middle), intracalcarine, temporal, occipital (pole), fusiform, crus, lingual *Cannabis > Flower (odor):* striatum (putamen), central (pre, post), ACC, supramarginal, angular, frontal (pole, operculum), juxtapositional, parietal (operculum, superior), temporal (middle, planum temporale), occipital (lateral), cerebellum (crus)	*Cannabis > flower (picture & odor):* parietal (superior, precuneus), occipital cortex (lateral superior), angular	_

Note: ROI = Region of interest, OFC = Orbitofrontal cortex, VTA = ventral tegmental area, ACC = anterior cingulate cortex, PCC = posterior cingulate cortex, PFC = prefrontal cortex, NAcc = nucleus accumbens, CB = cannabis, DMN = default mode network, CEN = central executive network SN = salience network.

[a] Baseline data for Vingerhoets et al. 2016

[b] Subgroup demographics not reported

Table 2.3

Associations Between Brain Function During Cue-Reactivity Tasks and Cannabis Craving, Dependence, Level of Use, Substance Use Level

Author, year	fMRI data analysis approach	Cannabis Cravings/Urges/Arousal	Cannabis Use/Dependence Level	Substance Use Level
Filbey, 2009	Whole brain	_	(+) *Marijuana Problem Scale* & mOFC, ACC, striatum (NAcc)	_
Charboneau, 2013	Whole brain	(NS) MCQ	_	_
Cousijn, 2013	Whole brain, ROI: Amygdala, OFC, ACC, striatum/VTA	*Frequent users:* (-) MCQ & striatum (putamen), DLPFC	(NS) grams/week, joints/lifetime, duration	(NS) Nicotine use duration, cigs/day, FTND, presence vs absence of tobacco use
Feldstein Ewing, 2013	Whole brain	(NS) VAS	_	_
Goldman, 2013	ROI: Amygdala, hippocampus, OFC, striatum (ventral), insula (ventral)	(+) MCQ & striatum (ventral), OFC (medial, lateral)	_	_
Bitter, 2014	ROI: Amygdala, thalamus, striatum (NAcc), vmPFC	(NS) MCQ	(NS) joints/mo	_
Filbey, 2014	Seed-based: Amygdala, hippocampus, OFC, ACC, striatum (NAcc, VTA), insula	(+) MCQ & connectivity in striatum (NAcc) – thalamus, pulvinar, cerebellum (+) MCQ & connectivity in amygdala – precentral, frontal (middle)	_	_
Wetherill, 2014	ROI: Amygdala, hippocampus, mOFC, periACC, striatum (ventral), insula	_	_	_

Wetherill, 2015	ROI: Amygdala, hippocampus, mOFC, periACC, striatum (ventral), insula	(NS) MCQ *Female:* (+) MCQ & insula (anterior) (-) MCQ & OFC (lateral) *Male:* (+) MCQ & striatum	_	_
Filbey, 2016	Whole brain	(+) VAS & striatum (caudate, globus pallidus), OFC, amygdala, ACC, thalamus, parahippocampus, frontal (inferior, middle), PCC, cerebellum, temporal (superior), insula (+) MCQ & insula, pre/postcentral, parietal (inferior), cuneus, temporal (superior)	(NS) N of DSM-IV cannabis dependence symptoms (+) urine THC (ng/ml) & lingual gyrus, cuneus	_
de Sousa Fernandes Perna, 2017	ROI: striatum (putamen, caudate, pallidum)	_	_	_
Vingerhoets, 2016	ROI: Amygdala, OFC, ACC, striatum/VTA	_	(+) CUDIT (cannabis problems) & striatum (putamen), VTA (NS) CB use (grams/weekly)	_
Wetherill, 2016	ROI: Amygdala, hippocampus, mOFC, periACC, striatum, insula	*Early onset:* (+) MCQ & striatum (dorsal) *Late onset:* (NS) MCQ	_	_
Karoly, 2019	ROI: amygdala, OFC, striatum (ventral)	(NS) MCQ	(NS) days/lifetime, episodes/day	(NS) AUDIT (Alcohol use)
Zhou, 2019	ROI: striatum (ventral, dorsal)	*Dependent:* (+) VAS & striatum (dorsal/ventral caudate) *Non-dependent:* (-) VAS & striatum (dorsal/ventral caudate)	_	_
Kuhns, 2020	Whole brain/ROI: Amygdala, OFC, ACC, striatum/VTA	_	*cannabis+tobacco users:* cannabis grams/wk & VTA[a] *cannabis+non-tobacco users:* (NS) cannabis grams/wk	(NS) Group x tobacco use interaction
Yoo, 2020	ROI: 347 regions, DMN, CEN, SN, amygdala, NAcc	(+) VAS & connectivity between striatum (NAcc) - networks (SN, DMN, CEN) (+) MCQ & connectivity within central executive network (+) MCQ & modularity of whole brain	_	_

Kleinhans, 2020	Whole brain, ROI: striatum (NAcc, pallidum, VTA)	*Cannabis > Flower:* (+) VAS &: striatum (caudate, putamen, pallidum), OFC, amygdala, insula, para/ACC, post/precentral, opercular (central), Heschl's, planum polare, temporal pole, parietal (operculum), temporal (planum temporale), frontal (inferior), lingual/cuneal, calcarine (intra, supra), precuneus, occipital (lateral), juxtapositional *All other contrasts:* (NS) VAS	–	–

Note: ROI = Region of interest, NS = not significant, OFC = Orbitofrontal cortex, VTA = ventral tegmental area, ACC = anterior cingulate cortex, PCC = posterior cingulate cortex, PFC = prefrontal cortex, NAcc = nucleus accumbens, SN = Salience Network, CEN = Central Executive Network, DMN = Default Mode Network, MCQ = Marijuana Craving Questionnaire, VAS = Visual Analogue Scale, CUDIT = Cannabis Use Disorders Identification Test, AUDIT = Alcohol Use Disorders Identification Test, FTND = Fagerstrom Test for Nicotine Dependence.

[a] Direction of association not specified

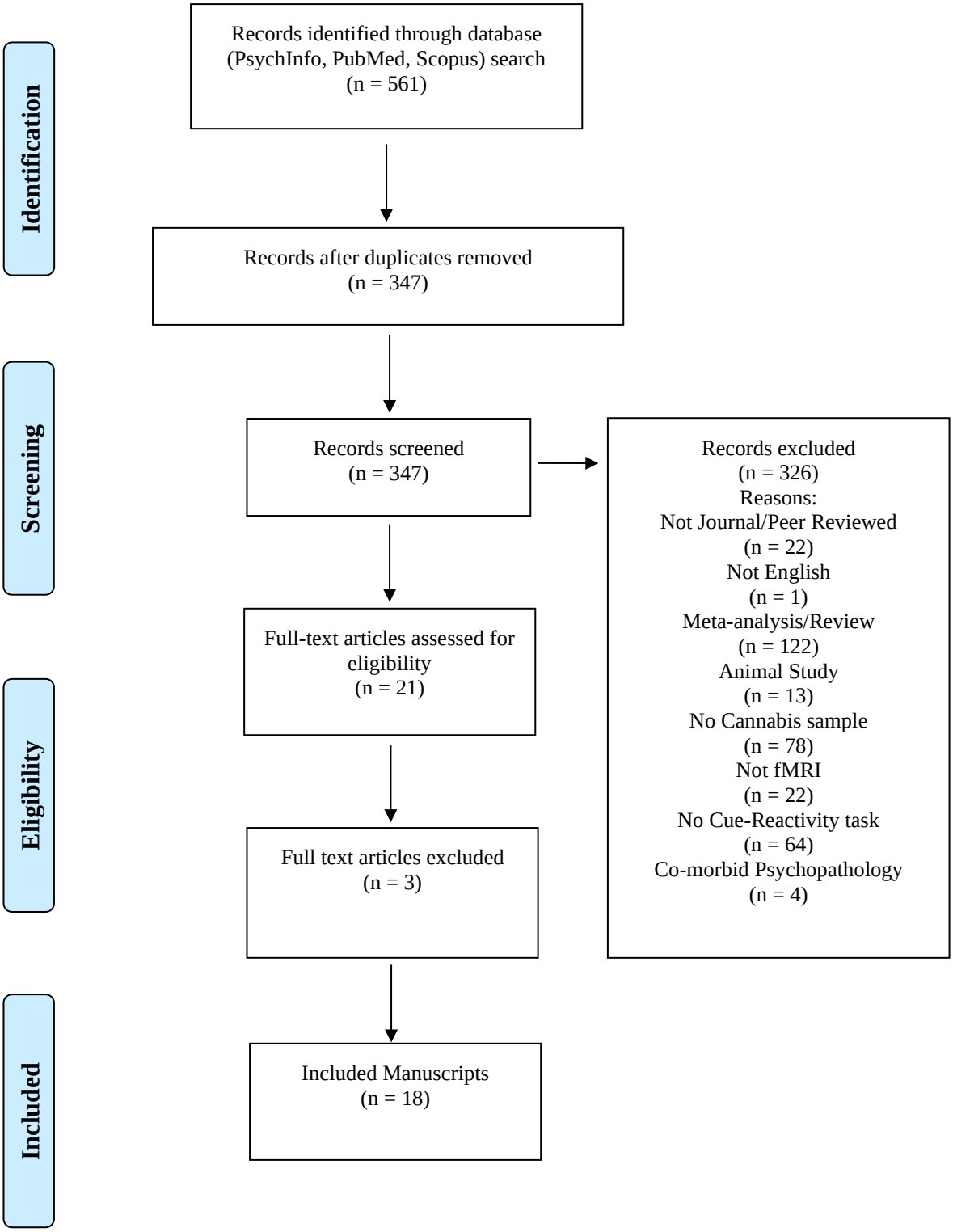


Figure 2.1. PRISMA Flowchart for Study Selection Process (based on Moher et al. 2009)

2.7 Supplementary Material

2.7.1 Overview of Participants Inclusion and Exclusion Criteria

2.7.1.1 Overview of Exclusion of Psychopathologies

Ten of the eighteen studies excluded participants with a co-morbid DSM-4 axis-1 disorder other than cannabis abuse/dependence (81-84, 86-88, 135, 139, 141, 153-155). Additional exclusion criteria included a lifetime history of psychotic symptoms (4 studies; (79, 138, 140, 156) or receiving a diagnosis of or treatment for schizophrenia or other psychotic disorder, bipolar disorder, or depression within the past 6 months (85). Further, single studies excluded high levels of depression – i.e., Beck's Depression Inventory scores > 20 (157), and known prenatal alcohol or illicit substance exposure (139).

The reviewed studies used different criteria for excluding levels of comorbid illicit substance use, alcohol and nicotine. These included a positive uranalysis for any illicit substance (9 studies; 79, 81, 86, 134, 138, 139, 142, 156, 158) self-report of a substance use disorder in the past 6-months plus any use of psychoactive substances in the past 4-weeks (2 studies; (84, 86), in the past 60 days (134), current use of prescribed or illicit psychoactive drugs (142), > 100 lifetime occasions of any substance (other than cannabis; 2 studies; 139, 142), and reported moderate-to-high risk use of other illicit substances (ASSIST Substance Involvement Score ≥ 4 for each substance reported; (85). Alcohol dependence was excluded using DSM-4 criteria (2 studies; 140, 156), Alcohol Use Disorder Identification Test (AUDIT) scores > 10 (1 study; 82) and scores > 12 (142) and CAGE score >2 (85).

Eleven studies did not exclude any level of nicotine use (79, 81, 135, 138, 143) (83, 84, 87, 134, 139, 141), whereas varying cut-off levels were reported across six studies – i.e., > 20 cigarettes/day (82, 86, 88, 142); > 15 cigarettes/day (140); > 1 pack/month (156). One study reported screening IQ > 79 (84). A single study screened for history of learning or developmental disability (139).

2.7.1.2 Overview of Exclusions of Medical-Related Criteria

Majority of the included studies (except 5 studies; 79, 85, 138, 143, 156) excluded participants with an unstable/major medical condition or neurological disorder. Similarly, history of traumatic brain injury was excluded in all but seven studies (79, 85, 86, 88, 140, 142, 158). Use of medications were excluded if they affect the central nervous system (5 studies; (83, 85-87, 141), classed as psychotropic (4 studies; 81, 85, 135, 140), anti-psychotic or anti-convulsant (1 study; 143), and vasoactive (1 study; 81). Other medical exclusions reported by single studies were cardiovascular abnormalities, hypertension, unhealthy BMI (140), hyposmia, anosmia (85), regular exercise > 2-hours/week over the past month (81), a new tattoo in the past month (143), and premature or low birth weight and sensory problems (139). All studies screened for MRI contraindications (e.g. floating metal in body), and studies comprising of female participants screened out individuals who were pregnant or breastfeeding.

2.7.1.3 Overview of Inclusion Criteria for Types of Groups

Criteria related to cannabis use levels for inclusion in the cannabis, cannabis subgroups, and non-using control groups varied across the reviewed studies and have been summarised below.

2.7.1.3.1 Cannabis Group

In eight of the 18 studies, inclusion in the cannabis sample was based on meeting DSM-4 criteria for cannabis abuse (2 studies; 81, 138), or cannabis dependence (5 studies; 81, 83, 84, 87, 135), with one study specifying a minimum length of time (i.e., past 12-months; 135). Eleven of the eighteen studies set specific cannabis use patterns as inclusion criteria which

varied across studies (79, 83, 84, 134, 138-140, 142, 143, 156, 158), four of which were in addition to DSM-4 abuse/dependence criteria being met (84, 87, 135, 138). A single study screened out cannabis users that had a current and/or history of cannabis abuse/dependence (158), and another did not report on initial inclusion criteria for their cannabis group (86). One study used Cannabis subtest of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) to determine the inclusion in the cannabis dependence group (ASSIST Substance Involvement Score ≥ 4; 85).

Eleven studies included non-treatment seeking cannabis users (79, 81-84, 134, 135, 138, 142, 143, 156), five studies recruited treatment seeking samples (83, 86, 87, 139, 141), and two studies did not report on treatment status (85, 140). The minimum period of abstinence from cannabis for inclusion varied across studies from 0-hours/normal use (1 study; 84), 12-hours (1 study; 139), 24-hours (8 studies; 82, 83, 86, 87, 135, 141-143), 48-hours (1 study: 85), 72-hours (4 studies; 79, 134, 138, 156) to 168-hours (1 study; 140).

2.7.1.3.2 Cannabis Subgroups

Dependent vs Non-Dependent Cannabis Users. Two of the 18 studies performed a sub-group analysis comparing dependent and non-dependent cannabis users (86, 138). One analysed brain activity (86), and the other, brain connectivity (138). Both studies characterised dependence according to DSM-4 criteria using the Structured Clinical Interview for DSM-4- (SCID-4; (138) and Mini International Neuropsychiatric Interview (MINI; (86).

High-Problem vs Low-Problem Cannabis Users. A single study carried out a sub-group analysis comparing cannabis users characterised as high-problem users vs low-problem users according to their Cannabis Use Identification Test (CUDIT) scores (82).

Frequent vs Sporadic Cannabis Users. Two groups were included in a study based on their cannabis use patterns. Frequent was defined as cannabis use > 10-days/month for at least 2-years, whereas sporadic cannabis use was defined as 1-50 lifetime occasions (82).

Early Onset vs Late Onset Cannabis Users. One study compared dependent cannabis users based on their age of onset. Participants were grouped as early onset if their cannabis use commenced < 16 years old and late onset if their use started > 16 years old (141).

Male vs Female Cannabis Users. In one study cannabis users were compared based on their sex (male and female; (87)). Of the 44 participants, 20 were also included in the study conducted by (83), which performed a within group analysis of dependent cannabis users.

Baseline vs 3-year Follow-up Frequent Cannabis Users. A single study examined associations between frequent cannabis baseline measures of brain function during a fMRI cue-reactivity task with cannabis use patterns at 3-year follow-up (88).

Co-users of cannabis and cigarettes vs users of cannabis only. A single study compared cannabis users and co-users of cannabis and tobacco (142).

2.7.1.3.2 Non-Using Control Group

Eight of the 18 studies included a non-using control group (85, 86, 134, 135, 142, 156, 158). The non-using control groups were included and excluded against the same criteria as the cannabis groups, with the exception of their cannabis use which varied across all six studies. One study excluded participants if their lifetime cannabis use exceeded 10 occasions and/or 15 grams (86). Another study screened for any lifetime occasions of cannabis use (82), whereas one excluded individuals with any lifetime period of daily use (156). One study included controls based on the absence of daily cannabis use at any period in their lifetime, as well as an absence of current illicit drug use in the past 60 days (134). A single study

compared users of cannabis only and co-users of cannabis and tobacco with controls using only tobacco or not (142). In this study controls were allowed to have used cannabis up to 50 times in their life, but not during the past year (142). One study did not report these details (135).

2.7.2 Overview of Substance Use Assessment

2.7.2.1 Assessment of Substance Use Levels

Majority of the studies measured the quantity (e.g. grams, cigarettes, drinks), frequency (e.g. number of days, occasions), and duration (e.g. years, age of onset) of cannabis (18 studies), alcohol (16 studies), nicotine (13 studies), and other illicit substance use (2 studies; 81, 86) using the Timeline Follow Back (TLFB) calendar (148) or a similar self-report method. Presence and/or levels of recent substance use was assessed via urine sample (10 studies; 79, 81, 82, 86, 134, 138, 139, 142, 156), breath (2 studies; 81, 139), and blood (1 study; 140).

2.7.2.2. Assessment of Substance Dependence Severity

All studies (except two; (139, 140) assessed cannabis dependence severity levels. Cannabis dependence severity was verified via a variety of semi-structured clinical interviews and questionnaires. Semi-structured clinical interviews included the MINI (4 studies, (81, 84, 86, 88), the SCID-4 (3 studies; 79, 138, 156); and the Addiction Severity Index (ASI; 4 studies; 83, 87, 135, 141). Self-report measures included the CUDIT (4 studies; 82, 85, 88), Marijuana Dependence Scale, and Cannabis Problems Questionnaire-Adolescents (1 study; 143).

Alcohol dependence severity was assessed in nine of 18 studies (81- 83, 85, 87, 88, 135, 141, 156). Four used the Semi-Structured Assessment for the Genetics of Alcoholism,

Adolescent Version (83, 87, 135, 141), four used the Alcohol Use Identification Test (AUDIT; 82, 85, 88, 142), and single studies used the SCID (156) and the MINI (81).

Nicotine dependence severity was assessed by six of 18 studies. Four used the Fagerstrom Test for Nicotine Dependence (FTND; 82, 83, 88, 142), single studies used the SCID (156), the ASI (141), and the ASSIST—Tobacco (85).

2.7.3 Overview of Group Matching (Cannabis vs Control and Sub-Groups)

In studies that had a non-using control (8 studies; 85, 86, 134, 135, 142, 156, 158). Groups were matched on age and sex, and one study on age only. Groups were matched on IQ in three studies (82, 135, 142), and cannabis users had lower IQ than controls in one study (135). Similarly, in single studies groups were matched in years of education (86), with one cannabis group having less years of education than non-using controls (156). Where reported, dependent vs non-dependent cannabis using samples in two studies were matched on age, sex, IQ, years of education, duration of cannabis use (years), and lifetime cannabis (grams). However, in one study the dependent group had an earlier age of onset than the non-dependent (86), and in the other, the non-dependent group smoked more cigarettes/day than the dependent group. High-problem and low-problem groups were matched across reported demographics except for levels of problems (82). Frequent and sporadic cannabis users had matching demographics, however frequent users reported smoking more cigarettes/day, longer lifetime cigarette smoking and greater nicotine dependence (82). In one study that compared male and female samples (87), groups were matched on reported demographics other than sex. One study reported matching on alcohol use and problems, other substance use, anxiety, depression, ADHD (142).

2.7.4 Overview of Subjective Craving Ratings During the Cue-Reactivity fMRI Task

Supplementary Table 2.7.1 overviews the measures used to rate participants' subjective level of cannabis craving during the cue-reactivity fMRI task, when these measures were administered, and any changes in subjective craving in relation to cannabis and neutral cues. The relevant results are summarised below.

2.7.5 Overview of Self Report Measures Used to Rate Craving

Two measures were used to rate craving during the cue-reactivity fMRI task. Most studies measured self-reported subjective craving using either Marijuana Craving Questionnaire (MCQ; 6 studies; 79, 85-87, 138, 139), or the Visual Analogue Scale (VAS; 6 studies; (81, 82, 88, 135, 142, 156). Two studies used both the MCQ and VAS (84, 134), and three studies did not report the measure (81, 87, 143). Craving was measured either both pre and post the fMRI task (8 studies), or after every stimulus in the fMRI task (6 studies).

2.7.6 Overview of Self-Reported Level of Craving in the Samples

Changes in cannabis users' self report craving assessed pre and post the fMRI task, showed increased in craving in seven studies and not change in craving in three studies. Other four studies reported that participants' that craving levels in relation to cannabis stimuli were higher than those reported in relation to neutral stimuli.

2.7.7 Overview of fMRI Cue-Reactivity Task Stimuli

Supplementary Table 2.7.2 overviews the characteristics of the stimuli used in the literature as cues during the cue-reactivity fMRI tasks, which were described in all studies but one. *Cannabis related cues* included different types of stimuli: images in 11 studies (e.g., showing paraphernalia [e.g. pipe, joint], cannabis plant matter, and people using/holding

cannabis); images *and* tactile stimuli (e.g., pipe) to be held by participants in the scanner in 5 studies, images and odor in one study, and audio-visual marketing clips in one study. *Neutral cues* (i.e. defined as non-cannabis, non-rewarding cues) included different types of stimuli: images (e.g., of people interacting with objects, nature, building facades, stationary, cars and faces). Cannabis and control stimuli were *matched* for basic perceptual features in all but two studies, and three more studies did not provide any information on matching. Eight studies included *additional control stimuli*: rewarding images (related to food and sex), aversive images, control images (blurred, animals), and alcohol marketing clips.

2.7.8 Overview of the Methodological Characteristics of the Cue-Reactivity fMRI Task

Supplementary Table 2.7.3 overviews the design and characteristics of the cue-reactivity fMRI tasks (e.g., duration, number of stimuli, runs, blocks, stimuli/block, presence of a fixation cross). Most studies used a *block design* (n = 11), and the others used an *event-related* design (n = 7). The *duration* of the fMRI cue-reactivity task ranged from 8 to 33 minutes (average of 15 minutes) and varied widely with no more than four studies using a task of the same duration. The *order of stimulus presentation* was pseudo, -random or quasi order the studies. All but three studies presented cannabis stimuli first (79, 134, 135). Other fMRI task characteristics varied widely between studies: i) the *number of stimuli* (cannabis and neutral) ranged from 1-to-96, ii) the *number of task runs* varied between 1 and 3; iii) there were 2-to-24 *blocks*; iv) the *number of stimuli used per block* ranged from 5 to 96, and v) the duration of stimulus' presentation was 0.33 to 20 seconds. Most studies presented a *fixation cross* between stimuli for a heterogeneous duration (from 0.5-20 seconds), and a rest between blocks for 20 seconds.

Supplementary Table 2.7.1

Overview of measures and outcomes of self-reported Craving during the Cue-Reactivity fMRI Task

Author, year	Self-Reported Craving Rating			
	Measure	Time Administered	Pre vs Post cue reactivity fMRI Task	
			Cannabis	Control
Filbey, 2009	VAS (0-10)	Post each cue	CAN > NEU (craving)	_
Charboneau, 2013	MCQ	Pre & Post MRI	Post > Pre	_
Cousijn, 2013	MCQ	Pre & Post MRI	Post > Pre	_
Feldstein Ewing, 2013	_	_	_	_
Goldman, 2013	VAS (0-10)	Post each cue	Post = Pre	_
Bitter, 2014	MCQ	Pre & Post MRI	Post = Pre	Post = Pre
Filbey, 2014	VAS (0-10)	Post each cue	CAN > NEU (craving)	_
Wetherill, 2014	_	_	_	_
Filbey, 2016	MCQ	Pre & Post MRI	Post = Pre	Post = Pre
Wetherill, 2015	VAS (0-10)	Post each cue	CAN > NEU (craving)	_
de Sousa Fernandes Perna, 2017	_	_	_	_
Vingerhoets, 2016	MCQ	Pre & Post MRI	Post > Pre	_
Wetherill, 2016	_	_	_	_
Karoly, 2019	VAS (0-5)	Post each block	CAN > NEU (wanting)	_
Zhou, 2019	VAS (0-100)	Pre & Post MRI	Post > Pre	_
Kuhns, 2020	MCQ	Pre & Post MRI	Post > Pre	Post = Pre
Yoo, 2020	VAS (0-10), MCQ	Post each cue	Post > Pre	_
Kleinhans, 2020	VAS (0-10)	Pre & Post MRI	Post > Pre	Post = Pre

Note: VAS = Visual Analogue Scale, MCQ = Marijuana Craving Questionnaire, CAN = Cannabis Stimuli, NEU = Neutral Stimuli; * Not analysed for significance

Supplementary Table 2.7.2

Overview of type of cannabis and neutral stimuli presented as cues during the fMRI Cue-Reactivity Task

	Type	Cannabis		Neutral		Control	
		Source	Content Example	Source	Content Example	Source	Content Example
Filbey, 2009	Tactile pipe & mirrored image	_	Pipe	_	Pencil	_	_
Charboneau, 2013	Image	_	Close-up whole plant, dried, joints, bong, pipe, papers with/without people	_	Landscapes, animals, or insects both close-up & far away	_	Food; Gaussian blurred
Cousijn, 2013	Image	[a]	Whole plant, dried, joints, bong, pipe, papers with/without people	[a]	Individuals & objects	[a]	Animals
Feldstein Ewing, 2013	Tactile pipe & mirrored image	_	Pipe	_	Pencil	_	_
Goldman, 2013	Image	_	Whole plant, dried, joints, bong, pipe, papers with/without people	_	Stationary, keys	_	_
Bitter, 2014	Image	Public	Whole plant, dried	IAPS	Faces, cars, nature	_	_
Filbey, 2014	Tactile pipe & mirrored image	_	Pipe	_	Pencil	_	_
Wetherill, 2014	Image	_	Whole plant, dried, joints, bong, pipe, papers with/without people	Authors laboratory archive	Building facades, people engaged in everyday activities	IAPS	Sexual & aversive images

Wetherill, 2015	Image	_	Whole plant, dried, joints, bong, pipe, papers with/without people	Authors laboratory archive	Building facades, people engaged in everyday activities	IAPS	Sexual & aversive images
Filbey, 2016	Tactile method & mirrored image	_	Preferred method of use (e.g. pipe, bong, blunt, joint)	_	Pencil	Fruit (preferred)	Tactile piece of fruit & mirrored image
Vingerhoets, 2016	Image	b	Whole plant, dried, joints, bong, pipe, papers with/without people	b	Individuals & objects	b	Animals
Wetherill, 2016	Image	_	Whole plant, dried, joints, bong, pipe, papers with/without people	Authors laboratory archive	Building facades, people engaged in everyday activities	_	_
de Sousa Fernandes Perna, 2017	Audio-visual clip	_	CB marketing clips included adverts for CB paraphernalia & a selection of short film fragments portraying CB use & marketing practices at CB selling points	_	Not described		Alcohol marketing clips
Karoly, 2019	Image	_	Whole plant, dried, joints, bong, pipe, papers with/without people	_	Non-food objects and plants	_	Blurred images
Zhou, 2019	Image	c	Whole plant, dried, joints, bong, pipe, papers with/without people	IAPS + NAPS	*Not described*		
Kuhns, 2020	Image	c	flower nuggets, joints, and individuals smoking cannabis.	c	Office supplies	*Not described*	*Not described*

Yao, 2020	Tactile method & mirrored image	Preferred method of use (e.g. pipe, bong, blunt, joint)	_	Pencil	_	Fruit (preferred)	Tactile piece of fruit & mirrored image
Kleinhans, 2020	Image + odor	_	paraphernalia, the cannabis odorant, non-psychoactive garden-variety flowers and related products, pure phenylethyl alcohol which smells like roses	_	Cross	*Not described*	*Not described*

Note: IAPS = International Affective Picture System (159), NAPS = The Nencki Affective Picture System (160).

[a] Adapted (62)

[b] Same task as Cousijn et al. 2013

[c] Adapted from Cousijn et al. 2013

Supplementary Table 2.7.3

fMRI Cue-Reactivity Task Duration, Design, and Stimuli Presentation Protocol

1st author, year	Total task duration	Task Design	Stimuli, *n*		Runs, *n*	Blocks, *n*	Stimuli shown/Block, *n*	Stimulus Presentation, *sec*	Inter Stimulus Interval		Rest between Blocks, *sec*	Random Stimuli Presentation	VAS presentation, *sec*
			Cannabis	Neutral					Duration, *sec*	Fixation cross			
Filbey, 2009	19 min & 12 sec	Block	1	1	2	2	6	20	20	✓	20	Pseudo	5
Charboneau, 2013	~9 min	Block	30	30	3	6	10	3	_	_	_	Random	_
Cousijn, 2013	11 min	Event-related	30	30	1	1	75	4	jittered b/n 2-6	✓	_	Quasi	_
Feldstein Ewing, 2013	~21 min & 20 sec	Block	1	1	1	4	5	20	20	✓	20	Pseudo	5
Goldman, 2013	8 min	Block	60	60	1	12	10	1.5	0.5	✓	20	Semi [a]	_
Bitter, 2014	_	Block	6	6	1	5	6	4.75	2.5	✓	20	Quasi	_
Filbey, 2014	19 min & 12 sec	Block	1	1	2	2	6	20	20	✓	20	Pseudo	5
Wetherill, 2014	8.5 min	Event-related	96	33	2	1	96	0.33	10-to-20 *jittered*	✓	_	Random / quasi	_
Wetherill, 2015	8.5 min	Event-related	96	33	2	1	96	0.33	10-to-20 *jittered*	✓	_	Random / quasi	_
Filbey, 2016	28 min	Block	1	1	3	3	6	20	20	✓	20	Pseudo	5
de Sousa Fernandes Perna, 2017	33 min	Block	10	10	1	30 sec blocks	_	_	_	_	_	Random	_
Vingerhoets, 2016	11 min	Event-related	30	30	1	1	75	4	2-to-6 *jittered*	✓	_	Quasi	_

Wetherill, 2016	8.5 min	Event-related	96	33	2	1	96	0.33	10-to-20 *jittered*	✓	_	Random / quasi	_
Karoly, 2019	_	Block	36	36	1	24	6	6	_	_	_	Pseudo	6
Zhou, 2019	_	Block	45	45	1	9	5	3	0.5-1.5 & 14.5-15.5 inter-block	✓	_	Random	5
Kuhns, 2020	_	Event-related	10	10	2	1	_	4	2-to-6 *jittered*	✓	_	Quasi	_
Yao, 2020 [b]	13 min & 30 sec (x 2)	Block	1	1	2	12	_	20	_	_	_	Pseudo	5
Kleinhans, 2020	12 min & 10 sec	Event-related	77	77	1	_	_	0.85	0.25	✓	_	_	_

Note: min = minute, sec = second, VAS = Visual Analogue Scale, N = number, Ctrl = Control Stimuli.
[a] as per Gellermann series (161).
[b] cue exposure task originally described in Filbey et al. (2016), which was modified from Filbey et al. (2009)

Supplementary Table 2.3.4

Technical Characteristics of Imaging Data Acquisition

Author, Year	MRI scanner	N head coil' channels	T1 acquisition parameters	fMRI acquisition parameters
Filbey, 2009	3T Siemens Trio	*NA*	MPRAGE, TR= 2300 ms, TE = 2.74 ms, TI = 900 ms, slab thickness = 176 mm, FOV =256 x 256 mm, matrix = 256 x 256 x 176, voxel size = 1 x 1 x 1 mm, number of echos = 4, pixel bandwidth = 650 Hz	EPI, TR = 2000 ms, TE = 27 ms, 32 slices, matrix size = 64 x 64, voxel size = 3 x 3 x 4 mm, FA = 70°
Charboneau, 2013	3T Philips Intera Achieva	*NA*	*NA*	*NA*
Cousijn, 2013	3T Philips Intera Achieva	8	TFE, TR= 9600 ms, TE = 4.6 ms, 182 slices, slice thickness =1.2 mm, FOV = 256 x 256 mm, in-plane resolution = 256 x 256, FA = 8°	EPI, TR = 2290 ms, TE = 30 ms, 38 slices, slice thickness = 3 mm, interslice gap = 0.3 mm, FOV = 220 x 220 mm, in-plane resolution = 96 x 96, FA = 80°
Feldstein Ewing, 2013	3T Siemens Trio	12	MPRAGE, TR = 2300 ms, TE = 2.74ms, TI = 900 ms, FOV = 256 x 256 mm, slab thickness = 176 mm, matrix = 256 x 256 x 176, voxel size = 1 x 1 x 1 mm, FA = 8°, number of echos = 4, pixel bandwidth = 650 Hz	EPI, TR = 2000 ms, TE = 27 ms, 32 slices, matrix size = 64 x 64, voxel size = 3 x 3 x 4 mm, FA = 70°
Goldman, 2013	3T Siemens Trio	*NA*	MPRAGE, TR = 1620ms, TE = 3 ms, FOV = 250 x 250 mm, matrix = 192 x 256, slice thickness = 1mm	EPI, TR = 2000 ms, TE = 30 ms, 33 slices, slices thickness = 3mm (no gap), FOV = 192 mm, matrix = 64 x 64 FA = 90°
Bitter, 2014	4T Varian Unity Inova	*NA*	*NA*	EPI, TR = 2000 ms
Filbey, 2014	3T Siemens Trio	12	MPRAGE, TR = 2300 ms, TE = 2.74 ms, TI = 900 ms, 192 slices, FOV = 256 x 256 mm, slab thickness = 176 mm, matrix = 256 x 256 x 176, voxel size = 1 x 1 x 1 mm, FA = 8°, number of echos = 4, pixel bandwidth = 650 Hz	EPI, TR = 2000 ms, TE = 27 ms, 32 slices, matrix = 64 x 64, voxel size = 3 x 3 x 4 mm, FA = 70°
Wetherill, 2014	3T Siemens Trio	8	MPRAGE, TR = 510 ms, TE = 3.7 ms, 160 slices, FOV = 192 × 256 mm, slice thickness = 1 mm, FA = 90°	EPI, TR = 2000 ms, TE = 30 ms, 32 slices, slice thickness = 4.5 mm, FOV = 64 × 64 mm, FA = 90°
Wetherill, 2015	3T Siemens Trio	8	MPRAGE, TR = 510 ms, TE = 3.7 ms, 160 slices, FOV = 192 × 256 mm, slice thickness = 1 mm, FA = 90°	EPI, TR = 2,000 ms, TE = 30 ms, 32 slices, slice thickness = 4.5 mm. FOV = 64 x 64 mm, FA = 90°
Filbey, 2016	3T Philips	*NA*	MPRAGE, TR = 8.2 ms, TE = 3.7 ms, TI = 1100ms, FOV = 256 x 256 mm, slab thickness = 160 mm, voxel size = 1 x 1 x 1 mm, FA = 12°	EPI, TR = 2000 ms, TE = 29 ms, 39 slices, matrix = 64 x 64, voxel size = 3.44 x 3.44 x 3.5 mm, FA = 75°
de Sousa Fernandes Perna, 2017	3 T Siemens Magnetom Allegra	*NA*	MPRAGE, TR = 9.7 ms, TE = 4 ms, matrix = 256 ×256, voxel size = 1 × 1 × 1 mm, FA = 12°	EPI, TR =2000 ms, TE= 30 ms, FOV = 224 mm, matrix = 64 x 64, voxel size = 3.5 x 3.5 x 3.5 mm, FA = 90°
Wetherill, 2016	*NA*	*NA*	*NA*	*NA*

Karoly, 2019	3T Siemens Trio	*NA*	*NA*	EPI, TR = 2200 ms, TE = 35 ms, 37 slices, FOV = 192 mm, slice thickness = 3 mm, Matrix = 64 × 64, voxel size = 3 × 3 mm, FA =90°;
Zhou, 2019	3T Siemens Trio	*NA*	TR = 1660 ms, TE = 2.54 ms, 208 slices, FOV = 256 mm, voxel size = 0.8 × 0.8 × 0.8 mm	EPI, TR = 2500 ms, TE = 30 ms, 37 slices, FOV = 192 mm, voxel size = 2 × 2 × 3 mm, FA = 90°
Kuhns, 2020	3T Philips Intera Achieva	32	TFE, TR = 8200 ms, TE = 3.8 ms, 220 slices, FOV = 240 × 188 mm, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm, FA = 8°	EPI, TR = 2000 ms, TE = 27.63 ms, 37 slices, FOV = 240 × 240 mm, slice thickness = 3 mm, voxel size = 3 × 3 × 3 mm, slice gap = 3 mm, flip angle = 76.1°
Yoo, 2020	3T Philips	*NA*	MPRAGE, TR = 8.1 ms, TE = 3.7 ms, voxel size = 1 × 1 × 1 mm, matrix = 256 × 256, FOV = 256 × 256 mm, FA = 12°	EPI, TR = 2000 ms, TE = 29 ms, FOV = 220 × 220 mm, matrix = 64 x 64, voxel size = 3.44 × 3.44 × 3.50 mm, FA = 75°
Kleinhans, 2020	3T Philips Intera Achieva	32	MPRAGE, TR = 7.6 ms, TE = 3.6 ms, TI = 910.5 ms, FOV = 256 × 256 × 176 mm, matrix = 176 × 256 matrix, voxel size = 1 × 1 × 1 mm, FA = 7°	EPI, TR = 2000 ms, TE = 24 ms, 39 slices (no gap), FOV = 240 × 240 × 156 mm, matrix = 80 × 78, voxel size = 3 × 3 × 4 mm, FA = 79°

Note: EPI = echo-planar imaging, TR = repetition time, TE = echo time, TI = inversion time, FOV = field of view, FA = flip angle, TFE = Turbo Field Echo, MPRAGE = magnetization-prepared gradient echo, *NA* = not available

Supplementary Table 4.7.5

Overview of the Risk of Bias: National Heart, Lung, and Blood Institute – Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Author, year	1	2	3	4a	4b	5	6	7	8	9	10	11	12	13	14
Filbey, 2009	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (cannabis dependence severity, cannabis problems, cannabis dosage, frequency, duration, age of onset)
Charboneau, 2013	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Cousijn, 2013	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (cannabis problems, cannabis lifetime use, cigarette/day)
Feldstein Ewing, 2013	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (Age, gender, IQ, alcohol frequency)
Goldman, 2013	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Bitter, 2014	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (IQ)
Filbey, 2014	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y(cannabis problems, MCQ, cigarette/day)
Wetherill, 2014	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Wetherill, 2015	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (age, cigarettes & drinks/day, depression)
Filbey, 2016	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (drinks per drinking day, education years)
de Sousa Fernandes Perna, 2017	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Vingerhoets, 2016	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	Y	***Y*** (baseline CUDIT, AUDIT, FTND, MCQ, cigarettes/day, lifetime use of other psychotropic substances)
Wetherill, 2016	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Karoly, 2019	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***
Zhou, 2019	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (abstinence duration, age of onset)
Kuhns, 2020	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (alcohol dependence)
Yoo, 2020	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	Y (age)

Kleinhans, 2020	Y	Y	Y	Y	Y	***N***	Y	Y	n/a	Y	n/a	Y	***N***	n/a	***N***

Note: 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 cues as a dichotomous variable (i.e. cannabis vs neutral cues) was a key inclusion criteria.

Note: Item 10. was deemed ‘not applicable’ as single trials in fMRI analysis are considered a non-reliable method and requires multiple trials for a single reliable measure of cue-reactivity.

Note: Item 13. was deemed ‘not applicable’ as all studies (but one) were cross-sectional.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	n/a
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 5-6
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 5-6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 6-7
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 6-7
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 6-7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 6-7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 6-7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 6-8
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 8
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	n/a
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 8-14
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 22-32

Section and Topic	Item #	Checklist item	Location where item is reported
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	n/a
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	n/a
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	n/a
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 8
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 7 & 32
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	no
Study characteristics	17	Cite each included study and present its characteristics.	Page 8 & 23 & Supp Material
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supp Material
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	n/a
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 8-14
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	

Section and Topic	Item #	Checklist item	Location where item is reported
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 14-16
	23b	Discuss any limitations of the evidence included in the review.	Page 17-18
	23c	Discuss any limitations of the review processes used.	Page 17
	23d	Discuss implications of the results for practice, policy, and future research.	Page 18-19
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 3 & 6
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	n/a
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	n/a
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 2
Competing interests	26	Declare any competing interests of review authors.	Page 2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 6

Chapter 3: General Experimental Methods

3.1 Chapter Guide

This chapter provides a comprehensive overview of the experimental methodology for the cross-sectional experiment in Study 2 (Chapter 4) and intervention experiment in Study 3 (Chapter 5). The two experiments were written for publication. As such, the method sections in both studies comprise the level of detail and word count reflecting publishing limits of targeted journals. The following chapter will outline general study information, including ethics approval and recruitment. Participant information is summarised with inclusion and exclusion criteria, screening procedures and diagnostic and clinical assessment measures employed. A detailed outline of the administration of the overall protocol is described, including procedures related to the neuroimaging component and statistical programs used for data analyses.

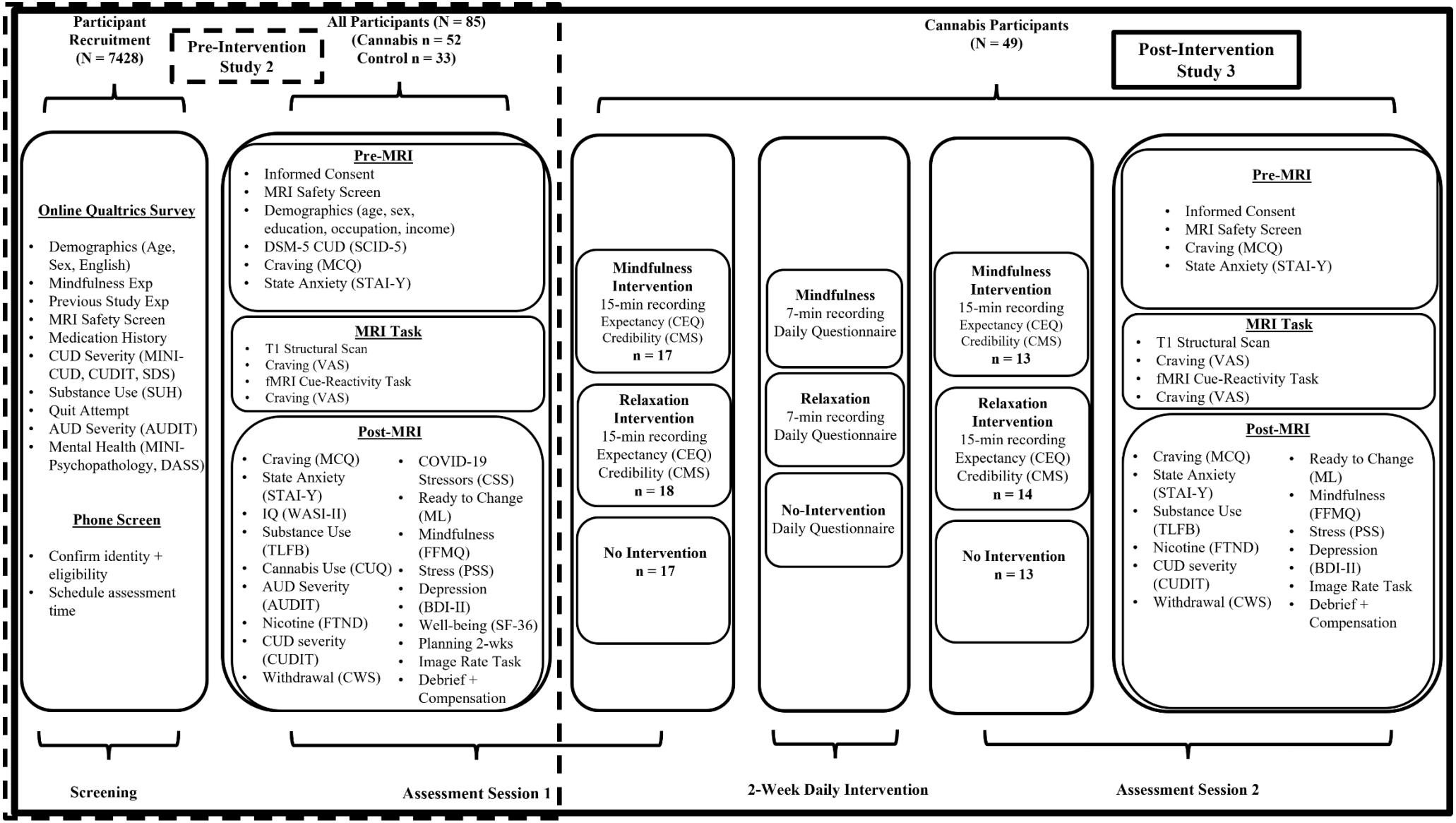
3.2 General Study Overview

This PhD research study protocol 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 (Ethics ID: 2019-71H; see Appendix C).

This PhD project is nested within a larger study: ”Mapping short-term brain changes in cannabis users: A fMRI Study” that was pre-registered (ID: ISRCTN76056942; see Appendix D). Therefore, only the measures relevant to the experiments comprised in this PhD project will be described. Figure 3.1 overviews the measures used to screen participants for the two experiments of this PhD. Some measures were relevant for both experiments and will be described only once for succinctness. Informed consent was confirmed prior to participation in all assessment sessions (see Appendix E). All assessment sessions were conducted at the Monash Biomedical Imaging Centre in Clayton, Victoria, utilising the MRI and behavioural testing facilities.

Figure 3.1

Overview of PhD Project Screening and Assessment Measures and Protocol



Note. The assessment measures and protocol included in the Pre-Intervention Study (Chapter 4) is outlined in the dashed line. The assessment measures and protocol included in the Post-Intervention Study (Chapter 5) is outlined in the solid line. MRI = Magnetic Resonance Imaging, CUD = Cannabis Use Disorder, MINI = Mini International Neuropsychiatric Interview, CUDIT = Cannabis Use Disorders Identification Test, SDS = Severity of Dependence Scale, SUH = Substance Use History, AUD = Alcohol Use Disorder, AUDIT = Alcohol Use Disorders Identification Test, DASS = Depression, Anxiety, and Stress Scale, DSM-5 = Diagnostic and Statisitacal Manual for Mental Health Disorders – Fifth Edition, SCID-5 = Structured Clinical Interview for DSM-5 Research Version, MCQ = Marijuana Craving Questionnaire, STAI-Y = State-Trait Anxiety Index – Y Form, VAS = Visual Analogue Scale, IQ = Intelligence Quotient, WASI-II = Weschler Abbreviated Standardised Intelligence – II, SF-36 = The 36-Item Short Form Survey Instrument, TLFB = Timeline Follow-Back, CUQ = Cannabis Use Questionnaire, FTND = The Fagerström Test for Nicotine Dependence, CWS = Cannabis Withdrawal Scale, PSS = Perceived Stress Scale, BDI-II = Beck Depression Index – II, ML = Marijuana Ladder, FFMQ = Five-Factor Mindfulness Questionnaire, CEQ = Credibility/Expectancy Questionnaire, CMS = Comprehension/Manipulation Check.

3.3 Assessment Protocol: Sample Selection and Intake

3.3.1 Recruitment

Eighty-five participants were recruited from Melbourne, Australia via public platforms (e.g., Google Ads, Gumtree, Facebook, university websites, community flyers, and others). See Appendix F for example of advertisements.

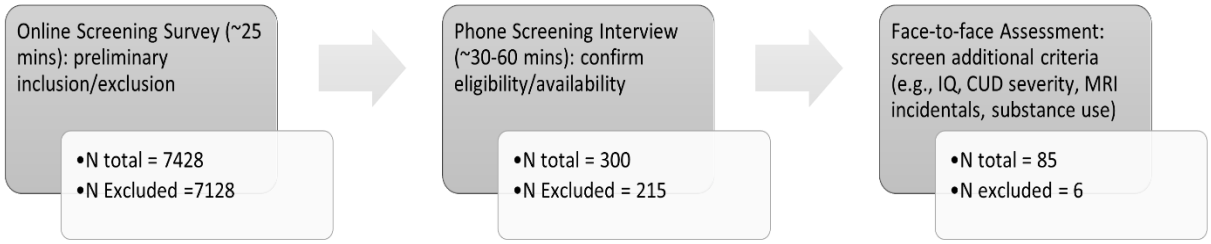
Pre-Intervention Study. Fifty-two cannabis users meeting criteria for a moderate-to-severe CUD were recruited for the CUD group and 33 non-cannabis using participants were recruited for the control group.

Post-Intervention Study. Participants in the CUD group were equally allocated to three different intervention groups (i.e., n = 18 mindfulness, n = 17, relaxation, n = 17 no intervention).

Advertisements for study participation included a link that directed ~7,428 interested members of the community to an online screening survey. Of these, 7,128 people were deemed ineligible or did not fully complete the online screening survey. A comprehensive interview script was used to contact the ~300 people who were potentially eligible in the study, of which 85 were confirmed as eligible and attended the face-to-face assessment session. Any queries about participants' eligibility were resolved via discussion with the study CI and research team, before confirming inclusion or exclusion in the study. Figure 3.2 provides an overview of the recruitment/screening process.

Figure 3.2

Overview of Recruitment and Inclusion/Exclusion Screening Process



Note. Flowchart depicts the recruitment and eligibility screening process and number of people excluded at each stage, as per 31 January 2022.

IQ = Intelligence Quotient. CUD = Cannabis Use Disorder. MRI = Magnetic Resonance Imaging.

3.3.2 Inclusion and Exclusion Criteria

Inclusion criteria for all participants were: age between 18 and 56 years, proficient in English, have normal or corrected-to-normal vision.

CUD participants were included if they: i) endorsed DSM-5 criteria for a moderate-to-severe CUD, ii) used cannabis daily/almost daily for minimum past 12-months, and iii) reported at least one attempt to quit or to reduce their use in the past 24 months.

All participants were excluded based on the following criteria: i) significant medical conditions, history of acquired brain injury or loss of consciousness > 5 minutes, ii) history of psychopathology (except for depression and anxiety) ascertained by the Mini International Neuropsychiatric Interview; iii) significant alcohol use or dependence; or iv) illicit drug use (other than cannabis in the cannabis group) in the past 4-weeks or above recreational levels (i.e., > 50 lifetime episodes, or > weekly use over a 3-month period), or v) any illicit drug and alcohol use self-reported in the 12-hours before testing; vi) current use of prescription medication that affects the central nervous system (except for anti-depressants – e.g., SSRI's, SSNI's, due to increased prevalence of depression and anxiety in CUD populations and our

inclusion of these mental health disorders; (162); v) MRI contraindications (e.g., pacemaker, pregnancy); viii) IQ scores < 80 determined by the Weschler Abbreviated Standardised Intelligence-II (Vocabulary and Matrix Reasoning; (163); and ix) regular mindfulness or relaxation experience (i.e., formal training and/or ≥ 1 x month or > 10 lifetime occasions in past year) assessed by qualified mindfulness instructors and trained researchers.

3.3.3 Online Survey for Participant Screening

An approximately 25-minute online screening survey using Qualtrics software was used to select eligible participants according to inclusion and exclusion criteria. The survey collected information relevant for participants eligibility including demographics, MRI safety contraindications, medical conditions and medication, history of illicit substance use, alcohol consumption and related problems, psychopathology, mindfulness and relaxation experience, as well as previous cannabis quit attempt via the measures listed below.

3.3.3.1 MRI Screening Questionnaire (MSQ)

The MSQ was provided by Monash Biomedical Imaging Centre; https://www.monash.edu/researchinfrastructure/mbi/forms-and-policies/mbi-policies. This questionnaire screens for any contraindication for undergoing an MRI scan (i.e., currently pregnant, weight, metal in the body, etc.).

3.3.3.2 Alcohol Use Identification Test (AUDIT)

The AUDIT (164) is a screening tool comprised of 10-items developed to assess alcohol consumption and related problems. The AUDIT also provides diagnostic cutoffs (i.e., alcohol dependence with scores ≥ 19).

3.3.3.3 Cannabis Use Identification Test – Revised (CUDIT-R)

The CUDIT-R (165) is an 8-item cannabis misuse-screening tool. It has good psychometric properties (Cronbach's $\alpha = 0.72$) based on clinical and community-based populations. It has DSM-5 diagnostic cutoffs for mild, moderate, and severe CUD (i.e., 9-10; 11; 12-13) respectively (16).

3.3.3.4 Substance Use History (SUH)

The SUH is adapted from the Drug History Questionnaire (166) and has been validated in drug users with Cronbach alpha coefficients ranging from 0.66-0.93. The SUH provided details on illicit substance use across the lifespan and was used to assess recreational levels and time since last use.

3.3.3.5 Mini International Neuropsychiatric Interview (MINI)

The MINI (167) 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 (2-3 = mild; 4-5 = moderate; 6-11 = severe).

3.3.3.6 Depression, Anxiety, and Stress Scale-21 (DASS-21)

The DASS-21 (168) is a 21-item clinical questionnaire that provides a quantitative measure of distress along the three axis of depression, anxiety and stress. Responses are given via a 4-point Likert scale (0 = Did not apply to me at all, 4 = Applied to me very much, or most of the time). Whilst the DASS is not recommended as an isolated diagnostic tool, it does characterise the degree of severity relative to the population. As such, scores represent

normal, mild, moderate, severe and extremely severe levels of depression, anxiety, and stress, respectively.

3.3.3.7 Cannabis Quit Attempt

To determine if cannabis users had previously attempted to reduce or cease their cannabis use in the past 12-24 months – single question was asked: "Have you attempted to cut down and/or quit using cannabis in the past 12-24 months?"

3.3.4 Follow-Up Phone Call to Confirm Eligibility and Assessment Time

Participants suitable for either the CUD or control group were contacted via a follow-up phone call to confirm their interest and availability to participate in the study. A comprehensive script was used to provide research aims and details of study participation, as well as questions to clarify details required to verify eligibility (e.g., substance use, medication, mindfulness experience, mental health and MRI safety). See Appendix G for full script.

3.4 Assessment Protocol: Pseudo-Randomised Group Allocation

A study co-ordinator not involved in data collection or analysis pseudo-randomly and equally assigned CUD participants with a stratification based on age, sex, and education years to one of three groups: i) mindfulness intervention (MBI); ii) relaxation active-control intervention; iii) no intervention passive control. Age stratification was based on three age ranges in years: i) 18-24; ii) 25-35; iii) 36-55. Sex was based on male and female, and years of education was monitored to ensure groups were matched.

3.4.1 Double-Blind Design

To minimise the risk of bias, researchers that conducted the face-to-face assessment for collection of MRI and behavioural data unrelated to the intervention were blinded to participant group assignment (concealed for the duration of data collection). Unblinded researchers only conducted intervention related-data collection and were responsible for confidential and secure storage of relevant data (password protected online servers and locked filing systems) .

To prevent expectancy effects specifically related to the engagement in one of the three conditions, participants remained blind to their group allocation and the specific study aims related to mindfulness. As such, there was no mention of the term “mindfulness” (or “relaxation”) made in any experimental or recruitment material to reduce expectancy effects relating to the increasing popularity and public discussion of complementary medicine approaches.

3.5 Assessment Protocol: Pre-Intervention Session 1

The pre-intervention assessment session was conducted in two parts over ~4.5-6 hours. A blinded and an unblinded tester were present to administer specific parts of the study protocol. The first part, conducted by the blinded tester, included all experimental procedures and assessments of socio-demographic variables, substance use, mental health, cognitive performance and MRI protocol. All participants (CUD and non-using controls) completed a battery of validated self-report measures related to mindfulness, substance use, and mental health. In addition, participants underwent a cognitive task to assess IQ, and a fMRI cue-reactivity task – which measured the main dependent variable of this study (i.e., brain activity during presentation of cannabis vs neutral stimuli) in the CUD group compared to controls. The assessment finished with a debrief and reimbursement for the non-using control group

(CUD participants were reimbursed at the end of the second session 2-weeks later). Participants in the CUD group underwent a planning session to schedule the 2-week daily task (i.e., intervention practice and/or completion of the Daily Questionnaire). The blinded tester then left and the unblinded tester conducted the second part. In this part of the assessment, the unblinded tester administered the relevant components depending on group allocation. Participants allocated to either the MBI or relaxation were guided through a 15-min intervention including completion of brief measures related to the comprehension and expectancy of the intervention. The session ended with instructions on how to practice the intervention task either via an online link or file stored on a USB, followed by a debrief.

3.5.1 Measures

3.5.1.1 Sociodemographic Information

3.5.1.1.1 Sociodemographic Survey. This was administered to confirm participant demographics (i.e., age, sex), and collect information on employment status (e.g., full/part time, homemaker), occupation, income, and level of education in years.

3.5.1.2 Substance Use

3.5.1.2.1 Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV). The SCID-5-RV (169) is an 11-item semi-structured interview that measures cannabis dependence according to specific DSM-5 criteria for CUD. This was administered at the first assessment session (pre-intervention) to confirm moderate-to-severe CUD by a staff or student research trained in SCID-5-RV administration. The severity of a CUD was determined by the number of criteria endorsed (i.e., 2-3 = mild; 4-5 = moderate; 6-11 = severe).

3.5.1.2.2 Marijuana Craving Questionnaire – Short-Form (MCQ-SF). The MCQ-SF (170) is a 12-item questionnaire that assesses cannabis craving. Each item is rated on a seven-point Likert-type scale ranging from "strongly disagree" to "strongly agree." Higher scores indicate stronger intensity of subjective craving.

3.5.1.2.2 Cannabis Withdrawal Scale (CWS). The CWS (171) is a 19-item diagnostic instrument for regular monitoring of negative impact of cannabis withdrawal symptoms on daily activity in clinical and research settings. Responses are measured on a 10-point scale from ‘Not at all’ to ‘Extremely’. Cannabis withdrawal scores range from 0-190, with higher scores indicating greater impact of withdrawal symptoms on functioning.

3.5.1.2.3 Cannabis Use Interview (CUI). Lifetime history of cannabis use was measured with a semi-structured interview and has been utilised for the testing of cannabis users in research settings (172-174). The CUI captures years of regular use, age of onset, changes in cannabis use patterns over time (e.g., frequency, dosage, abstinence periods). Details of typical cannabis administration method (e.g., joint, bong, ingested), as well as type (e.g., dried flower vs hashish resin) and strength consumed (rated ‘0’ = weak to ‘10’ strong) were collected for all intervals of time reflecting changes in use frequency (e.g., monthly, weekly, daily) across the individual’s lifetime period of use.

3.5.1.2.4 Timeline Follow-Back (TLFB). The TLFB (148) is a method that has been validated to quantify estimates of substance use including alcohol, cigarettes, cannabis, and other drugs, as well as measure change in substance use levels over time. It involves asking participants to retrospectively estimate their substance use using tools such as calendar events and social media logs in a specified timeframe prior to the assessment date (175). The TLFB was used to capture all illicit substance use as well as alcohol and nicotine in the past 30-days prior to the first assessment session and days prior to the second assessment session.

3.5.1.2.5 The Marijuana Ladder (ML). The ML (176) is a self-report visual scale measuring motivation/readiness to change cannabis using habits, adapted from cigarette smokers for marijuana. Eleven rungs and five statements represent stages of change. Ratings range from (0) = least motivated to (10) = most motivated. Scores ranging from 1-3 correspond with the stage of pre-contemplation (e.g., no plan to change cannabis use). Scores 4-6 correspond with the stage of contemplation (e.g., think about use, but no current plan to change it). Scores 7-8 correspond with the stage of preparation (e.g., planning on making changes/starting to reduce cannabis use). A score of 9 corresponds with the stage of action (e.g., I have made changes but worry about slipping back) and a score of 10 corresponds with the stage of maintenance (e.g., made changes and will never go back). The ML has demonstrated predictive validity of future cannabis use and treatment engagement among incarcerated adolescents (177).

3.5.1.2.6 The Fagerström Test for Nicotine Dependence (FNTD). The FTND (178) is a standardised measurement tool used to assess the intensity of physical addiction to nicotine. The multiple choice six-item test was designed to provide an ordinal measure of nicotine dependence related to cigarette smoking (i.e., quantity, compulsion to use, dependence). Items are scored 0-3 with a total score ranging from 0-10. Scores ≥ 3 indicate nicotine dependence.

3.5.2 Neuroimaging

3.5.2.1 MRI

All participants underwent an MRI session at Monash Biomedical Imaging Centre in Clayton, Victoria. MRI images were acquired on a Siemens Skyra 3 Tesla scanner (for data acquisition parameters for structural [T1] and functional [task] MRI scans see Appendix H). The total MRI scanning time was about 45-minutes and required

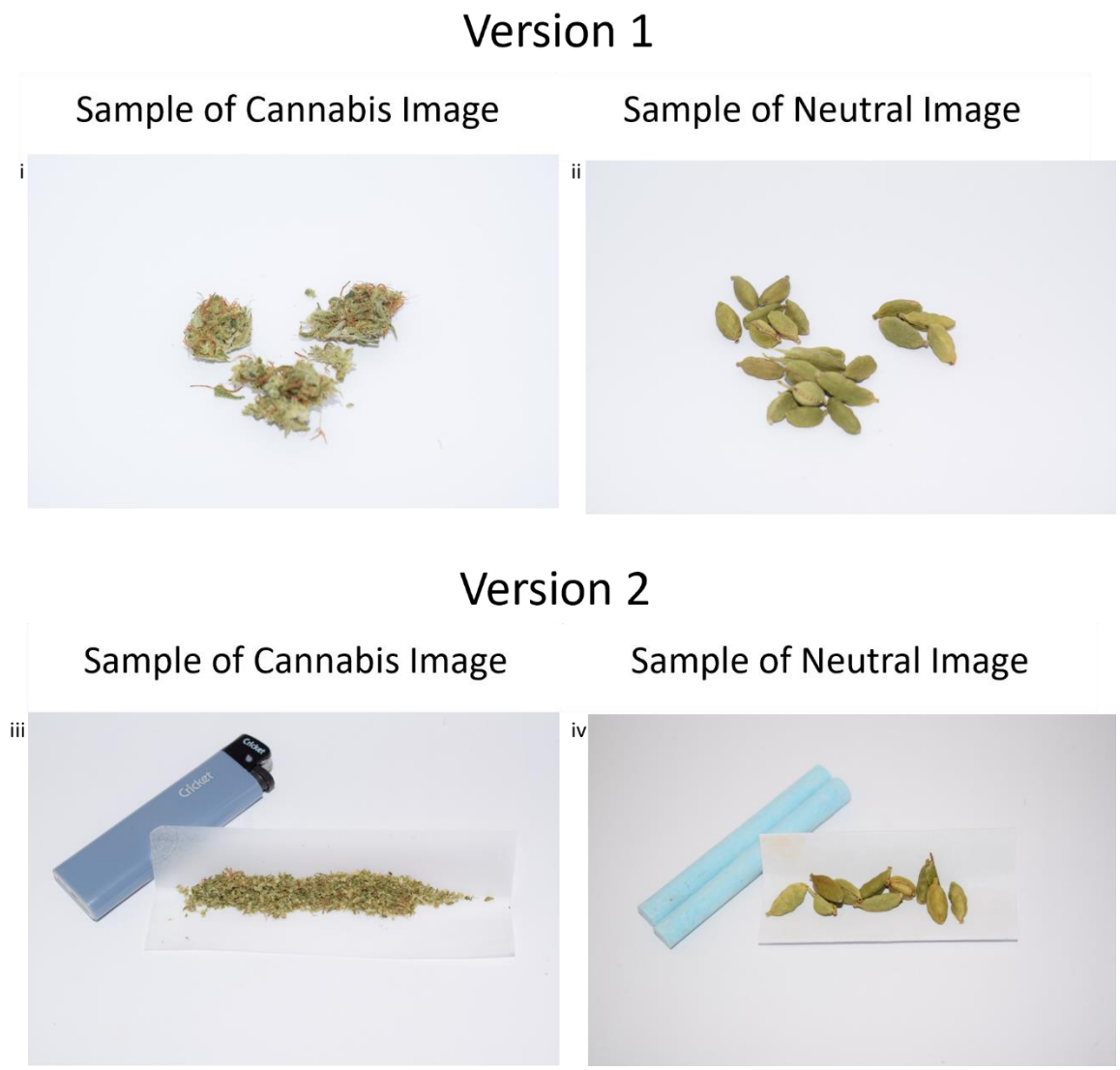
participants to lay still inside the scanner. A head-mask was placed on their head with a mirror attached enabling them to view the in-scanner tasks displayed on a screen positioned behind the MRI scanner.

3.5.2.2 fMRI Cue-Reactivity Task Description

An event-related cue-reactivity fMRI task was used to measure brain activity while participants passively watched 30 cannabis and 30 neutral non-cannabis images used in previous experiments from our extended team (Cousijn and colleagues, *Addictive Behaviors*, 2013; 82) and new images of comparable quality, complexity and luminosity. The images were visuals of cannabis-related paraphernalia and smoking behaviours and were controlled for visual valence to neutral cues, such as stationary items or cooking utensils. Cannabis and non-cannabis cues were comparable in level of complexity, type of activity, size, brightness, and luminance (see Figure 3.3 for an example).

Figure 3.3

Samples of Cannabis and Neutral Images in the fMRI Cue-Reactivity Task: Version 1 and 2



Note. Version 1 cannabis image: i) dried cannabis cluster. Version 1 neutral image: ii) a cluster of de-shelled pistachios. Version 2 cannabis image: iii) a lighter with dried cannabis on joint rolling paper. Version 2 neutral image: iv) chalk and de-shelled pistachios on paper.

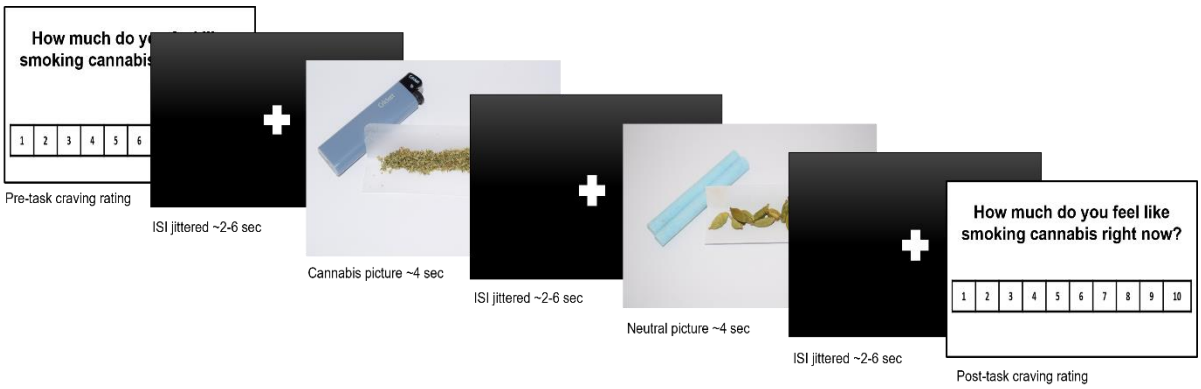
Participants were instructed to pay close attention to the images. To ensure they maintained attention, participants' alertness was monitored via an MRI compatible camera (provided by imaging facility), and any occasions of sleepiness prompted a re-start of the scan to ensure engagement. Images were presented using E-prime3 software (Version 3.0

Build 3.0.3.80, E-Studio Build 3.0.3.82, Psychology Software Tools Inc.) onto a rear projection screen positioned behind the MRI scanner. There were two versions of this task, which were identical in procedure but contained different pictures (matched for complexity, object size, colours and brightness) in order to minimise the compounding impact of memory and recognition on cue-reactivity at the second assessment session. The two versions were delivered in a counterbalanced order at both assessment sessions (pre-and-post intervention), via a pseudorandomised procedure (max three images of the same category in a row). Each image was presented for 4 seconds preceded by a fixation-cross that lasted on average 4 seconds, jittered between 2 and 6 seconds. Total task duration was ~10 minutes.

3.5.2.2.1 Visual Analogue Scale – Subjective Craving Rating (VAS). The VAS is a tailored measurement instrument that was used to measure the momentary craving for smoking cannabis on a 10-point scale. The question "How much do you feel like smoking cannabis right now?" with '0' indicating "Not at all" and 10 indicating "Extremely" was presented pre and post the cue-reactivity task. See Figure 3.4 for an example of the fMRI cue-reactivity task stimulus presentation and craving rating.

Figure 3.4.

Example of fMRI Cue-Reactivity Task Stimulus Presentation and Craving Rating



Note. Example of fMRI cue-reactivity task stimulus presentation. ISI = inter-stimulus interval. Sec = seconds. A craving rating was presented at the start and completion of the task

asking participants to rate “How much do you feel like smoking cannabis right now?” on a scale of 1-10. A total of 60 images were presented with an ISI (black screen with a centred white ‘+’). Display times illustrated.

3.5.2.3 Post-MRI Rating of Images Used in the fMRI Cue-Reactivity Task

On a computer, participants were re-presented each picture in the fMRI cue-reactivity task and were asked to rate the arousal and affective valence of the cannabis and neutral images using VAS scales from 0 to 10. Arousal was rated from 0 representing “calm” to 10 “excited”; and affective valence was rated on a scale from 0 “unpleasant”, to 10 “pleasant” with a rating of 5 representing “neutral”.

3.5.2.4 Cognition

3.5.2.4.1 Weschler Abbreviated Standardised Intelligence - II (WASI-II). The WASI-II (163) is a standardised measure of IQ. Two-subtests – Vocabulary and Matrix Reasoning were administered at the first assessment session (pre-intervention) to assess IQ and characterise the sample.

3.5.2.5 Mental Health

3.5.2.5.1 State-Trait Anxiety Index – Y Form (STAI-Y). The STAI-Y (179) is a 20-item questionnaire for assessing state anxiety. All items are rated on a 4-point scale (e.g., from “Not at all” to “Very much so”). Higher scores indicate greater anxiety, which can range from 20-80. Scores ranging between 20-37 indicate “no or low anxiety”, 38-44 “moderate anxiety” and 45-80 “high anxiety”.

3.5.2.5.2 Perceived Stress Scale (PSS). The PSS (180) measures the degree to which situations in one’s life are appraised as stressful. Items are designed to tap into how

unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of explicit questions about current levels of experienced stress. Scores on the PSS are not considered a diagnostic tool for a mental health disorder; however, they can be used to differentiate between low (0-13), moderate (14-26) and high (27-40) levels of perceived stress.

3.5.2.5.3 Beck Depression Index - II (BDI-II). The BDI-II (157) is a 21- item self-report measure of depression symptomology which aligns with DSM-4 criteria – and is able to discriminate between a clinical and general populations (181). Each item is rated on a 4-point Likert scale. Total scores reflect depression symptomology considered normal (1-10), mild mood disturbance (11-16), borderline clinical depression (17-20), moderate (21-30), severe (31-40) and extreme depression (40+).

3.5.2.5.4 The 36-Item Short Form Survey Instrument (SF-36). The SF-36 (182) is a set of generic, coherent, and easily administered quality-of-life measures encompassing physical, emotional and social functioning. These measures are widely utilized by research, managed care and government organizations for routine monitoring and assessment of clinical outcomes in adults.

3.5.2.6 Mindfulness

3.5.2.6.1 Five-Factor Mindfulness Questionnaire (FFMQ). The FFMQ (183) is a widely used psychological measurement of mindfulness comprising 39-items derived from an exploratory factor-analysis of items from five independently developed mindfulness questionnaires. Based on the results of the analysis mindfulness can be conceptualised as a construct comprising of five related dimensions: observing, describing, acting with awareness, non-judging of inner experience, and non-reactivity to inner experience, as well as an overall mindfulness score. All items are rated on a 5-point Likert-type scale ranging from

1 (never or very rarely true) to 5 (very often or always true). Cronbach's alpha coefficients for each factor ranges from .72 - .92. There is not cut off score indicating one is mindful or not. The scores represent a spectrum of mindfulness, with higher scores indicating greater mindfulness.

3.5.2 Intervention Protocol

At the first assessment session, prior to the intervention, participants underwent a *planning session* to schedule time to complete the 2-week intervention described as "daily tasks". To enhance compliance participants were encouraged to problem-solve potential barriers (e.g., put reminders in their phone).

3.5.2.1 Experimental Conditions: MBI and Active Control (Relaxation)

Using headphones, the MBI group then listened to a 15-minute mindfulness script and the active control (relaxation) group listened to a comprehension complexity, word count and time-matched relaxation script (validated in alcohol studies and adapted for cannabis; Kamboj et al., 2017; see Appendix I). Specifically, the two groups listened to a 3-minute explanation of the relevant strategy for managing cannabis cravings. They were then administered *The Credibility/Expectancy Questionnaire (CEQ).* The CEQ (184) measured the participant's belief that the received therapy will help to reduce cannabis cravings. The questions address what the participants both "*think"* and "*feel"* will happen. The MBI and relaxation group then listened to a 4-minute audio to introduce the practice followed by an extended 7-minute practice. To assess compliance/comprehension of the intervention the two groups were administered the *Comprehension/Manipulation Check (CMC*; 116) which is a 9-item questionnaire of intervention specific questions.

At the end of the first assessment session, participants in *all three groups* were instructed to complete their "daily tasks" via a link sent to them via SMS. The relevant "daily tasks" were also provided on a USB to ensure any technical issues did not prevent compliance.

3.5.2.2 Two-Week Daily Intervention

The two-week daily intervention was completed autonomously by participants via online Qualtrics links. The MBI and relaxation groups were asked to practice the brief intervention daily for 7-minutes in the two-weeks between assessment sessions (pre- and post-intervention).

3.5.2.3 Daily Questionnaire (DQ). All three groups were asked to complete a daily questionnaire (~3-minutes) which provided data on key behavioural variables to aid interpretation of the findings. The questions in the DQ asked participants in the MBI and active relaxation control group to report on daily 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 cravings to smoke cannabis, have you practiced the strategy you have been listening to on the audio track?") and provided metrics of engagement time (i.e., if and how long they listened to the audio). The questions in the DQ asked participants in *all three groups* via a VAS to rate the intensity (1-10) of their cannabis craving (i.e., physiological feeling) and urges (i.e., see something that makes them want to use cannabis), as well as daily ability to "step back and be aware of cravings/urges without being taken over by them". Participants were also asked to rate their mental state, level of relaxation-tension, nervousness/stress, and judgement of thoughts as "good or bad". Questions related to daily cannabis use provided information on 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?").

3.5.2.3 Monitoring of Participants' Compliance to Daily Task

An unblinded researcher monitored the participants' completion of the daily tasks relevant to each intervention group. Via Qualtrics, the unblinded researcher was able to observe if the participant had opened the provided link. An SMS reminder was sent to the participant if they did not access the link after one and two days. A telephone call to the participant was made to confirm if they were experiencing any issues in completing the task/s after missing more than two consecutive days of accessing the link (and receiving two SMS reminders).

3.5.3 Experimental Conditions

3.5.3.1 Mindfulness Script

Mindfulness instructions 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." Participants were 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."

3.5.3.2 Relaxation Script

By contrast, during the explanation of the strategy, the relaxation group was told, for example, that craving intensity can be reduced by "softening the muscles...and calming and unwinding the mind…releasing tension in your body". It was also emphasized that this is a way of gaining control over craving. Participants were also instructed that relaxation enables transformation of sensations into more calming, less unpleasant experiences.

3.5.3.3 No Intervention

Participants allocated to the no intervention condition were asked to complete the Daily Questionnaire, to minimize discernment of allocation to the control group.

3.6 Post-Intervention Assessment Session 2

The second assessment session was identical to the first with a few exceptions. First, at the first session, both CUD participants and non-using controls were included. Only the CUD group attended the second session in order to investigate differential effects of the three interventions over time. Second, the order of the assessment items was rearranged. The intervention was moved to immediately after consent, in order to boost possible effects of the brief intervention before the MRI scan, cognitive testing and all questionnaires.

There were also a few measures administered at the first session that were not readministered at the second and are outlined below:

- No measure of IQ was included at the second session as it was only needed at the beginning of the study to describe and match the groups, as well as screen for IQ < 80. Due to practice effects, it is recommended to re-administer a minimum of 2 years.
- "Trait" variables already assessed at the first session (i.e., socio-demographic data, CUI, AUDIT, CUD module of SCID, and SF-36) were not repeated as they are validated for measuring variables over periods of time longer than 2 weeks and not sensitive to detect changes within a 2-week timeframe.
- The 2-week planning session was not included in the second session as the intervention had been completed.

3.7 Software Packages and Statistical Analysis

Detailed description of data software packages and statistical analysis for the two experimental studies are outlined in their respective chapters (Chapters 4 and 5). As such, a brief overview of the software packages used in this thesis for data analysis will be described.

All MRI data was transferred and managed on MBI-XNAT archive system. Raw data in DICOM format were downloaded on a cloud-based cluster-computational platform, MASSIVE (massive.org.au). Data were then converted into BIDS format using BIDScoin (python toolkit) and dcm2niix (mricrogl). All quality checks and analyses were performed on MASSIVE. MRI quality checks and data pre-processing for both experimental studies was conducted using fMRIPrep (version 1.1.1) to ensure they could be used for the fMRI analysis (https://fmriprep.org/en/stable/index.html).

MATLAB Version r2018a and Statistical Parametric Mapping (SPM) Version 12 were used to conduct first and second level analyses. FMRIB's Software Library (FSL version 6.0.0;(185) were used to create ROIs for the post-intervention study (Chapter 5). Statistical Package for the Social Sciences (SPSS) version 28 was used to conduct all other statistical analyses.

Chapter 4: Mapping the Brain Functional Correlates of Cue-Reactivity in Moderate-to-Severe Cannabis Use Disorder: A Functional Neuroimaging Study

Study 2

4.1 Chapter Guide

This chapter presents Study 2, which is an experiment that investigated the neural mechanisms associated with cannabis cue-reactivity in CUD. The experiment aimed to address the limitations of the fMRI literature in the neurobiology of cannabis cue-reactivity in cannabis users reviewed in Chapter 2. We aimed to i) compare brain function during exposure to cannabis and neutral images in individuals with a moderate-to-severe CUD who have tried to reduce or cease use in the past 2 years, compared to non-using controls, and ii) explore correlations between brain functional differences between the CUD and control group, and levels of cannabis exposure (i.e., cannabis grams/month, years of regular use, hours from last use), and anxiety and depression levels.

Due to COVID-19 related disruptions to recruitment and face-to-face data collection, while facing the timeline to complete my PhD, the sample included in this experiment comprises a portion of the sample recruited thus far (N = 85). As this experiment is intended for publication, the analyses will be re-run on the complete sample (N = 120) post-submission of this thesis. As such, the findings are considered preliminary.

4.2 Abstract

Background: Cannabis use disorder (CUD) is characterised by high reactivity to cannabis cues, which can trigger habitual/compulsive use despite attempts to cut down or quit. The fMRI evidence to date reported differences between cannabis users and controls in prefrontal, striatal and parietal activity when viewing cannabis vs neutral stimuli, with emerging correlations with subjective craving. No study has measured if cannabis users endorsed a CUD using DSM-5 criteria. We aim to examine brain function during cue-reactivity for the first time in CUD compared to controls, and any differences in brain function in relation to cannabis use parameters, craving and mental health symptoms scores.

Methods: We used a fMRI cannabis (vs neutral) cue-reactivity task to compare brain activity between 49 people with a CUD (14 female) and 30 controls (15 female) aged 18-56 years; and correlated any functional differences with subjective craving, CUD severity, arousal ratings of cannabis images, cannabis exposure metrics (i.e., dosage, duration of regular use, age of onset, abstinence duration), depression and anxiety symptom scores. Correlations accounted for age, nicotine and alcohol dependence scores.

Results: The CUD group compared to controls, showed greater brain activation to cannabis vs neutral images in the lingual gyrus most strongly (*FWE*-corrected), followed by the middle frontal gyrus, medial orbitofrontal cortex and the cerebellum ($p < .001$; cluster $k > 10$). The middle frontal gyrus activity positively correlated with more cannabis grams.

Conclusion: The findings suggest that CUD has a (partly) overlapping neurobiology with that of other SUDs and consistent with neuroscientific theories of addiction. Different brain function during cannabis cue-reactivity may reflect alterations in reward processing, including salience evaluation and attention pathways resulting from regular exposure to cannabis/related cues; or predating CUD. Interventions that target these regions, may be effective at reducing cue-reactivity/craving in CUD.

Keywords: *cannabis use disorder, addiction, cue-reactivity, craving, fMRI, neuroimaging*

4.3 Introduction

Globally ~22 million people meet criteria for a cannabis use disorder (CUD; 186) and almost half (47%) of these meet criteria consistent with a moderate-to-severe CUD (1, 4, 5). These statistics are concerning as CUD is associated with a loss of control over cannabis consumption, with continued use despite adverse outcomes. They can include: physical and mental health problems in relation to cannabis consumption, relationship conflict due to prioritising cannabis use, risk-taking behaviours such as driving and operating machinery while intoxicated (6). CUD can be associated with a number of symptoms, such as tolerance to the effects of cannabis, whereby people may require greater quantities of cannabis over time, in order to achieve the same psychoactive effects of cannabis (6); withdrawal symptoms (e.g. sleep disruption, irritability, and/or mood disturbance) when cannabis is inaccessible (6); continued use despite repeated attempts to cut down or quit (6); as well as the experience of cravings – intense desire/preoccupation to use cannabis (6) - that can trigger continued cannabis use despite the experience of harms (7).

Neuroscientific theories of addiction posit that cue-elicited craving is a key factor for compulsive substance use and relapse (53). Cue-reactivity would develop via classical conditioning: with repeated substance use, the rewarding effect of substance exposure (i.e., dopamine increase) become paired with substance-related stimuli (i.e., images of substances, paraphernalia, contexts associated with substance use; 50) and over time people can experience reward when exposed to substance related cues. Exposure to substance-related cues can elicit craving and trigger relapse (substance seeking/taking), via conditioning of cognitive processes involved in reward evaluation, motivation and habit formation/learning (50, 53). For example, exposure to cannabis cues such as seeing a bong in a shop window or smelling it at a social gathering, can trigger craving for cannabis use and subsequently relapse in people with a CUD who are trying to reduce or cease their cannabis use (8, 24, 53).

Functional magnetic resonance imaging (fMRI) studies of cue-reactivity in regular cannabis users (187) show increased activity to cannabis cues (compared to neutral control cues) in key brain regions involved in cognitive processes known to be altered in CUD, such as reward processing (i.e., nucleus accumbens), habit formation/learning (i.e., dorsal striatum/caudate); motivation and disinhibition (i.e., middle frontal gyrus [MFG], anterior cingulate cortex [ACC]); and self-monitoring and awareness of environmental stimuli (i.e., posterior cingulate cortex [PCC], precuneus; 188). Importantly, the fMRI evidence from cue-reactivity studies in cannabis users, show alteration in partly overlapping brain pathways that are implicated in prominent neuroscientific theories of addiction (e.g., striatum, PFC, PCC; 53). Further, this evidence shows associations between brain function and behavioural indices of CUD. Specifically, correlations were observed between striatal, orbitofrontal, amygdala, occipital and insular function and greater subjective craving, severity of problems with use (e.g., loss of control over use) and cannabis dosage (i.e., grams/months, THC levels; for a systematic review, see Sehl and colleagues, *Psychopharmacology*, 2021; 187).

Overall, this evidence suggests that cannabis use is associated with brain functional differences during cue-reactivity which may be associated with symptoms of CUD. However, methodological limitations of these studies prevent an understanding of the neurobiological correlates of cue-reactivity in cannabis users. First, no study assessed CUD using the DSM-5 CUD criteria. Functional brain activation differences have been previously demonstrated between dependent vs non-dependent samples (86). Therefore it is unresolved whether the reported cue-reactivity alterations generalise to those who endorse a CUD.

Second, less than half of the studies to date included a non-using control group. Patterns of cue-elicited brain function were varied between studies with and without a control group, as well as both within and between group activations (80, 82, 85, 135). As such, more

studies that include both a CUD *and* a non-using control group are required to substantiate the findings in the literature to date and to enable future meta-analyses with adequate power.

Further, the association between brain function during cue-reactivity and subjective craving, CUD severity, cannabis exposure levels or entrenched confounders (e.g., mental health, concurrent nicotine and alcohol use) is unclear. Only a few studies have run correlations between cue-elicited brain function and behaviour (187). A lack of brain-behaviour correlation analyses in the current literature limits our understanding of how neural cue-reactivity may relate to behaviour and subgroups of cannabis using populations.

Lastly, in studies that included a non-using control group, only one included a treatment seeking sample (86) and no study reported whether the cannabis sample had attempted to reduce or cease their cannabis use (187). Different patterns of cue-elicited brain function may be observed depending on treatment-seeking status (189). To identify treatment targets, it is important to examine the neurobiology of cannabis cue-reactivity in people with a CUD of who have previously attempted to reduce or cease use (65).

This study aimed to overcome the limitations of the literature to date by examining for the first time the neural mechanisms of cannabis cue-reactivity in people with a moderate-to-severe CUD compared to control. Specifically, we examined brain function during a fMRI cue-reactivity task comprising cannabis and neutral images in a sample of 85 people aged 18-56 years, of which 52 met criteria for a moderate-to-severe CUD and had tried to cut down or quit (henceforth termed CUD) and 33 were non-using controls. In line with the emerging literature (9) and with neuroscientific theories of addiction (53), we hypothesised that the CUD compared to control group would show greater activity during cannabis vs neutral stimuli in the striatum, the PFC (i.e., orbitofrontal cortex [OFC], ACC, MFG), and parietal regions (i.e., precuneus, PCC).

Our secondary aim was to explore the association between group differences in brain function and cannabis use parameters and mental health symptom scores. We hypothesised that greater subjective cannabis craving, CUD symptom scores and arousal rating of the cannabis images shown in the fMRI task will be associated with altered brain function in CUD. We also explored the association between brain activation to cannabis cues in the CUD group and levels of cannabis exposure (i.e., cannabis grams/month, years of regular use, hours from last use), and anxiety and depression levels.

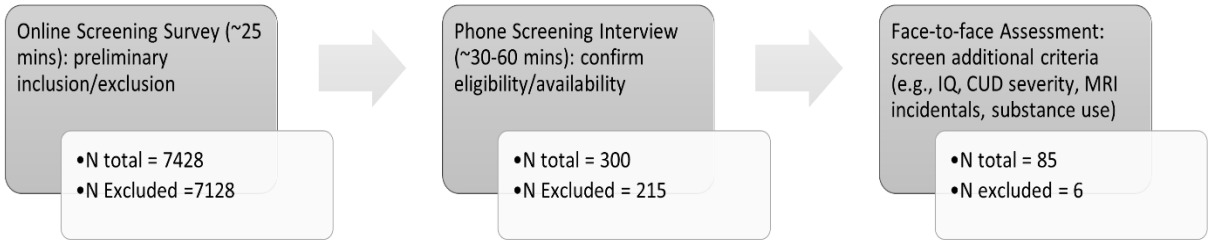
4.4 Method

4.4.1 Recruitment

Eighty-five participants were recruited from the Melbourne metropolitan area, Australia via public platforms (e.g., Google, Gumtree, Facebook, university websites, flyers in the general community and in university campuses, and others). Advertisements for study participation included a link that directed ~7,428 interested members of the community to an online screening survey. The tools used to screen participants are described in Supplementary Method, section 1.7.1.2. About 7,128 people were deemed ineligible or did not fully complete the online screening survey. A comprehensive interview script was used to contact the ~300 people who were potentially eligible in the study, of which 85 attended the face-to-face assessment session. Any queries about participants' eligibility were resolved via discussion with the study CI and research team, before confirming inclusion or exclusion in the study. Figure 4.1 provides an overview of the recruitment/screening process.

Figure 4.1

Overview of Recruitment and Inclusion/Exclusion Screening Process



Note. Flowchart depicts the recruitment and eligibility screening process and number of people excluded at each stage, as per 31 January 2022.

4.4.2 Inclusion and Exclusion Criteria

Inclusion criteria for all participants were: age between 18 and 56 years; proficiency in English; and normal or corrected-to-normal vision. CUD participants were included if they: i) endorsed DSM-5 criteria for a moderate-to-severe CUD based on the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV; 169), ii) consumed cannabis daily/almost daily for at least the past 12-months, and iii) reported at least one attempt to quit or to reduce their use in the past 24 months.

All participants were excluded based on the following criteria: i) significant medical conditions, history of acquired brain injury or loss of consciousness > 5 minutes; ii) history of psychopathology (except for depression and anxiety) ascertained by the Mini International Neuropsychiatric Interview (MINI; 190); iii) illicit drug and alcohol use self-reported in the 12-hours before testing; iv) significant alcohol use or dependence (i.e., Alcohol Use Disorder Identification Test (AUDIT; 164; score ≥ 19); or v) illicit drug use (other than cannabis in the CUD group) in the past 4-weeks or above recreational levels (i.e., > 50 lifetime episodes, or > weekly use over a 3-month period); or any illicit drug and alcohol use self-reported in the 12-hours before testing; vi) current use of prescription medication that affects the central nervous system (except for anti-depressants – e.g., SSRI's, SSNI's, due to increased prevalence of

depression and anxiety in CUD populations and our inclusion of these mental health disorders (6, 162); vii) MRI contraindications (e.g., pacemaker, pregnancy; and viii) IQ scores < 80 determined by the Weschler Abbreviated Standardised Intelligence-II (Vocabulary and Matrix Reasoning; 163).

4.4.2.1 Sample Included for Face-to-Face Assessment and Exclusion of Additional Participants

After the online and phone screener criteria, 85 people aged 18-56 years (32 female) were included and underwent face-to-face behavioural and MRI testing. Of these, one participant (male, aged 19 years), with an 11-month history of cannabis use was included as they met eight DSM-5 criteria for a CUD (i.e., severe) and used daily. Six participants (3 CUD and 3 controls) were excluded due to subsequently meeting exclusion criteria when face-to-face testing (See Supplementary Method, section 1.7.1.2).

4.4.3 Assessment Procedure

All assessment sessions were conducted at the Monash Biomedical Imaging facility in Clayton, Victoria. All participants gave written informed consent prior to participation. Assessments lasted ~4-to-6 hours and were conducted by experienced researchers and trained student researchers. Participants completed a battery of validated questionnaires delivered online through Qualtrics, face-to-face semi-structured interviews for detailed substance use and mental health profiling, and cognitive testing (e.g., IQ). The MRI scan included a structural T1 image acquisition, and a fMRI cue-reactivity task outlined below. The completion of assessment comprised of a debrief and reimbursement via Coles/Myers vouchers of $100 for controls and of $150 for CUD (because they underwent additional testing beyond the scope of this study).

4.4.3.1 Sociodemographic Information

Questionnaires measured socio-demographic variables (i.e., age, sex, education years, occupation, income).

4.4.3.2 CUD and Related Problems

CUD severity was confirmed with the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV; (169). Cannabis craving was measured pre-and post the fMRI cue-reactivity task via a visual analogue scale (VAS) asking participants to rate on a 10-point Likert scale *"how much [they] feel like smoking cannabis right now",* with '0' indicating *"not at all"* to '10' *extremely".* Symptoms of withdrawal were measured using the Cannabis Withdrawal Scale (CWS; 171).

4.4.3.3 Cannabis Exposure

Lifetime history of cannabis use (e.g., years of regular use, age of onset) was measured with a semi-structured interview used in previous studies (172-174). We extracted the following cannabis use parameters over the past month: days of use, dosage, strength, hours since last use, method of use, grams purchased using the Timeline Follow-Back (TLFB; 148).

4.4.3.4 Exposure to Other Substances

We measured exposure to any illicit or prescription substances as well as alcohol and nicotine (e.g., days of use, dosage, strength/type, hours since last use, method of use) over the past 30-days via the TLFB. Alcohol and nicotine dependence were assessed via the AUDIT and the Fagerström Test for Nicotine Dependence (FTND; 178).

4.4.3.5 Mental Health Symptom Scores

State anxiety symptoms were assessed using the State-Trait Anxiety Index – Y Form (STAI-Y; 179), and stress levels via the Perceived Stress Scale (PSS; 180). Depression symptoms was indexed using Beck's Depression Index – Second Edition (BDI-II; Beck et al., 1996). The experience of COVID-19 related stress was assessed using the COVID-19 Stress Scales (CSS; 191). The CSS is comprised of six subscales to capture COVID-19 related: 1) Danger, 2) Socio-economic Consequences, 3) Xenophobia, 4) Contamination, 5) Traumatic Stress, 6) Compulsive Checking.

4.4.3.6 Cue-Reactivity Image Rating Task

Participants' rated the arousal and affective valence of the cannabis and neutral images used in the fMRI cue-reactivity task at the end of the testing session using VAS scales from 0 to 10. Arousal was rated from 0 representing "calm" to 10 "excited"; and affective valence was rated on a scale from 0 "unpleasant", to 10 "pleasant" with a rating of 5 representing "neutral" .

4.4.4 MRI Data Acquisition

4.4.4.1 Structural MRI

MRI images were acquired on a Siemens Skyra 3 Tesla scanner using a 32-channel head coil at the Monash Biomedical Imaging facility. Brain images were acquired in coronal view, from anterior to posterior. Structural MRI data was acquired using T1-weighted MPRAGE scan. The acquisition parameters were: TE = 2.07ms, TR = 2300ms, flip angle = 9°, 192 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 and 20 seconds.

4.4.4.2 fMRI Cue-Reactivity Task

fMRI data for the fMRI cue-reactivity task was acquired using T2* weighted EPI scans. Acquisition parameters were: TR = 2240ms, TE = 30ms, flip angle = 90°, field of view = 192mm, matrix = 64, voxel size 3 x 3 x 3mm^3, 40 slices, with 227 total volumes. The task total acquisition time was ~8 minutes and 37 seconds.

4.4.5 fMRI Cue-Reactivity Task Description

An event-related cue-reactivity fMRI task was used to measure brain activity while participants passively watched 30 cannabis and 30 neutral non-cannabis images used in previous experiments from our extended team (Cousijn and colleagues, *Addictive Behaviors*, 2013; 82) and new images of comparable quality, complexity and luminosity. The images were visuals of cannabis-related paraphernalia and smoking behaviours and were controlled for visual valence to neutral cues, such as stationary items or cooking utensils. Cannabis and non-cannabis cues were comparable in level of complexity, type of activity, size, brightness, and luminance (see Figure 4.2 for an example).

Figure 4.2

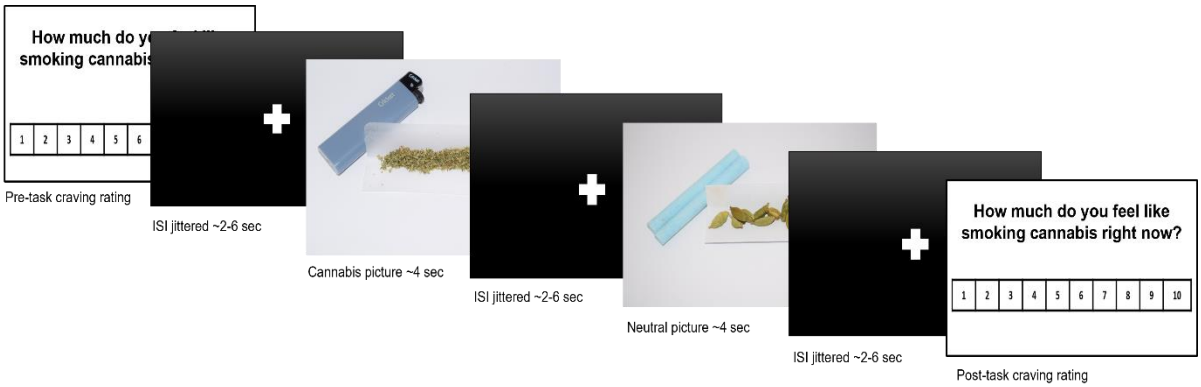
Example of Cannabis and Neutral Images in the Cue-Reactivity Task

Note. Samples of images used in the cue-reactivity fMRI task: cannabis images of i) dried cannabis cluster, ii) a bong, and iii) a lighter with dried cannabis on joint rolling paper. Neutral images of iv) a cluster of de-shelled pistachios, v) stacked plastic cups, and vi) chalk and de-shelled pistachios on paper.

Participants were instructed to pay close attention to the images. To ensure they maintained attention, participants' alertness was monitored via an MRI compatible camera (provided by imaging facility), and any occasions of sleepiness prompted a re-start of the scan to ensure engagement. Images were presented using E-prime3 software (Version 3.0 Build 3.0.3.80, E-Studio Build 3.0.3.82, Psychology Software Tools Inc.) onto a rear projection screen positioned behind the MRI scanner. Each image was presented for 4 seconds preceded by a fixation-cross that lasted on average 4 seconds, jittered between 2 and 6 seconds. The cannabis and neutral images were presented in the same semi-random order (max three images of the same category in a row) for each participant. Total task time was ~10 minutes. See Figure 4.3 for an example of fMRI cue-reactivity task stimulus presentation.

Figure 4.3

Example of fMRI Cue-Reactivity Task Stimulus Presentation



Note. Example of fMRI cue-reactivity task stimulus presentation. ISI = inter-stimulus interval. Sec = seconds. A craving rating was presented at the start and completion of the task asking participants to rate "How much do you feel like smoking cannabis right now?" on a scale of 1-10. A total of 60 images were presented with an ISI (black screen with a centred white '+'). Display times illustrated.

4.4.6 MRI Data Pre-processing

MRI quality checks and data pre-processing was conducted using fMRIPrep (version 1.1.1) to ensure they could be used for the fMRI analysis (https://fmriprep.org/en/stable/index.html). fMRI data preprocessing steps included: distortion correction; head motion correction; slice timing; spatial normalization to standard space (i.e., Montreal Neurological Institute [MNI] space); and smoothing with 6mm gaussian kernel. Image quality was assessed (i.e., motion, signal-noise ratio, artifacts) via review of Framewise Displacement (FD; indicator of motion); Derivatives of Root Mean Square Variance over Voxels (DVARS; indicator of noise level) as well as carpet plot (voxel-wise signal plot).

FD parameters were systematically assessed against conservative criterion validated for resting-state fMRI (192). Cases where motion exceeded the set criterion (n = 14) were further checked to ascertain if the FD parameters correlated with task-related events. No participant were excluded as a result of fMRI quality checks.

4.4.7 Statistical Analyses

4.4.7.1 fMRI Data Analysis

First level analyses were run via Matlab (version r2018a) using SPM version 12. They were conducted with a general linear model (GLM) to quantify the relationship between the observed event-related blood-oxygen level dependent (BOLD) signals and two regressors (i.e. cannabis and neutral cues) encoding cue-reactivity task conditions (i.e., cannabis > neutral and cannabis < neutral). The six motion estimates (i.e., translation and rotation on three axis: x, y, z) and their derivatives were entered as covariates of no interest for both cannabis and neutral cues. First, we examined a sample wide contrast (cannabis > neutral and cannabis < neutral) to determine there was a main effect of the task (cluster size was set k ≥

10, *FWE* corrected, *p* value < 0.001). Second, we investigated group differences (CUD vs controls) in cue-reactivity (cannabis > neutral and cannabis < neutral) using Independent Samples *t*-tests. A whole brain approach was run using GLM with group as a factor (CUD vs controls), cluster size was set k > 10, *p* value > 0.001.

4.4.7.2 Behavioural Data

All behavioural data and correlation analyses were run using SPSS version 28. Chi-squared tests were run to compare groups for categorical data (i.e., sex, employment status, income). T-tests or Mann-Whitney *U* tests were run to compare groups for normally or non-normally distributed variables (i.e., IQ, education years, substance use, mental health, craving, withdrawal).

To analyse group differences in the mean affective valence and arousal ratings of images used in the fMRI cue-reactivity task, we first calculated the inter-rater reliability of all of the cannabis and all of the neutral images respectively. We did this to determine the fit of utilising the two groups' mean scores of self-reported ratings of the images. Cronbach's α >.95 indicating that all of the cannabis and all of the neutral images were strongly correlated respectively. Therefore, the use of participants' mean scores for all image ratings was appropriate to create a variable of affective valence and arousal rating of cannabis and neutral images respectively.

Differences between mean ratings were examined using non-parametric paired Wilcoxin signed ranks tests. This was due to the data distributions (i.e., limited variance in the control groups' arousal rating of cannabis images) that violated the assumption of normality.

4.4.7.3 Brain-Behaviour Correlations

We used a series of Pearson's correlations for normally distributed data, and Spearman correlations for non-normally distributed data to address the secondary aim. Specifically, we explored the association between β coefficients reflecting differences in brain activation in the CUD group (vs controls); and variables directly relevant for cue-reactivity (i.e., number of CUD symptoms, subjective craving, and arousal ratings in relation to the cannabis images used in the cue-reactivity task); cannabis exposure levels (i.e. cannabis use days and grams/past month, age of onset, duration of regular cannabis use in years, and abstinence hours since last use, withdrawal symptoms [CWS]); and symptoms scores for anxiety (STAI-Y), depression (BDI-II) and stress (PSS), as well as, the positive, negative and depressive symptoms of psychosis (CAPE) and general well-being (SF-36).

A correlation matrix was run and examined to assess strength of correlation coefficients between variables (see correlation matrix used for variable selection, in Supplementary Material, Figure 4.7.1). Therefore cannabis frequency (number of days/past month) was removed due to strong correlation with cannabis dosage (grams/past month). Withdrawal symptoms (CWS), general well-being (SF-36), perceived stress (PSS) and the positive, negative and depressive symptoms of psychosis (CAPE) were removed due to strong correlation with depression (BDI-II) and/or anxiety (STAI-Y).

Importantly, brain-behaviour correlations accounted for the influence of age, alcohol (AUDIT) and nicotine (FTND) dependence levels. This was done by residualizing the β coefficients extracted from significant cue-induced activation clusters that differed between groups. Specifically, the standardised residuals were computed from linear regressions using age, AUDIT and FTND as predictors for each significant cluster in the CUD group for cannabis > neutral condition.

Hypothesis-driven correlations were run to examine the association between cue-induced brain function (i.e. measured with standardised residuals accounting for age, AUDIT and FTND scores) and i) subjective craving post-cue-reactivity task (VAS), ii) number of CUD symptoms (SCID), iii) arousal rating of cannabis images.

In the same way, exploratory correlations were run to examine brain-behaviour associations between cue-induced brain function and i) cannabis dosage (i.e., grams/past month), ii) cannabis use duration in years, iii) age of onset, iv) hours since last cannabis use, v) depression (BDI-II), and vi) anxiety (STAI-Y).

4.5 Results

4.5.1 Sample Characteristics

Table 4.1 overviews sample characteristics for sociodemographic data, mental health symptom scores, alcohol and nicotine exposure and dependence levels.

Table 4.1

Sample Characteristics: Sociodemographic Data, Education, IQ, Mental Health, Alcohol and Nicotine Use and Dependence Levels

Variable (Measure)	CUD		Control		Group Differences	
	M (*SD*)	Range	*M* (*SD*)	Range	*Z* /*t* [a]/ χ [b]	*p*
Total *N* [F]	49 [14]		30 [15]		2.81 [b]	.093
Age	26.87 (8.54)	18–56	28.56 (10.21)	18–55	-.471	.638
Education, (years)	14.94 (2.45)	10.17-21	15.96 (4.17)	6.50-25	-1.18 [a]	.245
IQ (WASI-II)[c]	106.12 (10.97)	83-129	106.15 (12.68)	82-128	0.10 [b]	.992
Depression (BDI-II)	10.69 (7.00)	0-34	4.76 (5.58)	0-22	-4.04	.001***
Anxiety (STAI-Y)	33.36 (8.13)	20-54	28.26 (6.68)	20-44	2.88 [a]	.005**
Stress (PSS)	16.06 (6.55)	4-33	12.23 (6.75)	1-25	2.48 [a]	.015*
Alcohol Days/month (TLFB)	5.45 (6.84)	0-29	2.50 (2.80)	0-11	-2.19	.028*
Drinks/month (TLFB)	26.94 (68.07)	0-206.80	8.68 (14.11)	0-65.90	-2.72	.006**
Alcohol dependence (AUDIT)	6.20 (4.42)	0-17	2.73 (2.75)	0-13	-3.68	_
Smoke Days/month (TLFB)	9.31 (13.30)	0-30	_	_	_	_
Cigarettes/month (TLFB)	60.61 (115.32)	0-525	_	_	_	_
Nicotine dependence (FTND)	1.12 (1.73)	0-6	_	_	_	

Note. SD = Standard Deviation. Z = nonparametric Mann-Whitney *U* test. F = Female. WASI-II = Wechsler Abbreviated Scale of Intelligence, 2nd Edition. STAI-Y = State-Trait Anxiety Index. BDI-II = Becks Depression Inventory – 2nd Edition. PSS = Perceived Stress Scale. TLFB = Timeline Followback. AUDIT = Alcohol Use Disorder Identification Test. /month = in the past month. FTND = Fagerström Test for Nicotine Dependence.

[c]CUD IQ (n = 48). Control IQ (n = 26).

*$p < .05$. **$p < .01$. ***$p < .001$

The sample included 79 people aged a mean of 27 years (range: 18-56 years), of which 29 were female. Participants included 49 people with a moderate-to-severe CUD who tried to cut down or quit in the past 2 years, and 30 controls.

CUD and controls did not differ significantly in sex, age, employment status (p = .831), household income (p = .674), IQ, years of education, and number of alcohol use days in the past month.

Compared to controls, the CUD group had higher levels of depression, state anxiety, and perceived stress, however, average CUD scores indicated low-to-moderate levels across all three measures. CUD vs control group had higher AUDIT scores, standard drinks/past month, FTND scores and cigarettes past month. Eight CUD participants (16.4%) met criteria for a nicotine dependence (i.e., FTND score ≥ 3), and no control used nicotine in the past month.

The CUD group also had higher incidence of positive, negative and depressive symptoms associated with psychosis and lower scores related to general well-being. The groups were matched on experience of COVID-19 related stressors (see Supplementary Material Table 4.7.1).

Eleven CUD (n = 8 males) and 1 control (male) used illicit substances less than 4 weeks prior to assessment. Substances included cocaine, mushrooms, MDMA, Xanax, ketamine, nitrous oxide, dexamphetamine and modafinil. Time since last use before assessment was a median of 156 hours (ranged 16-648 hours). Overview of details provided in Supplementary Results, Table 4.7.2.

4.5.2 CUD Groups' Cannabis Exposure and Subjective Craving

Table 4.2 overviews the CUD group's levels of cannabis exposure and subjective craving.

Table 4.2

CUD Groups' Cannabis Exposure Levels and Subjective Craving

Cannabis Exposure (Measure)		CUD (*N* = 48)	
		M (*SD*)	Range
CUD symptoms (SCID-5-RV)		7.10 (1.97)	4-11
Days/month (TLFB)		25.12 (5.34)	13-31
Grams/month (TLFB)		25.71 (20.91)	.90-85.50
Age onset, years (CUI)		16.73 (3.06)	12.83-32.08
Duration of regular use, years (CUI)		8.82 (8.89)	.91-40.60
Abstinence, hours (TLFB)		22.46 (13.38)	11.88-73.25
Withdrawal (CWS)		31.40 (26.43)	0-98
fMRI CR task Craving (VAS)	Pre	3.77 (2.67)	1-10
	Post	4.69 (2.60)	1-10

Note. SCID-5-RV = Structured Clinical Interview for DSM-5 Diagnoses Research Version (n = 49). TLFB = Timeline Followback. CUI = Cannabis Use Interview. CWS = Cannabis Withdrawal Scale (n = 49). CR = cue-reactivity. VAS = Visual Analogue Scale.

All cannabis users met criteria for a moderate-to-severe CUD with an average of 7 out of 11 criteria (range: 4-11) and had attempted to cut down and/or quit use in the past 12-24 months. The CUD group used cannabis almost daily in the past month, consuming an average of ~1 gram/day (range: 0.04-2.85). CUD participants started using cannabis on average at age ~17 years (range: 12.83-32.08), with most participants using regularly for an average of ~9 years (range: .91-40.60).

Participants abstained from cannabis for a mean of ~22.5 hours before testing (range: 11.88-73.25). Cannabis withdrawal scores were on average ~31 of a possible 190, which indicates relatively low experience and/or impact of withdrawal symptoms; and ranged from a minimum of 0, representing no withdrawal, to a maximum of 98, signalling relatively moderate withdrawal; indicating only a small proportion of cannabis users endorsed withdrawal symptoms. Subjective craving significantly increased pre-to-post the fMRI cue-reactivity task (Z = -4.08, p < .001).

Assessment of cannabis type showed that ~80% of CUD participants used strong cannabis, followed by weak (~12%). Joints and bongs were the most common method for consuming cannabis, and ~40% of the sample consuming their cannabis with tobacco (see Supplementary Results, Table 4.7.3).

4.5.3 *Group Differences in Arousal and Affective Valence Rating of Images in the fMRI Cue-Reactivity Task*

Table 4.3 overviews group differences in arousal and affective valence rating of cannabis and neutral images that were observed in the fMRI cue-reactivity task.

Table 4.3

Group Differences in Arousal and Affective Valence Ratings of Cannabis and Neutral Images

Variable		CUD (n = 43) Mean (SD)	Control (n = 29) Mean (SD)	Z	*p*
Arousal[a]	Cannabis	4.43 (2.15)	1.27 (.748)	-5.90	.001***
	Neutral	3.07 (1.86)	2.37 (1.95)	-1.60	.108
Affective Valence	Cannabis	6.12 (1.08)	4.09 (1.64)	-5.45	.001***
	Neutral	4.00 (1.46)	3.81 (1.81)	-.289	.772

Note. SD = Standard Deviation. Z = nonparametric Wilcoxon signed ranks test.

Arousal scores ranged '0' = calm to '10' = excited.

Affective valence[b] scores ranged '0' = unpleasant to '5' = neutral to '10' = pleasant.

*$p < .05$. **$p < .01$. ***$p < .001$.

CUD participants rated cannabis images higher in arousal and affective valence compared to controls. There were no group differences in ratings for neutral images. CUD participants rated cannabis images to elicit higher arousal and more "pleasant" affective valence compared to neutral images ($Z = -3.85$, $p < .001$; $Z = -4.95$, $p < .001$; respectively). The control group rated neutral images to elicit higher arousal compared to cannabis images ($Z = -2.63$, $p = .008$), but rated both cannabis and neutral images similar in affective valence ($Z = -.371$, $p = .710$).

1.5.4 Results on fMRI Cue-Reactivity Task: Brain Activity While Viewing Cannabis Vs Neutral Images

There was a main effect of task (see Supplementary Results, Table 4.7.4, Figure 4.7.2 and Table 4.7.5, Figure 4.7.3). Table 4.4 and Figure 4.4 overview the results on brain activation associated with cue-reactivity (cannabis > neutral images).

Table 4.4

Overview of Location and Strength of Peak Clusters Showing Greater Brain Activity in CUD than Control Participants While Viewing Cannabis vs Neutral Images

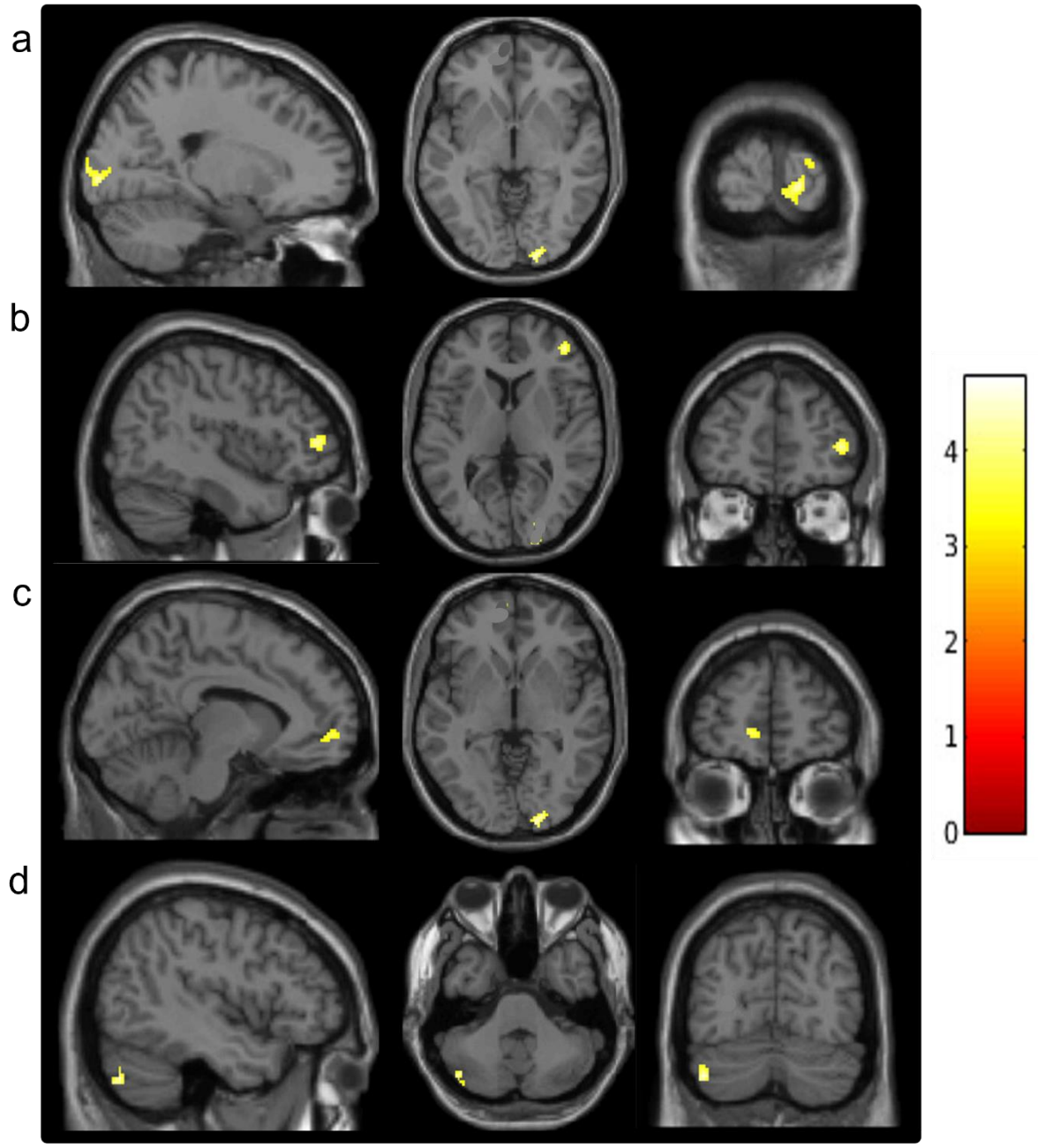
Cluster Size (# voxels)	Brain Regions	Hemisphere	MNI Coordinates			T score
			X	Y	Z	
161	Lingual Gyrus	Right	16	-100	-6	4.17
62	Middle Frontal Gyrus	Right	44	48	6	3.91
40	Medial Orbitofrontal Cortex	Left	-10	56	-2	3.60
52	Cerebellum	Left	-44	-72	-38	4.20

Note. All coordinates presented in Montreal Neurological Institute (MNI) space. Brain regions identified using AAL Atlas.

All clusters, uncorrected $p < 0.001$, except Lingual Gyrus *FWE* corrected $p < .05$.

Figure 4.4

Overview of Significantly Greater Brain Activity in CUD Compared to Controls During Exposure to Cannabis > Neutral Images in the fMRI Cue-Reactivity Task



Note. Greater activity related to cannabis vs neutral images in cannabis users: a = lingual gyrus, b = MFG, c = OFC, d = cerebellum. Clusters of significant activation (cluster threshold k >10 voxels, $p < 0.001$). The colour bar represents *t*-score.

CUD participants compared to controls showed greater BOLD activity while viewing cannabis vs neutral images in the following areas: lingual gyrus, MFG, medial OFC, and the cerebellum ($p < 0.001$, k > 10). The lingual gyrus was the only region of significantly greater activity after *FWE* correction ($p < 0.001$, k > 10). There was no significant group difference in brain function for the contrast Neutral > Cannabis images.

4.5.5 Brain-Behaviour Correlations

Within the CUD group, greater MFG activity was positively and moderately correlated with greater consumption of cannabis grams in the past month ($r_s = .302$, $p = .039$). There were no other significant correlations.

4.6 Discussion

This is the first study to examine brain function during a fMRI cannabis cue-reactivity task in people with a DSM-5 diagnosis of moderate-to-severe CUD comprehensively characterised for cannabis use levels, in comparison to controls. We found that CUD participants compared to controls had greater brain activity *FWE*-corrected in the lingual gyrus, and uncorrected in the MFG, the medial OFC, and the cerebellum while watching cannabis vs neutral images. We also found that in the CUD group, greater MFG activity correlated with higher consumption of cannabis grams in the past month.

The most robust, *FWE*-corrected finding was that CUD vs control participants showed greater lingual gyrus activity while watching cannabis images. The finding is consistent with previous work showing greater lingual gyrus activity in cue-reactivity fMRI tasks in cannabis users (187) and in users of other substances (i.e., nicotine, alcohol, cocaine, opiates; (193). The lingual gyrus is implicated in the processing of higher order visual stimuli (i.e., perception and recognition of familiar scenes, encoding and recall of complex pictures; (194-

196). Increased activation of the lingual gyrus has also been associated with internally directed attention (197) and mediating between competing attentional demands (198). With respect to our results, the increased activation in the lingual gyrus to cannabis cues may reflect a habituated attentional bias arising from paired association between cannabis cues and reward (199). Future studies using an eye tracker as a metric of attention (e.g., fixation time) to cannabis cues could examine correlations with visual cortex activity to test this notion, as previously done in smokers (200).

CUD participants compared to controls had greater MFG activity and this was significantly correlated with greater cannabis dosage (i.e., grams past month). The MFG is well-established for playing a key role in motivation to use substances, inhibition (53) and substance-related cue-reactivity (77, 187). Interestingly, the MFG is also part of the Ventral Attention Network and serves as a junction site for attentional processes to reorient attention from external to endogenous stimuli (201). Therefore, greater MFG activity may reflect reduced top-down attentional control, as evidenced in MFG resection compared to controls (202). Taken together, patterns of greater activation in the lingual gyrus and MFG in response to cannabis cues may underly attention-regulatory abilities, which could lead to poor reorientation of attention from cannabis to neutral stimuli. In other words, individuals with a CUD desiring to reduce and/or quit use or remain abstinent, may find it difficult to shift attention from experiences of craving (i.e., become preoccupied) in the presence of cannabis cues (53). This may also be exacerbated if cues are unexpected (203).

There was a correlation between MFG activity and cannabis dosage. Greater cannabis dosage has been associated with decreased gray matter in the MFG (204), suggesting that activity in the MFG during cue-exposure may be resultant of substance-induced alteration. Therefore, greater MFG activity to cannabis cues may be driven by the effects of repeated cannabis intoxication and/or attentional bias. However, studies employing a causal modelling

analysis (205) or longitudinal design may provide clearer understanding of the cause-and-effect relationship between MFG cue-reactivity and cannabis dosage.

We found that CUD compared to the control group had greater activation in the medial OFC. This finding is consistent with our hypothesis and with previous evidence from cue-reactivity studies of cannabis users (9), other substance users (8, 206) and neuroscientific theories of addiction (53). The OFC is posited to regulate limbic-striatal regions implicated in cognitive processes including reward processing (207) and effortful control (208). The observed greater activation in the OFC during cannabis cue-reactivity may reflect an increased attribution of salience and reward expectation to cannabis cues in CUD. It may also reflect a reduced ability to control/inhibit prepotent responses to cannabis-related cue-exposure as previously demonstrated in both animal and human fMRI studies (206, 208).

We did not find any correlation between OFC activity and craving or CUD severity despite prior work showing robust associations (206). This also contrasts previous findings in cannabis users of an association with craving (80, 84, 85, 87). The discrepant finding may be due to differences in methodologies in analysing brain-behaviour correlations. OFC-craving correlations were only reported in activation patterns to cannabis cues not compared to neutral/control stimuli. Studies that reported greater cue-elicited OFC activity to cannabis vs neutral/control stimuli, including the present study, did not find a correlation with craving (86) (82).

CUD vs controls had greater cerebellar activation while watching cannabis vs neutral images. This is in line with previous evidence in cannabis users (14), and in SUDs (i.e., cocaine; 209, alcohol; 210). Notably, the cerebellum is increasingly recognised as an important part of addiction neurocircuitry (53, 211, 212), having prefrontal-striatal connectivity via direct innervation of the ventral tegmental area (213). The cerebellum is a brain region with high concentration of cannabinoid (CB_1) receptors, to which Δ9-

tetrahydrocannabinol (THC) binds to exert its psychoactive effect (214). The cerebellum is also implicated in prediction processing (i.e., drug-associated cue-memory; 215, 216). Therefore, cue-elicited cerebellar activity may reflect an anticipatory response to cannabis cues in the absence of cannabis intoxication (217). Future work comparing cannabis intoxication vs sober in CUD during cannabis cue-exposure may provide a better understanding of the role of the cerebellum in relation to cue-reactivity.

There was no group difference in brain activity in the striatum. (77, 187). The striatum is a key hub for reward processing and part of the addiction-related neurocircuitry (53, 77, 187). Our null finding of a difference in striatal activity contrasts with previous work in similar cannabis cue-reactivity studies (82, 86). Indeed, the striatum is one of the most consistently implicated regions in fMRI cue-reactivity studies in people using cannabis and other substances. However, some cue-reactivity studies in cannabis users did not report different striatal activity (85, 142). Methodological variables (e.g., fMRI analyses approach, sample demographics, cannabis use patterns) may influencing the heterogeneous striatal activation patterns. Indeed, sex differences have been established in the patterns of cannabis use and neurobiology of cannabis users (218, 219). Both sex and age of cannabis use onset have also been shown to impact cue-elicited striatum-behaviour correlations (87, 141). As such, further investigation of cannabis cue-reactivity that examine cannabis subgroups and other methods of potential influence (e.g., fMRI analyses) may explain the inter-study differences in striatal activation patterns.

The activity of parietal regions (i.e. PCC and precuneus) did not differ between groups, which is in contrast with findings in previous addiction fMRI cue-reactivity studies (187, 220, 221) and addiction theory (53). The precuneus and the PCC comprise part of the Default Mode Network which is activated during mind-wandering or wakeful rest (73). As such, variation in task engagement was considered as a possible confounder. For example,

some tasks required passive vs active engagement, subliminal vs perceptually conscious, and different sensory engagement (i.e., visual, tactile, olfactory; see Sehl at al., 2021 for a review; 187). However, the variation in distinct methodological aspects of the cannabis cue-reactivity fMRI tasks makes it difficult to parse a part the impact of task engagement on neural cue-reactivity. Investigating whether certain sensory modalities are associated with neural cue-reactivity may help to understand if and how these parietal regions are involved in addiction neurobiology. Future work using cannabis stimuli delivered via different sensory modes may elucidate specific neurobiological effects and inform on the distinct and/or common underlying mechanisms of cue-reactivity.

Whilst our cue-reactivity task was shown to increase subjective craving, this did not correlate with the activity of any of the regions implicated in cue-reactivity. This is consistent with previous studies of cannabis users (81, 141), but inconsistent with others that reported significant brain-craving correlations (85, 86). The relationship between cue-elicited activity and subjective craving may be modulated by other factors associated with perceived availability of /expectancy to use cannabis post-presentation of cues (177, 222, 223). This can include varying hours of abstinence and treatment seeking status/motivation to not use cannabis use (224, 225), which have been associated with both neural cue-reactivity and levels of craving and withdrawal symptoms (226). To date, few studies have reported their sample's average abstinence hours (n = 4; 85, 86, 134, 135), or included a treatment seeking sample (compared to control; n = 1; 86), making it difficult to compare with our study. Future studies may elucidate how subjective craving relates to neural cue-reactivity by including varying abstinence durations (225) and/or comparing treatment and non-treatment seeking samples (224).

Further, the clinical context in which neural cue-reactivity is measured may impact on the experience of craving. Specific contexts (e.g., social settings) and routines of use (e.g.,

afterwork, bedtime) are also key stimuli for cue-reactivity (8, 223). Thus, it is possible that for some participants, specific cues elicit craving more than others (80, 85). Future work comparing different types of cannabis cues (olfactory, tactile, routine/contexts) and correlations with subjective craving will help to delineate the relationship between neural and behavioural cue-reactivity.

4.6.1 Limitations and Future Directions

The findings from this study need to be considered in light of several methodological limitations. First, the cross-sectional design prevents the understanding of whether group differences predated CUD and constitutes a neurobiological vulnerability for CUD, as previously suggested (227). Longitudinal study designs are required to elucidate if the neurobiology of cue-reactivity predates CUD or changes over time as people progress to greater or lower CUD severity, relapse or prolonged abstinence.

Second, analysis (e.g., urine, saliva, plasma) of cannabis cannabinoids (i.e., THC metabolites) was outside of the scope this study. It is unclear if THC levels drove brain function in this study as there is evidence that creatinine-weighted THC ng/ml can affect lingual gyrus activity in cannabis users during fMRI cue-reactivity tasks (80). Some cannabis users may include those using more potent and addictive cannabis varieties with high level of THC and low level of CBD with known distinct properties on brain function (147). Also, people with more severe CUDs can use more potent cannabis products with greater THC levels due to tolerance (228), which may in part be driving neural cue-reactivity. Measurement and analysis of THC metabolites and levels is required in future studies to understand the relationship with brain functional alterations in CUD. This will also inform psychopharmacological intervention and public health legislature with global trends towards the legalisation of cannabis.

4.6.2 Clinical Implications

Vulnerability to relapse has been associated with neural cue-reactivity in other SUDs (229). The findings of this study provide a foundation for informing targets for interventions for individuals with more severe CUDs who are vulnerable to relapse (24). Neural cue-reactivity in the lingual gyrus and MFG may diminish with interventions such as attentional bias retraining via cue-exposure therapy (230). Similarly, mindfulness-based "urge surfing" relapse prevention strategies (105, 110) may mitigate prefrontal and lingual gyrus hyperactivation to cannabis cues, as it is posited to strengthen top-down executive control including attention (231). Our findings also support the investigation of interventions that subjectively devalue cannabis by reducing its expected reward whilst increasing the relative valuations of non-drug rewards (232).

4.6.3 Conclusion

In sum, the findings of this study suggest that cannabis cue-reactivity in CUD is associated with greater activity of the lingual gyrus implicated in higher order visual/attention processing, and additional areas, some of which were dose-related (MFG). The findings suggest that cue-reactivity in CUD shares (partly) common neurobiological correlates as other SUDs (i.e., alcohol, nicotine, opioids, cocaine; (229), in line with prominent neuroscientific theories of addiction. Our findings may have implications for the development of psychological and of brain-based interventions that target the function of these regions in order to decrease craving, cannabis dosage and the severity of CUD.

4.7 Supplementary Materials

4.7.1 Supplementary Methods

4.7.1.1 Online Survey for Participant Screening

An ~25-minute online screening survey using Qualtrics software was used to select eligible participants according to inclusion and exclusion criteria. The survey collected information relevant for participants eligibility including demographics, MRI safety contraindications, medical conditions and medication, history of illicit substance use, alcohol consumption and related problems, psychopathology, experience in practicing mindfulness and relaxation strategies, as well as previous cannabis quit attempt via the measures listed below.

4.7.1.1.1 MRI Screening Questionnaire (provided by Monash Biomedical Imaging Centre; https://www.monash.edu/researchinfrastructure/mbi/forms-and-policies/mbi-policies). This questionnaire screens for any contraindication for undergoing an MRI scan (i.e., currently pregnant, weight, metal in the body, etc.).

4.7.1.1.2 Alcohol Use Identification Test (AUDIT). The AUDIT (164) is a screening tool comprised of 10-items developed to assess alcohol consumption and related problems. The AUDIT also provides diagnostic cutoffs (i.e., alcohol dependence with scores ≥ 19).

4.7.1.1.3 Cannabis Use Identification Test – Revised (CUDIT-R). The CUDIT-R (165) is an 8-item cannabis misuse-screening tool. It has good psychometric properties (Cronbach's $\alpha = 0.72$) based on clinical and community-based populations. It has DSM-5 diagnostic cutoffs for mild, moderate, and severe CUD (i.e., 9-10; 11; 12-13) respectively (16).

4.7.1.1.4 Substance Use History (SUH). The SUH is adapted from the Drug History Questionnaire (166) and has been validated in drug users with Cronbach alpha coefficients

ranging from 0.66-0.93. The SUH provided details on illicit substance use across the lifespan and was used to assess recreational levels and time since last use.

4.7.1.1.5 Mini International Neuropsychiatric Interview (MINI). The MINI (167) 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).

4.7.1.1.6 Depression, Anxiety, and Stress Scale-21 (DASS-21). The DASS-21 (168) is a 21-item clinical questionnaire that provides a quantitative measure of distress along the three axis of depression, anxiety and stress. Responses are given via a 4-point Likert scale (0 = Did not apply to me at all, 4 = Applied to me very much, or most of the time). Whilst the DASS is not recommended as an isolated diagnostic tool, it does characterise the degree of severity relative to the population. As such, scores represent normal, mild, moderate, severe and extremely severe levels of depression, anxiety, and stress, respectively.

4.7.1.1.7 Cannabis Quit Attempt. To determine if cannabis users had previously attempted to reduce or cease their cannabis use in the past 12-24 months – single "yes" or "no" question was asked: "Have you attempted to cut down and/or quit using cannabis in the past 12-24 months?"

4.7.1.2 Overview of Participants Excluded from Analyses

Six participants (3 CUD and 3 controls) were excluded due to subsequently meeting exclusion criteria when face-to-face testing. The people with a CUD who were excluded were: i) a female aged 25 years with an IQ score < 80 (i.e., FSIQ-2 = 61), ii) one male aged 22 years endorsing a neurological disorder (i.e., history of seizures), and iii) a male aged 31 years with an incidental finding determined by a neurologist that conducted comprehensive

checks of MRI images. The three controls excluded reported > 50 lifetime occasions of cannabis use. They were: i) a female aged 32 years, who reported ~4000 lifetime occasions for 13.5 years, ii) a female aged 51 years, who reported 176 lifetime occasions since the age of 18, and one male aged 35 years, who reported 420 lifetime occasions in 14-months and endorsed a history of a diagnosed psychiatric condition in adolescence.

4.7.2 Supplementary Results

Supplementary Table 4.7.1

Sample Characteristics: Psychotic Symptoms, General Well-being and COVID-Related Stressors

Variables	Measure	CUD		Control		Group Differences	
		M (*SD*)	Range	*M* (*SD*)	Range	*Z* /*t* [a]/ χ [b]	*p*
Psychotic Symptoms (+)	CAPE-42	38.39 (11.19)	20-73	29.26 (9.93)	0-56	3.65 [a]	.001***
Psychotic Symptoms (-)		40.33 (14.50)	14-82	27.83 (12.20)	0-50	-2.56	.010**
Depressive Symptoms		23.14 (7.92)	8-43	18.13 (7.13)	0-32	-3.69	.001***
Wellbeing	SF-36	2626.22 (541.03)	1100-3345	2981.66 (383.40)	1820-3420	-3.30	.001***
COVID-Danger		4.32 (5.49)	0-23	2.91 (2.64)	0-8	-.279	.781
COVID-Socioeconomic		0.32 (0.91)	0-4	0.75 (1.76)	0-6	-8.33	.405
COVID-Xenophobia	CSS	0.44 (1.13)	0-5	1.50 (2.74)	0-7	-8.49	.396
COVID-Contamination	(*N*=34, *N*=12)	1.88 (3.31)	0-14	1.66 (2.49)	0-6	-0.82	.935
COVID-Traumatic Stress		0.79 (2.11)	0-8	0 (0)	0-0	_	_
COVID-Checking		2.67 (3.73)	0-16	1.91 (2.10)	0-5	-.359	.720

Note. Z = nonparametric Mann-Whitney *U* test. CAPE-42 = Community Differences in Positive Psychotic Experiences. SF-36 = 36-Item Short Form Survey. CSS = COVID Stress Scales. Dependence.

*$p < .05$. **$p < .01$. ***$p < .001$.

Supplementary Table 4.7.3

Details of Included Participants with Illicit Substance Use within Four Weeks of Assessment

Substance	Group	Age	Sex	Hours[a]	Dosage
Cocaine					
	CUD	35	Male	24	1g
	CUD	22	Male	624	1g
	CUD	20	Female	504	1g
068	CUD	29	Male	38	3.5g
Mushrooms					
	CUD	21	Male	50	1g
	CUD	18	Male	648	2g
	CUD	20	Male	NS	4g
MDMA					
	CUD	20	Male	NS	0.2g
	CUD	27	Female	16	0.15g
	CUD	20	Female	480	0.2g
	Control	29	Male	60	~75mg
Xanax					
	CUD	18	Female	360	1mg
	CUD	19	Male	156	0.5mg
Ketamine					
	CUD	20	Male	240	0.5g
Nitrous Oxide					
	CUD	21	Male	50	8g
Dexamphetamine					
	CUD	35	Male	28.5	20mg
Modafinil					
	CUD	29	Male	96	200mg
	Control	29	Male	432	50mg

Note. NS = Not specified. g = grams. mg = milligrams.

Matching colours (excluding black) indicate the same participant.

[a] Hours last consumed before assessment.

Supplementary Table 4.7.3

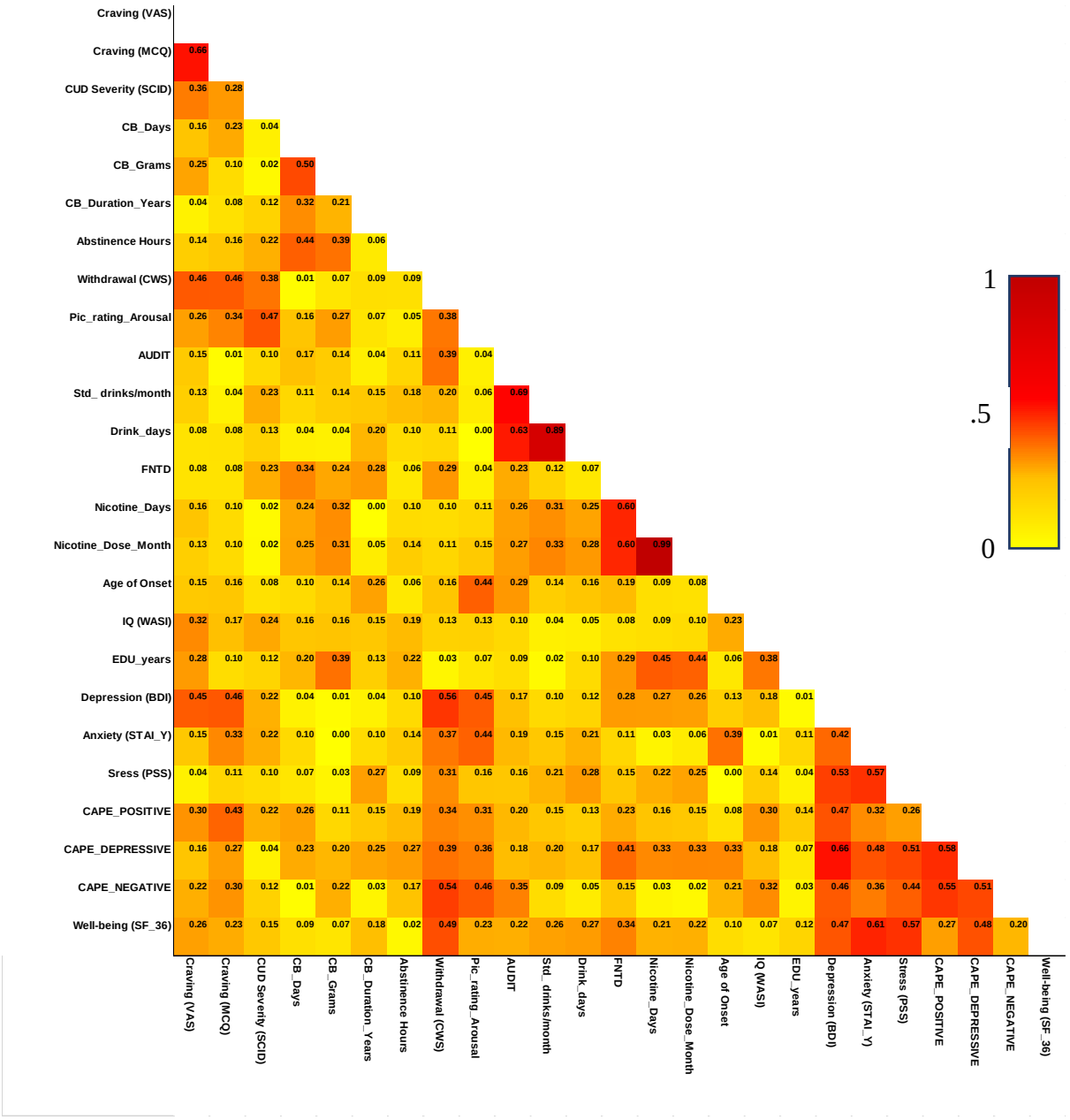
Cannabis Users' Patterns of Cannabis Strength and Method of Use

Characteristic	Cannabis Exposure	CUD (*N* = 48)
Cannabis Type *N* (%)	Strong	40 (81.6)
	Weak	6 (12.2)
Cannabis Use Method *N* (%)	Joint with tobacco	14 (17.4)
	Joint no tobacco **n* = 3 x herb/tea	16 (19.9)
	Bong with tobacco	16 (19.9)
	Bong no tobacco **n* = 2 x herb/tea	9 (11.4)
	Pipe with tobacco	0 (0)
	Pipe no tobacco	2 (2.5)
	Vape	6 (7.6)
	Ingested	4 (5.1)

Note. herb/tea = cannabis mixed with herbs or tea leaves.

Supplementary Figure 4.7.1

Correlation Matrix of Variables of Interest for Analyses



Note. CB = Cannabis. SCID = Structured Clinical Interview for DSM-5. AUDIT = Alcohol Use Disorder Identification Test. Std = Standard. /month = in the past month. FTND = Fagerström Test for Nicotine Dependence. WASI-II = Wechsler Abbreviated Scale of Intelligence, 2nd Edition. STAI-Y = State-Trait Anxiety Index. BDI-II = Becks Depression Inventory – 2nd Edition. PSS = Perceived Stress Scale. CAPE-42 = Community Differences

in Positive Psychotic Experiences. SF-36 = 36-Item Short Form Survey. CSS = COVID Stress Scales.

Note. Colour bar ranging from yellow representing correlation coefficient = 0 to red = ≥ .5 to deep red = 1.

Table 4.7.4

Overview of Location and Strength of Peak Clusters of Brain Activation for Main Effect of Task Condition Cannabis > Neutral

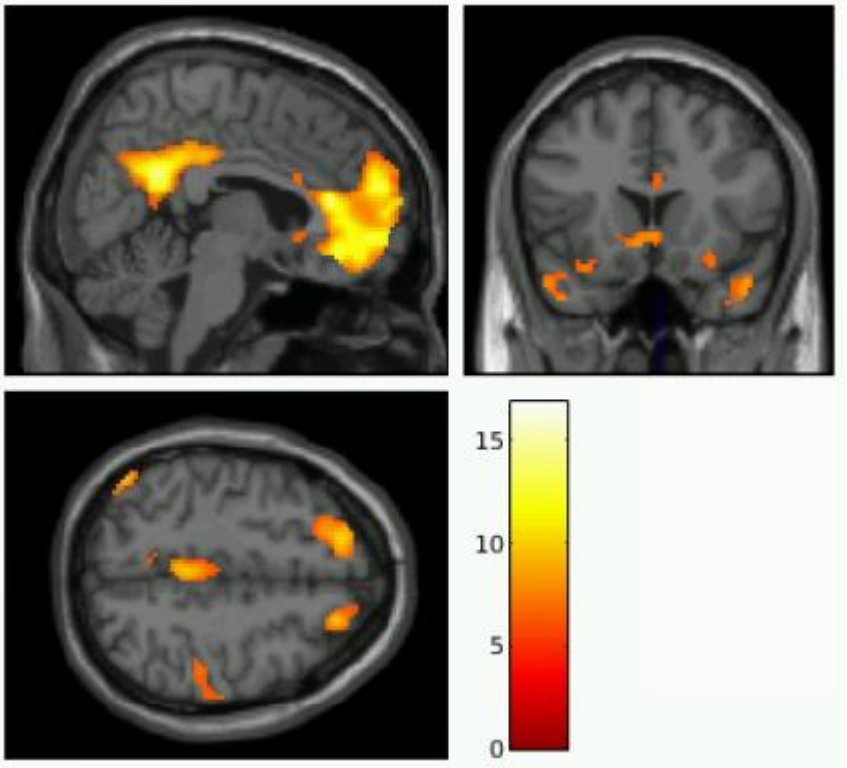
Cluster Size (# voxels)	Brain Regions	Hemisphere	MNI Coordinates			T score
			x	y	z	
6320	Medial Orbitofrontal Cortex[1]	Left	-2	54	-6	16.85
	Cingulum_Ant	Left	-4	42	-2	15.53
	Cingulum_Ant	Left	-	50	-2	14.27
3676	Precuneus	Left	-8	-54	32	14.65
	Precuneus	Right	6	-50	26	14.26
	Cingulum_Post	Left	-4	-46	30	13.71
	Angular	Right	50	-68	36	12.52
333	Temporal_Pole_Sup	Left	-	8	-	11.4
227	Insula	Left	-	10	-8	8.71
	Insula	Left	-	14	-	7.93
188	Frontal_Sup	Right	22	36	48	10.81
608	Temporal_Mid	Left	-	-6	-	10.59
	Temporal_Mid	Left	-	-6	-	9.35
	Temporal_Pole_Mid	Left	-	14	-	9.02
161	Hippocampus	Right	28	-22	-	10.53
	Hippocampus	Right	24	-36	2	8.2
	ParaHippocampus	Right	28	-30	-	7.84
773	Angular	Left	-	-66	46	9.83
	Angular	Left	-	-74	32	9.53
	Occipital_Mid	Left	-	-76	42	9.11
148	Lingual	Right	20	-	-8	9.83
	Lingual	Right	12	-92	-8	7.68
	Calcarine	Right	18	-	4	7.58
107	Hippocampus	Left	-	-20	-	9.78
	Calcarine	Left	-	-	-4	9.59
	Calcarine	Left	-4	-96	-6	6.43
47	Insula	Right	38	10	-	9.53
362	Postcentral	Right	62	-14	34	9.19
	SupraMarginal	Right	54	-16	30	8.96
	SupraMarginal	Right	64	-18	42	7.19
85	Temporal_Pole_Mid	Right	46	20	-	8.92
	Temporal_Pole_Mid	Right	40	18	-	6.83
136	Temporal_Mid	Right	62	-8	-	8.86
	Temporal_Mid	Right	66	-10	-	7.65
17	Cerebelum_Crus2	Right	42	-48	-	7.69
33	Insula	Right	30	16	-	7.63
22	Insula	Right	42	0	4	7.6
13	Insula	Right	40	-6	-4	7.51

10	Thalamus	Left	-8	-30	0	6.99
18	Inferior Orbitofrontal Cortex[2]	Right	36	28	-	6.84
14	Amygdala	Right	26	2	-	6.8
14	Precuneus	Right	12	-36	4	6.79
12	Putamen	Right	16	12	-4	6.64

Note. All coordinates presented in Montreal Neurological Institute (MNI) space. Brain regions identified using AAL Atlas. [1]Medial Orbitofrontal Cortex = Frontal_Med_Orb. [2]Inferior Orbitofrontal Cortex = Frontal_Inf_Orb All clusters, *FWE* corrected, p < .001.

Supplementary Figure 4.7.2

Greater Activity in Whole Sample for Main Effect of Task Condition Cannabis > Neutral



Note. Map activation of greater activity for main effect of task condition cannabis > neutral. Clusters of significant activation (*FWE* corrected, cluster threshold k >10 voxels, $p < 0.001$). The colour bar represents *t* -score.

Supplementary Table 4.7.5

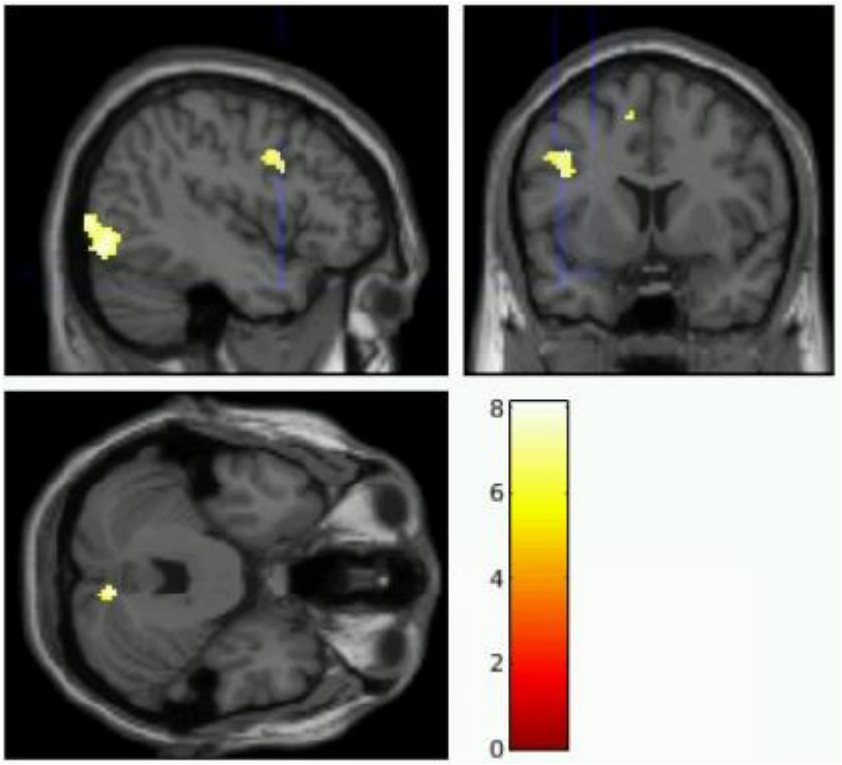
Overview of Location and Strength of Peak Clusters of Brain Activation for Main Effect of Task Condition Neutral > Cannabis

Cluster Size (# voxels)	Brain Regions	Hemisphere	MNI Coordinates			T score
			x	y	z	
231	Occipital_Inf	Left	-44	-76	-6	8.12
	Occipital_Inf	Left	-38	-86	-8	8.02
	Occipital_Mid	Left	-44	-84	4	7.58
62	Cerebelum_Crus1	Right	8	-74	-28	8.1
169	Occipital_Inf	Right	42	-70	-10	8.02
	Occipital_Inf	Right	44	-80	-10	7.35
	Occipital_Inf	Right	42	-76	-2	6.98
140	Precentral	Left	-40	6	36	7.48
	Frontal_Inf_Oper	Left	-38	6	28	7.05
	Frontal_Inf_Oper	Left	-56	16	34	6.57
20	Insula	Left	-30	26	0	7.34
25	Supp_Motor_Area	Left	-4	14	52	6.64
17	Frontal_Inf_Tri	Left	-50	30	22	6.45
15	Occipital_Mid	Left	-30	-72	34	6.44
	Parietal_Inf	Left	-30	-66	42	6.12

Note. All coordinates presented in Montreal Neurological Institute (MNI) space. Brain regions labelled using AAL Atlas. All clusters, *FWE* corrected, $p < .001$.

Supplementary Figure 4.7.3

Greater Activity in Whole Sample for Main Effect of Task Condition Neutral > Cannabis



Note. Map activation of greater activity of main effect of task condition neutral > cannabis.

Clusters of significant activation (*FWE* corrected, cluster threshold k >10 voxels, $p < 0.001$).

The colour bar represents *t*-score.

Chapter 5: Does a Brief Mindfulness-Based Intervention Reduce Cue-Reactivity Related Brain Function in Cannabis Use Disorder? An Active and Passive Placebo Controlled, Pseudo-Randomised fMRI Study

Study 3

5.1 Chapter Guide

This chapter presents Study 3. It examines for the first time whether a brief MBI with individuals who have a moderate-to-severe CUD significantly reduces cannabis cue-related neural activation in the brain regions identified in Chapter 4 (Study 2). Participants were assigned to one of three groups: MBI, an active placebo-controlled intervention (relaxation), and a passive (no intervention) control condition. The aim of this study was twofold: i) to investigate whether changes in cue-related activation significantly decreased pre-to-post a brief 2-week MBI compared to relaxation and no intervention, and ii) to explore whether changes in brain function pre-to-post MBI are associated with those in behaviour (e.g., cannabis dosage, craving). We hypothesised that decreased cue-elicited brain activity would be observed pre-to-post MBI.

Due to COVID-19-related disruptions to recruitment and face-to-face data collection, recruitment is still ongoing (43 out of target sample of 60), thus the findings reported in this chapter should be considered preliminary. As this experiment is intended for publication, all analyses will be re-run on the complete sample post-submission of this PhD manuscript.

5.2 Abstract

Background: Cannabis use disorder (CUD) affects ~22 million people globally and is associated with altered brain function during exposure to cannabis compared to neutral cues. Preliminary evidence shows that mindfulness-based interventions (MBIs) reduce cannabis use and craving, as well as altered brain function in prefrontal-striatal pathways during fMRI cue-reactivity tasks in substance users. No study to date has examined the underlying neural mechanisms of MBIs for cannabis cue-reactivity in CUD.

Method: Our previous experiment demonstrated greater cannabis cue-elicited brain activity in lingual, prefrontal and cerebellum regions in people with a moderate-to-severe CUD with past attempts to cut down or quit compared to controls. In this double-blind pseudo-randomised experiment, we measured how the activity of the aforementioned regions of interest [ROI] changed pre-to-post a brief 2-week MBI (n =13) vs active placebo control intervention (relaxation; n =14) and a passive control condition (n =13). All interventions included daily monitoring of cannabis use and subjective craving. We hypothesised that a reduction in cannabis cue-reactivity (relative to pre-intervention differences in ROIs) would be observed in the MBI but not the other intervention groups. We also explored how changes in ROI activity pre-to-post intervention correlated with those in substance use, mental health and mindfulness measures.

Results: In contrast to our hypothesis, there was a significant decrease in OFC activity pre-to-post all three interventions, as well as a significant decrease in subjective craving and arousal rating of cannabis images.

Conclusion: The brief MBI employed in this study compared to the control intervention did not reduce neural activation to cannabis cues in moderate-to-severe CUD. As all three interventions had a shared component of self-monitoring of daily cannabis use, self-monitoring may have influenced OFC cue-reactivity by increasing conscious awareness of

habitual patterns of use. This study lays the groundwork for future research aimed at understanding MBI-specific neural mechanisms in CUD.

Keywords: *cannabis use disorder, mindfulness-based intervention, cue-reactivity, craving, fMRI, neuroimaging*

5.3 Introduction

Worldwide ~ 22 million people meet criteria for a cannabis use disorder (CUD; 1), which is broadly defined as the continuation of cannabis use despite the experience of physical or psycho-social harms (6). Despite experiencing harms related to use, individuals with a CUD often report difficulty maintaining abstinence (4), with craving being a major maintaining factor despite a desire to quit/reduce use (6, 8, 24). Specifically, craving in CUD is defined as an intense desire and/or preoccupation to use cannabis during periods of abstinence or exposure to cannabis-related cues (i.e., paraphernalia, affective states, contexts; (8, 9). As such, many pharmacological (e.g., *N*-acetylcysteine) and psychological interventions for CUD (e.g., CBT) are predominantly aimed at reducing craving and associated arousal states related to either cannabis cue-exposure, withdrawal or both (96).

Prominent neuroscientific theories of addiction posit that cue-elicited craving is driven by neuroadaptations in brain regions associated with reward processing (53). Emerging evidence from a functional Magnetic Resonance Imaging (fMRI) study examined the functional correlates of cue-reactivity in moderate-to-severe CUD compared to non-using controls (see Sehl and colleagues in preparation; Chapter 4). Specifically, the study reported greater activity during exposure to cannabis vs neutral cues in regions associated with reward evaluation, motivation, inhibition and attention orientation. These regions include the orbitofrontal cortex [OFC], middle frontal gyrus [MFG] and lingual gyrus, as well as the cerebellum. Notably cue-induced hyperactivity in the OFC has also been associated with subjective craving in regular cannabis users in the literature to date (187). Further, studies on other substances suggest alteration in OFC functional brain activation to cues may be associated with craving and subsequently relapse (229). As such, from a public health perspective, individuals with a moderate-to-severe CUD are an important group for whom interventions that reduce brain reactivity to cannabis cues may be helpful.

A meta-analysis of Mindfulness-Based Interventions (MBIs) for SUDs (including one study on cannabis users) found that MBIs were more effective than treatment as usual in reducing substance use, craving, and stress, as well as in improving cognitive control (99). The development of psychological interventions are influenced by addiction models which posit that maladaptive drug-taking behaviours are preceded by cognitive and affective states (i.e., craving, arousal; (100, 101). Therefore, a reduction in the intensity of these states may reduce impulsive/reactionary substance use and promote behaviour change (102). The treatment approaches for SUDs including CUD often employ relaxation techniques for emotion regulation to reduce the intensity of arousal states associated with craving and stress (103, 104). In contrast, MBIs do not aim to eliminate or alter craving or associated arousal including thoughts, feelings, and sensations (100, 101). Instead, MBIs for SUDs address craving by training individuals to become familiar with their experience of craving in the mind and body and observe the transient nature of it (e.g., noticing how the craving changes and dissipates over time; 100). This is often termed "urge surfing" and uses the analogy of a wave rising to a peak and eventually crashing and disappearing (105).

Prominent neurobiological theories posit that the mental training involved in "urge surfing" strengthen prefrontal regions (e.g., OFC, MFG) involved in cognitive control (i.e., attention, awareness, evaluation, and inhibition) and emotion regulation (100, 231). As prefrontal control is regained, individuals desiring to reduce or quit using are able to respond to their craving deliberately and adaptively instead of automatically and maladaptively (100, 231). Of relevance, three pioneering studies in cigarette smokers have investigated the neural mechanisms of MBIs for cue-reactivity. Two studies using a whole brain analysis approach, observed that post-MBI there was a reduction in cue-elicited activations in prefrontal regions including the MFG and anterior cingulate cortex (ACC)-OFC connectivity (122, 123). A reduction in weekly cigarettes consumed and levels of self-reported stress was also reported

(123), with a relationship between changes in dosage and affect with changes in ACC-OFC connectivity (122). A study also reported pre-to-post changes in cue-induced activations via ROI analyses approach in the posterior cingulate cortex (PCC) and a correlation between PCC changes and a reduction in cigarette dosage (121). Taken together, the preliminary findings suggest that MBIs reduce neural activation associated with cue-reactivity, however this notion remains to be tested in CUD.

While the emerging evidence is informative and suggests that MBIs may have clinical utility for CUD, there are some methodological limitations. First, MBI's employed for SUDs include varying levels of complexity, structure (i.e., length in weeks, session duration), format (i.e., individual vs face-to-face, guided vs self-directed) and number of components (e.g., required engagement and homework; for review see Li et al., 2017; [99] and Cavichiolo et al., 2018; 125). Reviews of the dissemination of evidenced-based treatments (including MBIs) from controlled settings to clinical care suggest that MBIs delivered in easily accessible (e.g., self-administered) and flexible (e.g., brief) formats may prove more effective (e.g., uptake, retention (126, 127). Mindfulness is considered a form of cognitive training which requires consistent practice – much like physical training – to produce sustained results (e.g., changes in cognitive function; (128). However, there have been promising effects of brief MBIs (e.g., ~10-minutes/day) over 1-week in at-risk drinkers (116) and a single session in smokers (123). Therefore, it is important to determine if a brief MBI is effective in changing the neural correlates of cannabis cue-reactivity in CUD, with potential for improved clinical significance in treatment uptake and retention.

Second, in laboratory studies of MBIs control conditions are varied, with no known study including both an active and passive control; therefore limiting the ability to parse apart intervention specific changes and control for placebo effects (129). Further, very few studies have employed imaging techniques to elucidate brain-behaviour relationships. The lack of

imaging data limits our understanding of the underlying mechanisms of change needed to inform the development of more relevant and target-specific treatments.

These aforementioned limitations demonstrate the need for a double-blind randomised-control trial employing fMRI techniques to compare a condition matched MBI vs treatment as usual (i.e., relaxation) vs no intervention for people with mild-to-moderate CUD. Such a design will enable the highest level of experimental control to determine efficacy and unveil underlying neural mechanisms.

The aim of this pseudo-randomised fMRI experiment is to investigate for the first time in adults with moderate-to-severe CUD, how a brief MBI targeting cannabis craving compares to active and passive placebo control interventions in terms of reduced activation during a cannabis cue-reactivity task. The active control (relaxation) will inform on MBI-specific effects on brain function, and the passive control, will parse a part potential placebo effects.

In line with preliminary evidence of MBI-related changes in cue-elicited brain activity in cigarette smokers (122, 123) and neuroscientific theories of MBIs for SUD implicating widespread changes via top-down prefrontal influence (100, 231), an intervention-by-time effect on cue-elicited brain activity is expected (identified pre-intervention; see Sehl et al, *in preparation,* Chapter 4) is expected. Specifically, it is hypothesised that the activity in the MFG, OFC, lingual gyrus and cerebellum in CUD will significantly decrease pre-to-post the MBI, but not pre-to-post relaxation and no intervention control conditions.

Our secondary aim was to explore if changes in brain activity pre-to-post MBI was associated with changes in behaviour related to CUD. These include subjective craving, cannabis consumption (average daily grams), withdrawal symptoms, abstinence duration, arousal ratings of cannabis images, and CUD severity. Changes in mental health (e.g.,

anxiety, depression, stress), nicotine dependence and mindfulness and emotion regulation scores will also be explored.

Based on both behavioural and neuroimaging MBI evidence for SUD populations (99, 121-123), we expect that changes in the aforementioned behavioural measures will be related to changes in MBI-related brain function.

5.4 Methods

5.4.1 Experimental Procedures

This study was nested within a larger pre-registered experiment (registration ID: ISRCTN76056942) on the neurobehavioral mechanisms of MBIs in moderate-to-severe CUD. As such, detailed description of recruitment, inclusion/exclusion screening and pre-intervention data analyses of group differences (CUD compared to controls; Sehl and colleagues *in preparation*) are reported in Chapter 4. Ethics approval was obtained (ID: 2019-71H) by the ethics committee of the Australian Catholic University, Melbourne, Australia.

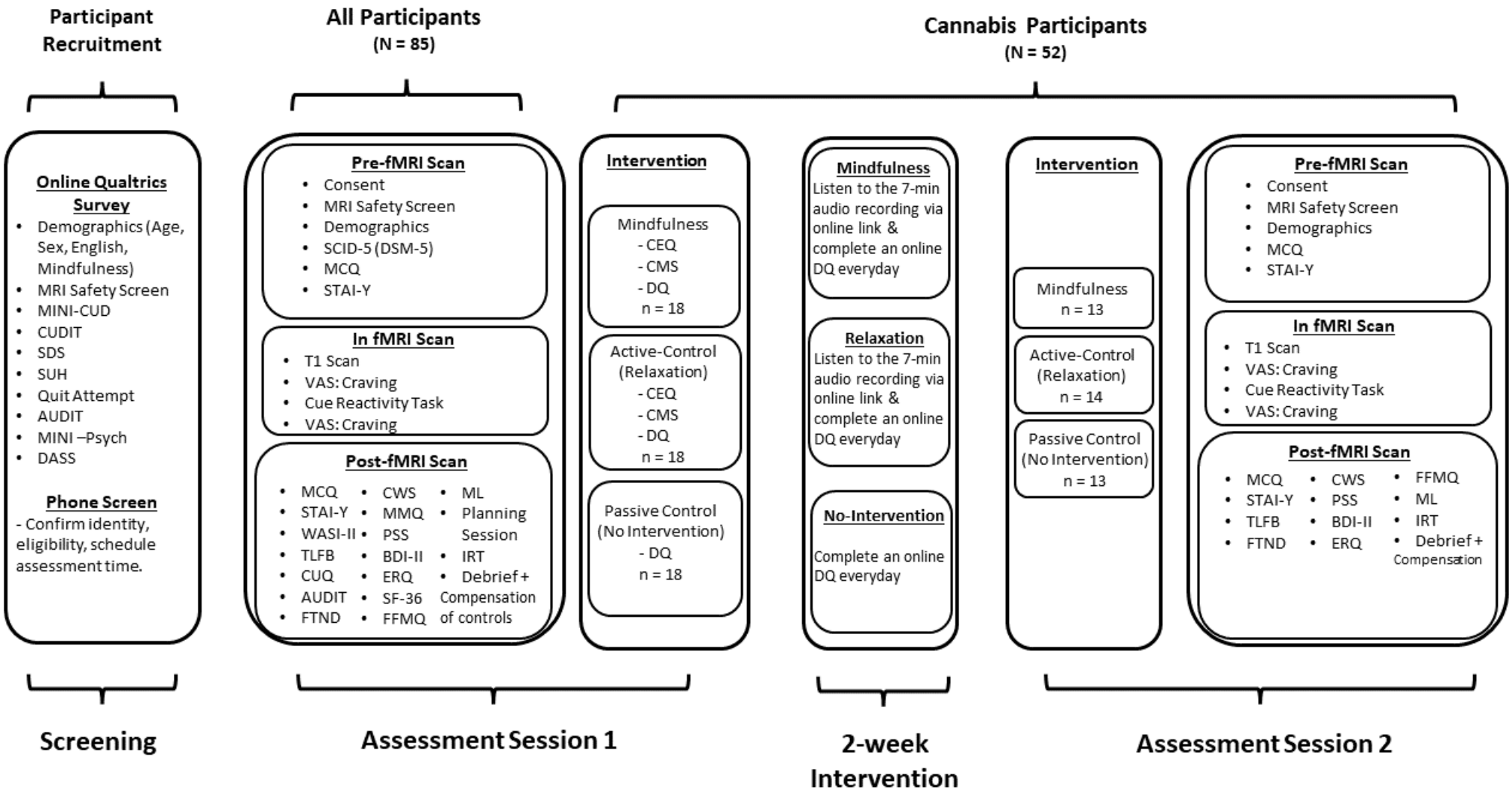
5.4.2 Experimental Design

A double-blind, pseudo-randomised controlled design was used to examine the effects of MBI compared to an active (i.e., relaxation) and passive (i.e., no intervention) control on neural cannabis cue-reactivity and behaviours associated with CUD. Following online and telephone screening, a study co-ordinator not involved in data collection or analysis pseudo-randomly and equally assigned CUD participants with a stratification based on age, sex, and education years to one of three groups: i) MBI; ii) relaxation active-control intervention; iii) no intervention passive control. Participants attended the assessment centre for session 1 (pre-intervention) and again about two weeks later (CUD only) for assessment session 2 (post-intervention). CUD participants were blind to intervention group allocation and study aims

related to mindfulness (or relaxation) specifically. Trained researchers conducting the non-intervention component of the face-to-face assessments were blind to participant group allocation (which remained concealed until all data was collected), with the intervention component conducted by a second unblinded researcher (responsible for confidential and secure storage of relevant data). Figure 5.1 provides and overview of the experimental methods.

Figure 5.1

Overview of Experimental Methods



Note. The figure overviews the protocol and assessment measures employed for screening participants against inclusion/exclusion criteria and face-to-face data-collection for analyses to address the aims of this study. MRI = Magnetic Resonance Imaging. MINI = Mini International Neuropsychiatric Interview. CUD = Cannabis Use Disorder. CUDIT = Cannabis Use Disorders Identification Test. SDS = Severity of Dependence Scale. SUH = Substance Use History. AUDIT = Alcohol Use Disorders Identification Test. DASS = Depression, Anxiety, and Stress Scale. SCID-5 = Structured Clinical Interview for DSM-5 Research Version. DSM-5 = Diagnostic and Statisitacal Manual for Mental Health Disorders – Fifth Edition. MCQ = Marijuana Craving Questionnaire. STAI-Y = State-Trait Anxiety Index – Y Form. VAS = Visual Analogue Scale. WASI-II = Weschler Abbreviated Standardised Intelligence – II. SF-36 = The 36-Item Short Form Survey Instrument. TLFB = Timeline Follow-Back. CUQ = Cannabis Use Questionnaire. FTND = The Fagerström Test for Nicotine Dependence. CWS = Cannabis Withdrawal Scale. PSS = Perceived Stress Scale. BDI-II = Beck Depression Index – II. ERQ = Emotion Regulation Questionnaire. FFMQ = Five-Factor Mindfulness Questionnaire. ML = Marijuana Ladder. IRT = Image Rating Task. CEQ = Credibility/Expectancy Questionnaire. CMS = Comprehension/Manipulation Check. DQ = Daily Questionnaire.

5.4.3 Participants

Eighty-five participants – of which 52 had a CUD and 33 were controls, were recruited from the Melbourne metropolitan area, Australia via public platforms (e.g., Google, Gumtree, Facebook, university websites, flyers in the general community and university campuses).

Six participants (3 CUD, 3 Controls) were excluded from analyses of group differences (CUD compared to controls) pre-intervention due to meeting exclusion criteria during face-to-face testing. Details reported in Supplementary Material Section 5.7.1. Therefore, analyses of group differences pre-intervention included 49 CUD and 30 controls.

Three CUD were excluded from analyses of group differences (MBI vs control conditions) post-intervention due to i) meeting exclusion criteria during face-to-face testing, and ii) fMRI technical issues. Details reported in Supplementary Material Section 5.7.1. Six CUD participants withdrew and did not take part in the second assessment session (post-intervention). Details reported in Supplementary Material Section 5.7.2. A total of 40 CUD participants completed both pre-intervention assessment, one of the three interventions and post-intervention assessment and were included in analyses of CUD group differences pre-to-post intervention.

5.4.4 Inclusion and Exclusion Criteria

Inclusion criteria for all participants were: age between 18 and 56 years, proficient in English, have normal or corrected-to-normal vision.

CUD participants were included if they: i) endorsed DSM-5 criteria for a moderate-to-severe CUD, ii) used cannabis daily/almost daily for minimum past 12-months, and iii) reported at least one attempt to quit or to reduce their use in the past 24 months.

All participants were excluded based on the following criteria: i) significant medical conditions, history of acquired brain injury or loss of consciousness > 5 minutes, ii) history of psychopathology (except for depression and anxiety) ascertained by the Mini International Neuropsychiatric Interview; iii) significant alcohol use or dependence; or iv) illicit drug use (other than cannabis in the cannabis group) in the past 4-weeks or above recreational levels (i.e., > 50 lifetime episodes, or > weekly use over a 3-month period), or v) any illicit drug and alcohol use self-reported in the 12-hours before testing; vi) current use of prescription medication that affects the central nervous system (except for anti-depressants – e.g., SSRI's, SSNI's, due to increased prevalence of depression and anxiety in CUD populations and our inclusion of these mental health disorders; 162); v) MRI contraindications (e.g., pacemaker, pregnancy); viii) IQ scores < 80 determined by the Weschler Abbreviated Standardised Intelligence-II (Vocabulary and Matrix Reasoning; 163); and ix) regular mindfulness or relaxation experience (i.e., formal training and/or ≥ 1 x month or > 10 lifetime occasions in past year) assessed by qualified mindfulness instructors and trained researchers.

Any queries about participants' eligibility were resolved via discussion with the study CI and the research team, before confirming their inclusion or exclusion in the study.

5.4.5 Face-to-Face Assessment Protocol

All assessment sessions were conducted at the Monash Biomedical Imaging facility in Clayton, Victoria, Australia. All participants gave written informed consent prior to participation. Assessments lasted ~4-to-6 hours and were conducted by experienced researchers and trained student researchers. Supervised by a researcher blinded to group allocation, participants completed a battery of validated questionnaires delivered online through Qualtrics, face-to-face semi-structured interviews for detailed substance use, mindfulness and mental health profiling, and cognitive testing (e.g., IQ). The MRI scan

included a structural T1 image acquisition, and a fMRI cue-reactivity task outlined below. A second researcher unblinded to group allocation guided CUD participants through the intervention-related tasks and questionnaires outlined below. The completion of assessment comprised of a debrief and reimbursement via Coles/Myers vouchers of $150 for CUD at the end of the second assessment (post-intervention).

5.4.6 Questionnaires/Measurement Tools

5.4.6.1 Socio-Demographic Data

At the first assessment session (pre- intervention), questionnaires measured socio-demographic variables including age in years, sex (i.e., male, female), education years, occupational status (i.e., full/part time; homemaker), and income range (i.e., < $10K; >$10-$50K; >$50-$100K; >$100K).

5.4.6.2 Substance Use and Diagnostic Assessment

5.4.6.2.1 SUD Severity Levels. The presence and severity of CUD was confirmed pre- intervention using the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV; 169). The Cannabis Use Identification Test (CUDIT; 165) was used to measure pre-to-post changes in CUD severity. Alcohol dependence was assessed at Session 1 (pre-intervention) via the AUDIT and nicotine dependence at both assessment sessions (pre- and post-intervention) via the Fagerström Test for Nicotine Dependence (FTND; 178).

5.4.6.2.2 Current Substance Exposure. Exposure to cannabis and other substances (i.e., alcohol, nicotine, illicit and prescription drugs) were measured at both assessment sessions (pre- and post-intervention) using the Timeline Follow-Back (TLFB; 148). We extracted the following substance use parameters over the past month at the first assessment session (pre- intervention) and past number of days between assessment sessions (pre- and

post-intervention): hours since last use, days of use, dosage, strength and method of use (e.g., joint, bong). The concurrent use of tobacco with cannabis (e.g., mixed together) and quantity ratio (in grams) was also obtained.

5.4.6.2.3 Lifetime Cannabis Use. Lifetime cannabis exposure (i.e., age of onset, duration in years, prolonged abstinence periods) was measured with a semi-structured interview (referred to as the Cannabis Use Interview; CUI) used in previous studies (172-174).

5.4.6.2.4 Subjective Craving. Subjective cannabis craving was assessed at both sessions (pre- and post-intervention). It was measured in-vivo pre and post the fMRI cue-reactivity task using a visual analogue scale (VAS) asking participants to rate on a 10-point Likert scale *"how much [they] feel like smoking cannabis right now",* with '0' indicating *"not at all"* to '10' *extremely".*

5.4.6.2.5 Motivation/Readiness to Change Cannabis Use. At the first assessment session (pre- intervention) we measured motivation, intention/desire and readiness to change cannabis using habits via The Marijuana Ladder (ML; 176). Scores ranging from 1-3 correspond with the stage of pre-contemplation (e.g., no plan to change cannabis use). Scores 4-6 correspond with the stage of contemplation (e.g., think about use, but no plan current to change it). Scores 7-8 correspond with the stage of preparation (e.g., planning on making changes/starting to reduce cannabis use). A score of 9 corresponds with the stage of action (e.g., I have made changes but worry about slipping back) and a score of 10 corresponds with the stage of maintenance (e.g., made changes and will never go back).

5.4.6.3 Mental Health, Well-Being and Mindfulness

Depression, anxiety and stress symptoms were measured at both assessment sessions (pre- and post-intervention) via Beck's Depression Index – Second Edition (BDI-II; 157), the

State-Trait Anxiety Index – Y Form (STAI-Y; 179), and Perceived Stress Scale (PSS; 180), respectively. The tendency to regulate emotions via *cognitive reappraisal* or *expressive suppression* was measured using the Emotion Regulation Questionnaire (ERQ; 233).

General well-being and quality of life was measured at the first assessment session (pre- intervention) using total scores of eight domains capturing physical, mental, and emotional health via the 36-Item Short Form Survey Instrument (SF-36; 182).

Mindfulness was measured at both assessment sessions (pre- and post-intervention)using the total score comprised of five related dimensions: observing, describing, acting with awareness, non-judging of inner experience, and non-reactivity to inner experience of the Five-Factor Mindfulness Questionnaire (FFMQ; 183). All items are rated on a 5-point Likert-type scale ranging from 1 (never or very rarely true) to 5 (very often or always true). There is no cut off score indicating one is mindful or not. The scores represent a spectrum of mindfulness, with higher scores indicating greater mindfulness.

5.4.7 MRI Data Acquisition

MRI data acquisition was conducted at both assessment sessions: i) prior to intervention and ii) post-intervention.

5.4.7.1 Structural MRI

MRI images were acquired on a Siemens Skyra 3 Tesla scanner using a 32-channel head coil at the Monash Biomedical Imaging facility. Brain pictures were acquired in coronal view, from anterior to posterior. Structural MRI data was acquired using T1-weighted MPRAGE scan. The acquisition parameters were: TE = 2.07ms, TR = 2300ms, flip angle = 9°, 192 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 and 20 seconds.

5.4.7.2 fMRI Cue-Reactivity Task

fMRI data for the CR fMRI task was acquired using T2* weighted EPI scans. Acquisition parameters were: TR = 2240ms, TE = 30ms, flip angle = 90°, field of view = 192mm, matrix = 64, voxel size 3 x 3 x 3mm^3, 40 slices, with 227 total volumes. The task total acquisition time was ~8 minutes and 37 seconds.

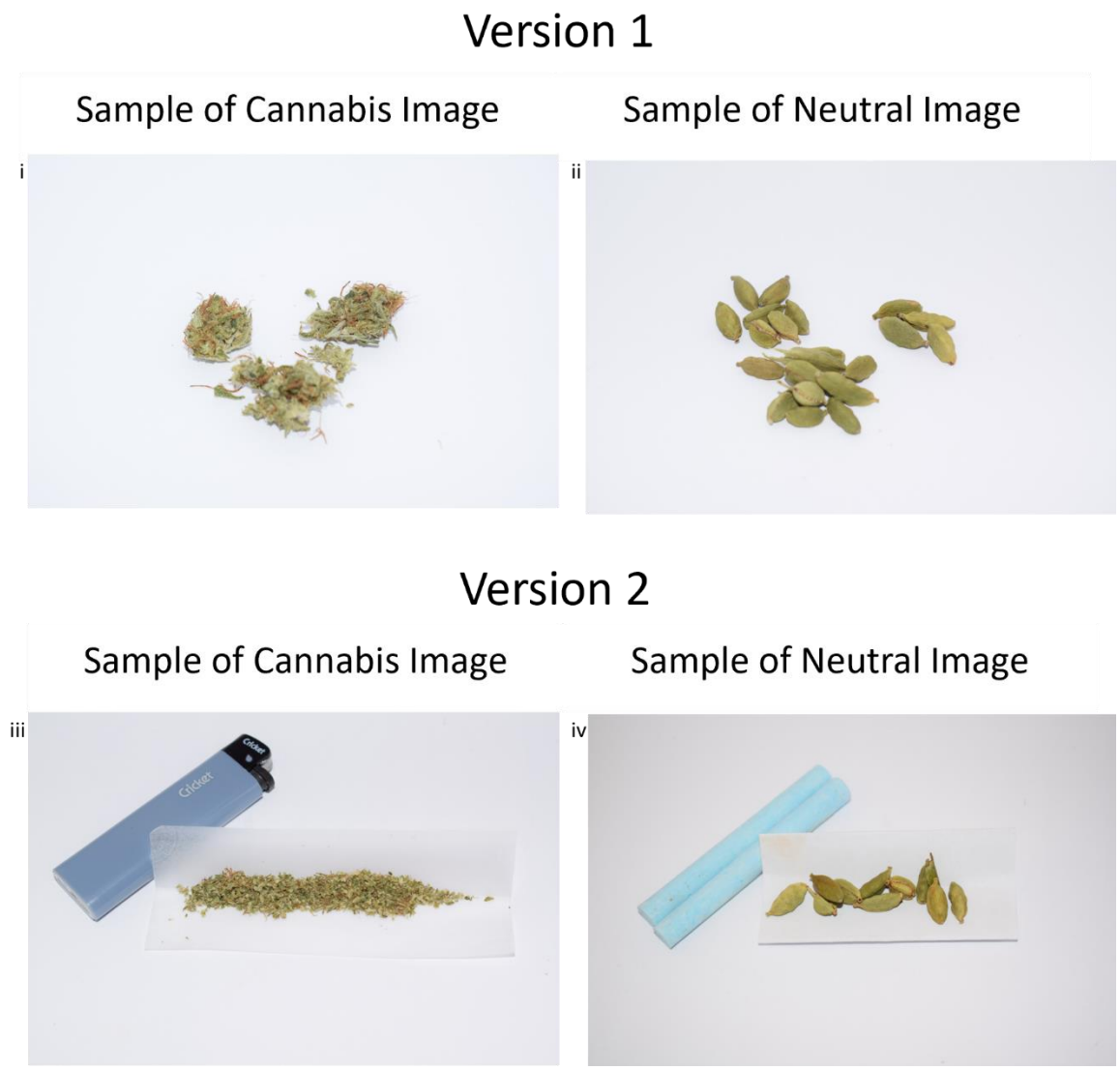
5.4.8 fMRI Cue-Reactivity Task Description

An event-related cue-reactivity fMRI task was used to measure brain activity while participants passively watched 30 cannabis and 30 neutral non-cannabis pictures used in previous experiments from our extended team (Cousijn and colleagues, *Addictive Behaviors*, 2013; 82) and new pictures of comparable quality, complexity and luminosity. The pictures were visuals of cannabis-related paraphernalia and smoking behaviours and were controlled for visual valence to neutral cues, such as stationary items or cooking utensils. Cannabis and non-cannabis cues were comparable in level of complexity, type of activity, size, brightness, and luminance.

There were two versions of this task, which were identical in procedure but contained different pictures (matched for complexity, object size, colours and brightness). The two versions were delivered in a counterbalanced order, so that each participant viewed a different version at each session. This was done in order to minimise the compounding influence of memory processes affecting overlapping brain pathways during cue-reactivity. See Figure 5.1 for an example of cannabis and neutral images from version 1 and 2.

Figure 5.2

Samples of Cannabis and Neutral Images in the fMRI Cue-Reactivity Task: Version 1 and 2



Note. Version 1 cannabis image: i) dried cannabis cluster. Version 1 neutral image: ii) a cluster of de-shelled pistachios. Version 2 cannabis image: iii) a lighter with dried cannabis on joint rolling paper. Version 2 neutral image: iv) chalk and de-shelled pistachios on paper.

5.4.9 Intervention Protocol

At the first assessment session, prior to the intervention, participants underwent a *planning session* to schedule time to complete the 2-week intervention described as "daily

tasks". To enhance compliance participants were encouraged to problem-solve potential barriers (e.g., put reminders in their phone).

5.4.9.1 Experimental Conditions: MBI and Active Control (Relaxation)

Using headphones, the MBI group then listened to a 15-minute mindfulness script and the active control (relaxation) group listened to a comprehension complexity, word count and time-matched relaxation script (validated in alcohol studies and adapted for cannabis; Kamboj et al., 2017 (116). Specifically, the two groups listened to a 3-minute explanation of the relevant strategy for managing cannabis cravings. They were then administered The Credibility/Expectancy Questionnaire (CEQ). The CEQ (184) measured the participant's belief that the received therapy will help to reduce cannabis cravings. The questions address what the participants both "*think"* and "*feel"* will happen. The MBI and relaxation group then listened to a 4-minute audio to introduce the practice followed by an extended 7-minute practice. To assess uptake/comprehension of the intervention the two groups were administered the *Credibility/Manipulation Check (CMC*; 116) which is a 9-item questionnaire of intervention specific questions.

At the end of the first assessment session, participants in all three groups were instructed to complete their "daily tasks" via a link sent to them via SMS. The relevant "daily tasks" were also provided on a USB to ensure any technical issues did not prevent compliance.

5.4.9.2 Two-Week Daily Intervention

The two-week daily intervention was completed autonomously by participants via online Qualtrics links. The MBI and relaxation groups were asked to practice the brief

intervention daily for 7-minutes in the two-weeks between assessment sessions (pre- and post-intervention).

5.4.9.2.1 Daily Questionnaire (DQ). All three groups were asked to complete a daily questionnaire (~3-minutes) which provided data on key behavioural variables to aid interpretation of the findings. The questions in the DQ asked participants in the MBI and active relaxation control group to report on daily 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 cravings to smoke cannabis, have you practiced the strategy you have been listening to on the audio track?") and provided metrics of engagement time (i.e., if and how long they listened to the audio). The questions in the DQ asked participants in *all three groups* via a VAS to rate the intensity (1-10) of their cannabis craving (i.e., physiological feeling) and urges (i.e., see something that makes them want to use cannabis), as well as daily ability to "step back and be aware of cravings/urges without being taken over by them". Participants were also asked to rate their mental state, level of relaxation-tension, nervousness/stress, and judgement of thoughts as "good or bad". Questions related to daily cannabis use provided information on 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?").

5.4.9.3 Monitoring of Participants' Compliance to Daily Task

An unblinded researcher monitored the participants' completion of the daily tasks relevant to each intervention group. Via Qualtrics, the unblinded researcher was able to observe if the participant had opened the provided link. An SMS reminder was sent to the participant if they did not access the link after one and two days. A telephone call to the participant was made to confirm if they were experiencing any issues in completing the task/s

after missing more than two consecutive days of accessing the link (and receiving two SMS reminders).

5.4.10 Experimental Conditions

5.4.10.1 Mindfulness Script

Mindfulness instructions 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." Participants were 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."

5.4.10.2 Relaxation Script

By contrast, during the explanation of the strategy, the relaxation group was told, for example, that craving intensity can be reduced by "softening the muscles...and calming and unwinding the mind…releasing tension in your body". It was also emphasized that this is a way of gaining control over craving. Participants were also instructed that relaxation enables transformation of sensations into more calming, less unpleasant experiences.

5.4.10.3 No Intervention

Participants allocated to the no intervention condition were asked to complete the Daily Questionnaire, to minimize discernment of allocation to the control group.

5.4.11 Statistical Analyses

5.4.11.1 MRI Data Pre-Processing and Analysis

5.4.11.1.1 Pre-Intervention Analysis. MRI data pre-processing and pre-intervention analysis has been described in detail Sehl and colleagues, *in preparation*, Chapter 4. Briefly, all MRI raw data was converted from DICOM to BIDS format. All quality checks and data pre-processing were conducted using fMRIPrep (version 1.1.1). No subjects were excluded due to acquisition quality.

First level analyses were run via Matlab (version r2018a) using SPM (version 12). A general linear model (GLM) was conducted to encode for cue-reactivity task condition (cannabis vs neutral). We investigated group differences (CUD vs controls) in cue-reactivity (cannabis > neutral and cannabis < neutral) using Independent Samples *t*-tests. A whole brain approach was run using GLM with group as a factor (CUD vs controls), cluster size was set k > 10, *p* value > 0.001.

5.4.11.1.2 Post-Intervention Analysis. Region of interest (ROI) analyses were performed in the lingual gyrus, the MFG, the medial OFC, and the cerebellum. These regions were selected because CUD showed significantly different activity compared to controls during fMRI cue-reactivity task at the first assessment session (pre-intervention) to cannabis vs neutral stimuli. This analysis has been reported in Sehl and colleagues, *in preparation*, Chapter 4. ROI masks were created by constructing 5mm radius spheres around peak region MNI coordinates. The mean β values from the first-level analysis for each ROI were extracted for each subject and used as the dependent variable in a repeated measures analysis of variance (ANOVA). Variables that differed significantly between groups were entered as covariates in the relevant field (i.e., cannabis use duration in years, alcohol dependence scores, intervention compliance). If covariates were not significant ($p > .05$) and there was no impact on statistical significance of the model they were removed from the analysis to

preserve statistical power. Therefore no covariates have been reported. All data was inspected to ensure that the assumption of parametric statistics was met.

5.4.11.2 Behavioural Data

Chi-squared tests were run to compare CUD groups for categorical data (i.e., sex, employment status). ANOVAs were run to compare groups for normally distributed variables (i.e., income, IQ, education years, mental health, mindfulness, arousal rating of cannabis images). Kruskal-Wallis tests were run to compare groups for non-normally distributed variables (i.e., substance use, craving, readiness for change, intervention compliance).

5.4.11.3 Longitudinal Effects of Intervention Group on Behaviour

Changes in behaviour and comparison between groups from pre-to-post intervention was analysed using repeated measures ANOVAs. Behavioural variables included subjective craving, cannabis dosage (average daily grams), withdrawal symptoms, abstinence duration, arousal ratings of cannabis images, and CUD severity (CUDIT), mental health (e.g., anxiety, depression, stress), nicotine dependence (FTND) and mindfulness and emotion regulation scores.

5.4.11.3.1 Creating a Variable for Cannabis Dosage (Average Daily Grams). To analyse changes in cannabis dosage we created a variable of *average daily grams* by dividing the total number of cannabis grams reported in the past month prior to the first assessment session (pre-intervention) and the total number reported between the two assessment sessions by the number of days respectively.

5.4.11.3.2 Creating a Group Variable for the Cue-Reactivity Image Rating Task. To analyse group differences and changes in the arousal rating of cannabis images used in the fMRI cue-reactivity task we first calculated the inter-rater reliability of all of the cannabis

images. We did this to determine the fit of utilising the three groups' mean scores of self-reported arousal ratings of the cannabis images at both assessment sessions (pre- and post-intervention). Cronbach's $\alpha = .995$ indicating that the images were strongly correlated, and the use of participants' mean scores for all image ratings was appropriate to create a pre- and post-intervention variable of arousal rating of cannabis images.

5.4.11.4 Exploratory Brain-Behaviour Correlations

Exploratory partial correlations were run for each group to examine the association between pre-to-post intervention changes in cue-reactivity function and changes in behaviour. Behavioural variables included: pre-to-post intervention changes in subjective craving and arousal rating of cannabis images. Covariates included years of cannabis use, pre-intervention AUDIT and changes in FTND scores, readiness for change and intervention compliance. All behavioural data and brain-behaviour correlations was run using SPSS (version 28).

5.5 Results

5.5.1 Sample Characteristics

Table 1 provides an overview of sample characteristics and group differences for sociodemographic data, substance use levels, well-being and intervention-related measures. The sample were 40 people with a moderate-to-severe CUD (9 females) aged between 18-55 years. Of these, 13 (2 females) were assigned to the MBI group, 14 (4 females) to the active (relaxation) control and 13 (3 females) to the passive (no intervention) control.

Table 5.1

Overview of Sample Characteristics and Group Differences

Variable	MBI	Relaxation	No Intervention	Group Differences	
	Median [Range]/ *Mean (SD)*	Median [Range]/ *Mean (SD)*	Median [Range]/ *Mean (SD)*	$X^a/H^b/F$	*p*
Total *N* [Females]	13 [2]	14 [4]	13 [3]	0.68[a]	.713
Age	*28.92 (8.35)*	*6.21 (8.37)*	*24.62 (5.77)*	1.06	.356
IQ	*108.08 (12.62)*	*103.50 (10.04)*	*106.54 (9.30)*	0.63	.540
Education, years	*14.06 (2.09)*	*15.55 (1.78)*	*15.54 (2.73)*	1.96	.155
CUD symptoms[1]	*7.15 (1.86)*	*7.21 (2.15)*	*7.00 (2.04)*	0.11	.949
CB Duration, years	4.25 [28.15]	5.23 [32.25]	5.30 [15.09]	.521[b]	.771
Age of onset, years	*16.06 (1.66)*	*16.59 (2.48)*	*17.99 (4.75)*	1.44	.486
CB days/month	*27.38 (4.74)*	*24.43 (5.65)*	*23.62 (6.17)*	3.30	.192
CB grams/month	28 [74.62]	18.29 [80.62]	15 [58.85]	1.59[b]	.452
CB x Tob *N* = Yes [No]	10 [3]	9 [5]	6 [7]	2.66[a]	.265
Alcohol days/month	2 [21]	3 [12]	5 [28]	5.42[b]	.067
Std drinks/month	14 [206.8]	4.4 [119.4]	24.8 [193]	8.72[b]	**.013***
Alcohol dependence	5 [13]	4.5 [11]	7 [13]	6.67[b]	**.036***
Cig days/month	0 [30]	0 [30]	1 [30]	1.98[b]	.371
Cigarettes/month	0 [300]	0 [210]	0.5 [525]	1.61[b]	.448
General Well-being	2670 [1625]	2447.5 [1530]	2850 [1800]	1.44[b]	.486
Readiness for change	5 [7]	4 [8]	6 [5]	.954[b]	.621
Intervention length, days	*15.54 (5.14)*	*14.50 (3.94)*	*13.77 (5.23)*	.449	.642
Intervention compliance, days	6 [14]	1.5 [14]	10 [8]	13.16[b]	**.001****

Note. Median [Range] reported for non-normally distributed data and Mean (SD) = Standard Deviation reported for normally distributed data. *X* = Chi Squared test. *H* = nonparametric Kruskal Wallis H test. *F* = ANOVA test. IQ = Intelligence Quotient. CB = Cannabis. Tob = Tobacco.

[1]CUD = cannabis use disorder, Symptoms measured using the Structured Clinical Interview for DSM-5 Research Version.

* $p < .05$. ** $p < .01$.

Table 5.2 provides an overview of sample characteristics pre- and post- intervention, group differences and main effects of time and intervention by time interactions in behavioural variables of interest (CUD related measures as well as nicotine dependence, mental health and mindfulness).

Table 5.2

Pre-to-Post Intervention Changes in Cannabis Exposure, Subjective Craving, CUD Severity, Nicotine Dependence Levels, Mental Health and Mindfulness Variables

Variable		MBI	Relaxation	No Intervention	Group Differences		Time		Group*Time	
		Median [Range]/ *Mean (SD)*	Median [Range]/ *Mean (SD)*	Median [Range]/ *Mean (SD)*	H^b/F	*p*	*F (df)*	*p*	*F (df)*	*p*
CB avg daily grams	Pre	0.93 [2.49]	0.61 [2.69]	0.50 [1.96]	1.57[b]	.452	3.50 (1,37)	.069	0.11 (2,37)	.897
	Post	0.73 [2.96]	0.44 [3.59]	0.29 [1.29]	2.30[b]	.317				
Craving Post CR	Pre	*4.30 (2.09)*	*4.92 (2.95)*	*4.46 (2.73)*	*0.21*	.814	6.00 (1,35)	**.019***	0.76 (2,35)	.477
	Post	4 [7]	3 [9]	2 [6]	1.34[b]	.511				
CUD Symptoms[1]	Pre	15 [20]	15 [18]	14 [18]	0.01[b]	.996	2.24 (1,37)	.143	1.67 (2,37)	.203
	Post	16 [16]	15.5 [17]	15 [23]	0.82[b]	.664				
CR Image Rating	Pre	*3.87 (2.28)*	*4.93 (2.29)*	*4.53 (1.98)*	*0.73*	.490	7.18 (1,33)	**.011***	0.02 (2,33)	.985
	Post	2.57 [7.07]	4.79 [6.47]	4.92 [6.40]	1.67[b]	.434				
Abstinence, hours	Pre	15.70 [30.75]	18.13 [52.62]	19.5 [30]	3.57[b]	.168	1.50 (1,37)	.228	0.91 (2,37)	.413
	Post	15 [100.85]	16.25[90]	18 [745.83]	0.84[b]	.657				
Withdrawal	Pre	26 [75]	26.5 [83]	25 [97]	1.20[b]	.550	2.70 (1,37)	.109	0.90 (2,37)	.432
	Post	43 [72]	31 [110]	27 [97]	1.40[b]	.500				
Nicotine dependence	Pre	0 [6]	0.5 [5]	1 [5]	0.58[b]	.747	1.58 (1,37)	.216	5.57 (2,37)	**.008****
	Post	0 [5]	1.5 [5]	1 [7]	3.69[b]	.158				
Depression	Pre	*10.08 (4.94)*	*12.86 (8.83)*	*9.38 (6.09)*	*.972*	.388	0.42 (1,37)	.523	0.20 (2,37)	.822
	Post	8 [39]	10 [42]	6 [22]	2.12[b]	.347				
State Anxiety	Pre	29 [35]	29.5 [49]	26 [24]	2.94[b]	.230	0.96 (1,37)	.333	0.29 (2,37)	.750
	Post	34 [57]	34 [22]	31 [27]	4.00[b]	.135				
Emotion Regulation	Pre	*42.69 (12.02)*	*41.79 (8.55)*	*44.23 (8.77)*	*0.21*	.811	0.04 (1,37)	.848	0.27 (2,37)	.763
	Post	*41.31 (8.34)*	*42.21 (8.79)*	*44.54 (5.87)*	*0.60*	.557				

Perceived Stress	Pre	*18.54 (7.57)*	*15.21 (6.49)*	*13.92 (6.30)*	*1.60*	.216	0.09 (1,37)	.770	0.96 (2,37)	.393
	Post	*18.78 (7.36)*	*17.14 (6.90)*	*12.62 (5.56)*	*2.99*	.063				
Mindfulness	Pre	*110.08 (14.33)*	*119.43 (14.28)*	*119.92 (15.00)*	*1.91*	.162	0.36 (1,37)	.552	0.79 (2,37)	.462
	Post	113 [50]	115 [60]	116 [51]	2.16[b]	.340				

Note. Median [Range] reported for non-normally distributed data and Mean (SD) = Standard Deviation reported for normally distributed data. *H* = nonparametric Kruskal Wallis H test. *F* = ANOVA test. *P* = significance value. *df* = degrees of freedom. CB = Cannabis. CR = Cue-Reactivity.

[1]CUD = cannabis use disorder, Symptoms measured using the Cannabis Use Identification Test.

* $p < .05$. ** $p < .01$.

5.5.1.1 Socio-Demographic Data and IQ

The three CUD groups were matched by sex and age with non-significant differences in IQ, years of education, or employment status (p = .155), or household income, (p = .872).

5.5.1.2 Substance Use and Dependence Levels

All groups were non-significantly different in CUD symptoms, cannabis duration in years, cannabis dosage (grams total and daily average grams) and frequency (days) in the past month pre-intervention and days between the two assessment sessions measured post-intervention, abstinence hours (pre- and post-intervention), and age of onset. The passive (no intervention) control group had higher AUDIT scores and consumed more standard drinks in past month pre-intervention compared to the other groups. There were no differences between the three groups in the number of drinking days or in nicotine exposure in the past month pre-intervention, or FTND scores (pre- and post-intervention).

5.5.1.2.1 Participants Included with Exposure to Other Substances. Eleven CUD (n = 3 females) and 1 control (male) used illicit substances less than 4 weeks prior to assessment and four CUD (n = 0 females) used illicit substances during the intervention period between the two assessment sessions. Substances included cocaine, mushrooms, MDMA, Xanax, ketamine, nitrous oxide, dexamphetamine and modafinil. Time since last use before the first assessment session (pre-intervention) was a median of 156 hours (ranged 16-648 hours). and a median of 100 hours (ranged 20-277 hours) before the second assessment session (post-intervention). Overview of details provided in Supplementary Material, Table 5.7.1.

5.5.1.3 Subjective Craving and Arousal Ratings of Cue-Reactivity Cannabis Images

All groups were matched in subjective craving and arousal ratings of cue-reactivity cannabis images measured at both pre-and post-intervention assessment sessions.

5.5.1.4 Mental Health and Mindfulness

There were no significant differences mental health symptom scores, psychological well-being and mindfulness scores between the three CUD groups (pre- and post-intervention).

5.5.1.5 Intervention-Related Measures

The intervention length (number of days between assessment sessions) was matched across groups; however, the no intervention group completed the daily task over more days. There was no difference between groups on readiness to change cannabis use, but more than half of the sample (62.5%) reported no plan to change their cannabis use.

5.5.1.5.1 Credibility/Expectancy of Intervention. The two experimental conditions (i.e., MBI and relaxation) did not differ on credibility or expectancy of the strategy effects (*p* values > .05). The groups endorsed the strategy as "logical" for managing cannabis cravings – with an average rating of 6.8/9 (SD =1.92, range: 1-9), and rated it an average of 3.5/9 (SD = 1.81, range: 1-7) in thinking the strategy could successfully reduce their cravings, estimating an average 25.6% improvement of their cravings (SD = 18.46, range: 0-70). Both groups provided equivalent ratings on understanding the goal of the intervention (*p* values > .05).

5.5.1.5.2 Comprehension/Manipulation Check. The two experimental conditions (i.e., MBI and relaxation) did not differ on uptake/comprehension of the strategy (*p* values >

.05), indicating that participants in both groups equally understood the relevant components of the intervention.

5.5.2 Changes in Behavioural Variables Pre-to-Post Interventions

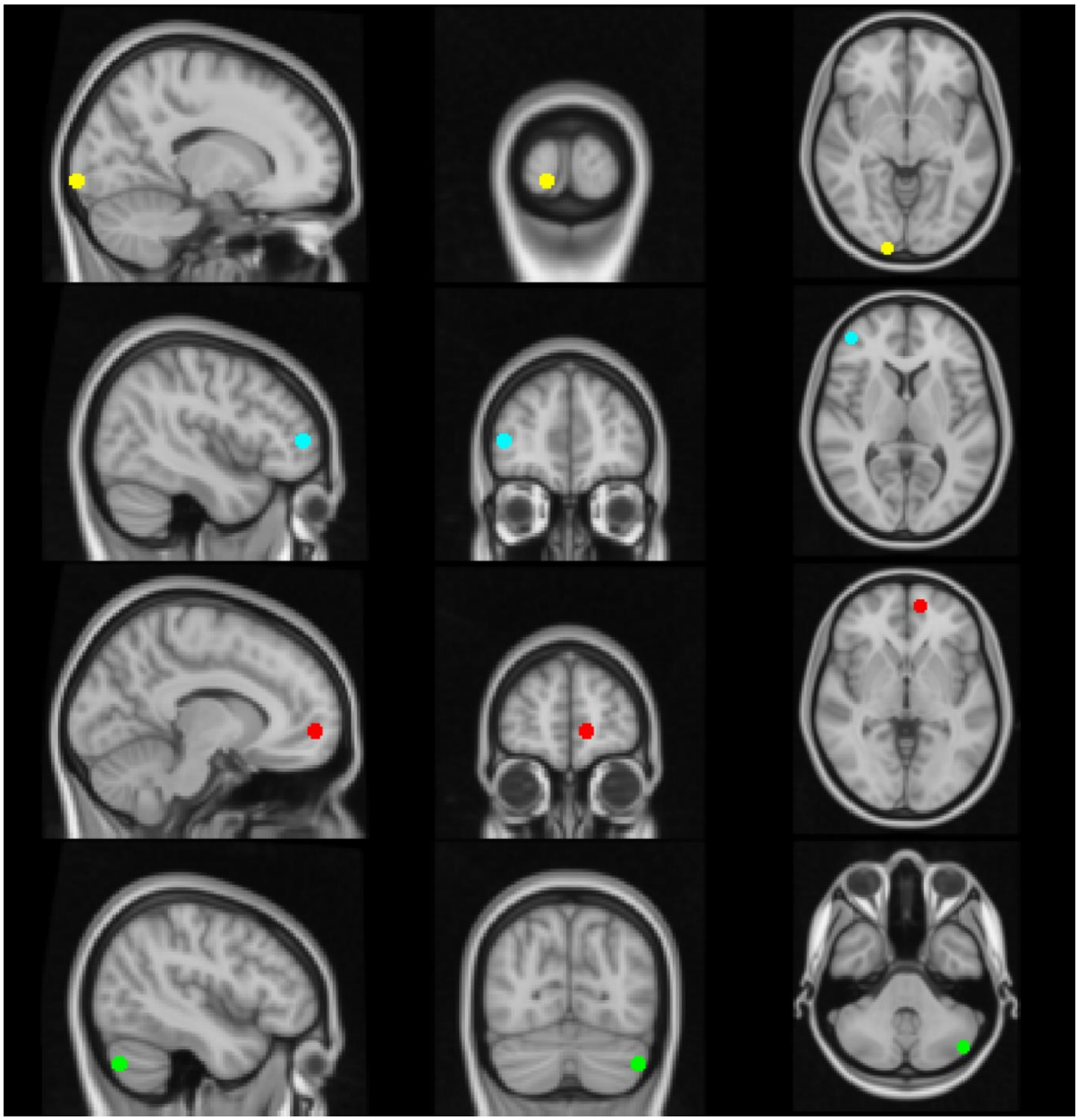
There was a significant decrease of subjective craving and arousal rating of cannabis images, pre-to-post intervention with large effect sizes (Cohen's d = 0.84, 0.94; respectively). There was a significant intervention-by-time interaction on FTND scores (Cohen's d = 1.09), which increased in the relaxation group. There was no significant change in cannabis dosage (average daily grams), CUD severity, withdrawal symptoms, abstinence duration, mental health or mindfulness scores (reported in Table 5.2).

5.5.3 fMRI Results: Changes in Cue-Reactivity Brain Function Pre-to-Post Intervention

Figure 5.3 depicts the ROI (5mm spheres of peak activations) used to measure intervention related brain changes based on group differences (CUD compared to controls) in cue-elicited activity (cannabis > neutral) at assessment session 1 (pre-intervention).

Figure 5.3

Region of Interest (ROI) Masks of 5mm Spheres of Peak Activation Region MNI Coordinates



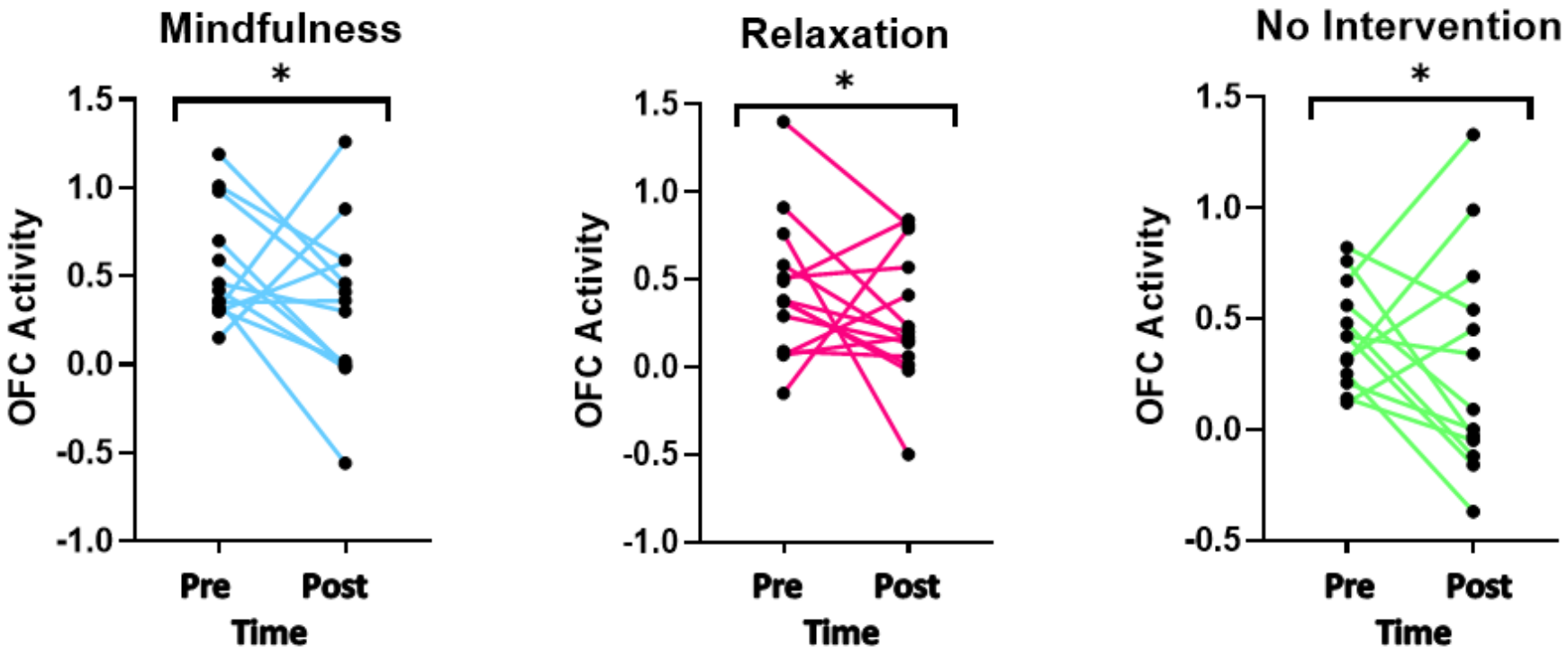
Note. The coloured dots indicate the Region of Interest (ROI) Masks of 5mm Spheres of Peak Activation Region MNI Coordinates: Yellow = lingual gyrus, Aqua = MFG, Red = OFC, Green = cerebellum.

Result on changes in cue-reactivity brain function over time from pre-to-post intervention are shown in Figure 5.4. There was a significant main effect of time on

participants' fMRI activation in the OFC, $F(1,36) = 7.67$, $p = .009$, with large effect size (Cohen's d = 0.94). There was a no-significant effect of group $F(2,36) = 0.32$, $p = .727$, or group*time interaction $F(2,36) = 0.45$, $p = .642$.

Figure 5.4

Changes in OFC Activity Pre-to-Post the Mindfulness, Relaxation and Passive Intervention



Note. OFC = Orbitofrontal Cortex.

Data points represent individual participants change over time.

OFC activity significanlty decreased from pre- *M* = 0.47, *SE* = 0.05, 95% CI [0.36-0.58] to-post *M* = 0.29, *SE* = 0.07, 95% CI [0.16-0.43] intervention in all groups.

There was no significant effect on the MFG, lingual gyrus, or cerebellum. Detailed results and visual display are reported in Supplementary Material Table 5.7.2 and Figure 5.7.1.

5.5.4 Exploratory Brain-Behaviour Correlations

Longitudinal changes in cue-elicited OFC function was not correlated with changes in subjective craving or arousal ratings of cannabis images (*p* values > .05). Correlation coefficients are reported in Supplementary Material Table 5.7.3.

5.6 Discussion

This is the first study to examine the functional neural correlates of a MBI in CUD. We employed an active (relaxation) and passive (no intervention) placebo controlled, pseudo-randomised design using fMRI to measure pre-to-post changes in brain activity during exposure to cannabis (vs neutral) images.

We found a significant decrease in the activity of the OFC in all three groups from pre-to-post intervention. In relation to our hypothesis, this indicates a null finding since the reduction in OFC activation cannot be attributed solely to the MBI. The finding suggests that there may be a shared therapeutic component to engagement in a trial which involves daily monitoring of cannabis use, subjective craving and mood (234).

The OFC has been shown to be an important brain region for self-monitoring processes (235) in relation to reward processing (i.e., salience attribution; 236-239). In humans, the OFC underscores the flexible shifting of evaluation based on conditioned outcomes, such as the ability to adapt behaviours based on whether positive or negative outcomes match the past experiences (206). For example, if a person's cannabis use is problematic (e.g., related to mood disturbance), but was positive in the past (e.g., social

connections) they are able to adjust their expectations and decision-making in relation to benefit vs consequence of using cannabis. In both animal and human studies, the structural impairment in the OFC results in a failure to exhibit this effect of outcome devaluation (206, 235).

Different properties of the integrity of the OFC (e.g. structure, function, molecular), are affected by repeated cannabis use e.g. (206, 240-242). Therefore, OFC functional alterations may impair one's ability to appropriately attribute salience to associated cannabis cues (243). It may be that bringing conscious awareness to one's daily cannabis use, cravings and mood may attenuate the OFC's conditioned response to cues (231, 239). As such, self-monitoring may be considered a methodological limitation in treatment studies (especially MBIs) as it can bring conscious awareness to automatic behaviours (231, 244). Future work, that includes a measure of a person's motivation for using cannabis, as well as their expectancy of outcomes (and adverse consequences) related to their cannabis use may assist to disentangle the effect of MBI from that of self-monitoring specific effects on functional OFC cue-reactivity. The use of an implicit vs explicit cannabis cue-exposure paradigm (see Wetherill et al., [2014; 83] for an example) may reveal if changes in OFC function are related to increases in conscious awareness or expectancy outcomes.

Interestingly, we also found that subjective craving and arousal rating of cannabis images significantly decreased from pre-to-post intervention across all three groups. This finding is consistent with a previous study comparing a similar intervention (MBI compared to relaxation) in at-risk drinkers (116). It is also consistent with fMRI studies in smokers who did not find a correlation between changes in cue-elicited brain function and subjective craving (122). As such, decreases in subjective craving and arousal rating of cannabis cues may be explained by factors such as re-exposure effects (e.g., reduced novelty; 245) due to repeated engagement in assessment. It may also be related to subjective craving methodology

including the self-report measure and timing of administration, as different brain-craving correlations were reported in the same sample when assessed by different subjective craving measures administered at different times during the task (i.e., pre-post scan, post every image presented in the task; 134, 156). Further, some studies ran brain-behaviour correlations using cue-elicited activity within cannabis users (84, 156), whereas brain behaviour (e.g., subjective craving) correlations were only run with group differences in cue-elicited activity. Therefore, it is important for future work to consider methodological approaches when conducting and interpreting brain-behaviour relationships.

We did not observe any changes pre-to-post MBI in the activity of the other examined brain regions – the lingula gyrus, the MFG, or the cerebellum. MBI-related effects have been observed in these regions in smokers (118-120), however they included heterogeneous cognitive tasks including resting state and emotion processing. As such, it is difficult to determine if MBI-related effects on brain function are task specific. However, two studies did observe decreases in cue-elicited MFG activity, with state changes observed in a "mindful vs passive" looking intervention during a cue-reactivity task (123) and pre-to-post changes observed after a 10-week MBI (122). Thus, changes in cue-elicited brain function may reflect an effect of intervention dosage. It may be that the brief MBI in our study was not long enough for potential state changes to effect more stable changes (108). Future work could test this notion by examining the effects of varying amounts of MBI exposure on the neural correlates of cannabis cue-reactivity, including duration of practice, number of days and occasions practiced.

Similarly, there was no change in cannabis dosage (i.e., average daily grams) from pre-to-post intervention. Changes in substance use dosage observed post-MBI is expected (99), however there are inconsistent findings (115). Motivation/readiness to change use has been shown to predict changes in substance use (246) including cannabis (176). More than

60% of the current sample reported being in the pre-contemplative and contemplative stage, indicating no plan to change their cannabis use. As such, investigating MBIs in CUD populations with greater motivation for change (i.e., ML scores > 8) may lead to a reduction in dosage as seen in smokers (121).

5.6.1 Limitations and Future Directions

There are several limitations in this experiment that must also be acknowledged. First, the sample size, is considered adequate (e.g., ≥ 40) for detecting large effect sizes, however it may be too small to detect moderate effect sizes (247). Whilst we detected changes in cue-elicited OFC function comparable to a similar study in smokers (122), a larger sample size is necessary to confirm our findings (that had large effect sizes), or to reveal additional changes in other regions (e.g., lingual gyrus, MFG) that may be subtler but detectable in a larger sample.

Further, the size of the included sample limited the number of correlations that could be run with adequate power to mitigate false positives (247). Demographic variables that may have an impact on the effect of MBIs on neural changes, such as sex, as evidenced in smokers (121), could be investigated with a larger sample. Examining other behavioural measures relevant for CUD populations would enable a more comprehensive understanding of how changes in brain function relate to behaviour and whether MBIs are more suitable for specific demographics. For example, concurrent nicotine use, varying levels of cognitive function (e.g., IQ, working memory), mental health (e.g., psychotic symptoms) and well-being (e.g., physical activity) may represent subgroups of cannabis users that MBIs may be more (or less) effective for. Further, as MBIs vary in structure and format, there may be specific approaches driving the previously reported effects of MBIs on SUDs. Investigating

more specific measures of mindfulness (e.g., awareness vs non-judgement, informal vs formal) may reveal more effective strategies or CUD-problem specific approaches.

It must be noted that the sample included in this study is smaller than planned due to COVID-19 related disruptions to recruitment and face-to face data collection. Despite this limitation, this is the first study to examine the underlying neural mechanisms of MBIs for cue-reactivity in CUD using a robust design, including careful control of treatment effects (i.e., active and passive control), monitoring and accounting for intervention engagement and expectancy and ensuring low risk of bias (i.e., Cochrane Risk of Bias = low in all five domains; (248). Analyses will be rerun on a larger sample (N = 60; 20 per group) once recruitment is complete.

Second, the method used to measure brain function in this study was an *a priori* ROI, based on previous findings in this sample that the ROI regions (lingual gyrus, MFG, OFC, cerebellum) selected were associated with cue-reactivity in CUD (see Sehl and colleagues *in preparation*; Chapter 4). As such, an ROI approach was appropriate to address our hypotheses. However, neural changes associated with mindfulness-based training that targets subjective cannabis craving may happen in additional brain regions and circuitry (113) and might have been missed using the current approach. The function of mindfulness-related regions could be revealed by future work using other data-driven analytical methods such as functional connectivity and whole brain analyses.

Lastly, whilst we monitored and supported compliance of daily tasks, the participants in the MBI and relaxation group engaged significantly less than the no intervention group, with an average of ~ 8 days. As previously discussed, it is possible that greater compliance (i.e., 14 days) or a longer intervention may have shown effects of mindfulness on cue-elicited brain function (i.e., reduced activity), as observed in smokers (122). The current sample had a low level of motivation to change cannabis use, despite having tried to cut down or quit

previously. Therefore, the low motivation to change may have led to the lower compliance rates with the experimental tasks in the MBI and relaxation groups, which may not have been long enough to effect detectable changes in cue-elicited brain activity. Future studies screening for cannabis users who are in the action stage (i.e., score > 8 on Marijuana Ladder) may have greater engagement with the experimental tasks in the MBI and relaxation conditions, and subsequently provide greater understanding of intervention effects on the brain (121). The inclusion of self-monitoring as an experimental condition and/or controlling for its effects (e.g., waitlist control) should also be considered in future work (244).

5.6.2 Clinical Implications

With global trends towards the legalisation of cannabis, the presence of cues (i.e., medical and non-medical) in the general community (e.g., commercial outlets, advertisements, public consumption) and positive views (e.g., greater benefit than harm) towards cannabis use may increase further (4). Greater availability of cannabis related cues may have detrimental impact on therapeutic goals (i.e., reducing/ceasing cannabis use) among those who are vulnerable to cue-reactivity, including those with a moderate-to-severe CUD. As such, our investigation of interventions that target the underlying neural mechanisms of cannabis cue-reactivity is important work.

Neuromodulation techniques including Repetitive Transcranial Magnetic Stimulation (rTMS), Transcranial Direct Current Stimulation (tDCS), and Deep Brain Stimulation (DBS) have shown promise for SUD treatment (249, 250). A systematic review of 60 studies investigating rTMS, tDCS and DBS for SUDs including CUD, report evidence for effects of neuromodulation on reducing craving and substance use, particularly when stimulation targets the dorsolateral PFC (249), which is implicated in cannabis cue-reactivity (187). In people with CUD and schizophrenia, rTMS in the dorsolateral PFC compared to a sham

condition, showed a clinically significant effect on reducing cannabis craving, improving attention, and supressing increased tobacco use that was associated with cannabis reductions (251).

The findings of this study also support the investigation of additional interventions that subjectively devalue cannabis by reducing its expected reward whilst increasing the relative valuations of non-drug rewards (232). Interventions that aim to directly reduce cue-reactivity, arousal, or attentional allocation may also prove helpful for preventing relapse given the ubiquitous nature of cues in places where it is legalised. Notably, a reduction in cue-elicited amygdala activity has been reported after Cognitive Bias Modification (vs a sham-training control condition), with the reduction significantly correlated with reduced subjective craving scores and arousal ratings of cues (151). Whilst this finding was in alcohol-dependent participants, a small pilot trial of Approach Bias Modification with cannabis users showed a blunted cannabis cue-induced craving at the end of the active vs sham training condition (152). As such, greater brain function in CUD compared to controls during cue-reactivity (i.e., in the lingual gyrus, MFG, OFC and cerebellum) may diminish with interventions such as neuromodulation, cognitive/approach bias retraining or via cue-exposure therapy (230), and should be considered in future work.

This is the first study to date to examine how MBIs effect cue-elicited brain function in CUD. Whilst our hypothesis was not supported, our study provides essential information for continued research to expand upon, including the potential confounding and/or therapeutic effects of self-monitoring practices on neural cue-reactivity. Null findings are equally as valuable for developing the effectiveness of current interventions and guiding the allocation of resources (e.g., funding) to worthwhile advances in treatment research and community access.

5.6.3 Conclusion

In summary, the findings of this study demonstrate that altered brain function in the OFC associated with cannabis cue-reactivity in CUD can be reduced during a brief engagement of MBI, relaxation and/or monitoring of daily cannabis use, cravings and mood. However the specific treatment effects remain unclear. The requirements of the study with respect to participants recording their daily cannabis use, cravings and mood may have impacted OFC activity over and above any intervention effects. Methodological factors, such as intervention length and sample characteristics (e.g., motivation/readiness to change), as well as sample size and fMRI analysis approach may limit the detection of MBI-related changes in cue-elicited activity. Future work in a larger sample, with higher levels of motivation for change using additional analyses approaches (e.g., whole brain) and varying intervention lengths (e.g., longer) is required to understand the efficacy of MBIs for neural cue-reactivity in CUD further. The findings of this study (i.e., main effect of time on OFC changes in *all groups*) highlights the importance of employing highly controlled experimental designs, and offers a template for continued work in this area.

5.7 Supplementary Material

5.7.1 Overview of Participants Excluded from Analyses

Nine participants (6 CUD and 3 controls) were excluded due to subsequently meeting exclusion criteria when face-to-face testing. The people with a CUD who were excluded were: i) a female aged 25 years with an IQ score < 80 (i.e., FSIQ-2 = 61); ii) one male aged 22 years endorsing a neurological disorder (i.e., history of seizures); iii) a male aged 31 years with an incidental finding determined by a neurologist that conducted comprehensive checks of MRI images; iv) a female aged 21 who consumed 2 grams of hallucinogen (psilocybin) during the intervention period less than 48 hours prior to assessment; v) a male aged 26 years with fMRI technical issues; and vi) a female aged 39 years, who reported no desire to reduce or cease cannabis use (ML score =1). The three controls excluded reported > 50 lifetime occasions of cannabis use. They were: i) a female aged 32 years, who reported ~4000 lifetime occasions for 13.5 years, ii) a female aged 51 years, who reported 176 lifetime occasions since the age of 18, and one male aged 35 years, who reported 420 lifetime occasions in 14-months and endorsed a history of a diagnosed psychiatric condition in adolescence.

5.7.2 Overview of Participants Not Assessed Post-Intervention

Six CUD participants did not take part in the post-intervention assessment session for various reasons. They included: i) a female aged 55 years who was unable to attend due to covid-related restrictions; ii) a male and a female aged 19 years withdrew due to work commitments; iii) two females aged 22 and 29 years and a males aged 22 years withdrew (were no longer interested/no reason provided).

Supplementary Table 5.7.1

Details of Included Participants with Illicit Substance Use Within Four Weeks of Assessment and During Intervention

Substance	Group	Age	Sex	Hours[a, b]	Dosage
Cocaine					
	CUD	35	Male	24[a], 100[b]	1g, 0.25g
	CUD	30	Male	250[b]	0.85g
	CUD	22	Male	624[a]	1g
	CUD	20	Female	504[a]	1g
	CUD	21	Male	84[b]	0.40g
	CUD	29	Male	38[a], 277[b]	3.5g, 0.85g
Mushrooms (Psilocybin)					
	CUD	21	Male	50[a]	1g
	CUD	18	Male	648[a]	2g
	CUD	20	Male	NS[a]	4g
MDMA					
001	CUD	20	Male	NS[a]	0.2g
031	CUD	27	Female	16[a]	0.15g
035	CUD	20	Female	480[a]	0.2g
004	*Control*	29	Male	60[a]	~75mg
052	CUD	21	Male	84[b]	0.40g
Xanax					
030	CUD	18	Female	360[a]	1mg
053	CUD	19	Male	156[a]	0.5mg
Ketamine					
033	CUD	20	Male	240[a]	0.5g
052	CUD	21	Male	84[b]	~60-120mg
Nitrous Oxide					
079	CUD	21	Male	50[a]	8g
Dexamphetamine					
076	CUD	35	Male	28.5[a], 20[b]	20mg, 30mg
Modafinil					
068	CUD	29	Male	96[a]	200mg

004	*Control*	29	Male	432[a]	50mg

Note. NS = Not specified. g = grams. mg = milligrams.

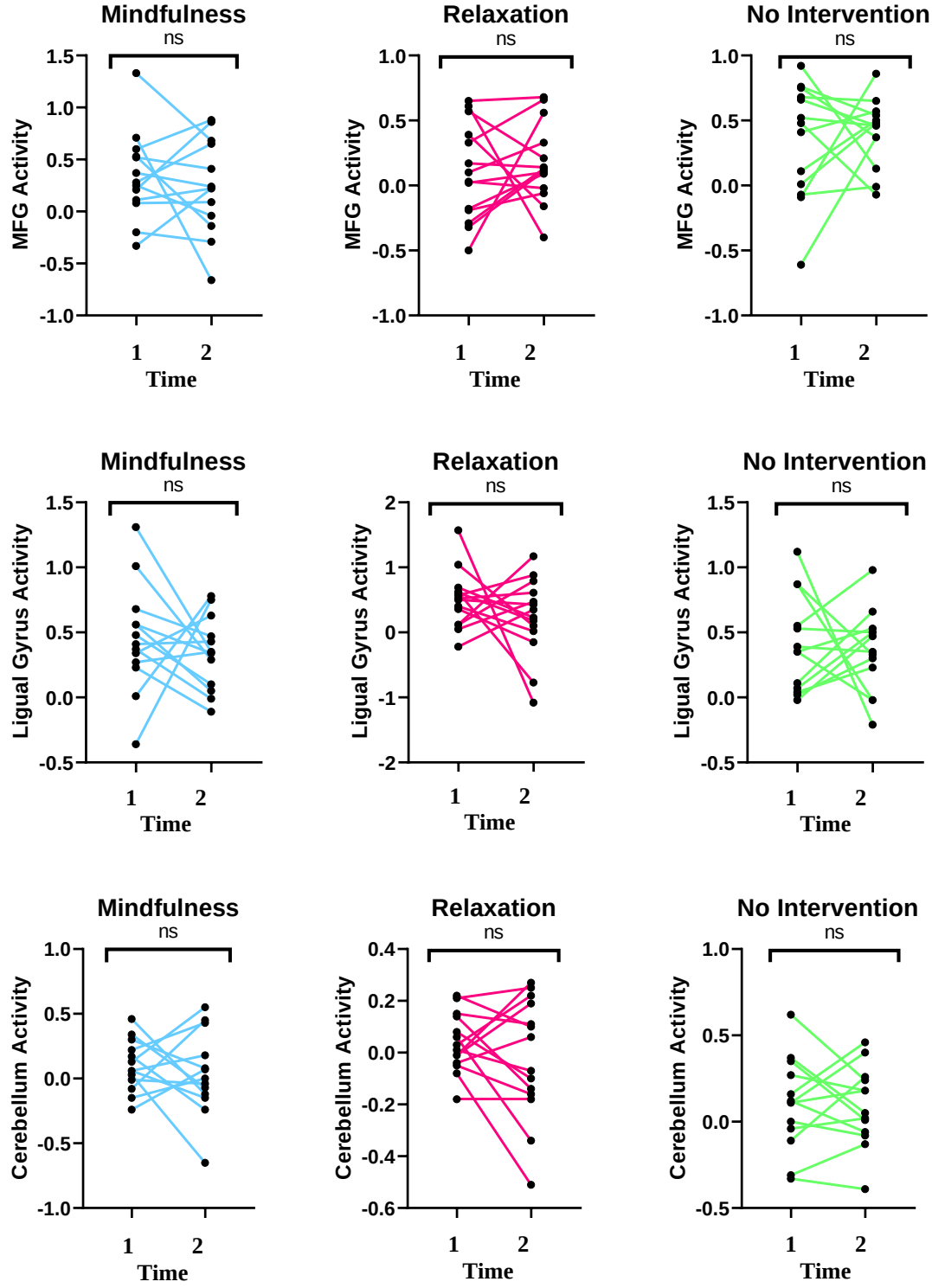
Matching colours (excluding black) indicate the same participant.

[a] Hours last consumed before the first assessment session (pre- intervention).

[b] Hours last consumed before the second assessment session (during intervention period).

Supplementary Figure 5.7.1

Within and Between Group Changes in MFG, Lingual Gyrus and Cerebellum Activity Pre-to-Post Intervention



Note. MFG = Middle Frontal Gyrus. 1 = First Assessment Session (Pre-Intervention). 2 = Second Assessment Session (Post-Intervention).

Data points represent individual participants change over time.

ns = not significant.

Supplementary Table 5.7.2

Pre-to-Post Intervention Changes in Cue-Elicited Brain Activity in the Middle Frontal Gyrus, Lingual Gyrus, Cerebellum

Variable		MBI	Relaxation	No Intervention	Group		Time		Group*Time	
		Mean (SD)	Mean (SD)	Mean (SD)	F (df)	p	F (df)	p	F (df)	p
MFG	Pre	0.34 (0.42)	0.10 (0.37)	0.35 (0.45)	2.66 (2,37)	.083	0.01 (1,37)	.921	0.45 (2,37)	.640
	Post	0.24 (0.46)	0.17 (0.31)	0.41 (0.26)						
Lingual Gyrus	Pre	0.45 (042)	0.50 (0.44)	0.40 (0.37)	0.08 (2,37)	.928	1.50 (1,37)	.229	0.32 (2,37)	.732
	Post	0.34 (0.28)	0.23 (0.61)	0.35 (0.32)						
Cerebellum	Pre	0.10 (0.20)	0.04 (0.11)	0.10 (0.27)	0.81 (2,37)	.454	0.91 (1,37)	.346	0.12 (2,37)	.888
	Post	0.08 (0.32)	-0.02 (0.23)	0.09 (0.23)						

Note. SD = Standard Deviation. CI = 95% Confidence Interval. F = repeated ANOVA test. df = degrees of freedom. P = significance value. MFG = Middle Frontal Gyrus.

Supplementary Table 5.7.3

Correlation Matrix of Pre-to-Post Intervention Change in OFC Activity with Behavioural Variables in the MBI, Relaxation and No Intervention Group

	MBI			Relaxation			No Intervention		
1									
2	.493			-.209			.272		
3	.218	.780*		-.011	.086		.503	.152	
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**

Note. 1 = OFC Activity Change. 2 = Subjective Craving Change. 3 = Arousal Rating Change.

Covariates included in the partial correlations: Readiness for change; Intervention Compliance; Nicotine Dependence Change; pre- intervention Alcohol Dependence Scores.

Degrees of freedom (MBI and No Intervention Group) = 0 (OFC); = 7 (Craving); = 7 (Arousal Rating).

Degrees of freedom (Relaxation Group) = 0 (OFC); = 6 (Craving); = 6 (Arousal Rating).

* $p < .05$.

Chapter 6: General Discussion

6.1 Chapter Guide

The overall objective of this thesis was to better understand the neurobiological mechanisms implicated in CUD to identify neurobiological treatment targets and to test how interventions can reduce brain changes in individuals with moderate-to-severe CUD. This was achieved, by studying the patterns of brain function associated with cannabis cue-reactivity in cannabis users and how it relates to behaviour (e.g., subjective craving, cannabis use), as well as the effects of a mindfulness-based intervention (MBI) targeting craving on brain function during cue-reactivity.

First, a systematic review of the literature on the patterns of brain function in regular cannabis users was conducted (Chapter 2). Next, two experimental studies were run using functional magnetic resonance imaging (fMRI). The first fMRI study examined differences in brain activity during a cue-reactivity task (presentation of cannabis vs neutral images) in adults with moderate-to-severe CUD compared to non-using controls (Chapter 4). The second fMRI study investigated how brain function during cannabis cue reactivity can be reduced with a brief MBI compared to active relaxation and passive (no-intervention) controls (Chapter 5). This chapter will summarise the main results from these three studies and provide an overview of the strengths, contributions and clinical implications of the findings. It will also discuss the limitations of this research with future research directions proposed.

6.2 Summary of Aims and Main Findings

6.2.1 Study 1: Systematic Review

The systematic review conducted in Study 1 (Chapter 2) aimed to synthesise the evidence to date on the functional neural correlates of cannabis cue-reactivity in regular cannabis users examined using fMRI tasks, which entail participants' exposure to cannabis *vs* neutral stimuli. The secondary aim of this review was to summarise the evidence on the associations between brain function associated with cannabis cue-reactivity in cannabis users and behavioural variables including subjective cannabis craving, cannabis exposure (e.g., duration, dosage, frequency), cannabis use related problems (e.g., CUD) and exposure to substances other than cannabis. An additional aim was to critically examine the methodologies used to measure cannabis cue-reactivity using fMRI in regular cannabis users in order to inform on the methodological standards in this area of research.

The reviewed literature comprised of 18 studies. It consistently reported greater brain activity in cannabis users in three key brain areas: the striatum, the PFC (ACC, MFG), and the parietal cortex (PCC/precuneus; relative to controls; $n = 8$) when viewing cannabis cues. Additional brain regions were activated (e.g., hippocampus, amygdala, thalamus, occipital cortex) among cannabis users while exposed to cannabis vs neutral cues in studies without controls/within group analyses ($n = 13$). Early evidence showed associations between greater brain function in similar brain regions (e.g., dorsal striatum, OFC, amygdala, insula) during cannabis cue-reactivity and higher subjective cannabis craving.

Further, evidence from brain-behaviour correlational analyses suggested that the results may be driven by subgroups of cannabis users with greater cannabis use related problems. Such subgroups may explain some of the heterogeneity in the results from the literature, which may be driven by variations in cannabis use parameters known to effect brain function, such as cannabis dependence severity (e.g., non-dependent users, mild use

disorder) and cannabis use history (e.g., later age of onset). The methodologies used to assess cannabis users and cue-reactivity using fMRI tasks varied widely between studies (e.g., cannabis exposure details, diagnostic criteria, cue-reactivity paradigm, fMRI analysis approach) which may also have influenced the heterogeneity in the findings. Overall, the evidence points to greater brain function during cannabis cue-reactivity in regular cannabis users which may drive stronger cannabis craving in response to exposure to cannabis related cues, and to a need for improved standardised assessment (e.g., DSM-5 diagnostic criteria to recruit CUD sample, lifetime patterns of cannabis exposure) of the neurobiology of cue-reactivity in cannabis users.

6.2.2 Study 2: Experiment 1

The primary aim of Study 2 (Chapter 4) was to overcome the limitations of the literature to date. It examined for the first time, brain function during cannabis cue-reactivity in a sample of adults with a moderate-to-severe CUD (who had tried to cut down or quit) compared to non-using controls (N = 85). The secondary aim was to explore the association between differences in brain function in the CUD group and relevant behavioural variables (e.g., subjective craving, cannabis exposure).

Group differences were observed in brain activity to cannabis vs neutral cues. Specifically, *FWE*-corrected results show that CUD participants compared to controls had greater brain activity in the lingual gyrus ($p < .05$, k > 10). Uncorrected results show greater activity in the MFG, the medial OFC, and the cerebellum ($p < .001$, k > 10) while watching cannabis vs neutral images. In the CUD group, greater MFG activity correlated with higher consumption of cannabis grams in the past month, suggesting that activity in this region may (in part) be dose dependent. These preliminary findings suggest that moderate-to-severe CUD may reflect common neurobiological correlates of cue-reactivity across SUDs (i.e., alcohol,

nicotine, opioids, cocaine; (229) and share a pattern of brain functional differences postulated to be core in addiction neurobiology (53). However, the robust activation of a region involved in higher order visual processing (i.e., lingual gyrus) suggests that the location of brain functional differences during cue-reactivity in more severe forms of CUD may be a partially distinct neural pattern relative to milder presentations and other substances.

6.2.3 Study 3: Experiment 2

Study 3 (Chapter 5) investigated for the first time how a brief MBI reduced cue-reactivity brain function shown in Chapter 4, in the same sample of adults with moderate-to-severe CUD (N = 43). Specifically, it examined how an MBI targeting cannabis craving changed brain function compared to both an active (relaxation) and passive (no intervention) placebo control. Brain functional changes were measured as differences in brain activation during a fMRI cannabis cue-reactivity task (cannabis vs neutral pictures) pre-to-post intervention (Study 2). A secondary aim was to explore if changes in brain activity pre-to-post MBI were associated with those in behaviour.

A significant decrease in the activity of the OFC was observed in all three intervention groups from baseline to follow-up. In relation to our hypothesis, this indicates a null finding since the reduction in OFC activation was not specific to the MBI. A decrease over time in subjective craving and arousal rating of cannabis images was also observed across all three groups, however these changes were not correlated with those in OFC activity. The findings suggest that there may be a shared therapeutic component to engagement in the three interventions. All of the interventions involved daily monitoring of cannabis use, subjective craving and mood (234), however the specific treatment effects remain unclear.

6.3 Strengths and Contributions of Research in this Thesis

6.3.1 Contribution to the Understanding of the Neurobiology of CUD

Altogether, the findings of the systematic review (Chapter 2) and two experimental fMRI studies (Chapter 4 and 5) contribute to furthering the understanding of the functional brain correlates of CUD, as discussed in this section.

6.3.1.1 Neural Correlates of Cue-Reactivity in CUD

6.3.1.1.1 The Lingual Gyrus

The findings of the studies comprised in this thesis advance the understanding of the neural correlates of cue-reactivity in CUD. Specifically, the studies suggest that the lingual gyrus may be a key neurobiological vulnerability for moderate-to-severe CUD in the presence of cannabis cues compared to neutral cues. The lingual gyrus is implicated in the processing of higher order visual stimuli (i.e., perception and recognition of familiar scenes, encoding and recall of complex pictures; (194-196). Increased activation of the lingual gyrus has also been associated with internally directed attention (197) and with mediating attentional processes between competing demands (198). Whilst not being a key brain region implicated in prominent addiction models (53), the lingual gyrus has been associated with cue-reactivity in users of other substances (i.e., nicotine, alcohol, cocaine, opiates; (193), and within group analyses (cannabis vs neutral stimuli) in cannabis users (i.e., not compared to a non-using control group; (81, 85). Further, the lingual gyrus has also been associated with subjective cannabis craving (85) and cannabis dosage (i.e., greater urinary THC/creatinine metabolites (e.g., THC-COOH ng/ml; (156). With respect to our results, the increased activation in the lingual gyrus to cannabis cues may reflect a habituated attentional bias arising from paired association between cannabis cues and reward (199).

6.3.1.1.2 The Middle Frontal Gyrus

The research in this thesis also advances the understanding of the role of the MFG during cue-reactivity in CUD. The MFG is a prefrontal brain region consistently implicated in cannabis cue-reactivity in the studies of this thesis. In the systematic review (Study 1), hyperactivation in the MFG was consistently associated with cannabis cue-reactivity, including the high-quality controlled studies. In Study 2, CUD participants compared to controls had greater MFG activity and this was dose dependent, with a significant correlation with greater cannabis dosage (i.e., monthly grams). The function of the MFG is well-established as playing a key role in motivation to use substances, disinhibition (53) and substance-related cue-reactivity (77, 187). Interestingly, the MFG is also part of the Ventral Attention Network. It serves as a junction site for attentional processes to reorient attention from external to endogenous stimuli (201), with evidence of reduced top-down attentional control associated with a MFG tumour resection (202). Therefore, greater MFG activity may reflect reduced top-down attentional control in the presence of cannabis compared to neutral cues.

6.3.1.1.3 The Lingual and Middle Frontal Gyrus

Taken together, greater activity in the lingual gyrus and MFG in response to cannabis cues may reflect a hyperactivation underlying attention-regulatory abilities, which in turn can lead to poor reorientation of attention from cannabis to neutral stimuli. As such, individuals with a CUD who desire to reduce and/or quit use or remain abstinent, may find it difficult to shift attention from experiences of craving (i.e., become preoccupied) in the presence of cannabis cues (53), which may be exacerbated if cues are unexpected (203). This highlights an increased vulnerability for individuals with a CUD, as the presence of cues may increase with changes in legalisation. Further, the current lack of legislated regulation of THC levels

in cannabis products presents a concern for all cannabis users, due to different cannabinoid ratios and potency levels influencing the rewarding properties (i.e., psychoactive effects) and addiction liability of cannabis. As THC levels may (in part) be driving both lingual and MFG cue-reactivity, future work should consider them as key regions for further investigation in cannabis cue-reactivity and targeted interventions for reducing hyperactive brain function during cue-reactivity in CUD.

6.3.1.1.4 The Orbitofrontal Cortex

A further contribution of this thesis relates to the role of OFC in cannabis cue-reactivity in CUD. The OFC is considered to play integral role in multiple aspects of SUDs, including cue-reactivity (53). Greater activity in the OFC was consistently implicated in the findings across the three studies in this thesis. In regular cannabis users (Study 1), cue-elicited OFC function was greater in those reporting more problems related to their cannabis use and lower connectivity (with pre/postcentral and superior frontal gyri) in dependent compared to non-dependent users. The OFC was also the most consistent region to be correlated with subjective craving, which was inconsistent with the findings in this thesis. In the first experiment (Study 2), the CUD compared to the control group had greater activation in the OFC during presentation of cannabis cues, and its function was not correlated with subjective craving. Compared to prior work, the correlation between OFC activity and craving was only reported in studies without a non-using control comparison or was not associated with cannabis cue presentation. In similar studies to Study 2 (i.e., non-using control group) that reported cue-elicited OFC activity, there was no correlation with subjective craving, suggesting that differing methodologies may drive findings in OFC-craving correlations. Additionally, in the second experiment (Study 3), cue-elicited activity in the OFC significantly decreased from baseline to follow-up after engagement in three distinct

intervention conditions which all involved a daily task that included a key characteristic of self-monitoring (i.e., reporting of daily cannabis use, subjective craving and mood). Pre-to-post intervention changes in OFC cue-reactivity were not related to those in subjective craving, reflecting a consistent pattern across the literature in cannabis users (Study 1) and Study 2.

The OFC has been shown to be an important brain region for self-monitoring processes (235) in relation to reward processing (i.e., salience attribution; (236-239)) and effortful control (208). Greater OFC function during cannabis cue-reactivity may reflect hyperactivity in people with a CUD, during increased attribution of salience and reward expectation when exposed to cannabis cues. It may also reflect a reduced ability to control/inhibit conditioned responses to cannabis-related cue-exposure as previously demonstrated in both animal and human fMRI studies (206, 208). Taken together, results showing greater OFC activation may reflect a neurobiological vulnerability of CUD in the context of cue-reactivity. However, OFC hyperactivation to cannabis cues may not be related to the conscious experience of craving. Instead it may reflect the habituation of reward evaluation that may be therapeutically impacted by increased self-awareness related to daily cannabis use, cravings, and mood. As such, the OFC should be a region of interest for future studies, as it may be an important neural biomarker for treatment approaches to target cannabis cue-reactivity in severe forms of CUD.

6.3.1.1.5 The Cerebellum

Lastly, the findings from the research in this thesis inform on the neural correlates of cue-reactivity in CUD pertaining to the cerebellum. In Study 2 (Chapter 4), CUD vs controls had greater cerebellar activation while watching cannabis vs neutral images. This is in line with previous (but modest) evidence from the systematic review (Study 1) in regular cannabis

users, as well as in SUDs (i.e., cocaine; (209), alcohol; (210), despite not being explicitly implicated in neuroscientific theory of addiction (53). Notably, the cerebellum is increasingly being recognised as an important part of addiction neurocircuitry (211, 212), with prefrontal-striatal connectivity via direct innervation of the ventral tegmental area (213). The cerebellum is a brain region with high concentration of cannabinoid (CB_1) receptors, to which Δ9-Tetrahydrocannabinol (THC) binds to exert its psychoactive effect (214). The cerebellum is also implicated in prediction processing (i.e., drug-associated cue-memory; (215, 216). Therefore, greater cue-elicited cerebellar activity in CUD may reflect an anticipatory response to cannabis cues (i.e., thinking of future cannabis use) in the absence of cannabis intoxication (217). Future work could test this notion by comparing cannabis intoxication vs abstinence in CUD during cannabis cue-exposure.

6.3.2 Implications for Neuroscientific Models of Addiction for CUD

The findings from this thesis significantly contribute to the research field in the context of validating neuroscientific models of addiction in CUD, as proposed by Volkow and colleagues, *Physiological Reviews*, 2019 (53). Specifically, the theory postulates that with repeated substance use, neuroadaptations occur within limbic-prefrontal brain regions involved in motivation and reward as people become sensitised to drugs and their related stimuli (53, 55, 56). Exposure to such stimuli or "cues" (e.g., substance-related paraphernalia) can be a trigger when drug access is not immediately available or after periods of abstinence. It can elicit a powerful form of craving driven by alterations in brain regions ascribed to the *Preoccupation/Anticipation stage and Interoception stage of addiction.* This stage involves brain regions implicated in motivation and attention (i.e., prefrontal cortex; PFC), cognitive control and reward evaluation (i.e., anterior cingulate cortex; ACC, OFC), response inhibition (i.e., medial prefrontal cortex; mPFC), conditioned learning (i.e., hippocampus), and

interoception/awareness (i.e., insula, precuneus, posterior parietal cortex; PCC; (52). Thus, the findings of the research in this thesis suggest that whilst regular cannabis use may share a common neurobiology with other SUDs, moderate-to-severe CUD may have unique underlying mechanisms related to cue-reactivity.

The findings from the systematic review (Study 1) show that cue-elicited activations in cannabis users are partly similar with users of other substances. However, heterogeneous methodologies make it difficult to generalise the model to individuals with a CUD. The findings of the pre-intervention experiment (Study 2) suggest that CUD shares a pattern of brain functional differences implicated in addiction neurobiology, particularly in the OFC and MFG. However, the robust activation of a region involved in higher order visual processing (i.e., lingual gyrus) and activation in the cerebellum suggests that cue-reactivity associated with more severe CUD also may have a (partially) distinct neural pattern.

6.3.3 Novelty of the Research

6.3.3.1 First Systematic Review of the fMRI Literature on Cannabis Cue-Reactivity

This thesis includes the first systematic review of the evidence on the brain functional correlates of cannabis cue-reactivity. The benefits of the systematic review are, (i) it provided a comprehensive overview of the literature which enabled identification of research gaps in our current understanding and methodological concerns that can be used to improve future work and (ii) it provided answers for some research questions, thereby reducing the need for further investigation and unnecessary use of resources (252). As such, the systematic review conducted in this thesis significantly accelerated our understanding of the neurobiological mechanisms that may be driving cannabis cue-reactivity and provided a clear rationale and methodological guidance for future research.

6.3.3.2 First fMRI study on Cannabis with a Moderate-to-Severe CUD Assessed using DSM-5 Diagnostic Criteria

To date, no known study has examined the neural correlates of cannabis cue-reactivity in a sample of moderate-to-severe CUD assessed using DSM-5 diagnostic criteria. CUD places a considerable treatment burden on the commnuity, as it is associated with higher risk of poor mental health (e.g., psychoses, depression), cognitive deficits (e.g., working memory) and psychical harms (e.g., bronchitis, road crashes; (1). Approximately 10% of cannabis users develop a CUD, of which 47% meet criteria for a moderate-to-severe CUD (5). Only a minority (13%) of individuals with a CUD seek treatment to cut down or cease cannabis use, with most presenting to treatment for problems related to their use (e.g., poor mental health; (253). Further, the relationship between craving and continued substance use despite experiencing harms/desire to quit is considered integral to the maintenance of SUDs including CUD (6, 8, 9). This is reflected in "craving" being added as a criterion for all SUDs in the latest edition of the DSM (i.e., 5th Edition; 6). Differences in functional brain activations to cannabis cues have been previously demonstrated between dependent vs non-dependent samples (86). Therefore, it was unknown whether the reported cue-reactivity differences observed in the fMRI studies to date in regular cannabis users (Study 1) generalised to those who endorse a CUD. Study 2 addressed this issue via examining cue-reactivity with fMRI for the first time in cannabis users with a DSM-5 diagnosis of moderate-to-severe CUD. Therefore, it creates new knowledge about the neurobiology of CUD and informing potential treatment targets to reduce neurobiological reactivity to cannabis cues in those with moderate-to-severe CUD.

6.3.3.3 First Neuroimaging Study of MBI for CUD

This was the first study to date to examine how MBIs reduces cue-elicited brain function in CUD. The study showed that cue-elicited function decreased in all intervention groups not just the brief MBI as hypothesised. This result contrasts previous evidence that MBIs attenuate neural cue-reactivity in smokers (121-123). However, the study results provide world first data on a population of moderate-to-severe CUD, using a community sample of individuals who have tried to cut down or cease their cannabis consumption. Together with the robust methodology (e.g., double-blind, placebo controlled and pseudo-randomised design), the research conducted in this study provides essential information for continued research to expand upon (discussed further in section 3.6 below).

6.3.4 Strengths of the Experimental Study Design

6.3.4.1 Low Risk of Bias

Another strength of this thesis is its use of and adherence to validated risk of bias assessments (Study 1; [254], Study 2 and 3; 248). Evaluating the risk of bias of the employed study designs in this thesis reduced the likelihood that the conduct or design of the studies produced misleading and/or false results. This is important for minimising any risk of harms (e.g., for cannabis users) associated with providing ineffective interventions and through the unnecessary use of resources including monetary funding and oversight of prospective interventions with greater efficacy.

Importantly, the intervention study (Chapter 5) had a low risk of bias as measured by Cochrane Risk of Bias scoring of ‘low’ in all five domains (248). This includes using a double-blind design to reduce any influence of expectancy biases (e.g., demand characteristics). Group allocation was pseudo-randomly assigned with a stratification based on age and sex, and monitored for variance measures of known confounding variables

(education in years, cannabis and alcohol use) to ensure well-matched groups (255). The use of both an active and passive control group is considered "gold standard" for ensuring the credibility of observed treatment effects (i.e., eliminating placebo effects; 129). Whilst a similar study in smokers found MBI associated changes in cue-induced brain activity compared to control, their design did not include an active control (122). Therefore, some of the observed changes may be due to placebo/expectancy effects. For example, if participants are aware they are meant to be reducing use and/or finding cannabis (and its cues) less appealing, their evaluation of the presented cues may be lowered and result in reduced cue-elicited neural activation. The findings of Study 3 (i.e., main effect of time on OFC changes in *all groups*) highlights the importance of employing highly controlled experimental designs, and the robust active-placebo-controlled double-blind design offers a template for continued work in this area.

6.3.4.2 Monitoring of Intervention Uptake and Compliance

The level of acceptability (e.g., uptake and compliance) of an intervention by the targeted population is key to interpreting the clinical significance of its related effects (e.g., reducing neural cue-reactivity) . Whilst there is evidence that MBIs may be effective for reducing substance use, craving, stress and for increasing the rate of abstinence in SUD populations (99, 115), MBIs have also been associated with high levels of attrition (99). The low retention levels of MBIs may be due to distinct MBI parameters, including complexity of instructions, structure (e.g., length in weeks, session duration), format (e.g., individual vs face-to-face, guided vs self-directed) and number of components (e.g., required engagement and homework; for a review see Li et al., 2017; [99] and Cavicchioli et al., 2018; 125). It may be that differing MBI parameters are associated with levels of uptake and compliance, and subsequently MBI-related effects. Indeed, in cigarette smokers, the number of modules

completed in an app-based MBI predicted changes in cue-elicited brain activity (121). As such, an objective quantification of engagement is an important component for MBI studies to enable synthesis and interpretation of this growing body of research. The objective and detailed monitoring of intervention compliance (e.g., number of days the intervention was completed) via an online survey platform (Qualtrics) in Study 2 provided a reliable (compared to self-report) and quantifiable measure of MBI (and control condition) dosage (i.e., number of days completed). This enabled additional analyses to assist in interpretation of the findings (e.g., correlational, group differences). For example, a between group difference was observed in the number days of intervention compliance (i.e., passive control engaged significantly more days than MBI and relaxation groups). As such, intervention compliance was added as a covariate in correlation analyses to control for its potential effects on changes in cue-elicited OFC activity and behaviour variables (e.g., subjective craving and arousal rating of cannabis images). Further, the monitoring of compliance as conducted in Study 3 provides essential information for future synthesis and analyses (e.g., meta-analytic) with future work, which will help to inform on the clinical utility of MBIs for CUD populations.

CUD has been associated with impaired cognitive function (e.g., attention, working memory, IQ), which may limit the acceptability of MBIs for distinct CUD subgroups (255b). Therefore, it is important for CUD interventions that involve psychological strategies (e.g., MBIs) to measure levels of uptake/comprehension (255c). A measure of intervention comprehension was also included in Study 3. It confirmed the acceptability of the two experimental conditions (MBI and relaxation) and that there were no group differences in uptake/comprehension.

6.4 Clinical Implications of Research in this Thesis

The past decade has seen a continuous increase in cannabis use and prevalence of CUD, which constitutes a considerable social burden on health and treatment services (1, 5). The research in this thesis pertaining to the identification of the neural correlates of cannabis cue-reactivity in CUD and examination of how a MBI that targets cannabis craving effects cue-elicited brain activity, has significant clinical implications.

6.4.1 Implications for Cannabis Legalisation on Cue-Reactivity in CUD

The findings from the research in this thesis have implications for cannabis legalisation/decriminalisation. Cue-reactivity presents a unique vulnerability for relapse in SUD populations (229). With global trends towards the legalisation and decriminalisation of medical and nonmedical cannabis, there has been a quantifiable increase in cannabis use (1). Despite the prevalence of a CUD being comparable in medical vs non-medical cannabis users, cannabis legalisation has been associated with more positive views towards cannabis use (i.e., more perceived benefits than harms associated with use; (1, 4). As such, the presence of cannabis cues in the general community, such as commercial outlets, advertisements, public consumption of cannabis may become more prevalent (4). This may have detrimental impact on therapeutic goals (i.e., reducing/ceasing cannabis use) among those who are vulnerable to cue-reactivity, including those with a moderate-to-severe CUD (8, 256).

Whilst treatment interventions for CUD (psychological and pharmacological), may lead to reduced cannabis use in the short-term, it may be that cue-reactivity contributes to relapse without concurrent reductions in underlying functional brain reactivity to cannabis cues (24, 53, 101). As such, cessation strategies that do not specifically target cue-reactivity may leave individuals with a CUD vulnerable to the motivational properties of relevant cues.

6.4.2 Identifying Neurobiological Targets for Attenuating Cannabis Cue-Reactivity in CUD

The findings of the research in this thesis inform the development of interventions designed to alter or minimise neural function associated with cue-reactivity in CUD. Progressing the identification of and/or improving the magnitude and longevity of treatment effects for CUD requires an address of the gaps in knowledge regarding "how" and for "whom" interventions works at the level of the brain (257). Neuroimaging tools, as employed in this thesis, provide a robust methodology for revealing neural mechanisms underlying CUD (e.g., cue-reactivity), as well as potential treatment effects of targeted interventions (e.g., MBIs). This is highlighted by the identification of a unique pattern of brain function (e.g., lingual gyrus) associated with cannabis cue-reactivity in a DSM-5 moderate-to-severe CUD sample (Study 2). The findings from the research in this thesis can be used to inform future work to further identify the neural targets for treatment including analyses of imaging data to predict and monitor outcomes (e.g., longitudinal neural changes, brain-behaviour relationships) and to identify individual specifiers (e.g., demographics, diagnostic profiles) of whom an intervention will be effective (257).

6.4.3 Investigation of Self-Monitoring as an Intervention for Neural Cue-Reactivity in CUD

In the context of CUDs there is very limited neuroimaging research investigating the neural correlates of interventions that target the neurobiology of cannabis cue-reactivity. Study 3 is the first research to date to examine if MBIs are effective for changing cue-elicited brain function in CUD. Whilst our hypothesis that changes in cue-elicited function would decrease in the MBI group only was not supported, our findings suggest that self-monitoring may have therapeutic effects on cue-elicited brain function. Specifically, the daily monitoring

of cannabis use, craving and mood as done in all three groups, may reduce reward identification/expectation (e.g., encoding value) when exposed to cannabis cues, reflected in reduced OFC cue-reactivity. There is no known neuroimaging research informing on neural correlates of self-monitoring practices in the context of CUD or other SUDs. However, there is preliminary support of the notion that self-monitoring may have therapeutic effects (234). A recent descriptive systematic review of the impact of self-monitoring on substance use outcomes found that the act of documenting one's substance use can reduce substance use (234). Self-monitoring was also shown to be more helpful in reducing substance use in non-treatment seekers, such as the sample examined in this thesis (234). Indeed, the significant change in OFC activity in such a brief period of time (~ 2 weeks) in Study 3, suggests the potential effectiveness of simple monitoring practices (e.g., daily use, cravings) on reducing neural cue-reactivity in a non-treatment seeking CUD population. The feasibility of self-monitoring practices has clinical implications for providing low-cost and accessible interventions for managing CUD in both treatment and community settings.

6.4.4 Investigation of Additional Interventions for Neural Cue-Reactivity in CUD

The findings of this thesis also support the investigation of additional interventions that target the neural mechanisms associated with cue-reactivity. Neuromodulation techniques including Repetitive Transcranial Magnetic Stimulation (rTMS), Transcranial Direct Current Stimulation (tDCS), and Deep Brain Stimulation (DBS) have shown promise for SUD treatment (249, 250). For example, a systematic review of 60 studies investigating rTMS, tDCS and DBS for SUDs including CUD, report evidence for effects of neuromodulation on reducing craving and substance use, particularly when stimulation targets the dorsolateral PFC (249), which is implicated in cannabis cue-reactivity (187). In people with CUD and schizophrenia, rTMS in the dorsolateral PFC compared to a sham

condition, showed a clinically significant effect on reducing cannabis craving, improving attention, and supressing increased tobacco use that was associated with cannabis reductions (251).

Further, interventions that aim to directly reduce cue-reactivity, arousal, or attentional allocation may also prove helpful for preventing relapse given the ubiquitous nature of cues in places where it is legalised. Interventions that subjectively devalue cannabis by reducing its expected reward whilst increasing the relative valuations of non-drug rewards may also attenuate neural cue-reactivity (232). Notably, a reduction in cue-elicited amygdala activity has been reported after Cognitive Bias Modification relative to a sham-training control condition, with the reduction significantly correlated with reduced subjective craving scores and arousal ratings of cues (151). Whilst this finding was in alcohol-dependent participants, cue-elicited amygdala activity has also been implicated in cannabis cue-reactivity and subjective craving (Study 1). There has only been one small pilot randomised controlled trial of Approach Bias Modification with cannabis users to date (96). Interestingly, those receiving the active intervention showed blunted cannabis cue-induced craving at the end of training compared to those in the sham-training control condition, though greater reductions in cannabis use were only observed among male participants in the active-condition (152). As such, greater brain function in CUD compared to controls during cue-reactivity (i.e., in the lingual gyrus, MFG, OFC and cerebellum) may diminish with interventions such as neuromodulation, cognitive/approach bias retraining or via cue-exposure therapy (230), and should be considered in future work.

6.5 Limitations and Directions for Future Research

The findings of the research in this thesis indicate that moderate-to-severe CUD is associated with region-specific brain activity in response to cannabis images which may be

associated with cannabis exposure. However, this work had a number of limitations that must be considered when interpreting the thesis findings. This section discusses the methodological limitations that pertains to the conducted research and proposes directions for future research to address these concerns.

6.5.1 Cross-Sectional Study Design

The findings from the experimental research in Study 2 (Chapter 4) relied on a cross-sectional design to examine the neural correlates of CUD and brain-behaviour relationships. However, the cross-sectional design prevents the understanding of whether group differences in cue-elicited brain function constitutes a neurobiological vulnerability for CUD, as previously suggested (227). It could not be established if the observed differences pre-exist cannabis use onset, further exacerbate with continued use, or persist and/or attenuate with varying durations of abstinence from cannabis use. Longitudinal designs are required to elucidate the time course of neurobiological differences associated with cue-reactivity in CUD.

6.5.2 Sample Size (Statistical Power)

6.5.2.1 Study 1: Systematic Review (Chapter 2)

The fMRI literature to date on the neural correlates of cannabis cue-reactivity is limited by a low number of studies using a controlled design. A comparison control group of non-cannabis users included in less than half of the studies included in the systematic review (N = 8 of 18), which limits the ability to confirm the location and direction of the group differences. Further, a meta-analysis of the fMRI literature of cue-reactivity in cannabis users could not be run as only one study (39) met criteria for inclusion in a meta-analysis (i.e., reported all coordinates and utilised a whole brain approach; 258). More research is needed

that employ methods and report details that allow for inclusion in meta-analysis to provide a systematic synthesis of findings to further our understanding of the neurobiology of reactivity to cannabis cues in CUD.

6.5.2.1 Experimental Research: Study 2 and 3 (Chapter 4 and 5)

The sample size in the second experimental study that measured pre-to-post intervention changes in three groups was N = 43). This sample size is considered adequate (e.g., ≥ 40) for detecting large effect sizes, however it may be too small to detect moderate effect sizes (247). Whilst we detected changes in cue-elicited OFC function comparable to a similar study in smokers (122), a larger sample size is necessary to confirm our findings (that had large effect sizes), or to reveal additional changes in other regions (e.g., lingual gyrus, MFG) that may be subtler but detectable in a larger sample.

Further, the sample size included in both of the experimental studies in this thesis limited the number of correlations that could be run with adequate power to reduce the chances of reporting false positives (247). Demographic variables that may have an impact on group differences and/or the effect of MBIs on neural changes, such as sex, as evidenced in cannabis users (87) and smokers (121), could be investigated with a larger sample. Examining other behavioural measures relevant for CUD populations, such as concurrent nicotine use, mental health (e.g., psychotic symptoms) and well-being (e.g., physical activity), cognitive function (e.g., IQ, working memory) and more specific measures of mindfulness (e.g., awareness vs non-judgement) would enable a more comprehensive understanding of how differences and/or changes in brain function relate to behaviour and whether MBIs are more suitable for specific demographics.

6.5.2.1.1 Exposure to Other Substances in CUD Group. Eleven people with a CUD and one control with illicit substance use other than cannabis in the four weeks prior to the

first assessment session and four with a CUD in the days between the first and second assessment sessions were included. Due to COVID-19 related disruptions to recruitment and data collection the current sample size (N = 49 CUD) is smaller than projected (N = 90 CUD). The 11 participants were included conditionally with a sensitivity analysis planned prior to submission for publication. A summary of the participants' demographic (i.e., age, sex) and illicit substance use details (i.e., substance, hours last used prior to assessment, dosage) was provided in the Supplementary Material section of each chapter. As such, the findings of this thesis should be considered preliminary as the results may change post sensitivity analysis and/or in the complete sample.

6.5.3 Examination of CUD Subgroups

Demographic characteristics of subgroups of CUD populations may have exerted an independent or interactive effect on the patterns of brain function associated with cannabis cue-reactivity and brain-behaviour correlations, as well as intervention related effects. Key CUD subgroups are outlined below with considerations for future work to address the potential impact of these variables on neural cue-reactivity discussed.

6.5.3.1 Sex Differences Associated with CUD

The role of sex membership was not addressed in the conducted analyses due to unbalanced male to female ratios and small sample size. There are well established sex (male and female) differences in cannabis use patterns and CUD (1, 259). Cannabis use occurrence is higher in men, however the progression from first use to a CUD is faster in women (259). Women report greater subjective abuse-related effects (e.g., will "take again") in the context of cannabis intoxication, and more intense withdrawal symptoms in the context of abstinence

(260), suggesting a sensitivity to cannabis which may contribute to their vulnerability in developing a CUD (261).

Sex differences have also been established in the neurobiology of cannabis users, with significantly smaller volumes of the OFC and cerebellar white matter in cannabis dependent women (218) and different patterns of cortical thickness between males and females with a CUD (219). In the context of cue-reactivity, few studies have examined sex differences (154). The findings of only two known studies indicates that there may not be any sex differences associated with cue-elicited brain activity, however patterns in cue-elicited brain activity was differentially related to subjective craving based on sex (87, 154). In men, subjective craving was correlated with ventral striatal activity compared to the OFC and insula activity in women (87). In a principal components analysis, PC2, which explains the second greatest source of variance in the data (i.e., brain activity during a cue-reactivity task), was correlated with subjective craving levels which was significantly stronger in women (154).

Sex differences in CUD treatment outcomes have also been observed (260). Motivational and readiness to change factors demonstrate a vulnerability for women (262), as well as CUD severity (e.g., craving and withdrawal) and comorbid psychiatric conditions (260). Interestingly, the effects of a MBI on neural cue-reactivity in smokers demonstrated stronger effects in women (e.g., reduced neural cue-activity correlated with reductions in cigarettes; (121). With consideration of increasing cannabis use amongst women (UNODC, 2022) and lower inclusion of them in cue-reactivity studies (Study 1), accounting for sex differences (i.e., balanced male-female CUD samples) in future work is needed. Not only to understand the underlying neural mechanisms better, but to improve the efficacy of treatment interventions that may require differential approaches (e.g., psychological, pharmacological) and targets (e.g., craving, withdrawal) based on sex (260).

6.5.3.2 Concurrent CUD and Nicotine Use

Nicotine exposure may have had a considerable impact on the neural correlates of cue-reactivity in CUD, even though nicotine dependence was controlled for in the correlation analyses. Concurrent cannabis and nicotine use (i.e., tobacco products), either as cigarettes or in combination (i.e., mixed together with cannabis) is common (263-265), with ~ 25% of the current CUD sample smoking cigarettes and 40% using tobacco in combination with their cannabis use. Nicotine dependence has been reported in 37.5% of individuals with a CUD, with 16.4% of the sample in this thesis also meeting criteria for nicotine dependence. The included non-using control group did not report any nicotine use, therefore comparison between the groups on effects of nicotine (e.g., those with and without use) was not feasible.

The common co-use of cannabis with tobacco products represents a research confound the neural correlates of cannabis cue-reactivity, with the effects of concurrent tobacco use on the underlying neural mechanisms of CUD representing a gap in the current literature (as per review in Study 1; 187). Two studies have shown differential effects on cannabis vs tobacco vs co-use on brain functioning (47, 266). In the context of cue-reactivity in cannabis only users (vs cannabis and cigarette co-users), an interaction was found between cannabis and concurrent cigarette use on cue-elicited brain function (i.e., amygdala, striatum, frontal pole, inferior frontal gyrus), with a positive correlation between cue-elicited activity in the ventral tegmental area and cannabis grams per week (142).

Further, tobacco use may influence CUD treatment outcomes (267-269). Motivational factors underlying single vs co-use, as well as varying pathways progressing to cannabis, tobacco, or co-use may have implications for treatment efficacy and approach (270). Co-use (compared to cannabis only) is associated with more severe CUD symptoms (271), higher incidence of comorbid psychiatric conditions (264), and increased sensitivity to drug reward which may impact reactivity to cue-exposure and subjective craving (142). Notably, the

inclusion of tobacco in cannabis preparations may induce stronger effects of THC, as it can almost double the release of THC into the smoke compared to cannabis alone (272). Despite the added research burden of capturing data and added analyses that pertains to co-using populations, it is warranted due to the implications on treatment outcomes (269).

Conducting future studies that assess CUD and non-using controls matched by tobacco exposure can reduce the potential confounding effects of nicotine from that of cannabis on neural cue-reactivity. Further, examination of the independent and interactive effects of nicotine exposure on the neurobiology of CUD and treatment outcomes could be achieved by following the design employed by Kuhns and Colleagues (2020; 142), (i.e., cannabis and controls with and without tobacco use), but in a moderate-to-severe CUD sample.

6.5.3.3 Motivation/Readiness to Change

In the context of the experimental research in this thesis, levels of motivation/readiness to change may have had an impact on the findings. The measurement of motivation or "readiness" to change has been used to predict outcomes in alcohol and drug treatments with moderate effects (d = 0.41; 246), and demonstrated good concurrent and predictive validity on predicting cannabis use and related problems at 1-, 6- and 12-months follow-up (273, 274). The heuristic was originally developed in the context of behaviour change in general and adapted for addiction (275). Readiness to change is described as a dynamic motivational state influenced by internal (e.g., cognitive, affective) and external (environmental, interpersonal) states (276). Compartmentalised into five stages: i) pre-contemplation, ii) contemplation, iii) preparation, iv) action, v) maintenance, each reflecting a greater likelihood of change (e.g., cannabis use; 275).

More than half of the sample (62.5%) were in the pre-to-contemplation stages reflecting no plan to change their cannabis use (176). As there was very little variance in the data it was difficult to quantitatively determine its effects on neural cue-reactivity and engagement levels in the MBI and should be considered in future work. As brain regions involved in motivation and reward processing are associated with cannabis cue-reactivity, there may be differences in neural cue-reactivity in subgroups of CUD populations at different stages of readiness to change. Further, specific treatment approaches (including MBIs) may be more effective for individuals at different stages of readiness to change (246). Future studies that screen for levels of motivation/readiness to change (to compare and/or control for interactive effects), will help to parse a part any effects on the neurobiology of CUD and treatment outcomes.

6.5.3.4 Treatment Seeking Status

Treatment seeking status/motivation to change use may modulate factors associated with cue-reactivity. Inclusion in the examined CUD sample, required a past attempt to cut down or quit their cannabis use in the past 12-24 months, to reflect a sample motivated to engage in an intervention targeted to manage cravings (274). However, as discussed in the section above, the current sample also reported low levels of motivation/readiness for change (i.e., pre-to-contemplation stages). Treatment seeking substance users report significantly higher stages of readiness to change compared to non-treatment seekers (277). It is possible that the metric used in this thesis for recruiting a sample motivated for change in their cannabis use, does not explicitly reflect a treatment seeking sample, which may have impacted on both the brain-behaviour findings in this thesis due to motivational influences on levels of intervention compliance (i.e., days completed). Further, treatment seeking status/motivation to change use may modulate other factors associated with cue-reactivity

such as perceived availability of /expectancy to use cannabis post-presentation of cues (177, 222-225). Differing characteristics in addition to consumption of substance use have been associated with treatment seeking status (i.e., psychopathology, physical well-being, interpersonal challenges and socioeconomic factors; 278, 253), which may also influence neural cue-reactivity. As such, clear characterisation of treatment seeking status (e.g., explicit endorsement and/or in past 30-days) of CUD samples in future work may reduce potential noise that may be associated with treatment seeking status.

6.5.4 Cannabis Dosage

There is potential that the measurement and inter-individual variability in cannabis dosage associated with THC levels and abstinence duration in the examined CUD sample may have influenced the findings of the experimental studies in this thesis.

6.5.4.1 Measure of THC Levels

The analysis (e.g., urine, saliva, plasma) of cannabis cannabinoids (i.e., THC metabolites) was not included in this thesis. It is unclear if THC levels drove brain function in this study as there is evidence that creatinine-weighted THC ng/ml can affect lingual gyrus activity in cannabis users during fMRI cue-reactivity tasks (80). Detailed data collection of cannabis dosage, frequency, strength, method of consumption and combination with tobacco products (including ratios), as well as lifetime patterns of use (i.e., age of first use, age of onset of regular use, periods of abstinence) was conducted. However, some cannabis users may include those using more potent and addictive cannabis varieties with high level of THC and low level of CBD with known distinct properties on brain function (147). Also, people with more severe CUDs can use more potent cannabis products with greater THC levels due to tolerance (228), which may in part be driving neural cue-reactivity. Measurement and

analysis of THC metabolites and levels is required in future studies to understand the relationship with brain functional alterations in CUD. This will also inform psychopharmacological intervention and public health legislature with global trends towards the legalisation of cannabis.

6.5.4.2 Duration of Abstinence from Cannabis

Duration of abstinence from cannabis prior to assessment was variable among the examined CUD sample (i.e., mean 22.5 ± 13.4 hours; range 12 hours to 3 days); and may have influenced indices of neural (cue-elicited brain function) and associated behavioural (craving, withdrawal, affective states) cue-reactivity. Indeed, acute abstinence from cannabis has been related to increased subjective craving and withdrawal symptoms (226), suggesting that abstinence periods may have influential effects on neural cue-reactivity and subjective craving. Withdrawal symptoms were minimal in the examined CUD sample, which may explain the lack of correlation with abstinence duration or withdrawal symptoms on neural cue-reactivity. However, investigating varying levels of abstinence durations (e.g., longer follow-up time points of ≥ 3, 6, 12 months) can provide a greater understanding of the role abstinence duration on functional brain and behaviour indices during cue-reactivity in CUD.

6.5.5 Ecological Validity of Cannabis and Neutral Cues

The cannabis and neutral cues presented in the fMRI cue-reactivity task employed in the experimental studies to measure neural cue-reactivity may have lacked ecological validity. Whilst our cue-reactivity task was shown to increase subjective craving, we did not find a correlation between any of the activity in brain regions implicated in cue-reactivity and subjective craving. This is consistent with previous studies of cannabis users (81, 141), but inconsistent with others that reported significant correlations (85, 86). The cannabis

compared to neutral images in our cue-reactivity task were rated higher in levels of subjective arousal and affective valence which is considered a reliable measure of stimulus validity (279). However, the laboratory context in which neural cue-reactivity is measured may impact on the experience of cue-elicited craving and subsequent neural reactivity.

Specific contexts and routine use are also key stimuli for cue-reactivity (8, 223), and different patterns of cue-elicited brain function have been reported in studies that employed different types of cannabis cues (visual, olfactory, tactile), as reviewed in Study 1. Thus, it is possible that for some participants, specific cues elicit craving more than others (80, 85). Future work which includes different types of cannabis cues including sensory, affective states, routine (e.g., before sleep) and environmental contexts (e.g., social) will help to delineate the relationship between neural and behavioural cue-reactivity and treatment approaches that are relevant for individual susceptibility for specific cues.

6.5.6 Intervention Considerations for Future Research of MBIs for CUD

6.5.6.1 MBI Duration

The duration (i.e., number of days) of the MBI may not have been long enough to reduce cue-elicited brain function in moderate-to-severe CUD. The long-term effects of MBIs on both neural cue-reactivity and behaviour (i.e., prolonged abstinence) has yet to be determined. Longer MBIs (i.e., 10 weeks), have been shown to reduce cue-elicited brain function and cigarette consumption in smokers, however they did not conduct further follow-up assessments to assess abstinence rates (122). The findings of Study 3 in this thesis supports the investigation of longer mindfulness-based "urge surfing" relapse prevention strategies (105, 110). A longer MBI may reduce prefrontal and lingual gyrus hyperactivation to cannabis cues, as it is may take longer than a 2-week MBI to strengthen top-down executive control including attention (231) in moderate-to-severe CUD. Future work

examining longer MBIs with follow-up assessments at different time-points (e.g., 3-, 6-, 12-months) are needed to test this notion and inform on clinical utility for abstinence.

Further, whilst we monitored and supported compliance of daily intervention tasks, the participants in the MBI and relaxation group engaged significantly less than the no intervention group, with an average of ~ 8 days. As previously discussed, it is possible that greater compliance (i.e., 14 days) or a longer intervention may have shown effects of mindfulness on cue-elicited brain function, as observed in smokers (122). The current sample's low level of motivation to change cannabis use may have been reflected in the lower compliance rates with the experimental tasks in the MBI and relaxation groups. Future studies screening for cannabis users who are in the action stage (i.e., score > 8 on Marijuana Ladder) may observe greater engagement with the experimental tasks in the MBI and relaxation conditions, and subsequently reveal a more definitive intervention effect on the brain (121).

6.5.6.2 Can Self-Monitoring of Daily Cannabis Use and Cravings Reduce Neural Cue-Reactivity in CUD?

All three groups were asked to complete a daily questionnaire (~3-minutes) which provided data on daily intervention compliance (objective and self-report), subjective craving levels, cannabis use (occasions and quantity), risk behaviour (e.g., dangerous use) and mood. This data was collected to support interpretation of intervention effects, identify potential confounders and to minimize discernment of allocation to the passive no intervention control group. A descriptive systematic review of the impact of self-monitoring on substance use outcomes found that the act of documenting one's substance use can have an effect on reducing substance use (234). As such, the self-monitoring conducted by completing the daily questionnaire may be considered a methodological limitation in Study 3, as it may have

brought conscious awareness to automatic behaviours (244, 231). Future studies that use the same interventions in this thesis without daily monitoring (e.g., monitor at the end of the intervention via TLFB methods), or include self-monitoring practices as a defined experimental condition, could prove useful to unpack monitoring vs intervention effects. Additionally, a waiting list could be a suitable control to tease apart the effect of monitoring.

6.5.7 fMRI Data Analyses

Analyses approach for fMRI data is driven by research aims (280). Exploratory aims may start with a whole brain voxel wise analysis, whereas ROI analysis may be logical for certain theory driven hypotheses of specific areas that could be involved (280). However, research is done not only to confirm theories but also expand them, therefore a whole brain analysis may provide information that may not have been expected. Further, analyses of region-specific vs network circuitry are also important to answer specific research questions (280). The fMRI analyses employed to address the aims of the research in this thesis are discussed below in the context of how future work could employ additional fMRI analyses approaches to further the understanding of the neurobiology associated with cue-reactivity and MBI-related effects in CUD.

6.5.7.1 Whole Brain and ROI Methods

The use of whole brain and/or ROI analyses method varied within the fMRI literature on cue-reactivity in cannabis users and may have contributed to the heterogeneity in findings across the reviewed studies (Study 1). The whole brain fMRI analysis method conducted in Study 2, enabled an exploratory approach that was appropriate due to the novelty of a moderate-to-severe CUD sample. However, future work using an ROI approach may be able to detect more subtle differences in subcortical regions (e.g., striatum) where signal can be difficult to detect due to the location deep in the cortex and high iron concentration (281).

In contrast, an ROI approach was conducted in Study 3 based on previous findings that specific regions are consistently associated with cue-reactivity in CUD in Study 2. As such, an ROI approach was appropriate to address the hypothesis. However, neural changes associated with MBIs that targets subjective cannabis craving may happen in additional brain regions and circuitry associated with addiction and mindfulness training (113), that could be revealed using other exploratory analyses such as whole brain analyses or ROI analyses of mindfulness-specific regions/networks.

6.5.7.2 Functional Connectivity and Network Circuitry

A greater understanding of the neurobiology of CUD in the context of cue-reactivity may be achieved by examining patterns of brain function at a network level. Many brain regions are interconnected and form dynamic networks that integrate to enable specific functions (e.g., reward, self-regulation, perception, attention; (56). There is evidence to suggest that cue-reactivity in cannabis users is not only associated with differences in isolated regions but also with network-level alterations between regions (86, 134, 138). As such, region-specific activation patterns in Study 2 may be interconnected with each other and/or additional networks, which may be important targets for CUD treatment.

Similarly, MBIs have been associated with changes in cue-elicited functional connectivity in smokers (122) and brain networks including the executive control, salience, and default networks (282-284). Therefore, examining the functional connectivity of cue-elicited brain activations and network circuitry is warranted. Functional connectivity analyse may reveal additional underlying mechanisms that are associated with CUD treatment effects. For example, observed changes in the OFC in Study 3 may have been driven by connectivity with other regions.

6.6 Summary and Conclusion

The research conducted in this thesis has significantly contributed to the understanding of the neurobiology of cue-reactivity in CUD and of how a brief MBI targeting craving reduces greater brain function during cue-reactivity. This was achieved by conducting the first systematic review of fMRI cue-reactivity literature in cannabis users to date. The review informed the two experimental studies, with specific aims examining whether:

Aims of Experiment 1:

i) Patterns of brain function associated with cannabis cue-reactivity generalise to individuals with a moderate-to-severe CUD compared to a non-using control group.
ii) Patterns of cue-elicited brain function relate to behavioural indices associated with CUD (e.g., subjective craving, cannabis exposure, mental health).

Aims of Experiment 2:

i) Cue-elicited brain function in individuals with a moderate-to-severe CUD can be changed pre-to-post a brief MBI compared to both an active (relaxation) and passive (no intervention) control condition.
ii) Changes in cue-elicited brain function are associated with changes in behavioural indices associated with CUD (e.g., subjective craving, cannabis exposure, mental health).

The two experiments performed in this thesis led to the identification of the following key findings:

Key Findings of Experiment 1:

i) In moderate-to-severe CUD compared to controls, hyperactivation to cannabis (vs neutral) cues was detected *FWE*-corrected in the lingual gyrus ($p < .05$, $k > 10$). Uncorrected results show greater activity in the MFG, the medial OFC, and the cerebellum ($p < .001$, $k > 10$).

ii) Greater MFG activity correlated with higher consumption of cannabis grams in the past month.

Key Findings of Experiment 2:

i) A significant decrease in the activity of the OFC was observed in all three intervention groups from baseline to follow-up. In relation to our hypothesis, this indicates a null finding since the reduction in OFC activation cannot be attributed solely to the MBI.

ii) A decrease over time in subjective craving and arousal rating of cannabis images was also observed across all three groups, however these changes were not related to changes in OFC activity.

The data emerging from this thesis indicate that moderate-to-severe CUD may reflect common neurobiological correlates of cue-reactivity across SUDs (i.e., alcohol, nicotine, opioids, cocaine; (229). In line with this notion, cue-induced activation in reward processing regions suggests that CUD does share a pattern of brain functional differences implicated in addiction neurobiology (53). However, the robust activation of the lingual gyrus suggests that cue-reactivity associated with more severe CUD may have a partially distinct neural pattern, which may be related to cannabis exposure levels (i.e., THC, number of grams consumed).

As such, the findings provide preliminary focus for the development of interventions that target cue-reactivity in these regions and/or pathways.

Additionally, the findings from the research in this thesis demonstrates that altered brain function in the OFC associated with cannabis cue-reactivity in CUD can be reduced during a brief engagement of MBI, relaxation and/or monitoring of daily cannabis use, cravings and mood. However the specific intervention effects that drove changes in brain function remain unclear. Future work in a larger treatment seeking sample using additional analyses approaches (e.g., whole brain, functional connectivity), control groups (e.g., waitlist) and varying intervention lengths (e.g., longer) is required to test this further.

Globally, cannabis use is on the rise despite being associated with a range of adverse psychosocial outcomes including a CUD. As such, ongoing research should continue to examine both current and novel treatments (pharmacological, psychological) that can 'normalise' patterns of brain function associated with cannabis cue-reactivity in efforts to improve health outcomes for CUD.

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Appendices

Appendix A. Publication: Statement of Contribution by Others

The following statement of contribution is made regarding Chapter 2 (Study 1) of this thesis, which was published as:

First author: Hannah Sehl
I acknowledge that my contribution to the above paper is 70%.
Extent of contribution: H.S. 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: 18/08/2022

Second author: Gill Terrett
I acknowledge that my contribution to the above paper is 4%.
Extent of contribution: G.T. was involved in the conceptualisation of the work.

Signature: 

Date: 18/08/2022

Third author: Lisa Greenwood
I acknowledge that my contribution to the above paper is 5%.
Extent of contribution: L.G. provided feedback on manuscript drafts.

Signature: 

Date: 18/08/2022

Fourth author: Magdalena Kowalczyk
I acknowledge that my contribution to the above paper is 2%.
Extent of contribution: M.K. provided proofreading on manuscript drafts.

Signature: 

Date: 18/08/2022

Fifth author: Hannah Thomson
I acknowledge that my contribution to the above paper is 2%.
Extent of contribution: H.T. performed re-screening of search results to ensure accuracy of included/excluded articles.

Signature: 

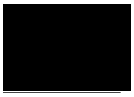
Date: 18/08/2022

Sixth author: Govinda Poudel
I acknowledge that my contribution to the above paper is 2%.
Extent of contribution: G.P. provided feedback on manuscript drafts.

Signature: 

Date: 18/08/22

Seventh author: Victoria Manning
I acknowledge that my contribution to the above paper is 5%.
Extent of contribution: V.M. provided feedback on manuscript drafts.

Signature: 

Date: 18/08/2022

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:18/8/2022

Citation:

Sehl, H., Terrett, G., Greenwood, L. M., Kowalczyk, M., Thomson, H., Poudel, G., . . . 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. doi:10.1007/s00213-021-05973-x

Appendix B. Publication: PROSPERO Pre-Registration

PROSPERO
International prospective register of systematic reviews

Systematic review

Please complete all mandatory fields below (marked with an asterisk *) and as many of the non-mandatory
fields as you can then click *Submit* to submit your registration. You don't need to complete everything in one
go, this record will appear in your *My PROSPERO* section of the web site and you can continue to edit it until
you are ready to submit. Click *Show help* below or click on the icon
to see guidance on completing each section.
This record cannot be edited because it has been rejected

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.

Patterns of brain function associated with cue-reactivity in regular cannabis users: A systematic review of
fMRI studies

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.

18/02/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

31/08/2021

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 Yes 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 Sehl

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.sehl3@myacu.edu.au

8. Named contact address

Give the full postal address for the named contact.

Australian Catholic University, 115 Victoria Parade, Fitzroy VIC 3065

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

0406872029

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:

https://www.acu.edu.au/

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 Sehl. Australian Catholic University
Dr Valentina Lorenzetti. Australian Catholic University
Ms Alexandra Gorelik. Australian Catholic University
Assistant/Associate Professor Gill Terrett. Australian Catholic University
Miss Hannah Thomson. 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.

Which patterns of brain function are implicated during fMRI cue-reactivity tasks (i.e., viewing cannabis vs
control stimuli) in i) regular cannabis users, and ii) regular cannabis users compared to controls?
Is there an association between patterns of brain function implicated in the fMRI cue-reactivity tasks (i.e.,
viewing cannabis versus control stimuli) in cannabis users and behavioural measures? These include the
severity of cannabis use (e.g., dependence, craving, dosage), mental health (e.g., psychopathology symptom scores) and cannabinoid levels (e.g., THC, CBD) as measured by analyses of hair, urine, saliva, or
breathsamples.

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.)

- Datae boaf sseesa: rSchceosp:u 2s,7 PAupbrMil 2e0d2, 0PsycINFO

- Search terms include ("cannabis use disorder" OR cannabis OR marijuana) AND (fMRI OR "functional
magnetic resonance imaging" OR MRI OR "magnetic resonance imaging" OR "brain activity" OR "brain
function" OR connectivity OR "neural activity") AND ("cue-reactivity" OR "cue-salience" OR craving OR
reward OR sensitization)
- Database searches will be re-run just before the final analyses is conducted and any further studies identified will be included.
- References of primary studies and review articles meeting the inclusion criteria will be searched manually
to identify further eligible studies.
- Unpublished studies will not be sought.

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.

Regular cannabis use. Patterns of brain function during a fMRI cue-reactivity task (i.e., viewing cannabis
versus control stimuli) in i) regular cannabis users, and ii) regular cannabis users compared to controls.

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.

I-n Icnlduisviiodnu:als who regularly use cannabis (as defined by each study criteria).
- Participant's aged 13 and 66 years.

Exclusion:
- Participant's 14 years and 65 years.
- Co-morbid diagnosis of a mental health disorder (except anxiety and depression)
- Co-morbid substance use disorder or dependence (except cannabis and tobacco).

- Co-morbid neurological disorders (e.g., Epilepsy, Multiple Sclerosis)
- The primary substance of use was not cannabis (e.g., cocaine, methamphetamines)

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the nature of the interventions or the exposures to be reviewed.

All publications included in this systematic review will have conducted an fMRI scan on all participants to
acquire measures of cue-induced brain function. During the fMRI scan all participants will be exposed to a
cue-reactivity task which includes the presentation of both cannabis and control stimuli. Examples of cannabis stimuli include cannabis in various forms (grass, hash, plant), apparatus for use (bongs, joints,
grinders, rolling papers, lighters), cannabis being prepared and consumed, etc. Examples of control stimuli
include neutral (nature scenes, stationary, etc.) and/or reward (food, sex, etc.).

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.

Control stimuli. The fMRI cue-reactivity task must include both cannabis and non-cannabis/control stimuli.

Any study that does not include control stimuli will not be included.

Control groups.1) Non-cannabis users; 2) other cannabis usering groups (e.g., irregular cannabis users).

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.

For the systematic review: Studies will be included if they met the following eligibility criteria:
- The full-text was published in the English language;
- Peer reviewed
- Used only human participants;
- Measure brain function using fMRI;
- Include the use of a fMRI cue-reactivity task comparing cannabis versus control stimuli

Articles will be excluded that:
- Used a single case-report, book chapter, or conference abstract only;
- Were reviews or meta-analyses of the literature;
- Used other imaging techniques (e.g., EEG, CT, PET, SPECT, structural neuroimaging).

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.

The main outcome is brain function measured during a fMRI cue-reactivity task. Specifically this will be
measured by contrasting brain function during the presentation of cannabis stimuli versus that of control
stimuli for the following samples: 1) within regular cannabis users and 2) between regular cannabis users
compared to controls (e.g., non-cannabis users and other cannabis using groups such as irregular users).

* 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.

Brain function will be measured isung the following parameters: strength (e.g., Beta/*t*), extent (e.g., cluster
size), and location (e.g., brain areas, MNI coordinates).

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

Associations between patterns of brain function implicated in the fMRI cue-reactivity task (i.e., viewing cannabis versus control stimuli) and behavioural variables, such as the level of cannabis use (e.g., dependence, craving, dosage), mental health problems (e.g., psychopathology symptom scores) and cannabinoids (e.g., THC, CBD).

These will be examined within in i) regular cannabis users, and ii) other cannabis using groups such as
irregular users.

* 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).

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 the PRISMA guidelines (Moher, Liberati, Tetzlaff, & Altmann, 2009).

To determine which studies will be included, two individuals (blinded to each others decisions) will screen

titles and abstracts and the resultant full-texts articles that have not been excluded and select studies for
inclusion in the review. Disagreements between individual judgements will be resolved via discussion with a
senior staff member.

The following data will be extrcted:

Study characteristics:
- First author.
- Year of publication.
- Recruitment strategy.
Participant characteristics:
- Sample size
- Age
- Sex
- Treatment status
- 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 speciman (e.g., urine, saliva, hair, breath)
- Other substance use (e.g., tobacco, alcohol)
- Psychopathology (e.g., symptom scores)
- Recruitment location
Cue-reactivity fMRI :
- Task developer and version
- Task parameters (e.g., task and stimuli duration, type and number of stimuli, block/event-related design,
ISI, number of runs)
- Acquisition parameters (e.g., TR, TE, GFE, FOV, matrix)
- Analysis method (e.g., whole brain, ROI, seed-based)
- MRI scanner strength/manufacturer
- Head coil (number of channels)
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., peak voxel coordinates in MNI space), and extent (e.g.,
cluster size). For consistency, for all studies where peak voxels were originally reported in Talairach coordinates, we will use the Brainmap.org software (icbm2tal; http://www.brainmap.org/icbm2tal/) to perform
a nonlinear transformation of Talairach coordinates to MNI space.

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 given there is enough power (i.e., a sufficient number of studies) and will use
a quantitative, random-effects meta-analytic method known as activation likelihood estimation (ALE; brainmap.org/ale) implemented in the software GingerALE 2.3.6. to synthesise coordinates.

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-analysis will be performed on associations between brain function during the
cue-reactivity task and key variables (e.g., sex, IQ and/or education, age, and 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 the 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.

A paper will be submitted to a leading journal in this field.

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.

Systematic review; meta-analysis; cannabis; cue-reactivity; cue-salience; fMRI; brain activity.

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).

This field should be left empty until details of the completed review are available.

Give the link to the published review.

Appendix C. Ethics Approval

From: Kylie Pashley on behalf of Res Ethics
To: Valentina Lorenzetti
Cc: Bernardo Jarrin; Res Ethics
Subject: 2019-71H Ethics application approved!
Date: Monday, 10 June 2019 1:27:21 PM

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

THIS IS AN AUTOMATICALLY GENERATED RESEARCHMASTER EMAIL

Appendix D. ISRCTN Study Registration

http://www.isrctn.com/ISRCTN76056942

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.

<u>OUTCOMES</u>

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 utilized 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.

OVERALL STRUCTURE OF THE ASSESSMENT AND INTERVENTION PROTOCOL

1. THREE MAIN PHASES

The testing protocol comprises three main phases:

1.1 Face-to-face baseline assessment, ~4 hours (here on referred to as 'baseline assessment')

1.2 ~2-week daily off-site, online intervention, ~10-15 minutes daily

1.3 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, 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).

PLAIN ENGLISH SUMMARY

Aims

This randomised, double-blind, placebo-controlled study aims to examine (i) the brain, cognitive and mental health correlates of moderate-to-severe cannabis use disorder compared to non-cannabis use, and (ii) how these correlates change with a brief mindfulness intervention relative to an active control relaxation intervention, and to no intervention. The intervention has been successfully tested in hazardous drinkers by Co-Investigators Professor Kamboj and Dr Freeman (please see PMID: 29016995).

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

Appendix E. Participant Information Letter and Consent Form

CUD Group Example:

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, Ms Kelly Van Egmond
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 Student & 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

BASELINE CONSENT FORM

One Copy for Researcher and Participant

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, Miss Kelly Van Egmond

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:

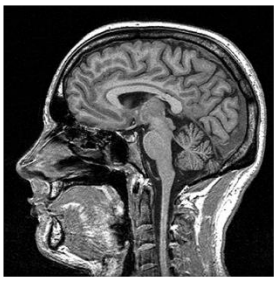
SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR): …….. DATE:

SIGNATURE OF STUDENT RESEARCHER: ……..... DATE:

Appendix F. Advertisement Flyer for CUD Group

ACU
AUSTRALIAN CATHOLIC UNIVERSITY

PLEASE NOTE: **our face-to-face data collection sessions are run in accordance with the most recent social distancing guidelines.**



Calling CANNABIS USERS

This research may interest you!

Research Volunteers Needed

We are seeking volunteers to take part in a study to map brain changes over time.

We are looking for people:

* Aged **18-55** years
* **Regularly use cannabis**
* Tried to cut down or quit cannabis at least once in the last 2 years
* Fluent in English
* Normal or corrected vision
* Willing to participate in **two testing sessions 2-weeks apart,** including a **MRI** brain scan, **cognitive tasks,** and **questionnaires** related to your experience with cannabis
* Willing to practice brief instructions for **10 minutes daily for two weeks**

Study Name: Mapping short-term brain changes in cannabis users: An fMRI study
Project ID: 2019-71H

All participants will receive:

- Reimbursement for your time
- A copy of your brain scan

If you have any questions, feel free to contact us on 0490391342 or cannabis@acu.edu.au

Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab

Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab
Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab
Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab
Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab
Research volunteers needed
Follow this link ☐ https://cutt.ly/IkBdFe6
NB: Contact details on reverse side of tab

If interested in being a part of our study please complete our online survey, either by

- scanning the QR code

or

- using the link below (or in the tear off tab)

https://cutt.ly/IkBdFe6

Appendix G. Phone Screening Interview Script

Group ☑ Cannabis
☐ Control

Screening Number _____
Initials _____
(person making screening decision)

Date ___ /___ /___ (online screening decision)

☐ Male
☐ Female

Age _____

☐ 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

v1.28_October_2021, Cannabis

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; 0490 391 342.

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	Duration/ other questions
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*		……

……
……
……
……
……
……
……
……
……
……

			2. Monthly **1.** Less than monthly	**0.** None in the past 3 months	**0.** None in the past 3 months		years ago	
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 H: MRI Data Acquisition Parameters

\\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

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

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

\\USER\Valentina\new_protocol_use_this\Brain_cann\A-P_V2_CR_Task_2_ep2d_p2_3mm_gap

TA: 8:37 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	40
Dist. factor	10 %
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	2240 ms
TE	30.0 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	HEA;HEP

Contrast - Common

TR	2240 ms
TE	30.0 ms
MTC	Off
Flip angle	80 deg
Fat suppr.	Fat sat.

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	227
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

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	40
Dist. factor	10 %
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	2240 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

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	2240 ms
Concatenations	1

BOLD

BOLD

Meas[18]	Active
Meas[19]	Active
Meas[20]	Active
Motion correction	Off
Spatial filter	Off
Measurements	227
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

Appendix I: Intervention Scripts

PART A – Administered at Baseline

TRACK 1/3 (A,B) 3 MIN 40 SEC	
Mindfulness **TRACK 1_A**	**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	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]	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]	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]	**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.**

Expectancy/credibility questionnaire about 3 mins	
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	[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

5 seconds]. **Notice sensations** in other parts of your body. **[pause 5 sec]**	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. *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].*	*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].*
As you keep this image in mind you, may notice some craving or urges to smoke. [2 sec] As you **notice** these feelings, focus	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

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]**	**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**

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]	**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 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].	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 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	*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].	*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**	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**

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.**	**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.**	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 **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].	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.*** ***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 J. Permission to Reproduce CUD Criteria from the APA

RE: Request to Obtain Permission from APA/APP. Permission Request Reference ID: PL19021

Robin Allan <RAllan@psych.org> 23 August 2022 at 04:43
To: Hannah Sehl [redacted]
Cc: permissions <permissions@psych.org>

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