The Neurobehavioral and Psychophysiological Mechanisms of Cannabidiol for Alcohol Use Disorder

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Thesis Abstract

Alcohol use disorder (AUD) is characterised by compulsive alcohol seeking despite negative consequences. Although AUD is associated with a large proportion of the total global disease burden, there remains a paucity of effective medications available for this indication. This is in part due to the heterogeneity of AUD and the need pharmacological agents that are effective through various mechanistic pathways to address diverse alcohol-induced neurobiological dysregulations that precipitate and maintain the disorder.

Cannabidiol (CBD), is a promising novel pharmacotherapeutic agent that has been suggested by preclinical literature to have potential to treat AUD; however, relatively little research has been conducted in human samples with AUD. This thesis addresses this gap through the synthesis of previous literature examining the effects of CBD on neurobiology in healthy human samples and subsequently presents a proof of concept cross-over double-blind randomised controlled trial of subacute 800mg/day dosing of CBD versus placebo. The data from this research is then considered in three separate empirical chapters in which CBD-mediated effects on psychophysiology, regional brain activity, and neurometabolites are detailed.

Specifically, Chapter 4 demonstrates that CBD administration is associated with elevated parasympathetic nervous system (PNS), reductions in self-report anxiety during cue exposure stages and improved self-report craving recovery following cue exposure relative to placebo sessions. Chapter 5 demonstrates that CBD administration is associated with the hypoactivation of the precuneus, a region associated with cue-induced alcohol craving, during control and alcohol cue exposure. Chapter 6 demonstrates that CBD administration is associated with the restoration of Glutamate (Glu) and γ-Aminobutyric acid (GABA) and significant increases in glutamine + glutamate (Glx) concentrations in the dorsal anterior

cingulate cortex relative to placebo for individuals who consume alcohol the previous day compared to those that do not.

Together, results from these three empirical chapters which consider three interdependent biological systems, provide sufficient evidence to discuss the potential therapeutic mechanisms of CBD in the AUD indication and provides preliminary signs of efficacy for the use of CBD in the treatment of AUD. In Chapter 7, these preliminary signs of efficacy are proposed to be driven by CBD anxiolytic-like effect that may be particularly suited to target tension reduction motivations to consume alcohol by reducing high arousal states and ameliorating negative affect.

In conclusion, this body of work contributes to the literature that considers the potential role of CBD in the treatment of AUD and provides valuable insight into the mechanisms of CBD. Subsequently this paves the way for a much-needed large parallel randomised controlled trial to further understand CBD-associated therapeutic mechanisms and establish efficacy of CBD to improve treatment outcomes for those with AUD.

Preface

All empirical chapters (2-6) were written for publication and have either been accepted (Chapter 2), accepted with revisions (Chapter 3) or have been submitted for publication in peer-reviewed scientific journals (Chapters 4-6). For consistency, each chapter has been reformatted to abide by APA 7 format throughout this thesis. Contributions are outlined as per below.

Chapter 2

Published as: *Hurzeler, T., Watt, J., Logge, W., Towers, E., Suraev, A., Lintzeris, N., ... & Morley, K. (2024). Neuroimaging studies of cannabidiol and potential neurobiological mechanisms relevant for alcohol use disorders: a systematic review. Journal of Cannabis Research, 6(1), 15.*

I (T.H.) designed the study, performed the search and wrote the manuscript. The risk of bias assessment was conduced by J.W. and K.M. K.M. is my supervisor and was the senior researcher. All authors edited the manuscript and approved the final version for submission.

Chapter 3

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I (T.H.) developed the study protocol with contributions from W.L., K.M. and P.H. with regards to study design, methodology and analytic design. All authors edited and read the manuscript and approved the final version for submission.

Chapter 4

Submitted for publication: *Hurzeler, T., Logge, W., Watt, J., DeMayo, M., Suraev, A., McGregor, I., Haber, P., & Morley, K*

I (T.H.) coordinated the study and completed the data collection, extracted the data, conducted data analysis and wrote the manuscript. W.L. supervised the analytic design. P.H. provided clinical oversight and site governance. J.W. was the site physician. K.M. was the senior researcher for the study and provided overall supervision. All authors contributed to manuscript editing, provided guidance and approved final version for submission.

Chapter 5

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I (T.H.) coordinated the study and completed the data collection, extracted the data, conducted data analysis and wrote the manuscript. W.L. supervised the analytic design. P.H. provided clinical oversight and site governance. J.W. was the site physician. K.M. was the senior researcher for the study and provided overall supervision. All authors contributed to manuscript editing, provided guidance and approved final version for submission.

Chapter 6

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I (T.H.) coordinated the study and completed the data collection, extracted the data, conducted data analysis and wrote the manuscript. M.D. supervised the methodology and supervised the analytic design. W.L. contributed to the analytic design. P.H. provided clinical

oversight and site governance. J.W. was the site physician. K.M. was the senior researcher for the study and provided overall supervision. All authors contributed to manuscript editing, provided guidance and approved final version for submission.

Statement of Originality

I certify that, to the best of my knowledge:

1. The content presented in this thesis is my original work and has not been submitted for any other degree or purposes. The intellectual content of this thesis is my own and, as indicated in the preface, any assistance provided by other authors have been duly acknowledged.
2. This thesis contains fewer than 100,000 words, exclusive of figures, tables, footnotes, references, and appendices.
3. Due acknowledgement has been made for all other material used.

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Tristan Hurzeler | July 03, 2024

Student ID: 430194247

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

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Kirsten Morley | July 03, 2024

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To my fellow PhD students and colleagues on level 6 at the DHS, thank you. During the first few years of my candidature, I felt completely isolated, COVID-19 limited our access to the DHS, making it a very lonely period. Eventually, I can't remember exactly when, the reopening of the DSH brought with it a community of new PhD candidates and postdocs. Although many of my days were spent with headphones on, stressing over the words on the screen, your presence was calming, and all our little chats made things so much easier. Thank you all for your help and friendliness; I hope to repay all the kindnesses in the coming few years.

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List of Abbreviations

5-HT	5-Hydroxytryptamine (Serotonin)
ACC	Anterior Cingulate Cortex
AEA	Anandamide
AIHW	Australian Institute of Health and Welfare
A1R	Adenosine Receptor
ANS	Autonomic Nervous System
ArLD	Alcohol-Related Liver Disease
AUD	Alcohol Use Disorder
BART	Balloon Analogue Risk Task
BOLD	Blood Oxygenation Level Dependent
CAN	Central Autonomic Network
CBD	Cannabidiol
CB	Cannabinoid Receptor
CCT	Columbia Card Task
CEN	Central Executive Network
Cho	Choline
CONSORT	Consolidated Standards of Reporting Trials
CR	Creatine
CNS	Central Nervous System
DBRCT	Double-Blind Randomized Controlled Trial
DMN	Default Mode Network
DSH	Division of Systems Health
EAATs	Excitatory Amino Acid Transporters
ECS	Endocannabinoid System
EF	Executive Function
EPI	Echo Planar Imaging
FAAH	Fatty Acid Amide Hydrolase
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
Glx	Glutamine + Glutamate
GSH	Glutathione

GSR	Galvanic Skin Response
HF-HRV	High-Frequency Heart Rate Variability
HERMES	Hadamard Encoding and Reconstruction of MEGA-Edited Spectroscopy
MI	Motivational Interviewing
MRS	Magnetic Resonance Spectroscopy
NAA	N-Acetylaspartate
NAc	Nucleus Accumbens
NMDA	N-Methyl-D-Aspartate
PFC	Prefrontal Cortex
PNS	Parasympathetic Nervous System
PRESS	Point-Resolved Spectroscopy
PTSD	Post-Traumatic Stress Disorder
RSA	Respiratory Sinus Arrhythmia
SCL	Skin Conductance Level
SD	Standard Deviation
SN	Salience Network
SNS	Sympathetic Nervous System
TGA	Therapeutic Goods Administration
THC	Tetrahydrocannabinol
TMT	Trail Making Test
TN	Triple Network
TRPV	Transient Receptor Potential Vanilloid
VAS	Visual Analogue Scale
VTA	Ventral Tegmental Area

Chapter 1: General Introduction

1.1 Alcohol Use Disorder

Alcohol use disorder (AUD) is a chronic relapsing disorder, particularly when moderate to severe, and is characterised by compulsive alcohol-seeking and consumption despite severe negative repercussions to both physical and mental health (Haber, Riordan, & Morley, 2021). The consequences of AUD in terms of clinical and social outcomes are major global public health concerns. Harmful alcohol consumption alone is responsible for around 5.3% of the global burden of disease (Griswold et al., 2018; World Health Organization, 2018, 2019, 2021). This burden is approximately five times greater than the combined global disease burden of all other illicit drug use (Degenhardt et al., 2013). Additionally, the global rate of AUD is estimated to be 1.4% (Grant et al., 2017; Ritchie & Roser, 2018) making it among the most prevalent mental disorders in the world (Puddephatt et al., 2022). In Australia, estimates suggest that 22.7% of the population is at lifetime risk of developing an AUD, which is the highest globally (Glantz et al., 2020). Such high rates of alcohol use place a significant burden on both healthcare systems and the economy. While alcohol use is declining in younger age groups, it remains responsible for 4.6% of the overall Australian burden of disease (Australian Institute of Health & Welfare, 2023) and an economic burden equating to approximately $67 billion a year in direct and indirect costs (Australian Institute of Health & Welfare, 2023).

Acute alcohol consumption has a dose-dependent impact on functioning with a range of behavioural and cognitive effects. These effects include disinhibition, a feeling of wellbeing, impaired judgment and movement, slurred speech, impaired coordination and perception, nausea and vomiting, possible loss of consciousness, difficulty breathing, coma,

and death (Australian Government Department of Health, 2022). Chronic heavy alcohol use, that is characteristic of AUD, is associated with a significant decline in physical and psychological health (Kushner et al., 2000; Schwarzinger et al., 2018). A multitude of AUD-related physical health complications such as neurodegeneration, cardiovascular disease, cognitive decline and liver cirrhosis are precipitated by chronic alcohol-induced oxidative stress, inflammation, immune dysfunction, and neurotoxicity (Arteel, 2003; Fernández-Solà, 2020; Harper, 2009). Additionally, AUD is a major risk factor for other mental disorders including anxiety, depression, other substance use disorders and degenerative disorders (Grant et al., 2017). Studies have estimated that approximately 37% of those with a lifetime diagnosis of AUD also experience at least one other mental disorder (Kranzler & Soyka, 2018). Furthermore, alcohol use is also a significant risk factor for self-harm and suicidal behaviour (including completed attempts and ideation) in addition to harm to and by others, including domestic violence (Conner & Bagge, 2019). These consequences of chronic, heavy alcohol use contribute to high mortality rates (3.38 and 4.57 relative risk for male and female treatment seekers respectively (Roerecke & Rehm, 2013)) and reduced quality of life (Witkiewitz et al., 2018).

Unfortunately, treatment options for AUD are limited and the efficacy of available interventions are modest (Haber, Riordan, & Morley, 2021; Morley, Perry, et al., 2021). This is partly due to the challenges in novel treatment development as well as the complexity of the disorder. AUD is a heterogeneous disorder with various neurobiological, genetic, comorbid, environmental, and psychological factors thought to precipitate and maintain the disorder (Kendler et al., 2011; Volkow et al., 2016). In an attempt to facilitate treatment development, addiction medicine research employs a neurobiological model of addiction to provide a framework in which neurobiological adaptions precipitate AUD symptomatology.

By elucidating the neurobiological dysregulation associated with AUD, neurobiological models aid in identifying key pharmacotherapeutic targets for novel pharmacotherapy.

1.2 **Neurobiological Model of AUD**

The mid-20th century marked new research into key brain regions associated with reward processes (Olds & Milner, 1954), the discovery of dopamine (Carlsson et al., 1957), and dopamine's implication in reward processes (Ungerstedt, 1971). The neurobiological model of addiction subsequently arose from this work and suggests that allostatic adaption or neurobiological dysregulation is triggered by chronic heavy alcohol use. This neurobiological dysregulation precipitates downstream effects on behaviour, cognition, and psychology, and subsequently key characteristics of AUD including craving, withdrawal, vulnerability to relapse and impaired executive controlling of seeking behaviours (Kalivas & Volkow, 2005; Volkow et al., 2016). A comprehensive understanding of these downstream effects on behaviour, cognition and psychology with respect to the stages of addiction (Koob & Volkow, 2016) provides context for the neurobiological changes that are characteristic of AUD. Therefore, a brief overview of each stage of addiction will be provided in the following section (Section 1.2.1). This will be followed by a detailed description of the neurobiological dysregulation underlying each of these stages (Section 1.2.2).

1.2.1 **Stages of Addiction**

AUD can be understood as being comprised of three cycling and distinct phases: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (Koob & Volkow, 2016). Binge intoxication stages are characterised by heavy alcohol consumption leading to intoxication. During bingeing episodes, alcohol consumption elicits euphoric and rewarding effects through the activation of reward neurocircuitry which reinforces alcohol-seeking behaviours (Koob & Moal, 1997). In the early phases of addiction, these positive

effects are generally the primary driver to consume alcohol. Subsequently, individuals may seek contexts in which there is increased access to alcohol which therefore increases alcohol exposure (Koob & Le Moal, 2001). However, additional models provide alternative drivers to initially seek alcohol. For example, the tension reduction hypothesis (Kushner et al., 2000) suggests that individuals may consume alcohol primarily to alleviate stress or other emotional states because of discomfort experienced either psychologically or physiologically. Additionally, the self-medication hypothesis suggests that individuals may seek alcohol in order to alleviate underlying symptoms of additional mental health disorders (Khantzian, 1987, 1997).

With persistent heavy use, alcohol-induced neurobiological changes lead to tolerance to the effects of ethanol (Hoffman & Tabakoff, 1989), requiring individuals to increase their volume of alcohol intake to continue to experience associated reinforcing and euphoric effects (Volkow et al., 2016). More marked developments of tolerance are associated with more rapid progression and severe presentations of AUD (Schuckit, 1994). Additionally, following these neurological adaptions, the absence of alcohol intake may precipitate unpleasant withdrawal symptoms (Gilpin & Koob, 2008; Yip, 2011), including nausea or vomiting, hallucinations, psychomotor agitation and anxiety, paired with negative affect (Heilig et al., 2010). Withdrawal symptoms are characteristic of the withdrawal/negative affect stage and generally commence within 6-8 hours, peak at around 72 hours, and diminish between 5 to 7 days if abstinence is maintained (Kosten & O'Connor, 2003; Mainerova et al., 2015). During this period, individuals experiencing withdrawal commonly have strong motivational urges to consume alcohol to obtain relief from these withdrawal symptoms (Volkow et al., 2016). In order to limit consumption while experiencing these urges, individuals must employ behavioural inhibition systems, however, these are additionally dysregulated following regular alcohol consumption (Tahaney et al., 2014). If abstinence is

maintained throughout this relatively short period, individuals remain at risk of relapse to the binge/intoxication stage during the preoccupation/anticipation stage.

Preoccupation/anticipation stages may persist for up to or longer than 4-6 months (Bahji et al., 2022) providing a protracted period in which individuals may relapse. This stage may follow periods of withdrawal in cases where individuals are attempting to abstain from alcohol, however, they may also occur between drinking sessions to a lesser degree in cases where individuals are not attempting to maintain abstinence. This stage is characterised by strong motivational urges to seek alcohol, a preoccupation with obtaining alcohol, and increases in the anticipation of reward obtained following consumption (Koob & Volkow, 2016). These strong motivational urges to seek alcohol, otherwise referred to as craving, are associated with negative affect and physiological symptoms that additionally cause discomfort (Kotov et al., 2010).

During this preoccupation/anticipation stage, particularly following multiple cycles of these stages, individuals may experience a transition from "liking" to "wanting" alcohol (Robinson & Berridge, 2008). As the rewarding and euphoric effects of alcohol become desensitised, tension reduction and cue-elicited motivational urges to seek alcohol begin to drive individuals to consume alcohol (Everitt et al., 2008). These strong motivational urges to seek alcohol may be precipitated by various factors, including alcohol context cues (such as environmental, emotional, or cognitive cues) (Koob & Volkow, 2010; Robinson & Berridge, 2001), physiological or psychological symptoms from comorbid disorders, or stress-related cues. This process, termed cue-induced craving, is a key characteristic of this preoccupation/anticipation stage. Cue-induced craving is a major contributor to relapse and an important intervention point for both psychosocial and pharmacological interventions (Vafaie & Kober, 2022).

These three stages provide a framework for which many individuals may develop AUD, however, these stages may present in a non-linear fashion. Especially in earlier stages of addiction, individuals may demonstrate protracted periods of abstinence and relapse. For example, certain individuals may not consume alcohol throughout the week (abstinence) and drink higher volumes of alcohol on weekends (relapse) or experience pronounced preoccupation/anticipation in the absence of withdrawal/negative affect stages. However, regardless of the presentation of these stages or the motivations that drive individuals to persistent exposure to alcohol, alcohol-elicited neurobiological alterations may eventually entrench regular drinkers to compulsively seek and consume alcohol despite negative consequences.

1.2.2 **Neurobiological Alterations Associated with AUD**

Previous literature has identified diverse neurobiological alterations to various neurotransmitter systems, as well as to brain regions and circuitry that may be responsible for the behavioural, cognitive, and psychological characteristics of addiction stages (Koob & Volkow, 2016; Quaglieri et al., 2020). Primary neurotransmitter systems influenced by prolonged and heavy alcohol consumption include multiple receptor systems implicated in optimal functioning in the central nervous system (CNS). These include the systems that implicate dopamine, γ-aminobutyric acid (GABA), glutamate (Glu), serotonin (5-HT), and opioid receptors (Chastain, 2006; Vitale et al., 2021). The dysregulation of these neurotransmitter systems further dysregulates regions associated with reward circuitry, memory retrieval, reinforced learning, and salience attribution processes including the striatum, prefrontal cortex (PFC), caudate, amygdala, insula, ventral tegmental area (VTA), hippocampus, nucleus accumbens (NAc) and anterior cingulate cortex (ACC) (Koob & Volkow, 2010; Zeng et al., 2021). Furthermore, these regions are implicated in major

neurocircuitry including mesocorticolimbic, fronto-cortico-striatal, and striato-pallido-thalamo-cortical networks which have been demonstrated to be implicated in reward and reinforcement effects of alcohol, impulse control, decision-making, and habit formation respectively (Jentsch & Taylor, 1999; Koob & Volkow, 2010). In the following paragraphs, some of these neuroadaptations will be considered within the context of the aforementioned stages of addiction.

During binge/intoxication phases there are marked increases in opioid and dopamine 1 (D1) signalling into the ventral striatum which is associated with the initial euphoric feelings associated with alcohol intoxication (Everitt et al., 2008; Volkow et al., 2007). This D1 signalling within the striatum is a component of the mesocorticolimbic dopaminergic network. The mesocorticolimbic network employs dopaminergic pathways extending from the VTA and NAc up into limbic and PFC regions and is implicated in identifying rewarding stimuli, reinforced learning, and motivational salience which drives goal-directed behaviour (Yamaguchi et al., 2011). Additionally, during this binge/intoxication stage, alcohol-induced increases in inhibitory transmission via GABA and dampened excitatory Glu signalling systems produce anxiolytic and depressant-like effects (Koob, 2003).

Following chronic heavy administration and repetition of the binge/intoxication stage, individuals begin to experience baseline alterations in inhibitory and excitatory baseline signalling to maintain allostasis and regulated brain function (Koob, 2003). These glutamatergic and GABAergic adaptions are in part responsible for the development of tolerance. Subsequently, when access to alcohol is limited, hyper-glutamatergic and hypo-GABAergic signalling leads to hyper-excitatory withdrawal symptoms characteristic of the withdrawal stage (Becker, 1998; Littleton, 1998). Additionally, during this stage, heavy chronic alcohol use shifts dopaminergic signalling primarily in the ventral striatum to the dorsal striatum. This shift from ventral to dorsal signalling is thought to precipitate transitions

from “liking” to “wanting” alcohol (Robinson & Berridge, 2008) and from reward to relief drinking (Burnette et al., 2021).

Alcohol-induced modulations of dopaminergic signalling extend throughout mesocorticolimbic networks. Subsequently, dopamine signalling is dampened in response to the pharmacological effects of ethanol and naturally appetitive stimuli and conversely is increased during the presentation of alcohol-associated cues (Koob & Volkow, 2016). These changes precipitate increased incentive salience attribution toward alcohol-related cues, rendering them to be perceived as motivationally significant, and subsequently eliciting strong motivational urges to seek alcohol. In detail, this increased incentive salience is a result of hyper-dopaminergic signalling within mesocorticolimbic pathways connecting VTA/NAc to limbic and PFC regions. These alcohol-elicited modulations in broad dopaminergic signalling further contribute to tolerance, precipitate anhedonia (Koob, 2014), and increase the risk of relapse (Koob & Volkow, 2010). Further, changes in dopaminergic signalling within the striatum and striatum-mediated recruitment of striato-pallido-thalamo-cortical loops are thought to drive observed habitual compulsive alcohol-seeking behaviours. Moreover, alcohol-induced perturbations of the frontostriatal and frontocortical networks (Jentsch & Taylor, 1999), may also limit the capacity to implement executive functions like response inhibition, mental flexibility, and attention (Day et al., 2015) further reducing the capacity for individuals to control alcohol-seeking despite negative consequences.

1.3 **Pathological, Psychological, and Psychiatric Correlates of AUD**

Clinical profiles observed in AUD populations are generally heterogeneous. However, there are a range of consistent neuropathological (Section 1.3.1), neuropsychological (Section 1.3.2), and psychiatric (Section 1.3.3) correlates that have been associated with AUD. These

correlates are precipitated by persistent exposure to alcohol and the neurobiological impairments described above. These correlates of AUD are detailed below.

1.3.1 **Neuropathological Correlates of AUD**

Chronic heavy alcohol use can cause a variety of neuropathology through its detrimental effects on brain structure and function. Alcohol-induced cortical neural dendritic arborization and white matter loss (Harper & Corbett, 1990) can occur through a variety of mechanisms including nutritional deficiency (commonly a B1 vitamin deficiency) (Toledo Nunes et al., 2019), reduced liver functioning and/or the direct effect of ethanol on the brain (Dennis et al., 2014). Ethanol has direct effects on oxidative stress (Alfonso-Loeches & Guerri, 2011) whereby during the metabolism process acetaldehyde and reactive oxygen species are generated which both cause significant damage to human tissues (Zakhari, 2006) and direct cytotoxicity (Baker & Kramer, 1999). Additionally, alcohol modulates the immune system by increasing the circulation of pro-inflammatory cytokines which may cause inflammatory responses in the gastrointestinal tract and the liver (Crews et al., 2006). This inflammation causes both neuronal damage directly as well as through indirect processes causing deficits in function and overwhelms the liver and gastrointestinal tract, exacerbating inflammation at both peripheral and central sites (Bishehsari et al., 2017).

In approximately 20-35% of cases with prolonged heavy alcohol use, those with AUD develop some level of alcohol-related liver disease (ArLD) which greatly reduces the ability to metabolise alcohol and increases inflammation in the body (Kawaratani et al., 2013). Liver damage additionally increases the volume of pro-inflammatory cytokines causing further damage in the body. Subsequently, this cytokine modulation can lead to acute respiratory distress in the lungs by increasing the expression of transforming growth factor (Bechara et al., 2004). Additionally, preclinical studies indicate that an influx of proinflammatory

cytokines expressed due to oxidative products of alcohol (Dong et al., 2016) may be closely linked with an increased risk of degenerative disorders (Qin et al., 2008), cancer (Boffetta & Hashibe, 2006) and heart disease (Imhof & Koenig, 2003). Furthermore, inflammation and demyelination caused by alcohol neuroinflammation in the CNS has been shown to cause brain atrophy and long-term cognitive decline (Lanquetin et al., 2021; Pascual et al., 2014). Oxidative stress and neuroinflammation are tightly associated with chronic alcohol intake and relapse (Quintanilla et al., 2018), and are particularly exacerbated in heavy drinkers with liver injury (Kawaratani et al., 2013).

Alcohol-induced chronic pro-inflammatory states are further associated with perturbation of certain neurometabolites that are implemented in neuronal and neurobiological integrity including N-acetyl aspartate (NAA), Choline (Cho), creatine (Cr), glutathione (GSH), as well as major excitatory (Glu) and inhibitory (GABA) neurometabolites (Leclercq et al., 2017). Abnormal levels of these metabolites have previously been demonstrated to be characteristic of AUD clinical profiles (Chitty et al., 2014; Kirkland et al., 2022). Subsequently, alcohol-induces chronic inflammation is believed to perturb global neural function (Crews et al., 2015), contributing to neurobiological dysregulation, as well as the precipitation of neuropsychological impairments and psychiatric comorbidities.

1.3.2 **Neuropsychological Impairments Associated with AUD**

Protracted heavy use of alcohol is associated with a variety of neurocognitive impairments across various cognitive domains including executive functioning (EF) (Le Berre et al., 2017). These defects may be precipitated through common comorbidities associated with AUD including (but not limited to) Wernicke-Korsakoff syndrome (Victor, 1989), degenerative disorders (Sachdeva et al., 2016), and psychiatric disorders (Trivedi,

2006). Deficits are additionally a consequence of alcohol-induced inflammation, cytotoxicity, neurotransmitter modulation (Section 1.3.1), and/or modulations in fronto-striatal functional connectivity (Section 1.2.2) (Baker & Kramer, 1999; Galandra et al., 2019; Pascual et al., 2014).

Regardless of their aetiology, these deficits exist both as a risk factor for the development of AUD (Norman et al., 2011; Wetherill et al., 2013) and also for the exacerbation of AUD severity (Grenard et al., 2008). Around half of AUD patients experience cognitive deficits and, as a consequence, experience more impulsive and habitual alcohol-seeking (Czapla et al., 2016; Martins et al., 2018), generally worse clinical outcomes (Blume et al., 2005; Dalley et al., 2011) and everyday function vital for engaging with treatment (Eckardt & Martin, 1986). Specific EF deficits that have been demonstrated to be associated with long-term alcohol use include poor response inhibition, verbal and visuospatial learning, abstract problem solving, memory function, distractor interference control, perceptual motor skills, motor function, attention, planning and decision-making, and emotion regulation (Le Berre et al., 2017; Wilcox et al., 2014). Certain cognitive deficits are more persistent than others, including deficits in verbal learning, working memory, attention, impulse control, planning and decision making, and each varies in their contribution to maintaining AUD or inhibiting recovery (Manning et al., 2017; Velayudhan & Saraswathy, 2020).

Impairments that are particularly relevant for AUD treatment include impulse control and decision-making given that these lead to deficits in controlling strong motivational urges. Evaluations of risky options with increased reward, delayed discounting (the devaluation of future rewards), and reduced learning from punishment in addition to deficits in goal-directed behaviour and planning underpin deficits in decision-making and lead to impulsive alcohol-seeking (Scarfe et al., 2023; Verdejo-Garcia et al., 2018). Additionally, emotional regulation

deficits may precipitate alcohol consumption in the form of self-medication (Colder, 2001). These neuropsychological impairments have been demonstrated to limit AUD pharmacotherapy adherence (Butler & Le Foll, 2019). Therefore, addressing these neuropsychological impairments are considered to be an important consideration in the management of AUD.

1.3.3 **Psychiatric Correlates of AUD**

AUD is associated with various psychiatric comorbidities. Studies estimate that in individuals with AUD there is a lifetime prevalence of the following comorbid disorders: 27-40% have major depressive disorder; 20-40% have anxiety disorders including generalized anxiety disorder, social anxiety disorder, and panic disorder; 15-30% have Post Traumatic Stress Disorder (PTSD) (NIAAA, 2022) and 40-47% have other substance use disorders. As with neuropsychological impairments, AUD can be both a risk factor for the abovementioned psychiatric comorbidities due to alcohol-induced neurobiological changes and a consequence of these comorbidities. Indeed, as previously mentioned, the self-medication hypothesis suggests that alcohol is consumed to manage symptoms stemming from other disorders (Khantzian, 1987). In this model, alcohol consumption may acutely reduce psychological and physiological symptoms relating to anxiety, PTSD, and depression and also lead to a mutual maintenance pattern that can impede recovery (McHugh & Weiss, 2019).

While the above psychiatric comorbidities are the most commonly presented with AUD, additional comorbidities include personality disorders (Helle et al., 2019), impulse control disorders (Luderer et al., 2021) and sleep disorders (Romano et al., 2023). Sleep disorder comorbidities are particularly important given that sleep disturbances have been demonstrated to exacerbate alcohol-elicited neurobiological alterations (Colrain et al., 2014), other psychiatric symptoms (Carton et al., 2018), quality of life (Piekarski et al., 2022),

cognitive impairment (Zhang et al., 2021), and lead to increased relapse to alcohol (Brooks et al., 2021; Brower, 2003; Brower et al., 1998).

1.4 **Interventions for AUD**

There is an urgent need to improve strategies to mitigate the societal and individual impacts of AUD (Brady, 2000). There are various methods to managing the prevalence of AUD ranging from public prohibition, health messaging, education, psychosocial interventions, and pharmacotherapy. Alcohol use is deeply culturally ingrained, therefore, policy which limits alcohol use and availability is often considered to be paternalistic and a violation of individual self-determination (Martin & Brady, 2004). Therefore, the enforcement of these prohibitive laws can have political and social repercussions. Community-based educational programs can explain the risks of heavy acute and chronic alcohol use. However, these awareness and educational interventions have been demonstrated to either have a small but generally favourable effect (Strøm et al., 2014) or are ineffective (Casswell & Thamarangsi, 2009; Mialon & McCambridge, 2018). Alternatively, psychosocial and pharmacotherapy can be used as effective tools for harm reduction and management for individuals with moderate to severe AUD.

Australian treatment guidelines (Haber, Riordan, Winter, et al., 2021) suggest that non-pharmacological approaches to AUD treatment and management should focus on cognitive behavioural therapy (CBT) as the first line of psychosocial intervention. CBT has been shown to have moderate to significant effect sizes when compared to minimal treatment (Magill et al., 2019). They additionally suggest that brief interventions apply motivational interviewing (MI) for those with less severe cases of AUD. MI focuses on increasing a sense of autonomy, readiness to change, and reducing ambivalence to change alcohol-seeking behaviours (Miller & Rollnick, 2012). While MI is more effective than no treatment it is generally associated with small treatment effect sizes (Haber, Riordan, Winter, et al., 2021).

In conjunction with psychosocial support, pharmacotherapy is considered in moderate to severe AUD cases and may reduce core symptoms, facilitate abstinence, and reduce consumption (Morley, Perry, et al., 2021). Some medications may target relieving stress and agitation associated with withdrawal symptoms (relief craving) while others may reduce craving and alcohol-seeking behaviours (reward craving). Pharmacotherapy may also be particularly useful when psychosocial interventions may not be accessible, for example in rural communities where there is limited access to psychologists despite risky drinking, alcohol-related harms and alcohol-related suicide being at a higher rate per capita (Hurzeler et al., 2021; Miller et al., 2010). Nonetheless, optimal treatment combines both psychosocial and pharmacological treatments (Milena et al., 2024).

1.5 **Current Targets of Pharmacotherapy**

Over the previous 20 years, there have been significant advancements in the understanding of the neurobiological determinants of AUD (Section 1.2.2), however, there continues to be a paucity of medications that are available for clinicians to prescribe (Morley, Perry, et al., 2021). Only three "first-line" medications are currently approved by the Therapeutic Goods Administration (TGA) in Australia (Section 1.5.1), however, additional second-line and novel medications are emerging as potential options for the management of AUD (Section 1.5.2). These pharmacological treatments will be discussed below.

1.5.1 **First-Line Medications**

Currently, there are three medications approved in several countries for the management of AUD including Naltrexone, Disulfiram (Antabuse), and Acamprosate (Haber, Riordan, Winter, et al., 2021; Petrakis, 2006; Pettinati & Rabinowitz, 2006; Zindel & Kranzler, 2014). Disulfiram inhibits the function of the liver enzyme aldehyde dehydrogenase

and is used to dis-incentivise alcohol use due to the severe effects when alcohol is consumed including nausea, dizziness, flushing, and vomiting (Moreels et al., 2012; Roache et al., 2011). Conversely, naltrexone and acamprosate modulate neuro-signalling. Naltrexone is an opioid receptor selective competitive antagonist that blocks alcohol-induced opioidergic activity which subsequently dampens the reinforcing effects of alcohol (Sinclair, 2001; Swift et al., 1994). Acamprosate is a weak NMDA antagonist that may modulate GABAergic signalling during the early stages of abstinence from alcohol (Rammes et al., 2001; Spanagel & Zieglgänsberger, 1997). Although these treatments have been demonstrated to have some efficacy in the management of AUD, the number needed to treat is still over 12 patients for acamprosate and naltrexone (Jonas et al., 2014).

1.5.2 **Second-Line and Emerging Medications**

Second-line medications include baclofen and topiramate. Baclofen is a selective $GABA_b$ antagonist with few side effects (Addolorato et al., 2007). Several high-quality trials have demonstrated reduced alcohol intake and higher abstinence rates following baclofen administration (Addolorato et al., 2002; Morley, Baillie, et al., 2018) while some studies have not demonstrated any sign of efficacy (Garbutt et al., 2010). These differences in results have been suggested to be due to varying levels of AUD severity and/or baseline alcohol consumption, withdrawal status, or anxiety (Rombouts et al., 2019). Topiramate is another second-line medication that is just as effective as naltrexone (Morley, Kranzler, Luquin, Jamshidi, Adams, Montebello, Tremonti, Dali, Logge, Baillie, et al., 2024) although requires gradual titration due to a complex side effect profile. Topiramate facilitates GABAergic neurotransmitter function (White et al., 2000), reduces dopamine in extracellular sites in mesocorticolimic regions (Moghaddam & Bolinao, 1994), and is a Glu antagonist at kainite and AMPA receptors (Angehagen et al., 2005). There are limited effect sizes for all first-line

and second-line medications for AUD and a large degree of heterogeneity in treatment response (Morley, Perry, et al., 2021) and low prescribing rates (Hallgren et al., 2020). New pharmacotherapeutic options are thus required (Burnette et al., 2022). Treatment success is limited due to the complexity and heterogeneity of the disorder and may be modulated by treatment goals, psychological comorbidities, pharmacogenetics, and safety considerations (Morley, Perry, et al., 2021). Subsequently, recent calls have been made for personalised treatment catering to the specific needs, along with specific neurobiological and neuropsychological profile of each patient (Boness & Witkiewitz, 2023). Emerging medications consider diverse mechanistic pathways which may normalise various dysregulated systems and, if identified as effective in normalising these processes, may provide additional options for personalised treatments. Indeed, there are numerous novel agents currently being explored for AUD management such as varenicline, gabapentin, N-acetylcysteine, and probenecid (for review see, (Morley, Perry, et al., 2021)). However, none of these emerging medications have been approved and made available in Australia in the last two decades.

1.6 **Cannabidiol**

There has been an increasing number of studies considering cannabidiol (CBD) as a potential pharmacotherapy for a variety of indications due to its various therapeutic properties (Crippa et al., 2018). CBD is the second most abundant chemical constituent of the *cannabis sativa* plant and, unlike tetrahydrocannabinol (THC), it lacks unwanted psychotropic effects and has been shown to have a low side effect profile (Bergamaschi et al., 2011; Haney, Malcolm, Babalonis, Nuzzo, Cooper, Bedi, Gray, McRae-Clark, Lofwall, & Sparenborg, 2016; F. Leweke et al., 2012). Side effects that have been observed are mild and include somnolence (Cunha et al., 1980), headache, or gastrointestinal upset (Haney,

Malcolm, Babalonis, Nuzzo, Cooper, Bedi, Gray, McRae-Clark, Lofwall, Sparenborg, et al., 2016). Treatment with CBD for longer periods (Consroe et al., 1991) and higher doses (Zuardi et al., 1995) have been conducted without serious adverse effects in various populations.

1.6.1 **Therapeutic Use of CBD**

CBD was rescheduled from a TGA schedule 3 to a schedule 4 medication in Australia on February 1st, 2021 (TGA, 2020) following various literature demonstrating that CBD possesses various therapeutic properties including anti-seizure (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, & Filloux, 2016), anxiolytic (Berger et al., 2022; Bhattacharyya et al., 2018; Jadoon et al., 2017), neuroprotective (Fernández-Ruiz et al., 2013), anti-inflammatory and antioxidant effects (Mandolini et al., 2018). This diverse set of therapeutic properties additionally suggests CBD may thus be well suited to address gaps in pharmacological management for AUD.

1.6.2 **Potential Mechanism of CBD for AUD Management**

CBD has previously been shown to modulate various elements that are characteristic of addiction. Preclinical studies have demonstrated that acute CBD administration leads to reductions in withdrawals; anxiety, stress, and drug cue alcohol reinstatement (Skelley et al., 2020); voluntary alcohol consumption; opiate-seeking behaviour (Hurd et al., 2019; Ren et al., 2009); as well as withdrawal symptoms and alcohol-induced relapse behaviours in a rat model (Hurd et al., 2019; Ren et al., 2009; Viudez-Martínez, García-Gutiérrez, et al., 2018a, 2018b); as well as withdrawal symptoms and alcohol-induced relapse behaviours in a rat model (Viudez-Martínez, García-Gutiérrez, et al., 2018a, 2018b). CBD has a poly-pharmaceutical action and has been shown to modulate over 65 pharmacological sites (see

reviews; (Ibeas Bih et al., 2015; Peng et al., 2022)). These implicated sites are not solely restricted to the endocannabinoid system (ECS) (Corroon & Felice, 2019), but modulate neurotransmission for various systems including serotoninergic, dopaminergic, glutamatergic, GABAergic (Chastain, 2006; Scopinho et al., 2011; Vitale et al., 2021). These neurotransmitter systems are implicated in substance use disorders (Hermann & Schneider, 2012; Mandolini et al., 2018) and dysregulation in these systems are associated with various stages and characteristic properties of AUD as described above (Section 1.2.2). Given the diverse nature of these CBD-mediated effects, CBD may manage specific stages of addiction and clinical profiles through distinct pharmacological mechanisms (Prud'homme et al., 2015). These potential mechanisms will be outlined below with respect to the stages and characteristics of AUD.

Alcohol Withdrawal

Preclinical and clinical research suggests that CBD modulates Glu and GABA signalling (Gomes et al., 2015; Long et al., 2006; Moreira & Guimarães, 2005) which is a feature of AUD withdrawal. CBD limits the degradation of endocannabinoids by blocking fatty acid amide hydrolase (Pisanti et al., 2017) which may modulate GABA and Glu signalling (Gururajan & Malone, 2016). Previous supporting research suggests that CBD administration is effective in reducing seizure severity in other indications (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, & Filloux, 2016), reductions in severity of alcohol withdrawal in mice (Viudez-Martínez, García-Gutiérrez, et al., 2018a) and modulation of GABA and Glu in human basal ganglia and cortex (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, & Heasman, 2019). CBD could reduce relapse rates in AUD by targeting these symptoms during withdrawal/negative affect stages of addiction.

Oxidative Stress

CBD has previously been suggested to modulate oxidative stress through its anti-oxidant and pro-oxidant effects. CBD has been demonstrated to both regulate redox function and scavenge free radicals (for review see; (Pereira et al., 2021)). CBD can increase the presence of antioxidant enzymes including GSH peroxidase and superoxide dismutase (SOD), through modulating redox-sensitive transcription factors such as Nrf2, and upregulating natural antioxidant defence which subsequently maintains GSH levels. CBD may therefore protect individuals from cellular damage caused by the alcohol-elicited influx of reactive oxygen species (ROS). Additional mechanisms by which CBD may show anti-inflammatory effects include interactions with the ECS, cytokine production, reduced excitotoxicity caused by excessive neuronal excitation, and transient receptor potential vanilloid 1 (TRPV1) receptors (for review see, (Henshaw et al., 2021; Peyravian et al., 2020)). For example, CBD has been shown to reduce reuptake of anandamide (AEA) in presynaptic neurons which serves to increase the availability of AEA (Pisanti et al., 2017), which has anti-inflammatory and neurogenic properties (Pflüger-Müller et al., 2020; Rettori et al., 2012). These antioxidant and anti-inflammatory properties of CBD may aid in protecting against the development of cognitive deficits and other complications precipitated by alcohol which may aid in reducing the severity of clinical outcomes (Maillard et al., 2020).

Cognitive Functioning

It has been suggested that CBD, either directly or indirectly, may improve EF (Batalla et al., 2021). CBD may indirectly improve EF through associations with improved sleep

(Shannon et al., 2019), reduced inflammation and oxidative stress (Mandolini et al., 2018; Pereira et al., 2021), and anxiolytic properties (Berger et al., 2022; Bhattacharyya et al., 2018; Jadoon et al., 2017). Further, CBD-normalisation of neurometabolites in regions experiencing alcohol-elicited dysregulation may aid in improving cognitive function specific to AUD. If CBD administration does demonstrate the rescuing of preservation of EFs, CBD may serve to facilitate reduced impulsive alcohol-seeking during preoccupation/anticipation stages of AUD (MacKillop et al., 2010) and treatment adherence.

Affect

CBD additionally may possess anxiolytic effects that may be relevant for heightened arousal states which precipitate relief drinking. While the specific pharmacological mechanisms of the anxiolytic effects of CBD remain elusive, various mechanisms have been proposed. The ECS, which is modulated by CBD via cannabinoid receptors 1 and 2 (CB1 and CB2; respectively) receptors (Galaj et al., 2020; Luján & Valverde, 2020), is heavily implicated in stress and anxiety (Viveros et al., 2005). CB1 density is high in regions associated with emotion regulation including the hippocampus, cortex, and amygdala (Glass et al., 1997; Hájos & Freund, 2002; Pistis et al., 2004). Further, CBD is a 5-HT antagonist (Russo et al., 2005) and has been shown to inhibit synaptic uptake of adenosine and dopamine in rat and mouse striatum (Pandolfo et al., 2011), These effects, paired with the potential to modulate withdrawal symptoms, may be beneficial to relief motivated drinking and reduce symptoms of comorbidities such as anxiety, depression and PTSD. Indeed, acute doses of CBD modulate anxiety symptoms (Skelley et al., 2020); have been shown to reduce stress cue reinstatement in preclinical models (Viudez-Martínez, García-Gutiérrez, et al., 2018a, 2018b); and reduce opiate cue-induced anxiety for those with opioid use disorder (Hurd et al., 2019). Therefore, CBD may aid in improving clinical outcomes for comorbid

groups (Ipser et al., 2015) through targeting tension reduction/relief drinking (Greeley & Oei, 1999).

Salience Attribution and Anticipation

CBD is a partial agonist for dopamine D2 receptors (Seeman, 2016) whereby modulation of dopaminergic signalling essential in networks implicated in reward processing and salience attribution is likely to be relevant to AUD (Calpe-López et al., 2021; Galaj et al., 2020; Gasparyan et al., 2020; Renard et al., 2016). Furthermore, CBD may have alternative mechanistic pathways to the modulation of salience and reward processing through its effects on 5-HT1a receptors (Katsidoni et al., 2013), alpha-1 adrenergic receptors (α1AR) , TRPV1 and V2 (TRPV2) (Qin et al., 2008), GABAergic and glutamatergic systems (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, & Heasman, 2019), and CB1 and CB2 (Galaj et al., 2020). The capacity to modulate these systems is explain downstream effects on neurotransmission within aforementioned regions associated with salience attribution and mesocorticolimbic networks (Hassanlou et al., 2021; Karimi-Haghighi et al., 2020; Katsidoni et al., 2013; Yang et al., 2020)including the VTA, dorsal raphé nucleus, hippocampus, NAc, ACC and PFC. This mechanism has been corroborated by a preclinical study in which intra-NAc administration of CBD prevented the acquisition of a methamphetamine-induced condition place preference paradigm in rats (Hassanlou et al., 2021) and decreased attentional bias to cigarettes for those with nicotine dependence (Hindocha, Freeman, Grabski, Stroud, et al., 2018). CBD may therefore aid in reducing alcohol cue-induced craving and impulsive alcohol-seeking by normalising neurobiological and subsequent behavioural responses to cues. This may facilitate abstinence during preoccupation/anticipation stages, limit the number of heavy drinking days, and limit drinking profile severity.

In summary, taken together, previous research suggests that CBD would be well suited to manage symptoms relating to AUD through a wide range of diverse mechanisms. While there are some studies registered that aim to examine the effects of CBD in AUD using various paradigms (e.g. NCT03252756, NCT04873453) nothing has been published to date. Further research using human psychopharmacological models investigating both preliminary signals of efficacy and the direct mechanisms of CBD in AUD populations will address this gap. Clinical psychopharmacology research requires sophisticated measures that can examine the CBD-mediated modulations of cognitive, psychophysiological, and neurobiological determinants of AUD.

1.7 **Clinical Psychopharmacology Models**

Formal clinical trials are costly and can take 4–5 years. A more efficient approach to screening novel medications for efficacy is via clinical experimental paradigms (Koob et al., 2009). These psychopharmacology models use randomised double-blind experimental designs (DBRCT) assessing acute or sub-acute responses to medication. The impacts of novel agents are commonly assessed via the restoration and regulation of i) brain regions and circuitry function associated with cue-elicited salience attribution, reward anticipation, and impulse control through functional magnetic resonance imaging (fMRI) cue reactivity paradigms (Logge et al., 2019); ii) dysregulated neural biomarkers such as neurometabolite concentrations including GABA+, NAA, Glu, combined glutamate and glutamine (Glx), and GSH using magnetic resonance spectroscopy (MRS) (Morley, Lagopolous, et al., 2018); iii) cue-elicited psychophysiology responses through indices including heart rate variability (HRV) and galvanic skin response (GSR) during psychophysiological cue reactivity paradigms; iv) cue-elicited changes in alcohol craving and mood following alcohol-cue or stress-cue exposure (Logge et al., 2019); v) EF as measured with a range of cognitive tasks.

1.7.1 **Magnetic Resonance Imaging (MRI)**

MRI is a versatile, non-invasive imaging technique that can establish the structure, activity, and functional connectivity between brain regions. To achieve this MRI manipulates the orientation of protons in the brain by creating strong magnetic fields within a bore. Pulsing radiofrequencies disorient protons out of alignment with this field and the relaxation time taken for protons to return to alignment, which releases energy in the form of radiofrequency signals, is recorded. Given that different tissue types emit different signal intensities, tissue types can be detected, and a detailed reconstruction of the brain can be created (Brown et al., 2014).

Functional MRI

fMRI acquisitions like Single echo planar imaging (EPI) or Multi-Echo EPI measure changes in blood oxygenation on the assumption that oxygenated blood is transported to neurons to meet metabolic demands following activation (referred to as hemodynamic response). The blood oxygenation level-dependent (BOLD) signal is therefore used as a proxy for brain activation. fMRI is either used to measure brain function associated with specific cognitive or behavioural demands by measuring BOLD signal during tasks to establish patterns of functioning relative to these processes (Worsley and Friston, 1995, Worsley 1997; Linden DE et al., 1999; Heeger and Ress, 2002), or during task-free resting-state (Raichle et al., 2001; Fox et al., 2007). Resting state fMRI is conducted during resting, in the absence of a task, allowing for the evaluation of intrinsic connectivity within the brain during resting states. Conversely, common task paradigms in fMRI include oddball tasks, sensorimotor tasks, memory tasks, EF tasks and cue reactivity tasks.

fMRI cue-reactivity tasks are commonly implemented in addiction research due to their capacity to modulate regions underlying AUD neuropathology (Ekhtiari et al., 2016) associated with interoception, habit formation, executive control, memory, incentive salience, and reward evaluation (Heitzeg et al., 2015; Sangchooli et al., 2024). Modulation within regions associated with this neurocircuitry has previously been demonstrated to predict outcomes in AUD (Beck et al., 2012; Zilverstand et al., 2018).

Magnetic Resonance Spectroscopy

MRS acquisitions like *Point RESolved Spectroscopy* (PRESS), MegaPRESS, or Hadamard Encoding and Reconstruction of MEGA-Edited Spectroscopy (HERMES) can quantify neurometabolites within a prespecified voxel in the brain. Similarly to other MRI techniques, MRS measures the resulting distinct electromagnetic signals emitted by different neurometabolites when exposed to radiofrequency pulses. The spectra emitted by various metabolites can be analysed and specific neurometabolites can be quantified following preprocessing and suppression of chemicals that are more abundant like water or Cr. Common neurometabolites used in addiction research include GSH, Glu, Glx, GABA, NAA, Cr, and Cho-containing compounds. These neurometabolites can be established through distinct peaks across electromagnetic spectra and are quantified in parts per million (ppm). Given the neurobiological alterations associated with AUD (Section 1.2.2), pharmacological modulation of these neurometabolite concentrations, as measured using MRS, can provide insight into the mechanisms and potential efficacy of pharmacotherapy. For example, therapeutic mechanisms for gabapentin (Prisciandaro et al., 2021) and baclofen (Morley, Lagopolous, et al., 2018) but not N-acetylcysteine (Kirkland et al., 2023) have been shown to modulate neurometabolites in AUD.

1.7.2 **Psychophysiology**

Psychophysiological cue-reactivity paradigms allow for the investigation of cue-elicited psychophysiological responses via indices of the sympathetic nervous system and parasympathetic nervous system recruitment. Following exposure to an appetitive cue, cue salience is subsequently appraised by salience networks. Once a cue is established as salient, anterior executive networks mobilize the autonomic nervous system (ANS) to guide rapid responses to changes in the external environment. To do this, the central autonomic network, consisting of sympathetic and parasympathetic derivative systems, modulate neuroendocrine and visceromotor systems and implement goal-directed behaviour (Benarroch, 1993). During this process, the sympathetic nervous system is upregulated and the parasympathetic nervous system is downregulated precipitating emotional, hedonic or affective states (Thayer & Lane, 2000). Following chronic, heavy alcohol use there are neurobiological alterations (Section 1.2.2) that establish strong associations between cues that proceed alcohol consumption and the reinforcing effects of alcohol. These alterations increase salience attribution, reward anticipation, and attentional bias towards cues which elicits strong motivational urges to seek alcohol (Koob & Volkow, 2016). Subsequently, these neurobiological alterations may lead to exacerbated and prolonged recovery of these increased sympathetic and decreased parasympathetic nervous system responses (Cooney et al., 1997). This subsequently results in protracted periods of strong motivational urges to seek alcohol, decreased regulatory capacity, and increased impulsive alcohol-seeking. The parasympathetic and sympathetic nervous systems can be observed through measures of variation in high-frequency HRV (HF-HRV) and skin conductance levels (SCL), respectively.

High-Frequency Heart Rate Variability

HRV is a measure of beat-to-beat variability in heart rate that is mediated by the ANS. The parasympathetic nervous system modulates heart rate and HRV through neural projections via the vagus nerve from the CNS to the peripheral nervous system (Rajendra et al., 2006). Although sympathetic neural activity can slightly alter cardiac behaviour (Levy et al.,1993), HRV is directly modulated by the baroreflex (indicated by low-frequency HRV) and respiratory sinus arrhythmia (RSA) (indicated by HF-HRV) (Rajendra et al., 2006). Therefore, RSA measured through HF-HRV is used as an index of changes in parasympathetic nervous system activity.

HF-HRV can be considered as a proxy for EF recruitment to regulate responses to the environment (V. M. Karpyak et al., 2014). Specifically, HF-HRV reflects the capacity of the central autonomic network (CAN) including the PFC, central nucleus of the amygdala, hypothalamus, and brainstem – to meet and adapt to environmental demands (Thayer and Friedman, 2002). Furthermore, abnormal ACC functioning and dysregulated functional connectivity between the insula/amygdala and the ACC (Koob & Volkow, 2016), have previously been identified in AUD. These forms of neurobiological dysregulations are associated with decreased emotional and physiological processing (Wilcox et al., 2016). Increased HF-HRV reflects an individual's capacity to regulate their emotions (Geisler et al., 2010), and may be a key indication of psychological flexibility. Psychological flexibility refers to the capacity to respond to changing situation demands, and maintain control over competing needs, life domains and desires and is relevant to resilience to triggers, employing coping strategies and value driven behaviours (Kashdan & Rottenberg, 2010). Subsequently, HF-HRV is a valuable indicator of regulatory functions that are implicated during cue-elicited alcohol-seeking (Garland, 2011).

Skin Conductance Level

In contrast to HF-HRV, SCL is measured through GSR and is associated with sympathetic nervous system recruitment. SCL provides an index for the modulation of the sympathetic nervous system by the CNS via preganglionic neurons in the spinal cord. As increased sympathetic nervous system recruitment precipitates perspiration on the skin, improved conductance elicited by sweat serves as a proxy for sympathetic nervous system activity. Primarily, this modulation occurs via intervention by the amygdala, hypothalamus, and PFC (ventromedial and ACC) which can reflect changes to decision-making and emotion regulation processing. High sympathetic nervous system responses have been demonstrated to be associated with impaired inhibitory control, reward, and punishment sensitivity (Arnsten, 2009; Herman & Cullinan, 1997). In severe AUD cases, often co-occurring with liver disease, the sympathetic nervous system is dampened in response to alcohol cues which reflects dysregulation of ANS responses (Logge et al., 2023).

1.7.3 **Cognitive Functioning**

Cognitive tasks including the Number–letter task (adapted from (Rogers & Monsell, 1995)), Stroop task (Stroop, 1935), N-back task (Braver et al., 1997; Jonides et al., 1997; Kirchner, 1958), Balloon Analogue Risk Task (BART, (Lejuez et al., 2002)), Columbia Card Task, Hot Version (CCT; (Figner et al., 2009)) and the Trail Making Test (TMT: Part A and B (Reitan & Wolfson, 1985)) all provide insight into EF relevant for the regulation of alcohol consumption. These tasks may be used to examine functioning capacity domains including decision-making, working memory, inhibitory control, self-regulation, attention, and cognitive flexibility. As described above, impaired EF in AUD predicts treatment outcomes and relapse rates (Wilcox et al., 2014) such that laboratory models that seek to develop

pharmacotherapy should examine improvements in EF or, at least to ensure that there are no exacerbations of these deficits.

1.8 The Neurobehavioral and Psychophysiological Mechanisms of Cannabidiol (CBD) for AUD

1.8.1 Overall Aims

This thesis aims to determine signals of efficacy and to examine potential underlying mechanisms of CBD in the treatment of AUD. The first objective is to determine whether CBD modulates neurobiological and psychophysiological responses to alcohol-related cues and neurometabolites in individuals with AUD. My second objective is to examine whether acute CBD treatment modulates subjective alcohol craving, mood, and, in addition, does not impede cognitive functioning.

Firstly, existing research into the effect of CBD in neuroimaging paradigms will be evaluated through a systematic review (Chapter 2 - published in *Journal Cannabis Research. 2024 Mar 21;6(1):15*). Once the experimental paradigms and relevant neurobiological and cognitive effects have been evaluated, a cross-over study examining CBD versus placebo in AUD will be designed (Chapter 3 – conceptual and protocol paper, accepted with revisions in *Contemporary Clinical Trials Communications*). The rationale behind the design of the overall study and a description of the various paradigms is articulated in this conceptual protocol paper. The results of this large study stemming from several paradigms are then presented in this thesis including the effect of CBD on psychophysiological measures of arousal during an alcohol cue (Chapter 4 - submitted for publication), activation of brain areas associated with alcohol cue-induced craving during an fMRI alcohol cue task (Chapter 5 – submitted for publication) and brain levels of neurometabolites (Chapter 6 submitted for publication). In the final chapter (Chapter 7) the results from these previous chapters will be discussed in light of mechanisms by which CBD may be promising as a tool to manage AUD.

1.8.2 **Hypotheses**

The following hypotheses are tested in this thesis:

In AUD individuals, CBD administration relative to placebo will lead to:

i) Significantly reduced HF-HRV to alcohol cues;

ii) Significantly less change in HF-HRV levels during recovery periods after cue offset;

iii) Significantly lower SCL arousal during cues

iv) Significantly attenuated mesocorticolimbic brain activation (e.g., NAc, striatum, ACC, ventromedial prefrontal areas) when exposed to an alcohol cue;

v) Significantly elevated brain GABA+, NAA and GSH and reduced Glx;

vi) Significantly lower craving, and elevated mood with no impairments in cognition.

1.8.3 **Summary**

Currently, there is a gap in the literature regarding the role of CBD in the management of AUD. This thesis will evaluate how CBD modulates psychophysiological and neurobiological processes implicated in AUD and determine early signals of the efficacy of CBD as a novel treatment for AUD.

Chapter 2: Neuroimaging Studies of Cannabidiol and Potential Neurobiological Mechanisms Relevant for Alcohol Use Disorders: A Systematic Review

Abstract

The underlying neurobiological mechanisms of cannabidiol's (CBD) management of alcohol use disorder (AUD) remains elusive. Aim: We conducted a systematic review of neuroimaging literature investigating the effects of CBD on the brain in healthy participants. We then theorise the potential neurobiological mechanisms by which CBD may ameliorate various symptoms of AUD. Methods: This review was conducted according to the PRISMA guidelines. Terms relating to CBD and neuroimaging were used to search original clinical research published in peer-reviewed journals. Results: Of 767 studies were identified by our search strategy, 16 studies satisfied our eligibility criteria. The results suggest that CBD modulates γ-Aminobutyric acid and glutamate signaling in the basal ganglia and dorso-medial prefrontal cortex. Furthermore, CBD regulates activity in regions associated with mesocorticolimbic reward pathways; salience, limbic and fronto-striatal networks which are implicated in reward anticipation; emotion regulation; salience processing; and executive functioning. Conclusion: CBD appears to modulate neurotransmitter systems and functional connections in brain regions implicated in AUD, suggesting CBD may be used to manage AUD symptomatology.

2.1 **Introduction**

The medical, psychological, and social sequelae of alcohol use disorder (AUD) are major global public health concerns. Harmful alcohol consumption is linked to many physical and mental health complications and is responsible for 5.1% of the global burden of disease (Griswold et al., 2018; WHO, 2018, 2021). AUD, particularly when moderate to severe, is a chronic relapsing disorder, characterized by compulsive alcohol-seeking and consumption despite negative repercussions to both physical and mental health (Haber, Riordan, Winter, et al., 2021). A wealth of research suggests that neurobiological changes to various neurotransmitter systems and brain circuits underpin the behaviour and psychology which maintains AUD (Koob & Volkow, 2016). Primary neurotransmitter systems influenced by prolonged and heavy alcohol consumption include dopaminergic, γ-aminobutyric acidergic (GABA)-ergic, glutamatergic, serotonergic, and opioidergic (Chastain, 2006; Vitale et al., 2021). Pharmacotherapy can be useful, in conjunction with psychosocial support, for reducing the core symptoms of AUD (such as reducing craving, habitual seeking behaviours, and withdrawal) and achieving abstinence or aiding the control of consumption (Morley, Perry, et al., 2021). However, there currently exists a paucity of medications available to treat AUD (Morley, 2021).

Neuroimaging literature has identified specific neurocircuit and biochemical alterations thought to be responsible for the observed cognitive and behavioural changes associated with AUD. Changes to mesocorticolimbic reward pathways, following steep increases in opioid and D1 signalling into the ventral striatum, leads to increases in reward anticipation and salience attribution to drug-related cues which leads to increased drug-seeking behaviours (Koob & Volkow, 2016). Further, reduced signaling of dopaminergic systems in reward and limbic networks leads to negative emotion, anhedonia, and heightened stress (Koob & Volkow, 2016). Finally, fronto-striatal network and fronto-cortical

dysregulation leads to reduced executive functioning and emotion regulation (Jentsch & Taylor, 1999). Understanding the brain correlates of AUD and implementing neuroimaging techniques to identify the methods by which novel pharmacotherapies may modulate these correlates provide a method for more effective and tailored treatments.

Over the past few years there has been an influx of research exploring CBD as a potential pharmacotherapy for a variety of indications due to its wide-ranging therapeutic effects and favourable safety profile (Crippa et al., 2018). CBD is the second most abundant chemical constituent of the *Cannabis sativa* plant and, unlike Δ9-tetrahydrocannabinol (THC), is non-intoxicating and has nil potential for abuse or dependence (Arout et al., 2022; Bergamaschi et al., 2011; Haney, Malcolm, Babalonis, Nuzzo, Cooper, Bedi, Gray, McRae-Clark, Lofwall, & Sparenborg, 2016; F. Leweke et al., 2012; McCartney et al., 2022; Schoedel et al., 2018). CBD has shown to possess affinity for multiple targets including the modulation of serotoninergic, dopaminergic, glutamatergic, GABAergic (Scopinho et al., 2011) and endocannabinoid signalling (Corroon & Felice, 2019). This multi-target action of CBD may explain the various therapeutic properties including antiepileptic (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016; Talwar et al., 2022), anxiolytic (Berger et al., 2022; Bhattacharyya et al., 2018; Borgwardt et al., 2008; Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016; Fusar-Poli et al., 2010; Jadoon et al., 2017; Talwar et al., 2022; Wilson et al., 2019), anxiolytic (Berger et al., 2022; Bhattacharyya et al., 2018; Borgwardt et al., 2008; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019), neuroprotective (Crippa et al., 2018), and anti-inflammatory and antioxidant effects (Mandolini et al., 2018; Mechoulam et al., 2007; Ren et al., 2009). This combination of potential therapeutic effects suggests that CBD might be particularly well suited to management of alcohol use disorder. In fact, CBD may modulate drug craving and seeking behaviours. CBD has been shown to

reduce craving and anxiety in heroin users (Hurd et al., 2019), as well as stress and drug cue alcohol reinstatement, voluntary alcohol consumption, withdrawal symptoms and alcohol induced relapse behaviours in preclinical models of alcohol dependence (Viudez-Martínez, García-Gutiérrez, Fraguas-Sánchez, et al., 2018; Viudez-Martínez, García-Gutiérrez, et al., 2018a). This suggests that CBD could protect from further damage of alcohol due to its neuroprotective and anti-oxidant properties which could improve executive functioning, but may also modulate key disorder characteristics which precipitate relapse such as heightened anxiety (Skelley et al., 2020) and craving in response to alcohol cues and stressors (Hurd et al., 2019). Neuroimaging techniques provide valuable insights into the structure and function of the brain and may explain the relationship between the pharmacological action of CBD and its behavioural and psychological effects (Hargreaves et al., 2015; Nathan et al., 2014; Wong et al., 2009). However, there has currently been no attempt to compile and compare neuroimaging studies to examine whether the converging neurobiological effects of CBD are relevant to AUD.To establish the current understanding of the neurobehavioral mechanisms of action of CBD on the human brain, and its pharmacotherapeutic potential for AUD, we examined common neuroimaging techniques including, magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI, including both functional and structural imaging), single photon emission computed tomography (SPECT) and positron emission tomography (PET). MRI is a non-invasive technique that produces anatomical images of the brain used to investigate both structural and functional aspects of the brain. Structural MRI provides a snapshot of brain anatomy in time while functional MRI (fMRI) can identify brain activity occurring during a variety of cognitive and functional activities of the brain in real-time. Specific cognitive phenomena can be targeted by presenting participants with specific tasks, known as task fMRI (tfMRI) (Heeger & Ress, 2002; Linden et al., 1999; Worsley & Friston, 1995) or also conducted in task-free paradigms known as resting state fMRI

(rsfMRI) (Fox & Raichle, 2007; Raichle et al., 2001). Magnetic resonance spectroscopy (MRS) is an imaging modality that can identify the presence and density of a variety of neurometabolites in the brain. Finally, nuclear imaging techniques PET and SPECT use radiotracers which are absorbed by the body and the resulting emission of positrons (in the case of PET) and gamma rays (in the case of SPECT) provides a measure of cellular and molecular function. The destination of the radiotracers indicates the location of changes in metabolic and other physiological processes such as blood flow, and regional chemical absorption.

This review aimed to systematically examine studies using these imaging techniques to elucidate the neurobehavioural and neuropsychological effects of CBD, as well as provide insights into the potential mechanism of CBD in the management of key symptoms of AUD.

2.2 **Methods**

This review follows the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines for systematic reviews (Moher et al., 2009). Prior to the commencement of the data extraction, this review was registered with the international prospective register of systematic reviews (PROSPERO # CRD42021272561). The original protocol can be accessed on the PROSPERO website.

2.2.1 **Search Strategy**

Terms relating to CBD, and neuroimaging were used to search EMBASE, PubMed, Medline and PsycINFO databases. This search strategy used a combination of MeSH heading and key words and used two main sections. These sections related to "cannabidiol" and a section relating to imaging techniques i.e. (MRS OR Magnetic Resonance Spectroscopy OR Spectroscopy OR Metabolite Concentrations OR magnetic resonance spectroscopy OR MRS OR functional magnetic resonance imaging OR fMRI OR resting state functional OR magnetic resonance imaging OR rsfMRI OR structural magnetic resonance imaging OR MRI OR magnetic resonance spectroscopy OR PET OR positron emission tomography). The search was re-run in June 2022 to capture any new publications (See Appendix A).

2.2.2 **Study Selection**

Once all the database searches had been completed and duplicate studies removed, a multi-stage screening process was performed by one author (TH). Studies were screened in the following order i) title ii) abstract iii) full-text article. Titles were screened to ensure studies used CBD as the active medication and that neuroimaging outcomes were the key measure of interest. Abstracts were then further assessed to ensure only human studies were

included. In the final stage, all remaining studies had a full-length text review to ensure that the study satisfied more specific inclusion criteria.

2.2.3 **Eligibility Criteria**

Studies which investigated the effect of CBD on the brain using either MRI, fMRI, MRS, SPECT or PET in human subjects were included. All eligible studies also had to include an experimental group that received CBD which did not fit diagnostic criteria for a mental health disorder. Studies were excluded if they were post-mortem, animal investigations, non-brain MRI studies, or examined the effect of cannabis rather than CBD. Review and non-English articles were excluded. The reference list of all eligible studies was also manually searched to identify any additional publications.

2.2.4 **Data Extraction**

The following data was extracted from all eligible studies: author, year of publication, number of participants in patient and control groups, age, proportion of males and females, clinical condition and diagnosis (patients only), matching factors in controls, neuroimaging paradigm, scanner specifics, outcome variables including: i) structural brain changes; ii) CBD-modulated brain activity (as measured through the blood oxygen level dependent [BOLD] response) or functional connectivity; iii) CBD-induced alterations in metabolites such as glutamate, GABA, and glutamine; iv) CBD-induced alterations in metabolism of blood PET.

2.2.5 **Quality Assessment**

Risk of bias was assessed by the AXIS for cross-sectional studies or the Cochrane Risk of Bias (Sterne et al., 2019) for randomised trials with both crossover and parallel

designs. The risk of bias assessment was assessed independently by two authors (KM and JW) and any discrepancies were resolved by discussion between the two authors with consultation available from a third party if required.

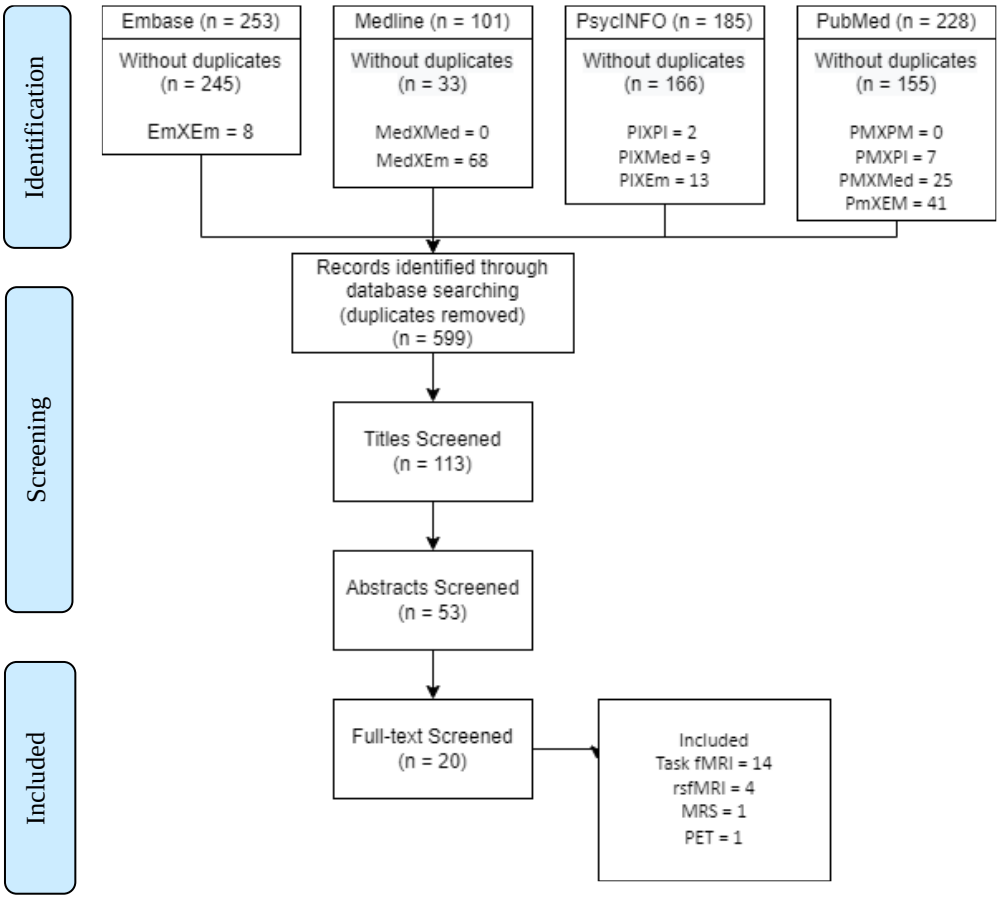
2.3 **Results**

2.3.1 **General Overview of Study Selection Process**

The primary search identified 767 records from four databases (Figure 2.1). After removing duplicates, 599 items remained. Titles were screened first and items that did meet eligibility criteria were removed leaving 113 studies. After screening abstracts, 53 studies remained. Finally, the full-texts were screened leaving 16 studies for inclusion in the review. Before publication, a secondary search in June 2022 identified four new studies that were included in the final version of the review. The 20 included studies all administered CBD orally with 19 studies dosing 600 mg CBD and one 400 mg CBD. Fourteen of the 20 included studies were task-based fMRI, four were rsfMRI, one study was an MRS study, and one study used SPECT imaging Although search terms related to SPECT were not included in the search strategy, this study was included due to its pertinence to the focus of this review. Additionally, various studies were used the same or similar participant samples as depicted by the colour categorisations of the outer ring of the sunburst plot (Figure 2.2). A summary of pharmacotherapy schedule, study design, and sample characteristics from extracted studies can be found in Appendix A (Table 1 and 2). In section 2.3.2 we detail functional (subdivided into rsfMRI and different task paradigms) and neurochemical findings.

Figure 2.1

PRISMA Flow Chart



Note. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram of the study selection process. EM = Embase; Med = Medline; PI = PsycINFO; PM = PubMed; MRI = magnetic resonance imaging; fMRI = functional MRI; rsfMRI = resting state fMRI; MRS = magnetic resonance spectroscopy; PET = positron emission tomography

Figure 2.1

Sunburst Chart

Note. The proportion of studies using each neuroimaging modality in the inner ring. Each colour in the outer ring indicates a different participant sample.

2.3.2 **Functional MRI (fMRI)**

Resting State fMRI

Four resting state fMRI studies (Bloomfield et al., 2020; Grimm et al., 2018; Pretzsch, Voinescu, et al., 2019; Wall et al., 2022) were identified. These studies examined brain activation by either measuring spontaneous low frequency fluctuations in BOLD signal while participants remain at rest in a MRI machine or cerebral blood flow (CBF) via the use of a technique called arterial spin labelling (ASL; (Barbier et al., 2001)).

Firstly, in a double-blind, randomised, placebo-controlled (DBRCT) crossover study, 13 males with autism spectrum disorder (ASD) and 17 neurotypical males (mean [SD] age= 30.85 [9.79] years and 28.47 [6.55] years for ASD and neurotypical participants, respectively) received a single dose of 600 mg CBD or placebo (Pretzsch, Voinescu, et al., 2019). Participants were scanned 2 h after drug administration. CBD significantly increased spontaneous fluctuations in BOLD signal across both groups in the right fusiform gyrus (p=0.041) and in the cerebellar vermis (p=0.048) which post-hoc analyses demonstrated were driven by an effect in participants with ASD (p_{FWE}=0.029 and p_{FWE}=0.045; for fusiform and vermis clusters, respectively). Following a post-hoc seed-based analysis of functional connectivity in these regions of interest (ROIs), CBD was not shown to have a significant effect on vermal or fusiform functional connectivity with any other regions for neurotypical participants.

In another study (Grimm et al., 2018), 16 healthy male participants (demographic information not available) were included in a DBRCT crossover study with three separate arms and one-week intervals between scans. Participants were scanned 75mins following the consumption of either 10 mg THC, 600 mg CBD, or matched placebo. Seed-based analysis on four ROIs in the striatum, including the caudate (left and right) and putamen (left and right) were examined for connectivity with the rest of the brain. CBD administration led to an increase of fronto-striatal functional connectivity relative to placebo. Specifically, relative to placebo, those who were administered CBD showed a significant increase in connectivity between the right putamen seed (p<0.03), and three clusters in the right prefrontal cortex (PFC). However, the analysis did not establish directionality.

In a crossover DBRCT, 15 participants (mean [SD] age=24.1 [5.0] years, female = 60%) were administered either 600 mg CBD or placebo on separate days. The washout period was not reported, however, based on previous studies using the same sample, we can

infer that the washout period was ≥1-week. Regional CBF was measured at rest 3 h after drug administration (Bloomfield et al., 2020). Compared to placebo, CBD administration significantly increased CBF in the hippocampus (15 mL/100 g/min [CI 5.78–24.21, p=0.004]). CBF increased in the orbitofrontal cortex (p=0.019) by 10.04 mL/100 g/min (CI, 1.90–18.19). However, only the effect in the hippocampus survived Bonferroni correction.

Finally, a crossover DBRCT was used to examine the effects of CBD on the striato-cortical connectivity of 23 healthy participants administered 600 mg CBD or a matched placebo (≥1-week washout period) 150 minutes before an MRI scan (mean [SD] age 23.8 [4.3] years, female=53%)(Wall et al., 2022). CBD significantly decreased functional connectivity between subregions of the striatum, including limbic striatum activity with the lateral frontal cortex and the right hemisphere insula (p<0.05), and between the sensorimotor striatum and cerebellum (p<0.05). However, increased connectivity was observed between the associative striatum (regions receiving information from the associative areas of the cortex) and posterior parietal lobes (extending into the parieto-occipital sulcus and into the left posterior cingulate) (p<0.05).

Task based fMRI

Task-fMRI was the most common paradigm used to investigate the effect of CBD, with 14 of the 20 included studies employing task-fMRI. However, these 14 task fMRI studies comprised of data pertaining from three participant samples. In eight studies (Bhattacharyya et al., 2012; Bhattacharyya et al., 2014; Bhattacharyya et al., 2009; Bhattacharyya et al., 2010; Borgwardt et al., 2008; Fusar-Poli et al., 2010; Fusar-Poli et al., 2009; Winton-Brown et al., 2011), 15 male participants (mean [SD] age 26.7 [5.7]) were scanned using a crossover DBRCT, pseudo randomisation and a within group study design. Participants were either given THC 10 mg, CBD 600 mg or placebo 1 h prior to

a task-fMRI scan with 1-month intervals between scanning sessions. Furthermore, four studies (Bhattacharyya et al., 2018; Davies et al., 2020; Wilson et al., 2019) scanned one sample consisting of 19 healthy controls (HC) and 33 clinical high risk (CHR) for psychosis (mean [SD] age of 23.4 [4.8] and 24.3 [4.73]; 49% and 42% female, respectively). In these DBRCT parallel-arm studies, CHR participants were given 600 mg CBD or placebo, while HC did not receive any medication, 3 h prior to a scan. Finally, two more studies (Bloomfield et al., 2022; Lawn et al., 2020) examined 24 participants (mean [SD] age 23.6 [4.12], female = 50%), however, the study by Lawn et al. (2020) excluded one participant because they did not complete the MID task correctly (mean [SD] age 23.74 [4.2], female = 52%). In this crossover DBRCT, participants were given 600 mg oral dose of CBD or matched placebo and were scanned 150 minutes later with a 7-day washout period. Experimental tasks applied across all three participant samples included go/no-go (Bhattacharyya et al., 2010; Borgwardt et al., 2008), oddball tasks (Bhattacharyya et al., 2012; Bhattacharyya et al., 2014), verbal paired memory (Bhattacharyya et al., 2009; Bhattacharyya et al., 2010), fearful faces tasks (Bhattacharyya et al., 2010; Bloomfield et al., 2022; Davies et al., 2022; Davies et al., 2020; Fusar-Poli et al., 2010; Fusar-Poli et al., 2009), monetary incentive delay (Lawn et al., 2020; Wilson et al., 2019), and passive visual and auditory presentations (Winton-Brown et al., 2011). The results are summarised by experimental task here.

Go/No go and oddball tasks

In go/no go tasks, participants are required to respond to appropriate, target "go" stimuli and not respond to inappropriate, "no-go" stimuli (Rubia et al., 2006). The number of false responses to "no-go" indicates inhibition capacity. Go/no-go tasks can be combined with oddball tasks to measure participants' responses to novel stimuli, and ability to discriminate between salient or non-salient information. To do this, participants are presented

with a series of repetitive stimuli that are irregularly interrupted by novel stimuli (the oddball stimulus) thereby providing information about how participants respond to novelty. In an additional analysis of the same data, no significant drug effects on the combined go/no-go and oddball task performance, although there were different activation patterns on the 'no-go' relative to oddball trials between placebo and CBD conditions . Placebo administration revealed significant hyperactivation in the inferior and medial frontal gyri, the anterior insula, the anterior cingulate gyrus, and the supplementary motor area for 'no-go' compared to oddball condition (p<0.0025). CBD administration showed hyperactivation in middle and superior temporal gyrus, insula, and posterior cingulate gyrus for 'no-go' compared to oddball condition (p<0.0025). In comparison to placebo and for 'no-go' relative to oddball trials, CBD was associated with reduction in activity in the left insula and left superior and transverse temporal gyri (p<0.01). Bhattacharyya et al. (2012), in a secondary analysis (Borgwardt et al., 2008) of reported results from a go/no-go task with added oddball stimuli to account for the novelty of 'no-go'stimuli. Response latencies across all task conditions were significantly reduced in CBD groups compared to placebo (p=0.01) with a trend towards higher reduction in response latency to oddball than standard stimuli (p>0.01). During the task, CBD attenuated activation in clusters in the left medial PFC (p=0.01) and augmented activation in clusters in the right caudate, parahippocampal gyrus, insula, precentral gyrus, and thalamus (p=0.02), relative to placebo. In a follow-up analysis, seed clusters in the inferior frontal, dorsal, striatal and posterior hippocampal foci were selected as ROIs due to their involvement in processing deviant, rare or novel stimuli (Rubia et al., 2007) and were shown to be functionally connected to multiple brain regions during the oddball task (Bhattacharyya et al., 2014). CBD attenuated functional connectivity from the inferior frontal gyrus seed cluster with a cluster with peaks in the left anterior lobe of the cerebellum, left thalamus, and lingual gyrus (p<0.001) and attenuated functional connectivity with the

right insula (p=0.043). In the dorsal striatum seed cluster CBD augmented the functional connectivity of the left dorsal striatum with the body of the left caudate nucleus and the left inferior frontal gyrus (p=0.008) and attenuated functional connectivity with the left anterior cingulate and the left medial frontal gyrus (p=0.007). In the hippocampal seed cluster, functional connectivity of the left posterior hippocampal cluster with the left parahippocampal gyrus was augmented by CBD (p=0.0045), whereas the functional connectivity between the right parahippocampal gyrus, the left posterior cingulate, and the tail of the left caudate was attenuated in the CBD condition (p=0.004).

Verbal paired memory task

The verbal paired memory tasks used in the selected articles were adapted from the paired associate learning subtest of the Wechsler Memory Scale–Revised (Wechsler, 1987). This task primarily assesses episodic memory and induces activity in various areas associated with memory. Bhattacharyya et al. (2009) investigated the impact of CBD on mediotemporal and PFC activation during a verbal paired association task. Performance on the task was not significantly affected by treatment. However, CBD administration did modulate regions associated with memory consolidation and including insula, mediotemporal gyrus, lingual gyrus, precuneus, and precentral gyrus activation during repeated encoding (p<0.05) and the hippocampus during recall blocks relative to placebo (p=0.01).

A similar study incorporating the verbal paired memory task by task (Bhattacharyya et al., 2018), in which CHR participants who received CBD demonstrated greater activation in the precentral gyrus compared to placebo, coupled with reduced activation in the parahippocampus extending to the superior temporal gyrus and cerebellum (p=0.003) and precentral gyrus (p≤0.003) during encoding phases. Additionally, CHR participants who received CBD showed greater activation than placebo in regions including the medial frontal

gyrus, right precentral gyrus and adjacent cingulate gyrus, and the left cingulate gyrus and caudate body ($p \leq 0.002$) during the recall phase of the task (Bhattacharyya et al., 2018). Generally, these activation patterns signified a trend towards the normalisation of activity in these regions and resembling activation patterns observed in HC.

Fearful faces

During the fearful faces task, images of faces that exhibit varying levels of fearful expressions are presented to the participants to elicit activity associated with emotional processing and anxiety responses (Keedwell et al., 2005; Morris et al., 1996). Fusar-Poli et al. (2009) demonstrated that CBD reduced activity in the amygdala (p=0.0012) and the anterior and posterior cingulate cortex (p=0.00065 and p=0.000432 respectively) while participants were processing intensely fearful faces. Moreover, CBD reduced activity in the posterior lobe of the cerebellum for moderately fearful face stimuli compared to placebo. Concurrently recorded electrodermal psychophysiological responses also demonstrated reduced skin conductance response (SCR) fluctuations for intensely fearful expression stimuli (p<0.05) but not neutral or mildly fearful faces. This reduction of SCR fluctuations is a proxy for physiological arousal (Bach et al., 2010). The suppression of amygdala as well as the anterior cingulate covaried with the reductions in the number of SCR fluctuations (r=0.524; p=0.049) and, as reported in a later study (Bhattacharyya et al., 2010), a trend level anxiolytic effect as indexed by the State Trait Anxiety Inventory (r=0.551, p=0.017). Finally, the effect of CBD in modulating prefrontal-subcortical connectivity during emotion processing was investigated in a follow-up analysis (Fusar-Poli et al., 2010). CBD treatment led to significant disruption of forward connectivity between the amygdala and anterior cingulate observed in the placebo group while participants responded to fearful faces (p=0.035).

In a DBRCT parallel arm study, the effect of CBD on both the mediotemporal and striatal function (Davies et al., 2020) was examined. Subsequently, the relationship between mediotemporal function and serum cortisol level during the fearful faces paradigm was examined in the same sample but using different techniques (Davies et al., 2022). During the processing of fearful faces, CHR participants in the placebo condition experienced greater activity in parahippocampal gyrus ($p \leq 0.003$) and reduced activity in the striatum ($p \leq 0.002$) compared to HC. Moreover, CHR participants receiving CBD, versus those who received placebo, showed hypoactivation in the parahippocampal gyrus and amygdala ($p \leq 0.002$) and greater activation in the putamen ($p \leq 0.001$). In the healthy control group, higher cortisol induced by social stress led to lower parahippocampal activation ($p=0.023$). CHR participants who received placebo showed a statistically significant difference between parahippocampal activation and cortisol when compared to controls who did not receive any treatment ($p=0.033$). When CHR participants received CBD, they showed a similar relationship between cortisol and parahippocampal activation compared to healthy controls ($p=0.67$). Conversely, one study demonstrated no significant drug effects on brain responses to emotional faces from any category (open-mouth happy/angry/neutral) when comparing CBD to placebo administered groups(Bloomfield et al., 2022). However, this task did slightly differ from the previous face task as it used happy, fearful and neutral faces from the NimStim stimulus set (Tottenham et al., 2009).

Monetary Incentive Delay

Monetary Incentive Delay (MID) tasks present stimuli as cues that precede a monetary reward stimulus and can be used to measure the anticipation and feedback phases of reward processing (Knutson & Greer, 2008). One study demonstrated that CBD attenuated the observed hyper-activity in the left insula/parietal operculum in the CHR group which

occurred during reward and loss anticipation stages of the task (p=0.035) (Wilson et al., 2019). Additionally, (Lawn et al., 2020) revealed that a whole brain analysis resulted in insufficient statistical evidence to suggest that CBD modulated reward-related brain activity to a greater degree than placebo.

Passive listening and viewing of stimuli

Viewing and listening passively during an fMRI scan allows for the investigation of the neural correlates of visual and auditory processing (Brown et al., 2004). Winton-Brown et al. (2011) investigated the effect of CBD on visual (checkboards) and auditory processing (speech). During passive auditory processing, CBD increased activation in temporal cortex bilaterally extending medially to the insulae and caudally to the hippocampi and parrahipocampal gyri compared to placebo ($p \leq 0.007$). During auditory processing, CBD also reduced activation in posterolateral parts of the left superior temporal gyri-incorporating portions of supramarginal gyrus, the insula, and posterior middle temporal gyrus (p=0.002). During passive visual processing, CBD increased activation in the right occipital lobe, with the largest increases in the lingus gyrus, cuneus, and middle and inferior occipital gyrus (p=0.0065). This study demonstrates that CBD modulates a variety of regions during passive visual and auditory processing.

2.3.3 **Magnetic Resonance Spectroscopy (MRS)**

(Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, Heasman, et al., 2019) investigated the effects of 600 mg of CBD on GABA and Glx (glutamine/glutamate) (N=34 with ASD, mean [SD] age of 28.47 [6.55] years; N=17 neurotypical controls) measured 2 h after drug administration. In the basal ganglia (BG), CBD increased Glx in both groups (p_{uncorr}=0.070); in the DMPFC, CBD decreased Glx in

both groups (p_{uncorr}=0.055). There was a significant voxel × drug interaction effect (p_{uncorr}=0.033) in both groups, CBD increased Glx in the BG and decreased Glx in the DMPFC (this effect did not survive Bonferroni-correction). CBD increased GABA+ in the control group (surviving Bonferroni-correction (p_{corr}=0.004)). This group × drug interaction was largely driven by changes in the DMPFC (p_{uncorr}=0.038).

2.3.4 **SPECT**

The search revealed only one study that had utilised SPECT methodology. Crippa et al 2004 examined 400 mg of CBD versus placebo in a crossover DBRCT (N = 10, 7 day washout period) on resting blood flow using SPECT 110 mins post-drug administration (Crippa et al., 2004). ROIs associated with limbic and paralimbic networks were selected a priori. Compared to placebo, CBD decreased uptake of a radiotracer contrast in clusters in the medio portion of the left amygdala-hippocampal complex and uncus extending into the hypothalamus and the superior section of the left posterior cingulate gyrus (p<0.001). CBD also showed comparably increased activity in a cluster in the mediotemporal cortex including the left parahippocampal gyrus extending to include the left fusiform gyrus (p<0.001). CBD was also associated with decreased subjective anxiety and increased mental sedation (p<0.001) however there was no correlation between the mood scales and the ECD uptake.

2.3.5 **Quality Assessment**

Table 2.1 depicts the risk of bias as per each domain of the Cochrane RoB (Sterne et al., 2019). The randomisation processes for all studies were rated as having a *low risk of bias* (Domain 1). *Some concerns* were noted with respect to period and crossover effects (domain S) whereby some studies reported limited washout periods (e.g. 1-week (Bhattacharyya et al., 2009; Bhattacharyya et al., 2010; Bloomfield et al., 2022; Crippa et al., 2004; Grimm et al., 2018; Lawn et al., 2020; Wall et al., 2022) or did not provide sufficient information regarding

the washout period (Bloomfield et al., 2020). Although the half-life of CBD has previously been suggested to be up to 32 hours {Ujváry, 2016 #131} suggesting that 7 days may be a sufficient washout period, recent work showed that CBD has a long window of detection in plasma of up to 4 weeks post-drug administration (McCartney et al., 2022). No studies were considered to have risk of bias due to deviations from intended interventions (Domain 2) and there was low risk of bias due to missing data (Domain 3). Some potential concerns of bias in the outcomes that were measured (Domain 4) only were due to the potential of residual response due to test design. Concerns noted in the selection of reported results (Domain 5) were due to the majority of studies having no published pre-determined statistical analysis plan which may suggest potential vulnerability to selective analyses and reporting biases particularly relevant to fMRI data.

Table 2.1

Cochrane Risk of Bias

Reference	Domain 1; bias arising from the randomization process	Domain S; Risk of bias arising from period and carryover effects	Domain 2; bias due to deviations from the intended intervention	Domain 3; bias due to missing outcome data	Domain 4; bias in measurement of the outcome	Domain 5; bias in selection of the reported results	Overall risk of bias judgement
Crossover							
Bhattacharyya et al 2009	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
Bhattacharyya et al 2010	Low	Low	Low	Low	Low	Some concerns	Low
Bhattacharyya et al 2012	Low	Low	Low	Low	Some concerns	Some concerns	Low
Bhattacharyya et al 2015	Low	Low	Low	Low	Low	Some concerns	Low
Bhattacharyya et al 2018	Low	Low	Low	Low	Low	Low	Low
Bloomfield et al 2020	Low	Some concerns	Low	Low	Low	Some concerns	Low
Bloomfield et al 2022	Low	Low	Low	Low	Low	Low	Low
Borgwardt et al 2008	Low	Low	Low	Some concerns	Low	Some concerns	Some concerns
Crippa et al 2004	Low	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Fusar Poli et al 2009	Low	Low	Low	Some concerns	Low	Some concerns	Some concerns
Fusar-Poli et al 2010	Low	Low	Low	Low	Low	Some concerns	Some concerns
Grimm et al 2018	Low	Some concerns	Some concerns	Some concerns	Low	Some concerns	Some concerns
Lawn et al 2020	Low	Some concerns	Low	Some concerns	Low	Low	Some concerns
Pretszch et al 2019	Low	Low	Low	Low	Low	Low	Low
Pretzsch 2019	Low	Low	Low	Low	Low	Low	Low
Wall et al 2022 (Study 1)	Low	Low	Low	Low	Low	Low	Low
Wall et al 2022 (Study 2)	Low	Low	Low	Low	Low	Low	Low
Winton-Brown et al 2011	Low	Low	Low	Some concerns	Low	Some concerns	Some concerns
Parallel							
Davies et al 2020	Low	Low	Low	Low	Low	Low	Low
Davies et al 2022	Low	Low	Low	Low	Low	Low	Some concerns
Wilson et al 2019	Low	Low	Low	Low	Low	Some concerns	Some concerns

2.4 **Discussion**

This review synthesised neuroimaging literature examining the effects of CBD on neurobiology in healthy subjects and to further consider whether CBD may have promise in the management of AUD. We identified 20 neuroimaging studies that examined CBD in a healthy sample since 2004 which revealed broad modulatory effects across several brain regions and networks. Below we synthesise these results according to neuroimaging modality and then in light of converging neurobiological correlates associated with addictive behaviours.

Functional MRI was by far the most common neuroimaging modality accounting for 90% of the studies reviewed. Resting state fMRI was the focus of four studies presented in this review. Three studies demonstrated that CBD significantly modulated functional connectivity (Grimm et al., 2018) (Wall et al., 2022) and CBF (Bloomfield et al., 2020). CBD was shown to increase fronto-striatal coupling, from a seed in the right putamen to the PFC (Grimm et al., 2018); as well as increasing connectivity between "associative" striatum and parietal regions (Wall et al., 2022). Furthermore, CBD was observed to increase CBF to the hippocampus (Bloomfield et al., 2020). CBD was also demonstrated to produce minor decreases in functional connectivity in limbic and sensorimotor regions (Wall et al., 2022). However, one study showed non-significant differences between CBD and placebo on whole brain BOLD activity (Pretzsch, Voinescu, et al., 2019). Fourteen task-based fMRI articles, published between 2008 – 2022, used task paradigms to examine reward processing, salience attribution, emotion regulation and executive functioning following CBD administration. During go/no-go and oddball tasks, which tests response inhibition and salience attribution, CBD was found to reduce activity in the left insula and left superior and transverse temporal gyri (Borgwardt et al., 2008). Further, while reducing response latencies, CBD was demonstrated to attenuate activation in left medial PFC and augment activation in right caudate, parahippocampal gyrus, insula, precentral gyrus

and thalamus (Bhattacharyya et al., 2012). Increased fronto-striatal connectivity and reduced mediotemporal-prefrontal connectivity was also reported during attentional salience tasks following CBD administration (Bhattacharyya et al., 2014). During a learning and memory, verbal paired task, CBD was observed to modulate insula, midtemporal gyrus, lingual gyrus, precuneus, and precentral gyrus during repeated encoding phases and modulated hippocampus during recall. However, none of these results reached threshold for less than one false positive cluster (Bhattacharyya et al., 2009). During an emotional regulation and processing task, CBD administration led to a lower number of SCR fluctuations for intensely fearful stimuli, but not neutral or mildly fearful stimuli. This lower SCR covaried with reduced activity in the amygdala and anterior and posterior cingulate cortex (Fusar-Poli et al., 2009). Additionally, CBD was found to disrupt forward connectivity between the amygdala and anterior cingulate while participants responded to fearful faces (Fusar-Poli et al., 2010). This result is supported by another study by (Davies et al., 2022) whereby CBD administration decreased activation in the parahippocampal gyrus and amygdala and increased activation in the putamen during emotion processing in a CHR sample, and also normalised the relationship between cortisol and parahippcampal activation. This effect of CBD on brain activity during emotional processing was not replicated in a later study (Bloomfield et al., 2022), however, this study did not yield a significant task effect in response to neutral vs fear faces unlike previous studies which may explain the conflicting results. Functional MRI during MID tasks, which probe anticipation and feedback of reward processing, yielded mixed results. While CBD slowed reaction times in one study with attenuation of the hyperactivation of left insula/parietal operculum in a CHR sample (Wilson et al., 2019), another study failed to observe any significance differences in whole brain modulation (Lawn et al., 2020). Only two other studies were identified by the search strategy, focusing on neurometabolie presence and cerebral blood flow. One study used MRS (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers,

Ajram, Ivin, Heasman, et al., 2019). This study demonstrated that CBD modulates primary inhibitory and excitatory neurometabolites by increasing the inhibitory neurotransmitter GABA+ in BG and DMPFC while increasing the excitatory Glx (glutamate + glutamine) in the BG but decreasing in the DMPFC relative to placebo-treated individuals. Additionally, one SPECT imaging study satisfied the inclusion criteria to be included in this review (Crippa et al., 2004). These authors found that CBD decreased cerebral blood flow to clusters in the medio portion of the left amygdala-hippocampal complex and uncus extending into the hypothalamus and the superior section of the left posterior cingulate gyrus. CBD was also shown to increase activity in a cluster in the mediotemporal cortex including the left parahippocampal gyrus extending to include the left fusiform gyrus.

These results suggest that CBD may modulate certain neurobiological correlates of addictive behaviors. There is a well-researched link between chronic heavy alcohol use impairs reward processing, salience attribution, emotion regulation and executive functioning (including inhibition control, working memory and self-monitoring) through the perturbation of various brain networks implicated in the development and maintenance of AUD (Koob & Volkow, 2016). Some of these networks include the mesocorticolimbic (MCL), salience, fronto-striatal, and the limbic networks (Koob & Volkow, 2016). These networks rely on various neurotransmitter systems including dopamine, opioid, endocannabinoid, serotonin, GABA, and glutamate systems. It has previously been suggested that CBD may normalise this perturbed neurocircuitry and subsequently support positive behavioural changes (Fagundo et al., 2013). Here, neuroimaging findings support the notion that CBD may modulate neurocircuitry implicated in the maintenance of AUD.

Mesocorticolimbic and salience attribution networks, which are responsible for reward processing and salience attribution, are functionally and anatomically linked (McCutcheon et al., 2019). The cannabinoid 1 receptors (CB_1R), of which CBD is a negative

allosteric modulator (NAM), are commonly located on the presynaptic terminals of dopaminergic neurons (Fitzgerald et al., 2012; Laprairie et al., 2015). Therefore, CBD may normalise the increased reward and salience attribution to alcohol associate cues by down regulating dopaminergic signalling in both the MCL and salience network. Evidence of this can be seen through CBD's effect on the insula which is a major junction for both the mesolimbic (McCutcheon et al., 2019) and salience networks (Goulden et al., 2014; Peyron et al., 2000; Seeley et al., 2007). Various studies presented in this review suggest that CBD may attenuate both insula activity (Goulden et al., 2014; Peyron et al., 2000; Seeley et al., 2007; Wilson et al., 2019). Various studies presented in this review suggest that CBD may attenuate both insula activity (Bhattacharyya et al., 2012; Wilson et al., 2019) and functional connectivity (Wall et al., 2022). Thus, CBD may act to normalise hyper-signalling in the insula found in those with AUD, reducing both salience attribution and the reward processing. Indeed, the insula has been shown to have a major role in interoception (Critchley, 2004) and patients with lesions in the insula have been observed to show attenuated craving and abstinence from cigarettes (Naqvi et al., 2007). Furthermore, CBD was shown to modulate the hypothalamus, the amygdala, the thalamus, the anterior cingulate cortex, and the hippocampus which may be an indication that CBD may not only modulate the salience and reward processing but also emotion regulation.

Prolonged alcohol use can commonly lead to negative emotional states and impairment in limbic neurocircuitry and emotional processing (Jansen et al., 2019; Oscar-Berman & Marinković, 2007). Several studies have observed heightened activation in the amygdala in those with AUD relative to controls during fMRI affect reactivity tasks (Gilman et al., 2008; O'Daly et al., 2012). Across the studies included in this review, CBD induced modulation of the hippocampus during recall (Borgwardt et al., 2008); attenuation of the amygdala and ACC during fearful faces paradigms (Bhattacharyya et al., 2010; Bloomfield et

al., 2022; Davies et al., 2022; Davies et al., 2020; Fusar-Poli et al., 2010; Fusar-Poli et al., 2009); decreased connectivity between the amygdala and the anterior cingulate during emotion processing (Fusar-Poli et al., 2010); normalisation of parahippocampal activity during encoding processes (Bhattacharyya et al., 2018) and fear processing (Davies et al., 2020; Fusar-Poli et al., 2010); and the relationship between cortisol and parahippocampal activity in CHR participants during fear processing (Davies et al., 2022). These results support the idea that CBD administration demonstrates interactions with limbic, particularly amygdala and ACC, activity as well as the functional connectivity between the amygdala and ACC. This modulation of the limbic network may be due to a number of mechanisms such as NAM action on CB_1Rs (Campos & Guimarães, 2008; Russo et al., 2005), alterations 5-HT1A in the amygdala and hippocampus and/or the release of pro-opiomelanocortin, corticotropin-releasing factor and glucocorticoid receptor gene expression following acute stress exposure (Viudez-Martínez, García-Gutiérrez, Fraguas-Sánchez, et al., 2018; Viudez-Martínez, García-Gutiérrez, et al., 2018b).

Finally, CBD may induce improvements in reward processing, salience attribution and emotion regulation due to top-down control through increased fronto-striatal functional connectivity. In this review, several studies demonstrated increased fronto-striatal connectivity following CBD administration (Bhattacharyya et al., 2014; Grimm et al., 2018) (Wall et al., 2022) and therefore, improved executive functioning. In the context of AUD, deficits in executive functioning have been thought to be due to deficits of GABAergic signalling from the PFC (George et al., 2012). Further, glutamatergic projections from the PFC to the VTA in rats controls dopaminergic activity in the mesocortical pathway (Geisler & Wise, 2008). This excitatory signalling to the VTA has been suggested to be involved in increasing conditioned behaviour and incentive salience in the presence of alcohol related cues (Lapish et al., 2006). To this degree, one study demonstrated CBD to increase

GABAergic but decrease glutaminergic signalling from the DMPFC (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, Heasman, et al., 2019) which may therefore be relevant to alcohol recovery by improving both executive functioning and reducing cue induced craving and conditioned alcohol-seeking behaviour.This review identified several limitations in the studies that have utilised neuroimaging methods to examine the effect of CBD on the brain. Firstly, with regards to fMRI studies, there was a lack of consistency of imaging tasks and substantial methodological heterogeneity across the studies which therefore limit conclusions regarding CBD-induced neurobiological modulations to be relatively task specific. In addition, the 20 studies found in our search were obtained from only 6 different participant samples following completion of long neuroimaging protocols (see the outer ring of the sunburst plot, Figure 2). Thus, it is possible that the literature base may be subject to some bias due to sample specific effects and limited heterogeneity. Comprehensive and longer neuroimaging protocols may be vulnerable to task fatigue (Wylie et al., 2020) and poorer data collection due to movement artifacts and scanner drift (Kopel et al., 2019). Moreover, there was diversity with regards to the timing between drug administration, scan time and also washout periods between sessions in crossover studies. In addition, the lack of pre-published protocols may lead to selective analyses which may be particularly relevant for fMRI studies. Additionally, as a meta-analysis could not be conducted due to the number of outcome variables, there may be involuntary bias in reporting results which were unintentionally favoured. Finally, while this review provides evidence for CBD's modulation of neurocircuitry implicated in AUD-related behaviours, certain results suggest some non-significant results (Bloomfield et al., 2022; Lawn et al., 2020) and some are conflicting (Bloomfield et al., 2020; Crippa et al., 2004). Further, as results presented here may not translate to effects in AUD clinical profiles, directly examining the effect of CBD in AUD participants is required before determining the mechanisms by which CBD

may function as a therapeutic use in this population. Recommendations for future research include publication of protocols to reduce deviation from protocol bias and selective analyses, optimised study design to reduce participant fatigue, ensuring a sufficiently long washout period between the crossover sessions, and consistent drug-scan administration time relative to peak plasma CBD concentrations.

In conclusion, previous research suggests that CBD may affect salience, reward, emotion generation and regulation and executive control (including inhibition control, working memory and self-monitoring) processes. These processes are highly relevant to alcohol seeking behaviours, suggesting that CBD may have potential in the management of alcohol use disorder. Although not supported by all the studies presented, the majority of the neuroimaging literature presented in this systematic review suggests that CBD may normalise these processes through its effect on mesocorticolimbic, limbic, salience and fronto-striatal signalling. Various limitations may explain some of the discrepancy in results including heterogenous methodological designs, the same or similar participant samples being used across different studies, variable drug administration times, possible carryover effects and participant fatigue due to long imaging protocols. Given the relevance of the networks affected by CBD in this review in alcohol seeking behaviour and relapse, research into the effect of CBD on brain and behaviour in populations with AUD to determine any potential role for management is warranted.

Chapter 3: The Neurobehavioural Effects of Cannabidiol in Alcohol Use Disorder: Study Protocol for a Double-blind, Randomised, Cross-over, Placebo Controlled Trial

Abstract

Current treatments for alcohol use disorders (AUD) have limited efficacy. Recently, Cannabidiol (CBD) has been examined in a multitude of clinical settings. Preclinical and clinical results suggest that CBD might be particularly well suited for the treatment of AUD and may reduce alcohol cue and stress-induced craving and alcohol seeking. This study aims to investigate this new pharmacotherapy with a particular focus on neurobiological and physiological indicators of craving. *Methods:* In this double-blind, within-subject, randomized, placebo-controlled, cross-over study, non-treatment seekers will be randomly allocated to three days of four 200mg CBD gel capsules (800 mg/day) or placebo, with an 18-day washout period. Cognitive, clinical, and neuroimaging assessments will be completed during these three days. The CBD and placebo assessments will be compared. The primary outcomes are i) BOLD signal as a proxy for regional activity during a cue reactivity and a fear response task measured with functional magnetic resonance imaging (fMRI), ii) heart rate variability and skin conductance levels as a proxy for psychophysiological responses to alcohol stimuli. The secondary outcomes are: i) neurometabolite levels (γ-Aminobutyric acid, ethanol, glutathione, and glutamate+glutamine (combined signal)) using magnetic resonance spectroscopy (MRS); ii) functional connectivity using resting state fMRI (rsfMRI); iii) executive functioning task results; iv) clinical outcomes such as craving, anxiety, and sleep. *Discussion:* This study will improve the understanding of the mechanisms of action of CBD and provide early signals of efficacy regarding the therapeutic potential of CBD in the treatment of alcohol use disorder.

ClinicalTrials.gov Identifier: NCT05387148.

3.1 **Introduction**

Alcohol use disorder (AUD) is a chronic and relapsing disorder that is a major public health concern due to the associated medical, psychological, and social sequelae. Although pharmacological is now a widely accepted approach to the management of AUD, current treatments have modest efficacy whereby new treatment approaches are required (Morley, Perry, et al., 2021). Cannabidiol (CBD), a cannabis compound that lacks any intoxicating effects, has been suggested as a potential pharmacotherapeutic in a multitude of clinical contexts (Gaston et al., 2017; F. M. Leweke et al., 2012) and may have therapeutic potential in managing AUD. CBD inhibits fatty acid-binding proteins (FABP) catabolism of anandamide (AEA) and reduces cellular uptake of endocannabinoids (Elmes et al., 2015). Previous research suggests that the endocannabinoid system may underlie psychiatric and substance use disorders (Mandolini et al., 2018). Indeed, the endocannabinoid system has been found to be perturbed following chronic heavy alcohol use (Hungund & Basavarajappa, 2004) and is implicated in reward processing (Paulus et al., 2022).

CBD modulates the endocannabinoid system through negative allosteric modulation of the cannabinoid 1 receptor (CB1R (Corroon & Felice, 2019). Preclinical research has demonstrated that CBD not only modulates the endocannabinoid system but also serotoninergic, dopaminergic, glutamatergic, and γ-aminobutyric acidergic (GABA) signalling (Ibeas Bih et al., 2015; Scopinho et al., 2011)). This poly-pharmaceutical action of CBD may explain the various therapeutic properties including anti-seizure (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016), anxiolytic (Berger et al., 2022; Bhattacharyya et al., 2018; Borgwardt et al., 2008; Devinsky, Marsh,

Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019), anxiolytic (Berger et al., 2022; Bhattacharyya et al., 2018; Borgwardt et al., 2008; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019), neuroprotective (Crippa et al., 2018), anti-inflammatory and antioxidant effects of CBD (Mandolini et al., 2018; Mechoulam et al., 2007; Ren et al., 2009). Preclinical research has demonstrated that CBD administration reduces stress and drug cue alcohol reinstatement, voluntary alcohol consumption, withdrawal symptoms, and alcohol-induced relapse behaviours (Gonzalez-Cuevas et al., 2018; Viudez-Martínez, García-Gutiérrez, et al., 2018a, 2018b). In clinical samples, CBD has been shown to reduce nicotine consumption in tobacco smokers (Morgan et al., 2013) and cue-induced craving and anxiety in opioid dependent individuals (Hurd et al., 2019). These results suggesting that CBD may modulate cue-elicited motivational urges and drug-seeking behaviours (de Carvalho & Takahashi, 2017; Parker et al., 2004).

Cue-induced craving is a substantial contributor to relapse (Vafaie & Kober, 2022). fMRI task-based studies that elicit craving implicate specific regions leading them to be considered to be associated with cue and stress-induced craving. These regions include the posterior insula, posterior and anterior cingulate, medial prefrontal areas, and the striatum which, when more active in cue-inducing imaging tasks, have also been associated with increased rates of relapse (Sinha & Li, 2007). Various studies have demonstrated that CBD modulates activity in these regions in samples of healthy participants (Hurzeler et al., 2023). While several trials investigating the potential therapeutic properties of CBD for AUD have recently been completed or are ongoing (NCT04873453 and NCT03252756), the effects of CBD on regional brain activity in those with AUD has yet to be comprehensively elucidated. Additionally, while cue induced-craving and drug cue-induced changes to heart rate and salivary cortisol have been shown to be modulated by 400mg CBD in a sample of heroin

users (Hurd et al., 2019) these experimental paradigms have not been explored in AUD samples.

We aimed to conduct a double-blind, within-subject cross-over, randomised trial in individuals with AUD to determine the effect of CBD versus placebo on i) blood-oxygen-level-dependent functional magenetic resonance imaging (BOLD fMRI) and ii) psychophysiological (heart rate variability and skin conductance) responses to alcohol and threat stimuli. Secondary objectives include examination of the effect of CBD versus placebo on i) neurometabolite levels using magnetic resonance spectroscopy; ii) functional connectivity using resting state fMRI (rsfMRI); iii) self-reported alcohol craving, mood, and sleep; and iv) cognitive functioning.

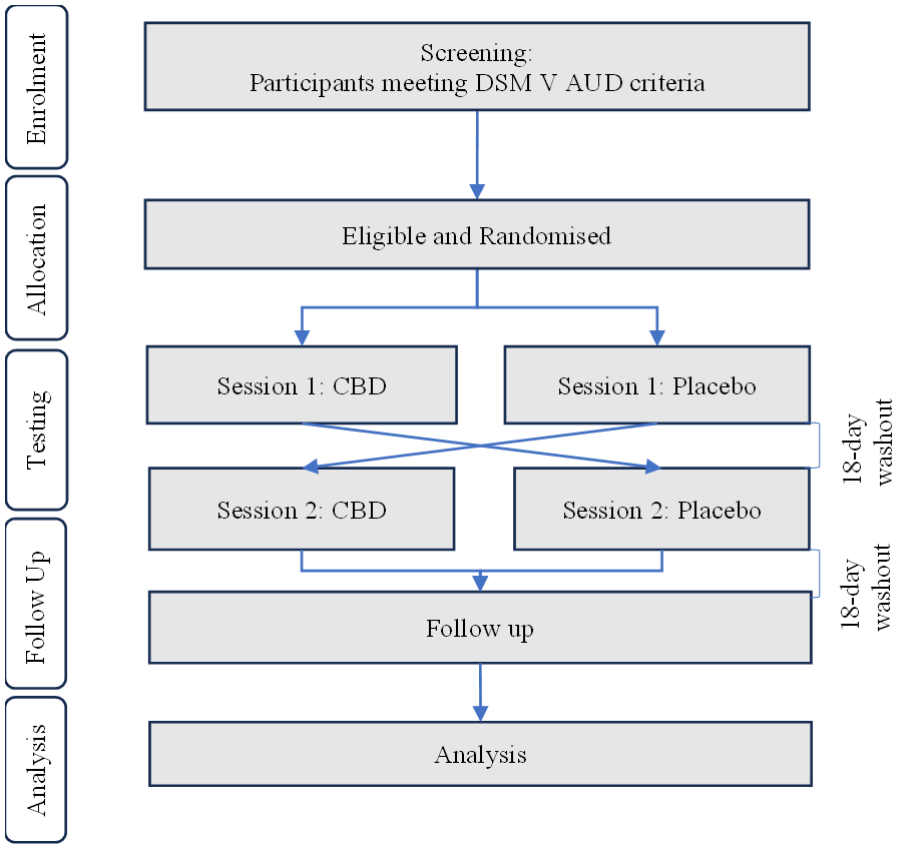
3.2 **Methods**

3.2.1 **Design**

This single-center, within-subject, cross-over, double-blind, randomised trial with 3 days of 800 mg CBD/matched placebo will be conducted at the Royal Prince Alfred Hospital (RPAH) in Sydney, NSW, Australia. 22 non-treatment-seeking individuals who meet DSM-V criteria for current alcohol use disorder, but are not engaged in any form of AUD treatment currently (< 60 days) nor seeking treatment for AUD at the time of recruitment, will be recruited to participate in the research. Participants will be recruited through clinical referral from treating physicians, nurses, and psychologists among RPAH outpatients as well as via flyers/community advertisements and through websites. On the second day of each arm, participants will be scanned 1.5 hours after treatment administration, corresponding with C_{max} occurring between 0 and 4 hours after CBD administration. Further, a conservative washout period of 18 days was selected between each testing session and follow-up as the half-life of CBD following chronic administration occurs between 2–5 days (Millar et al., 2018). More recent literature suggests that on average a 13-day washout period would reduce CBD plasma levels to 'zero' at a 300mg dose (McCartney et al., 2023). Figure 3.1 indicates the flow of participants throughout the study while Table 3.1 describes the spirit schedule of specific measures through the duration of the study.

Figure 3.1

CONSORT Flow Diagram



Note. Figure provides a visual representation of the intended progression of participants through each stage of the DBRCT including enrolment, allocation, follow-up and analysis, in adherence with CONSORT (Consolidated Standards of Reporting Trials) guidelines.

Table 3.1

Spirit Schedule

Study Period									
	Prescreen/ Screen	Allocation	Post-allocation						
Timepoint	T0		S1			S2			Follow-up
			T1	T2	T3	T1	T2	T3	
Enrolment									
Eligibility Screening	X								
PIS/Consent	X								
Randomisation		X							
Interventions									
CBD			←	—	→	←	—	→	X
Placebo			←	—	→	←	—	→	X
Assessments									
Background and demographics*			X						
Questionnaires on mood, sleep, and craving+			X			X			X
Timeline follow back			X			X			X
Ballon analogue risk task, Columbia card task				X			X		
Trail making task				X	X		X	X	
Bloods	X		X	X		X	X		
fMRI, rs-fMRI, MRS ^				X			X		
Visual analogue scale, Alcohol urge questionnaire			X	X	X	X	X	X	
Number Letter task, N-Back, Stroop					X			X	
Psychophysiology Cue Reactivity^					X			X	
Sleep ecological momentary assessment			X	X	X	X	X	X	
Medical Review									X

Note.

***** Demographics, medical history, personal and family history of AUD, and alcohol treatment history.

+ Timeline follow-back method (TLFB), Alcohol Dependence Scale (ADS), Penn Alcohol Craving Scale (PACS), the Drinker Inventory of Consequences (DrInC-L), Tension Reduction Alcohol Outcome Expectancies (TRAE), Depression Anxiety symptom scale DASS, Insomnia Severity Index (ISI), Alcohol Abstinence Self-Efficacy (AASE), consequences of drinking (DrInC); Obsessive Compulsive Drinking Scale (OCDS), Behavioural approach and avoidance (BIS/BAS), Intolerance of Uncertainty Scale (IUS), Impulsivity Behaviour Scale (UPPS), expectancy of alcohol effects and urges to drink (AUQ), Positive and Negative Affect Schedule (PANAS), Visual Analogue Scales (VAS) assessing alcohol craving, thirst, and anxiety.

^ See details in measures section

Leeds Sleep Evaluation Questionnaire (LSEQ), Consensus Sleep Diary (CSD-M) through the SEMA3 application.)

3.2.2 **Inclusion and Exclusion Criteria**

Inclusion criteria: a) Male and female patients between the ages of 18 and 65 meeting DSM-V criteria for current alcohol use disorder; b) Adequate cognition and English language skills to give valid consent and complete research interviews; c) A blood alcohol concentration (BAC) reading of 0.00; d) Must have a stable residence and be able to identify an individual who could locate subject if needed; e) Women of child-bearing potential must be non-lactating, using birth control and have a negative pregnancy test; f) Willingness to give written informed consent.

Exclusion criteria: a) Active major psychological disorder associated with psychosis, significant suicide risk; b) Pregnancy or lactation - Women shall be advised to use reliable contraception for the duration of drug therapy and a urine pregnancy test will be performed where necessary; c) Dependence on any substance other than nicotine; d) Diagnosis of epilepsy, and/or current use of anti-epileptic drugs (AED); e) Liver failure with jaundice or prolonged INR above 1.3; f) Medical complications such as liver failure, cardiac ischemia or conduction abnormalities, renal impairment or unstable elevated vital signs (systolic blood pressure > 180, diastolic blood pressure > 120 or heart rate > 150); g) Severe cognitive impairment or insufficient English or literacy to complete study processes; h) Concurrent use of drugs potentially exacerbated by CBD via CYP3A4, CYP2C9 and CYP 2C19 including cardiac medication (eg betablockers, calcium channel blockers and statins), macrolides and recent antihistamine use; i) Claustrophobia; j) Extreme obesity; k) Previous brain surgery; l) Ever employed as a machinist, a welder or a metal worker; m) Metal items such as

pacemakers; aneurysm clips in the brain; metal dental implants; metallic fragments in the eye or anywhere else; insulin pump; metal implants; hearing aid or a prosthetic device.

3.2.3 **Randomisation And Allocation Concealment**

Participants will be randomised to receive 3 days of 800mg CBD or matched placebo arms in a cross-over, double-blind, randomised trial. A random allocation procedure will be conducted by computer-generating a random table, using R (A programming language for statistical computing; RStudio Team (2020)). This randomisation table will be applied using REDCap (Research Electronic Data Capture; a secure web application for building and managing online surveys and databases) to randomly allocate participants to either treatment group. Pharmacists at the RPAH will be tasked with using REDCap's randomisation module to randomise participants to either active medication or placebo and subsequently dispense the medication. Prior to administration, participants will be required to detail any non-prescribed medication use or substance use each session before dispensing the medication by the research nurse.Researchers, clinicians, and participants will all be blinded to treatment allocation. In the event of a medical emergency that requires knowledge of the treatment condition in the opinion of the treating clinician at the time, the investigators will be able to contact the 24-hour telephone service at the NHMRC Clinical Trials Centre to break the randomisation code for that individual.

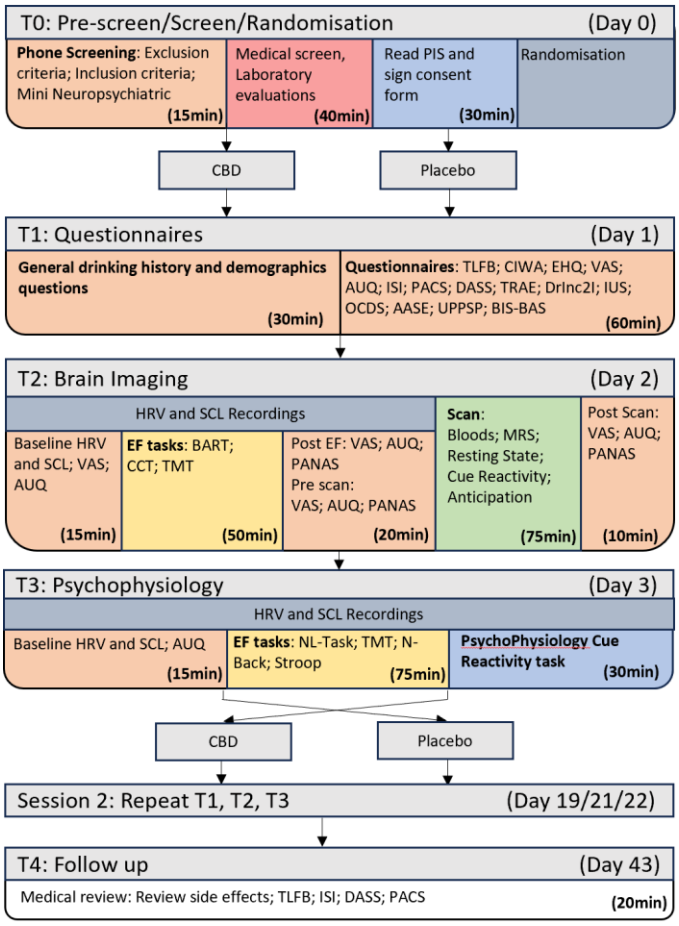
3.2.4 **Procedure**

Participants will participate in a screening phase (over the phone and in-person medical screening), 6 days of in-person testing (T1-T3, repeated in a cross-over fashion), and one day of follow-up questions over the phone. T1 consists of a variety of questionnaires; T2 will include physiological recording during executive functioning tasks as well as a brain

scan; T3 will consist of physiological recordings during executive functioning tasks and a cue-induced craving task. Specific measures are detailed in the measures section of this protocol paper. Figure 3.2 demonstrates the overview of the procedure including when each task will be conducted. The order of days occurs from top to bottom (T0, T1, T2, T3, washout, crossover repeat, T4) while the order of procedures occurs from left to right. Additionally, a blood sample will be taken at the commencement of T1 and T2 followed by drug administration occurring at the commencement of each testing day (T1, T2, T3).

Figure 3.2

Study Protocol Flowchart



Note. Timeline follow back (TLFB); Clinical Institute Withdrawal Assessment Alcohol Scale (CIWA); Edinburgh Handedness Inventory (EHQ); Visual analogue scale (VAS); Alcohol urge questionnaire (AUQ);

Insomnia severity index ISI; Penn Alcohol Craving (PACS); Depression anxiety symptom scale (DASS); Tension Reduction Alcohol Expectancy Questionnaire (TRAE-Q); Drinker inventory of consequences (DrInc2I); Intolerance of uncertainty scale (IUS); obsessive compulsive drinking scale (OCDS); Alcohol Abstinence Self-efficacy scale (AASE); Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency (UPPS-P); Behavior Inhibition System and Behavior Approach System Scale (BIS-BAS); Heart Rate Variability (HRV); Skin conductance level (SCL); Ballon Analogue risk task (BART); Columbia card task(CCT); Trail making task (TMT); Positive and Negative Affect Schedule (PANAS); Magnetic Resonance spectroscopy (MRS); Number letter task (NL-Task).

3.2.5 **Pharmacotherapy schedule**

Cannabidiol: Study medication will be administered onsite under observation in the form of four 200 mg, CBD oral soft gel capsules (manufactured by Linnea Natural Pharma Solutions) for a total dose of 800 mg per day, over three days. Medications will be delivered to the clinical trials pharmacy with accounting and reconciliation according to S4 principles according to established procedures. All dosage forms will be stored at 25°C and in accordance with S4 poisons schedule guidelines. The pharmacist will be provided with access to the REDCap randomisation module and dispense the study medication without revealing the allocation to the participants or the study coordinator. The placebo capsules will be identical in appearance, taste, and composition except for the active ingredient of pure CBD. 800 mg capsules of CBD or placebo will be administered in the laboratory under supervision/observation once daily for 3 consecutive days starting on the first test session day, session 1 (T1). and patients will be monitored for side effects through each testing day.

This study will conduct the majority of testing under observation and participants will be monitored closely for adverse events during these testing days. Open ended questions regarding AEs will be assessed each day before dosing by the physician and nurse and systematic assessment for adverse events will be completed on the first day of the second arm

(day 19) and at follow-up (day 43). If necessary, physician and/or psychiatric consultations will be arranged. Participants will be encouraged to call the investigator and/or the study physician if they experience any unusual or distressing symptoms during study involvement. Participants who experience mild or moderate side effects but wish to stay on the medication can have their dose reduced based on physician judgment. At the end of the study, participants will be asked to report any non-prescribed medication or substance use. In the event of a medical emergency where the participant needs to be unblinded, the lead investigator will be notified. Unblinding may occur by contacting the pharmacy to access the randomisation list. If a participant is unblinded, the details,date and reason for breaking the blind will be clearly documented.

3.2.6 **Measures**

Phone Pre-Screening: Before being invited to participate in the study, individuals who show interest in participating will be informed about the study in detail and initially screened for exclusion and inclusion criteria over the phone by the research team using a semi-structured interview. The study coordinator will then conduct further screening by administering the MINI Neuropsychiatric Interview (D.V. Sheehan et al., 1998).

Medical/laboratory tests: Participants will be screened for inclusion and exclusion criteria face to face and tested for objective measures of alcohol use. Firstly, participants will be assessed on medical history, including a history of any problems with alcohol withdrawal or risk factors for serious alcohol withdrawal such as unstable medical illness, concurrent medications, and psychiatric diagnoses. The physical examination at baseline will include blood pressure and cardiovascular observations, signs of alcohol-related liver disease, a brief neurological examination, and a mental state examination. Laboratory tests at baseline include urinalysis, urine toxicology, full blood count, liver function tests (LFTs: bilirubin,

gGT, ALP, AST, ALT, albumin, protein), coagulation tests (INR, APTT), creatinine and phosphatidyl ethanol (PEth). Bicarbonate and electrolytes are checked to screen for metabolic acidosis. Blood samples will be collected and stored for biochemical, genetic, and molecular analysis in the current trial and for future use in ancillary studies.

Blood sampling: Blood sampling will be conducted at additional periods throughout testing for peripheral markers of inflammation, cortisol, and CBD plasma concentration.

Questionnaires and assessment tools: A set of general demographics and background drinking history questions (30 minutes) will be administered including demographics, medical history, personal and family history of AUD, and alcohol treatment history, as obtained in previous work (Morley et al., 2014; Morley, Baillie, et al., 2013; Morley, Leung, et al., 2013; Morley et al., 2006). Additionally, Recent (last 28 days) alcohol consumption (frequency/quantity) assessed by the Timeline follow-back method (TLFB; (Sobell et al., 1988); (ii) severity of alcohol dependence assessed by the Alcohol Dependence Scale (ADS; (Skinner & Allen, 1982)); (iii) craving for alcohol measured by the Penn Alcohol Craving Scale (PACS; (Flannery et al., 1999); (iv) the Drinker Inventory of Consequences (DrInC-L: baseline; R: follow-up; (Miller et al., 1995) provides a measure of the social, physical, and emotional consequences of alcohol use; (v) the Tension Reduction Alcohol Outcome Expectancies (TRAE) measures expectancies regarding the outcome of alcohol use (Kushner et al., 1994); (vi) the Depression Anxiety Symptom Scale (DASS) measures the severity of symptoms of depression, anxiety and stress (Lovibond & Lovibond, 1995); (vii) sleep problems are assessed by the Insomnia Severity Index (Bastien et al., 2001); (viii) the Alcohol Abstinence Self-Efficacy (AASE) scale measures perceived self-efficacy (DiClemente et al., 1994); (ix) Obsessive Compulsive Drinking Scale (Anton et al., 1995) measuring drinking obsessionally and compulsivity; (x) the behavioural approach and avoidance (BIS/BAS; (Carver & White, 1994); (xi) the Intolerance of Uncertainty Scale

(IUS; (Freeston et al., 1994) measuring reactions to ambiguous situations (xii) the Urgency Premeditation Perseverance Sensation-Impulsivity Scale (Whiteside & Lynam, 2001) measuring impulsivity; (xiii) expectancy of alcohol effects and urges to drink (AUQ; (Bohn et al., 1995); (xiv) the Positive and Negative Affect Schedule measuring positive and negative mood states (PANAS; (Watson et al., 1988)); (xv) Visual Analogue Scales (VAS) assessing alcohol craving, thirst, and anxiety. Additionally, Participants will also be required to answer 10 questions from the Leeds Sleep Evaluation Questionnaire (LSEQ; (Parrott & Hindmarch, 1980) and 17 questions from the Consensus Sleep Diary (CSD-M; (Carney et al., 2012) administered through the SEMA3 application (Koval et al., 2019).

Cognitive and Executive Function

A suite of cognitive tasks will be used to assess cognitive capacity including the: (i) Number–letter task (adapted from (Rogers & Monsell, 1995)) to assess working memory; (ii) Stroop task (Stroop, 1935) to assess inhibitory control; (iii) N-back task (Braver et al., 1997; Jonides et al., 1997; Kirchner, 1958) to assess working memory capacity; (iv) Balloon Analogue Risk Task. (BART, (Lejuez et al., 2002)) to assess self regulation and adaptive risk-taking; (v) Columbia Card Task, Hot Version (CCT; (Figner et al., 2009)) assessing inhibition, working memory updating, task-set switching, and attention; (vi) Trail making test (TMT: Part A and B (Reitan & Wolfson, 1985)) assessing set-shifting flexibility, attention, and inhibition.

Imaging

MRI data will be acquired on a 3Tesla GE Discovery 750 scanner (GE Healthcare, Milwaukee, Wisconsin, USA), using a 32-channel phased array head coil. The imaging order

will be as follows i) 267 sec of T1-weighted (T1-w) structural scan, ii) 544 sec of MRS to determine levels of GABA+, glutathione, and ethanol, iii) 136 sec B_0 Map, iv) 620 sec multi-echo (ME) resting state), v) 645 sec ME fMRI alcohol cue reactivity task, vi) 633 sec single-echo fMRI anticipation using anxiety inducing/anticipatory and matched neutral cues stimuli.

Structural: A T1-weighted (1-mm^3 voxel resolution) structural scan will be acquired for each subject for voxel placement co-registration (TR, 7200 ms; TE, 2.7 ms; 176 sagittal slices; 1 mm thick; no gap; 256 × 256 × 256 matrix).

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS): An edited ^{1}H-MRS using Hadamard Encoding and Reconstruction of Mega-Edited Spectroscopy (HERMES; (Chan et al., 2016)) sequence will be acquired. This acquisition is optimized to simultaneously edit for gamma-aminobutyric acid, glutathione, and ethanol quantification (Gong et al., 2020; Saleh et al., 2020). The voxel will be placed in the dorsal ACC with the long edge of the voxel along the genu of the corpus callosum (voxel size 4 x 2 x 3cm^3). The acquisition parameters for the HERMES are TR/TE = 2000/80 ms, 256 VAPOR water-suppressed scan averages, 16 unsuppressed scan averages, number of data points = 4096, spectral width = 5000 Hz, acquisition time 544 s. The dorsal ACC is chosen given high relevance in the reward/motivation circuit (Volkow et al., 2011).

*Resting-state fMRI (rs-FMRI) sc*an: BOLD signal will be recorded using a Multiecho echoplanar imaging (ME EPI) sequence which enables improved signal to inferior regions (e.g., VMPFC etc) which generally suffer from signal loss (Deichmann et al., 2002). ME EPI allows for the BOLD signal to be recovered at multiple echo times per radio frequency excitation pulse. Hyperband Multi-echo EPI (HyperMEPI; GE Healthcare, Waukesha, WI, USA) will be used to capture multiphase volumes of whole brain, comprising 45 axial slices collected in an ascending interleaved fashion angled 15 degrees from the AC-PC line superior to inferior using a Gradient Echo pulse sequence with 3 echoes (TR: 2000 ms, TE_1 /TE_2 /TE_3

= 0.01/0.025/0.04 s, Flip Angle:70°, FOV:220 mm, matrix of 64× 64, slice thickness 3mm; slice gap 0.4mm; with a voxel resolution 3.44 × 3.44 × 3.44 mm3). The B_0 map will be acquired using the same parameters as above except the following: TR = 1000ms, TE = 4.6 ms, flip angle 30°, FOV: 220 mm, matrix 64 x 64, and bandwidth 62.5.

Functional Magnetic Resonance Imaging (fMRI)

Cue Reactivity Task: Participants will complete a visual cue reactivity task adapted from (Grüsser et al., 2004) to measure alcohol cue-elicited brain activity (See Appendix B, Figure 1 for visual depiction of task). Stimuli comprise two types: alcohol-related pictures depicting types of alcohol (larger/wine/spirits) and drinking situations; and a control type comprising validated neutral pictures matched for colour and complexity. Images will be presented for 6.6 s in blocks of 3 images of the same type. 10 alcohol stimuli and 6 neutral blocks will be presented throughout the experiment with stimuli and block order will be randomised (16 total blocks, 645 s). Following each block, an 11-point visual analogue craving scale is presented and participants respond using an MRI-compatible two-button response pad (Cedrus Corporation; San Pedro) within a 10 s window.

Fear Anticipation Continuous Performance Task (FCPT): A novel fear response task consisting of a continuous performance task (CPT) with an intermittent presentation of high-threat and low-threat cues will be used to evaluate brain activity elicited by anticipation to fear-inducing cues. This task utilises stimuli from the Nencki Affective Picture System (NAPS; (Marchewka et al., 2014) which is a validated set of images that reliably elicits a fear response. Specific images that will be selected for high and low-threat blocks are listed in Table 2 in the appendix. Participants also complete the CPT to maintain attention, indicating the direction of 3 to 6 arrows pointing left or right presented on a grey background, along

with a 440 Hz tone, using the response pad buttons (arrow block). These arrow blocks are followed by high-threat or low-threat blocks, in which participants are presented with 3 arrows along with either high-threat ('threat' 1000 Hz + Orange background) or a low-threat ('safe' 250 Hz + Blue background) signal stimulus followed by presentation of a fearful or neutral pictorial unconditioned stimulus (US) respectively. The US images comprise 18 fear cues (of animals, objects, humans, and faces) and 18 control images matched for valence and arousal dimensions. The number of arrows is randomised per arrow block, with ≤2 same blocks presented consecutively, and US block type order (either fearful or neutral) with ≤3 same US type presented consecutively, and US block order randomised per participant.

Psychophysiological alcohol cue reactivity: The psychophysiological cue reactivity (CR) task has been described previously (Logge et al., 2020a). The CR contains 5 consecutive stages including baseline, juice cue, recovery 1, alcohol cue, and recovery 2; (5 min stages, total = 25 min). In cue reactivity stages (cue exposure stages) either a bottle of a novel, non-sweet juice (carrot, control) or a bottle containing the participants preferred alcoholic drink will be placed in front of participants with relevant juice/beer/wine glasses. Alcohol cues will either be red/white wine or lager depending on which of these the participant most commonly consumes. Vignettes that provide context to enhance drink cue craving will be presented before the participants are instructed to pour, hold, and smell the beverage for 5 min (Appendix B). During this alcohol cue presentation, a previously hidden simulated bar will be revealed to enhance external contextual cue craving. During the five-minute baseline and recovery periods, participants will view neutral landscape videos set to classical music.

Throughout the psychophysiological CR paradigm, MLT117F GSR Electrodes (ADInstruments; Bella Vista, Australia) will be placed on the middle phalanges on the II and

III fingers of the participant's non-dominant hand. A FE116 GSR Amplifier (ADInstruments; Bella Vista, NSW, Australia) will then be used to amplify the skin conductance signal. Additionally, electrocardiogram (ECG) data will be recorded using a three-lead ECG with Ag/AgCl electrodes. These electrodes will be placed on the non-dominant wrist (as a ground electrode) and two above the cubital fossa on each arm. This ECG signal will then be amplified using an ML408 Dual Bioamp/Stimulator (ADInstruments; Bella Vista, NSW, Australia). Both amplifiers will be connected to a PC operating LabChart Pro 7.3.7 software (ADInstruments, 2012) via a PowerLab 8/25 System (ADInstruments; Sydney, Australia) and sampling at a rate of 1,000 Hz/s. Additionally, in-between cue exposure phases; AUQ, VAS and PANAS will be used to record craving as a self-measure and applied in data analysis.

Brain metabolites (MRS): The open-source "Osprey" toolbox (Oeltzschner et al., 2020) will be used in MATLAB to process and quantify the presence of metabolites (in ppm) for the HERMES acquisition. Metabolites will be corrected for tissue relaxation factors, (Gasparovic et al., 2018; Gasparovic et al., 2006), and in the case of GABA, the alpha correction will be applied (Harris et al., 2015). Signal-to-noise and fit metrics, along with visual inspection, will be used to ensure quality of the data. Generalised estimation equations (GEEs) will be implemented using the CrossCarry package (Cruz et al., 2023) using R software (Version 4.0.3), which allow for the estimation of both simple and complex carryover effects within the context of a crossover design with repeated measures. GEE models are advantageous compared to parametric approaches as they account for the order and carryover effects with effects averaged across groups (Cruz et al., 2023; Zeger & Liang, 1986). Session order and carryover effects will be explicitly modelled using Crosscarrys createCarry. Additionally, covariates reflecting previous day drinking (PDD) and percentage of heavy drinking day during the previous two weeks will be modelled given the effects of recent drinking on neurometabolite concentration (Prisciandaro et al., 2016).

fMRI

Preprocessing: fMRIPrep (Esteban et al., 2020; Esteban et al., 2019), will be used for both anatomical and structural image preprocessing. Intensity non-uniformity correction will be applied to T1-w images which will then be skull-stripped. After these T1-w images are used in brain surface reconstruction, volume-based structural images will be segmented and normalised into MNI space. fMRIPrep will additionally be used for cue reactivity and resting state ME EPI preprocessing. Images acquired during the ME EPI sequences will be distortion-corrected using the B0 field maps, motion-corrected, co-registered to the T1-weighted structural data, normalised to MNI space, and projected to cortical surface. Functional time series will then be resampled to FreeSurfer's (FreeSurfer 6.0.1, surfer.nmr.mgh.harvard.edu) fsaverage space. Further, distortion correction, motion correction, slice timing, co-registration, spatial normalisation, and smoothing will be applied for FCPT single-echo preprocessing. Task-based volumes will be excluded if they demonstrate framewise displacement of greater than 4mm.

Post Processing: Cue reactivity and FCPT data will be post-processed separately using SPM. To improve sensitivity during group analysis, functionally resampled images will be smoothed with a full-width half maximum (FWHM) 8 mm Gaussian kernel. eXtensible Connectivity Pipelines (XCP-D) (Ciric et al., 2017; Satterthwaite et al., 2013) will be used to further process resting state data. Resting-state volumes will be excluded if they demonstrate framewise displacement of greater than 4mm. Additionally, nuisance regressors based on the '36P' strategy (Ciric et al., 2017; Satterthwaite et al., 2013) will be regressed out. Using the XCP-D output from each resting state acquisition a Fisher's r-to-z transformation will be applied to Pearson correlation coefficients for each resultant ROI-to-ROI connectivity matrix

cell using the Schaefer 17-network 400 parcel atlas. This will provide us with 44 (22 participants with two sessions) symmetrical functional connectivity matrices with 400×400.

fMRI Statistical Analysis

Data analysis will be conducted using SPM12 with two levels. Within the first level (session-specific) analysis two conditions will be modelled for each task: alcohol for cue reactivity or high threat anticipatory cues for FCPT; and control cues (low threat and cue reactivity control cues; matched cues for novelty and visual complexity of the stimuli). These conditions will be modeled as a box-car function convolved with the canonical haemodynamic response. Additionally, for both tasks six motion correction parameters, VAS blocks, and CPT arrow (during FCPT analysis) blocks will be included as regressors of no interest, and fixation crosses will be considered an implicit baseline.

For our second-level random-effects analysis we will be using the MarsBar toolbox (Fox & Lancaster, 2002a) and extract unweighted beta estimates (β) within ROIs specific to each task. A priori regions of interest (ROIs) will be used, including the dorsolateral PFC, left and right caudate, and bilateral ventromedial PFC given these regions are shown to be relevant in alcohol cue studies (Grodin & Ray, 2019; Zeng et al., 2021). For the FCPT ROIs will include the amygdala, parahippocampal gyrus, insula, ventral striatum, and hypothalamus given their relevance to fear anticipation (Fullana et al., 2016) and modulation by CBD (Tristan Hurzeler: In publication). Regions will be defined using the Brainmap database (Brett et al., 2002) and relevant β will be collected for both sessions of each participant, and drug differences will be analysed using GEEs using the CrossCarry package in R software (Version 4.0.3). GEEs will be modelled for alcohol and control conditions for cue reactivity, and high and low threat cues for anticipation task will be across both sessions. Individual contrasts within condition will be selected as comparisons between conditions (eg

Alcohol against control) have been shown to have low reliability in repeated measures designs(Bach et al., 2022). Session order and carryover effects will be explicitly modelled using Crosscarrys createCarry, and PDD will be modelled as a covariate given its effects on functional brain activity (Zheng et al., 2015). We will then use the Simple Interactive Statistical Analysis Bonferroni tool (http://www.quantitativeskills.com/sisa/calculations/bonfer.htm) to account for correlation between ROIs. For task-based results we will report beta mean correlation coefficients, and resulting in an equivalent corrected alphas with a threshold of P<.05.

Resting-State Data Analysis

Using the CONN toolbox, functional connectivity will be extracted from the resting state data (Whitfield-Gabrieli & Nieto-Castanon, 2012) version 22a (Nieto-Castanon & Whitfield-Gabrieli, 2021). To compare CBD and Placebo (CBD > placebo [i.e., 1 -1]) functional connectivity we will use GLM, 44 (22 participants with two sessions) symmetrical functional connectivity matrices. Additionally, parametric multivariate statistics will then be applied to identify individual ROI contributions. Further, a FDR-corrected ROI-level p-value (P_{FDR} <.05, p<0.1) MVPA omnibus test (Benjamini & Hochberg, 1995) will be used to examine individual ROI-ROI connections.

Psychophysiology: GEEs will be used to assess session differences and treatment effects for continuous variables of psychophysiological indices HF-HRV and SCL, and AUQ craving scores, with planned contrasts comparing specific key stages of the cue reactivity task (i.e., baseline versus cue presentations, neutral and alcohol cues, cue presentations versus recovery periods, alcohol cue presentation versus alcohol recovery period). Session order and carryover effects will be explicitly modelled using Crosscarrys createCarry, andPDD will be modelled as a covariate given its effects on skin conductance (Stewart & Pihl, 1994),

cardiovascular responses (Victor M Karpyak et al., 2014; Romanowicz et al., 2011), and craving and mood measures (Koob & Le Moal, 2008).

Follow-up data: Outcomes variables (alcohol consumption, craving, mood, sleep) will be summarized by calculating means, standard deviations, and percentile ranges for all continuous variables and by calculating proportions for all categorical variables. A one-way analysis of variance (ANOVA) will be used to assess group differences for continuous variables. Nominal variables will be analysed via chi-square tests of independence. Mixed models will be conducted to examine differences from baseline (CBD vs PL as main group effects).

Sample Size: The sample size of N = 22 with 2 observations per participant is calculated based previous literature implementing a similar design and statistical analysis (Spinella et al., 2021). Given the complexity of conducting an a priori power analysis we used G*power to conduct a power analysis using an analytic approach with less statistical power (repeated-meausre ANOVA). The analysis indicated that a total sample size of 22 participants was required to achieve a power of 0.85. The critical F-value for this analysis was 4.32479. These results ensure that the study has an adequate sample size to detect a medium effect size with a high probability of success (power = 0.85) while maintaining an acceptable Type I error rate ($\alpha = 0.05$).

3.3 **Discussion**

This study intends to examine early signs of efficacy of CBD as a pharmacotherapy in the management of AUD by identifying modulation in relevant neurobiological and psychophysiological systems. The primary interest of the research is mechanistic and therefore MRI paradigms investigating brain metabolite levels (MRS), functional brain connectivity through (rsfMRI) and functional activity in areas associated with AUD-related characteristics will be applied. We will also use heart rate variability and skin conductance paradigms to investigate parasympathetic and sympathetic responses to alcohol cue presentations. Secondary outcomes, considering the changes to clinical measures associated with AUD (including alcohol consumption, craving, mood, and sleep), will additionally be measured.

CBD could reduce alcohol craving and seeking due to moderating responses to alcohol and stress cues, normalising dysregulated neurobiological systems and/or improving relevant clinical characteristics that lead to relapse such as sleep and mood disturbances. Compared to other medications used for the management of addiction, CBD has been demonstrated to be particularly safe with less severe side effects and few contraindications (Iffland & Grotenhermen, 2017) which may lead to better treatment adherence (Arnold et al., 2023). CBD may also offer potential protection from alcohol-related liver and brain damage due to anti-inflammatory and antioxidant properties. By improving our understanding of CBD in relation to AUD-related neurobiological, cognitive, and clinical characteristics, this study will provide key information with regards to the potential of CBD as a pharmacotherapy for the management of AUD.

Chapter 4: The Effect of Cannabidiol on Psychophysiological and Craving Responses to a Laboratory Alcohol Cue Reactivity Task in Individuals with Alcohol Use Disorder

Abstract

Preclinical studies have demonstrated that cannabidiol (CBD) reduces alcohol-seeking behaviours and may have potential for the management of alcohol use disorder (AUD). In this study, we examined the effect of CBD versus placebo on psychophysiological and craving responses to alcohol and appetitive cues. Twenty-two non-treatment seeking individuals with AUD (DSM-V) participated in a cross-over, double-blind, randomised trial (3 days of 800 mg CBD/matched placebo). Psychophysiological indices of autonomic nervous system activity (skin conductance, high-frequency heart rate variability (HF-HRV) and self-report (craving and anxiety) were recorded during an alcohol cue reactivity task to examine cue-elicited responses in addition to cognitive measures. This task comprised of cue exposure and subsequent recovery periods including baseline, neutral appetitive control cue, and alcohol cue conditions. CBD administration was significantly associated with elevated parasympathetic nervous system (PNS) activity across the task (as indexed HF-HRV) relative to placebo sessions, and reductions in self-report anxiety during cue exposure stages. Additionally, CBD facilitated reductions in craving for alcohol post-cue exposure. In conclusion, relative to placebo, administration of CBD reduces cue-elicited anxiety responses, facilitates PNS recruitment and modulates recovery of self-reported craving in individuals with AUD.

4.1 **Introduction**

Alcohol use disorders (AUD) are responsible for 5.3% of global burden of disease due to the direct effects of chronic, heavy alcohol use, along with its precipitating comorbidities(Griswold et al., 2018; WHO, 2018, 2021; World Health Organization, 2019). Chronic alcohol use disorders and acute alcohol use are associated with 5.3% of all global deaths (7.7%, 2.6% for men and women respectively) and implicated in 13.5% of deaths per year for those ages 20-39 years (World Health Organization, 2019). AUD, particularly when moderate to severe, is a chronic relapsing disorder characterized by compulsive alcohol-seeking and consumption despite negative repercussions to health (Haber, Riordan, Winter, et al., 2021). In conjunction with psychosocial support, pharmacotherapy can be used as a tool for reducing the core symptoms of AUD, achieving abstinence and reduce consumption (Morley, Rombouts, et al., 2021). However, there currently exists a paucity of medications available to treat AUD and these medications are only modestly effective (Morley, Rombouts, et al., 2021). There is a pressing need to improve treatment for this highly prevalent and debilitating health problem.

Cannabidiol (CBD) has been demonstrated to have various potential therapeutic properties that may be particularly well suited to the medical management of AUD. Preclinical and clinical research has demonstrated that CBD has various therapeutic properties including anti-seizure (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016), anxiolytic (Borgwardt et al., 2008; Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019), anxiolytic (Borgwardt et al., 2008; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019) neuroprotective (Crippa et al., 2018), anti-inflammatory and antioxidant effects (Mandolini et al., 2018; Mechoulam et al., 2007; Ren et al., 2009). Furthermore, preclinical evidence has demonstrated that CBD can

reduce alcohol self-administration (Maccioni et al., 2022) and reduced stress and alcohol cue-induced alcohol seeking in alcohol addiction rat models (Viudez-Martínez, García-Gutiérrez, et al., 2018a, 2018b). In humans, CBD has been shown to decrease attentional bias to cigarettes (Hindocha et al., 2018) and modulate arousal following conditioned cues which is characteristic of AUD and substance use disorder (Hurd et al., 2019). These results suggest that CBD may reduce motivational urges and drug seeking behaviours via modulation of attentional bias towards drug cues and/or hedonic states induced by drug cues.

Laboratory cue reactivity tasks can be used to identify appetitive and alcohol cue-elicited autonomic nervous system (ANS) responses and concomitant hedonic, and affective states (Mather & Thayer, 2018) such as craving and anxiety. Following cue exposure, key neurobiological (e.g. mesocorticolimbic) networks are implicated in salience attribution. Once cues are considered salient, central networks (executive and autonomic) mobilise rapid behavioural responses by modulating neuroendocrine and visceromotor systems (Thayer & Lane, 2000). This mobilisation coincides with downregulation of the parasympathetic division and upregulation of the sympathetic division of the ANS. Cue reactivity tasks measure indices of parasympathetic nervous system (PNS) activity via high frequency heart rate variability (HF-HRV), and sympathetic nervous system (SNS) activity via electrodermal conductivity or salivary cortisol (Garland, 2011; Hurd et al., 2019; Nesic & Duka, 2006). Through psychophysiological indices of HF-HRV and electrodermal conductivity, the cue reactivity task provides insight into appraisal of cue salience and cue-elicited motivational urges.

Individuals with AUD present with low baseline HF-HRV which subsequently drops following alcohol cue exposure and coincides with elevated electrodermal conductivity. These specific ANS modulations are thought to be due to the increased salience attributed by the rewarding effects of alcohol and recruitment of the mesocorticolimbic dopamine

networks (Koob, 2014) which increases the risk for habitual alcohol seeking behaviours (Ingjaldsson et al., 2003; Thayer & Brosschot, 2005). Following the removal of cues (recovery periods), patients may demonstrate protracted periods of elevated arousal and delayed recovery to baseline homeostasis leading to prolonged periods of strong urges to seek alcohol (Garland, 2011).

Potential pharmacotherapies aimed to manage addictive behaviours and to attenuate motivational urges can thus be evaluated through their ability to dampen SNS recruitment and facilitate PNS recruitment during cue exposure and recovery. Although occasionally inconsistent with self-report craving measures (Carter & Tiffany, 1999), ANS cue-elicitation may be able to predict drinking and treatment outcomes (Garland et al., 2012; Logge et al., 2020a; Logge et al., 2020b; Rohsenow et al., 1994). For example, baclofen, a GABAb agonist, with efficacy in the treatment of AUD (Morley, Baillie, et al., 2018) reduces recruitment of the PNS, during neutral (water) and active (alcohol cues) with greater recovery to baseline levels of arousal (Logge et al., 2020a). These baclofen mediated effects on the ANS were found to be associated with reduced heavy drinking days later in treatment (Logge et al., 2020a).

This study aimed to evaluate the effect of administration of CBD on modulating self-report craving and psychophysiological responses to alcohol cues, using a laboratory cue reactivity task. Specifically, we aimed to elucidate whether CBD normalised dysregulated physiological systems to alcohol cues, as measured by HF-HRV and SCL. We hypothesised that, during CBD conditions compared to placebo, participants would demonstrate i) reduced arousal (as indicated by higher HF-HRV and lower SCL) to alcohol cues but not neutral cues; and ii) greater ANS recovery responses during recovery periods.

4.2 **Methods**

4.2.1 **Design**

The trial was conducted over a 48-month period at the Royal Prince Alfred Hospital (RPAH) in Sydney, NSW Australia between 2021 and 2024. The study was approved by the Human Ethics Review Committee of the Sydney Local Health District (X19-0416). The trial was sponsored by the Sydney Local Health District and registered in the Clinical Trials Registry (NCT05387148). Twenty-two non-treatment seeking individuals who met the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V) (Diagnostic, 1994), criteria for current AUD were recruited to participate in a randomised, double-blinded, placebo-controlled, crossover trial of 3 days of dosing and experimental sessions. Here, we present results from the cue reactivity psychophysiology paradigm (day 3). Recruitment streams consisted of clinical referral from treating physicians, nurses, and psychologists among RPAH outpatients as well as via flyers/community advertisements at local general practitioners, newspapers, and websites. All participants provided written informed consent prior to randomisation and the commencement of the trial.

4.2.2 **Subjects**

Inclusion criteria: a) Male and female participants between the ages of 18 and 65 meeting DSM-V criteria for current alcohol use disorder; b) Adequate cognition and English language skills to give valid consent and complete research interviews; c) A breath alcohol concentration (BrAC) reading of 0.00; d) Women of child-bearing potential were required to be non-lactating, using birth control and have a negative pregnancy test; e) Willingness to give written informed consent.

Exclusion criteria: a) Active major psychological disorder associated with psychosis, significant suicide risk; b) Pregnancy or lactation - Women were advised to use reliable contraception for the duration of the study and a urine pregnancy test was performed where necessary; c) Dependence on any substance other than nicotine (e.g. methadone); d) Diagnosis of epilepsy, and/or current use of anti-epileptic drugs (AED); e) Liver failure with jaundice or prolonged INR above 1.3; f) Medical complications such as liver failure, cardiac ischemia or conduction abnormalities, renal impairment or unstable elevated vital signs (systolic blood pressure > 180, diastolic blood pressure > 120 or heart rate > 150); g) Severe cognitive impairment or insufficient English or literacy to complete study processes; h) Concurrent use of medication metabolised via CYP3A4, CYP2C9 and CYP 2C19 with potential to interact with CBD including cardiac medication (e.g. betablockers, calcium channel blockers and statins), macrolides and recent antihistamine use; i) Claustrophobia; j) Extreme obesity; k) Previous brain surgery.

4.2.3 **Procedure**

After meeting the eligibility criteria through structured interview and medical evaluations, participants provide informed consent. They were then randomly assigned to one of two treatment regimens: 800mg CBD (4 x 200mg gel capsules per day, days 1-3) or matched placebo (see Figure 3.2). Following an average washout of 29.2 days (SD = 11.25), participants received the alternate treatment allocation at the subsequent session. A washout period of >18 days was applied before participants received the alternate capsules during the subsequent testing session. This washout period was chosen given the half-life of CBD following chronic administration is 2–5 days (Millar et al., 2018), and requires a washout of 13-day for effective elimination of CBD from plasma (McCartney et al., 2023). On the first day of dosing (T1) participants were administered their first dose of CBD or matched placebo

and completed baseline sample characteristics questionnaires. On the second day (T2) participants completed a series of MRI tasks, reported elsewhere (Hurzeler et al., under review). The psychophysiology cue reactivity task was completed on the third day (T3). Participants were administered their third dose followed by questionnaires and cognitive tasks. Participants then completed the psychophysiology cue reactivity task (outlined below). Following completion of the repeated testing days, participants underwent telephone follow-up an average of 34 days (SD = 15.7) after the final testing day.

4.2.4 **Medication**

Softgel capsules containing Medium chain triglyceride (MCT) oil and 200mg CBD (manufactured by Linnea Natural Pharma Solutions; Linnea ECS315, 123 Cantonale, Lavertezzo 6595, Switzerland) and matching placebo (softgel capsules containing only MCT oil) was purchased from BOD Australia pharmaceuticals (Bod ECS100, 1/377 New South Head Road, Double Bay, NSW 2028, Australia). The matching placebo was identical in appearance, taste, and composition except for the presence of CBD. A three-day consecutive dose regimen was administered as follows: T1 (Day 1): 4 x 200 mg followed by T2 (Day 2 Experimental Session 1): 4 x 200 mg and then T3 (Day 3 Experimental Session 2): 4 x 200 mg. Participants were administered CBD and placebo orally with water, under supervision, to ensure adherence.

4.2.5 **Randomisation and Allocation Concealment**

A randomisation table was computer-generated by an independent researcher using R Studio (Team, 2020). This table was then implemented by an independent clinical trial pharmacists using RedCAP (Research Electronic Data Capture), a secure web application for

building and managing online surveys and databases (Harris et al., 2009b) to randomise participants to one of two treatment orders (CBD then PL; PL then CBD).

4.2.6 **Measures**

Questionnaires

A set of general demographics and background drinking history questions (30 minutes) were administered at baseline and follow-up (see Figure 3.2): (i) Demographics, medical history, personal and family history of AUD, and alcohol treatment history, as obtained in previous work (Morley et al., 2014; Morley, Baillie, et al., 2013; Morley, Leung, et al., 2013; Morley et al., 2006). Additionally, (ii) recent (last 28 days) alcohol consumption (frequency/quantity) assessed by the Timeline follow-back method (TLFB; (Sobell et al., 1988); (iii) severity of alcohol dependence assessed by the Alcohol Dependence Scale (ADS; (Skinner & Allen, 1982)); (iv) craving for alcohol measured by the Penn Alcohol Craving Scale (PACS; (Flannery et al., 1999); (v) the Depression Anxiety Symptom Scale (DASS) measures the severity of symptoms of depression, anxiety and stress (Lovibond & Lovibond, 1995); (vi) sleep problems were assessed by the Insomnia Severity Index (Bastien et al., 2001); (vii) Self-rated sedation was measured via a Likert scale (‘*between 1-10, how sedated do you feel right now where 1 is not at all and 10 is very sedated?*).

Subjective Craving and Mood

A set of craving and mood measures were administered at various points throughout testing days: (i) urges to drink (AUQ; (Bohn et al., 1995); (ii) the Positive and Negative Affect Schedule measuring positive and negative mood states (PANAS; (Watson et al.,

1988)); (iii) Visual Analogue Scales (VAS) assessing alcohol craving, thirst, and anxiety e.g. (0) indicating 'no craving' and (100) very severe craving.

Executive Functioning Tasks

A set of executive functioning tasks (30 minutes) were administered on day 2 (see Figure 3.2) using Inquisit (Millisecond Software version 4.0.10, 2021) unless otherwise stated. The tasks included: *(i) Trail making task (TMT), Part A and B* (Reitan & Wolfson, 1985). This is a pen and paper timed task in which participants trace between circles with consecutive numbers in part A and alternate between numbers and letters (1-A-2-B-3-C etc) in part B. (ii) *Number–letter task* (Rogers & Monsell, 1995): A number–letter pair is presented in one of four quadrants on the computer screen. The participants indicate whether a number is odd or even when a number–letter pair is presented in either of the top two quadrants and indicate whether the letter was a consonant or a vowel when the number–letter pair was presented in either of the bottom two quadrants. A switch cost was calculated as the average of trials in which trials shifted between numbers and letters compared to no shift trials. (iii) *Stroop task (voice activated)* (Stroop, 1935): Participants verbally indicate the colour (red, green, blue or yellow) over 2 trial types: congruent trials (e.g., 'red' in red text), and incongruent trials (e.g., 'red' in blue text). Higher Stroop interference scores (mean congruent trial response latencies - mean incongruent trial latencies) indicate poorer executive functioning. (iv) *Nback* (Braver et al., 1997; Jonides et al., 1997; Kirchner, 1958): Participants are presented different modalities of shapes and the participant indicated whether the current shape stimulus matched the stimulus 2 positions back in the sequence. The dependent variable is the proportion of (TotalHits - TotalFalseAlarms)/number of total stages, with higher values demonstrating better working memory capacity.

Psychophysiological Alcohol Cue Reactivity

The cue reactivity task was completed on the third day 90 minutes after the dose of CBD or placebo (see Figure 3.2 for Protocol Flowchart). The psychophysiological cue reactivity task applied here was adapted from a commonly implemented cue reactivity task (Logge et al., 2020a). This adapted cue reactivity task comprised of five, 5 min stages (25 min total) of cue exposure including baseline, juice cue, recovery 2, alcohol cue and recovery 3 stages. In appetitive cue exposure stages either a bottle of a novel, non-sweet juice (carrot, control) or an alcoholic beverage was placed in front of participants with relevant juice/lager/wine glasses. The alcoholic beverage cue was matched to participants historical preferences (red/white wine or lager). Audio scripts describing drinking contexts were played to enhance drink cue craving prior to participants being instructed to pour, hold, and smell the beverage for 5 min. During this alcohol cue presentation, a previously hidden simulated bar was revealed to improve contextual cues. During 5 min baseline, and recovery periods, participants watched neutral landscape videos set to classical music. Following each stage participants completed subjective urges, craving, and mood questionnaires (AUQ, VAS crave/anxiety/thirst and PANAS).

4.2.7 **Psychophysiological Acquisition and Transformation**

During the psychophysiological paradigm MLT117F GSR Electrodes (ADInstruments; Sydney, Australia) were placed on the II and III fingers of the non-dominant hand while electrocardiogram (ECG) electrodes were placed on the non-dominant wrist (as a ground electrode) and two above the cubital fossa on each arm. GSR and ECG signals were amplified using a FE116 GSR Amplifier (ADInstruments; Sydney, Australia) and a ML408 Dual Bioamp/Stimulator (ADInstruments; Sydney, Australia) respectively and connected to a

PC operating LabChart Pro 7.3.7 software (ADInstruments, 2012) via a PowerLab 8/25 System (ADInstruments; Sydney, Australia) and sampled at 1,000 Hz/s.

Mean skin conductance (SC) in microSiemens (μS) and R-wave for cardiovascular data were calculated for each stage using the following method. GSR artifacts were identified and removed manually using Labchart Pro. Following a low pass filter of 0.205 HZ, standard proportions of SCL were obtained using the following formula (Dawson et al., 2007):

$$\frac{SCL - SCLmin}{SCLmax - SCLmin}$$

SCL min = baseline minimum skin conductance value per stage

SCL max = maximum skin conductance value per stage

Mean SCL proportion per participant per stage were subsequently implemented i*n* further analysis. A low-pass filter was used to interpolate, and where possible replace, sections with ECG artifacts. Trend components were compensated for using smoothness priors method ($\lambda = 500$; (Tarvainen et al., 2002)). Fast Fourier transformation was then used to calculate HF-HRV during spectral analysis in 0.15-0.40 HZ frequency bands and mean HF-HRV per participant per stage was subsequently calculated and implemented in statistical analysis (See Appendix C for further Acquisition and Analysis details).

4.2.8 **Statistical Analysis**

The sample size of N = 44 (n=22 with two sessions each) was calculated based on our previous fMRI work (Logge et al., 2019) and previous MRS studies (Bendszus et al., 2001), where there was more than 80% power to detect a small effect of 0.2.

Baseline sample characteristics (mean, standard deviation and range) were extracted from baseline questionnaires using R studio Version 4.3.1 (RStudio Team; 2020) and described in Table 4.2. Given the crossover within subjects design, the modelling approach controlled for carryover effects of the treatment patterns spanning the washout period to identify treatment effects on dependent variables (Madeyski & Kitchenham, 2018). We employed generalised estimation equations (GEEs) implemented through the CrossCarry package, which allow for the estimation of both simple and complex carryover effects within the context of a crossover design with repeated measures. GEEs are advantageous compared to parametric approaches as it accounts for the order and carryover effects with effects averaged across groups (Cruz et al., 2023; Zeger & Liang, 1986). Session order and carryover effects are explicitly modelled using Crosscarrys createCarry. We added previous day drinking (PDD) as a covariate given its effects on skin conductance (Stewart & Pihl, 1994), cardiovascular responses (V. M. Karpyak et al., 2014; Romanowicz et al., 2011), and craving and mood measures (Koob & Le Moal, 2008). GEEs were applied to relevant questionnaires (PACs and PANAS), executive functioning tasks and all measures used throughout the psychophysiological alcohol cue reactivity task. Fitted values from these GEEs were used to depict variables where relevant to ensure accurate representation and interpretation of results.

Cue Reactivity Task: Data analysis was conducted using R studio Version 4.3.1 (RStudio Team; 2020). GEEs were applied to assess treatment differences (CBD vs placebo) for dependent variables of interest including HF-HRV, SCL proportion and mean standardised craving scores of VAS and AUQ. Planned contrasts (see Appendix C, Table 1) comparing specific key stages of the cue reactivity task (i.e., baseline versus appetitive cue exposure stages, juice versus alcohol appetitive cue, appetitive cue exposure versus recovery stages, alcohol cue exposure versus alcohol recovery stages) were included as a random factor. We included previous day drinking as well as session effects and crossover contrasts

(defined using crosscarry package (Cruz et al., 2023)) to account for potential cross over effects for each dependent variable.

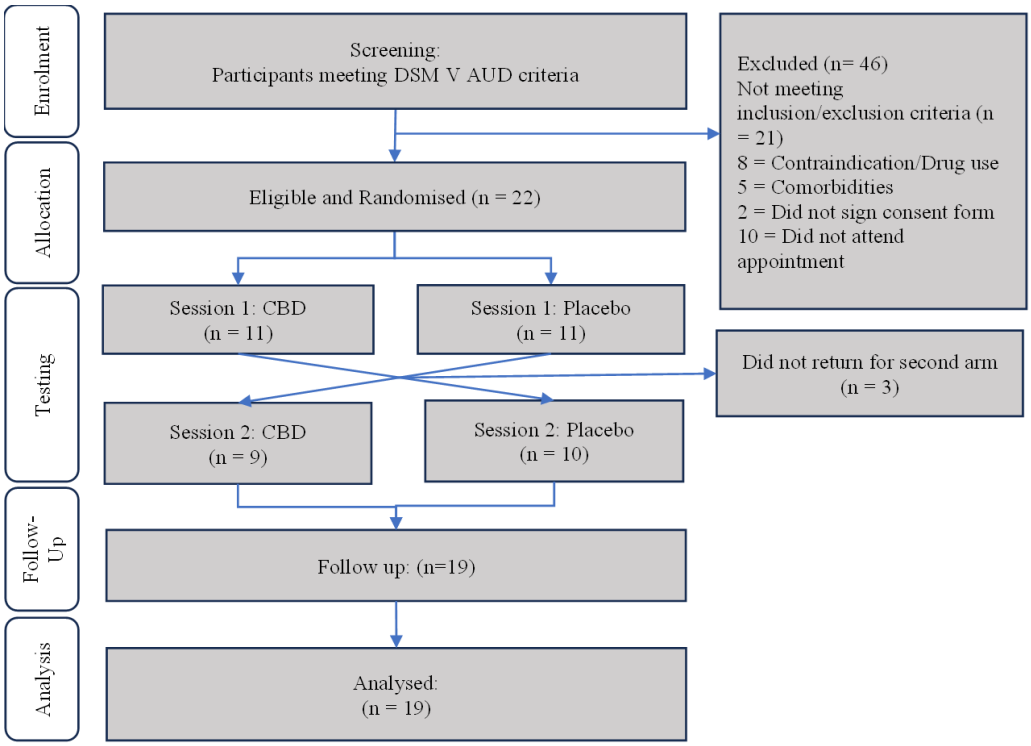
4.3 **Results**

4.3.1 **Sample Characteristics**

Figure 4.1 indicates the flow of participants through the study. Twenty-two non-treatment seeking individuals were recruited, with 19 participants completing both sessions of the study.

Figure 4.1

CONSORT Flow Diagram



Note. provides a visual representation of the progression of participants through each stage of the DBRCT including enrolment, allocation, follow-up and analysis, in adherence with CONSORT (Consolidated Standards

of Reporting Trials) guidelines and includes information regarding dropout. Of the 68 participants screened, 46 were excluded proceeding consent and randomisation, and 3 participants dropped out before completing their second session.

The mean washout period between placebo and CBD sessions was 28.2 days (SD = 10.25). The mean age of the participants was 28.29 (12.45). These participants had received an average of 14.54 (1.9) years of education and worked on average 6.53 months (4.86) over the preceding year. Seven of the 17 final participants experienced signs of comorbidities and 6/17 of participants used antidepressants. Participants, on average, consumed 3.55 standard drinks per drinking day (SD = 3.17), had a baseline ADS score of 16 (8.24) and had experienced 8.27 (10.15) years of problem drinking. The mean baseline PACS score was 11.69 (5.87). Five of the 17 participants regularly used nicotine over the preceding 28 days. In the SCL sample, participants had a mean age of 29.79 (12.59). They were highly educated with an average of 14.54 (1.9) years of education and worked 7.11 (4.9) months in the preceding year. Seven participants experienced signs of comorbidities, six of whom were prescribed antidepressants. These participants drank 3.55 (SD = 3.17) standard drinks a day, had a mean baseline ADS score of 15.89 (7.84), a mean PACS score was 11.89 (5.55) and had been experiencing problems with drinking for 8.47 (9.67) of problem drinking. 5 of the 19 participants regularly used nicotine over the preceding 28 days.

Table 4.1

Sample Characteristics

Participants (n = 19)	
Demographics	
Age (years)	29.79 ± 12.59
Education (years)	14.53 ± 1.77
Gender, (F/M)	13/19
Employment	
Employed within last year, %	84
Months employed in past year	7.11 ± 4.9
Clinical characteristics	
SDD	3.55 ± 3.17
Years since alcohol-related problems began	8.47 ± 9.67
ADS score	15.89 ± 7.84
PACs craving score	11.89 ± 5.55
ISI score	8.47 ± 6.39
DASS (21)	
Anxiety subscale	3.63 ± 3.06
Depression subscale	5.79 ± 5.15
Stress subscale	7 ± 4.74
Comorbidities, %	37
Anti-depressant use, %	32
Tobacco use, %	26

Note. Values presented are mean and standard deviation unless otherwise noted. SDD standard drinks per day of the past 28 days ADS alcohol dependence scale, PACs Penn Alcohol Craving scale, ISI insomnia severity index, DASS depression anxiety symptom scale. Comorbidities identified by the mini international neuropsychiatric interview. Antidepressant and tobacco use present here for the past 28 days.

4.3.2 **Adverse Events**

No serious adverse events were reported throughout the duration of the study. Eight individuals (36%) reported side effects including 4 during CBD administration and 4 following the placebo session. During the CBD sessions 4 participants (18%) reported

drowsiness (n = 2), somnolence (n = 1), lethargy (n = 2), or fatigue (n=1). During placebo sessions 4 participants (18%) reported similar side effects including drowsiness (n = 1), sedation (n = 1), lethargy (n = 1), or fatigue (n = 1) with an additional report of diarrhoea (n = 1).

4.3.3 **Cognitive Functioning and Sedation**

There were no significant differences between CBD and placebo groups on any measures of cognitive functioning including the TMT-A ($p = 0.200$), indicating that visual attention, processing speed, and basic motor function were not impaired following CBD administration. Similarly, there was no difference between CBD and placebo on TMT-B performance ($p = 0. 820$), or the TMT-difference score ($p = 0.262$) suggesting maintenance of task-switching, cognitive flexibility, and set-shifting executive function. There was also no significant difference between CBD and placebo for the remaining executive functioning tasks (Nback task, $p= 0.747$; Number Letter, p-value = 0.327; and Stroop, $p = 0.630$). Finally, CBD dosing sessions were not associated with significantly higher self-report sedation compared to placebo sessions ($p = 0.988$).

4.3.4 **Craving and Mood**

There were no significant differences between CBD vs PL groups for craving as measured by the PACS ($p = 0.43680$). There were also no significant differences between CBD and PL sessions on the positive subscale of the PANAS ($p = 0.607$). However, there were significantly reduced scored on the PANAS negative subscale ($p < 0.001$) in CBD sessions compared to placebo.

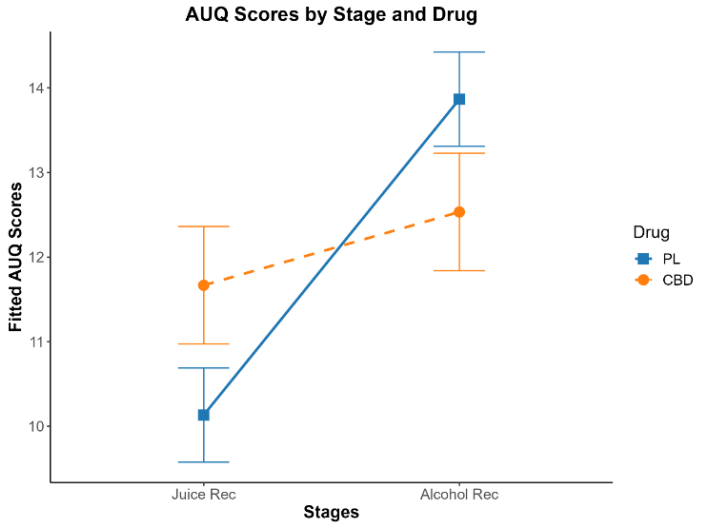
4.3.5 **Cue Reactivity**

Self-report Craving and Mood

Significantly higher AUQ scores were reported comparing all cues to baseline ($p < 0.001$), alcohol cue to juice cue ($p < 0.001$), alcohol recovery to juice recovery ($p = 0.019$), cue stages to recovery stages ($p < 0.001$) and alcohol cue to alcohol recovery ($p < 0.001$). No main effects of treatment (CBD vs PL), session order or carry over effects were identified across AUQ measures (See Appendix C Table 2 for raw analysis results). However, there was a significant interaction between treatment and contrast (Figure 4.2) comparing alcohol recover to juice recovery stages which indicated a reduced increase of AUQ score during juice recovery compared to alcohol recovery during CBD sessions compared to placebo ($p = 0.004$).

Figure 4.2

Treatment by Stage Interaction: AUQ Scores with Fitted Values



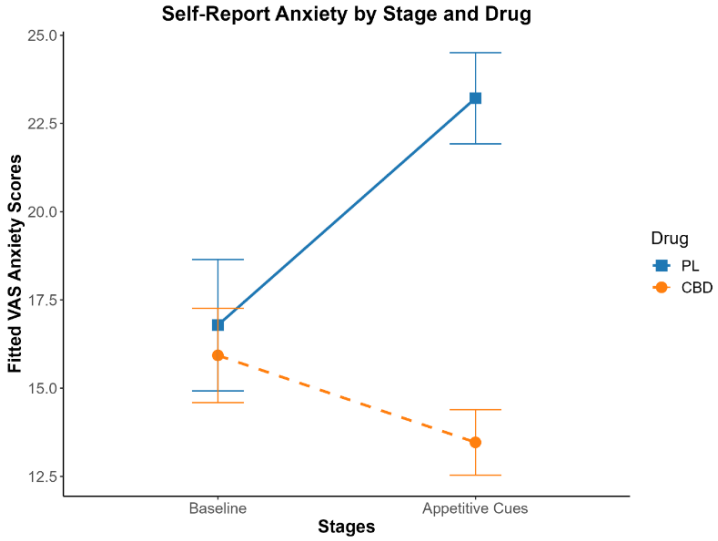
Note. Illustrates interaction between drug (cannabidiol (CBD) vs placebo (PL)) and stage (juice recovery vs alcohol recovery) with respect to fitted alcohol use questionnaire (AUQ) scores from a generalised estimated equations (GEE) model accounting for carry over, session effects and previous day drinking. Standard error and

means from relevant fitted values are indicated by error bars. This significant interaction (p = 0.004) suggests a differential effect of treatment on AUQ scores such that CBD, compared to PL administration, resulted in dampened increases in AUQ scores observed irrespective of group (est = 1.867; p =0.019).

Self-reported anxiety, measured via the VAS, significantly decreased during cue exposure compared to baseline stages (p = 0.045), recovery to cue exposure (p = 0.017), and alcohol recovery stages to alcohol cue exposure (p = 0.048). No main effects of treatment (CBD vs PL), session order or carry over effects were identified across VAS anxiety measures (See Appendix C, Table 3 for raw analysis results). However, interaction effects (Figure 4.3) revealed that anxiety scores significantly increased during cue exposure compared to baseline following placebo but decreased following CBD administration (p = 0.027).

Figure 4.3

Treatment by Stage Interaction: VAS Anxiety Scores with Fitted Values



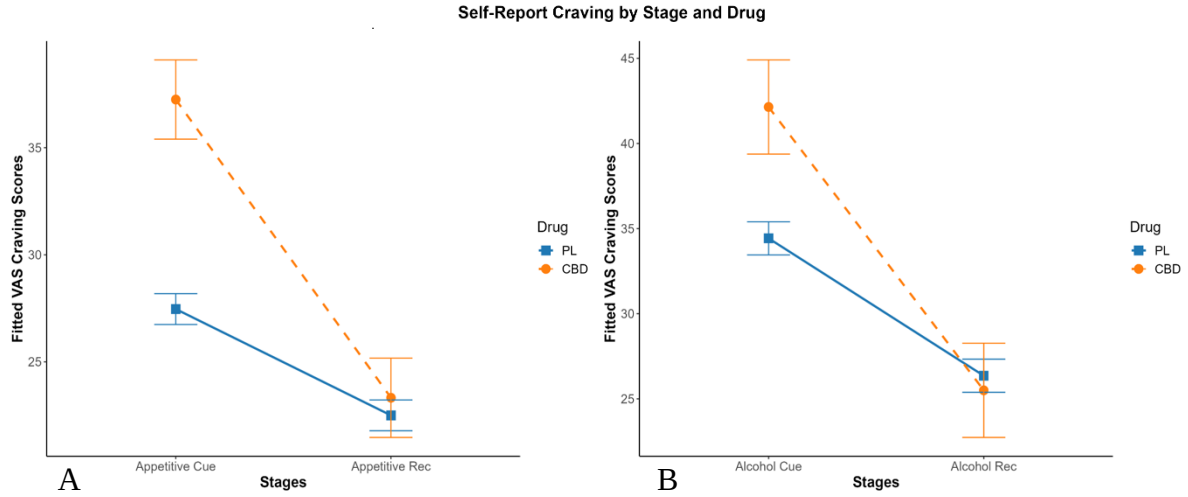
Note. Illustrates interaction between drug (cannabidiol (CBD) vs placebo (PL)) and stage (baseline vs appetitive cues) with respect to fitted visual analogue scale (VAS) anxiety scores from a generalised estimated equations (GEE) model accounting for carry over, session effects and previous day drinking. Standard error and means

from relevant fitted values are indicated by error bars. This significant interaction ($p = 0.027$) suggests a differential effect of treatment on VAS anxiety scores such that CBD, compared to PL administration, rescued increased VAS anxiety scores during cue exposure compared to baseline (est = 4.286; $p = 0.045$).

During the psychophysiological task, self-reported VAS craving significantly increased during appetitive cues compared to baseline ($p < 0.001$) and alcohol cue to juice cue ($p = 0.003$) but decreased during recovery compared to appetitive cues ($p = 0.033$) and alcohol recovery stages to alcohol cue ($p = 0.030$). There were no main effects for treatment ($0.061 < p\text{'s} < 0.226$; See Appendix C Table 4 for raw analysis results), session order or carry over effects identified across contrasts for VAS craving measures. Interaction effects between treatment and contrast (Figure 4.4) were observed whereby, compared to placebo, CBD sessions were associated with greater decreases in VAS craving scores when comparing recovery to appetitive cue exposure ($p = 0.026$) and alcohol recovery to alcohol cue ($p = 0.025$).

Figure 4.4

Treatment by Stage Interaction: VAS Craving Scores with Fitted Values



Note. Interaction between drug (cannabidiol (CBD) vs placebo (PL)) and stage (A: appetitive cues vs appetitive recovery; B: alcohol cue vs alcohol recovery) with respect to fitted visual analogue scale (VAS) craving scores from a generalised estimated equations (GEE) model accounting for carry over, session effects and previous day drinking. Standard error and means from relevant fitted values are indicated by error bars. This figure suggests a differential effect of treatment on craving such that CBD, compared to PL administration, resulted in more marked decreases in craving scores when comparing appetitive recovery to appetitive cue (A: $p = 0.026$) and alcohol recovery to alcohol cue exposure stages (B: $p = 0.25$). Main treatment effects within specific cue stages including appetitive and alcohol cue stages were not directly modelled, therefore, specific comparisons between CBD and PL within stage should not be evaluated.

Psychophysiology

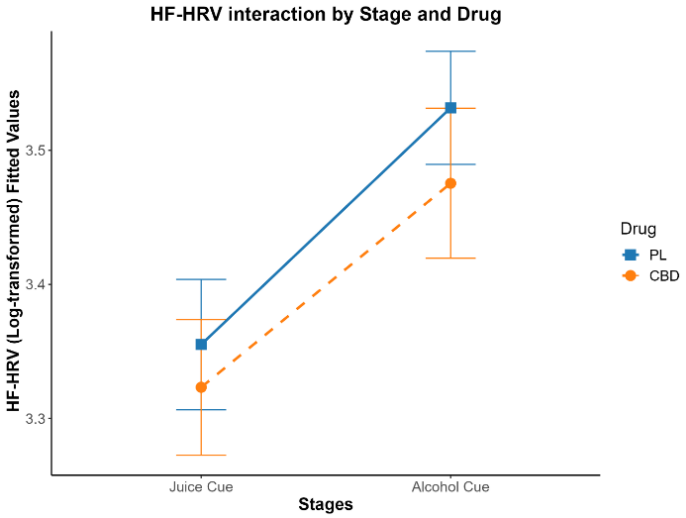
There were no significant treatment differences (p's > 0.422) or interactions between each contrast and treatment (p's > 0.164) across SCL measures (See Appendix C Table 5 for raw analysis results). Two participants were removed from HF-HRV data analysis due to signal loss and data artifacts. Independent of treatment, HF-HRV increased during cue stages compared to baseline ($p = 0.022$), increased during alcohol cue exposure compared to juice

cue exposure ($p = 0.013$), and increased during alcohol recovery compared to juice recovery ($p = 0.024$).

Across the entire cue reactivity task, HF-HRV was significantly higher during CBD sessions compared to placebo sessions (est = 0.619; $p < 0.001$). This CBD-mediated elevation in HF-HRV was observed across all contrasts (p's < 0.008) with more marked CBD-mediated increases during recovery periods (est = 0.788; $p<0.001$) compared to cue exposure stages (est = 0.497; $p =0.008$). Carry over effects (p's < 0.003) and session effects (p's < 0.024) were observed across all contrasts (See Appendix C Table 6 for raw analysis results). A significant interaction effect between treatment and contrast (Figure 4.5) was demonstrated whereby CBD sessions were associated with smaller increases in HF-HRV during alcohol cue compared to juice cue exposure stages compared to modest increases in placebo sessions (Figure 5; $p = 0.046$). This interaction was driven by increases in HRV during alcohol cues compared to juice cues in placebo sessions ($p = 0.013$) which were not statistically significant during CBD sessions ($p= 0.357$).

Figure 4.5

Treatment by Stage Interaction: Log-transformed HF-HRV Fitted Values



Note. Interaction between drug (cannabidiol (CBD) vs placebo (PL)) and stage (juice cue vs alcohol cue) with respect to log-transformed high frequency heart rate variability (HF-HRV) fitted values from a generalised estimated equations (GEE) model accounting for carry over effects (p = 0.0036), session effects (p = 0.024) and previous day drinking (p = 0.0134). Standard error and means from relevant fitted values are indicated by error bars. This significant interaction (p = 0.046) suggests a differential effect of treatment on HF-HRV such that CBD, compared to PL administration, resulted dampened increases in HF-HRV when comparing alcohol cue to juice cue exposure stages.

4.4 **Discussion**

This is the first study to examine the effects of CBD on psychophysiological responses to a cue reactivity paradigm in individuals with AUD. Our results demonstrated that HF-HRV is generally elevated following CBD administration compared to placebo. However, distinct treatment effects were demonstrated whereby CBD-mediated HF-HRV increases were attenuated during cue exposure compared to recovery stages and during alcohol cue exposure compared to juice cue stages. Interestingly, during CBD sessions compared to placebo, there was also significantly greater reductions of self-reported cue-elicited craving during recovery periods. These CBD-mediated modulations of PNS recruitment may have precipitated the changes in craving given their relationship with improved interoception (Leganes-Fonteneau et al., 2021) as well as improved behaviour and cognitive regulation (Thayer & Lane, 2000). The observed steeper decreases in VAS craving scores during recovery, as mirrored by the AUQ scores, compared to during cue exposure stages may be a result of this improved behavioural regulation and impulse control throughout the task (Quintana et al., 2013).

In the current study we also observed reductions in self-reported anxiety from baseline to cue exposure in CBD sessions, while increases in anxiety during cue exposure were observed in placebo sessions. The capacity for CBD to modulate negative affect has similarly been demonstrated in previous studies (Borgwardt et al., 2008; Fusar-Poli et al., 2010; Jadoon et al., 2017; Wilson et al., 2019). These anxiolytic effects have been proposed to be driven by CBDs modulation of cannabinoid receptor 1 (CB1r) (Austrich-Olivares et al., 2022) given the high density of CB1r in regions responsible for emotion regulation including the hippocampus, cortex and amygdala (Glass et al., 1997; Hájos & Freund, 2002; Pistis et al., 2004). These results are further supported by our finding that CBD sessions lead to significant reductions in PANAS negative subscale scores relative to the placebo sessions.

Taken together, the current results are in line with previous literature but also provide valuable enhancements to the prevailing interpretation of the mechanisms of CBD in AUD. For example, preclinical research demonstrates that CBD reduces stress and alcohol cue alcohol induced reinstatement (Viudez-Martínez, García-Gutiérrez, et al., 2018a) and HPA-related gene expression (Viudez-Martínez, García-Gutiérrez, et al., 2018b). Although our results do not directly measure alcohol seeking behaviours, they suggest that the modulation of parasympathetic nervous system may occur throughout cue exposure, but reductions of cue-elicited craving may primarily occur following the removal of appetitive cues in individuals with AUD. Further, one previous psychophysiological study in a clinical sample of patients with heroin use disorder (Hurd et al., 2019), demonstrated that craving and anxiety were shown to reduce following CBD administration during salient cue presentation. In contrast, the current set of results suggest that, in individuals with AUD, although CBD may induce reductions in cue-elicited anxiety during cue exposure, reductions in self-report craving for alcohol may only occur *post* cue exposure. The results presented here additionally suggest that CBD is well tolerated and does not impact cognitive functioning nor does it increase sedation relative to placebo. This limited side effect profile is further supported by relevant literature which employs CBD in clinical samples (Iffland & Grotenhermen, 2017; Machado Bergamaschi et al., 2011).

One limitation of the current study may be generalisability to treatment settings due to the lower number of males recruited. Sex mediated pharmacokinetic CBD effects have previously been demonstrated such that this may have also impacted on the results (Child & Tallon, 2022; Silote et al., 2021). The small sample size may also be a limitation by impacting on power, although consistent with similar experimental cross-over designs. Inclusion of non-treatment seekers may also limit generalisability to clinical AUD samples due to the potential of smaller treatment effects (Ray et al., 2017). Nonetheless, this study

also possesses certain strengths. To our knowledge, this study is the first examination of the effect of CBD on psychophysiological responses to alcohol cues. Moreover, the crossover design aided in limiting between group variance introduced by different baseline covariates and carry-over effects were assessed using a robust statistical analysis design. Additionally, our cue reactivity paradigm used tangible appetitive cues which accurately replicate naturalistic cues. Future research should ensure to implement statistically robust analysis designs to account for CBD-mediated novelty and carry over effects. Further we suggest research be conducted in treatment seeking samples and stratified by sex to better represent populations experiencing AUD.

In summary, this is the first study to indicate that, in AUD individuals, CBD facilitates parasympathetic nervous system recruitment in a non-cue-specific manner but somewhat diminishes during alcohol cue presentations. Moreover, CBD administration precipitates decreased self-reported anxiety during cue presentation and improved cue-induced craving recovery post alcohol and appetitive cue exposure. Furthermore, our results suggest that high dose CBD (800mg) is well tolerated within this population and did not impair executive function or elevate sedation. Thus, CBD may have therapeutic potential in the management of alcohol use disorder due to its capacity to facilitate PNS recruitment and effects on anxiety and craving during salient cue and during post cue recovery periods respectively.

Chapter 5: Cannabidiol Attenuates Precuneus Activation During Appetitive Cue Exposure in Individuals with Alcohol Use Disorder

Abstract

Cannabidiol (CBD) may have potential in the management of a range of psychiatric conditions including alcohol use disorder (AUD). This study investigated the effect of CBD on alcohol cue-induced activation of brain regions commonly associated with alcohol craving. Twenty-two non-treatment seeking individuals with AUD (DSM-V) participated in a cross-over, double-blind, randomised trial (800 mg CBD or matched placebo/day for three days). On day 2, cue-elicited activation to alcohol and matched control visual cues was measured using a functional magnetic resonance imaging alcohol cue reactivity task. Mood, craving, and cognitive functioning was also measured. Although region of interest analysis demonstrated non-significant differences in dorsolateral prefrontal cortex (PFC), left and right caudate, or bilateral ventromedial PFC, an exploratory whole brain analysis demonstrated a significant treatment effect of CBD on precuneus activation that was non-cue specific. There were no treatment effects of CBD vs placebo on acute craving, mood, or cognitive functioning. During cue exposure, high dose CBD modulated the precuneus, a region associated with cue-induced alcohol craving, suggesting that CBD may have promise attenuating alcohol-seeking behaviours.

5.1 **Introduction**

Alcohol use disorder (AUD) is characterised by compulsive, chronic alcohol use despite adverse social, occupational, or health consequences (Haber, Riordan, Winter, et al., 2021). Heavy, chronic alcohol use perturbs functioning within regions of the meso-corticolimbic (Cardenas et al., 2011; Ilari et al., 2022), salience networks (Makris et al., 2008), limbic networks (Suzuki et al., 2010) as well as fronto-striatal functional connectivity (Rogers et al., 2012) which are thought to underpin the compulsive seeking behaviour and cognitive processes which maintain AUD (Volkow et al., 2016). Pharmacotherapies targeting these dysregulated neurobiological systems aim to manage the core symptoms of AUD and improve clinical outcomes by facilitating behavioural change. However, TGA approved medication for the management of AUD (naltrexone, disulfiram (Antabuse), and acamprosate (Petrakis, 2006; Pettinati & Rabinowitz, 2006; Zindel & Kranzler, 2014)), are only modestly effective and there is a large degree of heterogeneity in treatment response (Morley, Perry, et al., 2021) wherein new pharmacotherapeutic options are required (Burnette et al., 2022).

Cannabidiol (CBD), a non-intoxicating component of *Cannabis sativa*, has emerged in recent years as a potential treatment target for AUD (Nona et al., 2019). CBD is a non-intoxicating phytocannabinoid with known anti-seizure properties (Devinsky, Marsh, Friedman, Thiele, Laux, Sullivan, Miller, Flamini, Wilfong, Filloux, et al., 2016) and a favourable side effect profile (Bergamaschi et al., 2011; Haney, Malcolm, Babalonis, Nuzzo, Cooper, Bedi, Gray, McRae-Clark, Lofwall, & Sparenborg, 2016; F. Leweke et al., 2012). It is a CB1R non-competitive allosteric modulator (Laprairie et al., 2015), mediating anandamide (AEA) transportation by targeting fatty acid-binding proteins (FABP). CBD inhibits FABP catabolism of AEA and reduces cellular uptake of endocannabinoids (Elmes et al., 2015) and modulates dopaminergic release (Cheer et al., 2004) in regions of networks involved in cue induced craving and regulatory behaviours (Hurzeler et al., in press)

including mesocorticolimbic, salience, and fronto-striatal networks in normative samples. CBD may therefore normalise functions characteristic of AUD such as reward anticipation, emotion regulation, salience processing, and executive functioning.

CBD has shown promise for the management of cue-induced craving in both preclinical models and clinical samples with substance use disorders. Preclinical studies have demonstrated that acute doses of CBD have been shown to reduce stress and alcohol cue reinstatement in preclinical models (Viudez-Martínez, García-Gutiérrez, et al., 2018a). The only previous clinical study of CBD in a substance use disorder to-date demonstrated that CBD, compared to placebo, reduced opiate cue-induced craving and anxiety for those with opioid use disorder (Hurd et al., 2019). Further, CBD induced decreases in attentional bias to cigarettes for those with nicotine dependence (Hindocha, Freeman, Grabski, Stroud, et al., 2018) suggests a capacity to normalise the appraisal of drug associated cues as hyper-salient. While THC disrupts salience network activation, neuroimaging studies have found that CBD restores salience network functionality (Wall et al., 2019). CBD also normalises insular dysfunction during motivational salience processing for those with clinical high risk of psychosis (Wilson et al., 2019). CBD may thus have potential to manage cue induced craving in AUD through the regulation of hyper-sensitivity to alcohol associated cues, however, the effect of CBD on alcohol cue reactivity and neurocircuitry in the AUD population has not been studied to-date.

Neuroimaging paradigms enable examination of neurobiological mechanisms associated with AUD and to identify signals of efficacy for medications that modulate activation. Cue reactivity tasks are a well-validated paradigm for investigating craving associated neuronal activity (Sangchooli et al., 2024). Previous research demonstrates that the dorsolateral PFC, left and right caudate, and bilateral ventromedial PFC, among others, have been shown to be relevant in alcohol cue studies in which participants are exposed to alcohol

cues (Grodin & Ray, 2019; Zeng et al., 2021). We have previously shown, with the use of fMRI cue reactivity paradigms, that placebo compared to baclofen resulted in hypoactivation in bilateral caudate nucleus and dorsal anterior cingulate cortex and that this attenuation resulted in a reduction of heavy drinking days (HDD) (Logge et al., 2021). Similarly, naltrexone has been shown to attenuate fronto-striatal regions associated with alcohol-cue induced craving (Myrick et al., 2008) and that the degree of this modulation predicts time to relapse (Mann et al., 2014). Cue-reactivity neuroimaging paradigms provide a method for examining the mechanism of pharmacotherapy and their effect on clinical outcomes.

Therefore, the aim of this study was to directly examine the effects of acute CBD versus placebo on alcohol cue elicited neural activity, as measured using the blood oxygen level dependent (BOLD) signal. We hypothesised that mesocorticolimbic brain activation (e.g., dorsolateral PFC, left and right caudate, and bilateral ventromedial PFC) when exposed to an alcohol cue will be significantly attenuated in CBD-treated AUD individuals compared to those on placebo. We also examined subjective craving, mood, and executive functioning.

5.2 **Methods**

5.2.1 **Design**

The trial was conducted over a 48-month period at the Royal Prince Alfred Hospital (RPAH) in Sydney, NSW Australia between 2021 and 2023. The study was approved by the Human Ethics Review Committee of the Sydney Local Health District (X19-0416). The trial was sponsored by the Sydney Local Health District and registered in the Clinical Trials Registry (NCT05387148). Twenty-two non-treatment seeking individuals who met the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V) (Diagnostic, 1994), criteria for current AUD were recruited to participate in a randomised, double-blinded, placebo-controlled, crossover trial including 3 days of data collection. Here, we present results from the cue reactivity fMRI paradigm (day 2). Recruitment streams consisted of clinical referral from treating physicians, nurses, and psychologists among RPAH outpatients as well as via flyers/community advertisements at local general practitioners, newspapers, and websites. All participants provided written informed consent prior to randomisation and the commencement of the trial.

5.2.2 **Participants**

Twenty-two participants were recruited following screening for inclusion and exclusion criteria. *Inclusion criteria*: a) Male and female patients between the ages of 18 and 65 meeting DSM-V criteria for current AUD; b) Adequate cognition and English language skills to be able and willing to give valid informed consent and complete research interviews; c) Must have a stable housing and be able to nominate a reliable contact person for locating them if necessary.

Exclusion criteria: a) Active major psychological disorder associated with psychosis or significant suicide risk; b) Pregnancy or lactation. Women were advised to use reliable contraception throughout the duration of drug therapy, and a urine pregnancy test was conducted as necessary; c) Dependence on any substance other than nicotine (e.g., methadone); d) Diagnosis of epilepsy and/or current use of anti-epileptic drugs; e) Liver failure with jaundice or prolonged INR above 1.3; f) Medical complications such as liver failure, cardiac ischemia or conduction abnormalities, renal impairment or unstable elevated vital signs (systolic blood pressure > 180, diastolic blood pressure > 120, or heart rate > 150); g) Severe cognitive impairment or insufficient English or literacy to complete study processes; h) Concurrent use of drugs potentially exacerbated by CBD via CYP3A5, including cardiac medication (eg betablockers, calcium channel blockers, and statins), macrolides and recent antihistamine use; i) Claustrophobia; j) Extreme obesity; k) Previous brain surgery; l) Unable to complete an MRI scan (e.g., metal implants, previous emoployment as a machinist, welder or metal worker).

5.2.3 **Procedure**

After meeting the eligibility criteria through structured interview and medical evaluations, participants provide informed consent. They were then randomly assigned to one of two treatment regimens: either receiving 800mg CBD (4 x 200mg gel capsules per day, days 1-3) or matched placebo for two consecutive days. Following an average washout of 29.2 days (SD = 11.25), participants received the alternate treatment allocation at the subsequent session.

On the first testing day (T1), participants presented to the RPAH to receive the first dose of CBD or Placebo and complete a series of questionnaires as detailed above. On the second day (T2), participants received their second dose under supervision then completed a

battery of executive functioning tasks, followed by assessments of craving and mood (see below). They were then escorted to an imaging facility for an MRI scan conducted 90 minutes after their second dose of CBD/matched placebo, aligned with literature suggesting C_{max} occurs between 1-4 hours post-dose (Guy & Robson, 2014; Haney, Malcolm, Babalonis, Nuzzo, Cooper, Bedi, Gray, McRae-Clark, Lofwall, & Sparenborg, 2016; Manini et al., 2015). Prior to imaging, participants underwent a blood alcohol content (BAC) reading (BAC=0.00 was required), followed by a structural scan and functional cue reactivity task-based acquisition. Craving was assessed after alcohol and control cue blocks, as well as before and after the scan. Following washout, participants subsequently received the alternate capsules and completed the same testing procedure. Following completion of the repeated testing days, participants underwent a follow-up over the phone, with an average of 34 days (SD = 15.7) between final testing day and follow-up.

5.2.4 **Interventions**

Softgel capsules containing MCT oil and 200mg CBD (manufactured by Linnea Natural Pharma Solutions) (Solutions, 2021) and matching placebo (softgel capsules containing only MCT oil) was purchased from BOD Australia pharmaceuticals (Pharmaceuticals, 2021). The matching placebo was identical in appearance, taste, and composition except for the active ingredient of pure CBD. A three-day consecutive dose regimen was administered as follows: T1 (Day 1): 4 x 200 mg followed by T2 (Day 2 Experimental Session 1): 4 x 200 mg and then T3 (Day 3 Experimental Session 2): 4 x 200 mg. Participants were administered CBD and placebo orally with water, under supervision, to ensure compliance with the dose regimen.

5.2.5 **Randomisation and Allocation Concealment**

This study was conducted under double-blind conditions such that participants and study staff were unaware of medication assignment. A computer-generated random allocation procedure was conducted through REDCap (Research Electronic Data Capture; a secure web application for building and managing online surveys and databases; (Harris et al., 2009a)) by the allocated RPAH pharmacist.

5.2.6 **Measures**

Sample Characteristics

The following questionnaires were presented on Day 1, and responses collected online using REDCap: (i) Demographics, medical history, personal and family history of AUD, and alcohol treatment history as collected in previous research (Morley et al., 2006); (ii) a structured psychiatric diagnostic interview using the Mini-International Neuropsychiatric Interview (MINI) (David V Sheehan et al., 1998) (iii) recent (last 28 days) alcohol consumption (frequency/quantity) assessed by the Timeline Followback Method (TLFB; (Sobell et al., 1988)); (iv) severity of alcohol dependence assessed by the Alcohol Dependence Scale (ADS; (Skinner & Allen, 1982)); (v) craving for alcohol measured by the Penn Alcohol Craving Scale (PACS; (Flannery et al., 1999); (vi) symptoms of depression, anxiety and stress as measured by the Depression Anxiety Symptom Scale (DASS) (Lovibond & Lovibond, 1995); (vii) sleep problems as assessed by the Insomnia Severity Index (ISI; Bastien, Vallieres, & Morin, 2001).

Craving and Mood

The following self-reported questionnaires were collected on Day 2 using REDCap unless preformed within the scanner for which Inquisit 4 (LLC., 2009) was used: (i) Positive and Negative Affect Schedule (PANAS; (Watson et al., 1988)), with higher scores indicating

higher positive and negative mood states pre- and post- scan; (ii) Alcohol Urge Questionnaire (AUQ) was used to assess expectancy of alcohol effects and urge to drink alcohol (Bohn et al., 1995); (iii) 11-point Visual Analogue Scale (VAS) was used to assess alcohol craving, thirst, and anxiety, and was captured before, during, and afte the scan.

Executive Functioning Tasks

A 30-minute neurocognitive battery of tasks measuring executive function was administered via Inquisit 4 before the scan on Day 2. These tasks included: (i) The Balloon Analogue Risk Task (BART; (Lejuez et al., 2002)). Participants are instructed to pump a balloon to earn as many points as possible, risking a pop that erases points for that balloon. Participants must complete 50 trials, with trial end signified either by a balloon pop (unsuccessful) or collection of trial earnings (successful). The overall number of pumps for successful trials assess self-regulation and adaptive risk taking; (ii) Columbia Card Task, Hot Version with delayed feedback (CCT; (Figner et al., 2009)): a task where 32 cards are presented facedown, participants are instructed to select as many cards they would like for points before requesting them to be revealed. All selected cards are then shown to the participants one-by-one with points being awarded for every "win" card until there is none left, or a "loss" card is encountered, whereby points are subtracted from the total gained thus far in the trial. The average number of cards turned over in the task is a score of inhibition, working memory updating, task-set switching, and attention.

Participants were also administered a paper version of the Trail Making Task (TMT), comprising of Part A and B (TMT-A and TMT-B) (Reitan & Wolfson, 1985). In TMT-A, participants must connect consecutively numbered circles as quickly as possible, while in TMT-B, they alternate between numbers and letters (1-A-2-B-3-C, etc). Difference scores

were derived by subtracting completion times for TMT-A from TMT-B, with shorter completion times indicating better executive functioning.

Cue Reactivity Task

An adapted cue reactivity task (Grüsser et al., 2004) was presented to measure alcohol cue-elicited brain activity. Stimuli comprised of two types: alcohol-related pictures depicting types of alcohol (lager/wine/spirits) and drinking situations; and a control type comprising of validated, neutral pictures matched for colour and complexity. Images were presented on an MRI-compatible screen for 6.6 s in blocks of three images of the same type. Ten alcohol and six neutral blocks presented throughout the experiment with stimuli and the block order was randomised (16 total blocks, 646 s). Following each block, an 11-point VAS was presented and participants responded using an MRI-compatible two-button response pad (Cedrus Corporation; San Pedro) within a 10 s window (see Appendix C for visual depiction of task).

5.2.7 **MRI acquisition**

Participants were scanned using a 3-Tesla GE Discovery scanner and a 32-channel head coil. A structural scan (T1 weighted 1-mm^3 voxel resolution) was acquired for use in pre-processing purposes (TR: 7200 ms, TE 2.7 ms, 176 sagittal slices, 1 mm thick, no gap, 256 × 256 × 256 matrix). For functional imaging, a GE Multiecho "HyperMEPI" echoplanar imaging sequence (Fernandez et al., 2017) that employs multiband (3 echo times) fMRI was used, enabling improved signal to inferior regions (e.g., VMPFC etc) which generally suffer from signal loss (Deichmann et al., 2002). Multiphase volumes of whole brain, comprising 45 axial slices were collected in an ascending interleaved fashion angled 15 degree from the AC-PC line superior to inferior using a Gradient Echo pulse sequence with 3 echoes (TR: 2000 ms, TE_1 /TE_2 /TE_3 = 10/25/40 ms, Flip Angle:70°, FOV:220 mm, matrix of 64× 64, slice

thickness 3mm; slice gap 0.4mm; with a voxel resolution 3.44 × 3.44 × 3.44 mm3). Additionally, A B_0 map was acquired (TR = 1000ms, TE = 4.6 ms, flip angle 30°, FOV: 220 mm, matrix 64 x 64 and bandwidth 62.5). Task acquisition was 646 seconds. 323 echoplanar image volumes comprising 45 axial slices were acquired from ventral to dorsal (963 images from three diff TEs). Participants' heads were fixed with foam pads and a set of MRI compatible headphones to minimise head movement and framewise displacement.

5.2.8 **Image processing**

Cue reactivity relevant functional scans were processed using FMRIPrep ((Esteban et al., 2020; Esteban et al., 2019), RRID:SCR_016216); a summary is provided here, with full preprocessing pipeline presented in Supplementary Material (See Appendix C for FMRIprep boiler plate). Intensity non-uniformity correction was applied to T1-w images which were then skull-stripped. These T1-w images were then used in brain surface reconstruction with volume-bases structural images segmented and normalised into MNI space.

For each participant, and session a reference volume was generated from the shortest echo of the BOLD run and skull-stripped. Head motion was estimated with respect to the BOLD reference. Respective field maps (B0-nonuniformity map) were co-registered to the EPI reference run and then converted to a displacements field map. A corrected EPI reference was calculated using the estimated susceptibility distortion for a more accurate co-registration with the T1-w image. This displacement field map was then used to better co-register the BOLD reference scan to the T1w. BOLD reference volumes were then co-registered to the T1w reference and the timeseries were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions resulting in a pre-processed BOLD timeseries (PP BOLD). fMRIPrep was then leveraged to optimally combine each of the three different echo image sets. To do this the

monoexponential signal decay model was fitted to a nonlinear regression to create a T2* map (using T2*/S0 estimates from a log-linear regression fit as initial values). Using the method described in (Posse et al., 1999), this T2* map was then used to combine PP BOLD across echoes. What remained were optimally combined PP BOLD time series. These were then distortion-corrected using the B0 field maps, motion correct, co-registered to the T1-w structural data, normalised to MNI space, projected to cortical surface and then resampled to FreeSurfer's (FreeSurfer 6.0.1, surfer.nmr.mgh.harvard.edu) fsaverage space. fMRI images were then resampled in SPM12 (citation) with spatial smoothing using a kernal of 8mm for subsequent fMRI subject modelling (See Appendix C for further details regarding Imaging data preprocessing).

5.2.9 **Statistical Analysis**

Mean, standard deviation, and proportions were calculated for all relevant sample characteristics using R software (Version 4.0.3). Further relevant state change covariates (previous day drinking, session order, and crossover contrasts defined using crosscarry package (Cruz et al., 2023)) were extracted for use as covariates in subsequent generalised estimation equations (GEE). For all other non-imaging measures including mood, craving and cognitive tasks, GEEs in R software (Version 4.0.3) were used to test for statistically significant differences between treatment (CBD vs placebo) and across time (pre/post scan and alcohol vs control blocks). Interactions were examined for treatment effects across time (ie treatment effects for comparisons between alcohol/control cue exposure blocks or pre/post scan).

Data analysis for the fMRI cue reactivity task was conducted using SPM12 with two levels. Within the first level (session-specific) analysis two conditions were modelled: alcohol cues and control cues (matched for novelty and visual complexity of the alcohol

stimuli). These conditions were modelled as a box-car function convolved with the canonical haemodynamic response. Additionally, six motion correction parameters and VAS blocks were included as regressors of no interest while fixation crosses were considered an implicit baseline.

A priori regions of interest (ROIs) were identified for analysis of activity associated with alcohol cue reactivity. These ROIs comprised the dorsolateral PFC, left and right caudate, and bilateral ventromedial PFC which are highlighted during alcohol cue reactivity in studies examining AUD (Courtney et al., 2016; Schacht et al., 2013) including treatment effects (Zeng et al., 2021). The dorsolateral and bilateral ventromedial PFC ROIs were be defined using probabilistic maps extracted from Brainmap database (Brett et al., 2002; Fox & Lancaster, 2002b), binarized with a threshold of ≥.90. While the Harvard-Oxford subcortical probability atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) was used here to define the caudate (caudate body) ROIs. ROIs were extracted using the MarsBar toolbox (Fox & Lancaster, 2002a) to obtain unweighted beta estimates within ROIs specific to each task. Relevant betas were collected for both sessions of each participant for each of these regions. As beta weights from Alcohol cue > Control cue contrasts have previously been demonstrated to have low reliability as a contrast when scans repeated (Bach et al., 2022), alcohol cue exposure blocks compared to implicit baseline (ALC) and control cue exposure blocks compared to implicit baseline (CON) were applied as contrasts at the second level. Generalised estimating equations (GEEs) were conducted in R software (Version 4.0.3). These equations consisted of beta estimates of both contrasts, including a within-subjects factor of treatment (placebo, CBD) and relevant covariates were included to ensure adequate balancing of potential confounding variables (drinking on the previous day, session order and dummy variables to account for cross over effects). By employing the Simple Interactive Statistical Analysis Bonferroni tool (http://

www.quantitativeskills.com/sisa/calculations/bonfer.htm) to balance type I and type II error associated with multiple comparisons correlation between ROIs, an adjusted bonferoni threshold was set at $p < .03$. For task-based results we report beta mean correlation coefficients.

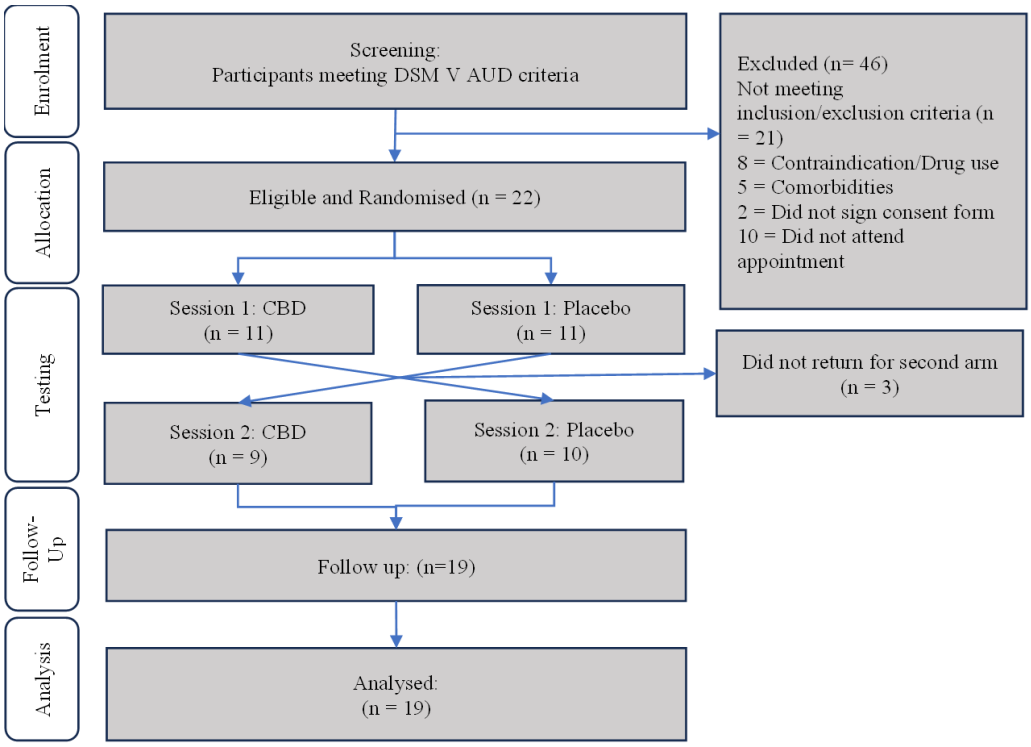
Post hoc whole brain analysis was then conducted to further investigate difference in BOLD signal between post placebo and CBD dosing imaging session using the Multivariate and Repeated Measures for Neuroimaging (MRM) toolbox (version 1.0). MRM is particularly suited to mixed effects statistical modelling of repeated measures designs. First-level ALC and CON contrasts were implemented using GLM at the second-level with 5000 permutations to account for between subject variances. Family-wise error ($p < 0.05$) at the cluster was corrected for by using a cluster-forming height threshold of $p = .001$ as recommended by (Eklund et al., 2016). x, y, z MNI coordinates are reported and the SPM Wake Forest University (WFU) Pickatlas toolbox (http://www.fmri.wfubmc.edu/cms/software, version 3.0.5) used to identify significant cluster regions.

5.3 **Results**

Nineteen participants completed both study sessions of the fMRI study (see Figure 5.1) with 18 participants yielding complete and usable neuroimaging data.

Figure 5.1

Consort Flow Diagram



Note. provides a visual representation of the progression of participants through each stage of the DBRCT including enrolment, allocation, follow-up and analysis, in adherence with CONSORT (Consolidated Standards of Reporting Trials) guidelines and includes information regarding dropout. Of the 68 participants screened, 46 were excluded proceeding consent and randomisation, and 3 participants dropped out before completing their second session.

5.3.1 **Sample Characteristics**

Baseline sample characteristics are depicted in Table 5.1. Overall, the mean age of the remaining participants was 29.39 years (SD = 12.83) and participants had received on average 13.95 years (SD = 2.41) of education. Participants, on average, drank 8.41 (SD =

6.02) standard drinks per drinking day, had an ADS score of 15.44 (SD = 7.81) with approximately 8.6 (SD = 9.97) years of problem drinking. Throughout the study, a total of eight side effects were recorded. Among these, four participants experienced side effects during CBD administration while the remaining four reported these following placebo administration. During CBD administration days one participant reported drowsiness, one reported somnolence, and two reported lethargy.

Table 5.1

Sample Characteristics

Participants (n=18)	
Demographics	
Age (years)	29.39 ± 12.83 [18 - 62]
Education (years)	13.94 ± 2.41 [11 – 17]
Gender % (F/M)	72% (13/18)
Employment	
Employed within last year	83% (15/18)
Months employed within last year	7.44 ± 4.8 [0-12]
Clinical Characteristics	
Standard drinks per drinking day	8.41 ± 6.02 [2.71 – 24]
Years since alcohol-related problems began	8.63 ± 9.97 [1 – 38]
ADS score	15.44 ± 7.81 [9-42]
PACs craving score	11.53 ± 5.50 [4-24]
ISI	8± 6.22 [1 -19]
DASS-21: Anxiety	3.28 ± 2.72 [0-9]
DASS-21: Depression	5.39 ± 4.98 [0-16]
DASS-21: Stress	7.06 ± 4.87 [0-17]
Comorbidities (Mini) %	33% (6/18)
Antidepressants (last 28 days) %	33% (6/18)
Tobacco use (last 28 days) %	22% (4/18)

Note. Mean ± *SD*s [Range] format used to represent data unless otherwise noted. ADS (Alcohol Dependence Score); PACS (Penns Alcohol Craving Score); ISI (Insomnia Severity Index); DASS (Depression Anxiety Symptom Scale)

5.3.2 **Cognitive Functioning**

For the BART (p = 0. 4) and CCT (p = 0. 57), there were no significant effects of CBD vs placebo. For the TMT, neither part-A, part-B or B-A times were significantly different between CBD and Pla dosing sessions (p>0.62).

5.3.3 **Mood and Subjective Craving**

There were no statistically significant CBD vs placebo treatment differences on the PANAS either before the scan (negative p = 0.170; positive p = 0.725) or after the scan (negative p = 0.69; positive p = 0.96). Overall, there was a significant increase between pre and post scan PANAS score for positive subscale (p < 0.001), but not negative subscale (p = 0.500), independent of treatment (negative p = 0.804; positive p = 0. 503).

There were no statistically significant differences in AUQ scores pre and post scan (p = 0.058) nor was this mediated by drug. No significant (CBD vs placebo) differences were observed pre or post scan timepoints (p = 0.40 and p = 0.49 respectively) nor was the difference between pre and post scan moderated by drug (p = 0.88). There was a significant increase in VAS scores following alcohol blocks vs control blocks (p < 0.001) but no interaction between treatment and block effect suggesting no differences in-scanner subjective craving (Alc vs Con p = 0.728).

Table 5.2

Cue Reactivity relevant AUQ and VAS scores.

Craving measures		Placebo	CBD
AUQ	Pre-scan	1.54 ±0.76	1.56 ±0.99
	Post-scan	1.73 ±1.13	1.75 ± 1.03
VAS craving	Alcohol images	2.44 ±2.15	3.19 ±2.84
	Control images	1.68 ± 2.18	2.19 ± 2.53

Note. Mean ± SDs format used to represent data unless otherwise noted. AUQ (Alcohol Urge Questionnaire); VAS (Visual Analogue Scale)

5.3.4 **fMRI Cue Reactivity**

ROI analysis

Compared to placebo, CBD non-significantly modulated activation in any a priori selected ROIs compared to placebo during alcohol cue ($p_{FWE} > 0.043$) or control cue presentations ($p_{FWE} > 0.13$). GEE results evaluating treatment effects on activation within a priori selected ROIs (left and right dorsolateral PFC, left and right caudate, and bilateral ventromedial) are presented in Appendix C Table 7.

Whole Brain analysis

There were clusters demonstrating overall alcohol cue reactivity for the sample, with increased activation to alcohol versus control cues, with one cluster (MNI peak coordinates -5, -91, -9, $p_{FWE} = 0.03$) spanning the left and right lingual gyrus as well as the cuneus and a second left superior parietal lobule cluster (MNI peak coordinates -35, -61, 56, $p_{FWE} = 0.042$) see Table 5.3.

Table 5.3

Whole Brain Identified Brain Regions with Significant Differences

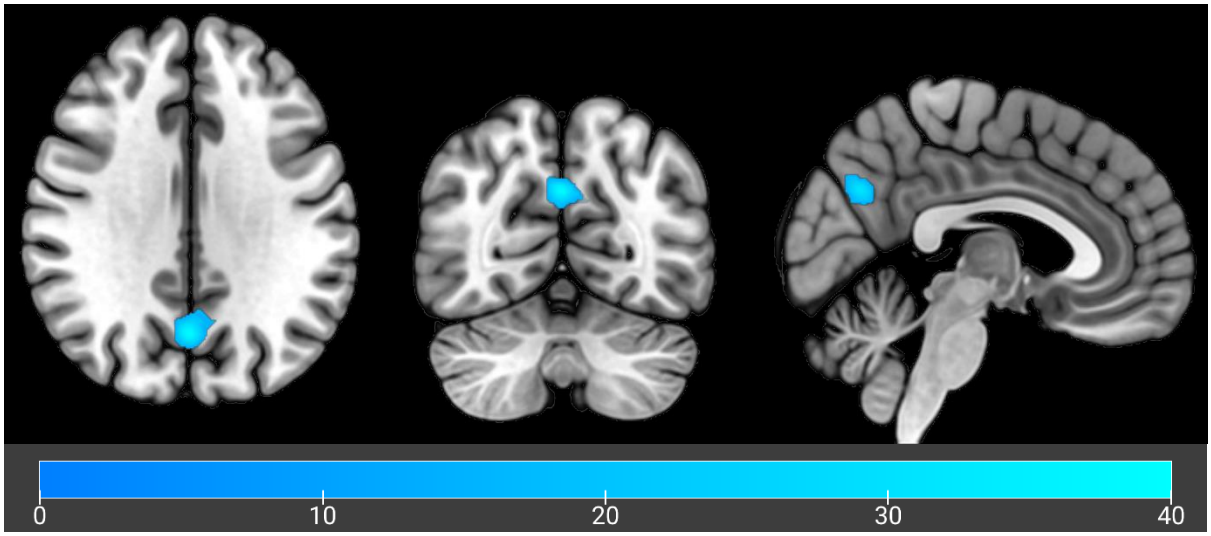
Side	Area	cluster size	MNI coordinates (max peak)			P_{FWEc}
			X	Y	Z	
Condition						
Left/Right	Lingual Gyrus, Cuneus,	329	-5	-91	-9	0.03
Left	Superior Parietal Lobule	276	-35	-61	56	0.042
Treatment						
Left	Precuneus	191	-1	-67	32	0.038

Note. Clusters with statistically significant treatment differences in BOLD signal across Alcohol and Neutral cues compared to fixation.

When evaluating treatment effects (CBD vs placebo), CBD sessions were associated with significant hypoactivation for sessions in a cluster within the precuneus (MNI peak coordinates: -1, -67, 32, $p_{FWE} = 0.038$). This hypoactivation was evident across both control and alcohol cues, suggesting a non cue specific reduction within this cluster following CBD administration (Figure 5.2).

Figure 5.2

Whole Brain Analysis Visualisation



Note. Cluster hypoactivation across Alcohol and Neutral cues when comparing CBD to Placebo. Colour bar indicates F-values with cooler colours indicating increasing hypoactivation.

5.4 **Discussion**

This is the first pharmaco-fMRI crossover design evaluating the neural effects of CBD on alcohol cue reactivity in non-treatment seeking participants with AUD. While there were no treatment effects seen for fMRI cue reactivity in the a priori defined regions (chiefly mesocorticolimbic) implicated in alcohol cue reactivity, whole brain analyses demonstrated CBD mediated reductions in cue reactivity relative to placebo, in a cluster of the precuneus during alcohol as well as control cue exposure blocks.

Non-cue-specific reduced activation observed following CBD administration suggests a general attenuation of brain activation in response to external cues. The precuneus is located between the sensorimotor cortices of the para-central lobule and the parieto-occipital cortex and is linked with subcortical and cortical structures. It is implicated as a hub serving the default mode network and the para-cingulate network, a subnetwork of the central executive network (Dadario & Sughrue, 2023). The precuneus is involved in episodic memory retrieval, visuospatial processing, and self-mental imagery (Cavanna & Trimble, 2006), which are key processes in alcohol cue reactivity (Courtney et al., 2014). The precuneus has also been implicated in higher-order aspects of craving, including the subjective experience of craving (Courtney et al., 2014). Previous studies have shown that CBD treatment can reduce subjective craving in individuals with opiate use disorder (Hurd et al., 2019). However, our current results are not consistent with this as we did not observe any significant effect of CBD versus placebo on subjective craving measures (i.e., VAS, AUQ) in AUD. Previous cue reactivity studies have identified a relationship between severity of dependence and activation of the precuneus (Courtney et al., 2014). Therefore, it is plausible that greater treatment differences may emerge in treatment seeking samples and among participants with greater dependence severity.

We found no evidence of performance differences between CBD and placebo across neuropsychological tests, including the BART, CCT, TMT-B or TMT-B-A tasks. These results align with previous literature demonstrating a lack of CBD-mediated modulation of executive functioning in individuals with opioid dependence (as measured using the Digit Symbol Substitution Task, Digit Span Test–Backward, and a Continuous Performance Task) (Hurd et al., 2019)or during tobacco abstinence (Hindocha, Freeman, Grabski, Crudgington, et al., 2018). Taken together, these results suggest that CBD has minimal impact on executive functioning in individuals with substance use disorders. This is relevant to note given that cognitive deficits are common among treatment-seeking individuals (Bruijnen et al., 2021) and can hinder medication compliance or response to psychological treatment (Stephan et al., 2017).

The present study has both strengths and limitations. It is the first study, to our knowledge, to examine the effects of CBD on individuals with AUD with a robust crossover design, which reduces within-participant variability. Our study design included a substantial washout period and used GEE to ensure no carry-over effects of CBD. Additionally, we employed multi-echoplanar imaging which performs better (Posse, 2012; Posse et al., 1999; Weiskopf et al., 2005) and recovers more orbito-frontal signal (Kirilina et al., 2016) compared to single band acquisitions. However, limitations include the potentially low generalizability of results due to the recruitment of non-treatment-seeking participants with less severe clinical presentations. The lack of statistically significant CBD-modulated changes in ROIs and clusters in our whole brain analysis may be attributed to reduced treatment effects observed in non-treatment seekers (Kiluk et al., 2018; Ray et al., 2017). Nonetheless, the current sample exhibited a baseline consumption of eight standard drinks per drinking day, which, although is substantially less than the AUD treatment-seeking population in Australian clinical trials (Morley, Baillie, et al., 2018; Morley, Lagopolous, et

al., 2018; Morley et al., 2006), exceeds the national alcohol consumption guidelines (Conigrave et al., 2021) and falls within the high risk category of World Health Organisation risk levels (Shmulewitz et al., 2021).

In conclusion, in individuals with AUD, high dose CBD (800 mg/day) modulates precuneus activation in response to non-specific cues relative to placebo. CBD did not significantly change subjective craving relative to placebo and did not impair executive function or change self-reports of positive or negative affect. High dose CBD may be promising for treatment of AUD and further trials in AUD treatment-seeking populations are warranted.

Chapter 6: The Effect of Cannabidiol on Neurometabolite Levels in Alcohol Use Disorder

Abstract

Background and Aims: Preclinical research suggests that cannabidiol (CBD) may have potential as a pharmacotherapy for alcohol use disorder (AUD) given the capacity to reduce alcohol-seeking behaviour. This study aimed to examine the effect of CBD versus placebo on modulating neurometabolites in individuals with AUD. Methods: Twenty-two participants were randomised to receive 800 mg/day CBD or matched placebo in a three-day experimental paradigm using a crossover double-blind, randomised design. Presence of GABA+, NAA, Glx, Cho and GSH in the dorsal anterior cingulate cortex (dACC) was measured on day two of the paradigm using *in vivo* proton magnetic resonance spectroscopy (^{1}H-MRS). Results: No significant main treatment effects were identified across neurometabolites. However, CBD was significantly associated with restoration of GSH and GABA relative to placebo for participants who consumed alcohol the previous day whereas this was not the case for individuals that did not. Similarly, Glx concentrations were significantly increased when comparing previous day drinking to no previous day drinking following CBD dosing but not placebo dosing. Conclusion: These results suggest that, for individuals with AUD, the role of CBD in modulating levels of neurometabolites may be contingent on recent alcohol consumption. Furthermore, the results presented here suggest a potential role of CBD in the management of AUD through restoration of alcohol-induced abnormal levels of neurometabolites.

6.1 **Introduction**

Alcohol Use Disorder (AUD) is a prevalent and chronic disorder that presents a significant burden for individuals and the community (Haber, Riordan, & Morley, 2021). Not only are 3.8% of global deaths attributed to alcohol (Rehm et al., 2009), but chronic heavy alcohol use is associated with significant detrimental effects on physical, social, and emotional domains (Tawa et al., 2016). The currently available medications for the management of AUD are limited and investigations into novel medications are required (Haber, Riordan, Winter, et al., 2021).

A key characteristic of AUD neuropathology is the dysregulation of various neurometabolites including glutathione (GSH), N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr) containing metabolites (Chitty et al., 2014; Kirkland et al., 2022). Furthermore, heavy chronic alcohol use leads to long-term depreciation of γ-Aminobutyric acid (GABA) has consistently been demonstrated in the anterior cingulate cortex (Abé et al., 2013; Prisciandaro et al., 2020; Prisciandaro et al., 2019; Prisciandaro et al., 2017). Findings regarding glutamate (Glu) in AUD has been inconsistent, potentially due to the dynamic fluctuations with alcohol use. For example, brain glutamate (Glu) levels in alcohol patients relative to healthy non-drinking controls are significantly lower during intoxication (Ende et al., 2013), and higher during initial withdrawal (Hermann et al., 2012). Finally, Glu-GABA-Gln cycling systems have been suggested to be dysregulated in those with AUD (McCunn et al., 2022; Thoma et al., 2011). Glu and GABA are the principal excitatory and inhibitory, respectively, neurotransmitters and subsequently a homeostatic balance is required to maintain stable cognitive and neurological functioning (Benarroch, 2010; Cherubini & Conti, 2001). Furthermore, dysregulation of these neurometabolite signalling contributes to the development and maintenance of AUD (Koob & Volkow, 2016).

In AUD, dysregulated neurotransmitter systems have been demonstrated to precipitate poorer clinical and treatment outcomes. Alcohol-induced adaptions to Glu and GABA signalling are responsible for withdrawal symptoms (De Witte, 2004), tolerance (Valenzuela, 1997) and the dysregulation of mesocorticolimbic and extended amygdala pathways which are heavily implicated in alcohol craving, and seeking behaviours (Rao et al., 2015). Further, lower levels of GSH suggest increased oxidative stress and poorer immune function that may precipitate cognitive deficits (Tan & Norhaizan, 2019), degenerative disorders that are common comorbidities in prolonged cases of AUD (Sullivan & Pfefferbaum, 2019), and increased percentage of heavy drinking days (Morley et al., 2018). Reduced levels of NAA, a marker of neuronal integrity, have also been reported in frontal lobes (Meyerhoff et al., 2013) and these have been found to negatively correlate with recent heavy alcohol consumption (Prisciandaro et al., 2016). Finally, dysregulation of Cho, responsible for membrane structure and synthesis (Gallo & Gámiz, 2023), along with depleted NAA, suggests poorer overall neurobiological health. Therefore, chronic alcohol use-induced imbalances to neurotransmitter systems provide a target for pharmacotherapy (Morley, Perry, et al., 2021).

Recent literature suggests that cannabidiol (CBD) may modulate the disruption of these systems. For example, in healthy participants, one study demonstrated that CBD precipitated increases GABAergic but decrease glutaminergic signalling from the dorsomedial prefrontal cortex (dmPFC), and increased Glx in the basal ganglia and cortically (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, & Heasman, 2019). Further, CBD has been demonstrated to increase the anti-inflammatory and neuroprotective metabolite GSH as well as decrease inflammatory mediators (IL -1β, CD68, TNF-α, MCP-1, and NF-κB) in a preclinical study (d'Almeida et al., 2020). Given the neuroprotective and anti-inflammatory properties of CBD, administration may indirectly restore/maintain NAA concentrations decreased due to inflammation, although the direct

effects of CBD on NAA have yet to be investigated. Through anti-inflammatory and neuroprotective properties, CBD may additionally counterbalance inflammatory-mediated reduction in Choline (Cho) and creatine (Cr) metabolite levels.

One method of observing neurometabolites in the brain is proton magnetic resonance spectroscopy (^{1}H-MRS). ^{1}H-MRS enables the *in vivo* detection of neurometabolites to parse the underlying mechanism of action of novel therapeutics. Investigations into the effects of alcohol pharmacotherapies on metabolite concentrations in alcohol use disorder have revealed significant amelioration of these abnormalities following treatment relative to placebo. For example, two studies have reported that acamprosate reduced glutamate concentrations in the anterior cingulate cortex (ACC) (Frye et al., 2016; Umhau et al., 2010) while one study observed baclofen to increase GSH and NAA concentrations (Morley, Baillie, et al., 2018). There have been no studies to date examining CBD-mediated changes to neurometabolite concentrations in AUD individuals.

In the current study, we aimed to investigate the effects of CBD on specific neurometabolites (GABA+, NAA, Glx, GSH, and Cho) in the dorsal Anterior Cingulate Cortex (dACC) using single voxel ^{1}H-MRS. The dorsal ACC was chosen given high relevance in the reward/motivation circuit (Volkow et al., 2011), of which is heavily implicated in GABA-related craving and reward-seeking (Hermann et al., 2012; Yeo et al., 2013). This region is commonly examined in novel pharmacotherapy for AUD due to its importance in craving and reward process (Zhao et al., 2021). We hypothesised that: GABA+, GSH, NAA, tNAA, and tCHO in the dACC would be significantly increased, while Glx would be decreased following CBD treatment relative to placebo in AUD patients.

6.2 **Methods**

6.2.1 **Design**

The trial was conducted over 48-months at the Royal Prince Alfred Hospital (RPAH) in Sydney, NSW Australia between 2021 and 2023. The study was approved by the Human Ethics Review Committee of the Sydney Local Health District (X19-0416). The trial was sponsored by the Sydney Local Health District and registered in the Clinical Trials Registry (NCT05387148). Twenty-two non-treatment-seeking individuals who met the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V) (Diagnostic, 1994), criteria for current AUD were recruited to participate in a randomised, double-blinded, placebo-controlled, crossover trial of 3 days of dosing and experimental testing sessions. Here, we present results from the MRS acquisition which was completed on day 2. Participants were made aware of the study via flyers/community advertisements at local general practitioners, newspapers, and websites as well as through clinical referral from treating physicians, nurses, and psychologists among RPAH outpatients. All participants were required to satisfy the following criteria (detailed below) and provide written informed consent prior to randomisation to either 800mg CBD (4 x 200mg capsules per day at the beginning of each testing session) or matched placebo per day.

6.2.2 **Participants**

Twenty-two participants were recruited following screening for inclusion and exclusion criteria. *Inclusion criteria*: a) Male and female patients between the ages of 18 and 65 meeting DSM-V criteria for current AUD; b) Adequate cognition and English language skills to be able and willing to give valid informed consent and complete research interviews; c) Must have stable housing and be able to nominate a reliable contact person for locating them if necessary.

Exclusion criteria: a) Active major psychological disorder associated with psychosis or significant suicide risk; b) Pregnancy or lactation. Women were advised to use reliable contraception throughout the duration of drug therapy, and a urine pregnancy test was conducted as necessary; c) Dependence on any substance other than nicotine (e.g., methadone); d) Diagnosis of epilepsy and/or current use of anti-epileptic drugs; e) Liver failure with jaundice or prolonged INR above 1.3; f) Medical complications such as liver failure, cardiac ischemia or conduction abnormalities, renal impairment or unstable elevated vital signs (systolic blood pressure > 180, diastolic blood pressure > 120, or heart rate > 150); g) Severe cognitive impairment or insufficient English or literacy to complete study processes; h) Concurrent use of drugs potentially exacerbated by CBD via CYP3A5, including cardiac medication (eg beta blockers, calcium channel blockers, and statins), macrolides and recent antihistamine use; i) Claustrophobia; j) Extreme obesity; k) Previous brain surgery; l) Unable to complete an MRI scan (e.g., metal implants, previous employment as a machinist, welder or metal worker)

6.2.3 **Medication**

Softgel capsules containing medium chain triglyceride (MCT) oil and 200mg CBD (manufactured by Linnea Natural Pharma Solutions) (Linnea Solutions, 2021) and matching placebo (softgel capsules containing only MCT oil) were purchased from BOD Australia pharmaceuticals (Pharmaceuticals, 2021). The matching placebo was identical in appearance, taste, and composition except for the active ingredient of pure CBD. A three-day consecutive dose regimen was administered as follows: T1 (Day 1): 4 x 200 mg followed by T2 (Day 2 Experimental Session 1): 4 x 200 mg and then T3 (Day 3 Experimental Session 2): 4 x 200 mg. Participants were administered CBD and placebo orally with water, under supervision, to ensure compliance with the dose regimen.

6.2.4 **Randomisation and Allocation Concealment**

A randomisation table was computer generated (Using R Studio; (RStudio Team, 2020)) by an independent researcher and then inputted into RedCAP (Research Electronic Data Capture; a secure web application for building and managing online surveys and databases (Harris et al., 2009b). This randomisation list was then implemented by RPAH pharmacist to randomly allocate participants to one of two treatment orders (CBD then PL; PL then CBD).

6.2.5 **Procedure**

After meeting the eligibility criteria through structured interviews and medical evaluations, participants provide informed consent. They were then randomly assigned to one of two treatment regimens: either receiving 800mg CD (4 x 200mg gel capsules per day, days 1-3) or matched placebo (*see* Figure 3.2). Following an average washout of 29.2 days (SD = 11.25), participants received the alternate treatment allocation at the subsequent session. A washout period of >18 days was applied before participants received the alternate capsules during the subsequent testing session. This washout period was chosen given the half-life of CBD following chronic administration occurs between 2–5 days (Millar et al., 2018), and requires a washout of 13-day for CBD plasma levels to reduce to 'zero' (McCartney et al., 2023). On the first day of dosing (T1) participants were administered their first dose of CBD or matched placebo and completed baseline sample characteristics questionnaires. On the second day (T2) participants completed a series of MRI tasks of which data is reported elsewhere (under review; (Hurzeler et al., 2024)).

6.2.6 **Measures**

Questionnaires

REDCap was used to collect responses sample characteristic questionnaires at baseline and during follow-up (see Figure 3.2) including (i) Demographics, medical history, personal and family history of AUD, and alcohol treatment history, as obtained in previous work (Morley et al., 2014; Morley, Baillie, et al., 2013; Morley, Leung, et al., 2013; Morley et al., 2006). Additionally, (ii) recent (last 28 days) alcohol consumption (frequency/quantity) assessed by the Timeline follow-back method (TLFB; (Sobell et al., 1988); (iii) severity of alcohol dependence assessed by the Alcohol Dependence Scale (ADS; (Skinner & Allen, 1982)); (iv) craving for alcohol measured by the Penn Alcohol Craving Scale (PACS; (Flannery et al., 1999); (v) the Depression Anxiety Symptom Scale (DASS) measures the severity of symptoms of depression, anxiety and stress (Lovibond & Lovibond, 1995); (vi) sleep problems were assessed by the Insomnia Severity Index (Bastien et al., 2001); (vii) Sedation was also measured via a Likert scale (‘*between 1-10, how sedated do you feel right now where 1 is not at all and 10 is very sedated?*).

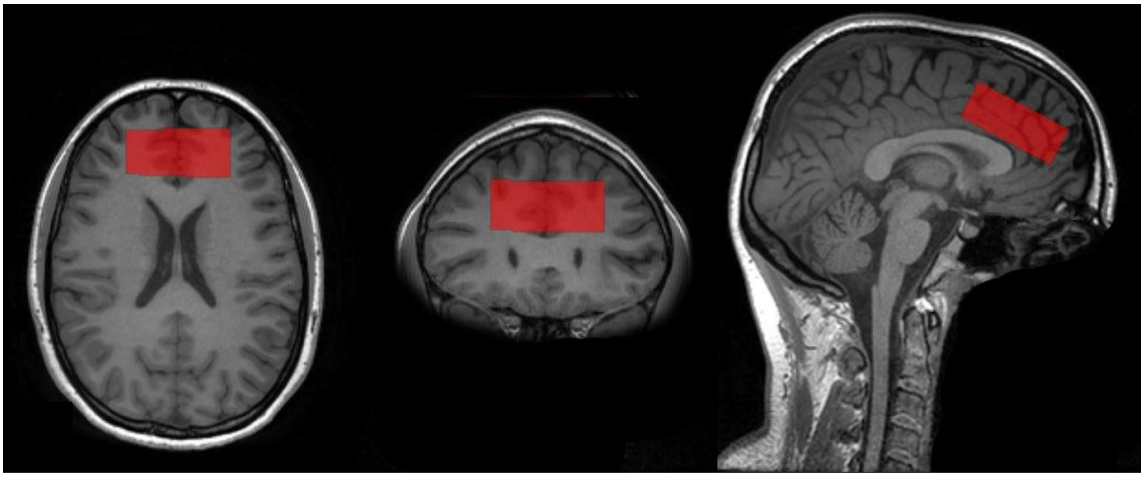
MRS Acquisition

MR scanning was conducted on a 3Tesla GE Discovery 750 scanner (i-MED, Brain and Mind Centre, Sydney), using a 32-channel phased array head coil. Firstly, A T1-weighted (1-mm^3 voxel resolution) structural scan was acquired for each subject for voxel placement co-registration (TR, 7200 ms; TE, 2.7 ms; 176 sagittal slices; 1 mm thick; no gap; 256 × 256 × 256 matrix). An edited ^{1}H-MRS using Hadamard Encoding and Reconstruction of Mega-Edited Spectroscopy (HERMES; (Chan et al., 2016; Saleh et al., 2016)) sequence placed in the dACC was completed to measure with editing for GABA+ and GSH, and ability

to quantify NAA, Glx and Cho from the unedited part of the experiment. In this editing scheme, the GABA signal is contributed to by macromolecules, and therefore is referred to as GABA+. This acquisition is optimised to simultaneously edit for GABA+ and glutathione (Gong et al., 2020; Saleh et al., 2020). The voxel was placed in the dACC (Figure 6.1) with the long edge of the voxel along the genu of the corpus callosum (voxel size 5 x 5 x 2cm^3). The acquisition parameters for the HERMES were: TR/TE = 2000/80 ms, 256 water-suppressed scan averages, 16 unsuppressed scan averages, number of data points = 4096, spectral width = 5000 Hz, acquisition time 544 s.

Figure 6.1

dACC Voxel Placement for MRS Acquisition



Note. Illustrates the placement of voxel (5 x 5 x 2cm3) for MRS acquisition presented on a T1-weighted image (A = horizontal; B = coronal sagittal; C = sagittal). The voxel placement was chosen to maximally cover the dorsal anterior cingulate (dACC) given its involvement in alcohol use disorder. Subsequently, an edited 1H-MRS using Hadamard Encoding and Reconstruction of Mega-Edited Spectroscopy (HERMES) acquisition was applied to quantify GABA+, NAA, Glx, Cho and GSH concentrations.

6.2.7 **MRS Processing**

The open-source "Osprey" MATLAB toolbox (Oeltzschner et al., 2020) was used to process and quantify the presence of GSH, GABA+, Glx, tNAA, and tCho in the HERMES acquisition. Firstly, SPM12 was used to segment T1-weighted images Osprey while we additionally extracted grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) voxel/tissue fractions. Water-referencing was used with metabolites were corrected for tissue relaxation factors, (Gasparovic et al., 2018; Gasparovic et al., 2006), and in the case of GABA+, the alpha correction was applied (Harris et al., 2015). Signal-to-noise (Cr) and fit metrics (Cr and water), along with visual inspection, were used to ensure quality of the data.

6.2.8 **Statistical Analysis**

Statistical analysis was conducted using R Studio Version 4.3.1 (RStudio Team; 2020). GEE was used to account for crossover effects (crosscarry package in r) to examine treatment effects on GSH, GABA+, Glx, tNAA, and tCho concentrations in the dACC. Metabolites and ID were used as random effects factors session order and carryover effects were explicitly modelled using Crosscarrys createCarry. Heavy drinking day percentage in proceeding fortnight (HDD%) prior to scanning and previous day drinking (PDD) were used as covariates given research indicating that alcohol has significant effects on neurometabolites. The variance inflation factor (VIF) between %HDD and PDD was calculated using the Companion to Applied Regression (CAR) package (Fox & Weisberg, 2018). A VIF threshold of greater than 5 (suggesting significant multicollinearity) was applied to ensure that multicollinearity between these two variables would not inflate parameter estimate variance. Finally, a post hoc analysis was conducted to examine interactions between treatment (CBD vs placebo) and previous day drinking (PDD) to

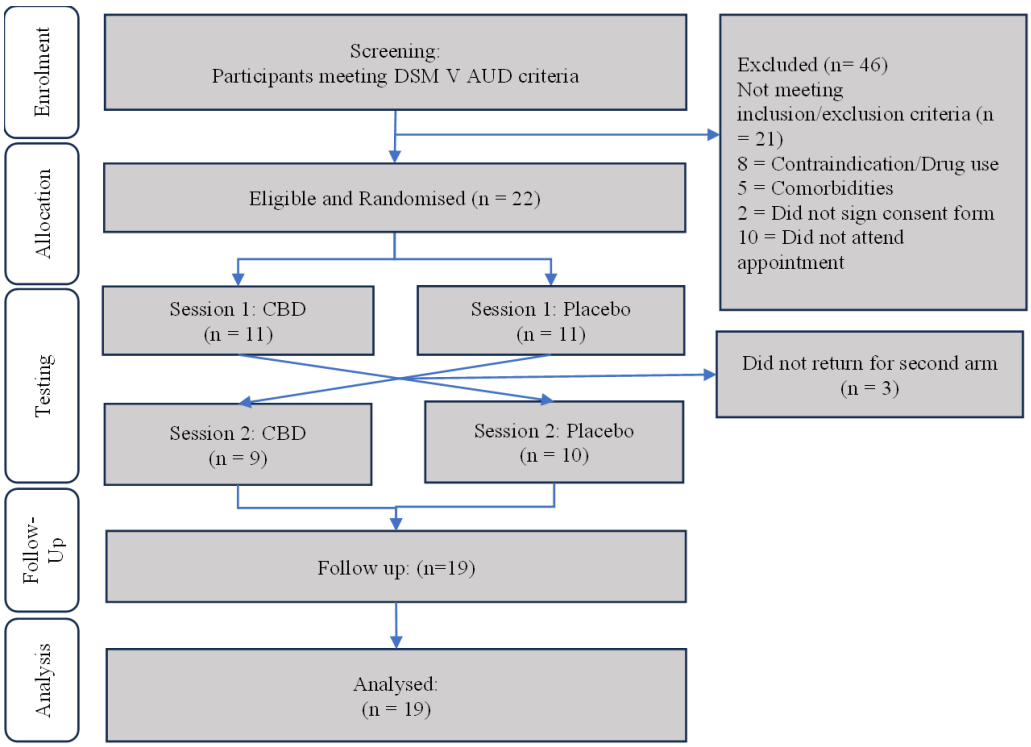
explore the role of recent alcohol use on the relationship between the effect of CBD on neurometabolites.

6.3 **Results**

Three participants did not complete both arms of the crossover design and therefore were removed from the final analysis (See Figure 6.2). Two additional participants were removed from the final analysis due to an error in voxel placement. One more participant was removed after a visual inspection of metabolite spectra abnormalities due to data quality issues.

Figure 6.2

Consort Flow Diagram



Note. Details the flow of participants through the cross-over design from recruitment to follow up including information regarding dropout. Of the 68 participants screened, 46 were excluded proceeding consent and randomisation, and 3 participants dropped out before completing their second session.

6.3.1 **Sample Characteristics**

Table 6.1 depicts the characteristics of the sample. The mean age of participants overall was 29.44 (SD = 13) with an average of 14.85 (SD = 1.68) years of education. Twelve out of 16 of the participants were female (75%). Participants on average drank 6.87 (SD = 3.99) drinks per drinking day, experienced a dependence severity of 13.69 (SD = 4.32) and had 7.93 (SD = 10.49) years of self-reported problems with drinking. Additionally, 4 out of the 16 participants reported using nicotine in the previous 28 days.

Table 6.1

Sample Characteristics

Participants (n=16)	
Demographics	
Age (years)	29.44 ± 13 [18 – 62]
Education (years)	14.85 ± 1.68 [11 – 17]
Gender %	75% (12/16)
Employment	
Employed within last year	87.5% (14/16)
Months employed	8.31 ± 4.35 [0 – 12]
Clinical Characteristics	
Drinks per drinking day	6.87 ± 3.99 [2.71 – 17.6]
Years since alcohol-related problems began	7.93 ± 10.49 [1 – 38]
ADS score	13.69 ± 4.32 [9 – 23]
PACs craving score	11.5 ± 5.68 [4 – 24]
ISI	7.31 ± 6.2 [1 – 19]
DASS-21: Anxiety	3.13 ± 2.85 [0 – 9]
DASS-21: Depression	5 ± 5 [0 – 16]
DASS-21: Stress	6.56 ± 4.9 [0 – 17]
Comorbidities (Mini) %	35% (6/17)
Antidepressants (last 28 days) %	31.25% (5/16)
Tobacco use (last 28 days) %	25% (4/16)

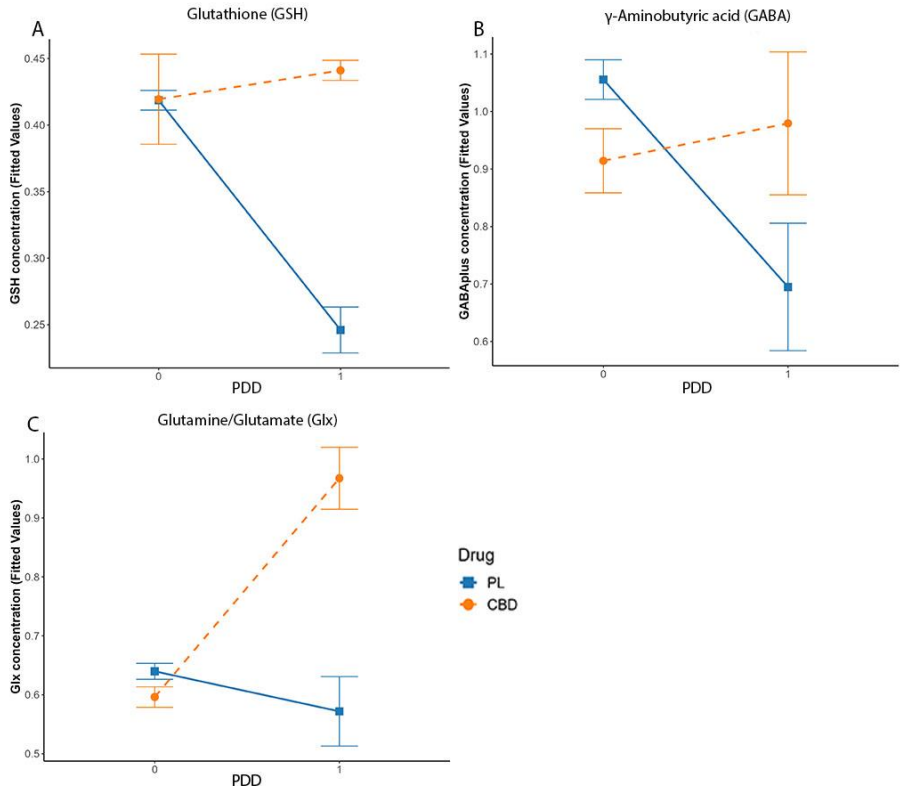
Note. Mean ± *SD*s [Range] format used to represent data unless otherwise noted. ADS (Alcohol Dependence Score); PACS (Penns Alcohol Craving Score); ISI (Insomnia Severity Index); DASS (Depression Anxiety Symptom Scale).

Effect of CBD vs Placebo on Neurometabolites

Spectroscopic analysis figures of GABA, GSH and other metabolites can be found in Appendix C, Figure 1-3. No significant treatment effects (Appendix C Table 8) were identified between neurometabolites, GSH, GABA, Glx, tNAA, and tCho (p's ≥ 0.28). However, there was a main effect of PDD for GABA (p = 0.0043 indicating a role of recent drinking on GABA+ concentration. Raw results from exploratory analysis examining the interaction terms of treatment x recent alcohol consumption (PDD) can be found in Appendix C Table 9. There were no significant interactions between PDD and treatment (CBD vs PL) for tNAA (p = 0.061) or tCho (p = 0.265). There were significant interactions between PDD and treatment (CBD vs PL) for GSH (p<0.001), Glx (p = 0.001), and GABA (p= 0.0024). For these neurometabolites, when comparing participants who had engaged in drinking the day before the scan to those that hadn't, concentration levels were reduced during placebo sessions but elevated in CBD sessions. This suggests the effect of CBD on modulating concentration of brain GSH, Glx, and GABA+ levels is associated with alcohol consumption within the previous 24 hours (see *Figure 6.3*).

Figure 6.3

Significant interactions effects between recent drinking and treatment for GSH, GABA, and Glx concentration



Note. Illustrates interaction effects between treatment (cannabidiol (CBD) vs placebo (PL)) and previous day drinking (PDD; coded 0 for no drinks and 1 for previous day drinking). Represented along the y axis are the fitted values from generalised estimated equations (GEE) models accounting for carry over, session effects and heavy drinking day percentage in proceeding two weeks with respect to neurometabolite concentrations. Standard error and means from relevant fitted values are indicated by error bars.

A: Glutathione (GSH); CBD, compared to placebo, rescues GSH concentrations comparing PDD vs no PDD.

B: γ-Aminobutyric acid (GABA); An inverse relationship such that GABA increased following CBD and decreased following PL when comparing PDD vs no PDD.

C: Glutamine + Glutamate (Glx); CBD, compared to placebo, was associated with a marked increase in Glx when comparing PDD vs no PDD.

6.4 **Discussion**

This is the first study investigating the effects of CBD on neurometabolite concentrations in a sample of individuals with AUD. Our results demonstrate that CBD may significantly modulate dACC GSH, Glx, and GABA+ concentrations relative to placebo but that this effect is contingent on alcohol consumption on the previous day. Specifically, when participants consumed alcohol the previous day before, relative to individuals who did not, CBD administration significantly replenished GSH concentrations and increased Glx and GABA+ levels. These interaction effects suggest that initial non-significant treatment effects were masked by variations in recent alcohol consumption and further emphasise that pharmaco-MRS studies in AUD populations should control for recent alcohol consumption. Our results suggest that CBD may normalise neurometabolite systems dysregulated by recent alcohol consumption within the dACC, specifically relating to oxidative stress as well as major excitatory and inhibitory neurotransmitter systems.

Our results indicating that CBD may replenish brain GSH levels following recent alcohol use is consistent with pre-clinical literature outlining the anti-oxidant and anti-inflammatory properties of CBD (for review, (Henshaw et al., 2021)). Heavy alcohol use can lead to oxidative stress, associated with reduced GSH, resulting in diminished neural function (Crews et al., 2015). Oxidative stress and neuroinflammation have been strongly associated with relapse (Quintanilla et al., 2018). Indeed, preclinical studies suggest that replenishing intracellular levels of GSH may play a role in reducing alcohol withdrawal (Schneider Jr et al., 2015) and relapse (Quintanilla et al., 2016). Thus, the current data suggest that CBD may have some promise in the management of AUD through ameliorating oxidative stress levels and the subsequent protection of clinical consequences associated with oxidative damage such as neuronal and liver injury and neuro-cognitive deficits (Ozburn & Spencer, 2023).

We additionally observed that CBD administration, in the context of recent alcohol consumption, precipitates both an increase in Glx and the restoration of GABA concentrations. Acute effects of alcohol typically precipitate an influx in inhibitory GABAergic signalling (Kumar et al., 2009). In cases of chronic alcohol consumption, alcohol cessation is then associated with a decrease in GABA concentrations which leads to psychological and physical withdrawal symptoms, and neuroadaptive dysregulation that precipitate and maintain AUD (Koob & Volkow, 2016). The current results indicating that CBD may be able to restore this imbalance indicates some promise for the management of AUD. This is supported by numerous preclinical studies wherein CBD has consistently been demonstrated to normalise GABA and glutamate dysregulation (Gomes et al., 2015; Long et al., 2006; Moreira & Guimarães, 2005; O'Neill et al., 2021). However, the current data regarding the effect of CBD on Glx are potentially more complex to interpret. Generally, increases in Glu signalling, typically indexed by Glx, precipitate decreased GSH concentrations due to hyper-excitation leading to excessive calcium ions in neurons precipitating oxidative stress (Fan et al., 2023). It is highly relevant to note, however, that our MRS analysis employed a proxy of Glu and Gln concentrations (Glx) which provides a measure of metabolite concentration within tissue but cannot differentiate between intra and extracellular sites whereby the specificity of the cycle is unknown. It is therefore plausible that, given the observed CBD-induced restoration of both GSH and GABA in the current study, CBD may increase the availability of Gln within metabolic pools modulating GABA and Glu synthesis and modulate Glu/GABA-Gln cycling (McCunn et al., 2022; Thoma et al., 2011).

An additional consideration when appraising results presented here is that the MRS voxel was restricted to measuring local changes to neurometabolite concentrations within the dACC. However, previous literature suggests that the effects of CBD on GABA+ and Glx

concentrations may differ by indication and brain region. For example, one clinical study utilising MRS demonstrated that CBD administration was associated with decreased cortical and increased subcortical Glx for both autism spectrum disorder (ASD) and neurotypical control groups relative to placebo-treated individuals. While GABA+ concentrations were increased in the DMPFC for controls but decreased for ASD (Pretzsch, Freyberg, Voinescu, Lythgoe, Horder, Mendez, Wichers, Ajram, Ivin, Heasman, et al., 2019). This suggests that changes to inhibitory and excitatory signalling may occur globally but in a distinct manner across regions. Further research is therefore required to disentangle CBD-elicited effects on additional regions associated with alcohol-induced neurobiological dysregulation. Furthermore, future research should explore whether CBD-induced neurometabolic changes differ across distinct neurobiological profiles within treatment-seeking AUD population.

The current study possess several strengths and limitations. Firstly, our analysis of interaction effects (treatment x recent alcohol consumption) is exploratory in nature and should thus be taken with caution. Further, a non-treatment seeking sample was recruited which may reduce generalisability to a clinical population. However, greater alcohol consumption observed in clinical samples would likely be associated with heightened imbalances of neurometabolites (Yeo et al., 2013) such that these results are likely to be generalisable to the treatment-seeking population. In regard to the acquisition, the voxel size is arguably both a strength and limitation. It was notably large ($50cm^3$) which limits the spatial specificity of our results as it was a highly inclusive dACC definition. This size, however, and the greater signal that comes from it, resulted in high quality data for the HERMES acquisition. The use of HERMES to quantify of GSH, Glx, GABA+, NAA, Cr, and Cho with only one scan reduces the risks of scanning artifacts associated with longer scan times and provides exciting insights into multiple relevant neurometabolites.

In conclusion, the results presented here demonstrate that, for AUD individuals that have recently consumed alcohol, CBD significantly modulates neurometabolite concentrations within the dACC relative to placebo. It is thus possible that dysregulation of GSH, Glx, and GABA following alcohol consumption could be normalised via treatment with CBD. CBD may repair oxidative stress and normalise dysregulated excitatory and inhibitory signalling resulting from alcohol consumption and therefore may have a role in the management of AUD.

Chapter 7: Discussion

7.1 Thesis Statement of Purpose

The overarching aim of this thesis was to identify preliminary signals of efficacy of CBD for the treatment of AUD in addition to examining potential mechanisms of action for this indication. Specifically, the impact of CBD administration on neurobiological and psychophysiological systems dysregulated in AUD was assessed in addition to craving, mood, and executive functioning (EF). Results from a systematic review (Chapter 2) of studies examining CBD in non-clinical human samples using various measures (e.g. task and resting state fMRI, MRS) then guided the design of the cross-over double-blind randomised controlled trial (DBRCT) of 800mg/day of CBD versus placebo on regional brain activity, psychophysiology and neruometabolites (Chapters 3, 4 and 5). This final chapter summarises (Section 7.2) and synthesises each of these results while providing insight into the potential efficacy and mechanism of CBD (Section 7.3). Subsequently, potential clinical implications are discussed (Section 7.4), in addition to overall limitations and methodological considerations (Section 7.5), and, finally, suggestions for further research regarding the use of CBD in the treatment of AUD (Section 7.6).

7.2 Overview of Empirical Chapters

7.2.1 Chapter 2

Chapter 2 (published in *J Cannabis Res. 2024 Mar 21;6(1):15*) reviews existing literature pertaining to CBD-mediated effects on neurobiology in non-clinical human samples as well as non-clinical samples relative to clinical samples (including clinical high risk of psychosis and autism spectrum disorder). This comprehensive literature synthesis demonstrated the diverse effects across the brain following CBD administration as measured

by fMRI (during both resting state and task-based paradigms), MRS, and SPECT measures. Studies reported that CBD administration relative to placebo modulated the following: fronto-striatal connectivity between the putamen/PFC (Grimm et al., 2018); associative striatum/posterior parietal lobes; limbic striatum/lateral frontal cortex/right hemisphere insula; and sensorimotor striatum/cerebellum (Wall et al., 2022) suggesting some potential to impact on EF (Galandra et al., 2019). Studies implementing go/no go tasks observed that CBD-modulated regions associated with discriminating between salient and non-salient cues including temporal gyrus, insula, posterior cingulate gyrus, left medial PFC, right caudate, parahippocampal gyrus, precentral gyrus and thalamus (Bhattacharyya et al., 2012; Borgwardt et al., 2008). Additionally, CBD-mediated increased functional connectivity was observed in regions of interest (ROIs) associated with processing novel stimuli and modulating salience attribution such as the dorsal striatum, hippocampus, and PFC (Bhattacharyya et al., 2014). Treatment with CBD was also associated with modulations in regions associated with memory consolidation including the insula, mediotemporal gyrus, lingual gyrus, precuneus, and precentral gyrus during encoding phases of a memory task and hippocampus during recall phases (Bhattacharyya et al., 2009). Further, several studies demonstrated the normalisation of regional brain activity during tasks associated with emotion regulation (Davies et al., 2020), memory retrieval (Bhattacharyya et al., 2018), and anticipation and reward processing (Wilson et al., 2019) for individuals at clinically high risk of psychosis. Using magnetic resonance spectroscopy (MRS), CBD administration was also associated with modulations of glutamate/glutamine (GLX) and γ-aminobutyric acid (GABA) concentrations relative to placebo in both the basal ganglia and the dorsomedial prefrontal cortex (DMPFC) for neurotypicals but not for those with autism spectrum disorder (Pretzsch, Voinescu, et al., 2019). Overall, this review highlights that brain regions modulated by CBD are relevant to AUD. Dysregulation within these identified regions underpins an array of

cognition and behaviour, such as perturbed decision-making, emotion regulation, salience attribution, reward processing, and habit formation, that precipitates and maintains AUD (See Chapter 1, Section 1.2.2). Accordingly, the next step to identify potential signals of efficacy and mechanism of action for AUD is research that directly examines the effect of CBD on AUD individuals.

7.2.2 **Chapter 3**

Chapter 3 (accepted with revisions in *Contemporary Trials Communication. 2024* June in press) presents the published protocol that outlines the methodology for the double-blind, within-subject, randomised, placebo-controlled, cross-over study wherein results are presented in Chapters 4, 5, and 6. This chapter includes a comprehensive and detailed description of the design, procedures, measures, and analysis. Briefly, non-treatment seekers were randomly allocated to three days of four 200mg CBD gel capsules (800 mg/day) or placebo, with an 18-day washout period. Cognitive, clinical, and neuroimaging assessments were completed during these three days. The CBD and placebo sessions were then compared across a range of predetermined outcomes. Primary outcomes included: i) BOLD signal as a proxy for regional activity during a cue-reactivity and a fear response task measured with functional magnetic resonance imaging (fMRI); and ii) high-frequency heart-rate variability and skin conductance levels as a proxy for psychophysiological responses to alcohol stimuli. The secondary outcomes included: i) neurometabolite levels (γ-Aminobutyric acid, glutathione, N-acetylaspartate, choline-containing compounds and glutamate+glutamine (combined signal)) in the dorsal Anterior Cingulate Cortex (dACC) using magnetic resonance spectroscopy (MRS); ii) functional connectivity using resting state fMRI (rsfMRI); iii) EF task results; iv) clinical outcomes such as craving and anxiety.

7.2.3 **Chapter 4**

Chapter 4 (submitted for publication) examines the CBD-mediated effects on cue-elicited psychophysiological, anxiety, and craving responses during a cue reactivity task. Indices of the parasympathetic and sympathetic nervous systems (high frequency heart rate variability (HF-HRV) and skin conductance level (SCL), respectively) along with self-report craving and anxiety were investigated during baseline, appetitive cue exposure (juice cue and alcohol cue exposure) and appetitive cue recovery periods (juice cue recovery and alcohol cue recovery). Results indicated that, compared to placebo, CBD administration was associated with increases in HF-HRV occurred throughout the cue reactivity task, decreased self-report anxiety during cue exposure compared to baseline, and more marked decreases in self-report craving when comparing cue exposure to recovery periods. An interaction between treatment and a contrast comparing control and alcohol cue exposure suggested that CBD-mediated increases of HF-HRV were dampened during alcohol cue exposure stages compared to juice cue exposure. Furthermore, significant session order effects demonstrated that treatment effects on the parasympathetic nervous system (PNS) were smaller when CBD followed a placebo dosing session. However, no treatment effects were identified on EF tasks (testing working memory, inhibitory control, task set-shifting flexibility, and attention) suggesting that treatment effects on PNS cue-elicited responses and self-report anxiety were not driven by sedative effects. These results add to the literature in several ways. These results are consistent with previous studies demonstrating that CBD is associated with reductions in anxiety (Masataka, 2019; Zuardi et al., 2017). However, the current results are somewhat inconsistent with studies finding CBD reduces cue-induced craving and sympathetic nervous system (SNS) recruitment to heroin-associated cues in heroin use disorder (Hurd et al., 2019). Instead, these results indicate that CBD may not decrease craving during drug cue exposure in AUD but that there are steeper decreases in self-report

craving during *recovery* periods following cue exposure which may be related to the *general* increased recruitment of PNS. These general increases in PNS recruitment may be driven by the anxiolytic properties of CBD given the current results showing reduced self-report anxiety during appetitive cue exposure and/or decreased salience attribution towards appetitive cues.

7.2.4 **Chapter 5**

Chapter 5 (submitted for publication) examined the effect of CBD versus placebo on ROIs within the mesocorticolimbic network during a neuroimaging alcohol cue reactivity paradigm and performance on EF tasks (Self-regulation, adaptive risk-taking, inhibition, working memory, task-set switching, and attention). An a priori analysis demonstrated that regions previously identified to be heavily implicated in cue-craving (dorsolateral prefrontal cortex (PFC), left and right caudate, or bilateral ventromedial PFC) were not modulated by CBD dosing in comparison to placebo during a common cue reactivity imaging paradigm. Conversely, an exploratory whole-brain analysis identified that the left precuneus was significantly hypo-activated during control and alcohol cue conditions in CBD versus placebo conditions. However, no treatment effects were identified on EF tasks suggesting that cue-elicited precuneus hypo-activation was not driven by sedative effects. Additionally, no treatment effects were identified on cue-elicited acute craving or mood. Similarly to Chapter 4, this indicates that CBD is associated with reductions in cue-elicited left precuneus activation during non-specific cue presentation. While this was not specific to alcohol-associated cues, rescuing left precuneus activation leads to reductions in arousal responses elicited by alcohol or novel cues which subsequently could modulate habitual alcohol seeking and be beneficial for AUD individuals.

7.2.5 **Chapter 6**

Chapter 6 (submitted for publication) examines the effect of CBD versus placebo on neurometabolites in the dACC. Results indicated that, within the dACC, metabolite concentrations were not modulated by CBD administration relative to placebo. However, exploratory analyses revealed that CBD significantly modulated neurometabolite concentrations depending on recent alcohol consumption. Specifically, in participants that consumed alcohol the previous day, CBD was associated with i) a rescuing of brain GSH that was decreased in placebo individuals; ii) an increase in GLX concentration relative to individuals that received placebo; and iii) a rescuing of GABA levels relative to placebo individuals. This suggests that CBD may modulate neurometabolite systems depending on the baseline severity of dysregulation and in this instance this dysregulation is due to recent alcohol consumption. These results are clinically relevant given the severity of drinking profiles and daily alcohol consumption characteristic of the AUD population. These CBD-induced effects that are dependent on varying neurobiological dysregulations have been previously identified and reported on in Chapter 2 (Bhattacharyya et al., 2018; Davies et al., 2020; Pretzsch, Voinescu, et al., 2019; Wilson et al., 2019). Furthermore, given that the dACC is a region strongly implicated in alcohol cue reactivity tasks, reward processing, and decision-making (Bechara, 2005; Noel et al., 2013; Sjoerds et al., 2013), these results suggest that CBD may be capable of modulating cue-elicited motivational urges to seek alcohol for those that have engaged in recent alcohol consumption. Finally, the results demonstrating CBD-induced increased brain GSH concentrations following recent alcohol consumption also support previous evidence that CBD has anti-oxidant properties (Pereira et al., 2021).

7.3 **Integration of Findings and Potential Mechanisms**

This thesis examined the potential pharmacotherapeutic effects of CBD across interdependent domains dysregulated by chronic alcohol consumption including neurometabolite concentrations, regional brain activity, the autonomic nervous system, EF, and affect. In the following paragraphs, an integration of findings from the empirical chapters with potential mechanisms of action will be concurrently discussed.

7.3.1 **Cellular Mechanisms**

Adenosine and Anandamide

Given the poly-pharmaceutical actions of CBD across numerous molecular targets a range of cellular-modulating mechanisms may underpin the current results (see Chapter 1, Section 1.6.2). The endocannabinoid system (ECS) is responsible for a vast number of homeostatic functions throughout the central and peripheral nervous system including the regulation of pain, stress responses, appetite, mood, immune function, and inflammation (Mechoulam & Parker, 2013). CBD-mediated effects on the ECS are precipitated via CBD's effects on endocannabinoid signalling through its mediation of the transportation of endo-cannabinoids AEA and adenosine. CBD inhibits the catabolism of AEA by fatty acid amide hydrolase (FAAH) and reduces the cellular uptake of endocannabinoids (Elmes et al., 2015). Furthermore, AEA is a ligand of CB1r in the central nervous system and has a low affinity for CB2r in peripheral sites, and subsequently CBD administration leads to increased activation of inhibitory CB1r and CB2r activity (Bisogno et al., 2001). However, CBD has also been demonstrated to be a negative allosteric modulator of CB1r (Laprairie et al., 2015). Further, CBD inhibits the reuptake of adenosine subsequently increasing the extracellular levels of adenosine which may contribute to neuroprotective and anti-inflammatory effects (Nichol et al., 2019). In the CNS, endocannabinoid CB1rs have been identified across various

brain regions as one of the highest-density G-protein-coupled receptors (Hirvonen, 2015). Indeed, CB1 receptor antagonism has been shown to decrease alcohol consumption in animal models (Turna et al., 2019). Furthermore, systemic downregulation of CB1 in the CNS has previously been demonstrated following protracted alcohol use (Hirvonen, 2015). CBD administration may therefore ameliorate this alcohol-induced dysregulation and alcohol-seeking behaviours via modulating the ECS.

The current results regarding the effect of CBD on neurometabolites (Chapter 6; Section 7.2.5 for summary) may be underpinned by these CBD-mediated effects on AEA and adenosine. For example, adenosine promotes excitatory amino acid transporters (EAATs) activity on astrocytes, facilitating the uptake of Glu and the conversion of Glu to Gln (Magi et al., 2019). Additionally, adenosine inhibits Glu release via its agonism of inhibitory adenosine receptor (A1r) activation (Magi et al., 2019). Through this capacity to lead to an influx of Gln within metabolic pools and inhibited Glu release, increases in adenosine may have encouraged increases in GABA production via conversion through the Gln-Glu-GABA cycle (Schousboe et al., 2014). These modulations of the ECS may have additionally contributed to the reported CBD-induced increases of the potent brain antioxidant GSH in participants who consumed alcohol recently. For example, modulation of CB1 and CB2 receptors at central and peripheral sites respectively may regulate reactive oxygen species generation through various signalling pathways (Pereira et al., 2021) and thus lead to these anti-oxidative effects. The observed general elevation in PNS activity and anxiolytic-like effects observed in Chapter 4 (see Section 7.2.3 for summary) may have additionally been due to these CBD-induced anti-oxidative effects within central and peripheral sites. Evidence of this is that the hippocampus, in which CB2rs are expressed (Gong et al., 2006), is responsible for the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Jacobson &

Sapolsky, 1991) and is particularly vulnerable to damage caused by oxidative stress (Salim, 2017). Furthermore, preclinical work has previously demonstrated the direct effects of oxidative stress in high arousal anxiety-like behaviours in mice (Bouayed et al., 2007), and previous pharmacotherapeutic studies additionally have demonstrated that anti-oxidative supplements significantly reduce depressive and anxiety symptoms (Wang et al., 2023).

Dopamine and serotonin

CBD-induced modulations of adenosine and AEA have also been demonstrated to inhibit dopaminergic and modulation of serotonergic signalling which could underpin the observed psychophysiological and functional brain activity responses to appetitive cues in Chapters 4 and 5. By inhibiting the reuptake of adenosine, CBD may indirectly decrease both dopaminergic and serotonergic signalling via similar agonism of inhibitory A1r activity (Choudhury et al., 2019). Additionally, CBD-induced increases in AEA may lead to decreased dopaminergic and serotonergic signalling through modulation of CB1 receptors found on dopaminergic neurons and the presynaptic terminal of serotonergic neurons (Kathmann et al., 1999; Nakazi et al., 2000). Furthermore, various other pharmacological mechanisms have been demonstrated whereby CBD may modulate serotonergic signalling including its action as a partial 5-HT1A receptor agonist (Russo et al., 2005) and via serotonin reuptake inhibition (de Mello Schier et al., 2014).

Preclinical studies have demonstrated that CBD administration is associated with the inhibition of amphetamine-elicited increases in dopamine activity (Renard et al., 2016). Furthermore, alcohol intake is reduced following CBD administration and reduces μ-opioid receptor gene expression (Viudez-Martínez, García-Gutiérrez, et al., 2018a), which is involved in dopamine biosynthesis (Schouten et al., 2024). However, CBD-induced

reductions in cocaine and alcohol use have previously been demonstrated to be inhibited by 5-HT1A antagonists (Galaj et al., 2020; Viudez-Martínez, García-Gutiérrez, Fraguas-Sánchez, et al., 2018). Finally, CBD anxiolytic-like effects have been tentatively suggested to be driven by indirect effects on CB1 and 5-HT1A receptors within the PFC and prelimbic cortex (Galaj et al., 2020; Sales et al., 2018; Sartim et al., 2016; Viudez-Martínez, García-Gutiérrez, Fraguas-Sánchez, et al., 2018) Therefore, although CBD may modulate dopaminergic activity CBD-mediated effects on drug seeking behaviour, more substantial effects on alcohol-seeking behaviours and alcohol use disorder characteristics including anxiety may be driven by changes in serotoninergic signalling.

7.3.2 **Regional Activity and Neurocircuitry Mechanisms**

Regional Brain Activity

Chapter 5 (Section 7.2.4 for summary) identified that the lingual gyrus, cuneus, and superior parietal lobule were hyperactivated across treatment conditions (CBD vs placebo) when comparing alcohol to control cue exposure. Additionally, the left precuneus was hypo-activated during control and alcohol cue-exposure for CBD vs placebo treatment sessions. This suggests that CBD administration was associated with a general dampening of a region sharing functional connectivity with regions with alcohol-cue-specific hyperactivation (Addis et al., 2004; Eustache et al., 2004; Gilboa et al., 2004). However, self-report measures of craving were not modulated following CBD administration during alcohol and control cue exposure in either of the cue-reactivity paradigms presented in Chapters 4 and 5.

The precuneus is responsible for self-referential processing, consciousness and awareness, memory retrieval, mental imagery, attention, and focus (Cavanna & Trimble, 2006; Northoff et al., 2006). Interestingly, in line with the non-significant CBD-mediated

changes to cue-elicited self-report craving, previous literature has indicated a consistent lack of relationship between precuneus activation and self-report craving (Chase et al., 2011) with only 2/13 (15.38%) alcohol cue reactivity studies identifying relationships between precuneus activation and self-report craving (Courtney et al., 2014; Park et al., 2007; Tapert et al., 2003). However, positive associations between severity and drug cue-elicited precuneus activation have previously been demonstrated (Claus et al., 2011). This relationship between severity, self-report craving, and precuneus activation has been proposed to reflect an indexing of broader addiction processes or "biological experience of craving" that are upstream to regions thought to underly subjective craving (Courtney et al., 2014) such as the insula (Garavan, 2010). Therefore, previous suggestions have been made that precuneus hypo-activation may lead to reduced habitual alcohol seeking in the absence of reductions in cue-induced self-report craving (Courtney et al., 2014).

Neurocircuitry

Hypo-activation of the precuneus during cue reactivity suggests the possible modulation of various neurocircuitry including the salience network (SN), default mode network (DMN), and central executive network (CEN), referred to in combination as the triple network (TN). The triple network model (Menon, 2011) suggests that the SN is tasked with evaluating external salient stimuli and subsequently mediating the switch between CEN and DMN-driven activation (Goulden et al., 2014) which have been shown to have a negative correlation (Sridharan et al., 2008). The CEN is responsible for various high-level executive processes which drive emotion regulation, goal-directed behaviour, decision-making, and cognitive flexibility (Bertocci et al., 2023; Sridharan et al., 2008) while increased DMN, along with the precuneus, is thought to be responsible for self-referential processing, autobiographical memory, interoception, thinking, and rumination (Zhou et al., 2020).

Through this capacity to switch between these circuits, the SN precipitates adaptive cognitive processing and psychological responses following cue exposure and precipitates downstream psychophysiological and behavioural responses to cues. However, these processes have previously been suggested to be perturbed by dysregulation of the DMN induced by drug and alcohol use, particularly during withdrawal and preoccupation stages of addiction (Gerhardt et al., 2022; Zhang & Volkow, 2019). Hypo-activation of the precuneus may therefore reflect CBD-mediated improved regulation of the DMN and reduced self-referential processing of cues which subsequently increases recruitment of the CEN and top-down executive control.

This CBD-induced reduction to the precuneus in the DMN may have been precipitated by serotonergic effects in the limbic network (LN). The SN contains numerous limbic structures and establishes the salience of stimuli through the incorporation of internal and external inputs (Craig, 2009; Uddin, 2016). Therefore, anxiolytic-like effects from CBD, as observed in Chapter 4, may have dampened salience attribution to cues throughout the cue reactivity task. CBD-induced precuneus hypo-activation potentially then contributes to this reduced salience attribution as a result of reduced cue-elicited rumination and retrieval of emotionally salient memories associated with the cues. Furthermore, CBD-elicited anxiolytic driven reduction of salience attribution is further supported by the dampening of general CBD-induced increases in PNS recruitment (as observed in Chapter 4) during alcohol cues. It is thus possible that CBD may be less suited to diminishing salience attribution to cues that have been encoded as salient through repeated associated dopaminergic release in mesocorticolimbic networks.

7.3.3 **Autonomic Nervous System Mechanisms**

CBD-induced anxiolytic-like modulations to LN may have additionally modulated brain regions directly responsible for regulating parasympathetic and sympathetic recruitment including the hypothalamus (Pecoraro & Dallman, 2010). Additionally, CBD-induced downregulation of the DMN and upregulation of the CEN may limit rumination-driven sympathetic arousal and improve top-down cognitive suppression of psychophysiological high arousal responses (Brosschot et al., 2006; Ochsner & Gross, 2005). The results from Chapter 4 seem to reflect this CEN-driven improved regulated autonomic cue-elicited response in a non-cue-specific manner during cue presentation, suppression of high arousal response post cue presentation and improved craving recovery post cue removal.

CBD-mediated modulation of the CNS may have led to top-down modulation of the autonomic nervous system observed in Chapter 4. However, CBD administration may have conversely led to modulations in the peripheral nervous system which may modulate higher-order processes driven by the CNS including salience attribution, and psychological experiences of anxiety and craving through bottom-up processes. This is because there exists a bidirectional relationship between the CNS and the ANS by which the hypothalamus and thalamus receive information regarding the status of the ANS via the vagus nerve and spinal cord following which the cortical and limbic regions integrate this autonomic feedback information through cognitive and emotional processes (Thayer & Friedman, 2002). Subsequently, the central autonomic network (CAN) responds accordingly to maintain autonomic nervous system homeostasis to complete the feedback circuit (Thayer & Friedman, 2002). Therefore, CBD-induced modulations of the PNS directly through CB1r and CB2r inhibition, are found both in the periphery such as in heart myocardium tissue (Bátkai et al., 2004) and cardiomyocytes (Defer et al., 2009) respectively, CBD-induce

inhibition of voltage-gated calcium channels (Ross et al., 2008) and sodium channels (Ghovanloo et al., 2018) or anti-inflammatory/anti-oxidant CBD-induced effects may modulate interoception, feedback, and autonomic action circuit at peripheral sites provides an additional mechanism for the results across Chapters 4 and 5.

7.3.4 **Mechanistic Summary**

In summary, tentative explanations for the results presented in this thesis could be related to anxiolytic-like effects in the central and/or in the peripheral nervous system. These anxiolytic-like effects may be underpinned by modulation of the ECS and anti-oxidative properties, modulation of dopaminergic, and to a greater degree serotonergic signalling, reduced salience attribution, improved SN innervation between the DMN and CEN, and corresponding effects on regions relevant for modulating the autonomic nervous system. Notwithstanding, however, certain results are incongruent with this theoretical network-based mechanism. These incongruencies may indicate an alternative mechanism may be driving the observed results or methodological limitations associated with the thesis. Firstly, if CEN recruitment was increased it would be expected that performance on cognitive tasks would be improved which was not demonstrated by the results presented in either Chapter 4 or 5. One potential cause of these non-significant improvements in EF may be due to the sample that was employed in these experiments as they were relatively young on average wherein cognitive impairment may be difficult to detect. Secondly, no additional regions that are commonly implicated in these networks were associated with CBD-induced modulations in activity during cue presentation in Chapter 5. However, this may suggest that precuneus hypoactivation is a consistent feature across all AUD individuals while modulations in additional regions may differ among individuals with varying clinical characteristics.

7.4 **Clinical Implications**

The following section will discuss the clinical implications of the current results and potential mechanisms of CBD in the management of AUD (Section 7.3). The results from Chapters 4-6 indicate that CBD may have the potential to target certain elements of the addiction cycle through anxiolytic-like processes. As previously discussed (Section 1.2.1), the binge/intoxication stage pertains to the acute effects of heavy alcohol consumption on the central and peripheral nervous system. Repeated periods of alcohol intake led to neurobiological allosteric adaptions and neurotransmitter dysregulation, as well as regional and network brain activation (Section 1.2.2). As demonstrated in Chapter 6, CBD may normalise neurometabolite dysregulation of GABA, GSH, and Glx following recent alcohol consumption. These changes may allow for the maintenance of homeostatic excitatory and inhibitory functioning and reductions in neuroinflammatory responses. CBD may have several therapeutic benefits for individuals in AUD such as aiding to reduce mesocorticolimbic dysregulation that precipitates various characteristic downstream traits of AUD (Section 1.2.2). Furthermore, the observed anti-inflammatory-like properties as observed via the CBD-induced GSH elevations additionally suggest CBD could be used to protect against alcohol-induced oxidative-stress-related complexities such as cognitive impairments and liver injury.

The results presented in Chapter 6 additionally have relevance to withdrawal/negative affect stages of AUD. Withdrawal symptoms associated with GABA imbalances may be alleviated with CBD administration given the demonstrated normalisation of GABA dysregulation caused by previous day alcohol consumption. Furthermore, CBD-induced anxiolytic effects demonstrated in Chapters 4 and 5 also suggest an additional potential to mitigate negative affect precipitated during the withdrawal stages and may aid individuals to

abstain during this stage whereby an influx of tension reduction motivated alcohol-seeking is often experienced.

Chapters 4-6 taken together suggest that CBD might also have additional treatment potential during preoccupation/anticipation stages. CBD-induced modulations to serotonergic signalling, hypoactivation in the precuneus, and increased PNS recruitment suggest that CBD administration may be associated with dampened cue-elicited motivational urges to seek alcohol, improved top-down cognitive control of alcohol seeking behaviours, and improved craving reduction post cue removal. This is highlighted by the general heightened PNS response throughout the psychophysiological cue reactivity task (Chapter 4) which is reflective of the dampening emotional, hedonic, or affective states and subsequent motivational urges to seek alcohol (Thayer & Lane, 2000). Furthermore, increased PNS response is associated with an improved capacity to respond to changing situational demands, employing coping strategies, and maintaining control over competing needs and desires (Kashdan & Rottenberg, 2010). Thus, CBD may not only dampen motivational urges to seek alcohol but may additionally facilitate craving *recover*y, as is supported by the results presented in Chapter 4. However, results in Chapter 4 additionally suggest that CBD may primarily modulate motivational urges to seek alcohol and seeking behaviours triggered by high arousal states rather than by alcohol-cues that have increased incentive salience due to alcohol-induced dysregulation of mesocorticolimbic dopaminergic signalling. Results from Chapter 4 are consistent with this hypothesis given that CBD appeared to be less effective in facilitating PNS recruitment during alcohol cue exposure compared to baseline, recovery, and control appetitive cue stages.

Results from this thesis indicate that CBD may be beneficial in targeting specific motivations for alcohol consumption. It has been suggested that distinct motivational drivers precipitate alcohol seeking behaviours, namely relief versus reward motivated drinking

(Koob & Volkow, 2010; Spanagel & Weiss, 1999). As discussed in Chapter 1, the underlying neurobiological dysregulation of the mesocorticolimbic network is believed to produce strong motivational urges to seek alcohol to obtain the rewarding effects of alcohol precipitated by D1 signalling influx within the ventral striatum release (Burnette et al., 2021). However, as is suggested by the tension reduction hypothesis (Greeley & Oei, 1999) and the self-medication hypothesis (Khantzian, 1987, 1997), individuals may also be motivated to drink to reduce physiological arousal and negative affect. These distinct drivers of alcohol consumption have been demonstrated to differ in their neural correlates (Milena Radoman et al., 2024), as well as having been demonstrated to lead to different pharmacotherapy treatment responses (Votaw et al., 2022). It has been suggested that reward and relief-motivated drinking behaviours may be differently modulated by treatment depending on an agents mechanism of action (Mann et al., 2018). The current results therefore suggest that CBD may particularly target motivations pertaining to tension reduction and self-medication but may be less beneficial for targeting motivations pertaining to dopaminergic-driven salience attribution.

In summary, based on the current data, there are several clinical implications hypothesised for the use of CBD in the management of AUD. Specifically, CBD may serve to normalise the neurobiological dysregulation that is characteristic of AUD and may aid in limiting negative affect and the severity of withdrawal symptoms associated with AUD. For individuals with tension reduction and self-medicating profiles, CBD may aid to limit preoccupation with alcohol seeking and alcohol seeking behaviours.

7.5 **Limitations and Methodological Considerations**

Relevant limitations have been discussed in each respective chapter. In the paragraphs below, limitations will be considered for the entire empirical program of PhD research. Firstly, the sample characteristics of individuals who participated in this research may not be

reflective of a typical treatment-seeking clinical population. Of the remaining 19 participants, the mean age of 29.79 is younger than reported in the majority of treatment trials (Logge et al., 2020a; Morley, Kranzler, Luquin, Jamshidi, Adams, Montebello, Tremonti, Dali, Logge, & Baillie, 2024; Morley, Kranzler, Luquin, Jamshidi, Adams, Montebello, Tremonti, Dali, Logge, Baillie, et al., 2024; Morley et al., 2023). Moreover, the majority of the sample was female (13/19; 68%) yet in treatment trials the majority are usually male (Logge et al., 2020a; Morley, Kranzler, Luquin, Jamshidi, Adams, Montebello, Tremonti, Dali, Logge, & Baillie, 2024; Morley, Kranzler, Luquin, Jamshidi, Adams, Montebello, Tremonti, Dali, Logge, Baillie, et al., 2024; Morley et al., 2023). This primarily female sample is particularly relevant given that previous literature has demonstrated sex differences in the ECS (Craft et al., 2013). CBD has been demonstrated to have different pharmacokinetic and pharmacodynamic profiles in men and women. For example, females tend to experience higher C_{max} and area under the curve than males which additionally vary depending on dose concentration (Child & Tallon, 2022; Matheson et al., 2022). Additionally, preclinical CBD studies have demonstrated significant sex by treatment effects wherein anti-depressive effects (Kaplan et al., 2021; Ledesma-Corvi et al., 2022; Silote et al., 2021) and reduced ethanol intake were demonstrated by male but not female mice (Viudez-Martínez et al., 2020). In clinical samples, however, no effects of sex on CBD treatment effects have yet been demonstrated (Matheson et al., 2022).

An additional consideration pertaining to the sample is the level of alcohol consumption (standard drinks per drinking day = 7.66 ± 5.70). While this level of alcohol consumption is commensurate with alcohol treatment trials in some regions globally (e.g. Europe, USA), treatment-seekers in Australia usually report an average of 10-14 standard drinks per drinking day. Nonetheless, this variability in drinking severity remains relevant to AUD management given that the level of alcohol consumption is still categorised as high-risk

(61-100g and 41-60g in a single drinking day for males and females respectively) according to the World Health Organisation Risk Levels (World Health Organization, 2000). Moreover, the CBD-induced normalisation of neurometabolites within the current sample suggests that one would expect greater therapeutic benefit observed in individuals with higher drinking severity given greater severity in neurometabolite abnormalities (Yeo et al., 2013).

A strength of this thesis includes the gold standard DBRCT design (Vieta & Cruz, 2012; von Mücke Similon et al., 2022). Further, by implementing a crossover design, sample limitations observed with between-group comparisons were eliminated allowing for a superior comparison of treatment vs placebo effects (Senn, 2002). An additional strength of design in this thesis was the use of generalise estimating equations which is the optimal statistical method of accounting for carry-over effects (Cruz et al., 2023; Zeger & Liang, 1986; Zorn, 2001).

An additional strength to this thesis included the pre-publishing of the experimental protocol (Chapter 3; published in *Contemporary Trials Communication. 2024 June in press*). Pre-publishing of the experimental protocol enhanced transparency, improved internal and external validity, and facilitated potential future replication (Chan & Hróbjartsson, 2018). However, an additional consideration is that exploratory post hoc analyses were implemented in Chapters 4 and 6 which could be considered to be both a limitation and a strength. Exploratory analysis is an important element of data exploration and, indeed, in the current thesis provided valuable evidence for future hypothesis generation. Nonetheless, results derived from exploratory analyses should be appraised with caution given the inflation of type 1 errors, reduced statistical power following the accounting for multiple comparisons, and researcher bias (Gelman & Loken, 2013; Srinivas et al., 2015).

Several additional limitations were intrinsic to the measures used in this thesis. Firstly, with regards to Chapter 5, although the novel HERMES MRS acquisition provided

additional capabilities to reduce scan times while allowing for the quantification of diverse neurometabolites (Chan et al., 2016; Saleh et al., 2016), the voxel used to measure metabolites concentration within the dACC was necessarily increased in size. This larger voxel is a strength given the improved signal-to-noise ratio but a limitation due to the reduced spatial specificity. An additional strength, however, was the application of HyperMEPI acquisitions for the fMRI in Chapter 5 (Fernandez et al., 2017) given the reduced scan times, improved signal-to-noise ratio and recovered clearer signal across ventral medial PFC (Deichmann et al., 2002).

Finally, Chapters 4 and 5 employed well-validated cue-reactivity paradigms and measures which are considered reliable methods for eliciting and recording cue elicited responses (Carter & Tiffany, 1999; Drummond, 2000). This ensures that the neurobiological and psychophysiological response to cues and subsequent results are grounded in robust, empirically supported approaches which increases the reliability and validity of these results and allows for comparisons with future studies. An additional strength was the implementation in Chapter 4 of HF-HRV as a measure of autonomic arousal is sensitive to rapid changes in autonomic tone (Shaffer & Ginsberg, 2017) which allowed for the investigation into changes in autonomic nervous system during recovery periods. Furthermore, in Chapter 4 naturalistic tangible appetitive cues including juice and alcoholic beverages, audio vignettes, and contextual cues (faux alcohol bar) were used to maximise the ecological validity of the task, such that cue-elicited responses would more similarly reflect response in out of lab contexts.

7.6 **Future Research Directions**

This thesis provides preliminary evidence to suggest the following potential mechanisms of CBD in AUD: i) reductions in anxiety; ii) hypoactivation of the precuneus

during cue exposure; iii) general elevation of PNS recruitment that is dampened during alcohol-associated cues compared to non-alcohol appetitive cues; iv) improved craving recovery post cue removal and v) modulation of neurometabolites that are dysregulated in the context of recent alcohol consumption. This thesis therefore provides some preliminary signals of efficacy and potential mechanisms of action regarding the therapeutic effect of CBD in AUD. Further pilot studies using these experimental paradigms may wish to examine a range of different doses (e.g. 400 mg/day, 600 mg/day). Ultimately, a large parallel randomised controlled trial of CBD versus placebo in an outpatient setting with AUD treatment seekers will be required. This thesis also suggests that stratification of the sample may be beneficial to determine any treatment effect in terms of alcohol consumption and drinking motivation (relief versus reward drinkers).

7.7 **Conclusion**

This thesis included a DBRCT crossover program of research in which CBD-mediated effects on cellular, regional brain activity, neurocircuitry, psychophysiology, mood, craving, and EF were investigated. This research program used state of the art measurement techniques including novel magnetic resonance acquisitions and well validated and robust cue-reactivity paradigms. The results suggest that CBD may facilitate more parasympathetic driven autonomic nervous system function, reduce cue-elicited precuneus activity, improve craving recovery post cue removal, and normalise neurobiological dysregulation. CBD may be particularly suited to target tension reduction motivations to consume alcohol by reducing high arousal states and ameliorating negative affect. Further research, including a large parallel randomised controlled trial, should be conducted in light of the results presented here to establish the therapeutic efficacy of CBD on long-term treatment outcomes.

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Appendix A (Systematic Review)

Table 2

Data Extraction Table

ID	title	Design	Medication	Scanner details	Results (CBD only)
	fMRI: Task Based				
1a	Borgwardt et al 2008	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD deactivated the left temporal cortex and insula. These effects were not related to changes in anxiety, intoxication, sedation, and psychotic symptoms. Trend for less anxiety following CBD relative to placebo (p =0 .06).
1b	Bhattacharyya et al 2009	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD induced trend in modulation of insula, mediotemporal gyrus, lingual gyrus, precuneus and precentral gyrus during encoding blocks and in hippocampus during recall blocks but did not survive threshold for less than 1 false positive.
1c	Bhattacharyya et al 2010	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD attenuated amygdala response while observing fearful which correlated with an anxiolytic effect (r=0.551, p=0.017). CBD also decrease amygdala response also correlated with reductions in galvanic skin response while and intensely fearful faces (r=0.524; p = 0.049). Right temporal cortex, parahipcampal gyrus, insula and caudate were augmented by CBD during response inhibition task. While listening to speech CBD the superior temporal cortex was augmented by CBD During visual processing the occipital lobe was augmented During verbal recall CBD was associated with a trend increase in activity in the striatum compared to placebo.
1d	Bhattacharyya et al 2012	DBPC pseudo-randomized, repeated measures, within-subject	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD attenuated activation in the left medial prefrontal cortex and augmented activation in the right caudate, parahippocampal gyrus, insula, precentral gyrus and thalamus, relative to placebo

		design. 1-month washout.			during oddball salience processing. CBD also reduced response latencies
1e	Bhattacharyya et al 2014	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD increased fronto-striatal connectivity but decreased mediotemporal-prefrontal connectivity during oddball salience processing.
1f	Fusar-Poli et al 2009	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD attenuated the BOLD signal in the amygdala and anterior and posterior cingulate cortex while subjects were processing intensely fearful faces, and its suppression of the amygdalar and anterior cingulate response was correlated with the concurrent reduction in SCR fluctionations. CBD also reduced activity compared to placebo in the posterior lobe of the cerebellum for 50% fearful face stimuli
1g	Fusar-Poli et al 2010	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	In the placebo condition, BMS identified a model with driving inputs entering via the anterior cingulate and forward intrinsic connectivity between the amygdala and the anterior cingulate as the best fit. CBD but not D9-THC disrupted forward connectivity between these regions during the neural response to fearful faces.
1h	Winton-Brown et al 2011	DBPC pseudo-randomized, repeated measures, within-subject design. 1-month washout.	THC 10mg or CBD 600mg or placebo	1.5 T Sigma (GE)	CBD had no significant symptomatic effects in anxiety, intoxication, and positive psychotic symptoms. CBD was associated with activation in right temporal cortex during auditory processing.
2a	Wilson et al 2019	DBRPC parallel-arm study.	CHR received 600 mg CBD or matched placebo, while HC received no treatment	GeneralElectric Signa HDx 3.0 T MRI scanner	CBD attenuated the hyperactivity in the left insula/parietal operculum for CHR participants and was associated with overall slowing of reaction time.
2b	Davies 2021	DBRPC parallel-arm study.	CHR received 600 mg CBD or matched placebo, while HC received no treatment	GeneralElectric Signa HDx 3.0 T MRI scanner	Healthy controls showed a significant negative relationship between cortisol and parahippocampal activation ($p = 0.023$). During fear processing, increases in cortisol levels induced by social stress led to lower parahippocampal activation. This relationship was

					significantly different in placebo compared to healthy controls (p = 0.033) but not CBD conditions vs healthy controls (p = 0.67).
2c	Bhattacharyya 2018	DBRCT, parallel-arm study	CHR received 600 mg CBD or matched placebo, while HC received no treatment	GeneralElectric Signa HDx 3.0 T MRI scanner	In the CBD group activation was greater than in the placebo group but lower than in the control group in the right caudate during encoding and in the parahippocampal gyrus and midbrain during recall. The level of activation in the CBD group was thus intermediate to that in the other 2 groups.
2d	Davies 2020	DBRCT, parallel-arm study	CHR received 600 mg CBD or matched placebo, while HC recived no treatment	GeneralElectric Signa HDx 3.0 T MRI scanner	During fear processing, CHR participants receiving CBD showed greater activation than HC but lower activation than those who received placebo in the parahippocampal gyrus. CHR participants receiving CBD showed lower activation than HC but higher activation than those who received placebo in the striatum.
3a	Lawn et al 2020	DBRPC repeated measures, crossover design.	600 mg oral dose of CBD and matched placebo	3-Tesla Siemens Prisma MRI Scanner	There was insufficient evidence to suggest that CBD altered reward-related brain activity.
3b	Bloomfeild 2022	DBRPC repeated measures, crossover design.	600 mg oral dose of CBD and matched placebo	3-Tesla Siemens Prisma MRI Scanner	There was insufficient evidence to suggest that CBD altered brain regions associated with emotional processing or responding to emotional faces.
	fMRI: Resting State				
3c	Bloomfield et al 2020	DBRPC repeated measures, crossover design.	600 mg CBD or placebo	3-Tesla Siemens Prisma MRI Scanner	CBD increased CBF in the hippocampus (p = 0.004). There was no effect on memory task performance, but there was a significant correlation whereby greater CBD-induced increases in orbitofrontal CBF were associated with reduced reaction time on the 2-back working memory task (r= −0.73, p = 0.005).
3d	Wall 2022	DBRPC repeated measures, crossover design.	600 mg CBD or placebo	3-Tesla Siemens Prisma MRI Scanner	Compared to placebo, CBD was associated with a relative increase between areas in the posterior parietal lobes, parietooccipital sulcus, the left posterior cingulate and areas of the striatum involved in association. CBD also led to decreased connectivity was found in the right hemisphere insula and

					lateral frontal cortex. Furthermore, CBD relatively decreased connectivity from the striatum sensorimotor seed-region and left cerebellum.
4	Grimm et al 2018	Subject observer-blinded, RCT Crossover	10 mg THC vs CBD 600mg vs placebo	3-Tesla Siemens Trio	Increase in front striatal coupling during intake of 600mg CBD. ROI-putamen showed increased activity with three clusters in the frontal lobe
5a	Pretzsch et al 2019	DBRPC, repeated-measures, cross-over study.	600mg CBD or placebo	3T GE Excite II	Primarily driven by the ASD group, with no significant change in controls, CBD significantly increased fALFF in the right fusiform gyrus ($p = 0.041$) and in the cerebellar vermis VI ($p = 0.048$). Within the ASD group only, CBD also significantly altered vermal functional connectivity with several of its subcortical (striatal) and cortical targets
	MRS				
5b	Pretzsch et al 2019	DBRPC, repeated-measures, cross-over study.	600 mg CBD or placebo	3T GE Excite II	Across regions, CBD increased GABA+ in controls, but decreased GABA+ in ASD; the group difference in change in GABA + in the DMPFC was significant.
	PET				
6	Crippa et al 2004	DBRCT	400mg CBD	Double-detector SOPHAs DST system	CBD was associated with an increased parahippocampal gyrus blood flow. CBD conditions also showed decreased blood flow to a mediotemporal cluster including the left amygdala-hippocampal complex, hypothalamus, and a cluster in the left posterior cingulate gyrus blood flow

Note. ASD=autism spectrum disorder; CBD=cannabidiol; DBRPC = double-blind, randomized, placebo-controlled; DBRCT ; Double blind, randomised control trial, MIDT = monetary incentive delay task; ROI = Region of interest; fLAFF = 'fractional amplitude of low-frequency fluctuations'; GABA = γ-aminobutyric acidergic

Table 3

Sample Demographics Per Included Study

Sample ID	Name	Participants	Age (mean [sd])	Sex (female%)	Recruited
	fMRI: Task Based				
1a	Borgwardt et al 2008	15 healthy men	26.7 [5.7]	0%	Recruited through advertisement in the local media
1b	Bhattacharyya et al 2009	15 healthy men	26.67 [5.7]	0%	Recruited through advertisement in the local media
1c	Bhattacharyya et al 2010	15 healthy men	26.7 [5.7]	0%	Recruited through advertisement in the local media
1d	Bhattacharyya et al 2012	15 healthy men	26.67 [5.7]	0%	Recruited through advertisement in the local media
1e	Bhattacharyya et al 2014	15 healthy men	26.67 [5.7]	0%	Recruited through advertisement in the local media
1f	Fusar-Poli et al 2009	15 healthy men	26.67 [5.7]	0%	Recruited through advertisement in the local media
1g	Fusar-Poli et al 2010	15 healthy men	26.67 [5.7]	0%	Recruited from advertisement in the local media.
1h	Winton-Brown, T. et al 2011	14 healthy men	26.7 [5.7]	0%	Recruited advertisements in local media.
2a	Wilson, R. et al 2019	19 HC and 33 CHR	23.9 [4.15]	41%	HC recruited by local advertisement while CHR were recruited from early intervention services in the UK
2b	Davies. C 2021	19 HC and 33 CHR	23.4 [4.8]; 24.3 [4.73]	49% ; 42%	HC recruited by local advertisement while CHR were recruited from early intervention services in the UK
2c	Bhattacharyya 2018	19 HC and 33 CHR	23.4 [4.8]; 24.3 [4.73]	49% ; 42%	HC recruited by local advertisement while CHR were recruited from early intervention services in the UK
2d	Davies C 2020	19 HC and 33 CHR	23.4 [4.8]; 24.3 [4.73]	49%; 47.4%	HC recruited by local advertisement while CHR were recruited from early intervention services in the UK
3a	Lawn, W. et al 2020	23 healthy participants	23.74 [4.2]	52%	Recruited through public advertisement
3b	Bloomfeild .M 2022	24 healthy participants	23.6 [4.12]	50%	Recruited through public advertisement
	fMRI: Resting State				

3c	Michael A P Bloomfield et al 2020	15 healthy participants	24 [5]	60%	Recruited through online adverts, posters and word-of-mouth
3d	Matthew B Wall 2022	23 healthy participants	23.8 [4.3]	52%	online adverts, posters and word-of-mouth.
4	Grimm, O. et al 2018	16 HC	NA	0%	Recruited via local advertisement
5a	Pretzsch, C. M. et al 2019	17 neurotypicals, 13 ASD	28.47 [6.55]; 30.85 [9.79]	0%	na
	MRS				
5b	Pretzsch, C. M. et al 2019	17 neurotypicals, 17 ASD	28.47 [6.55]; 31.29 [9.94]	0%	na
	PET				
6a	Crippa 2004	10 healthy volunteers	29.8 [5.1]	0%	Postgraduate students

Note. ASD=autism spectrum disorder; CBD=cannabidiol; HC=healthy controls

Search Terms Used For Systematic Review

EMBASE (253):

(MRS or Magnetic Resonance Spectroscopy or Spectroscopy or Metabolite Concentrations or magnetic resonance spectroscopy or MRS or functional magnetic resonance imaging or fMRI or resting state functional or magnetic resonance imaging or rsfMRI or structural magnetic resonance imaging or MRI or magnetic resonance spectroscopy or PET or positron emission tomography).mp. and (cannabidiol.mp. or (exp cannabidiol/ or exp cannabidiol derivative/)) [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

PUBMED (228):

Search: (MRS OR Magnetic Resonance Spectroscopy OR Spectroscopy OR Metabolite Concentrations OR magnetic resonance spectroscopy OR MRS OR functional magnetic resonance imaging OR fMRI OR resting state functional OR magnetic resonance imaging OR rsfMRI OR structural magnetic resonance imaging OR MRI OR magnetic resonance spectroscopy OR PET OR positron emission tomography) AND (cannabidiol)

PSYCINFO (185):

(MRS or Magnetic Resonance Spectroscopy or Spectroscopy or Metabolite Concentrations or magnetic resonance spectroscopy or MRS or functional magnetic resonance imaging or fMRI or resting state functional or magnetic resonance imaging or rsfMRI or structural magnetic resonance imaging or MRI or magnetic resonance spectroscopy or PET or positron emission tomography).mp. and (exp Cannabinoids/ or cannabidiol.mp.) [mp=title, abstract, heading word, table of contents, key concepts, original title, tests & measures, mesh]

Medline (101):

(MRS or Magnetic Resonance Spectroscopy or Spectroscopy or Metabolite Concentrations or magnetic resonance spectroscopy or MRS or functional magnetic resonance imaging or fMRI or resting state functional or magnetic resonance imaging or rsfMRI or structural magnetic resonance imaging or MRI or magnetic resonance spectroscopy or PET or positron emission tomography).mp. and (cannabidiol.mp. or exp Cannabidiol/)

[mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]]]

(last search on 06/10/22)

Appendix B (Protocol Paper)

Audio Vignettes for CR task scripts

Control Recording

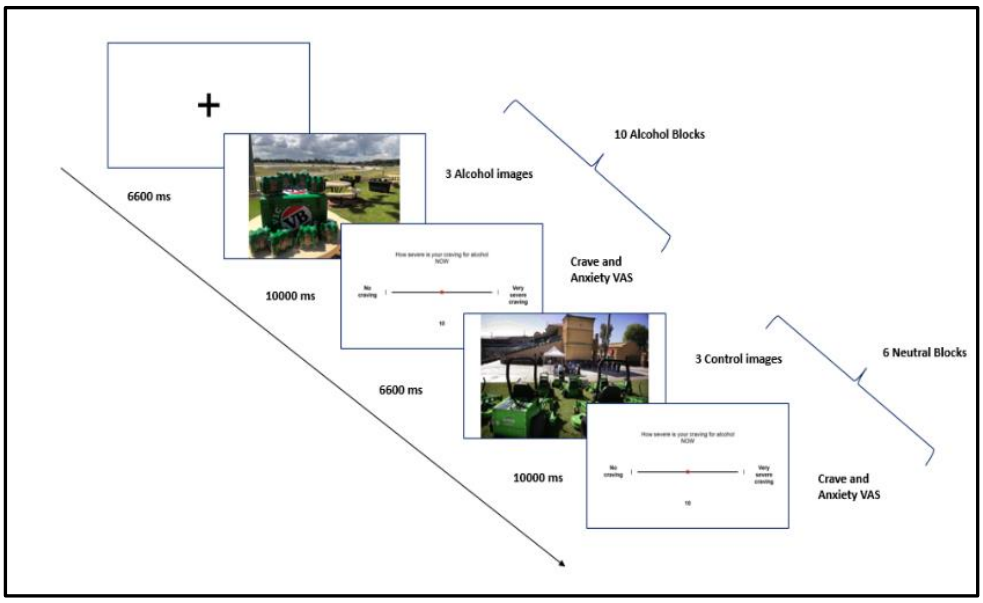
"You are sitting alone on the beach. You look up, and a good friend of yours is walking towards you! They tell you they've brought you a beach towel – you can see it in their hand. You notice the smell of the laundry soap coming from the orange beach towel. You sit on the towel, and it feels rough against your skin, but it reminds you of holidays so it relaxes you. You pick up a bottle of sunscreen and the bottle is slightly greasy in your hand. You squirt some more into your hand and you can smell it, it smells like coconut. The bottle makes a noise as you squirt more onto your palm. You want to put the sunscreen on quickly, you think you might get burnt soon. Suddenly you feel like everything's just right – here you are with your friend, sitting on the sand, and it's just so easy. The warmth from the sun is making your skin tingle a bit. This is such a relief, you've needed this break. You rub the cool, creamy sunscreen across your shoulders, onto your arms, down your legs, and it feels good against your skin. You stretch out on the sand and feel all of your muscles relaxing as the tension just melts away. You think about how much you enjoy being able to just take a break, and suddenly you can't wait to have more fun. This is exactly what you've needed; it feels better than anything has all week. You haven't even been here long before you're thinking about getting into the water."

Juice Recording

"You are sitting alone in a bistro. You look up and a good friend of yours is walking towards you! They tell you they've brought you your favourite alcoholic drink – you can see it in their hand. You think about how you weren't going to drink, you've tried so hard not to. Maybe you'll just leave it sitting there. You sit down together and start to have a chat. In spite of yourself, you find yourself reaching forward and taking the drink – you feel how smooth the glass feels against your hand, and the thought of it is making your mouth water. You bring it to your lips and suddenly you can smell it, its right there in front of you. You can hear the drink moving around in the glass, and you're really looking forward to it. You can't wait to taste it; your mouth is watering a lot now. Suddenly you feel like everything's just right – here you are with your friend, having a chat and a drink, and it's just so easy. The drink hits your tongue and it's wonderful – you can feel it moving around in your mouth and there's that taste, the taste you've been waiting for. Such a relief. You can feel the liquid all around your mouth, around the inside of your cheeks; haven't you tried so hard at being good? You swallow, and suddenly you can't wait for the next mouthful. You take another mouthful and it's just as good, no, better than the last. You haven't even swallowed this mouthful before you are thinking about the next."

Figure 1

Experimental Design of Cue Reactivity Imaging Task



Note. Figure visual depicts the experimental design used for the fMRI alcohol cue-reactivity task. Participants are initially presented with a fixation cross followed by Images presented for 6.6 s in blocks of 3 images of the same type. 10 alcohol stimuli and 6 neutral blocks will be presented with stimuli and block order randomised (16 total blocks, 645 s). Following each block, an 11-point visual analogue craving scale is presented and participants respond using an MRI-compatible two-button response pad within a 10 s window.

Appendix C (Empirical Chapters)

Psychophysiological Acquisition and Analysis: Additional Information

During each stage of the psychophysiological paradigm MLT117F GSR Electrodes (ADInstruments; Sydney, Australia) was placed on the middle phalanges on the II and III fingers of the participant's non-dominant hand. A FE116 GSR Amplifier (ADInstruments; Sydney, Australia) was then used to amplify the skin conductance signal. Additionally, electrocardiogram (ECG) data was recorded using a three-lead ECG with Ag/AgCl electrodes. Electrodes were placed on the non-dominant wrist (as a ground electrode) and two above the cubital fossa on each arm. This ECG signal was amplified using a ML408 Dual Bioamp/Stimulator (ADInstruments; Sydney, Australia). Both amplifiers were connected to a PC operating LabChart Pro 7.3.7 software (ADInstruments, 2012) via a PowerLab 8/25 System (ADInstruments; Sydney, Australia) and sampled at 1,000 Hz/s.

Data Transformation Additional Information

The mean skin conductance (SC) in microsiemens (μS) and R-wave for cardiovascular data were calculated for each stage. The R-wave was calculated using Kubios 2.2 HRV software (Biosignal Analysis and Medical Imaging Group, 2012). Artifacts in SC and ECG data were identified and removed manually using Labchart Pro. Participants with a significant amount of signal loss were removed from further analysis.

SCL: A low pass filter of 0.205 HZ was used to screen out phasic peaks for each cue and recovery stage. Remaining tonic changes in SCL were processed using labchart. Standard proportions of SCL were obtained using the following formula:

$$\frac{SCL - SCLmin}{SCLmax - SCLmin}$$

SCL min = baseline minimum skin conductance value per stage

SCL max = maximum skin conductance value per stage

This proportion value accounts for SCL heterogeneity between participants as it takes into account the variations in baseline and range of each participant (Dawson et al., 2007). The mean SCL for each stage was then calculated in respect to this proportional value for each participant per stage of the cue reactivity task.

ECG: A low-pass filter was used to interpolate and replace sections with ECG artifacts. Trend components were compensated for using smoothness priors method ($\lambda = 500$; (Tarvainen et al., 2002)). Fast Fourier transformation was used to calculate HF-HRV during spectral analysis in 0.15-0.40 HZ frequency bands. This process was applied for all 5 stages of the cue reactivity task. The distribution of HF-HRV across participants demonstrated positive skewing and were subsequently natural log-transformed for normality prior to statistical analysis.

Table 1

Timeline and All Contrasts

Stage name		Baseline 5 mins	Juice exposure 5 mins	Juice recovery 5 mins	Alcohol exposure 5 mins	Alcohol recovery 5 mins
Stimuli		Neutral video	Juice beverage + control audio script	Neutral video	alcohol matched to participant + script	Neutral video
Contrasts	Baseline vs Appetitive Cue Exposure	-1	1/2	NA	1/2	NA
	Juice Cue vs Alcohol Cue	NA	-1	NA	1	NA
	Juice Recovery vs Alcohol Recovery	NA	NA	-1	NA	1
	Appetitive Cues vs Recovery	NA	-1/2	1/2	-1/2	1/2
	Alcohol Cue vs Alcohol Recovery	NA	NA	NA	-1	1

Note. Details contrast applied during generalised estimating equation modelling.

Table 2

Modelling Results: Craving Measures (AUQ)

AUQ	Baseline vs Cue		Juice Cue vs Alcohol Cue		Juice Rec vs Alcohol Rec		Cue vs Rec		Alcohol Cue vs Alcohol Rec	
	Estimate (Std.err)	Pr(>|W|)	Std.err (Wald)	Pr(>|W|)	Std.err (Wald)	Pr(>|W|)	Std.err (Wald)	Pr(>|W|)	Std.err (Wald)	Pr(>|W|)
(Intercept)	12.5724 (2.0939)	**1.92E-09***	14.333 (2.386)	**1.90E-09***	8.154 (1.901)	**1.80E-05***	11.5 (2.19)	**1.50E-07*****	13.7948 (2.5551)	**6.70E-08*****
Treatment (CBD)	6.6251 (4.0543)	0.102238	7.371 (4.496)	0.10107	5.595 (3.127)	**0.0735.**	6.27 (3.8)	**0.099.**	5.2012 (4.546)	0.25
Contrast	4.7778 (1.3354)	**0.000347***	2.967 (0.803)	**0.00022***	1.867 (0.792)	**0.0185***	-5.03 (1.16)	**1.40E-05*****	-3.0667 (0.6829)	**7.10E-06*****
Session 2	-7.5124 (4.3828)	**0.086517.**	-8.855 (4.839)	**0.06728.**	-4.97 (3.276)	0.1293	-6.81 (4.01)	**0.089.**	-6.9441 (4.5696)	0.13
Carry (PL during ses2)	11.5114 (8.0889)	0.154703	14.23 (9.434)	0.13129	10.53 (6.762)	0.1194	12 (8.24)	0.145	12.935 (9.5102)	0.17
DDB	0.6175 (2.435)	0.799826	0.567 (2.637)	0.82988	3.753 (2.868)	0.1907	1.72 (2.63)	0.513	1.0284 (2.8645)	0.72
Treatment:Contrast	-1.2222 (1.3679)	0.371604	-1.5 (0.921)	0.10352	-1.433 (0.503)	**0.0044****	-1.96E-16	1	0.0333 (0.9098)	0.97

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on Alcohol Urge Questionnaire (AUQ) responses

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3

Modelling Results: Mood Measures

VAS Anxiety										
	Baseline vs Cue		Juice Cue vs Alcohol Cue		Juice Rec vs Alcohol Rec		Cue vs Rec		Alcohol Cue vs Alcohol Rec	
	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)
(Intercept)	19.47 (7.224)	**0.007***	24.9155 (9.5147)	**0.0088***	9.008 (4.797)	0.06	18.047 (7.137)	**0.011***	20.59 (8.51)	**0.015***
Treatment (CBD)	-0.626 (10.365)	0.952	-5.0259 (12.2463)	0.6815	4.905 (8.509)	0.56	-0.874 (10.247)	0.932	-2.77 (10.99)	0.801
Contrast	4.286 (2.136)	**0.045***	1.5 (1.5739)	0.3406	0.536 (1.225)	0.66	-5.321 (2.228)	0.017	-3.14 (1.59)	**0.048***
Session 2	-3.469 (9.893)	0.726	-0.9027 (11.8732)	0.9394	-5.905 (8.307)	0.48	-2.59 (9.848)	0.793	2.55 (11.08)	0.818
Carry (PL during ses2)	11.19 (19.747)	0.571	7.0586 (24.6657)	0.7747	21.04 (17.387)	0.23	12.15 (20.947)	0.562	7 (22.96)	0.761
DDB	-7.905 (7.167)	0.27	-16.7271 (9.2398)	**0.0702.**	4.611 (5.428)	0.4	-7.957 (6.464)	0.218	-13.29 (9.15)	0.147
Treatment:Contrast	-5.929 (2.686)	**0.027***	0.0357 (2.0155)	0.9859	0.571 (1.702)	0.74	4.464 (2.532)	0.078	2.5 (1.99)	0.21

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on self-report anxiety using Visual Analogue Scale (VAS)

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4

Modelling Results: Craving Measures (VAS)

Vas Crave	Baseline vs Cue		Juice Cue vs Alcohol Cue		Juice Rec vs Alcohol Rec		Cue vs Rec		Alcohol Cue vs Alcohol Rec	
	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)
(Intercept)	20.06 (9.89)	**0.04259***	22.04 (10.96)	**0.0443***	9.22 (6.08)	0.13	18.16 (9.03)	**0.044***	23.68 (11.23)	**0.035***
Treatment (CBD)	21.67 (12.86)	**0.092.**	26.17 (13.99)	0.0613.	18.18 (10.82)	**0.093.**	20.27 (12.5)	0.105	18.15 (14.99)	0.226
Contrast	7.98 (2.2)	**0.00028*****	6.96 (2.36)	**0.0031****	3.86 (1.99)	**0.053.**	-4.96 (2.33)	**0.033***	-4.04 (1.86)	**0.03***
Session 2	-22.05 (13.68)	0.10701	-26.24 (14.76)	0.0755	-20.11 (11.32)	**0.076.**	-21.27 (13.11)	0.105	-22.22 (14.03)	0.113
Carry (PL during ses2)	27.23 (25.34)	0.28264	33.63 (27.14)	0.2152	37.11 (22.54)	0.1	30.94 (25.94)	0.233	30.68 (28.06)	0.274
DDB	2.89 (11.82)	0.8066	6.06 (13.79)	0.6602	16.75 (7.95)	**0.035***	6.97 (9.84)	0.478	8.68 (12.01)	0.47
Treatment:Contrast	3.05 (2.47)	0.21781	-2.07 (2.15)	0.3344	-1.68 (2.5)	**0.502.**	-8.96 (4.04)	**0.026***	-4.29 (1.91)	**0.025***

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on craving

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 5

Modelling Results: Psychophysiological Measures SCL

SCL	Baseline vs Cue		Juice Cue vs Alcohol Cue		Juice Rec vs Alcohol Rec		Cue vs Rec		Alcohol Cue vs Alcohol Rec	
	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)
(Intercept)	-0.7071 (0.12023)	4.10E-09	-0.7026 (0.13852)	3.90E-07	-0.727 (0.1431 2)	3.80E-07	-0.72325 (0.11099)	7.20E-11	-0.6753 (0.12)	1.80E-08
Treatment (CBD)	-0.14979 (0.18652)	0.422	-0.12751 (0.20293)	0.53	-0.1459 (0.2559)	0.57	-0.13297 (0.20999)	0.53	-0.1118 (0.2449)	0.648
Contrast	-0.2133 (0.11005)	0.053	-0.00519 (0.1074)	0.96	0.0212 (0.0644)	0.74	0.0589 (0.09405)	0.53	0.0418 (0.0611)	0.494
Session 2	0.00794 (0.18189)	0.965	-0.03021 (0.2257)	0.89	0.0754 (0.2789)	0.79	-0.00411 (0.24441)	0.99	0.0936 (0.2732)	0.732
Carry (PL during ses2)	-0.31425 (0.3198)	0.326	-0.5615 (0.39143)	0.15	-0.4849 (0.4121)	0.24	-0.49059 (0.37719)	0.19	-0.6927 (0.3941)	**0.079.**
DDB	0.1126 (0.10155)	0.267	0.10593 (0.15308)	0.49	0.1133 (0.1764)	0.52	0.14741 (0.12764)	0.25	0.1169 (0.1561)	0.454
Treatment:Contrast	0.20815 (0.14951)	0.164	0.13701 (0.13937)	0.33	0.0478 (0.0911)	0.6	-0.04731 (0.13493)	0.73	-0.0674 (0.0962)	0.483

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on skin conductance level (SCL)

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 6

Modelling Results: Psychophysiological Measures HF-HRV

High Frequency HRV	Baseline vs Cue		Juice Cue vs Alcohol Cue		Juice Rec vs Alcohol Rec		Cue vs Rec		Alcohol Cue vs Alcohol Rec	
	Std.err (Wald)	Pr (>|W|)	Estimate (Std.err)	Pr (>|W|)	Std.err (Wald)	Pr (>|W|)	Std.err (Wald)	Pr (>|W|)	Std.err (Wald)	Pr (>|W|)
(Intercept)	3.2077 (0.142)	<2e-16	3.213 (0.1427)	<2e-16	3.1449 (0.1404)	2.00E-16	3.1518 (0.1514)	2.00E-16	3.259 (0.1425)	2.00E-16
Treatment (CBD)	0.502 (0.1871)	0.0073	0.4967 (0.1874)	0.008	0.7883 (0.1483)	**1.10E-07*** **	0.622 (0.1668)	**0.00019*** **	0.5117 (0.1537)	**0.00087*** **
Contrast	-0.1567 (0.0683)	**0.0218***	0.1167 (0.0472)	**0.0134***	-0.0769 (0.0341)	**0.024***	0.172 (0.0913)	**0.05958.**	-0.0108 (0.0526)	0.83692
Session 2	-0.4266 (0.188)	**0.0232***	-0.4223 (0.1872)	**0.0241***	-0.5705 (0.1147)	**6.50E-07*** **	-0.4987 (0.1282)	**1.00E-04*** **	-0.4866 (0.12)	**5.00E-05*** **
Carry (PL during ses2)	0.8729 (0.2969)	**0.0033***	0.8648 (0.2967)	**0.0036** **	1.363 (0.1792)	**2.80E-14*** **	1.1176 (0.2412)	**3.60E-06*** **	1.009 (0.2094)	**1.40E-06*** **
DDB	0.3159 (0.1278)	**0.0134***	0.3052 (0.1292)	**0.0182***	0.3549 (0.1676)	**0.034***	0.2943 (0.1325)	**0.02639***	0.1597 (0.1554)	0.30406
Treatment:Contrast	-0.073 (0.0711)	0.3042	-0.0691 (0.0346)	**0.0461***	-0.0261 (0.0672)	0.697	0.091 (0.1484)	0.53972	0.067 (0.0993)	0.49984

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on high frequency heart rate variability (High Frequency HRV)

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Imaging Data Preprocessing fMRIPrep Boiler Plate

A total of 2 T1-weighted (T1w) images were found within the input BIDS dataset. All of them were corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (Tustison et al. 2010), distributed with ANTs 2.3.3 (Avants et al. 2008, RRID:SCR_004757). The T1w-reference was then skull-stripped with a *Nipype* implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 5.0.9, RRID:SCR_002823, Zhang, Brady, and Smith 2001). A T1w-reference map was computed after registration of 2 T1w images (after INU-correction) using mri_robust_template (FreeSurfer 6.0.1, Reuter, Rosas, and Fischl 2010). Brain surfaces were reconstructed using recon-all (FreeSurfer 6.0.1, RRID:SCR_001847, Dale, Fischl, and Sereno 1999), and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle (RRID:SCR_002438, Klein et al. 2017). Volume-based spatial normalization to one standard space (MNI152NLin2009cAsym) was performed through nonlinear registration with antsRegistration (ANTs 2.3.3), using brain-extracted versions of both T1w reference and the T1w template. The following template was selected for spatial normalization: *ICBM 152 Nonlinear Asymmetrical template version 2009c* [Fonov et al. (2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym],

Functional data preprocessing

For each of the 2 BOLD runs found per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped

version were generated from the shortest echo of the BOLD run using a custom methodology of *fMRIPrep*. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9, Jenkinson et al. 2002). A B0-nonuniformity map (or *fieldmap*) was directly measured with an MRI scheme designed with that purpose (typically, a spiral pulse sequence). The *fieldmap* was then co-registered to the target EPI (echo-planar imaging) reference run and converted to a displacements field map (amenable to registration tools such as ANTs) with FSL's fugue and other *SDCflows* tools. Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using bbregister (FreeSurfer) which implements boundary-based registration (Greve and Fischl 2009). Co-registration was configured with six degrees of freedom. The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as *preprocessed BOLD in original space*, or just *preprocessed BOLD*. A T2* map was estimated from the preprocessed BOLD by fitting to a monoexponential signal decay model with nonlinear regression, using T2*/S0 estimates from a log-linear regression fit as initial values. For each voxel, the maximal number of echoes with reliable signal in that voxel were used to fit the model. The calculated T2* map was then used to optimally combine preprocessed BOLD across echoes following the method described in (Posse et al. 1999). The optimally combined time series was carried forward as the *preprocessed BOLD*. First, a reference volume and its skull-stripped version were generated using a custom methodology of *fMRIPrep*. The BOLD time-series were resampled into standard space, generating a *preprocessed BOLD run in MNI152NLin2009cAsym space*.

First, a reference volume and its skull-stripped version were generated using a custom methodology of *fMRIPrep*. Several confounding time-series were calculated based on the *preprocessed BOLD*: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, Power et al. (2014)) and Jenkinson (relative root mean square displacement between affines, Jenkinson et al. (2002)). FD and DVARS are calculated for each functional run, both using their implementations in *Nipype* (following the definitions by Power et al. 2014). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (*CompCor*, Behzadi et al. 2007). Principal components are estimated after high-pass filtering the *preprocessed BOLD* time-series (using a discrete cosine filter with 128s cut-off) for the two *CompCor* variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM and combined CSF+WM) are generated in anatomical space. The implementation differs from that of Behzadi et al. in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted a mask of pixels that likely contain a volume fraction of GM. This mask is obtained by dilating a GM mask extracted from the FreeSurfer's *aseg* segmentation, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from

consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al. 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with *a single interpolation step* by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos 1964). Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer).

Many internal operations of *fMRIPrep* use *Nilearn* 0.6.2 (Abraham et al. 2014, RRID:SCR_001362), mostly within the functional processing workflow. For more details of the pipeline, see the section corresponding to workflows in *fMRIPrep*'s documentation.

Table 1

ROI Analysis Table Alcohol Cue Generalised Estimated Equations

	BL vmpfc		L caudate		R caudate		L dlpfc		R dlpfc	
	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)
Intercept	0.13282 (0.14434)	0.3575	-0.2006 (0.2031)	0.32	-0.1174 (0.1444)	0.42	-0.0417 (0.1544)	0.787	-0.035 (0.2557)	0.89
Session	0.18983 (0.19855)	0.339	-0.0094 (0.1307)	0.94	0.0392 (0.1356)	0.77	-0.4353 (0.1976)	**0.028***	-0.3979 (0.3212)	0.22
Carry 2	-0.71172 (0.36728)	**0.0526.**	-0.2432 (0.3509)	0.49	-0.3503 (0.3554)	0.32	0.0508 (0.2922)	0.862	-0.5509 (0.5391)	0.31
Drug1	-0.45973 (0.22754)	**0.0433***	-0.0832 (0.2225)	0.71	-0.2153 (0.1799)	0.23	0.022 (0.2279)	0.923	-0.2282 (0.3726)	0.54
DDB_t2	0.02571 (0.14497)	0.8592	0.1309 (0.1736)	0.45	0.0224 (0.1498)	0.88	0.1115 (0.146)	0.445	0.0639 (0.2922)	0.83

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on BOLD signal within ROIs during alcohol cue presentation vs fixation cross.

DDB_t2 = drinking day before the scan; Carry_2 = carry over effects; Drug1 = CBD vs placebo; session1 = the second session relative to first session

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 7

ROI Analysis Table Control Cue Generalised Estimated Equations

	BL vmpfc		L caudate		r caudate		L dlpfc		R dlpfc	
	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)	Estimate (SD)	Pr(>|W|)
(Intercept)	-0.0767 (0.1633)	0.64	-0.25093 (0.2167)	0.25	-0.1772 (0.1484)	0.23	-0.0972 (0.1518)	0.52	-0.0621 (0.2452)	0.8
session1	0.1906 (0.1377)	0.17	0.00411 (0.11282)	0.97	0.0432 (0.1148)	0.71	-0.3846 (0.1958)	**0.05.**	-0.3518 (0.3595)	0.33
Carry_2	-0.5161 (0.3169)	0.1	-0.20678 (0.34229)	0.55	-0.2968 (0.3287)	0.37	0.0555 (0.2748)	0.84	-0.5645 (0.5475)	0.3
Drug1	-0.293 (0.1924)	0.13	-0.04763 (0.22535)	0.83	-0.1667 (0.1667)	0.32	0.0268 (0.2208)	0.9	-0.2298 (0.3972)	0.56
DDB_t2	0.1308 (0.1319)	0.32	0.13415 (0.17375)	0.44	0.0407 (0.1438)	0.78	0.1113 (0.1408)	0.43	0.0204 (0.2785)	0.94

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on BOLD signal within ROIs during control cue presentation vs fixation cross.

DDB_t2 = drinking day before the scan; Carry_2 = carry over effects; Drug1 = CBD vs placebo; session1 = the second session relative to first session

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Figure 1

Spectroscopic analysis of GABA signal

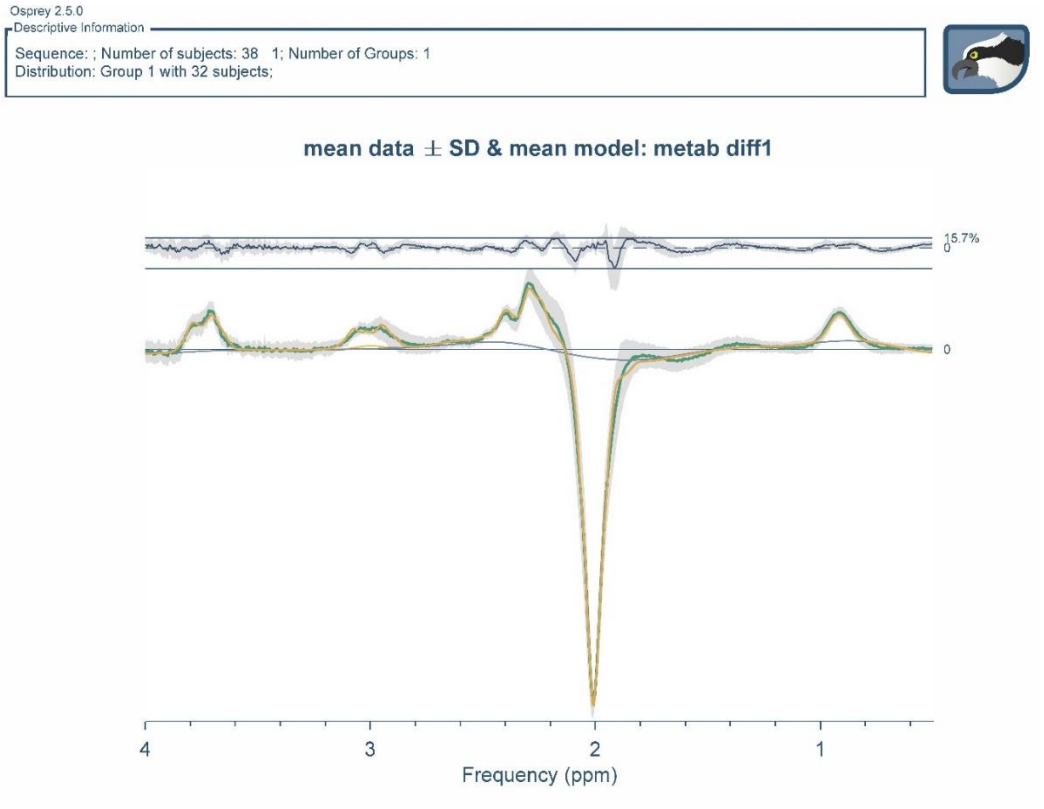


Figure 2

Spectroscopic analysis of GSH signal

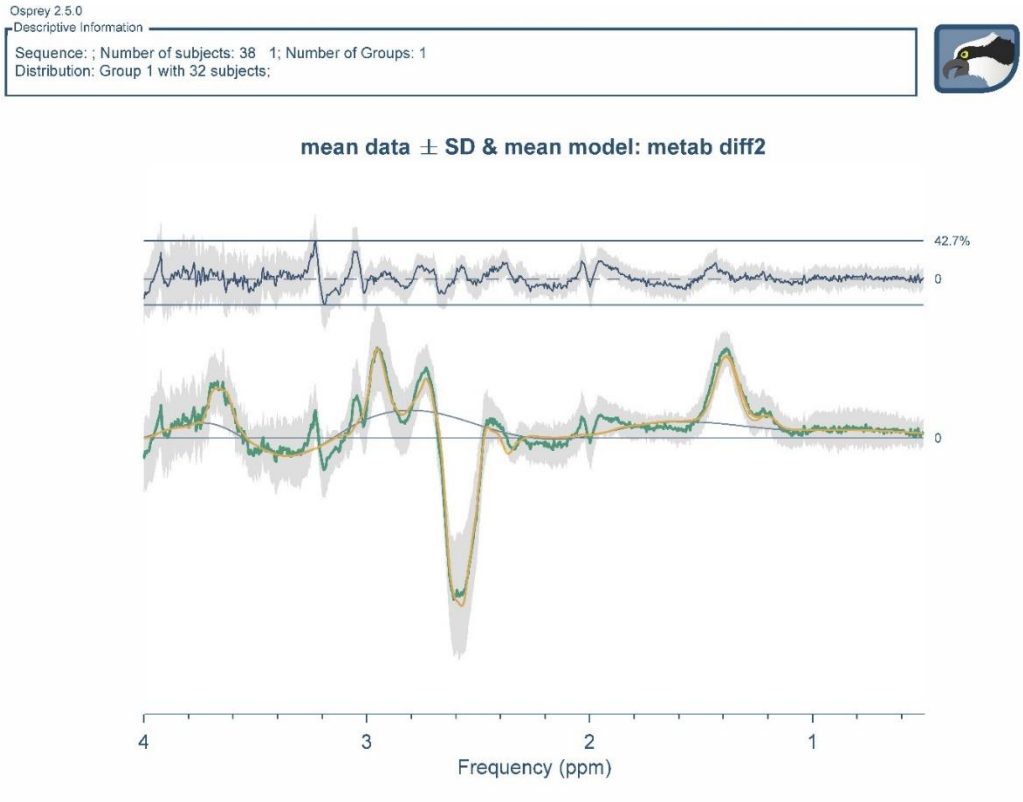


Figure 3

Spectroscopic analysis of Other Metabolites signals

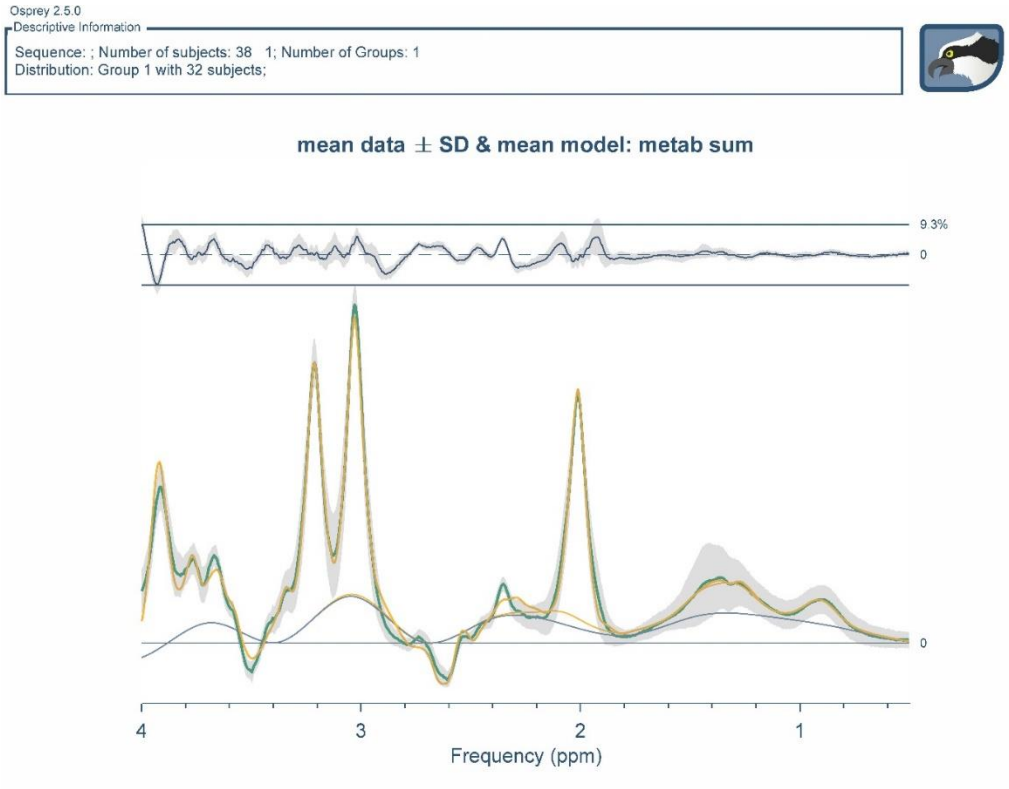


Table 8

Planned Analysis of Neurometabolite Concentration Model Results

	GSH		Glx		GABA		tNAA		tCho	
	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)
(Intercept)	0.34241 (0.07907)	1.50E-05***	0.47907 (0.10481)	**4.90E-06*** **	1.16938 (0.26605)	**1.10E-05*** **	3.57292 (0.27814)	**<2e-16*** **	0.612 (0.0588)	**<2e-16*** **
Drug (CBD)	0.13632 (0.12658)	0.28	0.08921 (0.14091)	0.53	-0.05752 (0.28446)	0.84	-0.16142 (0.33079)	0.63	0.00584 (0.0606)	0.9232
session2	-0.1261 (0.12168)	0.3	0.04524 (0.15118)	0.76	-0.09768 (0.21389)	0.65	0.31182 (0.28055)	0.27	-0.00196 (0.0553)	0.9718
Carry_1	0.13757 (0.18533)	0.46	0.00925 (0.19311)	0.96	-0.0682 (0.3912)	0.86	-0.3059 (0.51099)	0.55	0.0436 (0.0986)	0.6582
PDD	-0.05491 (0.05652)	0.33	0.05677 (0.09195)	0.54	0.04705 (0.15977)	0.77	0.02094 (0.21461)	0.92	0.0905 (0.0317)	**0.0043** **
HDD	0.00163 (0.00141)	0.25	0.00441 (0.00302)	0.14	-0.00629 (0.00482)	0.19	0.00139 (0.0065)	0.83	-0.0000805 (0.00132)	0.9515

Note. Presents generalised estimated equations model results regarding CBD-mediated effects on neurometabolite concentration for glutathione (GSH), glutamate + glutamine (Glx), γ-Aminobutyric acid (GABA), N-acetylaspartate (NAA), choline (Cho). Session order and carryover effects were explicitly modelled and covariates percentage of heavy day drinking over the previous two weeks (HDD) and previous day drinking (PDD) were included.

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 9

Exploratory Analysis Recent drinking modulations of CBD-mediated effects on neurometabolite concentration

	GSH		Glx		GABA		tNAA		tCho	
	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)	Estimate (Std.err)	Pr(>|W|)
(Intercept)	0.426806 (0.073156)	**5.40E-09*** **	0.55897 (0.10823)	**2.40E-07*** **	1.37484 (0.25151)	**4.60E-08*** **	3.646661 (0.294569)	**<2e-16*** **	0.616 (0.0607)	**<2e-16*** **
Drug (CBD)	0.066163 (0.126023)	0.6	0.01716 (0.13807)	0.9011	-0.22693 (0.28532)	0.4264	-0.224746 (0.346385)	0.516	0.000911 (0.0629)	0.988
PDD	-0.19073 (0.047101)	**5.10E-05*** **	-0.13113 (0.09182)	0.1533	-0.26967 (0.20274)	0.1835	-0.147006 (0.247465)	0.552	0.0726 (0.0397)	**0.067.**
HDD	0.000547 (0.001313)	0.677	0.00381 (0.00348)	0.2725	-0.00903 (0.00394)	**0.0219***	0.000594 (0.006344)	0.925	-0.0000641 (0.00131)	0.961
session2	-0.222664 (0.113186)	0.049*	-0.08679 (0.13491)	0.52	-0.30595 (0.20502)	0.1356	0.179553 (0.270257)	0.506	-0.0188 (0.0525)	0.721
Carry_1	0.181052 (0.173526)	0.297	0.09479 (0.18043)	0.5994	0.01003 (0.36125)	0.9779	-0.214672 (0.508908)	0.673	0.0581 (0.0966)	0.548
Drug (CBD): PDD	0.340045 (0.038949)	**2.00E-16*** **	0.45936 (0.14073)	**0.0011** **	0.76154 (0.25103)	**0.0024** **	0.462023 (0.246894)	**0.061.**	0.0513 (0.046)	0.265

Note. Presents generalised estimated equations model results regarding recent drinking modulations of CBD-mediated effects on neurometabolite concentration for glutathione (GSH), glutamate + glutamine (Glx), γ-Aminobutyric acid (GABA), N-acetylaspartate (NAA), choline (Cho). Session order and carryover effects were explicitly modelled and covariates percentage of heavy day drinking over the previous two weeks (HDD) and previous day drinking (PDD) were included. Further, interaction effects between treatment and PDD were included.

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1