THE EFFECTS OF CANNABIDIOL (CBD) EXPECTANCY ON SUBJECTIVE STRESS, ANXIETY,
AND RELATED NEURAL RESPONSES IN HEALTHY ADULTS

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

DEDICATION PAGE

The present thesis is dedicated to my late supervisor, mentor, and role model, Dr. Sean Barrett. I write this dedication with a heavy heart after Sean's recent passing following a year-long journey with cancer. Sean was a pioneer in this field of work, and in many ways this thesis is just as much his as it is mine. I am eternally grateful for Sean's careful mentorship, invaluable feedback, and unwavering support. Sean always believed in me, and he changed my life in immeasurable ways. As I carry his wisdom and passion for research with me, I feel confident knowing he has equipped me exceptionally well for the next chapter of my journey. This thesis is only the beginning of my commitment to continue his research legacy, and his influence will resonate in everything I do.

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ABSTRACT

Background: Cannabidiol (CBD) may be a promising treatment candidate for stress and anxiety disorders. Our group has previously shown that CBD expectancy alone is sufficient to impact subjective, physiological, and endocrine markers of stress and anxiety. This study aimed to delineate the extent to which CBD expectancy may alter subjective state and functional connectivity (FC) within and between the default mode network (DMN), salience network (SN), and central executive network (CEN).

Methods: Using a between-subject, repeated measures design, healthy adults (N=32, 47% female) were randomly assigned to receive accurate or misleading instructions regarding the CBD content of a CBD-free oil. The participants then underwent magnetic resonance imaging with resting state functional connectivity (rsFC) assessed at baseline and following a stress task. Subjective state was measured at multiple timepoints.

Results: Increased rsFC was observed within and between the DMN and CEN in the Told CBD condition. In the CBD-free expectancy condition, the SN showed increased rsFC with both the CEN and DMN. No significant between-group differences in voxel clusters were found after voxel-wise FDR correction. Significant main effects of time were identified for stress ($p<0.001$), anxiety ($p<0.001$), and energy ($p<0.001$). Planned pairwise comparisons revealed decreased stress ($p=0.017$) and anxiety ($p=0.021$) following oil administration, and significantly reduced stress ($p=0.024$) and anxiety ($p=0.017$) during recovery, in the Told CBD condition.

Conclusion: CBD expectancy may alter stress- and anxiety-related neural responses associated with its therapeutic properties. Further research is needed to examine the interactive effects of CBD's pharmacological and non-pharmacological factors.

LIST OF ABBREVIATIONS USED

ACC	Anterior Cingulate Cortex
AI	Anterior Insula
B-BAES	Brief Biphasic Alcohol Effects Scale
BIOTIC	Biomedical Translational Imaging Centre
BOLD	Blood-Oxygen-Level-Dependent
CBD	Cannabidiol
CEN	Central Executive Network
CSF	Cerebral Spinal Fluid
DASS	Depression Anxiety Stress Scale
DFAQ-CU	Daily Sessions, Frequency, Age of Onset and Quantity of Cannabis Use Inventory
dACC	Dorsal Anterior Cingulate Cortex
dlPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DVARS	Per-image standard deviation of the temporal derivative of the data
EPI	Echo Planar Imaging
FC	Functional Connectivity
FDR	False Detection Rate
FD	Framewise Displacement
FOV	Field of View
fMRI	Functional Magnetic Resonance Imaging
FWHM	Full Width Half Maximum

GM	Grey Matter
HPA	Hypothalamus-Pituitary-Adrenal Axis
INU	Intensity Non-Uniformity
MNI152	Montreal Neurological Institute 152 Standard Space Template
MRI	Magnetic Resonance Imaging
PCC	Posterior Cingulate Cortex
PSS	Perceived Stress Scale
ROI	Region of Interest
rsFC	Resting-State Functional Connectivity
rsfMRI	Resting-State Functional Magnetic Resonance Imaging
SBRefs	Single-Band Reference Sequence
SN	Salience Network
SSRI	Selective Serotonin Reuptake Inhibitor
STAI-T	State-Trait Anxiety Inventory
TE	Echo Time
THC	Tetrahydrocannabinol
T1w	T1-Weighted
T1w-ref	T1-Weighted Reference
T2w	T2-Weighted
TR	Repetition Time
TSST	Trier Social Stress Test
WM	White Matter

Weights and Measures

º	Degrees
kg	Kilogram
kHz	Kilohertz
mg	Milligram
min	Minute
mm	Millimeter
ms	Millisecond
ppm	Parts per million
s	Second

CHAPTER 1. GENERAL INTRODUCTION

The *Cannabis sativa* plant has been used both recreationally and for medical purposes for hundreds of years. Following the legalization of recreational cannabis in Canada in 2018, cannabis use amongst Canadians has steadily increased (Health Canada, 2022). In fact, in 2022, 27% of Canadians aged 16 years or older reported using cannabis in the last 12 months (Health Canada, 2022). Cannabidiol (CBD) is the most abundant non-psychoactive cannabinoid derived from the *cannabis sativa* plant. In contrast with tetrahydrocannabinol (THC), an abundant psychoactive cannabinoid in cannabis, CBD is considered generally safe and well tolerated as it is not believed to have intoxicating or reinforcing properties which may result in misuse (World Health Organization, 2018). Notably, among non-medical cannabis users with knowledge of relative CBD and THC concentrations of their regularly consumed products, 58% report using products that had equal or greater concentrations of CBD relative to THC (Health Canada, 2023). Given the relative safety profile, CBD has been considered by some a promising treatment candidate for psychiatric disorders (Dammann et al., 2024). Indeed, CBD administration appears to be largely free of serious side effects when administered either acutely or chronically (Crippa et al., 2010), across a range of doses and forms of administration (Blessing et al., 2015). Moreover, CBD is not believed to significantly affect critical physiological parameters such as heart rate, blood pressure, respiration, or body temperature (Bergamaschi et al., 2011; Iffland & Grotenhermen, 2017). Though CBD is not believed to impair cognitive or psychomotor performance, it has been demonstrated to have a sedative effect (Lo et al., 2024). Overall, most Canadian CBD users believe that it is more helpful than harmful to their health (Goodman et al., 2022).

There is some evidence, however, suggesting that CBD use may not be entirely risk-free. For instance, CBD has the potential to alter the pharmacokinetics of other medications metabolized through the CYP2D6, CYP2C9, CYP1A2, CYP2C19, CYP2C8, CYP2B6, CYP2E1, and CYP3A4 enzymes (Brown & Winterstein, 2019; Ujváry & Hanuš, 2016), has been suggested to potentially increase the risk of liver damage including development of jaundice, and can cause harm to the male[1] reproductive system (Gingrich et al., 2023). Recent literature has also suggested that non-medical CBD preparations might not always contain the advertised amount of CBD, and could include additional chemicals, potentially heightening the risk of harm (Moore et al., 2024). Though rates of adverse events are typically low, reported adverse drug events have included but are not limited to sedation, anemia, infection, and sleep disturbances (Brown & Winterstein, 2019). Given the prevalence of CBD use amongst the general population, it is important to understand the motivation for its administration and the potential for associated healthcare burdens.

Early evidence suggests that CBD might have therapeutic potential for myriad neurological and psychiatric disorders (Dammann et al., 2024). Though cannabis use has been associated with greater rates of mental health issues (e.g., psychosis; Bhattacharyya et al., 2009), and increased levels of state anxiety (Hall & Solowij, 1998; Tournier et al., 2003), individuals report using cannabis to help reduce stress and anxiety (Buckner et al., 2006). Indeed, in North America, mental health conditions for which consumers most commonly report using CBD have included anxiety, depression, and posttraumatic stress disorder (Goodman et al., 2022). CBD is suggested to exhibit a range of therapeutic effects including but not limited to

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[1] Sex refers to the biological attributes distinguishing males and females, whereas gender refers to the social roles, behaviours, and expectations, of men and women (Rich-Edwards et al., 2018). Throughout this text, the language used will reflect that reported in the original cited articles to maintain accuracy.

immunomodulatory, anxiolytic, anti-depressant, anti-convulsant, and antiemetic properties (Bergamaschi, Queiroz, Zuardi, et al., 2011; Campos et al., 2016; Martinez Naya et al., 2023).

The mechanisms of action of CBD are diverse and largely unknown (Martinez Naya et al., 2023); however, there is evidence that CBD may impact neural processes implicated in a variety of neuropsychiatric disorders, such as anxiety disorders (Campos et al., 2016). It is suspected that the proposed pharmacological mechanisms underlying CBD's ability to diminish stress and anxiety include its interactions with diverse targets including agonism of the serotonin 5HT-1A receptors, antagonism of the cannabinoid CB1 receptor, inverse agonism of the CB2 receptor, and agonism of the transient receptor potential vanilloid type 1 receptor (Blessing et al., 2015b; García-Gutiérrez et al., 2020). In contrast to THC, CBD demonstrates only minimal negative allosteric modulation of the cannabinoid CB1 receptor (Laprairie et al., 2015). THC's activity on the CB1 receptor is attributed to THC's psychomimetic properties (Laprairie et al., 2015). Interestingly, CBD is a partial agonist of the dopamine D2 receptor resulting in potential antipsychotic effects (García-Gutiérrez et al., 2020). Mesoamygdaloid dopaminergic projections may also characterize anxiety-related responses, and D2 agonists are believed to elicit anxiolytic effects via this pathway (de la Mora et al., 2010). Thus, CBD's action on diverse neural substrates may characterize its anxiolytic effects.

Drug effects in humans are considered to arise from a combination of the drug's direct pharmacological effects and other non-drug-related factors (Kirsch, 1997). In fact, beliefs about the content of substances (i.e., stimulus expectancies), as well as beliefs about the drug's related effects (i.e., response expectancies), may induce drug effects independently (Schlagintweit et al., 2020). Beliefs about drug content can be manipulated by the instructions provided to participants about the products they administer during the study (told active drug vs. told placebo), and may

be mediated through verbal information, conditioning, and/or observational learning (Kirsch, 2018). Given that CBD is devoid of intoxicating or overt subjective effects, it is a promising candidate for expectancy-related effects (referred to as "placebo effects"). In fact, our previous work has demonstrated that increased sedation, regulated heart rate, and decreased salivary cortisol following acute stress were seen in participants told they were receiving CBD (vs. told no CBD), although they had received a placebo (Spinella, Burdeyny, et al., 2023; Spinella et al., 2021; Zhekova et al., 2024). Likewise, in an unpublished study from our group using the dataset from the current investigation, CBD expectancy was associated with blunted functional connectivity (FC) between the dorsal ACC (dACC) and the amygdala (Perry et al., 2024, under review). Therefore, controlling for CBD-related response expectancies may lead to more accurate inferences regarding CBD's mechanisms of action and therapeutic effects for stress and anxiety in humans.

1.1 BEHAVIOURAL THERAPEUTIC EFFECTS OF CBD

CBD has gained considerable attention in the field of psychiatry for its reported therapeutic potential (Corroon & Phillips, 2018; Kirkland et al., 2022). Globally, there have been notable increases in the reported use of CBD for medicinal purposes (World Health Organization, 2018). Indeed, cannabis users tend to attribute cannabis' anxiety- and stress-relieving properties to CBD as opposed to THC (Spinella, Bartholomeusz, et al., 2023; Tran & Kavuluru, 2020; Wheeler et al., 2020). However, most of the literature regarding CBD's therapeutic effects remains pre-clinical, and there are very few well-controlled randomized studies (Khoury et al., 2019).

One potential application for CBD has been in the treatment of stress- and anxiety-related disorders. Preclinical animal models have suggested that CBD administration may be associated

with reduced stress- and anxiety-related responses across a diverse range of stress tasks including the elevated plus maze, the Vogel conflict test, and the elevated T maze (for review, see Blessing et al., 2015). CBD administration has also been associated with reduced heart rate and blood pressure induced by restraint stress (Blessing et al., 2015). Though CBD's anxiolytic effects are likely attributable to its interactions with a range of molecular targets, preclinical mouse models have revealed that its interactions with CB1 receptors may be mediating its anxiolytic properties (Austrich-Olivares et al., 2022). Evidence of CBD's anxiolytic and stress-reducing effects in humans remains limited; however, there is some support for CBD's purported anxiolytic effects in both healthy and clinical populations (García-Gutiérrez et al., 2020). For instance, CBD decreased the perceived anxiogenic effects of THC compared to placebo in a group of eight healthy participants (Zuardi et al., 1982). In a second double-blind, placebo-controlled, follow-up study, CBD was reported to reduce subjective anxiety in ten healthy participants performing a public speaking task (Zuardi et al., 1993). Another double-blind, placebo-controlled study identified similar reductions in anxiety during a public speaking task in a larger sample of fifty-seven healthy male participants (Linares et al., 2018).

Evidence of CBD's anxiolytic and stress-reducing effects has also been demonstrated in a range of clinical populations. For example, a study by Bergamaschi et al. (2011) compared the effects of CBD and placebo on subjective stress- and anxiety-related responses in participants diagnosed with social anxiety disorder completing a public speaking task. They reported that CBD (vs. placebo) significantly reduced subjective ratings of anxiety, alertness, cognitive impairment, and discomfort throughout the task (Bergamaschi, Queiroz, Zuardi, et al., 2011). Findings have also been extended to the treatment of anxiety in individuals with substance use disorders. Namely, CBD administration was found to reduce anxiety associated with drug cue

exposure in individuals with heroin use disorder (Hurd et al., 2019). However, the literature is mixed, and some double-blind trials have failed to detect effects of CBD on stress- and anxiety-related processes. For instance, one study found that 600 mg of oral CBD did not result in changes to anxiety-related emotional processing, subjective stress, or related neural responses in 24 healthy adults (Bloomfield et al., 2022). Similarly, another randomized placebo-controlled study failed to identify any effect of a range of doses of CBD (150 mg, 300 mg, 600 mg) on exam anxiety relative to placebo (Stanley et al., 2022). Additionally, Leen Feldner et al. (2022) examined the impact of CBD on subjective and physiological measures of fear prior to a carbon dioxide-enriched air-breathing challenge in 61 healthy young adults. This study failed to detect an effect of 150mg, 300mg, or 600mg of CBD on subjective or physiological measures of fear (Leen-Feldner et al., 2022). Further, in 40 individuals with cocaine use disorder randomized to receive 800mg of CBD daily for 12 weeks, CBD treatment did not reduce mean anxiety, or anxiety responses to stressful cues relative to the placebo condition (Mongeau-Pérusse et al., 2022). Taken together, though evidence suggests some promise for CBD's potential anxiolytic and stress-reducing effects, empirical evidence is mixed, and there is currently insufficient high-quality evidence to suggest a therapeutic benefit of CBD for the treatment of psychiatric disorders (Black et al., 2019). There remains an imminent need for controlled clinical trials and longitudinal studies to appropriately assess CBD's efficacy (Dammann et al., 2024). Additionally, the extent to which any therapeutic effects of CBD are due to pharmacological and/or non-pharmacological properties has not yet been systematically examined.

1.2 THE BIOLOGICAL STRESS RESPONSE

Stress and anxiety are innate responses to real or perceived threats in the environment. When individuals are faced with a stressor, the biological stress response may be activated to

address the stressor and restore homeostatic balance (Russell & Lightman, 2019). Acute stress may result in various physiological responses including but not limited to increased heart rate, skin conductance, and elevated cortisol secretion (Armario et al., 2020). In the Hypothalamus-Pituitary-Adrenal (HPA) axis-mediated stress response, glucocorticoids, such as cortisol, are released into the bloodstream. These may affect immune function, and metabolism, to cope with an imminent stressor (Ulrich-Lai & Herman, 2009). Cortisol also serves as a negative feedback regulator of the HPA axis through its binding to mineralocorticoid receptors and glucocorticoid receptors in the hippocampus and the paraventricular nucleus (Oyola & Handa, 2017). Various limbic forebrain structures have been associated with top-down regulation of the stress response. Indeed, the amygdala, hippocampus, and prefrontal cortex may process psychogenic and systemic stimuli, as they receive information from subcortical and cortical areas involved in higher-order sensory processing, memory, attention, and arousal (Ulrich-Lai & Herman, 2009). These brain regions have outputs to downstream relay sites allowing for the processing of this limbic information, which may potentially influence the HPA axis (Ulrich-Lai & Herman, 2009).

Among healthy individuals, stress is a common adaptive response to challenges of daily living. In fact, subjective perceived daily stressors, including having a disagreement, avoiding an argument, work/school-related overload, or home-related overload, have been reported by healthy individuals once every three days (Stawski et al., 2013). Anxiety is also a common response to stress and may be associated with worry and apprehension (Bystritsky & Kronemyer, 2014). A primary motivating factor for CBD use amongst non-medical users is believed to be its purported ability to reduce both stress and anxiety (Geppert et al., 2023). Indeed, CBD's anxiolytic effects may involve the attenuation of physiological stress-related responses. For example, CBD has been shown to attenuate heart rate associated with restraint stress in

preclinical animal models (Blessing et al., 2015). Thus, CBD's action on neural substrates associated with stress- and anxiety-related responses may facilitate its purported anxiolytic effects.

1.3 CBD'S NEURAL EFFECTS

Though CBD appears to reduce behavioural stress- and anxiety-related responses, the neural substrates underlying its mechanism of action remain understudied. An increasing number of neuroimaging studies have examined the impact of CBD on human brain function (Batalla et al., 2021). Resting-state functional connectivity (rsFC) is a widely used method to analyze human brain function. This method refers to the temporal correlation of spontaneous blood-oxygen-level-dependent signal fluctuations between different brain regions at rest or when no specific task or external stimuli are present (Biswal et al., 1995). This technique measures the degree of synchrony in neural activity of brain regions directly or indirectly structurally connected (Lv et al., 2018). A recent systematic review identified mixed findings in relation to CBD's effects on striatal prefrontal resting-state connectivity (Lorenzetti, Gaillard, et al., 2023). For instance, Grimm and colleagues (2018) identified that 600mg of CBD administration significantly increased fronto-striatal rsFC in healthy males (Grimm et al., 2018). However, another study reported decreased rsFC between the striatum insula, lateral frontal cortex, and cerebellum, but greater rsFC between the striatum and associative, limbic, and sensorimotor regions in healthy participants following administration of 600mg of oral CBD (Wall et al., 2022). Mixed findings are likely attributable to studies investigating CBD's effects on the brain being largely under-powered and lacking robustness (Lorenzetti, Gaillard, et al., 2023).

Though evidence is limited, CBD has been demonstrated to modulate stress- and anxiety-related neural substrates (Batalla et al., 2021). For instance, CBD's subjective anxiolytic effects,

compared to placebo, have been suggested to be mediated by action on limbic and paralimbic brain regions in both healthy males (Crippa et al., 2004) and males with social anxiety disorder (Crippa et al., 2011). Moreover, Fusar-Poli and colleagues (2009) examined the effect of 600mg of CBD on regional brain activation and skin conductance in 15 healthy adult men. CBD was found to attenuate regional brain activation within medial temporal structures and regions of the cingulate cortex when viewing fearful faces, and this attenuation was correlated with fluctuations in skin conductance response (Fusar-Poli et al., 2009). In a follow-up study (Fusar-Poli et al., 2010), 600mg of CBD was suggested to decrease effective connectivity between the left ACC and left Amygdala while viewing intensely fearful faces in 15 healthy adult men. Consistent with these findings, CBD has been found to attenuate amygdala responses to fearful faces in healthy males (Bhattacharyya et al., 2010). Thus, in line with observed anxiolytic behavioural effects, CBD may correspondingly impact stress- and anxiety-related neural substrates. However, methodological heterogeneity limits the conclusions that can be drawn (Stanciu et al., 2021).

1.4 INTRINSIC CONNECTIVITY NETWORKS

Literature examining changes in FC associated with CBD's anxiolytic effects have been mostly limited to seed-to-seed based approaches. Though limited by its dependence on seed selection, this method is hypothesis-driven, and findings have been widely used in clinical models (Lv et al., 2018). On the other hand, a whole-brain analysis is a more exploratory method, and is data-driven, potentially providing a more holistic view of brain connectivity (Lv et al., 2018). Thus, further analysis of the whole brain is warranted to delineate key networks associated with CBD's mechanism of action. Networks composed of communities of brain structures are believed to demonstrate patterns of connectivity at rest. These functional brain networks are believed to be the fundamental, organizational elements of the brain's architecture.

Functional relationships between distinct brain regions forming networks can be identified using whole brain methods. Evidence suggests that investigation of large-scale brain networks may provide valuable insight into ongoing neurological processes (Menon, 2011). Menon (2011) proposed a triple-network model that assumes aberrant functioning of three large-scale brain networks. These networks may serve as neurophysiological biomarkers underlying various stress-related psychiatric disorders. Dysfunctions of the default mode network (DMN), salience network (SN), and central executive network (CEN) are thought to characterize various affective and neurocognitive symptoms in psychopathology.

The salience network is comprised of two core nodes – the anterior insula (AI) and the dACC – alongside three subcortical structures: the amygdala, the ventral striatum, and the substantia nigra/ventral tegmental area (Menon, 2015). Together, this network is instrumental in coordinating myriad brain functions including but not limited to social behavior, communication, and self-awareness (Menon, 2015). However, the SN is perhaps best known for its role in integrating sensory, cognitive, and emotional information (Menon, 2015). Thus, the SN is believed to play a key role in stress, and has been demonstrated to be activated in response to salient stimuli and during emotional processing (Hermans et al., 2014; van Oort et al., 2017), serving to direct attention towards significant internal and external cues, and facilitate decision making and goal-directed behaviour (Menon, 2011). The SN is suggested to be the mediator between two other intrinsic connectivity networks: the DMN, and its antagonist, the CEN. The CEN plays an important role in working memory and attention, while facilitating higher level cognitive processes such as planning and decision making (Menon, 2011). The CEN is anchored in the dorsolateral prefrontal cortex (dlPFC) and the lateral posterior parietal cortex, and it also includes a portion of the dorsomedial prefrontal cortex and the frontal eye fields (Menon, 2011;

van Oort et al., 2017). Conversely, the DMN is typically deactivated during cognitively demanding tasks, and has been identified to be important in a range of functions involving self-referential mental activity such as episodic memory, autobiographical memory, semantic memory, value-based decision making, and emotional regulation (Menon, 2011). Key nodes of the DMN include the posterior cingulate cortex (PCC) and the ventromedial prefrontal cortex, with connections to the inferior parietal lobule, the parahippocampal gyrus, and the hippocampus (Menon, 2011; van Oort et al., 2017). The SN, CEN, and DMN have been shown to have a prominent role in the acute stress response (van Oort et al., 2017). Triple-network alterations in response to stress may vary widely between individuals, and such alterations may be indicative of underlying psychopathology (Schimmelpfennig et al., 2023). For instance, alterations in the SN, DMN, and CEN in posttraumatic stress disorder patients may be associated with heterogeneous symptom presentations (Nicholson et al., 2020).

Evidence suggests that acute stress may result in dynamic interactions within and among these functional networks, enabling comprehensive reallocation of neural resources according to cognitive demands (Hermans et al., 2014). In the context of acute stress, increased connectivity within and between both the SN and DMN have been demonstrated across a range of studies, whereas most of the literature details no change in CEN connectivity (van Oort et al., 2017). When faced with a potential threat, the SN may facilitate the orientation of attention toward salient stimuli and mobilize energy resources to take imminent action (Hermans et al., 2014; Paltoglou et al., 2024). This is suspected to occur during acute stress to promote hypervigilance at the cost of executive control, to later downregulate when the stressor subsides and reorient cognitive resources to the CEN for the purpose of higher-order cognitive processes (Hermans et al., 2014). Key nodes of the SN have been suggested to be activated following a range of stress

tasks. For instance, increased within-network SN functional connectivity was associated with increased heart rate and physiological arousal index in healthy adults when they viewed emotionally arousing cinematographic material (Young et al., 2017). Though this is similar to a resting-state paradigm since participants in Young et al. (2017) passively viewed cinematographic material, the observed neural effects associated with increased physiological arousal may be specifically attributed to the visual stimuli. However, similar findings have also been demonstrated at rest, for instance increased rsFC between regions of the SN (i.e., the amygdala and the ACC) has been associated with attentional bias to threatening stimuli in healthy controls (Jenks et al., 2020). Also, a strong positive correlation between rsFC of key nodes of the SN including the left AI and basolateral amygdala was associated with state anxiety (Baur et al., 2013). Thus, it is evident that the SN is a key network involved in stress- and anxiety-related responses.

The role of the DMN in acute stress is less evident, but it has been suggested to be activated when the stressor involves self-referential processing (i.e., script-driven imagery) (Seo et al., 2011), or if the stressor involves negative feedback resulting in rumination (van Oort et al., 2017). Indeed, across a range of studies, regions of the DMN have been demonstrated to be co-activated with the SN following acute stress. For example, when exposed to scripts depicting personally relevant stressful events as opposed to neutral and relaxing scripts, healthy participants have exhibited heightened activity in crucial nodes of the DMN, including the ventromedial prefrontal cortex and the PCC (Seo et al., 2011; Sinha et al., 2004). This activation was coupled with increases in SN activity within key nodes such as the dACC and the AI (Seo et al., 2011; Sinha et al., 2004). Similar findings have been identified in studies utilizing mental arithmetic tasks. For instance, in response to negative feedback following a mental arithmetic

task, healthy individuals have demonstrated increased activity in the PCC, and areas of the SN such as the thalamus (Dedovic et al., 2014). However, this co-activation may not necessarily be indicative of increased FC between the SN and DMN, and more research is needed to better understand their interactions following acute stress. Moreover, the opposite has also been reported, wherein stress is believed to increase activity within the SN but attenuate that of the DMN (Paltoglou et al., 2024). Though the DMN is typically inactive during stimulus-driven cognitive tasks, its role in self-referential processing may significantly impact stressor-related responses, and abnormalities within this network have been identified across many psychiatric disorders (Menon, 2011).

Contrary to the DMN, during higher order cognitive tasks neural resources are redirected to the CEN (Menon, 2011; van Oort et al., 2017). This network may be particularly active during tasks involving working memory, problem solving, and decision making and has been demonstrated to be impacted widely in psychiatric disorders involving deficits in executive function (Menon, 2011). However, the CEN as a network is less commonly studied than either the DMN or SN (Menon, 2011), and findings regarding its role in the acute stress response have been more equivocal, often resulting in no changes between conditions (van Oort et al., 2017). However, increases in CEN activity have been reported across some studies examining stress-tasks involving higher cognitive load (Fechir et al., 2010; Gianaros et al., 2008). It is believed that initially during stress there is a decrease in intra-network FC within the CEN, followed by a subsequent increase in within-network FC and between-network FC with the DMN to facilitate higher-order cognitive processing of the stressor (Paltoglou et al., 2024). It has been suggested that the CEN may be involved in acute stress in an inverted-U shaped manner (van Oort et al., 2017) in accordance with the Yerkes-Dodson law (Yerkes & Dodson, 1908). For instance, at

moderate levels of arousal, increased FC between the SN and CEN has been demonstrated in healthy adults (Young et al., 2017).

The literature examining the impact of CBD's pharmacological and non-pharmacological effects on neural networks remains limited. Consistent with CBD's purported behavioral anxiolytic effects, CBD-containing cannabis has been suggested to decrease FC within both the DMN and SN (Wall et al., 2019). In fact, when presented with fearful faces, healthy adult men who were administered CBD demonstrated attenuated activity in regions of the SN and DMN relative to neutral faces (Fusar-Poli et al., 2009, 2010). Taken together, evidence suggests that acute CBD administration may significantly impact the SN and DMN; however, the role of non-pharmacological factors on neural networks has yet to be systematically examined.

1.5 EXPECTANCY EFFECTS

Stimulus expectancies, or beliefs about the drug content of a substance, may greatly impact the substance's subjective and physiological effects. Given that CBD is not associated with any overt intoxicating effects, it is a promising candidate for placebo-related responses. Indeed, open-label trials among adults with diagnosed anxiety disorders suggest that daily oral CBD administration can lead to clinically significant decreases in anxiety symptoms (Dahlgren et al., 2022), even among those with treatment-resistant anxiety (Berger et al., 2022). Since participants in these trials were aware they were receiving CBD, it is possible that expectancy factors were wholly or partially implicated in the observed treatment effects. However, double-blind trials in which participants are informed that they have an equal chance of being assigned to active or placebo drug conditions have yielded both positive (Bergamaschi, Queiroz, Zuardi, et al., 2011; Masataka, 2019) and null results (Bolsoni et al., 2022; Gournay et al., 2023; Kwee, Baas, et al., 2022) relative to placebo. These findings suggest a potentially significant role for

expectancy effects in subjective relief from stress and anxiety following CBD administration. Therefore, considering participants' beliefs about their drug assignment is often not accounted for in blinded trials, expectancy-related factors cannot be disentangled from the observed outcomes using traditional placebo-controlled experiments.

CBD expectancy effects have been empirically examined in healthy adults. For example, CBD expectancy instructions were associated with increased analgesia and reduced pain unpleasantness in healthy adults (De Vita et al., 2022). Similarly, in users of edible cannabis, positive expectancies for cannabis to improve general health and to reduce depression were associated with greater enjoyment and reductions in pain following acute administration (Chen et al., 2024). Our group has also shown that CBD expectancy alone is sufficient to impact subjective, physiological, and endocrine markers of stress and anxiety (Spinella, Burdeyny, et al., 2023; Spinella et al., 2021). Participants who were told they were receiving CBD, but who received a placebo, were demonstrated to have increased heart rate variability during stress anticipation, indicative of increased stress response regulation relative to participants told they were receiving a CBD-free hemp seed oil and who received hemp seed oil (Spinella et al., 2021). CBD expectancy (vs. CBD-free expectancy) was also associated with dampened cortisol responsivity, suggesting an impact on the HPA axis (Spinella, Burdeyny, et al., 2023). However, this effect was largely driven by male as opposed to female participants (Spinella, Burdeyny, et al., 2023). Notably, it is currently unclear the extent to which neural activity is directly impacted by CBD-expectancy.

Though the literature is sparse, there is some evidence of placebo-related brain changes in the field of stress and anxiety. For instance, in a study by Zhang et al. (2011), participants underwent a sham magnetic treatment in which they were led to believe that it would alleviate

pain and negative emotions. Placebo-related anxiolytic effects following a pain stimulus were associated with significantly decreased activity within regions of the SN (i.e., amygdala, insula, dACC, thalamus) and the DMN (i.e., hippocampus) (Zhang et al., 2011). This effect was also positively correlated with an associated behavioral placebo-related effect, as participants had lower ratings of unpleasantness when observing negative images (Zhang et al., 2011). Moreover, evidence suggests that neural pathways associated with response to selective serotonin reuptake inhibitors (SSRI) may be shared with those of placebo-related anxiolytic effects (Faria et al., 2012). Indeed, reduced cerebral blood flow in regions of the amygdala have been identified in both treatment responders to SSRIs and placebo for anxiety, pointing towards common pharmacological and psychological targets in anxiolysis (Faria et al., 2012). Similarly, placebo responders and treatment responders to SSRIs have demonstrated decreased regional blood flow in the amygdala coupled with decreased activation of the posterior dACC (Faria et al., 2014). Therefore, expectancy-related factors involved in the stress-reducing and anxiolytic effects of CBD may share similar neural pathways as the pharmacological mechanism. Indeed, our group has identified that CBD expectancy alone may also alter the connectivity of brain regions associated with stress and anxiety. Preliminary analyses of data from the current neuroimaging study of CBD placebo effects on stress responses revealed that CBD expectancy was associated with blunted FC between dACC and the amygdala (Perry et al., 2024, under review). However, given that seeds were selected *a priori*, we cannot be certain our seeds were placed in the locale of peaks. Likewise, seed-based approaches could be used to define multiple networks for inter-network connectivity analysis. Since the dACC and amygdala are central nodes in the SN, our previous findings suggest that CBD-expectancy alone may attenuate stress-induced FC within this network. Activity within the DMN, including the PCC, has been suggested to be associated

with placebo anxiolysis (Huneke et al., 2022). Studies examining FC corroborate these findings. For example, enhanced FC within the DMN was found to predict anxiolytic placebo effects in 23 healthy adults (Meyer et al., 2019). However, the impact of CBD's placebo-related effects on this and other brain networks remains understudied.

In an experimental context, the impact of perceived drug assignment on drug-related outcomes can be assessed using a balanced placebo design (Rohsenow & Marlatt, 1981). Previous studies have utilized the balanced placebo design (or a variation of it) to determine the relative contributions of the pharmacological and placebo components of several substances including nicotine (Dar & Barrett, 2014), amphetamine (Cropsey et al., 2017), analgesics (Atlas et al., 2012), and THC (Metrik et al., 2009). In most cases, both pharmacological and expectancy effects were found to significantly contribute to the overall drug effects. Pharmacological and expectancy effects have also frequently been shown to interact, typically producing additive effects (i.e., the most robust drug effects are observed when a drug is both expected and received). For instance, nicotine and nicotine dose expectancy has been associated with increased positive smoking effects in an additive fashion (Juliano et al., 2011). However, it is not clear the extent to which such effects are dependent on the presence (or absence) of overt physiological sensations that follow substance administration since changes in physiological state can themselves evoke drug-related expectations.

1.6 THE PRESENT THESIS

Given the early evidence supporting the use of CBD as a novel treatment for stress- and anxiety-related disorders (Corroon & Phillips, 2018; Kirkland et al., 2022), it is important to understand its underlying mechanism of action, as well as the role of expectancy effects. My thesis aims to use archival data (Perry et al., 2024, under review) of a mixed between-subject,

repeated measures design, to assess the independent effects of CBD expectancy on anxiety- and stress-related neural activity in a sample of healthy adults. Participants received either accurate or inaccurate information regarding the CBD content of a CBD-free hemp seed oil (Told CBD, Told CBD-free). Notably, a full balanced placebo design was not employed in this study, but rather a half-balanced placebo design (e.g., Spinella et al., 2021), and thus the interactive effects of CBD's pharmacological and non-pharmacological effects cannot be inferred. The primary aim of my thesis was to examine the extent to which CBD expectancy independently alters FC within and between the DMN, SN, and CEN, and its impact on subjective stress and anxiety following acute psychosocial stress as compared to participants told they are not receiving CBD. I suspected that CBD expectancy would attenuate rsFC associated with stressor-related effects. Therefore, consistent with our preliminary analyses (Perry et al., 2024, under review), and studies examining stress effects on neural networks (van Oort et al., 2017), I anticipated increased FC within and between the SN and DMN in the CBD-free expectancy group, which would be attenuated in the CBD expectancy group. There were no changes expected within the CEN. Given the relative dearth of studies examining CBD's effects on the brain, this study represents the first of its kind to examine neural network alterations in relation to CBD-placebo effects, and therefore our findings will be primarily exploratory in nature. Finally, consistent with previous studies from our group (Spinella, Burdeyny, et al., 2023; Spinella et al., 2021; Zhekova et al., 2023), participants told they were receiving CBD were expected to report reduced stress, anxiety, and increased sedation following psychosocial stress.

The present thesis includes a manuscript of the latter-described study entitled "The Effects of Cannabidiol (CBD) Expectancy on Subjective Stress, Anxiety, and Related Neural Responses in Healthy Adults" which includes sections from a submitted manuscript to which I

contributed (Perry et al., 2024, under review) as well as novel material unique to this thesis. This will be followed by a general discussion elaborating on the findings, limitations, strengths, future directions in the field, and implications.

CHAPTER 2. THE EFFECTS OF CANNABIDIOL (CBD) EXPECTANCY ON SUBJECTIVE STRESS, ANXIETY, AND RELATED NEURAL RESPONSES IN HEALTHY ADULTS

This chapter presents the manuscript on which this thesis is based. This study is based on archival data from a previous study and includes subjective results from the associated manuscript (Perry et al., 2024, under review). Readers should be advised that Robin Perry, Mikaela Ethier-Gagnon, and Dr. Sean Barrett are responsible for the initial draft of the Perry et al. (2024, under review) manuscript, alongside their co-authors. This manuscript was peer reviewed at the *Journal of Psychopharmacology*. Robin and Mikaela have made revisions suggested by reviewers under the guidance of Drs. Sean Barrett and Sherry Stewart. The manuscript is currently resubmitted for review. For the purposes of the current thesis, sections of the write-up and associated figures and tables in Perry et al. (2024, under review) for which Mikaela Ethier-Gagnon was primarily responsible are included herein.

2.1 INTRODUCTION

A growing body of evidence has suggested that cannabidiol (CBD), a naturally occurring phytocannabinoid of the *cannabis sativa* plant, may be a promising treatment candidate for a range of psychiatric disorders (Dammann et al., 2024). CBD is devoid of psychomimetic properties and has been demonstrated to be relatively safe and well-tolerated across a range of doses and routes of administration (Blessing et al., 2015). Additionally, it is believed to have no intoxicating or rewarding properties which may result in misuse (Mechoulam et al., 2002), making CBD an attractive candidate for the treatment of mental health symptoms. The mechanisms of action of CBD are diverse and not yet completely understood (Martinez Naya et al., 2023); however, evidence suggests that CBD may impact neural processes implicated in a variety of neuropsychiatric disorders, including anxiety- and stressor-related disorders (Campos et al., 2016). CBD's ability to diminish stress and anxiety are believed to be associated with its interactions with a range of receptor targets, such as serotonin 5HT-1A receptors, cannabinoid CB1 and CB2 receptors, dopamine D2 receptors, and transient receptor potential vanilloid type 1 receptors (Blessing et al., 2015; de la Mora et al., 2010; García-Gutiérrez et al., 2020; Shahbazi et al., 2020). However, evidence examining the role of both pharmacological and non-pharmacological factors in CBD's anxiolytic and stress-reducing effects remains limited.

Though much of the literature examining CBD's purported effects is pre-clinical, and there are very few well controlled randomized studies (Khoury et al., 2019), there is some support for CBD's anxiolytic effects in both healthy and clinical populations. Indeed, cannabis users tend to attribute cannabis' anxiety- and stress-relieving properties to CBD as opposed to THC (Spinella, Bartholomeusz, et al., 2023; Tran & Kavuluru, 2020; Wheeler et al., 2020). Double-blind studies have also demonstrated reductions in reported anxiety in healthy adults that

received CBD (vs. placebo) completing a public speaking task (Linares et al., 2018; Zuardi et al., 1993). Similar findings have been reported in individuals with social anxiety disorder (Bergamaschi, Queiroz, Chagas, et al., 2011). Correspondingly, CBD has been suggested to modulate stress- and anxiety-related neural substrates (Batalla et al., 2021). As previously described in Chapter 1, CBD's subjective anxiolytic effects, relative to placebo, have been suggested to be mediated by action on limbic and paralimbic brain regions in both healthy males (Crippa et al., 2004) and males with social anxiety disorder (Crippa et al., 2011). Moreover, evidence suggests that CBD (vs. placebo) may attenuate effective connectivity between medial temporal structures and regions of the cingulate cortex when viewing fearful faces (Fusar-Poli et al., 2009, 2010). Consistent with these findings, in a study by Bhattacharyya and colleagues (2010), CBD was found to attenuate amygdala responses to fearful faces in healthy males. Thus, in line with observed anxiolytic behavioural effects, CBD may correspondingly impact stress- and anxiety-related neural substrates. However, methodological heterogeneity limits the conclusions that can be drawn (Stanciu et al., 2021).

Evidence suggests that investigation of large-scale brain networks may provide valuable insight into ongoing neurological processes as discussed in more detail in Chapter 1 (Menon, 2011). Networks composed of communities of brain structures are believed to demonstrate patterns of connectivity at rest. Key functional brain networks include the default mode network (DMN), salience network (SN), and central executive network (CEN). Dysfunction of these networks is thought to characterize various affective and neurocognitive symptoms in psychopathology (Menon, 2011). The SN is comprised of two core nodes – the anterior insula (AI) and the dorsal anterior cingulate cortex (dACC) – alongside three subcortical structures: the amygdala, the ventral striatum, and the substantia nigra/ventral tegmental area (Menon, 2015).

The SN may be best known for its role in the integration of sensory, cognitive, and emotional information, as it has been demonstrated to be activated in response to salient stimuli (Hermans et al., 2014; Menon, 2015; van Oort et al., 2017). The SN is suggested to be the mediator between the DMN, and its antagonist, the CEN. The CEN plays an important role in working memory and attention and may facilitate higher level cognitive processes such as planning and decision making (Menon, 2011). This network is anchored in the dorsolateral prefrontal cortex (dlPFC) and the lateral posterior parietal cortex, including a portion of the dorsomedial prefrontal cortex and the frontal eye fields (Menon, 2011; van Oort et al., 2017). On the other hand, the DMN is typically deactivated during cognitively demanding tasks, and has been identified to be important in a range of functions involving self-referential mental activity such as episodic memory, autobiographical memory, semantic memory, value-based decision making, and emotional regulation (Menon, 2011). Key nodes of the DMN include the posterior cingulate cortex (PCC) and the ventromedial prefrontal cortex, with connections to the inferior parietal lobule, the parahippocampal gyrus, and the hippocampus (Menon, 2011; van Oort et al., 2017).

Acute stress may result in dynamic interactions within and among these functional networks, enabling comprehensive reallocation of neural resources according to cognitive demands (Hermans et al., 2014). In the context of acute stress, increased connectivity within and between both the SN and DMN has been demonstrated across a range of studies, whereas most literature details no change in CEN connectivity (van Oort et al., 2017). However, some have suggested that initially during stress there is a decrease in intra-network functional connectivity (FC) within the CEN, followed by a subsequent increase in within-network FC and between-network FC with the DMN to facilitate higher order cognitive processing of the stressor (Paltoglou et al., 2024). Consistent with CBD's purported behavioral anxiolytic effects, CBD-

containing cannabis has been suggested to decrease FC within both the DMN and SN (Wall et al., 2019). In fact, when presented with fearful faces, healthy adult men who were administered CBD demonstrated attenuated activity in regions of the SN and DMN relative to neutral faces (Fusar-Poli et al., 2009, 2010). However, CBD's impact on between-network functional connectivity has not yet been determined. Taken together, evidence suggests that acute CBD administration may significantly impact intrinsic connectivity network FC; however, the role of non-pharmacological factors on neural networks has yet to be systematically examined.

As described in Chapter 1, given that CBD is devoid of psychoactive properties, it is a promising candidate for placebo-related effects. Indeed, open-label trials among adults with diagnosed anxiety disorders suggest that daily oral CBD administration can lead to clinically significant decreases in anxiety symptoms (Dahlgren et al., 2022), even among those with treatment-resistant anxiety (Berger et al., 2022). Since participants in these trials were aware they were receiving CBD, it is possible that expectancy factors were wholly or partially implicated in the observed treatment effects. However, double-blind trials in which participants are informed that they have an equal chance of being assigned to active CBD or placebo conditions have yielded both statistically significant decreases in anxiety (Bergamaschi, Queiroz, Zuardi, et al., 2011; Masataka, 2019) and null results (Bolsoni et al., 2022; Gournay et al., 2023; Kwee, Baas, et al., 2022) in the active drug condition relative to placebo. These findings suggest a potentially significant role for expectancy effects in subjective relief from stress and anxiety following CBD administration. Indeed, our group has previously shown that CBD expectancy alone is sufficient to impact subjective, physiological, endocrine, and neural markers of stress and anxiety (Perry et al., 2024, under review; Spinella, Burdeyny, et al., 2023; Spinella et al., 2021; Zhekova et al., 2024). In preliminary analyses of the current dataset, CBD expectancy was associated with

blunted FC between key nodes of the SN: the dACC and the amygdala (Perry et al., 2024, under review). However, given that this was a seed-based approach, we cannot be certain our seeds were placed in the locale of peaks, and we cannot infer inter-network connectivity. The literature examining the impact of placebo effects for stress and anxiety on intrinsic connectivity networks is scarce. One study found that enhanced FC within the DMN predicted anxiolytic SSRI placebo effects in 23 healthy adults (Meyer et al., 2019). However, the impact of CBD's placebo-related effects on this and other brain networks remains understudied. Thus, further analysis on the whole brain is warranted to delineate key networks associated with CBD expectancy effects.

The manuscript contained in this thesis uses archival data (Perry et al., 2024, under review) of a between-subject, repeated measures design, to assess the independent effects of CBD expectancy on anxiety- and stress-related neural activity in a sex-balanced sample of healthy adults. The primary aim of my thesis is to examine the extent to which CBD expectancy independently alters rsFC within and between the DMN, SN, and CEN, and its impact on subjective stress and anxiety following acute psychosocial stress as compared to participants told they were not receiving CBD. Consistent with our previous findings (Perry et al., 2024, under review), and studies examining CBD's effects on the brain (Lorenzetti, McTavish, et al., 2023), I anticipated increased FC within and between the SN and DMN in the CBD-free expectancy condition (vs. Told CBD). There were no anticipated changes expected within the CEN, or between the CEN and the SN or DMN. Finally, consistent with previous studies from our group (Spinella, Burdeyny, et al., 2023; Spinella et al., 2021; Zhekova et al., 2024), participants told they were receiving CBD were expected to report reduced stress and anxiety, and increased sedation, following psychosocial stress relative to those told they were receiving a CBD-free product.

2.2 METHODS

2.2.1 Participant Selection

Thirty-eight (54% female) healthy adults were recruited from the Halifax, Nova Scotia community using online advertisements and community bulletins (Appendix A). Interested participants were contacted by a trained research assistant to conduct a brief telephone screening interview to confirm eligibility (Appendix B) and to screen for substance use disorders with the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991), the Michigan Alcoholism Screening Test (Selzer., 1971) and the Drug Abuse Screening Test (Skinner., 1982). Eligible participants were between the ages of 19-65 years old, as this is the age of majority in Nova Scotia, and no older than 65 to reduce potential confounding age-related decline in neural activity (Andrews-Hanna et al., 2007; La Corte et al., 2016). Participants were also required to have used cannabis at least once in their lifetime to ensure they had some familiarity with cannabis constituents, and to reduce the risk of a potential allergic reaction. Participants were excluded if they had a current or past-year diagnosis of a psychiatric disorder including substance use disorder, and/or current prescription medication use, aside from birth control in females. These criteria were set to ensure neurophysiological or psychological conditions did not influence subjective and neural stress- and anxiety-related responses. Moreover, participants were excluded if they presented with any contraindication for magnetic resonance imaging (MRI) (e.g., metal implants, pacemakers, intense claustrophobia). All participants provided written consent (Appendix C) and were compensated CAD$20/hour for their time.

2.2.2 Measures and Apparatus

2.2.2.1 Carbon Monoxide Measurement

Participants were instructed prior to the experimental session that a breath sample would be used to verify cannabis and tobacco smoking abstinence requirements. Breath samples were collected with a carbon monoxide analyzer (Vitalograph, UK). This measure was used as a bogus pipeline to enhance compliance to abstinence requirements given there is no reliable carbon monoxide cutoff for cannabis abstinence. Bogus pipelines have been demonstrated to reliably reduce socially desirable responding and increase honesty (Roese & Jamieson, 1993; Tourangeau et al., 1997). As such, participants were not informed of their carbon monoxide readings.

2.2.2.2 Demographics

Participants were assessed for demographic information including their age, sex assigned at birth, gender identity, ethnicity, and level of education (Appendix D).

2.2.2.3 Substance Use

Current cannabis use frequency was assessed using the *Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory* (DFAQ-CU; Cuttler & Spradlin, 2017) (Appendix D). This measure has been demonstrated to be psychometrically sound with excellent internal consistency reliability (α =0.95) and statistically significant moderate-to-good convergent (r= 0.705- 0.856 with other measures of cannabis use frequency) and predictive (r= 0.625-0.849 with other measures of hazardous cannabis use) validity (Cuttler & Spradlin, 2017). This measure is statistically significantly but only mildly correlated with a measure of alcohol use (r=0.227) suggesting discriminant validity as a measure of current cannabis use frequency in a cannabis using population (Cuttler & Spradlin, 2017).

2.2.2.4 Trait Anxiety

Trait-level anxiety at baseline was measured using 20-item Trait version of the State-Trait Anxiety Inventory (Spielberger, 1983). This measure has excellent internal consistency

reliability (α =0.90), and moderate-to-good test-retest reliability (r= 0.73–0.86) in male and female high school and college students from the United States (Spielberger, 1983). Moreover, this measure has moderate concurrent validity with other measures of anxiety in non-clinical samples (mean r=0.75) (Clark & Watson, 1991). Participants indicated their level of agreement with each item (e.g., "I feel nervous and restless", "I lack self-confidence") on a 4-point scale ranging from 1 'Not at all' to 4 'Very much'. Items were summed to generate a total score, which fell within a range of 20-80, with higher scores indicating greater trait anxiety. Summed scores in our sample had good internal consistency (α=0.879).

2.2.2.5 Perceived Stress

Levels of perceived stress the month prior to the participant's experimental session were examined using the 10-item Perceived Stress Scale (PSS) (Cohen et al., 1998). This scale has been demonstrated to have moderate reliability (internal consistency reliability α=0.78) and statistically significant but small correlations with conceptually related measures (criterion validity ranges from r=0.32-0.39 for stress frequency; convergent validity r=-0.22 with perceived health status, r=0.28-0.34 with psychosomatic symptoms, and r=0.22 with health service utilization) in residents of the United States (Cohen et al., 1998) providing some evidence of validity. Participants were prompted to rate the frequency at which specific life events were appraised as stressful (e.g., "In the last month, how often have you been upset because of something that happened unexpectedly?", "In the last month, how often have you found that you could not cope with all the things that you had to do?") on a 5-point scale ranging from 0 'Never' to 4 'Very often'. Items assess the degree to which participants perceive their lives to be unpredictable, uncontrollable, or overloaded. Components were summed to produce a total score,

ranging from 0-40, with higher scores being indicative of greater perceived stress. Summed scores had good internal consistency in the present sample (α=0.873).

2.2.2.6 CBD Beliefs

A priori beliefs about CBD's purported effects on stress and anxiety (i.e., reduces stress, reduces anxiety) were assessed using an experimenter-compiled numerical rating scale ranging from 1 'Not at all' to 10 'Completely' (Zhekova et al., 2024; Perry et al., 2024, under review) (Appendix D). For relative specificity, beliefs regarding THC's effects on stress and anxiety were also assessed with this scale.

2.2.2.7 Subjective State

Participants were prompted to indicate acute subjective stress, anxiety, sedation, and energy on a set of four horizontal lines ranging from 1 'Not at all' to 10 'Extremely' at six timepoints throughout the study (Laboratory baseline, MRI baseline, pre-stress, post-stress, anticipation, post-recovery) (Appendix D). Elevated scores on the 'stress' item are suggested to be indicative of difficulties with relaxation, agitation, and irritability (DASS; Lovibond & Lovibond, 1995) whereas elevated scores on the 'anxiety' item are believed to be related to apprehension, tension, nervousness, and worry (STAI-S; Spielberger, 1983). The 'energized' and 'sedated' items are derived from the Brief Biphasic Alcohol Effects Scale (B-BAES; Rueger & King, 2013). These items are suggested to be indicative of potential stimulating (e.g., elated, excited) and sedative (e.g., slow thinking, sluggishness) drug effects. These single item descriptors facilitate measurement of acute subjective state in the neuroimaging environment, and similar scales have been demonstrated to have sound psychometric properties for the assessment of stress (Karekla et al., 2017; Lesage et al., 2012) and anxiety (Davey et al., 2007; Rossi & Pourtois, 2012).

2.2.2.8 CBD-Free Hemp Seed Oil

All participants were administered CBD-free hemp seed oil (Manitoba Harvest: Manitoba, Canada) sublingually at a dose of 0.3mg/kg. This dose was chosen to mimic reported doses which have been demonstrated to produce anxiolytic effects in humans (MacCallum & Russo, 2018). Hemp seed oil was deemed an adequate placebo as it is considered inactive and free of psychoactive properties (Kowal et al., 2016). The oil was delivered in a packaging that was consistent with their expectancy condition (e.g., commercial packaging in the Told-CBD condition, hemp seed oil packaging for the Told CBD-free condition.).

2.2.2.9 Stress Induction

Participants completed an adapted Trier Social Stress Task (TSST) (Kirschbaum et al., 2008; Wang et al., 2005), in attempt to induce mild psychosocial stress. This task was piloted by our group out-of-scanner and was found to induce mild stress- and anxiety-related subjective and physiological responses in healthy adults (Zhekova et al., 2024). Wang et al. (2007) have also found this stress-induction paradigm to be efficacious in the functional magnetic resonance imaging (fMRI) environment.

Participants completed a serial subtraction in increments of 13, starting from a four-digit number (i.e., 2043). Participants were provided with scripted negative feedback throughout the task if mistakes were made or if participants slowed down and/or stopped counting (e.g., "Could you please count faster", "That is incorrect, please start counting again from 2043"). For further emphasis on the social evaluative component of the test, participants were also informed that their performance would be compared to other study participants. Moreover, participants were erroneously informed that they would need to complete a second more difficult version of the counting task following brain activity measurements. This mild deception was implemented in

hopes of distinguishing between acute and anticipatory stress and anxiety (Spinella et al., 2021; Zhekova et al., 2024), and to prolong task-induced stress.

2.2.2.10 Perceived Task Difficulty

Participants were assessed for their perception of task difficulty immediately following the counting task using a single-item rating scale. Scores ranged from 1 ('Not at all') to 10 ('Extremely'), allowing for examination of group differences in perceived task difficulty across conditions as these may be associated with their appraisals of and responses to the stress task (Allen et al., 2014).

2.2.3 Procedure

The current investigation consisted of two sessions (baseline and experimental). Upon study inclusion, participants were invited to complete a 1-hour baseline session at Dalhousie University. During the baseline session, participants completed the demographic and substance use questionnaire (Appendix D), the STAI-T, the PSS, and the CBD-Beliefs and THC-Beliefs scale. Participants were then scheduled for a three-hour experimental MRI session at the Nova Scotia Health Biomedical Translational Imaging Centre (BIOTIC) facility. A visual representation of the experimental procedure is provided in Figure 2. Participants were required to abstain from smoking, drinking alcohol, recreational drug use, and caffeine consumption for a minimum of 12 hours prior to the experimental session. Participants were administered the carbon monoxide analyzer to encourage compliance to abstinence requirements and weighed to determine the adequate dosage of hemp-seed oil. Prior to entering the MRI, participants completed initial scores on the numerical analogue scale examining stress, anxiety, sedation, and energy (laboratory baseline) (Appendix D). Participant verbal responses to the visual analogue scale were recorded by the experimenter at all timepoints in which it was administered.

Participants then entered the MRI and completed a baseline anatomical and fMRI scan (~15 minutes) followed by the latter numerical analogue scale to assess baseline subjective state (MRI baseline). Then, participants completed a baseline 8-minute resting-state fMRI scan. Participants then exited the MRI and received CBD-free hemp seed oil (0.3mg/kg) with instructions consistent with their assigned condition (Told CBD vs. Told CBD-free). To enhance the believability of the study, all participants in the Told-CBD condition underwent a 10-minute sham absorption period and were erroneously informed that the effects of CBD on cognitive performance may emerge within this period. Following this waiting period, participants completed the numerical rating scales assessing subjective state for a third time (pre-stress). Prior to re-entering the scanner, participants were provided instructions stating that they would complete two 4-minute trials of a counting task involving serial subtraction, with a break between trials to measure brain activity. They were then instructed to re-enter the MRI and began the 4-minute stress-induction counting task by performing a serial subtraction in units of 13 from a four-digit number as quickly and accurately as possible. Immediately following the task, participants were assessed for perceived task difficulty using a single item numerical rating scale, followed by the subjective state rating scale for the fourth time (post-stress). Participants were then informed that they must wait eight minutes before beginning a 'second more difficult trial of the counting task'. Throughout this waiting period, participants completed a second resting-state scan. Following the waiting period, participants completed the subjective state scale for a fifth time (anticipation) and were informed that they would no longer be required to complete the second trial of the stress task. Finally, participants had a 10-minute recovery period outside of the scanner and completed the subjective state scale for a final time (post-recovery). Participants were assessed for whether they believed content instructions for their assigned condition (Told

CBD vs. Told CBD-Free), by asking them which product they had received with these response options: "CBD oil", "CBD-free hempseed oil", or "Unsure" (Appendix D). This served as a manipulation check to determine whether participants believed the information provided by the blinder about the CBD content of the oil they received, as participants who did not believe instructions would be removed from later analyses. To prevent the deceptive nature of the study from being revealed to future participants, participants were debriefed in full about the study aims and use of deception once the data collection phase of the study was completed (Appendix E).

2.2.4 MRI Data Acquisition and Preprocessing

Anatomical and functional data was collected using a 3.0 Tesla GE MR750 scanner with a 32-channel radiofrequency head coil. Pulse sequences and parameters closely matched those used in the Human Connectome Project (Glasser et al., 2013). Specifically, a 3D inversion recovery fast spoiled gradient recalled sequence was used to obtain T1-weighted (T1w) anatomical 1.0 mm isotropic images with the following parameters: field of view (FOV) 256 mm, 256×256 matrix, 184×1.0 mm sagittal slices, one signal average, repetition time (TR)=4 s, echo time (TE)=1.3 ms, flip angle=9°, inversion time=450 ms, bandwidth=62.5 kHz, scan time: 7 min 3 s. T2-weighted (T2w) anatomical 1.0 mm isotropic images were also collected using a 3D CUBE T2 Prepped fluid-attenuated inversion recovery sequence with the following parameters: FOV 256 mm, 256×256 matrix, 184×1 mm sagittal slices, TR=5 s, TE=15 ms, bandwidth=62.5 kHz, auto calibrating reconstruction for Cartesian imaging phase factor 1.75, scan time: 6 min 6 s. For resting state functional data, 3.0mm isotropic images were collected with a multi-band gradient-echo echo planar imaging (EPI) sequence obtained from Stanford CNI laboratory (https://cni .stanford.edu/wiki/MUX_EPI) with these parameters: FOV 216 mm,

72×72 matrix, 51×3.0 mm oblique-axial slices, generalized auto-calibrating partially parallel acquisition acceleration factor 2 in-plane, multiplexed acceleration factor 3 slice direction, TR=950 ms, TE=30 ms, 500 time points, scan time 7.9 min. EPI reference scans were obtained using phase-encode blip direction reversal with identical parameters to the resting state scans to facilitate field distortion correction (<1 min).

Preprocessing of anatomical and functional MRI data was conducted using *fMRIPrep* 22.0.1 (Esteban et al., 2019, 2020; RID:SCR 016216) which is based on *Nipype* 1.8.4 (Gorgolewski et al., 2011; 2018; RRID:SCR 002502).

For anatomical data, T1w images were corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (Tustison et al., 2010), distributed with ANTs 2.3.3 (Avants et al. 2008, RID:SCR 004757), and used as T1w-reference (T1w-ref) throughout the workflow. The T1w-ref 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 6.0.5.1:57b01774, RID:SCR_002823; Zhang et al., 2001). Brain surfaces were reconstructed using recon-all (FreeSurfer 7.2.0, RID:SCR_001847; Dale et al., 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-ref and the T1w template. The following template was selected for spatial normalization:

ICBM 152 Nonlinear Asymmetrical template version 2009c (RRID:SCR_008796;

TemplateFlow ID: MNI152NLin2009cAsym; Fonov et al., 2009).

For each of the two resting state Blood-Oxygen-Level-Dependent (BOLD) runs (MRI

baseline, post-stressor), a reference volume and its skull-stripped version were generated by

aligning and averaging 1 single-band references (SBRefs). Head-motion parameters with respect

to the BOLD reference (transformation matrices, and six corresponding rotation and translation

parameters) were estimated before spatiotemporal filtering using MCFLIRT (FSL

6.0.5.1:57b01774; Jenkinson et al., 2002). The field map was estimated based on two opposing-

direction phase-encoded EPI references using TOPUP (Andersson et al., 2003). The estimated

field map was then aligned with rigid-registration to the target EPI reference run. The field

coefficients were mapped on to the reference EPI using the transform. BOLD runs were slice-

time corrected to 0.4475 using 3dTshift from AFNI (RID:SCR_005927; Cox, 1996). The BOLD

reference was then co-registered to the T1w-ref using bbregister (FreeSurfer) which implements

boundary-based registration (Greve & Fischl, 2009). Co-registration was configured with six

degrees of freedom. 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), the per-image standard deviation of the

temporal derivative of the data (standardized DVARS) and three region-wise global signals. FD

was computed using two formulations following Power et al. (absolute sum of relative motions;

Power et al., 2012) and Jenkinson et al. (relative root mean square displacement between affines;

Jenkinson et al., 2002). FD and DVARS were calculated for each functional run, both using their

implementations in Nipype (following the definitions by Power et al., 2012). The three global

signals were extracted within the CSF, WM, and the whole-brain masks. 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 0.5mm FD or 1.5 standardized DVARS were annotated as motion outliers. 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*. 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 antsApplyTrans forms (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).

2.2.5 Blinding and Randomization

Using stratified sampling, healthy adults were divided into two lists based on biological sex. Within each sex stratum, participants were randomly assigned to one of two expectancy conditions (Told CBD vs. Told CBD-free) in a 1:1 ratio using an online list randomizer. Thus, half were misled that their oil contained CBD (Told CBD), whereas the other half was accurately informed of their assigned condition (Told CBD-free). To ensure experimenter observations were unbiased, an independent trained research assistant administered the hemp seed oil sublingually. In contrast to our previous work which employed a within-subjects design (Spinella et al., 2021), the current investigation utilized a between-subjects design. This experimental design was selected to prevent habituation to the stress task across multiple sessions. This

protocol was piloted in an out-of-scanner study from our group (Zhekova et al., 2024), which suggested adequate power to detect large subjective effects.

2.2.6 Power calculation

A power calculation for the present secondary analysis of archival data from Perry et al. (2024, under review) was not conducted. However, sample size estimates for the subjective outcomes in Perry et al. (2024, under review) included in this thesis were determined. Given the analytic approach (i.e., marginal linear models) in Perry et al. (2024, under review) we were unable to conduct an *a priori* power analysis for this specific method. To calculate necessary sample sizes for models with correlated data, knowledge about the specific within-subject correlation structure of the model is required, and this information is empirically derived from the model itself (Gueorguieva & Krystal, 2004). Sample size estimates were therefore based on a power calculation for a similar, but less powerful analytic approach (i.e., repeated-measures ANOVA) using G*Power. Assuming a small effect size of $f2 = 0.25$ in accordance with our previous work (e.g., Zhekova et al., 2024), a correlation of $r = 0.6$ among timepoints, and an alpha level of 0.05, we required a total of 30 participants to achieve a power of 0.95 to detect within-between-subject interactions (Told CBD vs. Told CBD-free).

2.2.7 Data analysis

2.2.7.1 Descriptive Statistics

Between-group independent t-tests were conducted on age, STAI-T, PSS, CBD-beliefs, cannabis use frequency, and task difficulty ratings, as well as chi square tests on sex, gender, and ethnicity, to determine any differences between conditions (i.e., Told CBD, Told CBD-free; included, excluded) with regards to individual characteristics which may systematically bias responses to oil administration and stressor induction.

2.2.7.2 MRI Data

Resting-state FC was assessed using a seed-to-voxel analysis implemented through *Nilearn* version 0.9.0. Regions of interest (ROIs) were located in the right posterior cingulate cortex for the DMN (PCC; 6, −54, 20), the right dorsal anterior cingulate cortex (dACC) for the SN (10, 24, 28), and the right dorsolateral prefrontal cortex for the CEN (dlPFC; 44, 36, 20) (see Figure 3 for ROIs in MNI space). The ROIs selected are well established as key nodes in their respective networks, and coordinates have been selected based on literature examining FC in stress- and CBD-related studies (Corr et al., 2022; Lorenzetti, McTavish, et al., 2023; Wall et al., 2019, 2022). ROIs were further confirmed by automated meta-analytic data on http://neurosynth.org/, using the 'default mode', 'salience network', and 'executive control' terms. ROIs were centered in spherical spatial masks with a 7mm radius. Confounding time series (i.e., WM, CSF, global signal), the 6 motion parameters (i.e., translation x, y, z; rotation x, y, z), and their temporal derivatives and quadratic terms were included as regressors of no interest in the first-level models. Spatial smoothing was completed at full width half maximum (FWHM) 5mm. Temporal filtering was achieved using two separate methods: 1) high-pass discrete cosine filters with a cutoff frequency of 0.008 Hz as confounding timeseries in the first-level model, and 2) a low-pass temporal filter with a cutoff frequency of 0.08 Hz. Motion outliers were identified as frames which exceed an established threshold of 0.5mm FD or 1.5 standardized DVARS (Nichols et al., 2017). A scrubbing step was performed to exclude motion outlier volumes within runs via censoring. Subjects were dropped if the proportion of motion outlier volumes exceeded 30% of total volumes in any run. In total, 5 subjects were excluded from group analysis, *n*=3 in the Told CBD condition, and *n*=2 in Told CBD-Free condition. The time series for each seed region was extracted and correlated to the time series for every other

voxel in the brain for both runs (baseline and post-stress) in both groups (Told CBD vs. Told CBD-free) by employing the ConnectivityMeasure module from Nilearn. Using the model coefficients (i.e., r correlations) we controlled for within-group individual differences unrelated to experimental manipulations using a second level within-participant general linear model (i.e., contrasting baseline values and post-stress values). Then, post-stress runs between expectancy conditions (Told CBD, Told CBD-free) were contrasted using a third level general linear model. We then controlled for multiple comparisons using a false discovery rate (FDR) <0.05 with a minimum cluster threshold of 20 voxels. Corrected z-scores achieving statistical significance (p<0.05) were determined.

2.2.7.3 Subjective Data

Marginal linear models using the linear mixed model function of SPSS version 28 (SPSS Inc., Chicago, Illinois, USA) were used to analyze subjective outcomes. To select the optimal covariance structure, model simplicity and likelihood ratio tests were conducted. Main outcomes included subjective ratings of stress, anxiety, sedation, and energy. Time (pre-stress, post-stress, anticipation, post-recovery) was a fixed repeated factor, and expectancy condition (Told CBD, Told CBD-free) was a fixed factor. Baseline subjective ratings (baseline) served as time-varying covariates to control for individual variability across participants. To control for familywise type 1 error, the Benjamini–Hochberg procedure was conducted with the false detection rate (FDR) set at 0.05; when $p < 0.05$ but $FDR > 0.05$, findings were deemed potential false positives.

2.3 RESULTS

2.3.1 Demographics

Thirty-eight participants were recruited for this investigation, of which one male participant randomized to the Told CBD condition opted to have their data removed following

debriefing, and one female randomized to the Told CBD-free condition was excluded due to an anxiety-related reaction during baseline MRI scanning procedures leading to excessive head motion artifact (i.e., > 30% total censored volumes), resulting in a sample of N=36 (n=18 Told CBD, n=18 Told CBD-free). Finally, four additional participants (3 told CBD; 1 told CBD-free) were excluded from group-level rsFC analyses due to excessive head movement (i.e., > 30% total censored volumes) in either baseline or post-stress scans, resulting in a final sample of N=32 (M_{age}=23.7 years, SD=8.3, $Range$=19-56; n=15 Told CBD, n=17 Told CBD-free). Participants excluded for head motion artifact (n=5) were all female, which significantly differed from the sex distribution of included participants X^2 (1, N = 37) = 4.91, p = 0.027). Also, excluded participants had significantly greater scores on the STAI-T (p=0.003) and the PSS (p=0.011) relative to included participants. Moreover, excluded participants reported using significantly less cannabis per month (p=0.004) compared to included participants. Between-group differences in participant characteristics of included participants and participants excluded for head motion artifact are presented in Table 1. Given that the present sample was intended to be non-clinical, and panic attacks are a phenomenon described in the *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; DSM–5; American Psychiatric Association, 2013), differences in baseline characteristics amongst included participants and participants excluded for head motion were also sought after exclusion of n=1 female participant that had an anxiety-related reaction (apparent panic attack) in the scanner. Findings remained significant for trait anxiety t(34) = 2.58, p = .014 (MD=11.78, SE=4.57) and perceived stress t(34) = 2.38, p = .023 (MD=7.47, SE=3.13), but not monthly cannabis use t(34) = -1.12, p = .273 (MD=-4.44, SE=3.98), after exclusion of the participant with the anxiety-related reaction in the scanner. Of the N=32 participants selected for inclusion, 46.9% were assigned female sex at birth (n = 15),

whereas 53.1% were assigned male sex at birth ($n = 17$), and all were cisgender. An average of 7% of total volumes per run per participant (M=36.06, SD=36.89, $Range$=0-148) were censored as motion outliers amongst the final sample of included participants. Participant characteristics are further presented in Table 2. Two participants in this sample (5.3%) reported concurrent (i.e., past-month) nicotine use during eligibility screening, with N=1 concurrent nicotine user randomly assigned to each of the two expectancy conditions. Both concurrent nicotine users received a total score of 0 on the Fagerstrom Test for Nicotine Dependence (Heatherton et al., 1991), which is indicative of non-dependent use and would not be sufficient to induce significant withdrawal symptoms following 12-hours of nicotine abstinence. There were no significant differences between expectancy groups (Told CBD vs. Told CBD-free) on any participant characteristics measured which may systematically bias results. The CBD manipulation was also deemed successful, as all participants reported believing the CBD content instructions they received during their experimental session.

2.3.2 Seed-Voxel Results

There were no voxel clusters which significantly correlated with the regions of interest following voxel-wise control of the FDR and cluster-wise significance based on the cluster-defining threshold of p=0.05 between groups (Told CBD vs. Told CBD-Free). Given that this study was largely underpowered to detect subtle effects based on the *a priori* power analysis for Perry et al. (2024, under review), exploratory analyses of clusters meeting the cluster-wise significance threshold prior to correction for multiple comparisons was conducted. Significant clusters meeting a Z threshold of 3.1; with a minimum cluster threshold of 20 voxels were reported. Individual regions associated with peak voxel coordinates were defined with a combination of the Harvard-Oxford cortical and sub-cortical atlases (Desikan et al., 2006;

Frazier et al., 2005; Goldstein et al., 2007; Makris et al., 2006) and the Probabilistic Cerebellar

Atlas (Diedrichsen et al., 2009). Cortical intrinsic connectivity networks associated with cluster

location were identified with the Schaefer Atlas, a parcellation of the human cerebral cortex

derived from a gradient-weighted Markov Random Field model using adult task-fMRI and rs-

fMRI data (Schaefer et al., 2018). Uncorrected findings should be interpreted with caution due to

a much-increased risk of false positives. To minimize the potential influence of noise, clusters

with peak voxel coordinates with greater than 50% probability of being located in white matter

or ventricles were excluded as white matter and cerebral spinal fluid were deliberately regressed

out of analyses as confounding variables.

Seed-voxel analyses of uncorrected correlation maps revealed altered intrinsic

connectivity network FC between expectancy conditions. In the Told CBD condition, the DMN

right PCC seed revealed increased FC with CEN clusters in the right angular gyrus, with the

DMN-associated right PCC, and other regions within visual and somatomotor networks, as well

as cerebellar regions. By contrast, the DMN seed in the Told CBD-free condition did not

demonstrate alterations in triple-network activity, but rather in other networks, as the PCC seed

was correlated with regions in the visual, dorsal attention, and somatomotor regions. The SN

right dACC seed in the Told CBD condition demonstrated increased FC with regions located in

the somatomotor, visual, and dorsal attention networks. This region was also demonstrated to be

functionally connected to various subcortical parcellations in this condition, such as the right

hippocampus, right amygdala, and the left cerebellum. Alternatively, in the Told CBD-free

group, altered FC of the triple network with the SN seed was exhibited, as evidenced by

increased connectivity with clusters in the CEN (i.e., left temporooccipital and posterior middle

temporal gyrus) and DMN (i.e., right anterior, inferior, and posterior middle temporal gyrus,

right superior frontal gyrus). The SN was also shown to have increased FC with regions in the limbic and dorsal attention networks, as well as a cluster in the cerebellum. The CEN right dlPFC seed in the Told CBD condition exhibited increased FC with clusters in the DMN (i.e., right temporal pole, left anterior middle temporal gyrus, left posterior middle temporal gyrus), and a cluster in the CEN (i.e., left frontal orbital cortex, left insular cortex), as well as a region in the dorsal attention network. This seed also correlated strongly with subcortical regions such as the left caudate, a cluster in the cerebellum, the brainstem, and the thalamus. However, in the Told CBD-free condition, the CEN seed only correlated strongly with clusters in the somatomotor network and the cerebellum. For a detailed list of brain regions and their associated networks found to be functionally connected to the ROIs, view Table 3. Uncorrected Z-maps of results are presented in Figure 4.

2.3.3 Subjective Effects

Marginal linear models were utilized to assess the effects of Time, Expectancy, and Time by Expectancy effects for subjective stress, anxiety, sedation, and energy. Estimated marginal means and standard errors for stress and anxiety are presented in Figure 5.

A significant main effect of time was identified for stress ($F(4,28)=26.27$; $p<0.001$), anxiety ($F(4,28)=13.80$; $p<0.001$), and energy ($F(4,28)=3.05$; $p<0.001$), as well as a trend-level main effect of time for sedation ($F(4,28)=2.42$; $p=0.070$). Notably, subjective stress and anxiety increased significantly immediately following the stressor (post-stressor), followed by a significant decrease during anticipation and again at recovery (see Figure 5).

There were no significant main effects of Expectancy or Expectancy by Time interactions for any of the subjective outcomes. However, further breakdown of *a priori* planned pairwise

comparisons within each expectancy condition showed a significant decrease in subjective stress (MD=-0.83, p=0.017) and anxiety (MD=-0.70, p=0.021) from the baseline scan to post-oil in the Told CBD condition, but not the Told CBD-free condition (stress: MD=-0.44, p=0.16; anxiety: MD=0.47, p=0.09). Further, a significant decrease in subjective ratings of stress (MD=-0.60, p=0.024) and anxiety (MD=-0.60, p=0.017) was also observed from anticipation to recovery in the Told CBD condition. These findings were not observed in the Told CBD-free condition for stress (MD=0.235, p=0.329; see Figure 5); however, a significant decrease in anxiety was observed (MD=-0.50, p=0.033), though the FDR exceeded 5% (adjusted p=0.068). *A priori* planned analysis of pairwise comparisons for both sedation and energy revealed no significant differences between timepoints within either expectancy condition that passed the FDR of 5%. Raw means and standard errors for all subjective outcomes can be found in Table 4.

2.4 DISCUSSION

The current investigation aimed to examine the degree to which CBD expectancy (vs. CBD-free expectancy) may influence rsFC within and between the DMN, SN, and CEN, and its effect on subjective stress and anxiety following an acute psychosocial stressor in healthy adults. Results indicated no significant differences between groups (Told CBD vs. Told CBD-free) in voxel clusters that correlated with the regions of interest after voxel-wise control of the FDR. However, seed-voxel analyses of uncorrected correlation maps revealed altered intrinsic connectivity network rsFC between expectancy conditions. For instance, CBD expectancy was associated with increased rsFC within and between the DMN and CEN, whereas the SN demonstrated increased rsFC with both the CEN and DMN in the CBD-free expectancy condition. In relation to subjective stress, anxiety, energy, and sedation, we observed a significant main effect of time. However, we did not find any statistically significant interactions

involving expectancy condition and time, nor did we identify a main effect of expectancy condition. *A priori* planned pairwise comparisons revealed effects suggestive of CBD expectancy effects in reducing anxiety and stress following oil administration and from stress anticipation to stressor recovery. This study is the first of its kind to examine the impact of CBD expectancy on neural networks. The findings contribute to a growing body of literature examining placebo-related effects and highlight the need for further investigation of the neural mechanisms underlying expectancy-driven alterations in intrinsic connectivity networks.

This study did not identify significant differences in rsFC between participants expecting CBD versus those expecting a CBD-free product following stringent voxel-wise FDR correction. The current investigation was likely underpowered to detect subtle network-based effects relevant to CBD expectancy as an *a priori* power analysis was not conducted for this secondary analysis. Though this study was powered to detect large subjective effects according to a pilot experiment from our group (Zhekova et al., 2024), studies examining rsFC likely require very large sample sizes to detect nuanced effects (Marek et al., 2022). Notably, several participants (*n*=5) were excluded from analyses for head motion artifact. Even small movements can introduce noise in rsFC data as head motion may disrupt the signal from primary and neighboring voxels (Power et al., 2011). Therefore, it is necessary to exclude participants with excessive head motion artifact following motion correction to ensure that movement is not accounting for variability in the data, which may result in false-positive conclusions. However, it has been suggested that exclusion of participants for head motion may be associated with sampling bias (Wylie et al., 2012). Even the anticipation of the fMRI environment may be a stressful experience for healthy adults, potentially impacting their behavior during the scan (Weldon et al., 2015). Participants excluded for head motion were found to have significantly

increased trait anxiety and perceived stress, but lower reported monthly cannabis use, relative to participants included in the final sample. After exclusion of a participant who had an anxiety-related reaction (apparent panic attack) in the scanner resulting in excessive head motion artifact, the remaining excluded participants still demonstrated significantly increased levels of trait anxiety and perceived stress relative to included participants. Thus, my final sample may not be generalizable to a subsection of the population with elevated baseline stress and anxiety levels. Moreover, the hypothesized placebo effects of CBD on neural networks may have been reduced via exclusion of the very participants who may benefit most from CBDs anxiolytic effects (i.e., those with increased levels of stress and anxiety at baseline) and who may therefore be most susceptible to CBD placebo effects, potentially contributing to the null rsFC results following FDR correction.

Most placebo effects are subtle, but large placebo effects likely require both conceptual belief in expectancy-related factors, and personal experiences benefitting from the treatment (Wager & Atlas, 2015). Accordingly, a previous study from our group suggested that CBD expectancy was related to anxiolytic effects particularly in participants who endorsed strong *a priori* beliefs that CBD has anxiolytic properties (Spinella et al., 2021). However, though we collected data regarding CBD-related beliefs for descriptive purposes, the current investigation was underpowered to test any potential moderating effects of these *a priori* beliefs. Moreover, the strict statistical threshold utilized in seed-voxel analyses may have limited our ability to detect meaningful network-based effects. This constraint potentially impacted our ability to reproduce our preliminary analyses using a seed-ROI approach which revealed significantly blunted dACC-amygdala rsFC in the Told CBD condition (vs. Told CBD-free) following acute stress (Perry et al., 2024, under review). Further, the dACC seed selected for the current study

differed from that of Perry et al. (2024, under review) to align with reported coordinates from studies investigating the salience network (e.g., Corr et al., 2022), so the ROI may not have been placed in the locale of peak activation.

Though results should be interpreted with extreme caution due to increased risk of Type 1 error, between-group differences in intrinsic connectivity network rsFC were revealed in less stringent analyses prior to correction for multiple comparisons. Notably, CBD expectancy was associated with increased rsFC within and between the DMN and CEN, whereas the Told CBD-free condition demonstrated increased rsFC between the SN and CEN, and consistent with our hypothesis, between the SN and DMN. Though stress has been associated with increases in functional connectivity within the DMN (van Oort et al., 2017), our study identified increases in FC within the DMN in the Told CBD condition, more specifically, within the PCC. Notably, increased activity within the PCC has been associated with placebo anxiolysis in patients with social anxiety disorder (Huneke et al., 2022). For instance, in a study by Faria et al. (2017), 24 patients with social anxiety disorder were randomized to receive an SSRI with accurate information regarding their treatment arm, whereas 22 patients were randomized to be misled that they were receiving an SSRI, though they received placebo. Both groups improved equally in terms of anticipatory speech anxiety, and reduced social anxiety was associated with BOLD reactivity in the PCC (Faria et al., 2012). Moreover, Meyer and colleagues (2019) induced anxiolytic expectancy effects by providing participants with fictitious brochures describing the effects of laughing gas as anxiolytic and relaxant, but no actual laughing gas was administered. Following laughing gas placebo, reductions in anxiety were predicted by increased FC within the DMN (Meyer et al., 2019). Given the DMN's role in episodic memory and self-referential mental processing, it may be a key network involved in placebo-related anxiolytic effects

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(Menon, 2011). Increases in within-network rsFC in the CEN in the Told CBD condition may be indicative of stressor recovery. During acute stress, the CEN is typically suppressed; however, it has been suggested that during stressor recovery, higher-order cognitive functioning is reinstated to support cognitive flexibility (Hermans et al., 2014). Accordingly, these findings align with the pattern of subjective reports of stress and anxiety in our sample. For instance, participants in the Told CBD condition reported significantly decreased stress and anxiety from post-anticipation to recovery relative to participants in the Told CBD-free condition. Typically, as mediated by the SN, the DMN and CEN generally exhibit opposing activation patterns (Hermans et al., 2014). Therefore, literature reporting increases in connectivity between the CEN and DMN is limited. However, we suspect that this may be indicative of internally focused attention. Therefore, it is possible that DMN-CEN coupling may be particularly useful for placebo-related expectation and appraisal, as placebo effects may be influenced by the evaluation of their current experience relative to a different reference point in memory (e.g., a memory of an anxiolytic effect following CBD administration, or having heard from a friend that CBD reduces anxiety) (Wager & Atlas, 2015).

As expected, increased FC between the SN and DMN was observed in the Told CBD-free group relative to the Told CBD condition. Findings are consistent with most literature examining alterations in intrinsic connectivity network FC following acute stress (van Oort et al., 2017). Indeed, FC of the DMN with the SN may be reflective of the psychosocial nature of the experimental stress task (i.e., the adapted TSST), resulting in self-referential mental activity and potentially rumination associated with negative feedback. Indeed, increases in activity within the DMN and SN have been identified in studies examining personalized script-driven stressful imagery (Seo et al., 2013; Sinha et al., 2004). Since this connectivity was observed in the Told

CBD-free group but not in the Told CBD group, CBD expectancy may have attenuated this process in our sample, potentially reducing stressor-related rumination. To our knowledge, only one other study specifically inferred network FC alterations following CBD administration. Wall and colleagues (2022) identified reductions in DMN and SN FC in 17 healthy volunteers who were administered cannabis containing both THC and CBD relative to placebo. Thus, expectancy effects in our study may account for similar network-based effects as CBD's pharmacological actions (Wall et al., 2019). Moreover, increased FC was observed between the SN and CEN in the Told CBD-free condition in our study. This finding may potentially reflect the SN redirecting cognitive resources to the CEN due to the computational nature of the stress task (i.e., serial subtraction), as increases in FC between these networks have been observed following cognitively demanding acute stress tasks (van Oort et al., 2017). Indeed, SN-CEN coupling is believed to occur optimally at moderate levels of arousal (Young et al., 2017). Therefore, these findings raise the possibility that CBD expectancy may mitigate the FC of neural substrates associated with stress- and anxiety-related processing.

While our task was found to significantly increase subjective stress and anxiety across conditions, there were no identified interactions involving expectancy condition and time in the omnibus analyses. However, further probing of planned pairwise comparisons revealed that participants in the CBD expectancy condition reported significantly reduced stress and anxiety from post-anticipation to recovery. Moreover, in the Told CBD condition only, participants reported decreased stress and anxiety from the baseline scan to post-oil administration. These findings are consistent with our previous work (Spinella et al., 2021; Zhekova et al., 2024); however, CBD expectancy in our sample appeared to be most effective in dampening stress anticipation or facilitating stressor recovery as opposed to diminishing the impact of the

magnitude of the stress task on the initial stress response. Given that the scanner environment may serve as a potential stressor (Madl et al., 2022; Muehlhan et al., 2011), this may have obscured subjective stress and anxiety assessment during the anticipation period itself resulting in potential ceiling effects. However, an impact of CBD expectancy was revealed during the recovery period outside the scanner. This study did not consider whether participants had previous MRI scans, which could have influenced their anxiety levels during the scan and potentially confounded the results. Though our study controlled for baseline levels of anxiety, future studies should consider controlling for history of previous MRI scanning. Further, in contrast to our hypothesis, no significant differences in subjective sedation across timepoints were identified within either condition that passed the FDR of 5%. Oral CBD administration has been reported to produce sedative effects (Zuardi et al., 1993). Consistent with these findings, our previous work demonstrated that CBD expectancy was associated with increased levels of subjective sedation (Spinella et al., 2021; Zhekova et al., 2024). However, given that these studies did not incorporate a neuroimaging component, it is likely that the potentially anxiogenic scanner environment may have obscured the anticipated sedative effects of CBD expectancy in our sample (Muehlhan et al., 2011). Further research is needed to delineate the impact of CBD's pharmacological and non-pharmacological factors on stress anticipation and recovery.

The existing literature exploring the impact of CBD on stress and anxiety is mixed. Due to heterogeneity in methodological procedures across studies, the reasons behind these divergent outcomes remain unclear. In fact, double-blind trials have yielded both positive (Bergamaschi, Queiroz, Zuardi, et al., 2011; Masataka, 2019) and null results (Bolsoni et al., 2022; Gournay et al., 2023; Kwee, Baas, et al., 2022) for CBD relative to placebo. Participants in placebo-controlled trials will often make guesses about their assigned condition, which may influence

subjective effects (Dar & Barrett, 2014). Given that perceived drug assignment is often not determined in these trials, the role of strong placebo effects being obscured in negative trials, or contributing to reductions in stress and anxiety in positive trials remains unclear. Accordingly, our results suggest a subtle effect of CBD expectancy on subjective stress and anxiety; however, contrary to our preliminary analyses suggesting CBD expectancy effects on neural responses (Perry et al., 2024, under review), these alterations in stress and anxiety appraisal may be too subtle to detect at a network level.

The current investigation's findings should be viewed in the context of the following methodological considerations. As mentioned previously, this study was powered to detect large effects based on our previous out-of-scanner work (Spinella et al., 2021; Zhekova et al., 2023), and was likely underpowered to detect subtle neural effects or to examine potential moderators like *a priori* expectancies. Lack of power may also be reflective of the study design, given that the current study used a between-subjects analysis for expectancy condition; within-subjects designs are much more powerful. Moreover, participants were primarily healthy young adults of European descent. In the United States, cannabis use rates vary across racial and ethnic groups, with the highest rates reported amongst American Indian/Alaska Native individuals and African American/Blacks (Montgomery et al., 2022). It is possible that racial/ethnic differences in sensitivity to placebo effects may contribute to variability in patterns of cannabis use. However, the present study could not assess differences in placebo responses across racial and ethnic groups due to the predominantly European-descent sample. Future studies should consider replicating these findings in larger, more diverse samples. Next, due to the verbal nature of the counting task, we were unable to take task-based measurements of acute stress due to potential head motion artifact. Therefore, we were unable to disentangle post-stress vs. anticipatory stress

effects. Given that post-stress task rsFC measurements were taken during an anticipatory stress phase in hopes of prolonging post-stress effects on neural activity, this may not be considered a true 'resting state' task due to instructed changes in mental state, and because it immediately followed an experimental manipulation (Cole et al., 2010). Future work may consider implementing a stress task better suited to MRI, such as the Montreal Imaging Stress Task, which was developed to induce stress and measure associated neural effects while overcoming the restraints in the imaging environment imposed by common stress induction protocols (e.g., TSST) (Dedovic et al., 2005). Further, our study utilized a seed-voxel approach to analyze rsFC data. Though seed regions were selected based on previous research examining stress, anxiety, and CBD effects on neural activity (Corr et al., 2022; Lorenzetti, McTavish, et al., 2023; Wall et al., 2019, 2022), and were cross-referenced with automated meta-analytic data at http://neurosynth.org/, this method relies heavily on seed selection which may introduce bias (Lv et al., 2018). Indeed, the interpretation of a spatial map generated from a single seed region as a network may disregard the richness of the data as selection of a seed region may bias connectivity findings towards specific, often smaller or overlapping subsystems, rather than capturing larger, distinct networks (Cole et al., 2010). Moreover, we are unable to infer directionality or causality with the current analytic approach. To further delineate the dynamic relationships between specific brain regions in intrinsic connectivity networks, lag-based methods may be employed, such as the Granger Causality Analysis (Goebel et al., 2003). Other potential analyses which may be utilized to better capture intrinsic connectivity networks include independent component analysis and graph theory (Lv et al., 2018). These methods are largely data-driven, reducing potential bias in seed selection, and they can model whole-brain interactions by detecting multiple intrinsic connectivity networks simultaneously (Lv et al.,

2018). Additionally, several scales utilized in this study may not be valid and reliable for the population of interest. For instance, the STAI-T and the PSS have not been validated in substance use populations. Also, the measures of subjective state and CBD beliefs are experimenter-compiled and utilize single-item measures from a range of valid scales. Though these scales were piloted by our group and found to be associated with physiological measures of stress and anxiety (Zhekova et al., 2024), the psychometrics of these assessments have not yet been determined. Finally, this study did not employ a full balanced placebo design, but rather a half-balanced placebo design, thereby limiting our ability to distinguish the individual and potentially interacting effects of CBD expectancy and pharmacology.

Our study is the first to examine the impact of CBD expectancy on neural networks in the context of stress and anxiety, and uncorrected exploratory analyses revealed a potential biological mechanism underlying CBD placebo effects in healthy adults. Though the use of CBD has been associated with minimal adverse events (Bergamaschi, Queiroz, Zuardi, et al., 2011), it is not entirely risk-free (e.g., Gingrich et al., 2023), so it is important to understand the factors which motivate its use. The literature examining anxiety-related outcomes following CBD administration across species is mixed (Kwee, Baas, et al., 2022), suggesting a potentially important role for expectancy-related factors. Our work highlights the need for future studies examining the relative contributions of both CBD's pharmacological and non-pharmacological effects on stress and anxiety in the brain. This work may have several clinical implications, for instance, given the impact of expectancy-related effects on subjective outcomes, resources should be allocated towards public education regarding CBD's effects. Merely 70% of Canadians report having access to trustworthy information about the health risks of cannabis (Health Canada, 2023). Therefore, education regarding the relative risks and benefits may allow

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Canadians to make informed decisions regarding their CBD use, and to take advantage of potential expectancy-related factors on anxiety-related outcomes. Moreover, the present findings demonstrate that various open-label trials may be plagued by significant placebo effects, and this must be accounted for in the interpretation of drug effects.

2.5 CONCLUSION

Our findings provide novel insight into the potential biological mechanisms through which CBD expectancy may impact stress and anxiety. Consistent with our previous work (Spinella et al., 2021; Zhekova et al., 2024), CBD-related placebo effects may be sufficient to reduce stress and anxiety in healthy adults. Prior to correction for multiple comparisons, CBD expectancy was associated with increased rsFC between the DMN and CEN, whereas the Told CBD-free condition demonstrated increased rsFC between the SN and CEN, and consistent with our hypothesis, the SN and DMN. However, CBD expectancy was not found to independently alter FC of intrinsic connectivity networks following correction for multiple comparisons. Future research may benefit from evaluating the main effects and interactions of CBD expectancy and pharmacology on neural networks in larger samples, and in individuals with stress- and anxiety-related disorders. Though CBD is believed to be a promising candidate treatment for stress and anxiety-related disorders, the literature examining its effects remains mixed. Our work highlights the need for future adequately powered studies examining the relative contributions of both CBD's pharmacological and non-pharmacological effects on stress and anxiety in the brain.

CHAPTER 3. GENERAL DISCUSSION

My Masters' thesis examined the degree to which CBD expectancy (vs. CBD-Free expectancy) may influence rsFC within and between the DMN, SN, and CEN, and participants' subjective experiences in response to an acute psychosocial stressor in healthy adults. My study is novel in its approach to explore the impact of CBD expectancy on neural networks in the context of stress and anxiety. The current findings contribute to a growing body of literature examining placebo-related effects, highlighting the need for further investigation of the neural mechanisms underlying expectancy-driven alterations in neural substrates associated with stress and anxiety. Moreover, the results suggest that open-label studies examining the potential medicinal effects of CBD on stress and anxiety may be plagued by significant placebo effects which must be considered in the interpretation of the findings.

The results of my thesis partially supported my hypotheses. Seed-voxel analyses of FC correlation maps, prior to correction for multiple comparisons, revealed altered intrinsic connectivity network rsFC between expectancy conditions. However, no significant differences between groups (Told CBD vs. Told CBD-free) in voxel clusters that correlated with the ROIs were identified following stringent voxel-wise FDR correction. In relation to subjective stress, anxiety, and sedation, contrary to our hypothesis, we did not find any statistically significant interactions involving expectancy condition and time, nor did we identify a main effect of expectancy condition. However, we observed a significant main effect of time for subjective stress, anxiety, and energy, and *a priori* planned pairwise comparisons revealed effects suggestive of a potential role for CBD expectancy in reducing anxiety and stress following oil administration and from stress anticipation to stressor recovery. Contrary to our hypothesis, no mean differences in subjective sedation were revealed.

3.1 ALTERATIONS IN INTRINSIC CONNECTIVITY NETWORK FC

Consistent with our previous findings (Perry et al., 2024, under review), and studies examining CBD's effects on the brain (Lorenzetti, McTavish, et al., 2023), I anticipated increased FC within and between the SN and DMN in the Told CBD-free condition, which would be attenuated in the Told CBD condition. There were no anticipated changes expected within the CEN. Contrary to my hypothesis, this study did not identify significant differences in rsFC between participants expecting CBD versus those expecting a CBD-free product following stringent voxel-wise FDR correction. As described in Chapter 2, this study was powered to detect large effects according to a pilot out-of-scanner experiment from our group (Zhekova et al., 2024). However, this study was likely underpowered to detect subtle network-based effects relevant to CBD expectancy. Indeed, studies examining rsFC likely require very large sample sizes to detect nuanced effects (Marek et al., 2022), particularly with regards to placebo effects. Most placebo effects are subtle, but large placebo effects likely require both conceptual belief in expectancy-related factors, and personal experiences in benefitting from the treatment (Wager & Atlas, 2015). Indeed, a previous study from our group suggested that CBD expectancy was related to anxiolytic effects, particularly in participants who endorsed strong *a priori* beliefs that CBD has anxiolytic properties (Spinella et al., 2021). However, though we collected data regarding CBD-related beliefs for descriptive purposes, the current investigation was underpowered to detect any potential moderating effects of these *a priori* beliefs. This inability to examine individual difference moderators could have potentially washed out any more subtle neural effects of expectancy condition. Additionally, the pharmacological effects of CBD on neural outcomes may already be very subtle. In fact, empirical evidence supporting CBD's stress- and anxiety-relieving effects is mixed, with many studies reporting null effects (Kwee,

van Gerven, et al., 2022). For instance, Bloomfield et al. (2022) found that 600 mg of oral CBD failed to alter anxiety-related emotional processing, subjective stress, or related neural responses in 24 healthy adults. Though their sample size was deemed sufficient to detect subjective effects of CBD given their study design, sample size estimates for imaging-related analyses were not analyzed via *a priori* power analysis, and oral administration of CBD may have very low bioavailability, potentially contributing to null results (Bloomfield et al., 2022). Thus, the potential neural effects of CBD might be very small or require more sensitive measures or statistical power to detect. Drug effects in humans may arise from a combination of both expectancy and drug-related factors (Kirsch, 2018). Given that the observed overall drug effects of CBD may be small, the anticipated expectancy effects analyzed in this thesis may be even smaller. Moreover, the strict statistical threshold utilized in seed-voxel analyses may have limited our ability to detect meaningful network-based effects. This constraint potentially impacted our ability to reproduce our preliminary analyses using a seed-ROI approach which revealed significantly blunted dACC-amygdala rsFC in the Told CBD condition (vs. Told CBD-free) following acute stress (Perry et al., 2024, under review). Further, the dACC seed selected for the current study (10, 24, 28) differed slightly from that of Perry et al. (2024, under review) (7.35, 19.98, 29.06) to align with reported coordinates from studies investigating the SN. Thus, the ROI may not have been placed in the locale of peak activation. To improve the reliability of MRI findings, future studies may consider employing a within-subject design to reduce potentially confounding between-subject variability when employing the seed-voxel analysis method. Moreover, we may consider repeating the current analysis with the same seed derived in Perry et al. (2024, under review) in a future study to replicate these findings.

Though results should be interpreted with extreme caution due to increased risk of type-1 error, as described in Chapter 2, between-expectancy-group differences in intrinsic connectivity network rsFC were revealed prior to correction for multiple comparisons. Notably, the Told CBD-free condition demonstrated increased rsFC between the SN and CEN, and consistent with our hypothesis, the SN and DMN. In contrast, CBD expectancy was associated with increased rsFC within and between the DMN and CEN. Notably, our study identified increased FC within the PCC of the DMN in the Told CBD condition. Several studies have identified that increased activity within regions of the DMN, including the PCC, has been associated with placebo-related anxiolytic effects in patients with social anxiety disorder (Huneke et al., 2022). The DMN's involvement in episodic memory and self-referential processing suggests it plays a crucial role in mediating placebo-related anxiolytic effects (Menon et al., 2011). However, the role of intrinsic connectivity networks in placebo effects is not widely studied (Meyer et al., 2019), and the DMN has several critical functions (Menon et al., 2011); thus its role in expectancy-related effects remains speculative. Future neuroimaging studies are necessary to better understand the role of the DMN in emotion processing and placebo effects. Additionally, we suspect that the observed increased within-network CEN FC in the Told CBD condition may be reflective of enhanced recovery from stress. Evidence suggests that the CEN is typically suppressed during acute stress; however, neural resources are reallocated towards the CEN during recovery from the stressor to restore cognitive functioning and support cognitive flexibility (Hermans et al., 2014). Accordingly, these findings align with subjective reports of stress and anxiety in our sample. Indeed, participants in the Told CBD condition reported significantly decreased stress and anxiety from post-anticipation to recovery relative to participants in the Told CBD-free

condition. Therefore, stressor recovery in our sample may be facilitated by the observed increased within-network connectivity in the CEN in the CBD expectancy condition.

In contrast to the broad literature examining stress effects on intrinsic connectivity networks, our study also identified increased FC between the DMN and the CEN associated with CBD expectancy. Typically, as mediated by the SN, the DMN and CEN generally exhibit opposing activation patterns (Hermans et al., 2014). Therefore, literature reporting increases in connectivity between the CEN and DMN are limited. However, we suspect that this may be indicative of internally focused attention. Placebo effects may be influenced by a variety of contextual factors, such as learned associations between cues and previous positive or negative experiences with the substance (Wager & Atlas, 2015). Placebo-related expectations are therefore often influenced by the evaluation of their current experience relative to a reference point in memory involving the substance (e.g., being told by a friend that CBD reduces anxiety) (Wager & Atlas, 2015). Indeed, the CEN is believed to be responsible for mediating attention, including the maintenance and manipulation of information in working memory; in contrast, the DMN is often associated with memory retrieval (Menon, 2011). Thus, we suspect that DMN-CEN coupling may be particularly useful for placebo-related expectation and appraisal through the retrieval of stimulus-relevant information from memory via the DMN, and the examination of one's current experience with respect to those memories using the CEN. However, the CEN is one of the least widely investigated intrinsic connectivity networks (Menon, 2011), and findings with respect to its role in CBD's pharmacological effects are limited. Notably, both decreases (Vaisvaser et al., 2016) and increases (Corr et al., 2022) in FC between regions of the DMN and CEN during a mental arithmetic task have been observed in healthy participants. Therefore, though it is possible that CBD expectancy may be associated with increased FC between the

DMN and CEN, the literature regarding the role of these networks in stressor-related processing and placebo effects remains mixed and conclusions are limited.

As hypothesized, in the Told CBD-free condition, increased FC was observed between the SN and DMN. Findings are consistent with most literature examining alterations in intrinsic connectivity network FC following acute stress (van Oort et al., 2017). Indeed, FC of the DMN with the SN may be reflective of the psychosocial nature of the experimental task. Specifically, participants were informed that their performance would be compared to other study participants, and participants were provided with scripted negative feedback throughout the task if mistakes were made or if participants slowed down and/or stopped counting. This psychosocial aspect of the stressor task could potentially have resulted in self-referential mental activity and rumination associated with the negative feedback. Indeed, increases in activity within the DMN and SN have been identified in studies examining personalized script-driven stressful imagery (Seo et al., 2013; Sinha et al., 2004). Therefore, given that such findings were only identified in the CBD free condition, CBD expectancy may have attenuated this process in our sample, potentially reducing stressor-related rumination. To our knowledge, only one other study specifically inferred network FC alterations following CBD administration. Wall and colleagues (2019) identified reductions in DMN and SN within-network FC in 17 healthy volunteers who were administered cannabis containing both THC and CBD relative to placebo. Though this study may not be directly comparable to the present study as the latter was not examined in the context of stress, between-network FC was not inferred, and they administered cannabis containing both THC and CBD, expectancy effects in our study may account for similar network-based effects as CBD's drug effects.

Though no hypothesized changes in CEN FC were anticipated, we identified increased FC between the SN and CEN in the told CBD-free condition in our study. This finding may potentially reflect the SN redirecting cognitive resources to the CEN due to the computational nature of the stress task, as increases in FC between these networks have been observed following cognitively demanding acute stress tasks (van Oort et al., 2017). Indeed, SN-CEN coupling is believed to occur optimally at moderate levels of arousal (Young et al., 2017). Given this increased functional connectivity was only observed in the Told CBD-free condition and not in the CBD expectancy condition, these findings raise the possibility that CBD expectancy may mitigate the FC of neural substrates associated with stress- and anxiety-related processing of computational stimuli specifically or cognitively demanding stressors generally. However, due to the verbal nature of the stress task, we are unable to disentangle post-stress vs. anticipatory stress effects. It is therefore possible that increased FC between the SN and CEN in the Told CBD-free condition may represent the process of the SN reallocating cognitive resources to the CEN to facilitate stressor recovery. In contrast, the observed increased CEN-CEN connectivity in the Told-CBD condition may indicate that the recovery process has occurred at a faster rate due to the expectancy of CBD effects.

3.2 SUBJECTIVE EFFECTS

In terms of the subjective effects of CBD expectancy, consistent with our previous out-of-scanner work (Spinella et al., 2021; Zhekova et al., 2024), I anticipated there to be decreased stress, anxiety, and sedation post-stressor in those assigned to the Told CBD condition relative to the Told CBD-free condition. As reported in Chapter 2, there were no identified interactions involving expectancy condition and time, nor were there any main effects of expectancy condition in the omnibus analyses. It may be that CBD placebo effects are particularly effective

in reducing stress and anxiety for socially evaluative stressors as opposed to cognitive stressors. Indeed, most of the literature identifying positive impacts of CBD on stress and anxiety examined socially evaluative tasks (Bergamaschi, Queiroz, Chagas, et al., 2011; Linares et al., 2018; Zuardi et al., 2017). Though our task involved negative feedback, and participants were informed that their performance would be compared to their peers, this task lacked the public speaking component of the classic TSST (Kirschbaum et al., 2008). Therefore, our adapted TSST could be considered more computationally than psychosocially stressful; thus, CBD's impacts on stress and anxiety may not have been as heavily involved. However, notably, a main effect of time was identified. We aimed to identify temporal trends within groups to understand the impact of CBD expectancy at various stages of the experiment. Specifically, we were interested in its impacts immediately following the stressor relative to other timepoints, and to ensure successful stressor manipulation. Thus, we planned *a priori* pairwise comparisons across timepoints within expectancy conditions. These pairwise comparisons revealed increased stress and anxiety across both conditions following the stressor relative to all other time points, suggesting that the computational task effectively increased stress and anxiety (i.e., an effective stress manipulation). However, partially consistent with my hypothesis, decreased stress and anxiety were observed following oil administration, and between anticipation and recovery, only in the Told CBD group but not in the Told CBD-free group. CBD expectancy in our sample appeared to be most effective in dampening stress anticipation or facilitating stressor recovery as opposed to diminishing the impact of the magnitude of the stress task on the initial stress response. Given that the scanner environment may serve as a potential stressor (Madl et al., 2022; Muehlhan et al., 2011), this may have obscured subjective stress and anxiety assessment during the anticipation period itself, resulting in a lack of differences between expectancy

conditions at this timepoint (i.e., due to potential ceiling effects). Notably, MRI naïve individuals tend to have increased cortisol reactivity post-scan and report more anxiety in anticipation of the fMRI scan relative to participants having had a previous MRI (Tessner et al., 2006). This study did not account for differences in MRI scanning experience, which may have confounded results. Though we controlled baseline anxiety levels in our analyses in hopes of accounting for scanner-related anxiogenic effects, future studies should consider measuring and controlling for history of previous MRI scanning. Further, in contrast to our hypothesis, no significant differences in subjective sedation across timepoints were identified within either condition that passed the FDR of 5%. These findings differ from that of literature examining oral CBD administration (Zuardi et al., 1993), as well as our previous work which demonstrated that CBD expectancy was associated with increased levels of subjective sedation (Spinella et al., 2021; Zhekova et al., 2024). However, this study incorporated a neuroimaging component, which may have resulted in decreased sedation due to the anxiogenic effects of the MRI environment (Muehlhan et al., 2011). Further research is necessary to delineate the impact of CBD's pharmacological and non-pharmacological factors on various dimensions of stress anticipation and recovery.

3.3 LIMITATIONS

The methodological limitations briefly presented in Chapter 2 will be expanded upon in the following section. First, the current investigation was likely underpowered to detect subtle CBD-related placebo effects on the brain, or to examine potential moderators such as *a priori* expectancies. Indeed, this study was designed as a pilot and was powered to detect large effects based on our previous out-of-scanner work (Spinella et al., 2021; Zhekova et al., 2024). However, several participants ($n=5$) were excluded from analyses due to excessive head motion artifact in the scanner, significantly reducing statistical power. Head motion disrupts fMRI

signal, as it is dependent on correlations of BOLD signal over time. Thus, movement may disrupt BOLD signal in primary and neighboring voxels, resulting in a violation of the assumption that a signal corresponds to a given brain voxel across the time series (Power et al., 2012). For instance, head motion may have significant effects on intrinsic connectivity network functional connectivity, particularly the DMN, which could be mistaken for real neuronal effects (Van Dijk et al., 2012). Though motion correction and exclusion of participants for excessive head motion artifact is a necessary step in prepossessing of fMRI data to reduce the impact of movement on variability in the data, it has been suggested that this may be systematically inducing sampling bias (Nebel et al., 2022; Wylie et al., 2012). In the present sample, participants excluded for head motion artifact demonstrated increased baseline trait anxiety and perceived stress, but decreased reported monthly cannabis use, relative to participants included in the final sample. Given that even the anticipation of the fMRI environment may evoke psychological distress (Weldon et al., 2015), excluded participants may have been particularly vulnerable to the stress of the MRI environment, potentially resulting in excessive movement in the scanner. For instance, in a study involving youth, boys with higher negative affectivity were more likely to show excessive head movement in the scanner (Johnson et al., 2021). Excluding these participants from analyses reduces the likelihood of spurious findings, while raising the possibility of sampling bias. Thus, results may not generalize to more stressed/anxious females (who use less cannabis). Moreover, placebo effects in our study may have been minimized as participants with elevated trait anxiety and perceived stress may have benefitted most from CBD's anxiolytic properties and thus been most susceptible to CBD placebo effects. Alternatively, the contrary has also been identified, wherein elevated state and trait anxiety did not predict head motion during resting-state fMRI (rsfMRI) (Ekhtiari et al., 2019). Additionally, readers should interpret these findings with caution

as the small sample size in the excluded group relative to the included group may increase the risk of error and thus findings require replication. Nonetheless, more advanced statistical methods should be employed in future fMRI research to limit these potential biases, such as robust targeted minimum loss-based estimation (Nebel et al., 2022). Also, with repeated MRI sessions anxiety levels have been shown to decrease, suggesting that habituation to the MRI environment using an MRI simulator may reduce MRI-related fluctuations in anxiety (Chapman et al., 2010). Finally, it is possible that differences in head motion across the study participants is related to an imperfect fit of the head coil. Future studies should consider the use of subject-specific head molds that fit inside the MRI head-coil as they may significantly reduce head motion (Power et al., 2019).

Though our sample size (N=32) is consistent with that of several fMRI studies (e.g., N=25), recent literature has suggested that these sample sizes may not be sufficient to produce replicable findings and may be resulting in potentially inflated effect sizes (Marek et al., 2022). Indeed, in rsfMR analyses, a sample size of n=40 has been suggested to be acceptable for test-retest reliability (Ma et al., 2024). In neuroimaging studies, power analyses to determine appropriate sample sizes are extremely complex given the multiple comparisons of several voxels correlated in 3D space and the type of analysis employed. Thus, a traditional power analysis designed for single outcome variables may not be sufficient. Though several advances in the field have been made to conduct power analyses for neuroimaging purposes, it is still commonplace to base effect size estimates on pilot data (Mumford, 2012). Given that my thesis was a secondary analysis of archival data, an *a priori* power analysis (prior to data collection) was not possible for this specific analysis. Thus, we cannot be certain whether the observed null results following the seed-voxel analysis with FDR are due to a true lack of effect or to

insufficient power. However, our group has demonstrated convergence of endocrine, subjective, and neural findings regarding CBD expectancy effects, all in directions consistent with CBD's anxiolytic and stress-reducing effects (Perry et al., 2024, under review; Spinella, Burdeyny, et al., 2023; Spinella et al., 2021; Zhekova et al., 2023). This suggests that our findings are replicable and that the null MRI findings following FDR correction in our sample are most likely due to insufficient power. Future studies with larger sample sizes are required to replicate the uncorrected MRI findings and potentially extend beyond the current findings.

Next, the planned comparisons of mean differences in stress and anxiety across time and the uncorrected FC findings in the current investigation suggest that CBD expectancy may at least partially explain CBD's anxiolytic effects in healthy adults. Though CBD is being used increasingly by the general population for its perceived stress- and anxiety-relieving properties (Health Canada, 2022), CBD has also been demonstrated to effectively reduce clinically significant stress and anxiety in individuals with psychiatric conditions (e.g., PTSD, social anxiety) (Dammann et al., 2024). However, the impact of CBD expectancy on these outcomes in these clinical populations has yet to be tested. In the present study, participants with pre-existing psychiatric disorders were excluded, as evidence suggests that neurophysiological or psychological conditions may influence subjective and neural stress- and anxiety-related responses. Therefore, inclusion of individuals with psychiatric disorders could potentially have increased between-subject variability, resulting in reduced statistical power. Given that stress is a common experience among healthy adults (Stawski et al., 2013), and that among non-medical cannabis users, stress relief and relaxation are often cited as a primary reason for CBD use (Geppert et al., 2023), we believe that there is merit to studying CBD expectancy effects in healthy adults alone. However, CBD has been considered a potentially effective treatment for

psychiatric disorders, and future work should replicate and extend our findings to this population to examine the relative role of CBD's pharmacological and non-pharmacological effects on treatment outcomes for stress and anxiety-related disorders.

Further, there were several methodological constraints imposed by the MRI environment itself. Due to the verbal nature of the counting task, we were unable to take task-based measurements of acute stress due to potential head motion artifact. Therefore, we were unable to disentangle post-stress vs. anticipatory stress effects. However, the current analysis utilized rsFC, which is an appropriate technique for the analysis of intrinsic connectivity networks as these communities of brain structures are believed to be functionally connected at rest. Nevertheless, given that the rsFC measurements were taken during an anticipatory stress phase in hopes of prolonging post-stress effects on neural activity, this may not be considered a true 'resting state' task due to instructed changes in mental state, and because it immediately followed an experimental manipulation (Cole et al., 2010). Another stress task that may be better suited to the MRI environment is the Montreal Imaging Stress Task (Dedovic et al., 2005). This task was designed to provoke brain activation in response to stress in the MRI, while overcoming constraints associated with common stress induction protocols (e.g., speaking which results in head motion artifact) (Dedovic et al., 2005).

Additionally, our study utilized a seed-voxel approach to analyze rsFC data. Though seed regions were selected based on previous research examining stress, anxiety, and CBD effects on neural activity (Corr et al., 2022; Lorenzetti, McTavish, et al., 2023; Wall et al., 2019, 2022), and were cross-referenced with automated meta-analytic data at http://neurosynth.org/, this method relies heavily on seed selection which may introduce bias (Lv et al., 2018). Therefore, other potential seed ROIs may have been more representative of the analyzed intrinsic connectivity

networks. For instance, though the dACC is a core node of the SN, the anterior insula is also a crucial hub of this network (Menon, 2011) and may have been more robustly functionally connected to other brain regions within the networks of interest. Regardless, the interpretation of a spatial map generated from a single seed region as a network may disregard the richness of the data as selection of a seed region may bias connectivity findings towards specific, often smaller or overlapping subsystems, rather than capturing larger, distinct networks (Cole et al., 2010). Other potential analyses that may be utilized to better capture intrinsic connectivity networks include and Graph Theory (Lv et al., 2018). These methods are largely data-driven, reducing potential bias in seed selection, and they can model whole-brain interactions by detecting multiple intrinsic connectivity networks simultaneously (Lv et al., 2018). Specifically, Graph Theory is a method employed to analyze the topology of brain networks by organizing the brain as a complex network of nodes and edges (Wang et al., 2010). Graph theory provides insight into various network characteristics, such as small worldedness (i.e., high local clustering), degree distributions (i.e., the connectivity of a node with the rest of the nodes within a network), degree distribution, network efficiency, hierarchy, and node centrality among others (Wang et al., 2010). Moreover, Independent Component Analysis mathematically divides BOLD signal into several independent functional networks to form temporally correlated spatial maps, allowing for the detection of all networks within a participant (Lv et al., 2018). Though these proposed methods are considered computationally advanced, and while results may be difficult to interpret, they allow for a more holistic view of network connectivity (Lv et al., 2018). A final limitation in assessing rsFC is the lack of inference regarding causality or directionality. Using this approach, we may only speculate about the dynamic relationships between regions of interest (Cole et al., 2010). To assess directionality, lag-based methods may be employed, such as the Granger

Causality Analysis (Goebel et al., 2003). Thus, more advanced analysis methods should be considered in future research to better represent FC of intrinsic connectivity networks. Further, in the present study, white matter signals were regressed out of analyses as nuisance variables. Despite lack of evidence against white matter fMRI, it has been considered controversial. This is because BOLD signal is dependent on blood flow, which is typically lower in the white matter; and fMRI signal is dependent on post-synaptic potentials which are believed to occur mainly in gray matter (Gawryluk et al., 2014). However, recent literature has suggested that BOLD signals similar to those in cortical gray matter are detectable in white matter, and white matter signal may be modulated by the gray matter regions that the white matter tracts innervate (Gore et al., 2019). Spontaneous BOLD fluctuations at rest have also been identified in white matter and are believed to be reflective of neural activity within the white matter and potentially adjacent cortical regions (Ding et al., 2018; Gore et al., 2019). White matter fMRI may provide valuable insight into the functional relationships of various brain networks and their roles in cognitive processes. Though the neurophysiological underpinnings of white matter fMRI remain understudied (Gawryluk et al., 2014), future research should consider evaluating the role of white matter in responses to stress and anxiety. Finally, several clusters which significantly correlated with the ROIs in the present study were not interpreted as they corresponded to networks outside of the triple network model (Menon., 2011). Though interpreted networks were limited to the SN, DMN, and CEN due to their relevance to stress- and anxiety-related processes (Van Oort et al., 2017), disregarding these results discounts the comprehensiveness of the collected seed-voxel data, potentially overlooking additional networks elucidating the observed findings. This selective interpretation effectively reduces the analysis to a seed-seed analysis without *a priori* selection of seeds anticipated to correlate strongly with ROIs. This approach

may have biased the interpretation of results and may have limited our understanding of the networks implicated in CBD expectancy.

Finally, several limitations associated with our experimental approach must be addressed. Notably, the current investigation utilized a between-subjects design, which significantly reduces power and reliability of MRI-related analyses. In contrast to our previous work which utilized a within-subjects design (Spinella et al., 2021), we were unable to investigate the potential moderating effects of CBD-related beliefs on the outcomes of interest. The between-subjects design was initially chosen to diversify the sample, and account for potential habituation of the stress task across sessions. This protocol was piloted by our group in an out-of-scanner environment, and findings from this study suggested sufficient power to detect large effects (Zhekova et al., 2024). However, in the present sample, this design choice may have introduced variability. Further, the age range of participants in the present sample was between 19-56. Alterations in intrinsic connectivity network functional connectivity have been observed in healthy elderly individuals relative to young adults. Notably, older adults have demonstrated reduced network segregation and distinctiveness relative to young adults, which was associated with poorer cognitive performance (Chong et al., 2019). Thus, given that age was not accounted for in our analyses, the broad age range of participants may have resulted in significant variability in BOLD responses. However, mean age did not significantly differ across expectancy conditions, making it unlikely to have confounded expectancy effects. Also, the level at which the MRI environment may induce anxiety or discomfort in participants may differ, potentially introducing more variability relative to Zhekova et al. (2024). Additionally, the 12-hour cannabis abstinence period may not have been sufficient to control for residual effects of THC. Given THC's lipophilic nature, it is slowly released from adipose tissue for several weeks following

administration and may remain pharmacologically active (Lucas et al., 2018). Participants in this study were pre-screened for drug dependence (including cannabis dependence) and no significant differences in past-month or past-week cannabis use frequency were identified between the two expectancy conditions (Told CBD vs. Told CBD-free), which helps mitigate this concern. Our pre-post design and analytic approach also enabled us to control for individual differences at baseline. However, because recency of THC exposure was not directly assessed in the present study, we cannot rule out residual THC-related effects as a possible confound. Moreover, the current investigation did not employ a full balanced-placebo design where expectancy and pharmacology are crossed in a four-cell design (Rohsenow & Marlatt, 1981). Thus, we were unable to infer the impact of CBD's pharmacological effects alone, or the potentially interactive effects of both expectancy and pharmacology on subjective and neural outcomes. Therefore, future research may consider employing a within-between-subjects balanced placebo design to delineate the relative influence of CBD expectancy, pharmacology, and their interaction on CBD drug effects.

3.4 STRENGTHS

Despite the aforementioned limitations, the present study also has several notable strengths. For instance, though we were underpowered to detect any sex differences, the sample for my study was relatively sex balanced, improving the generalizability of our findings to both females and males. The sample also consisted of a range of age demographics. Though cannabis use is prevalent amongst diverse groups of adults, the mean age of the current sample (*M*=23.7, *SD*=8.3) is reflective of the demographic of Canadians which has been reported to use non-medical cannabis most frequently (Health Canada, 2023). Moreover, advanced modern methodologies were employed to detect the temporal correlation of our ROIs with the rest of the

brain. The seed-to-voxel rsFC analysis utilized in this study allowed for a more comprehensive assessment of how selected seed regions may interact with a range of networks relative to traditional seed-seed analyses employed in our previous work (Perry et al., 2024, under review). Given the relative novelty of the body of literature examining CBD placebo effects, this method also allowed for a more exploratory approach when analyzing brain regions which may be associated with CBD-placebo effects. Next, my study used a rigorous randomized placebo-controlled design, increasing internal validity of the study and potentially generalizability to the population.

3.5 FUTURE DIRECTIONS

Given the novelty of the present work, it is important to consider its impacts within the context of existing literature. Indeed, there are several gaps in the current CBD-related literature which must be addressed in future research to fully understand the impacts of CBD expectancy on anxiolytic outcomes. For instance, the literature assessing CBD's anxiolytic effects remains mixed, and placebo effects may have a potential role in these mixed findings. In fact, double-blind trials, in which participants are informed that they have an equal chance of receiving either drug or placebo, have yielded both positive (Bergamaschi, Queiroz, Zuardi, et al., 2011b; Masataka, 2019) and null results (Bolsoni et al., 2022; Gournay et al., 2023; Kwee, Baas, et al., 2022) relative to placebo. However, open-label trials in which participants are aware of their assigned treatment condition have uniformly reported effects of CBD (Berger et al., 2022; Dahlgren et al., 2022; Elms et al., 2019; Gournay et al., 2023). Therefore, it is likely that CBD expectancy may play a significant role in the observed positive findings in open-label trials. Also, participants in placebo-controlled blinded trials will often make guesses about their assigned condition, which may influence subjective effects (Dar & Barrett, 2014). Given that

perceived drug assignment is often not assessed in these trials, the role of strong placebo effects being obscured in negative trials, or contributing to CBD-induced reductions in stress and anxiety in positive trials remains unclear. Therefore, future CBD trials should consider the potential role of placebo-related effects on study outcomes to determine the relative impact of its pharmacological effects. In an experimental context, the impact of perceived drug assignment on drug-related outcomes can be assessed using a balanced placebo design (Rohsenow & Marlatt, 1981). This method may aid in determining the relative contributions of the pharmacological and non-pharmacological components of CBD by comparing active vs. placebo drug conditions in combination with either accurate or inaccurate instructions regarding their assigned condition. Alternatively, in double-blind trials, perceived drug assignment should be assessed to account for how participant guesses about their assigned treatment condition might impact the outcomes of interest.

Next, the current evidence supporting the anxiolytic effects of CBD in healthy adults consists primarily of male-only samples (e.g., Crippa et al., 2004; Fusar-Poli et al., 2009, 2010). Evidence suggests that males may achieve higher CBD plasma levels compared to females following acute oral CBD administration (Spindle et al., 2020). Also, males have been demonstrated to be more susceptible to placebo effects than females (Vambheim & Flaten, 2017). Therefore, it is possible that these factors which improve the likelihood that males experience CBD-related effects may have contributed to the outcomes of these positive trials. On the other hand, females are believed to display a preference for CBD-containing products relative to males (Goodman et al., 2022; Matheson et al., 2022). The present study consisted of a relatively sex-balanced sample, but we were underpowered to detect any potential moderating

effects of sex on study outcomes. Thus, future research should consider the impact of biological sex on CBD-related placebo effects, and its associated pharmacological effects.

Finally, the current literature regarding CBD's effects on stress and anxiety are methodologically heterogeneous. Specifically, there are marked differences in CBD administration procedures across studies (Dammann et al., 2024). A large majority of studies utilize oral administration procedures via capsules (Dammann et al., 2024); however, CBD's bioavailability via this route is low (Bergeria et al., 2022). In contrast, inhalation of CBD leads to peak concentrations within minutes (Bergeria et al., 2022; Spindle et al., 2020). These differences in administration procedures may significantly impact expectancy-related effects and must be addressed in future work. Additionally, a recent meta-analysis reported that 90% of the reviewed studies across species demonstrated no effect of CBD on anxiety, but that these effects may be dose-dependent (Kwee, Baas, et al., 2022). However, dosing across currently available trials have varied widely (Dammann et al., 2024), and the anxiolytic properties of CBD are believed to occur in a bell-shaped (inverted U-shaped) dose-response curve (Linares et al., 2018). More controlled studies and clinical trials are necessary to establish the efficacy of CBD in reducing stress and anxiety, as well as the specific dose at which it is most effective, and in delineating its underlying biological mechanism(s), particularly with regards to placebo effects.

3.6 IMPLICATIONS

The present study may have several research and clinical implications in the field of anxiety-related treatments. Specifically, our work highlights the need for future studies examining the relative contributions of both CBD's pharmacological and non-pharmacological effects on stress and anxiety in the brain. Understanding CBD's associated placebo effects may facilitate the differentiation of actual pharmacological drug effects relative to psychological

benefits derived from patient expectations. Our findings suggest a potentially significant impact of CBD expectancy on brain regions associated with stress and anxiety and associated subjective outcomes. Though the use of CBD has been associated with minimal adverse events (Bergamaschi, Queiroz, Zuardi, et al., 2011), adverse drug effects have been reported (Brown & Winterstein, 2019) (e.g., anemia, infection), so it is important to understand the factors which motivate its use and whether the rewards outweigh the risks. Future CBD-related double-blinded trials may consider measuring perceived drug-assignment to account for the potential guesses participants make about their assigned treatment condition. This may ensure that perceived drug efficacy is not overestimated by placebo-related effects, thus improving reliability of trial results. Indeed, a recent systematic review identified a benchmark large effect size for placebo affects across treatment modalities ($g = 1.05$), which may be accounted for in drug development, and leveraged in clinical practice (Jones et al., 2021). Additionally, as alluded to in Chapter 2, given the observed impact of expectancy-related factors on subjective outcomes, education should be provided to health care providers and the public regarding the impacts of CBD to leverage placebo effects in treatment. CBD use has been associated with potential harms, including adverse effects on the male reproductive system and the liver (Gingrich et al., 2023). Since CBD is likely amenable to placebo effects, negative expectancies may reduce its therapeutic potential, or potentially result in adverse events. Thus, education regarding the relative risks and benefits of CBD may allow for Canadians to make informed decisions regarding their CBD use and having positive expectations regarding CBD's effects may improve health outcomes and enhance treatment efficacy.

3.7 CONCLUSION

My thesis is the first study to examine the impact of CBD-expectancy on neural networks in the context of stress and anxiety. My uncorrected MRI findings and planned comparisons of the subjective data suggest a potentially significant impact of CBD placebo-related factors on stressor recovery and/or anticipation, which may be influenced by increased rsFC within and between the CEN and DMN, as well as potential attenuation of rsFC between the SN-DMN and SN-CEN. These findings have demonstrated that CBD expectancy may be associated with established alterations in stress- and anxiety-related neural networks (e.g., van Oort et al., 2017). This work contributes to a sparse body of literature examining CBD's non-pharmacological effects and placebo effects generally. Thus, this study may potentially influence future trials to examine the relative impacts of pharmacological and placebo effects on drug efficacy.

BIBLIOGRAPHY

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience and Biobehavioral Reviews*, *38*, 94–124. https://doi.org/10.1016/j.neubiorev.2013.11.005

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Andersson, J. L., Skare, S., & Ashburner, J. (2003). How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. *NeuroImage, 20*(2), 870–888. https://doi.org/10.1016/S1053-8119(03)00336-7

Andrews-Hanna, J. R., Snyder, A. Z., Vincent, J. L., Lustig, C., Head, D., Raichle, M. E., & Buckner, R. L. (2007). Disruption of large-scale brain systems in advanced aging. *Neuron*, *56*(5), 924–935. https://doi.org/10.1016/j.neuron.2007.10.038

Armario, A., Labad, J., & Nadal, R. (2020). Focusing attention on biological markers of acute stressor intensity: Empirical evidence and limitations. *Neuroscience & Biobehavioral Reviews*, *111*, 95–103. https://doi.org/10.1016/j.neubiorev.2020.01.013

Atlas, L. Y., Whittington, R. A., Lindquist, M. A., Wielgosz, J., Sonty, N., & Wager, T. D. (2012). Dissociable influences of opiates and expectations on pain. *Journal of Neuroscience*, *32*(23), 8053–8064. https://doi.org/10.1523/JNEUROSCI.0383-12.2012

Austrich-Olivares, A., García-Gutiérrez, M. S., Illescas, L., Gasparyan, A., & Manzanares, J. (2022). Cannabinoid CB1 receptor involvement in the actions of CBD on anxiety and coping behaviors in mice. *Pharmaceuticals*, *15*(4), 473. https://doi.org/10.3390/ph15040473

Batalla, A., Bos, J., Postma, A., & Bossong, M. G. (2021). The impact of cannabidiol on human

   brain function: A systematic review. *Frontiers in Pharmacology*, *11*, 618184.

   https://doi.org/10.3389/fphar.2020.618184

Baur, V., Hänggi, J., Langer, N., & Jäncke, L. (2013). Resting-state functional and structural

   connectivity within an insula–amygdala route specifically index state and trait anxiety.

   Biological Psychiatry, *73*(1), 85–92. https://doi.org/10.1016/j.biopsych.2012.06.003

Bergamaschi, M. M., Queiroz, R. H. C., Chagas, M. H. N., de Oliveira, D. C. G., De Martinis, B.

   S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A. E., Martín-Santos,

   R., Hallak, J. E. C., Zuardi, A. W., & Crippa, J. A. S. (2011). Cannabidiol reduces the

   anxiety induced by simulated public speaking in treatment-naïve social phobia patients.

   Neuropsychopharmacology, *36*(6), 1219–1226. https://doi.org/10.1038/npp.2011.6

Bergamaschi, M. M., Queiroz, R. H. C., Zuardi, A. W., & Crippa, J. A. S. (2011). Safety and

   side effects of cannabidiol, a Cannabis sativa constituent. *Current Drug Safety*, *6*(4),

   237–249.

Berger, M., Li, E., Rice, S., Davey, C. G., Ratheesh, A., Adams, S., Jackson, H., Hetrick, S.,

   Parker, A., Spelman, T., Kevin, R., McGregor, I. S., McGorry, P., & Amminger, G. P.

   (2022). Cannabidiol for treatment-resistant anxiety disorders in young people: An open-

   label trial. *Journal of Clinical Psychiatry*, *83*(5), 21m14130.

   https://doi.org/10.4088/JCP.21m14130

Bergeria, C. L., Spindle, T. R., Cone, E. J., Sholler, D., Goffi, E., Mitchell, J. M., Winecker, R. E., Bigelow, G. E., Flegel, R., & Vandrey, R. (2022). Pharmacokinetic profile of Δ9-tetrahydrocannabinol, cannabidiol and metabolites in blood following vaporization and oral ingestion of cannabidiol products. *Journal of Analytical Toxicology, 46*(6), 583–591. https://doi.org/10.1093/jat/bkab124

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C., Allen, P., Seal, M. L., Fletcher, P. C., Mechelli, A., & Atakan, Z. (2009). Modulation of mediotemporal and ventrostriatal function in humans by Δ-9-tetrahydrocannabinol. *Archives of General Psychiatry, 66*(4), 442–451. https://doi.org/10.1001/archgenpsychiatry.2009.17

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O' Carroll, C. M., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., & McGuire, P. K. (2010). Opposite effects of Δ-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology, 35*(3), 764–774. https://doi.org/10.1038/npp.2009.184

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

Black, N., Stockings, E., Campbell, G., Tran, L.T., Zagic, D., Hall, W.D., Farrell, M., & Degenhardt, L. (2019). Cannabinoids for the treatment of mental disorders and symptoms of mental disorders: A systematic review and meta-analysis. *The lancet. Psychiatry, 6*(12), 995–1010. https://doi-org/10.1016/S2215-0366(19)30401-8

Blessing, E. M., Steenkamp, M. M., Manzanares, J., & Marmar, C. R. (2015). Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics*, *12*(4), 825–836. https://doi.org/10.1007/s13311-015-0387-1

Bloomfield, M. A. P., Yamamori, Y., Hindocha, C., Jones, A. P. M., Yim, J. L. L., Walker, H. R., Statton, B., Wall, M. B., Lees, R. H., Howes, O. D., Curran, V. H., Roiser, J. P., & Freeman, T. P. (2022). The acute effects of cannabidiol on emotional processing and anxiety: A neurocognitive imaging study. *Psychopharmacology*, *239*(5), 1539–1549. https://doi.org/10.1007/s00213-022-06070-3

Bolsoni, L. M., Crippa, J. A. S., Hallak, J. E. C., Guimarães, F. S., & Zuardi, A. W. (2022). Effects of cannabidiol on symptoms induced by the recall of traumatic events in patients with posttraumatic stress disorder. *Psychopharmacology*, *239*(5), 1499–1507. https://doi.org/10.1007/s00213-021-06043-y

Brown, J. D., & Winterstein, A. G. (2019). Potential adverse drug events and drug–drug interactions with medical and consumer cannabidiol (CBD) use. *Journal of Clinical Medicine*, *8*(7), 989. https://doi.org/10.3390/jcm8070989

Buckner, J. D., Schmidt, N. B., Bobadilla, L., & Taylor, J. (2006). Social anxiety and problematic cannabis use: Evaluating the moderating role of stress reactivity and perceived coping. *Behaviour Research and Therapy*, *44*(7), 1007–1015. https://doi.org/10.1016/j.brat.2005.08.002

Bystritsky, A., & Kronemyer, D. (2014). Stress and anxiety: Counterpart elements of the stress/anxiety complex. *Psychiatric Clinics of North America*, *37*(4), 489–518. https://doi.org/10.1016/j.psc.2014.08.002

Campos, A. C., Fogaça, M. V., Sonego, A. B., & Guimarães, F. S. (2016). Cannabidiol,

neuroprotection and neuropsychiatric disorders. *Pharmacological Research*, *112*, 119–

127. https://doi.org/10.1016/j.phrs.2016.01.033

Chapman, H. A., Bernier, D., & Rusak, B. (2010). MRI-related anxiety levels change within and

between repeated scanning sessions. *Psychiatry Research*, *182*(2), 160–164.

https://doi.org/10.1016/j.pscychresns.2010.01.005

Chen, M. Y., Kramer, E. B., Gibson, L. P., Bidwell, L. C., Hutchison, K. E., & Bryan, A. D.

(2024). Investigating the relationship between cannabis expectancies and anxiety,

depression, and pain responses after acute flower and edible cannabis use. *Cannabis and

Cannabinoid Research*. https://doi.org/10.1089/can.2023.0264

Chong, J. S. X., Ng, K. K., Tandi, J., Wang, C., Poh, J. H., Lo, J. C., Chee, M. W. L., & Zhou, J.

H. (2019). Longitudinal changes in the cerebral cortex functional organization of healthy

elderly. *Journal of Neuroscience*, *39*(28), 5534–5550.

https://doi.org/10.1523/JNEUROSCI.1451-18.2019

Clark, L. A., & Watson, D. (1991). Tripartite model of anxiety and depression: psychometric

evidence and taxonomic implications. *Journal of Abnormal Psychology*, *100*(3), 316–

336. https://doi-org.ezproxy.library.dal.ca/10.1037//0021-843x.100.3.316

Cohen, S., Kessler, R., & Gordon, L. (1998). *Measuring stress: A guide for health and social

scientists*. Oxford: Oxford University Press.

Cole, D. M., Smith, S. M., & Beckmann, C. F. (2010). Advances and pitfalls in the analysis and

interpretation of resting-state FMRI data. *Frontiers in Systems Neuroscience*, *4*, 8.

https://doi.org/10.3389/fnsys.2010.00008

Corr, R., Glier, S., Bizzell, J., Pelletier-Baldelli, A., Campbell, A., Killian-Farrell, C., & Belger, A. (2022). Triple network functional connectivity during acute stress in adolescents and the influence of polyvictimization. *Biological psychiatry. Cognitive neuroscience and neuroimaging*, *7*(9), 867–875. https://doi.org/10.1016/j.bpsc.2022.03.003

Corroon, J., & Phillips, J. A. (2018). A cross-sectional study of cannabidiol users. *Cannabis and Cannabinoid Research*, *3*(1), 152–161. https://doi.org/10.1089/can.2018.0006

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*, *29*(3), 162–173. https://doi.org/10.1006/cbmr.1996.0014

Crippa, J. A. de S., Zuardi, A. W., Garrido, G. E. J., Wichert-Ana, L., Guarnieri, R., Ferrari, L., Azevedo-Marques, P. M., Hallak, J. E. C., McGuire, P. K., & Busatto, G. F. (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology*, *29*(2), Article 2. https://doi.org/10.1038/sj.npp.1300340

Crippa, J. A. S., Derenusson, G. N., Ferrari, T. B., Wichert-Ana, L., Duran, F. L., Martin-Santos, R., Simões, M. V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Filho, A. S., Freitas-Ferrari, M. C., McGuire, P. K., Zuardi, A. W., Busatto, G. F., & Hallak, J. E. C. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: A preliminary report. *Journal of Psychopharmacology*, *25*(1), 121–130. https://doi.org/10.1177/0269881110379283

Crippa, J. A. S., Zuardi, A. W., & Hallak, J. E. C. (2010). Uso terapêutico dos canabinoides em psiquiatria. [Therapeutical use of the cannabinoids in psychiatry]. *Revista Brasileira de Psiquiatria*, *32 (Suppl 1)*, S56-66.

Cropsey, K. L., Schiavon, S., Hendricks, P. S., Froelich, M., Lentowicz, I., & Fargason, R. (2017). Mixed-amphetamine salts expectancies among college students: Is stimulant induced cognitive enhancement a placebo effect? *Drug and Alcohol Dependence*, *178*, 302–309. https://doi.org/10.1016/j.drugalcdep.2017.05.024

Cuttler, C., & Spradlin, A. (2017). Measuring cannabis consumption: Psychometric properties of the Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory (DFAQ-CU). *PLoS One*, *12*(5), e0178194. https://doi.org/10.1371/journal.pone.0178194

Dahlgren, M. K., Lambros, A. M., Smith, R. T., Sagar, K. A., El-Abboud, C., & Gruber, S. A. (2022). Clinical and cognitive improvement following full-spectrum, high-cannabidiol treatment for anxiety: Open-label data from a two-stage, phase 2 clinical trial. *Communications Medicine*, *2*(1), 139. https://doi.org/10.1038/s43856-022-00202-8

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. *NeuroImage*, *9*(2), 179–194. https://doi.org/10.1006/nimg.1998.0395

Dammann, I., Rohleder, C., & Leweke, F. M. (2024). Cannabidiol and its potential evidence-based psychiatric benefits—A critical review. *Pharmacopsychiatry*. https://doi.org/10.1055/a-2228-6118

Dar, R., & Barrett, S. P. (2014). The effects of beliefs regarding drug assignment in experimental and field studies of nicotine delivery devices: A review. *Journal of Psychopharmacology*, *28*(11), 1071–1079. https://doi.org/10.1177/0269881114548295

Davey, H. M., Barratt, A. L., Butow, P. N., & Deeks, J. J. (2007). A one-item question with a Likert or Visual Analog Scale adequately measured current anxiety. *Journal of Clinical Epidemiology*, *60*(4), 356–360. https://doi.org/10.1016/j.jclinepi.2006.07.015

de la Mora, M. P., Gallegos-Cari, A., Arizmendi-García, Y., Marcellino, D., & Fuxe, K. (2010).

    Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and

    anxiety: Structural and functional analysis. *Progress in Neurobiology*, *90*(2), 198–216.

    https://doi.org/10.1016/j.pneurobio.2009.10.010

De Vita, M. J., Maisto, S. A., Gilmour, C. E., McGuire, L., Tarvin, E., & Moskal, D. (2022). The

    effects of cannabidiol and analgesic expectancies on experimental pain reactivity in

    healthy adults: A balanced placebo design trial. *Experimental and Clinical*

    Psychopharmacology, *30*(5), 536–546. https://doi.org/10.1037/pha0000465

Dedovic, K., Duchesne, A., Engert, V., Lue, S. D., Andrews, J., Efanov, S. I., Beaudry, T., &

    Pruessner, J. C. (2014). Psychological, endocrine and neural responses to social

    evaluation in subclinical depression. *Social Cognitive and Affective Neuroscience*, *9*(10),

    1632–1644. https://doi.org/10.1093/scan/nst151

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005).

    The Montreal Imaging Stress Task: Using functional imaging to investigate the effects of

    perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry*

    and Neuroscience, *30*(5), 319–325.

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R.

    L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An

    automated labeling system for subdividing the human cerebral cortex on MRI scans into

    gyral based regions of interest. *NeuroImage*, *31*(3), 968–980.

    https://doi.org/10.1016/j.neuroimage.2006.01.021

Diedrichsen, J., Balsters, J. H., Flavell, J., Cussans, E., & Ramnani, N. (2009). A probabilistic MR atlas of the human cerebellum. *NeuroImage*, *46*(1), 39–46. https://doi.org/10.1016/j.neuroimage.2009.01.045

Ding, Z., Huang, Y., Bailey, S. K., Gao, Y., Cutting, L. E., Rogers, B. P., Newton, A. T., & Gore, J. C. (2018). Detection of synchronous brain activity in white matter tracts at rest and under functional loading. Proceedings of the National Academy of Sciences, 115(3), 595-600. https://doi.org/10.1073/pnas.1711567115

Ekhtiari, H., Kuplicki, R., Yeh, H., & Paulus, M. P. (2019). Physical characteristics not psychological state or trait characteristics predict motion during resting state fMRI. *Scientific Reports*, *9*(1), 419. https://doi.org/10.1038/s41598-018-36699-0

Elms, L., Shannon, S., Hughes, S., & Lewis, N. (2019). Cannabidiol in the treatment of post-traumatic stress disorder: A case series. *Journal of Alternative and Complementary Medicine*, *25*(4), 392–397. https://doi.org/10.1089/acm.2018.0437

Esteban, O., Ciric, R., Finc, K., Blair, R. W., Markiewicz, C. J., Moodie, C. A., Kent, J. D., Goncalves, M., DuPre, E., Gomez, D. E. P., Ye, Z., Salo, T., Valabregue, R., Amlien, I. K., Liem, F., Jacoby, N., Stojić, H., Cieslak, M., Urchs, S., … Gorgolewski, K. J. (2020). Analysis of task-based functional MRI data preprocessed with fMRIPrep. *Nature Protocols*, *15*(7), Article 7. https://doi.org/10.1038/s41596-020-0327-3

Esteban, O., Markiewicz, C. J., Blair, R. W., Moodie, C. A., Isik, A. I., Erramuzpe, A., Kent, J. D., Goncalves, M., DuPre, E., Snyder, M., Oya, H., Ghosh, S. S., Wright, J., Durnez, J., Poldrack, R. A., & Gorgolewski, K. J. (2019). fMRIPrep: A robust preprocessing pipeline for functional MRI. *Nature Methods*, *16*(1), Article 1. https://doi.org/10.1038/s41592-018-0235-4

Faria, V., Appel, L., Åhs, F., Linnman, C., Pissiota, A., Frans, Ö., Bani, M., Bettica, P., Pich, E. M., Jacobsson, E., Wahlstedt, K., Fredrikson, M., & Furmark, T. (2012). Amygdala subregions tied to SSRI and placebo response in patients with social anxiety disorder. *Neuropsychopharmacology*, *37*(10), 2222–2232. https://doi.org/10.1038/npp.2012.72

Fechir, M., Gamer, M., Blasius, I., Bauermann, T., Breimhorst, M., Schlindwein, P., Schlereth, T., & Birklein, F. (2010). Functional imaging of sympathetic activation during mental stress. *NeuroImage*, *50*(2), 847–854. https://doi.org/10.1016/j.neuroimage.2009.12.004

Fonov, V., Evans, A., McKinstry, R., Almli, C., & Collins, D. (2009). Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *NeuroImage*, *47*, S102. https://doi.org/10.1016/S1053-8119(09)70884-5

Frazier, J. A., Chiu, S., Breeze, J. L., Makris, N., Lange, N., Kennedy, D. N., Herbert, M. R., Bent, E. K., Koneru, V. K., Dieterich, M. E., Hodge, S. M., Rauch, S. L., Grant, P. E., Cohen, B. M., Seidman, L. J., Caviness, V. S., & Biederman, J. (2005). Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder. *American Journal of Psychiatry*, *162*(7), 1256–1265. https://doi.org/10.1176/appi.ajp.162.7.1256

Fusar-Poli, P., Allen, P., Bhattacharyya, S., Crippa, J. A., Mechelli, A., Borgwardt, S., Martin-Santos, R., Seal, M. L., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. (2010). Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. *International Journal of Neuropsychopharmacology*, *13*(4), 421–432. https://doi.org/10.1017/S1461145709990617

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2009). Distinct effects of delta-9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*(1), 95–105. https://doi.org/10.1001/archgenpsychiatry.2008.519

García-Gutiérrez, M. S., Navarrete, F., Gasparyan, A., Austrich-Olivares, A., Sala, F., & Manzanares, J. (2020). Cannabidiol: A potential new alternative for the treatment of anxiety, depression, and psychotic disorders. *Biomolecules*, *10*(11), Article 11. https://doi.org/10.3390/biom10111575

Gawryluk, J. R., Mazerolle, E. L., & D'Arcy, R. C. (2014). Does functional MRI detect activation in white matter? A review of emerging evidence, issues, and future directions. *Frontiers in Neuroscience, 8*(8), 239. https://doi.org/10.3389/fnins.2014.00239.

Geppert, J., Lietzow, J., Hessel-Pras, S., Kirsch, F., Schäfer, B., & Sachse, B. (2023). Usage and health perception of cannabidiol-containing products among the population in Germany: A descriptive study conducted in 2020 and 2021. *BMC Public Health*, *23*(1), 2318. https://doi.org/10.1186/s12889-023-17142-0

Gianaros, P. J., Sheu, L. K., Matthews, K. A., Jennings, J. R., Manuck, S. B., & Hariri, A. R. (2008). Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *Journal of Neuroscience*, *28*(4), 990–999. https://doi.org/10.1523/JNEUROSCI.3606-07.2008

Gingrich, J., Choudhuri, S., Cournoyer, P., Downey, J., & Muldoon Jacobs, K. (2023). Review of the oral toxicity of cannabidiol (CBD). *Food and Chemical Toxicology*, *176*, 113799. https://doi.org/10.1016/j.fct.2023.113799

Glasser, M. F., Sotiropoulos, S. N., Wilson, J. A., Coalson, T. S., Fischl, B., Andersson, J. L.,
Xu, J., Jbabdi, S., Webster, M., Polimeni, J. R., Van Essen, D. C., Jenkinson, M., & WU-
Minn HCP Consortium. (2013). The minimal preprocessing pipelines for the Human
Connectome Project. *NeuroImage*, *80*, 105–124.
https://doi.org/10.1016/j.neuroimage.2013.04.127

Goebel, R., Roebroeck, A., Kim, D.-S., & Formisano, E. (2003). Investigating directed cortical
interactions in time-resolved fMRI data using vector autoregressive modeling and
Granger causality mapping. *Magnetic Resonance Imaging*, *21*(10), 1251–1261.
https://doi.org/10.1016/j.mri.2003.08.026

Goldstein, J. M., Seidman, L. J., Makris, N., Ahern, T., O'Brien, L. M., Caviness, V. S.,
Kennedy, D. N., Faraone, S. V., & Tsuang, M. T. (2007). Hypothalamic abnormalities in
schizophrenia: Sex effects and genetic vulnerability. *Biological Psychiatry*, *61*(8), 935–
945. https://doi.org/10.1016/j.biopsych.2006.06.027

Goodman, S., Wadsworth, E., Schauer, G., & Hammond, D. (2022). Use and perceptions of
cannabidiol products in Canada and in the United States. *Cannabis and Cannabinoid
Research*, *7*(3), 355–364. https://doi.org/10.1089/can.2020.0093

Gore, J. C., Li, M., Gao, Y., Wu, T. L., Schilling, K. G., Huang, Y., Mishra, A., Newton, A. T.,
Rogers, B. P., Chen, L. M., Anderson, A. W., & Ding, Z. (2019). Functional MRI and
resting state connectivity in white matter: A mini review. *Magnetic Resonance Imaging,
63*, 1-11. https://doi.org/10.1016/j.mri.2019.07.017.

Gorgolewski, K., Burns, C. D., Madison, C., Clark, D., Halchenko, Y. O., Waskom, M. L., & Ghosh, S. S. (2011). Nipype: A flexible, lightweight and extensible neuroimaging data processing framework in Python. *Frontiers in Neuroinformatics*, *5,* 13. https://doi.org/10.3389/fninf.2011.00013

Gorgolewski, K. J., Esteban, O., Ellis, D. G., Notter, M. P., Ziegler, E., Johnson, H., Hamalainen, C., Yvernault, B., Burns, C., Manhães-Savio, A., Jarecka, D., Markiewicz, C. J., Salo, T., Clark, D., Waskom, M., Wong, J., Modat, M., Dewey, B. E., Clark, M. G., … Ghosh, S. (2017). *Nipype: A flexible, lightweight and extensible neuroimaging data processing framework in Python. 0.13.1* (0.13.1) [Computer software]. Zenodo. https://doi.org/10.5281/zenodo.581704

Gournay, L. R., Ferretti, M. L., Bilsky, S., Vance, E., Nguyen, A. M., Mann, E., Williams, P., & Leen-Feldner, E. W. (2023). The effects of cannabidiol on worry and anxiety among high trait worriers: A double-blind, randomized placebo-controlled trial. *Psychopharmacology*, *240*(10), 2147–2161. https://doi.org/10.1007/s00213-023-06437-0

Greve, D. N., & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*, *48*(1), 63–72. https://doi.org/10.1016/j.neuroimage.2009.06.060

Grimm, O., Löffler, M., Kamping, S., Hartmann, A., Rohleder, C., Leweke, M., & Flor, H. (2018). Probing the endocannabinoid system in healthy volunteers: Cannabidiol alters fronto-striatal resting-state connectivity. *European Neuropsychopharmacology*, *28*(7), 841–849. https://doi.org/10.1016/j.euroneuro.2018.04.004

Gueorguieva, R., & Krystal, J. H. (2004). Move over ANOVA: Progress in analyzing repeated-measures data and its reflection in papers published in the Archives of General Psychiatry. *Archives of General Psychiatry*, *61*(3), 310–317. https://doi.org/10.1001/archpsyc.61.3.310

Hall, W., & Solowij, N. (1998). Adverse effects of cannabis. *The Lancet*, *352*(9140), 1611–1616. https://doi.org/10.1016/S0140-6736(98)05021-1

Health Canada. (2022). *Canadian cannabis survey 2022: Summary* [Surveys]. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/canadian-cannabis-survey-2022-summary.html

Health Canada. (2023). *Canadian cannabis survey 2023: Summary* [Surveys]. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/canadian-cannabis-survey-2023-summary.html

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerström, K. O. (1991). The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *British journal of addiction*, *86*(9), 1119–1127. https://doi-org/10.1111/j.1360-0443.1991.tb01879.x

Hermans, E. J., Henckens, M. J. A. G., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences*, *37*(6), 304–314. https://doi.org/10.1016/j.tins.2014.03.006

Huneke, N. T. M., Aslan, I. H., Fagan, H., Phillips, N., Tanna, R., Cortese, S., Garner, M., & Baldwin, D. S. (2022). Functional neuroimaging correlates of placebo response in patients with depressive or anxiety disorders: A systematic review. *International Journal of Neuropsychopharmacology*, *25*(6), 433–447. https://doi.org/10.1093/ijnp/pyac009

Hurd, Y. L., Spriggs, S., Alishayev, J., Winkel, G., Gurgov, K., Kudrich, C., Oprescu, A. M., & Salsitz, E. (2019). Cannabidiol for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder: A double-blind randomized placebo-controlled trial. *American Journal of Psychiatry*, *176*(11), 911–922. https://doi.org/10.1176/appi.ajp.2019.18101191

Iffland, K., & Grotenhermen, F. (2017). An update on safety and side effects of cannabidiol: A review of clinical data and relevant animal studies. *Cannabis and Cannabinoid Research*, *2*(1), 139–154. https://doi.org/10.1089/can.2016.0034

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*, *17*(2), 825–841. https://doi.org/10.1006/nimg.2002.1132

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*, *62*(2), 782–790. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jenks, S. K., Zhang, S., Li, C.-S. R., & Hu, S. (2020). Threat bias and resting state functional connectivity of the amygdala and bed nucleus stria terminalis. *Journal of Psychiatric Research*, *122*, 54–63. https://doi.org/10.1016/j.jpsychires.2019.12.017

Johnson, C. A., Garnett, E. O., Chow, H. M., Spray, G. J., Zhu, D. C., & Chang, S. E. (2021). Developmental factors that predict head movement during resting-state functional magnetic resonance imaging in 3-7-year-old stuttering and non-stuttering children. *Frontiers in Neuroscience*, *15*, 753010. https://doi.org/10.3389/fnins.2021.753010

Jones, B. D. M., Razza, L. B., Weissman, C. R., Karbi, J., Vine, T., Mulsant, L. S., Brunoni, A. R., Husain, M. I., Mulsant, B. H., Blumberger, D. M., & Daskalakis, Z. J. (2021). Magnitude of the placebo response across treatment modalities used for treatment-resistant depression in adults: A systematic review and meta-analysis. *JAMA Network Open*, *4*(9), e2125531. https://doi.org/10.1001/jamanetworkopen.2021.25531

Juliano, L. M., Fucito, L. M., & Harrell, P. T. (2011). The influence of nicotine dose and nicotine dose expectancy on the cognitive and subjective effects of cigarette smoking. *Experimental and Clinical Psychopharmacology*, *19*(2), 105–115. https://doi.org/10.1037/a0022937

Karekla, M., Panayiotou, G., & Collins, B. N. (2017). Predictors of urge to smoke under stressful conditions: An experimental investigation utilizing the PASAT-C task to induce negative affect in smokers. *Psychology of Addictive Behaviour*, *31*(7), 735–743. https://doi.org/10.1037/adb0000309

Khoury, J. M., Neves, M. de C. L. das, Roque, M. A. V., Queiroz, D. A. de B., Corrêa de Freitas, A. A., de Fátima, Â., Moreira, F. A., & Garcia, F. D. (2019). Is there a role for cannabidiol in psychiatry? *World Journal of Biological Psychiatry*, *20*(2), 101–116. https://doi.org/10.1080/15622975.2017.1285049

Kirkland, A. E., Fadus, M. C., Gruber, S. A., Gray, K. M., Wilens, T. E., & Squeglia, L. M. (2022). A scoping review of the use of cannabidiol in psychiatric disorders. *Psychiatry Research*, *308*, 114347. https://doi.org/10.1016/j.psychres.2021.114347

Kirsch, I. (2018). Response expectancy and the placebo effect. In L. Colloca (Ed.), *International Review of Neurobiology* (Vol. 138, pp. 81–93). Academic Press. https://doi.org/10.1016/bs.irn.2018.01.003

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (2008). The 'Trier Social Stress Test': A

tool for investigating psychobiological stress responses in a laboratory setting.

Neuropsychobiology, *28*(1–2), 76–81. https://doi.org/10.1159/000119004

Klein, A., Ghosh, S. S., Bao, F. S., Giard, J., Häme, Y., Stavsky, E., Lee, N., Rossa, B., Reuter,

M., Neto, E. C., & Keshavan, A. (2017). Mindboggling morphometry of human brains.

PLOS Computational Biology, *13*(2), e1005350.

https://doi.org/10.1371/journal.pcbi.1005350

Kowal, M., Hazekamp, A., & Grotenhermen, F. (2016). Review on clinical studies with cannabis

and cannabinoids 2010-2014. *Cannabinoids*, *11*, 1–18.

Kwee, C. M., Baas, J. M., van der Flier, F. E., Groenink, L., Duits, P., Eikelenboom, M., van der

Veen, D. C., Moerbeek, M., Batelaan, N. M., van Balkom, A. J., & Cath, D. C. (2022).

Cannabidiol enhancement of exposure therapy in treatment refractory patients with social

anxiety disorder and panic disorder with agoraphobia: A randomized controlled trial.

European Neuropsychopharmacology, *59*, 58–67.

https://doi.org/10.1016/j.euroneuro.2022.04.003

Kwee, C. M., van Gerven, J. M., Bongaerts, F. L., Cath, D. C., Jacobs, G., Baas, J. M., &

Groenink, L. (2022). Cannabidiol in clinical and preclinical anxiety research. A

systematic review into concentration-effect relations using the IB-de-risk tool. *Journal of

Psychopharmacology*, *36*(12), 1299–1314. https://doi.org/10.1177/02698811221124792

La Corte, V., Sperduti, M., Malherbe, C., Vialatte, F., Lion, S., Gallarda, T., Oppenheim, C., & Piolino, P. (2016). Cognitive decline and reorganization of functional connectivity in healthy aging: The pivotal role of the salience network in the prediction of age and cognitive performances. *Frontiers in Aging Neuroscience*, *8,* 204. https://www.frontiersin.org/articles/10.3389/fnagi.2016.00204

Lanczos, C. (1964). Evaluation of noisy data. *Journal of the Society for Industrial and Applied Mathematics Series B Numerical Analysis*, *1*(1), 76–85. https://doi.org/10.1137/0701007

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

Leen-Feldner, E. W., Bynion, T.-M., Eglit, G. M. L., Bonn-Miller, M. O., Gournay, L. R., & Feldner, M. T. (2022). A double-blind, randomized, placebo-controlled test of the effects of cannabidiol on fear elicited by a 10% carbon dioxide-enriched air breathing challenge. *Psychopharmacology*. https://doi.org/10.1007/s00213-022-06258-7

Lesage, F.-X., Berjot, S., & Deschamps, F. (2012). Clinical stress assessment using a visual analogue scale. *Occupational Medicine*, *62*(8), 600–605. https://doi.org/10.1093/occmed/kqs140

Linares, I. M., Zuardi, A. W., Pereira, L. C., Queiroz, R. H., Mechoulam, R., Guimarães, F. S., & Crippa, J. A. (2018). Cannabidiol presents an inverted U-shaped dose-response curve in a simulated public speaking test. *Revista Brasileira de Psiquiatria*, *41*(1), 9–14. https://doi.org/10.1590/1516-4446-2017-0015

Lo, L. A., Christiansen, A. L., Strickland, J. C., Pistawka, C. A., Eadie, L., Vandrey, R., & MacCallum, C. A. (2024). Does acute cannabidiol (CBD) use impair performance? A meta-analysis and comparison with placebo and delta-9-tetrahydrocannabinol (THC). *Neuropsychopharmacology*, 1–12. Advance online publication. https://doi.org/10.1038/s41386-024-01847-w

Lorenzetti, V., Gaillard, A., Thomson, D., Englund, A., & Freeman, T. P. (2023). Effects of cannabinoids on resting state functional brain connectivity: A systematic review. *Neuroscience and Biobehavioral Reviews*, *145*, 105014. https://doi.org/10.1016/j.neubiorev.2022.105014

Lorenzetti, V., McTavish, E., Broyd, S., van Hell, H., Ganella, E., Kottaram, A. R., Beale, C., Martin, J., Galettis, P., Solowij, N., & Greenwood, L.-M. (2023). Daily cannabidiol administration for 10 weeks modulates hippocampal and amygdalar resting-state functional connectivity in cannabis users: A functional magnetic resonance imaging open-label clinical trial. *Cannabis and Cannabinoid Research*. https://doi.org/10.1089/can.2022.0336

Lovibond, S. H., & Lovibond, P. F. (1995). *Depression Anxiety Stress Scales*. APA PsycTests. https://doi.org/10.1037/t01004-000

Lucas, C. J., Galettis, P., & Schneider, J. (2018). The pharmacokinetics and the pharmacodynamics of cannabinoids. *British Journal of Clinical Pharmacology*, *84*(11), 2477–2482. https://doi.org/10.1111/bcp.13710

Lv, H., Wang, Z., Tong, E., Williams, L. M., Zaharchuk, G., Zeineh, M., Goldstein-Piekarski, A. N., Ball, T. M., Liao, C., & Wintermark, M. (2018). Resting-state functional MRI: Everything that nonexperts have always wanted to know. *American Journal of Neuroradiology, 39*(8), 1390–1399. https://doi.org/10.3174/ajnr.A5527

Ma, L., Braun, S. E., Steinberg, J. L., Bjork, J. M., Martin, C. E., Keen Ii, L. D., & Moeller, F. G. (2024). Effect of scanning duration and sample size on reliability in resting state fMRI dynamic causal modeling analysis. *NeuroImage*, *292*, 120604. https://doi.org/10.1016/j.neuroimage.2024.120604

MacCallum, C. A., & Russo, E. B. (2018). Practical considerations in medical cannabis administration and dosing. *European Journal of Internal Medicine*, *49*, 12–19. https://doi.org/10.1016/j.ejim.2018.01.004

Madl, J., Janka, R., Bay, S., & Rohleder, N. (2022). MRI as a stressor: The psychological and physiological response of patients to MRI, influencing factors, and consequences. *Journal of the American College of Radiology*, *19*(3), 423–432. https://doi.org/10.1016/j.jacr.2021.11.020

Makris, N., Goldstein, J. M., Kennedy, D., Hodge, S. M., Caviness, V. S., Faraone, S. V., Tsuang, M. T., & Seidman, L. J. (2006). Decreased volume of left and total anterior insular lobule in schizophrenia. *Schizophrenia Research*, *83*(2–3), 155–171. https://doi.org/10.1016/j.schres.2005.11.020

Marek, S., Tervo-Clemmens, B., Calabro, F. J., Montez, D. F., Kay, B. P., Hatoum, A. S., Donohue, M. R., Foran, W., Miller, R. L., Hendrickson, T. J., Malone, S. M., Kandala, S., Feczko, E., Miranda-Dominguez, O., Graham, A. M., Earl, E. A., Perrone, A. J., Cordova, M., Doyle, O., … Dosenbach, N. U. F. (2022). Reproducible brain-wide association studies require thousands of individuals. *Nature*, *603*(7902), 654–660. https://doi.org/10.1038/s41586-022-04492-9

Martinez Naya, N., Kelly, J., Corna, G., Golino, M., Abbate, A., & Toldo, S. (2023). Molecular and cellular mechanisms of action of cannabidiol. *Molecules*, *28*(16), 5980. https://doi.org/10.3390/molecules28165980

Masataka, N. (2019). Anxiolytic effects of repeated cannabidiol treatment in teenagers with social anxiety disorders. *Frontiers in Psychology*, *10*, 2466. https://doi.org/10.3389/fpsyg.2019.02466

Matheson, J., Bourgault, Z., & Le Foll, B. (2022). Sex differences in the neuropsychiatric effects and pharmacokinetics of cannabidiol: A scoping review. *Biomolecules*, *12*(10), 1462. https://doi.org/10.3390/biom12101462

Mechoulam, R., Parker, L. A., & Gallily, R. (2002). Cannabidiol: An overview of some pharmacological aspects. *Journal of Clinical Pharmacology*, *42*(S1), 11S-19S. https://doi.org/10.1002/j.1552-4604.2002.tb05998.x

Menon, V. (2011). Large-scale brain networks and psychopathology: A unifying triple network model. *Trends in Cognitive Sciences*, *15*(10), 483–506. https://doi.org/10.1016/j.tics.2011.08.003

Menon, V. (2015). Salience network. In A. W. Toga (Ed.), *Brain mapping: An encyclopedic reference, Volume 2,* 597–611. Elsevier/Academic Press.

Metrik, J., Rohsenow, D. J., Monti, P. M., McGeary, J., Cook, T. A. R., de Wit, H., Haney, M.,
    & Kahler, C. W. (2009). Effectiveness of a marijuana expectancy manipulation: Piloting
    the balanced-placebo design for marijuana. *Experimental and Clinical*
    Psychopharmacology, *17*(4), 217–225. https://doi.org/10.1037/a0016502

Meyer, B., Yuen, K. S. L., Saase, V., & Kalisch, R. (2019). The functional role of large-scale
    brain network coordination in placebo-induced anxiolysis. *Cerebral Cortex*, *29*(8), 3201–
    3210. https://doi.org/10.1093/cercor/bhy188

Mongeau-Pérusse, V., Rizkallah, E., Morissette, F., Brissette, S., Bruneau, J., Dubreucq, S.,
    Gazil, G., Trépanier, A., & Jutras-Aswad, D. (2022). Cannabidiol effect on anxiety
    symptoms and stress response in individuals with cocaine use disorder: Exploratory
    results from a randomized controlled trial. *Journal of Addiction Medicine*, *16*(5), 521–
    526. https://doi.org/10.1097/ADM.0000000000000959

Montgomery, L., Dixon, S., & Mantey, D. S. (2022). Racial and ethnic differences in cannabis
    use and cannabis use disorder: Implications for researchers. *Current Addiction Reports*,
    9(1), 14–22. https://doi.org/10.1007/s40429-021-00404-5

Moore, A., Straube, S., Fisher, E., & Eccleston, C. (2024). Cannabidiol (CBD) products for pain:
    Ineffective, expensive, and with potential harms. *Journal of Pain*, *25*(4), 833–842.
    https://doi.org/10.1016/j.jpain.2023.10.009

Muehlhan, M., Lueken, U., Wittchen, H.-U., & Kirschbaum, C. (2011). The scanner as a
    stressor: Evidence from subjective and neuroendocrine stress parameters in the time
    course of a functional magnetic resonance imaging session. *International Journal of*
    Psychophysiology, *79*(2), 118–126. https://doi.org/10.1016/j.ijpsycho.2010.09.009

Mumford, J. A. (2012). A power calculation guide for fMRI studies. *Social Cognitive and Affective Neuroscience*, *7*(6), 738–742. https://doi.org/10.1093/scan/nss059

Nebel, M. B., Lidstone, D. E., Wang, L., Benkeser, D., Mostofsky, S. H., & Risk, B. B. (2022). Accounting for motion in resting-state fMRI: What part of the spectrum are we characterizing in autism spectrum disorder? *NeuroImage*, *257*, 119296. https://doi.org/10.1016/j.neuroimage.2022.119296

Nichols, T. E., Das, S., Eickhoff, S. B., Evans, A. C., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M. P., Poldrack, R. A., Poline, J.-B., Proal, E., Thirion, B., Van Essen, D. C., White, T., & Yeo, B. T. T. (2017). Best practices in data analysis and sharing in neuroimaging using MRI. *Nature Neuroscience*, *20*(3), 299–303. https://doi.org/10.1038/nn.4500

Nicholson, A. A., Harricharan, S., Densmore, M., Neufeld, R. W. J., Ros, T., McKinnon, M. C., Frewen, P. A., Théberge, J., Jetly, R., Pedlar, D., & Lanius, R. A. (2020). Classifying heterogeneous presentations of PTSD via the default mode, central executive, and salience networks with machine learning. *NeuroImage: Clinical*, *27*, 102262. https://doi.org/10.1016/j.nicl.2020.102262

Oyola, M. G., & Handa, R. J. (2017). Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: Sex differences in regulation of stress responsivity. *Stress*, *20*(5), 476–494. https://doi.org/10.1080/10253890.2017.1369523

Paltoglou, G., Stefanaki, C., & Chrousos, G. P. (2024). Functional MRI techniques suggesting that the stress system interacts with three large scale core brain networks to help coordinate the adaptive response: A systematic review. *Current Neuropharmacology*, *22*(5), 976–989. https://doi.org/10.2174/1570159X21666230801151718

Perry, R. N., Ethier-Gagnon, M. A. S., Stewart, S. H., Tibbo, P. G., & Barrett, S. P. (2024). The impact of cannabidiol (CBD) placebo on amygdala-based neural responses to an acute stressor. *Journal of Psychopharmacology,* under review.

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage*, *59*(3), 2142–2154. https://doi.org/10.1016/j.neuroimage.2011.10.018

Power, J. D., Cohen, A. L., Nelson, S. M., Wig, G. S., Barnes, K. A., Church, J. A., Vogel, A. C., Laumann, T. O., Miezin, F. M., Schlaggar, B. L., & Petersen, S. E. (2011). Functional network organization of the human brain. *Neuron*, *72*(4), 665–678. https://doi.org/10.1016/j.neuron.2011.09.006

Power, J. D., Silver, B. M., Silverman, M. R., Ajodan, E. L., Bos, D. J., & Jones, R. M. (2019). Customized head molds reduce motion during resting state fMRI scans. *NeuroImage,* *189*, 141–149. https://doi.org/10.1016/j.neuroimage.2019.01.016

Reilly, D., Didcott, P., Swift, W., & Hall, W. (1998). Long-term cannabis use: Characteristics of users in an Australian rural area. *Addiction*, *93*(6), 837–846. https://doi.org/10.1046/j.1360-0443.1998.9368375.x

Rich-Edwards, J. W., Kaiser, U. B., Chen, G. L., Manson, J. E., & Goldstein, J. M. (2018). Sex and gender differences research design for basic, clinical, and population studies: Essentials for investigators. *Endocrine Reviews*, *39*(4), 424–439. https://doi.org/10.1210/er.2017-00246

Roese, N. J., & Jamieson, D. W. (1993). Twenty years of bogus pipeline research: A critical review and meta-analysis. *Psychological Bulletin*, *114*(2), 363–375. https://doi.org/10.1037/0033-2909.114.2.363

Rohsenow, D. J., & Marlatt, G. A. (1981). The balanced placebo design: Methodological considerations. *Addictive Behaviors*, *6*(2), 107–122. https://doi.org/10.1016/0306-4603(81)90003-4

Rossi, V., & Pourtois, G. (2012). Transient state-dependent fluctuations in anxiety measured using STAI, POMS, PANAS or VAS: A comparative review. *Anxiety, Stress, & Coping*, *25*(6), 603–645. https://doi.org/10.1080/10615806.2011.582948

Rueger, S. Y., & King, A. C. (2013). Validation of the brief Biphasic Alcohol Effects Scale (B-BAES). *Alcoholism: Clinical and Experimental Research*, *37*(3), 470–476. https://doi.org/10.1111/j.1530-0277.2012.01941.x

Russell, G., & Lightman, S. (2019). The human stress response. *Nature Reviews, Endocrinology*, *15*(9), 525–534. https://doi.org/10.1038/s41574-019-0228-0

Satterthwaite, T. D., Elliott, M. A., Gerraty, R. T., Ruparel, K., Loughead, J., Calkins, M. E., Eickhoff, S. B., Hakonarson, H., Gur, R. C., Gur, R. E., & Wolf, D. H. (2013). An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *NeuroImage*, *64*, 240–256. https://doi.org/10.1016/j.neuroimage.2012.08.052

Schaefer, A., Kong, R., Gordon, E. M., Laumann, T. O., Zuo, X.-N., Holmes, A. J., Eickhoff, S. B., & Yeo, B. T. T. (2018). Local-global parcellation of the human cerebral cortex from intrinsic functional connectivity MRI. *Cerebral Cortex*, *28*(9), 3095–3114. https://doi.org/10.1093/cercor/bhx179

Schimmelpfennig, J., Topczewski, J., Zajkowski, W., & Jankowiak-Siuda, K. (2023). The role of the salience network in cognitive and affective deficits. *Frontiers in Human Neuroscience*, *17*. https://doi.org/10.3389/fnhum.2023.1133367

Schlagintweit, H. E., Perry, R. N., Darredeau, C., & Barrett, S. P. (2020). Non-pharmacological considerations in human research of nicotine and tobacco effects: A review. *Nicotine & Tobacco Research*, *22*(8), 1260–1266. https://doi.org/10.1093/ntr/ntz064

Seo, D., Jia, Z., Lacadie, C. M., Tsou, K. A., Bergquist, K., & Sinha, R. (2011). Sex differences in neural responses to stress and alcohol context cues. *Human Brain Mapping*, *32*(11), 1998. https://doi.org/10.1002/HBM.21165

Seo, D., Lacadie, C. M., Tuit, K., Hong, K.-I., Constable, R. T., & Sinha, R. (2013). Disrupted ventromedial prefrontal function, alcohol craving, and subsequent relapse risk. *JAMA Psychiatry*, *70*(7), 727–739. https://doi.org/10.1001/jamapsychiatry.2013.762

Selzer M. L. (1971). The Michigan alcoholism screening test: the quest for a new diagnostic instrument. *The American journal of psychiatry*, *127*(12), 1653–1658. https://doi.org/10.1176/ajp.127.12.1653

Shahbazi, F., Grandi, V., Banerjee, A., & Trant, J. F. (2020). Cannabinoids and cannabinoid receptors: The story so far. *iScience*, *23*(7), 101301. https://doi.org/10.1016/j.isci.2020.101301

Sinha, R., Lacadie, C., Skudlarski, P., & Wexler, B. E. (2004). Neural circuits underlying emotional distress in humans. *Annals of the New York Academy of Sciences*, *1032*(1), 254–257. https://doi.org/10.1196/annals.1314.032

Skinner H. A. (1982). The drug abuse screening test. *Addictive behaviors*, *7*(4), 363–371. https://doi.org/10.1016/0306-4603(82)90005-3

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23 (Suppl 1)*, S208-219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Spielberger, C. (1983). *Manual for the State-Trait Anxiety Inventory STAI (form Y; "self-evaluation questionnaire")*. Consulting Psychologists Press Inc.

Spindle, T. R., Cone, E. J., Goffi, E., Weerts, E. M., Mitchell, J. M., Winecker, R. E., Bigelow, G. E., Flegel, R. R., & Vandrey, R. (2020). Pharmacodynamic effects of vaporized and oral cannabidiol (CBD) and vaporized CBD-dominant cannabis in infrequent cannabis users. *Drug and Alcohol Dependence*, *211*, 107937. https://doi.org/10.1016/j.drugalcdep.2020.107937

Spinella, T. C., Bartholomeusz, J., Stewart, S. H., & Barrett, S. P. (2023). Perceptions about THC and CBD effects among adults with and without prior cannabis experience. *Addictive Behaviors*, *137*, 107508. https://doi.org/10.1016/j.addbeh.2022.107508

Spinella, T. C., Burdeyny, V., Oprea, A., Perrot, T. S., & Barrett, S. P. (2023). The impact of cannabidiol expectancy on cortisol responsivity in the context of acute stress: Associations with biological sex. *Cannabis and Cannabinoid Research*. https://doi.org/10.1089/can.2022.0326

Spinella, T. C., Stewart, S. H., Naugler, J., Yakovenko, I., & Barrett, S. P. (2021). Evaluating

cannabidiol (CBD) expectancy effects on acute stress and anxiety in healthy adults: A

randomized crossover study. *Psychopharmacology*, *238*(7), 1965–1977.

https://doi.org/10.1007/s00213-021-05823-w

Stanciu, C. N., Brunette, M. F., Teja, N., & Budney, A. J. (2021). Evidence for use of

cannabinoids in mood disorders, anxiety disorders, and PTSD: A systematic review.

Psychiatric Services, *72*(4), 429–436. https://doi.org/10.1176/appi.ps.202000189

Stanley, T. B., Ferretti, M. L., Bonn-Miller, M. O., & Irons, J. G. (2022). A double-blind,

randomized, placebo-controlled test of the effects of cannabidiol on experiences of test

anxiety among college students. *Cannabis and Cannabinoid Research*. *8*(6), 1090–1099.

https://doi.org/10.1089/can.2022.0062

Stawski, R. S., Cichy, K. E., Piazza, J. R., & Almeida, D. M. (2013). Associations among daily

stressors and salivary cortisol: Findings from the national study of daily experiences.

Psychoneuroendocrinology, *38*(11), 2654–2665.

https://doi.org/10.1016/j.psyneuen.2013.06.023

Tessner, K. D., Walker, E. F., Hochman, K., & Hamann, S. (2006). Cortisol responses of healthy

volunteers undergoing magnetic resonance imaging. *Human Brain Mapping*, *27*(11),

889–895. https://doi.org/10.1002/hbm.20229

Tourangeau, R., Smith, T. W., & Rasinski, K. A. (1997). Motivation to report sensitive behaviors

on surveys: Evidence from a bogus pipeline experiment. *Journal of Applied Social

Psychology*, *27*(3), 209–222. https://doi.org/10.1111/j.1559-1816.1997.tb00629.x

Tournier, M., Sorbara, F., Gindre, C., Swendsen, J. D., & Verdoux, H. (2003). Cannabis use and anxiety in daily life: A naturalistic investigation in a non-clinical population. *Psychiatry Research*, *118*(1), 1–8. https://doi.org/10.1016/S0165-1781(03)00052-0

Tran, T., & Kavuluru, R. (2020). Social media surveillance for perceived therapeutic effects of cannabidiol (CBD) products. *International Journal on Drug Policy*, *77*, 102688. https://doi.org/10.1016/j.drugpo.2020.102688

Tustison, N. J., Avants, B. B., Cook, P. A., Zheng, Y., Egan, A., Yushkevich, P. A., & Gee, J. C. (2010). N4ITK: Improved N3 bias correction. *IEEE Transactions on Medical Imaging*, *29*(6), 1310–1320. https://doi.org/10.1109/TMI.2010.2046908

Ujváry, I., & Hanuš, L. (2016). Human metabolites of cannabidiol: A review on their formation, biological activity, and relevance in therapy. *Cannabis and Cannabinoid Research*, *1*(1), 90–101. https://doi.org/10.1089/can.2015.0012

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews, Neuroscience*, *10*(6), 397–409. https://doi.org/10.1038/nrn2647

Vambheim, S. M., & Flaten, M. A. (2017). A systematic review of sex differences in the placebo and the nocebo effect. *Journal of Pain Research*, *10*, 1831–1839. https://doi.org/10.2147/JPR.S134745

Van Dijk, K. R. A., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage*, *59*(1), 431–438. https://doi.org/10.1016/j.neuroimage.2011.07.044

van Oort, J., Tendolkar, I., Hermans, E. J., Mulders, P. C., Beckmann, C. F., Schene, A. H., Fernández, G., & van Eijndhoven, P. F. (2017). How the brain connects in response to acute stress: A review at the human brain systems level. *Neuroscience & Biobehavioral Reviews*, *83*, 281–297. https://doi.org/10.1016/j.neubiorev.2017.10.015

Wager, T. D., & Atlas, L. Y. (2015). The neuroscience of placebo effects: Connecting context, learning and health. *Nature Reviews, Neuroscience*, *16*(7), 403–418. https://doi.org/10.1038/nrn3976

Wall, M. B., Freeman, T. P., Hindocha, C., Demetriou, L., Ertl, N., Freeman, A. M., Jones, A. P., Lawn, W., Pope, R., Mokrysz, C., Solomons, D., Statton, B., Walker, H. R., Yamamori, Y., Yang, Z., Yim, J. L., Nutt, D. J., Howes, O. D., Curran, H. V., & Bloomfield, M. A. (2022). Individual and combined effects of cannabidiol and Δ9-tetrahydrocannabinol on striato-cortical connectivity in the human brain. *Journal of Psychopharmacology*, *36*(6), 732–744. https://doi.org/10.1177/02698811221092506

Wall, M. B., Pope, R., Freeman, T. P., Kowalczyk, O. S., Demetriou, L., Mokrysz, C., Hindocha, C., Lawn, W., Bloomfield, M. A., Freeman, A. M., Feilding, A., Nutt, D., & Curran, H. V. (2019). Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity. *Journal of Psychopharmacology*, *33*(7), 822–830. https://doi.org/10.1177/0269881119841568

Wang, J., Korczykowski, M., Rao, H., Fan, Y., Pluta, J., Gur, R. C., McEwen, B. S., & Detre, J. A. (2007). Gender difference in neural response to psychological stress. *Social Cognitive and Affective Neuroscience*, *2*(3), 227–239. https://doi.org/10.1093/scan/nsm018

Wang, J., Rao, H., Wetmore, G. S., Furlan, P. M., Korczykowski, M., Dinges, D. F., & Detre, J. A. (2005). Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(49), 17804–17809. https://doi.org/10.1073/pnas.0503082102

Weldon, A. L., Hagan, M., Meter, A. V., Jacobs, R. H., Kassel, M. T., Hazlett, K. E., Haase, B. D., Vederman, A. C., Avery, E., Briceno, E. M., Welsh, R. C., Zubieta, J.-K., Weisenbach, S. L., & Langenecker, S. A. (2015). Stress response to the fMRI environment in healthy adults relates to degree of limbic reactivity during emotion processing. *Neuropsychobiology*, *71*(2), 85. https://doi.org/10.1159/000369027

Wheeler, M., Merten, J. W., Gordon, B. T., & Hamadi, H. (2020). CBD (cannabidiol) product attitudes, knowledge, and use among young adults. *Substance Use & Misuse*, *55*(7), 1138–1145. https://doi.org/10.1080/10826084.2020.1729201

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., Beckmann, C., Jenkinson, M., & Smith, S. M. (2009). Bayesian analysis of neuroimaging data in FSL. *NeuroImage*, *45*(Suppl 1), S173-186. https://doi.org/10.1016/j.neuroimage.2008.10.055

World Health Organization. (2018). *Cannabidiol (CBD): Critical review report*. Geneva: World Health Organization.

Wylie, G. R., Genova, H., DeLuca, J., Chiaravalloti, N., & Sumowski, J. F. (2012). Functional magnetic resonance imaging movers and shakers: Does subject-movement cause sampling bias? *Human Brain Mapping*, *35*(1), 1–13. https://doi.org/10.1002/hbm.22150

Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-

formation. *Journal of Comparative Neurology and Psychology*, *18*(5), 459–482.

https://doi.org/10.1002/cne.920180503

Young, C. B., Raz, G., Everaerd, D., Beckmann, C. F., Tendolkar, I., Hendler, T., Fernández, G.,

& Hermans, E. J. (2017). Dynamic shifts in large-scale brain network balance as a

function of arousal. *Journal of Neuroscience*, *37*(2), 281–290.

https://doi.org/10.1523/JNEUROSCI.1759-16.2016

Zhang, W., Qin, S., Guo, J., & Luo, J. (2011). A follow-up fMRI study of a transferable placebo

anxiolytic effect. *Psychophysiology*, *48*(8), 1119–1128. https://doi.org/10.1111/j.1469-

8986.2011.01178.x

Zhang, Y., Brady, M., & Smith, S. (2001). Segmentation of brain MR images through a hidden

Markov random field model and the expectation-maximization algorithm. *IEEE*

Transactions on Medical Imaging, *20*(1), 45–57. https://doi.org/10.1109/42.906424

Zhekova, R. M., Perry, R. N., Spinella, T. C., Dockrill, K., Stewart, S. H., & Barrett, S. P.

(2024). The impact of cannabidiol placebo on responses to an acute stressor: A

replication and proof of concept study. *Journal of Psychopharmacology*, *(Oxford,*

England), *38*(1), 116–124. https://doi.org/10.1177/02698811231219060

Zuardi, A. W., Cosme, R. A., Graeff, F. G., & Guimarães, F. S. (1993). Effects of ipsapirone and

cannabidiol on human experimental anxiety. *Journal of Psychopharmacology*, *7*(Suppl

1), 82–88. https://doi.org/10.1177/026988119300700112

Zuardi, A. W., Rodrigues, N. P., Silva, A. L., Bernardo, S. A., Hallak, J. E. C., Guimarães, F. S.,

& Crippa, J. A. S. (2017). Inverted U-shaped dose-response curve of the anxiolytic effect

of cannabidiol during public speaking in real life. *Frontiers in Pharmacology*, *8,* 259. https://www.frontiersin.org/articles/10.3389/fphar.2017.00259

Zuardi, A. W., Shirakawa, I., Finkelfarb, E., & Karniol, I. G. (1982). Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology*, *76*(3), 245–250. https://doi.org/10.1007/BF00432554

Table 1. Sample characteristics and between-group comparisons of included participants and participants excluded for head motion artifact.

Variable	Included Mean (SD)	Excluded Mean (SD)	t (df)	p
Age	23.97 (8.30)	25.80 (12.43)	0.43 (35)	0.67
STAI-T	38.23 (8.82)	51.40 (6.12)	3.20 (35)	**0.00**
PSS	15.03 (6.07)	22.60 (3.36)	2.70 (30)	**0.01**
CBD Beliefs				
Reduces Stress	7.31 (1.47)	7.00 (1.73)	-0.43 (35)	0.72
Reduces Anxiety	7.03 (1.56)	7.40 (1.82)	0.48 (35)	0.63
THC Beliefs				
Reduces Stress	7.03 (1.80)	6.40 (2.07)	-0.71 (35)	0.48
Reduces Anxiety	6.13 (2.18)	4.60 (2.30)	-1.44 (35)	0.16
Past-Month Cannabis Use (Days)	5.69 (7.84)	1.00 (1.41)	-3.08 (33.89)	**0.00**
CO (ppm)	4.40 (3.02)	4.82 (3.70)	0.35 (30)	0.73
Task Difficulty	6.79 (1.67)	7.90 (1.34)	1.39 (35)	0.17

Variable	Included N (%)	Excluded N (%)	X^2 (df)	p
Sex (Female)	15 (46.9)	5 (100)	4.91 (1)	**0.027**
Gender (Woman)	15 (46.9)	4 (80)	9.98 (2)	**0.007**
Ethnicity			1.86 (5)	0.868
White (European)	23 (71.9)	5 (100)		
Middle Eastern	3 (9.4)	-		
South Asian	2 (6.3)	-		
Black	1 (3.1)	-		
Mixed Ethnicity	3 (9.2)	-		

Note. STAI-T: State-Trait Anxiety Inventory (Spielberger, 1983); PSS: Perceived Stress Scale (Cohen et al., 1994); CO: Carbon Monoxide. STAI-T scores have possible values from 20-80; PSS scores have a possible value of 0-40; Past-month Cannabis use is defined as the number of days cannabis was used in the past month; CBD beliefs, THC beliefs, and Task Difficulty were rated on a scale from 1 (not at all) to 10 (completely or extremely). The proportion of motion outlier volumes exceeded 30% of total volumes in any run for participants excluded for head motion artifact (*n*=5). Excluded participants are compared to participants included in the final sample (*N*=32).

Table 2. Sample characteristics and between-group comparisons.

Variable	Entire Sample Mean (SD)	Told CBD Mean (SD)	Told CBD-free Mean (SD)	*t (df)*	*p*
Age	23.67 (8.30)	22.0 (3.02)	25.7 (10.9)	1.35 (30)	0.19
STAI-T	38.21 (8.82)	39.53 (10.34)	37.06 (7.37)	-0.79 (30)	0.44
PSS	15.03 (6.07)	14.27 (4.76)	15.71 (7.11)	0.66 (30)	0.51
CBD Beliefs					
Reduces Stress	7.31 (1.47)	7.13 (1.60)	7.47 (1.37)	0.64 (30)	0.53
Reduces Anxiety	7.03 (1.56)	7.07 (1.79)	7.00 (1.37)	-0.12 (30)	0.91
THC Beliefs					
Reduces Stress	7.03 (1.80)	7.29 (1.36)	6.73 (2.22)	0.85 (22.62)	0.41
Reduces Anxiety	6.13 (2.18)	6.23 (2.17)	6.00 (2.27)	0.30 (30)	0.77
Past-Month Cannabis Use (Days)	5.69 (7.85)	6.30 (7.72)	5.15 (8.15)	-0.41 (30)	0.68
Past-Week Cannabis Use (Days)	1.19 (1.99)	1.13 (1.88)	1.24 (2.14)	0.14 (30)	0.89
CO (ppm)	4.62 (3.34)	4.40 (3.02)	4.82 (3.70)	0.35 (30)	0.73
Task Difficulty	6.80 (1.67)	6.67 (1.50)	6.91 (1.85)	0.41 (30)	0.69

Variable	Entire Sample N (%)	Told CBD N (%)	Told CBD-free N (%)	X^2 *(df)*	*p*
Sex (Female)	15 (46.9)	7 (46.7)	8 (47.1)	0.00 (1)	0.982
Gender (Woman)	15 (46.9)	7 (46.7)	8 (47.1)	0.00 (1)	0.982
Ethnicity (White/European)				4.27 (5)	0.511
White (European)	23 (71.9)	11 (73.3)	12 (70.6)		
Middle Eastern	3 (9.4)	1 (6.7)	2 (11.8)		
South Asian	2 (6.3)	-	2 (11.8)		
Black	1 (3.1)	1 (6.7)	-		
Mixed Ethnicity	3 (9.2)	2 (13.4)	1 (5.9)		

Note. STAI-T: State-Trait Anxiety Inventory (Spielberger, 1983); PSS: Perceived Stress Scale (Cohen et al., 1994); CO: Carbon Monoxide. STAI-T scores have possible values from 20-80; PSS scores have a possible value of 0-40; Past-month cannabis use is defined as the number of days cannabis was used in the past month; Past-week cannabis use is defined as the number of days cannabis was used in the last week; CBD beliefs, THC beliefs, and Task Difficulty were rated on a scale from 1 (not at all) to 10 (completely or extremely). Adapted from Perry et al. (2024, under review).

Table 3. CBD-expectancy effects on intrinsic connectivity network resting-state functional connectivity prior to voxel-wise correction of the FDR.

Seed	Peak Voxel Coordinate (MNI)			Region	Intrinsic Connectivity Network	Z-value	Cluster Size (mm^3)
	X	Y	Z				
Positive (TC>TCF)							
PCC	2.5	-30.5	35.5	R Posterior Cingulate Gyrus	DMN	4.93	11880
	17.5	-36.5	-18.5	R Posterior Parahippocampal Gyrus	Visual	3.94	1836
	-3.5	-12.5	74.5	Juxtapositional lobule[a]	Somatomotor	3.87	2943
				Precentral Gyrus[a]			
				Superior Frontal Gyrus[a]			
	-21.5	-48.5	-54.5	L Cerebellum VIIIb		3.35	837
	-3.5	-57.5	-0.5	L Lingual Gyrus	Visual	3.23	1323
	62.5	-54.5	41.5	R Angular Gyrus[a]	CEN		
dACC	-15.5	-69.5	-24.5	L Cerebellum VI	-	4.03	13041
	29.5	-15.5	74.5	R Precentral Gyrus	Somatomotor	3.94	5454
	23.5	-12.5	-15.5	R Hippocampus	-	3.42	783
				R Amygdala[a]			
	-18.5	-57.5	-9.5	L Lingual Gyrus	Visual	3.36	1863
				L Temporal Occipital Fusiform Cortex[a]			

Seed	Peak Voxel Coordinate (MNI)			Region	Intrinsic Connectivity Network	Z-value	Cluster Size (mm³)
	X	Y	Z				
				L Occipital Fusiform Gyrus[a]			
	-9.5	-30.5	71.5	L Precentral Gyrus	Somatomotor	3.32	2835
				L Postcentral Gyrus[a]			
	-33.5	-0.5	50.5	L Middle Frontal Gyrus	Dorsal Attention	3.18	1701
				L Precentral Gyrus[a]			
dlPFC	47.5	20.5	-33.5	R Temporal Pole	DMN	5.29	972
	-9.5	14.5	2.5	L Caudate	-	3.47	675
	-54.5	-6.5	-21.5	L Anterior Middle Temporal Gyrus	DMN	3.46	3294
				L Posterior Middle Temporal Gyrus[a]			
	-0.5	-78.5	-30.5	L Cerebellum Vermis Crus II	-	3.43	2808
				L Cerebellum Right Crus II[a]			
				L Cerebellum Vermis VI[a]			
	-9.5	-30.5	-6.5	L Brainstem	-	3.39	729
				L Thalamus			
	-30.5	20.5	-9.5	L Frontal Orbital Cortex	CEN	3.39	621

Seed	Peak Voxel Coordinate (MNI)			Region	Intrinsic Connectivity Network	Z-value	Cluster Size (mm³)
	X	Y	Z				
				L Insular Cortex[a]			
	-42	-0.5	56.5	L Middle Frontal Gyrus	Dorsal Attention	3.24	972
				L Precentral Gyrus			
	-30.5	-63.5	-51.5	L Cerebellum VIIb	-	3.21	972
				L Cerebellum VIIa			
Negative (TCF>TC)							
PCC	26.5	-90.5	-9.5	R Occipital Pole	Visual	-4.15	16902
				R Occipital Fusiform Gyrus[a]			
				R Lateral Occipital Cortex[a]			
	-33.5	-93.5	-9.5	L Occipital Pole	Visual	-4.14	8370
				L Inferior Lateral Occipital Cortex[a]			
	-12.5	-75.5	-48.5	L Cerebellum VIIb		-4.02	1647
	41.5	-48.5	-15.5	R Temporal Occipital Fusiform Cortex	Dorsal Attention	-3.14	972
				R Inferior Temporal Gyrus			
	62.5	-6.5	41.5	R Precentral Gyrus[a]	Somatomotor	-3.18	4023
				R Postcentral Gyrus[a]			

Seed	Peak Voxel Coordinate (MNI)			Region	Intrinsic Connectivity Network	Z-value	Cluster Size (mm³)
	X	Y	Z				
dACC	38.5	-9.5	-36.5	R Posterior Temporal Fusiform Cortex	Limbic	-4.29	675
				R Posterior Temporal Fusiform Cortex[a]			
				R Anterior Temporal Fusiform Cortex[a]			
				R Inferior Posterior Temporal Gyrus[a]			
				R Inferior Anterior Temporal Gyrus[a]			
	5.5	-54.5	53.5	R Precuneous Cortex	Dorsal Attention	-3.75	3483
	14.5	-48.5	-60.5	R Cerebellum VIIIb	-	-3.57	567
				R Cerebellum IX			
	-60.5	-45.5	-6.5	L Temporooccipital Middle Temporal Gyrus	CEN	-3.48	3672
				L Posterior Middle Temporal Gyrus[a]			
	50.5	-3.5	-27.5	R Anterior Middle Temporal Gyrus	DMN	-3.27	3672
				R Anterior Inferior Temporal Gyrus[a]			
				R Posterior Middle Temporal Gyrus[a]			
	20.5	23.5	65.5	R Superior Frontal Gyrus[a]	DMN	-3.14	918

Seed	Peak Voxel Coordinate (MNI)			Region	Intrinsic Connectivity Network	Z-value	Cluster Size (mm³)
	X	Y	Z				
dlPFC	41.5	-48.5	-39.5	R Cerebellum Crus I	-	-3.60	594
				R Cerebellum Crus II[a]			
	-51.5	-12.5	26.5	L Postcentral Gyrus	Somatomotor	-3.34	729
				L Precentral Gyrus[a]			

a) <25% probability of the region was identified at the peak voxel location

*Note.* Voxel threshold *p*<0.05, cluster threshold of 20 voxels, Z>3.1; TC: Told CBD condition; TCF: Told CBD-free condition; PCC: right posterior cingulate cortex; dACC: right dorsal anterior cingulate cortex; dlPFC: right dorsolateral prefrontal cortex; L: Left; R: Right; DMN: default mode network; SN: salience network; CEN: central executive network. Intrinsic connectivity networks were defined using the 7 network 400 parcel Shaefer atlas (Schaefer et al., 2018).

Table 4. Raw means and standard errors for subjective outcomes: group x time.

Outcome Measure	Time	Told CBD	Told CBD-free
		Mean (SE)	Mean (SE)
Stress	T1 (Baseline)	3.0 (0.51)	2.6 (0.45)
	T2 (MRI Baseline)	2.3 (0.60)	2.5 (0.36)
	T3 (Post-oil)	2.2 (0.42)	2.0 (0.41)
	T4 (Post-stressor)	5.4 (0.56)	4.5 (0.58)
	T5 (Anticipation)	3.3 (0.44)	2.4 (0.41)
	T6 (Recovery)	2.7 (0.47)	2.2 (0.42)
Anxiety	T1 (Baseline)	2.5 (0.42)	2.4 (0.40)
	T2 (MRI Baseline)	2.6(0.50)	2.6 (0.35)
	T3 (Post-oil)	1.9 (0.34)	2.1 (0.38)
	T4 (Post-stressor)	4.6 (0.63)	3.7 (0.52)
	T5 (Anticipation)	2.9 (0.47)	2.6 (0.42)
	T6 (Recovery)	2.3 (0.40)	2.1 (0.41)
Sedation	T1 (Baseline)	2.7 (0.70)	2.6 (0.64)
	T2 (MRI Baseline)	2.9 (0.62)	3.2 (0.67)
	T3 (Post-oil)	3.8 (0.71)	3.7 (0.60)
	T4 (Post-stressor)	2.6 (0.39)	2.6 (0.47)
	T5 (Anticipation)	3.4 (0.43)	2.9 (0.51)
	T6 (Recovery)	3.1 (0.44)	3.1 (0.60)
Energy	T1 (Baseline)	5.5 (0.58)	5.0 (0.66)
	T2 (MRI Baseline)	4.0 (0.51)	3.6 (0.45)
	T3 (Post-oil)	3.9 (0.51)	3.6 (0.44)
	T4 (Post-stressor)	4.9 (0.56)	4.6 (0.47)
	T5 (Anticipation)	4.5 (0.52)	4.0 (0.49)
	T6 (Recovery)	4.1 (0.34)	4.5 (0.53)

Note. Raw means correspond to a more restricted head motion sample of N=32. All subjective outcomes were rated on a scale from 1 (not at all) to 10 (extremely).

Figure 1. Intrinsic connectivity networks in MNI152 space.
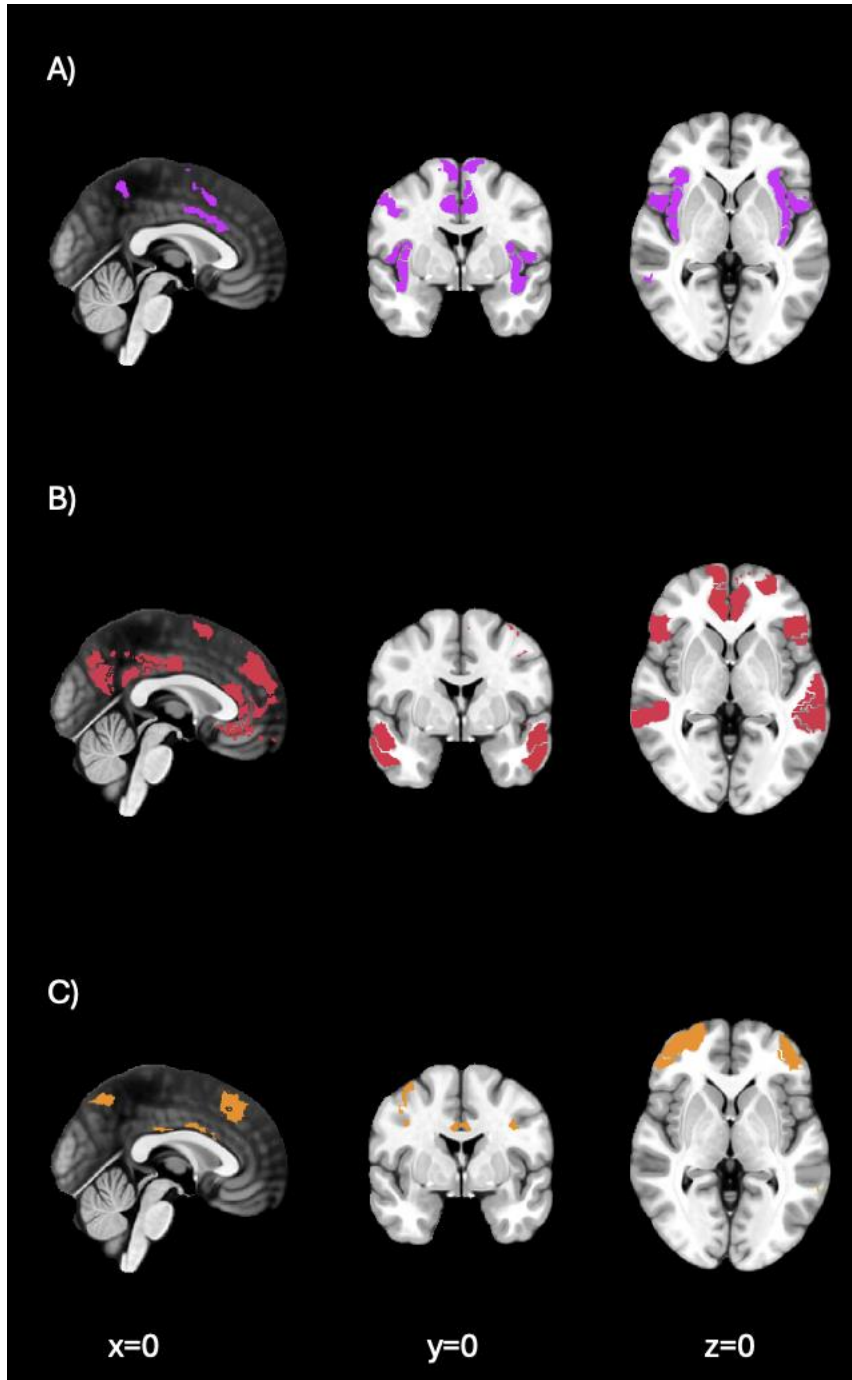
Note. Intrinsic connectivity networks as defined by the 7 network 400 parcel Shaefer atlas (Schaefer et al., 2018) in MNI152 space. A) The salience network. B) The default mode network. C) The central executive network. Plots were derived in FSL (Jenkinson et al., 2012; Smith et al., 2004; Woolrich et al., 2009).

Figure 2. Experimental session timeline.
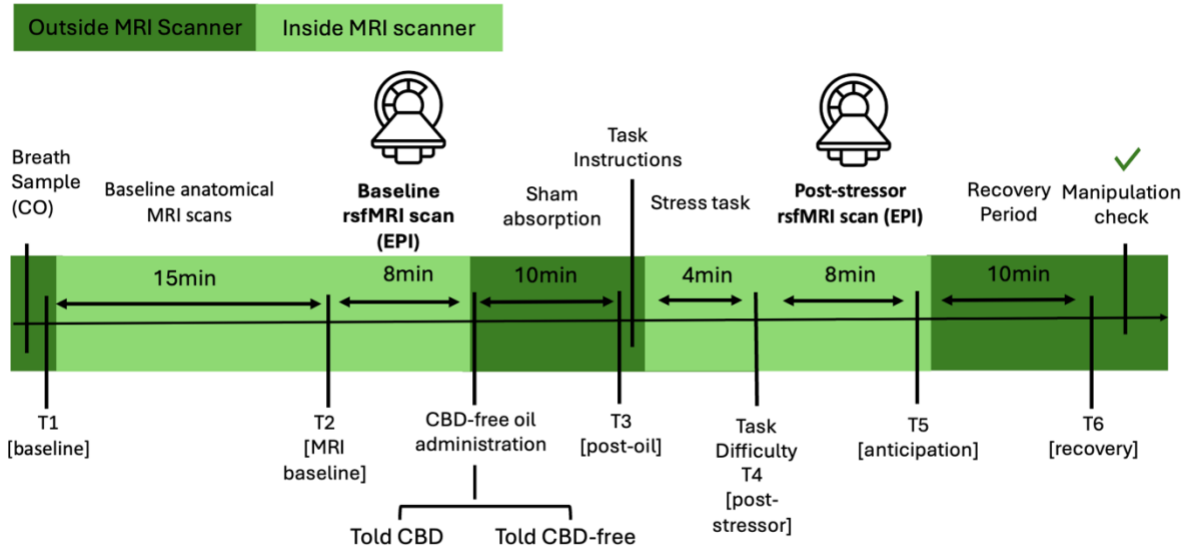
Note. T: Timepoint at which subjective measures were assessed, which included stress, anxiety, energy, and sedation, all measured on a scale from 1 (not at all) to 10 (extremely); CO: Carbon Monoxide; rsfMRI: resting-state functional magnetic resonance imaging; EPI: Echo Planar Imaging; Baseline and Post-Stressor rsfMRI scans are bolded, as these reflect the two primary time points of interest for the rsFC analysis. Light green and dark green shading differentiate measures taken outside vs inside the MRI scanner, respectively. Task difficulty was rated on a scale from 1 (not at all) to 10 (extremely). Adapted from Perry et al. (2024, under review).

Figure 3. ROIs in MNI152 space.
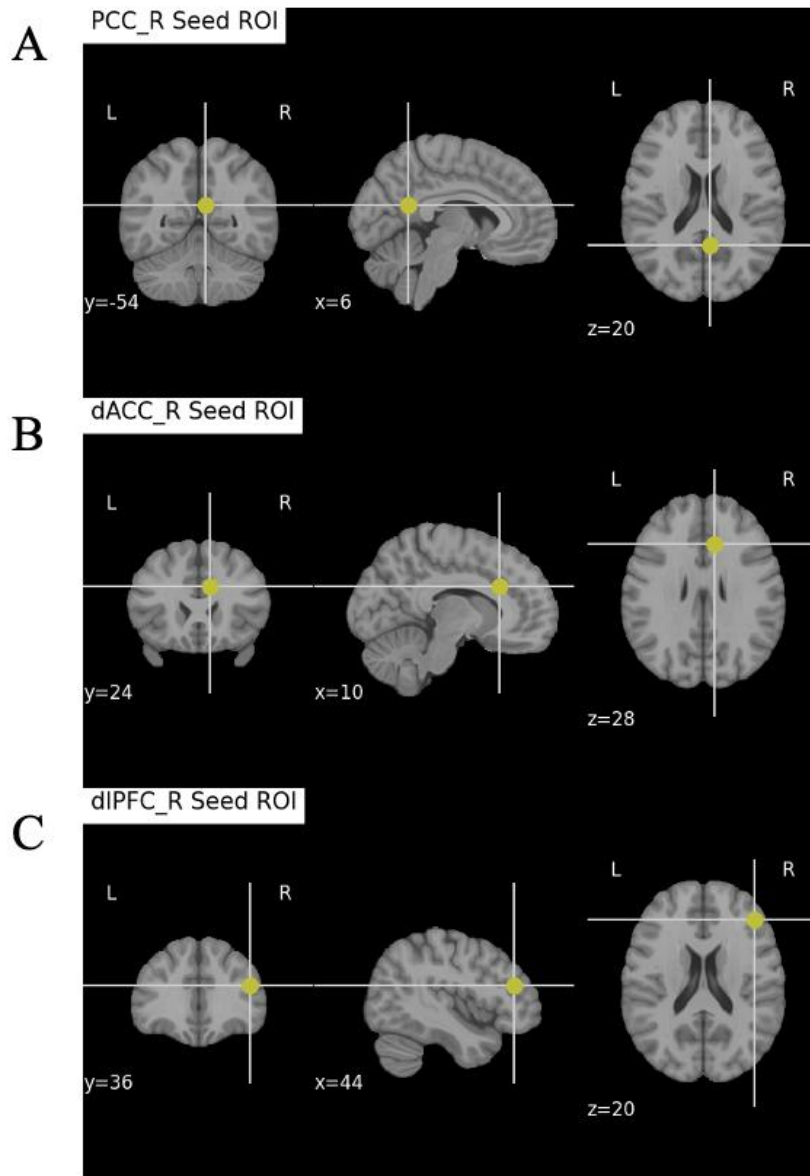
Note. 7mm Seed Regions of Interest (ROI) used for the rsFC analyses. R: Right; PCC: posterior Cingulate Cortex; dACC: dorsal Anterior Cingulate Cortex; dlPFC: dorsolateral Prefrontal Cortex; All seeds were defined as a 7mm sphere created using NiftiSphereMasker from *Nilearn* 0.9.0 in standard MNI152 template space. (A) R PCC [-54, 6, 20]. (B) R dACC [R: 24, 10, 28]. (C) R dlPFC [36, 44, 20]. Plots were derived in *Nilearn* version 0.9.0 with *nilearn.plotting*.

Figure 4. CBD expectancy effects on functional connectivity with distinct nodes of intrinsic connectivity networks prior to voxel-wise correction of the FDR.
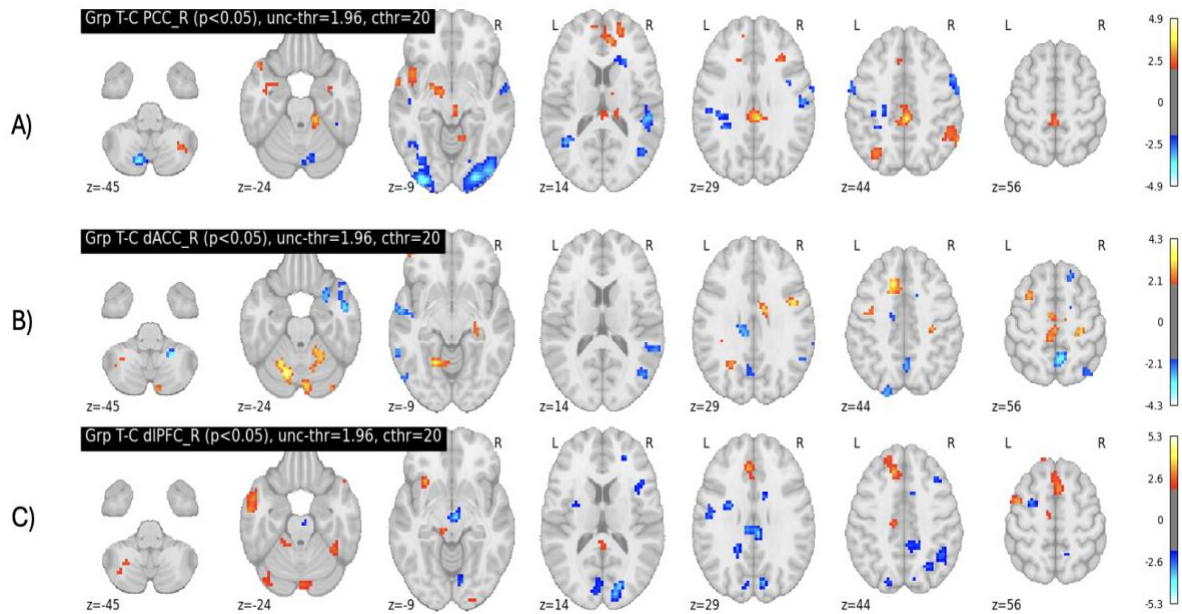
Note. Z-score map of sequential axial brain slices with a voxel threshold of $p<0.05$, a cluster threshold (cthr) of 20 voxels, and a Z-score threshold (unc-thr) of $Z>1.96$. Higher Z-scores represent stronger correlations between voxel clusters and regions of interest. Clusters in the red spectrum demonstrate increased functional connectivity in the Told CBD condition (Group T), whereas clusters in the blue spectrum demonstrate increased functional connectivity in the Told CBD-free condition (Group C). PCC: right posterior cingulate cortex; dACC: right dorsal anterior cingulate cortex; dlPFC: right dorsolateral prefrontal cortex; R: Right. Plots were generated using *Nilearn* version 0.9.0 with *nilearn.plotting.plot_stat_map*.

Figure 5. Estimated marginal means (± standard error) for subjective outcomes.
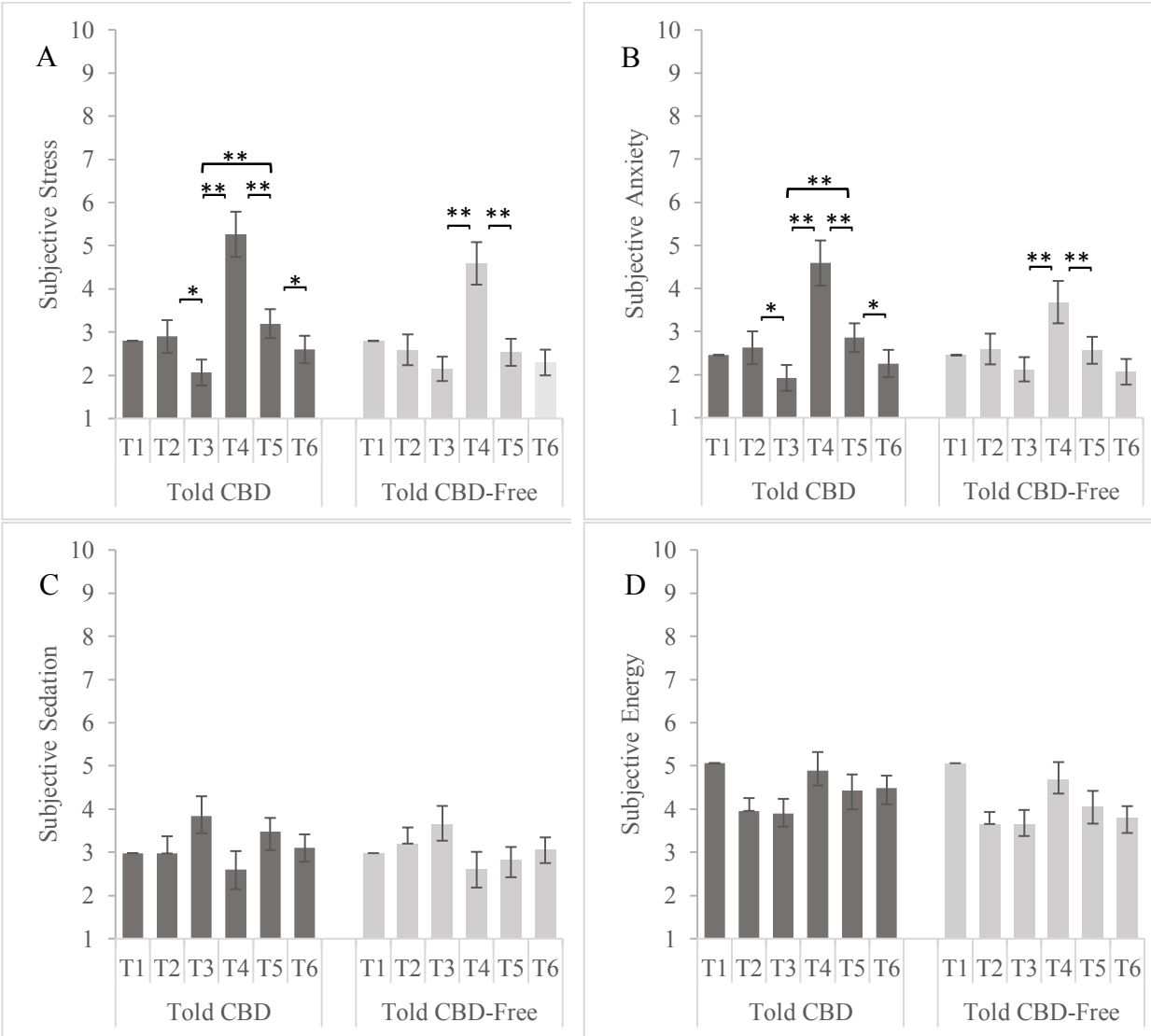
Note. Subjective results presented in panels A-D correspond to a more restricted head motion sample of N=32 with FDR correction. Stress, anxiety, sedation, and energy were rated on a scale from 1 (not at all) to 10 (extremely). T1-T6 refer to the time-points for subjective assessments (i.e., T1: Baseline; T2: in scanner; T3: post-oil; T4: post-stressor; T5: anticipation; T6: recovery). All panels show the pairwise breakdown of subjective ratings by time, within each of the two expectancy conditions (Told CBD vs. Told CBD-free). For panels A and B, timepoint 4 was significantly different from all other timepoints within each expectancy condition, indicating that the counting task effectively increased subjective stress and anxiety. Time varying covariates specified in each model are shown as baseline values. Adapted from Perry et al. (2024, under review). $**p<0.001$ $*p<0.05$.

<u>Participants needed for a 2-session CBD and brain imaging study</u>

If you:
 ➢ Are **19** years of age or older
 ➢ Have used cannabis at least once in your life

You may be eligible to participate in a research study examining the impact of CBD oil on brain activity and cognitive performance.

Each participant will be required to attend one orientation session at Dalhousie University (1 hour) and one experimental session (2 hours) at the Queen Elizabeth II Hospital. Participants will be asked to consume either hemp seed oil or CBD oil, engage in a brief cognitive task, and undergo brain imaging scans (functional MRI). All participants will be compensated for their time.

If you are interested and would like additional information please email us at **dal.substance.use.lab@gmail.com** with your name and a phone number where you can be reached. You can also contact us by telephone at **(902)-494-4596**.

Participation in the study is voluntary and all responses to this advertisement are strictly confidential.

Healthy participants needed for CBD & Brain Imaging study

If you:
1. Are **19 years** of age or older
2. Have used cannabis **at least once** in your life

You may be eligible to participate in a research study examining the impact of CBD oil on brain activity and cognitive performance.

Each participant will be required to attend one orientation session at Dalhousie University (1 hour) and one experimental session (2 hours) at the Queen Elizabeth II Hospital. Participants will be asked to consume either hemp seed oil or CBD oil, engage in a cognitive task, and undergo brain imaging scans (functional MRI). All participantes will be compensated **$20 per hour** for their time.

If you are interested and would like additional information, email us at **dal.substance.use.lab@gmail.com** with your name and phone number. You can also contact us by phone at **(902)494-4596**.

Participation in this study is **voluntary** and all responses to this advertisement are strictly **confidential**.

APPENDIX B: TELEPHONE SCREENING

Telephone Screening Interview: CBD fMRI Study

Date: _____   **Interviewer:** _____

Temporary Subject ID: _____

Hello, I am _____ calling from the Dalhousie Substance Use Laboratory about a study that you recently expressed interest in. Do you have some time right now to go over details about the study? First, I will tell you a little about the study, then I will ask you a few questions about your health and substance use history. The goal of this study is to improve our understanding of how one of the ingredients found in cannabis called cannabidiol, or CBD, impacts brain activity and thinking abilities. The study will take place over 2 sessions at Dalhousie University and the Queen Elizabeth II Hospital.

The first baseline session will take about one hour. You will be asked to provide basic demographic information, such as your age and marital status, as well as answering questionnaires about your drug use and beliefs about CBD. We will also need to measure your weight to figure out the exact dose of CBD or hemp seed oil to give you during the experimental session.

The second experimental session will take about 2 hours. During this session, your brain activity will be measured with MRI before and after you take a CBD oil or a CBD-free hemp seed oil, and you will be asked to complete a simple mental task while you are inside the MRI scanner.

Before coming to your experimental session, you will not be able to use cannabis, alcohol, nicotine, tobacco, or take any other drugs for 12 hours before your session start time. At the beginning of the experimental session you will be asked to give a breath sample, which will be used to measure how long ago you smoked. If the breath sample shows that you have smoked less than 12 hours before the session, we will reschedule the session for another day. We also ask that you eat your normal meals throughout the day, but avoid eating anything or drinking any caffeinated beverages for 2 hours before the session.

With this information in mind, are you willing to not use cannabis or take any other drugs before coming for the experimental session?

Circle one: YES   NO

If YES: proceed with the telephone interview; if NO: politely inform the participant that they do not meet the criteria to participate in this study.

If you find that you are unable to go without cannabis, alcohol, cigarettes or any other drugs for the required period of time, please contact us by phone or email, and we can reschedule the session for a later date.

Shortly after arriving at the lab, the study will be explained to you in detail and you will have an opportunity to choose whether or not you wish to continue with the study.

At the end of each study session, you will be given $20 per hour, or part thereof, for participating in this study. Therefore, total payment for completing both sessions will be $60. If you will be driving to the baseline study sessions, we can provide you with a free parking pass. Even though CBD does not have any intoxicating or serious side effects that may affect motor coordination, CBD oil may contain trace amounts of THC, so it is strongly recommended that you do not drive to the experimental session. We will not be able to provide a parking pass for this session but we will offer reimbursement for bus fare should you decide to take public transportation to and from the experimental session.

To ensure the health, safety and well-being of our research team and study participants, we are requiring that all participants be fully vaccinated against COVID-19. Participants will need to show proof of vaccination along with a piece of government-issued photo ID before beginning the first study session, however this information will not be recorded or stored.

Are you interested in participating in this study? If yes, I will need to ask you some questions to make sure that you meet the requirements to participate. This will include several questions about your health and medication use and will take about 10 minutes. Is this OK? If you do not feel comfortable with any question, you do not have to provide an answer and are free to end this telephone interview at any time. All information you provide is strictly confidential. This means that nobody aside from the study researchers will have access to your information.

Question	Response	Interviewer Response
Have you received at least 2 doses of a Government of Canada-accepted COVID-19 vaccine or at least 1 dose of the Janssen/Johnson & Johnson vaccine?	☐ Yes ☐ No	Reject if N
Are you taking any prescription medications: Specify:	☐ Yes ☐ No	Reject if Yes unless it is birth control in females
Do you have a **current** psychiatric diagnosis or a diagnosis of a psychological/mental illness?	☐ Yes Specify: ☐ No	Reject if current DSM-5 diagnosis
Do you have a **current** diagnosis of a serious medical condition?	☐ Yes Specify: ☐ No	Reject if Yes
Are you allergic to any of the following: • Cannabis • Hemp seed	☐ Yes Specify: ☐ No	Reject if Yes

1. How old are you?		Reject if under 19
2. What is your birthday?	YY:_____ MM:_____	** Only record the year and month **
3. What is your dominant hand?		Reject if Left
4. Have you ever used cannabis in your lifetime?	☐Y ☐N	Reject if N
5. Have you used cannabis in the last month? 5a. IF YES: How many days per week do you use cannabis?	☐Y ☐N _____days/week	
6. Have you ever used cannabis oil orally, in liquid form? (*Note:* this includes CBD and THC, alone or combined)	☐Y ☐N	
7. Please indicate on a scale from 1 (not at all) to 10 (very much so), how effective do you think cannabis is as a stress reliever?	Record #:	

8. Again on a scale from 1 to 10, where 1 is not at all and 10 is completely how much you agree with the following statement: "Stress relief from cannabis is caused by CBD"	Record #:	
9. Please indicate on a scale from 1 (not at all) to 10 (very much so), how effective do you think cannabis is as an anxiety reliever?	Record #:	
10. Again on a scale from 1 to 10, where 1 is not at all and 10 is completely how much you agree with the following statement: "Anxiety relief from cannabis is caused by CBD"	Record #:	
11. **For Females:** Are you currently pregnant, planning to get pregnant, or nursing a baby at this time?	☐Y ☐N	Reject if Y
12. Are you claustrophobic (fear of small spaces)?	☐Y ☐N	Reject if Y
13. Have you ever participated in a research study before? *Note. If they have participated in a research study, ask details to see if it was a balanced-placebo design or involved a stress induction paradigm using the Maastricht Acute Stress Test (e.g. what did you do in the study? Do you remember whose lab this was in? *DO NOT ask specifically if it was a BPD/MAST*)*	☐Y ☐N Specify:	Reject if balanced-placebo design (Barrett lab e.g. caffeine study, fMRI smoking study, etc.) Reject if previously exposed to MAST (Barrett lab e.g. Vaping & Stress study, CBD study, etc.)
14. Are you able to come in to the Brain Imaging Lab on Mondays, Wednesdays or Fridays? You will need to be available for approximately 3 hours. **IF YES:** please specify availability.	☐Y ☐N ☐ M: _____ ☐ W: _____ ☐ F: _____	Reject if N
15. Are you a current cigarette/tobacco smoker?	☐Y ☐N	**If Y proceed with FTND, If N skip FTND.**

MRI Pre-screen

Note: This prescreen does not replace the one done by the MRI technician

1. How much do you weigh? We need to know as the MRI has a set weight tolerance.
    _____ lbs / kg

2. Have you undergone surgery involving metal, such as: clips, rods, screws, pins, and/or wires?   Y   or   N.   Notes: _____

3. Do you have a heart pacemaker?   Y   or   N.

4. Do you have implanted electrodes, pumps or electrical devices?   Y   or   N.

5. Do you have cochlear (inner ear) implants?   Y   or   N.

6. Do you have eye implants?   Y   or   N.

7. Do you have any metallic foreign body, shrapnel or bullets embedded in your body? (Have you ever been a grinder, metal worker, welder, or wounded during military service?)
    Y   or   N.   Notes: _____

8. Do you have an intrauterine contraceptive device (IUD) or contraceptive diaphragm?
    Y   or   N.

9. Do you have any dental work held in place by magnets?   Y   or   N.

10. Do you have an non-removable dental braces and retainers?   Y   or   N.

11. Do you have metal dental work, not made of predominantly precious or semiprecious alloy or amalgam?   Y   or   N.   Notes: _____

12. Do you have tattooed eyeliner?   Y   or   N.

13. Do you have tattoos (some tattoos have metal fragments as pigments)?   Y   or   N.

    Where on your body? _____

    Are they recent within the last 2 weeks?   Y   or   N

14. Do you have an non-removable metal jewelry (body piercing)?   Y   or   N.

15. Are you using nicotine and/or contraceptive patches?   Y   or   N.

Passed MRI prescreen?   Y   or   N.   MRI prescreen failure?   Y   or   N.

RA initials _____

IF MEET REQUIREMENTS:

Schedule initial baseline session: _____
**ASK FOR/CONFIRM EMAIL ADDRESS (<u>DO NOT</u> WRITE ON THIS SHEET)

Is it okay if I keep your name in a database to be contacted regarding this study or other studies in the future?

☐Y   ☐N

In consideration of the ongoing COVID-19 pandemic, our lab is continuing to adhere to public health guidelines to ensure the health, safety, and well-being of our study participants and research team. In accordance with Nova Scotia Public Health, all faculty, staff, students, and visitors are asked to continue to wear masks in shared indoor common spaces. If you are feeling sick at any point while participating in the study, it is important to please stay home and closely monitor your health. To help us prevent the spread of COVID-19, we will ask that you answer a few standard screening questions prior to coming in for your study sessions.

In addition, we ask that you please bring your proof of vaccination along with a piece of government-issued photo identification to the baseline session. You will be required to show your proof of vaccination to the experimenter at the start of your baseline sessions; however, this information will not be recorded or stored.

IF DO NOT MEET REQUIREMENTS:
Currently you are not eligible to participate in the study, however because the requirements may change it is possible that you may be eligible at a later time or, that based on your answers you might be eligible for other studies that we are conducting in the lab. Is it okay if I keep you name and phone number in a database to be contacted regarding this study or other studies in the future?

☐Y   ☐N

May we have your permission to analyze the information which you have provided throughout this telephone interview? The information you have provided will be used in research about the selection criteria for this study.

Your data will not be connected to your personal identity. All of your information will be connected only with your participant ID, and no individual data will be used. All information will be kept for up to 7 years in a locked filing cabinet in Dr. Barrett's secure laboratory. All of your contact information and data will be recorded and stored separately.

☐Y   ☐N

Informed Consent Form Non-Interventional Study

STUDY TITLE:	**The impact of cannabidiol (CBD) on brain activity and cognitive performance.**
PRINCIPAL INVESTIGATOR:	Dr. Sean Barrett Department of Psychology and Neuroscience Life Science Center, Dalhousie University 1355 Oxford Street, Halifax, Nova Scotia CANADA (B3H 4H6) Telephone: (902) 494-2956
SUB-INVESTIGATOR:	Dr. Sherry Stewart Department of Psychiatry QEII Abbie Lane 5909 Veterans Memorial Lane HALIFAX, NS, CANADA (B3H 2E2) Telephone: (902)-494-3793 Dr. Phil Tibbo Nova Scotia Early Psychosis Program 5909 Veterans Memorial Lane AJLB Room 3030 Halifax, NS CANADA (B3H 2E2) Telephone: (902) 473-1678
FUNDER:	Canadian Institutes of Health Research (CIHR)

Introduction

You have been invited to take part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

The research team will tell you if there are any study timelines for making your decision.

Please ask the research team to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

The researchers will:
- Discuss the study with you
- Answer your questions
- Keep confidential any information which could identify you personally
- Be available during the study to deal with problems and answer questions

You are being asked to consider participating in this study because you have indicated you are 19 years of age or over, you have used cannabis at least one (1) time in your life.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.

2. Why Is There A Need For This Study?

The cannabis plant contains naturally occurring compounds called cannabinoids. One of these cannabinoids, cannabidiol (CBD) is found in rich quantities in cannabis plants. In recent years, there has been increased interest in using CBD as a therapeutic substance (e.g. treatment), particularly for a number of psychiatric conditions. Tetrahydrocannabinol (THC) is the main psychoactive ingredient in cannabis, meaning that it produces some degree of intoxication when it is consumed. On the other hand, CBD does not possess intoxicating effects when consumed, which makes it more desirable for use in treatment settings. However, we still don't completely understand how CBD works in the brain, or how it affects your thinking abilities. This research looks at how CBD impacts your brain activity and thinking.

3. How Long Will I Be In The Study?

This study involves a telephone screening (15 minutes), two (2) study sessions. The first session should take no more than 1 hour. The second session should be scheduled within two weeks of the first and will last approximately 2 hours.

Participants will also receive a follow-up telephone call to explain the study in further detail, which will take approximately 10 minutes once data collection is completed.

The study is expected to take about twelve (12) months to complete, and the results should be known in two (2) years.

4. How Many People Will Take Part in This Study?

It is anticipated that about forty-eight (48) individuals (19+) from throughout Halifax, NS, Canada will take part in the study. Participation for this study will take place in the Dalhousie Substance Use Lab and the Neuroimaging Research Laboratory (BIOTIC) at the Abbie J. Lane Building in the Queen Elizabeth II Hospital.

5. How Is the Study Being Done & What Will Happen If I Partake?

The purpose of this study is to understand how CBD consumption impacts brain activity and thinking. If you agree to participate, and you are eligible (as determined by a telephone screening interview), you will be required to take part in two (2) study sessions at Dalhousie University and the Queen Elizabeth II Hospital.

The first session will take less time (1 hour) and will mostly involve completing questionnaires. For the second session, participants will be randomly assigned to receive either a CBD or a CBD-free hemp seed oil during the session. A researcher will tell you whether the oil contains CBD before you consume it. You will also be asked to complete a simple counting task, fill out questionnaires that focus on your subjective state, and have your brain activity measured using MRI (i.e., brain imaging) at several time points throughout the session.

A. TELEPHONE SCREENING – 15 Minutes

To be a participant in this study and to sign this consent form, you must have agreed to and successfully completed a telephone screening interview. This telephone screening was done to make sure that you meet all the criteria to participate in this study. More specifically, you have told us that you are nineteen (19) years of age or older, you have used cannabis at least once in your life, your current cannabis use (i.e., in the past month) does not exceed two days a week, and you have never used any form of oral cannabis oil, including oral CBD oil. You have also told us that you are not currently taking any prescription medications (excluding birth control for females), and that you do not have any current serious health or psychiatric diagnoses.

B. BASELINE SESSION – 1 Hour

During the baseline session, you will be asked to complete a series of questionnaires that look at basic demographic information (e.g., your age, employment status, and education level), cannabis use history, mood and overall subjective state, and your knowledge about CBD. You will also be asked to fill out a week-long calendar assessing your substance use for the past week. We will also need to check your weight to determine the exact dose of CBD or hemp seed oil to give you, should you be assigned to receive the CBD oil.

At the end of session 1, you will be compensated for your time, and scheduled for your first magnetic resonance imaging (MRI) session (session 2). You will also have a chance to ask any questions you may have at this time about the MRI scanner session.

C. EXPERIMENTAL LABORATORY SESSIONS – 2 Hours

Before coming in for your experimental session, we expect you:
- **Not to consume cannabis** for at least 12 hours before coming into the lab.
- **Not to consume any tobacco, alcohol or use any illicit drugs** for 12 hours before coming into the lab.
- N**ot to eat or consume caffeine (coffee, pop, tea)** for 2 hours before coming into the lab.

- **Notify the research team** if you are running late, or if you need to miss or re-schedule your appointment for any reason. It is _especially important_ to try not to miss or re-schedule the MRI session as access to the facility is expensive. So, if you know you cannot make an appointment, please let us know as soon as possible (i.e., so there is a chance the MRI scanner can be used for someone else).

During the experimental session:
- You will first be asked to provide a breath sample by breathing through a sterile tube. This breath sample will be used to verify that you have not smoked or used cannabis for 12 hours before the session start time.
- You will then be asked to complete a series of questionnaires looking at your mood and subjective states. You will complete these same questionnaires at four time points throughout the session.
- You will have your brain activity measured using MRI at two time points throughout the session, both before and after consuming a CBD or the CBD-free hemp seed oil.
- You will be asked to consume **either** hemp seed oil or CBD oil contained in a syringe, provided by the researcher. You will be instructed to hold the oil under your tongue (i.e., sublingual) for 60 seconds. Following sublingual administration there will be a 10-minute absorption period. The CBD content of the oil you receive will be determined by random chance. You will be told the type of oil you have been assigned to receive before consuming it.
- After consuming the oil, you will complete a counting task, involving mental math, while you are inside the MRI scanner. The task will be completed in two 4-minute trials, with a rest period in between each trial.
- At the end of the session, you will be asked complete one additional questionnaire about your experience participating in the study.
- Once all study procedures are completed, you will be compensated accordingly.
- Upon completion of data collection, you will receive a final debriefing call to further explain the goals and nature of the study.

It is important that you tell the research team about any drugs or medicines you are taking or wish to take. You must also tell the research team about anything unusual that is happening with your health. This includes any medical problems that seem to be getting worse. If you need to see another doctor or have to go to a hospital, you should let the doctors know that you are in a research study. You should also tell your own doctor as quickly as possible, for your safety.

Of course, you may ask not to have further tests done, at any time. You will be free to leave at any time to take bathroom breaks or to withdraw from the study completely.

6. Are There Risks to The Study?

CBD oil: Research has shown that CBD is safe for human consumption, even at high doses with repeated use. Nevertheless, there is a very small possibility that you experience some side effects. All possible side effects that have been reported are temporary and will disappear once

CBD is excreted from your body. Side effects can include tiredness, diarrhea, changes in appetite, and altering how other drugs are metabolized in your body. If you at any point experience these side effects, you will be allowed any time you need to take a break or end the session completely.

Hemp seed oil: Hemp seed oil is an inactive substance often used as a nutritional supplement. It contains proteins and high-quality fatty acids which have been shown to have positive health effects and promote optimal human nutrition. There are no reported side effects for hemp seed oil.

Magnetic resonance imaging (MRI): The MRI scans require you to lie in a small space. Some people find this unpleasant. If you do, the examination can be stopped at any time. As well, we will supply you with soft earplugs to reduce the noise from the MRI scanner (the sound it produces is a loud knocking noise). The noise may make it difficult for you to communicate with the MRI technician. The technician can pause the machine and allow you to speak to him/her. Also, some people complain of temporary hearing problems that quickly disappear after the scan is finished. In addition, there are unknown risks associated with receiving a MRI scan during pregnancy. As a precaution, we will be excluding individuals who are currently pregnant, may be pregnant, or are trying to become pregnant.

In approximately 1-5% of MRI research scans on healthy volunteers, a researcher sees something which suggests the presence of a medical question. If this should occur in your case, the researcher will contact a medical specialist to review your scan. If the specialist decides that there is no indication of a problem, nothing further will be done. If the specialist decides that further medical follow-up should be considered, your doctor will be contacted. It is for this reason that we ask you to provide us with the name of your doctor in the unlikely event that it will be needed. If you do not have a regular physician, but have attended a walk-in clinic, you may indicate that clinic as your physician. If you do not specify the name of a physician, and we detect something that requires follow-up, a medical specialist who is part of the research team will contact you.

For certain people, medical investigations can be upsetting. For some, the existence of a diagnostic investigation (however it turns out) can affect insurance coverage. You should not take part in this research if you do not wish the remote possibility of future medical investigation.

It is important to understand that the MRI scans we will do in this study are for research purposes only. Although it is possible that we may detect an abnormality, if one exists, it is also possible that we would not detect such an abnormality. This is because the MRI scans we will do are different from the ones that might be done for clinical diagnosis.

Questionnaires: You may find the questionnaires you receive during this study upsetting or distressing. You may not like all the questions that you will be asked. You do not have to answer those questions you find too distressing.

Breach of confidentiality: As with all research, there is a small chance that confidentiality could be compromised; however, we are taking precautions to minimize this. More specifically, you will be given a de-identified code number when you are enrolled in the study. Only this code number will be connected to the information that you provide during the study. This is done to make sure that your study information cannot be linked back to any personally identifying information (e.g., your name or contact information). Participant names and contact information will be stored separately on a password-protected computerized datasheet, which will be locked in the Substance Use Research Laboratory at Dalhousie University. Only researchers directly involved with this study will have access to your study information or any personally identifying information.

7. Are There Benefits of Participating in This Study?

We cannot guarantee or promise that you will receive any benefits from this research. However, possible benefits include the generation of new information about cannabis and CBD. Your participation may contribute to novel findings about the impact CBD has on human behavior and brain activity.

8. What Happens at the End of the Study?

Once data completion is completed, you will receive a call detailing the study aims and will have an opportunity to ask the researcher any questions you may have, and the chance to withdraw your study data.

It is anticipated that the results of this study will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified.

9. What Are My Responsibilities?

As a study participant you will be expected to:
- Follow the directions of the research team or Principal Investigator.
- Report all medications being taken or that you plan on taking.
- Report any changes in your health to the research team or Principal Investigator.
- Report any problems that you experience that you think might be related to participating in the study.

10. Can My Participation in this Study End Early?

Yes. If you chose to participate and later change your mind, you can say no and stop the research at any time. If you wish to withdraw your consent, please inform the research team. If you choose to withdraw from this study, your decision will have no effect on your current or future medical treatment and healthcare. If you withdraw from the study, you will also have the option of withdrawing personal data collected up to that point, or of giving permission to the research team to analyze data collected from you to that point.

Additionally, the Canadian Institutes of Health Research (CIHR), the Nova Scotia Health Research Ethics Board (NS Health REB), or the Principal Investigator have the right to stop participant recruitment or cancel the study at any time.

Lastly, the principal investigator may decide to remove you from this study without your consent for any of the following reasons:

➢ You do not follow the directions of the research team or Principal Investigator.
➢ In the opinion of the Principal Investigator, you are experiencing side effects that are harmful to your health or well-being.
➢ There is new information that shows that being in this study is not in your best interests.

If you are withdrawn from this study, a member of the study team will discuss the reasons with you.

11. What About New Information?

You will be told about any other new information that might affect your health, welfare, or willingness to stay in the study and will be asked whether you wish to continue taking part in the study or not.

12. Will It Cost Me Anything?

<u>Compensation</u>

To thank you for your time, you will receive payment of $20 per hour or part thereof as compensation for taking part in this study. The baseline session will last approximately 1-hour, and the experimental session will last about 2 hours. Thus, you will be eligible to receive $60 for participating in both study sessions. Participants who will be driving to the baseline session will also be provided with a free parking pass. Importantly, even though CBD does not possess any intoxicating properties, nor does it have any serious side effects that may alter motor coordination, **it is strongly recommended that participants do not drive to the experimental session due to the possibility of increased feelings of tiredness or sedation**. We will therefore not be able to provide parking passes for these sessions; however, **we will offer reimbursement for bus fare should you decide to take public transportation to and from the experimental session**.

If you decide to leave the study early, you will receive a prorated payment for the time you did participate in the study.

<u>Research Related Injury</u>
If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a subject. In no way does this waive your

legal rights nor release the principal investigator, the research staff, the study sponsor or involved institutions from their legal and professional responsibilities.

13. What About My Privacy and Confidentiality?

Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made. If the results of this study are presented to the public, nobody will be able to tell that you were in the study.

However, complete privacy cannot be guaranteed. For example, the principal investigator may be required by law to allow access to research records.

If you decide to participate in this study, the research team will look at your personal health information and collect only the information they need for this study. "Personal health information" is health information about you that could identify you because it includes information such as your:
- Name,
- Address,
- Telephone number,
- Age or month/year of birth (MM/YY),
- Information from the study interviews and questionnaires,
- The types, dates, and results of various procedures.

Access to Records
Other people may need to look at your personal information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines. These people might include:
- The Canadian Institutes of Health Research (CIHR)
- The Nova Scotia Health Research Ethics Board (NS Health REB) and people working for or with the NS Health REB because they oversee the ethical conduct of research studies within the Nova Scotia Health.

Use of Your Study Information
Any study data about you that is sent outside of The Nova Scotia Health (NS Health) will have a code (i.e., a string of numbers) and will not contain your name or address, or any information that directly identifies you.

De-identified study data may be transferred to:
- Regulatory authorities within and outside Canada.

Study data that is sent outside of Nova Scotia Health will be used for the research purposes explained in this consent form.

The research team and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be eliminated completely.

The research team will keep any personal information about you in a secure and confidential location for seven (7) years and then destroy it according to NS Health policy. Your personal information will not be shared with others without your permission.

After your part in the study ends, we may continue to review your health records for safety and data accuracy until the study is finished or you withdraw your consent.

You have the right to be informed of the results of this study once the entire study is complete.

The Research Ethics Board (REB) and people working for or with the REB may also contact you personally for quality assurance purposes.

Your access to records
You have the right to access, review, and request changes to your study data.

14. Declaration of Financial Interest

The Canadian Institutes of Health Research (CIHR) is reimbursing the Principal Investigator and/or the Principal Investigator's institution to conduct this study. The amount of payment is sufficient to cover the costs of conducting the study.

15. What About Questions or Problems?
For further information about the study you may call the principal investigator, who is the person in charge of this study. The principal investigator is Dr. Sean Barrett,
Telephone: **902-494-2956**
Email: **sean.barrett@dal.ca**
If you cannot reach the Principal Investigator, please contact the research team at our contact number: (902) 494-4596, or email address: dal.substance.use.lab@gmail.com.

16. What Are My Rights?

You have the right to all information to help you decide whether or not to participate in this study. You also have the right to ask questions about this study and to have them answered to your satisfaction before you make any decision. You also have the right to ask questions and to receive answers throughout this study. You have the right to withdraw your consent at any time.

If you have questions about your rights as a research participant and/or concerns or complaints about this research study, you can contact the Nova Scotia Health Research Ethics Board Office:
- email: ResearchEthics@nshealth.ca
- Phone: 902-222-9263

17. Consent Form Signature Page

I have reviewed all the information in this consent form related to the study called:

The impact of cannabidiol (CBD) on brain activity and cognitive performance

I have been given the opportunity to discuss this study. All my questions have been answered to my satisfaction.

This signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time without affecting my future care.

In case of a possible abnormality showing up on the MRI scan, I give permission to the research team to disclose relevant information to a medical imaging specialist. I also give permission for a medical doctor who is a member of the research team to contact my physician. I understand that if I do not provide the name of a physician, one of the members of the research team who is a medical doctor would contact me directly if an abnormality was detected.

_____	_____	____ / _____ / ___
Signature of Participant	Name (Printed)	Year Month Day*
_____	_____	____ / _____ / ___
Signature of Person Conducting Consent Discussion	Name (Printed)	Year Month Day*
_____	_____	____ / _____ / ___
Signature of Investigator	Name (Printed)	Year Month Day*

Note: Please fill in the dates personally.

I will be given a signed copy of this consent form.

18. Consent Form Signature Page (LAB COPY).

I have reviewed all of the information in this consent form related to the study called:

The impact of cannabidiol (CBD) on brain activity and cognitive performance

I have been given the opportunity to discuss this study. All of my questions have been answered to my satisfaction.

This signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time without affecting my future care.

In case of a possible abnormality showing up on the MRI scan, I give permission to the research team to disclose relevant information to a medical imaging specialist. I also give permission for a medical doctor who is a member of the research team to contact my physician, Dr. _____ (print name). I understand that if I do not provide the name of a physician one of the members of the research team who is a medical doctor would contact me directly if an abnormality was detected.

_____   _____   _____ / _____ / ____
Signature of Participant   Name (Printed)   Year   Month   Day*

_____   _____   _____ / _____ / ____
Signature of Person Conducting   Name (Printed)   Year   Month   Day*
Consent Discussion

_____   _____   _____ / _____ / ____
Signature of Investigator   Name (Printed)   Year   Month   Day*

Note: Please fill in the dates personally

I will be given a signed copy of this consent form.

APPENDIX D: MEASURES

Demographic and Cannabis Use Questionnaire

DATE: _____   **SUBJECT ID**: _____

1. How old are you? _____

2. Please indicate your gender (i.e., the gender you identify with)?
   • Man • Woman • Non-binary • Prefer to self-describe: _____
   • Prefer not to answer

3. Please indicate your sex (i.e., the sex you were assigned at birth):
   • Male • Female • Intersex • Prefer not to answer

4. Please indicate your marital status:
   • Single • Common-Law • Married • Separated • Divorced • Widowed

5. Please indicate your highest level of education completed:
   • Some High School • High School Diploma • Some College/University
   • College/University Degree • Other (Please specify): _____

6. Which of the following best represents your ethnic background? Choose all that apply:
   • Prefer not to say
   • Black (e.g. African, Afro-Caribbean, African Canadian Descent)
   • South East Asian (e.g. Chinese, Korean, Japanese, Taiwanese descent or Filipino, Vietnemese, Cambodian, Thai, Indonesian, other Southeast Asian descent)
   • Indigenous (e.g. First Nations, Inuk/Inuit, Métis descent)
   • Latino (e.g. Latin American, Hispanic descent)
   • Middle Eastern (e.g. Arab, Persian, West Asian descent – i.e. Afghan, Egyptian, Iranian, Lebanese, Turkish, Kurdish)
   • South Asian (e.g. East Indian, Pakistani, Bangladeshi, Sri Lankan, Indo-Caribbean)
   • White (e.g. European descent)
   • Other (Please specify): _____

7. Are you currently enrolled in a post-secondary institution? • Yes/ • No

8. Are you currently employed? • Yes • No

9. How old were you when you <u>first</u> tried cannabis? _____ Years

10. Which of the following best captures the average frequency you **currently** use cannabis?

- I do not use cannabis
- Less than once a year
- Once a year
- Once every 3-6 months (2-4 times/year)
- Once every 2 months (6 times/year)
- Once a month (12 times/year)
- 2-3 times a month
- Once a week
- Twice a week
- 3-4 times a week
- 5-6 times a week
- Once a day
- More than once a day

11. Which of the following best captures how long you have been using cannabis **at this frequency**?

- less than 1 month
- 1-3 months
- 3-6 months
- 6-9 months
- 9-12 months
- 1-2 years
- 2-3 years
- 3-5 years
- 5-10 years
- 10-15 years
- 15-20 years
- More than 20 years

12. How many days of the past week did you use cannabis?

- 0 days
- 1 day
- 2 days
- 3 days
- 4 days
- 5 days
- 6 days
- 7 days

13. Approximately how many days of the <u>past month</u> did you use cannabis? _____ days

14. What is your preferred method of cannabis use?

- Joints
- Blunts (Cigar sized joints)
- Hand pipe
- Bong (Water pipe)
- Hookah
- Vaporizer (e.g., Volcano, Vape pen)
- Edibles (i.e., food)
- Beverages
- Concentrates (i.e., Oil, Wax, Shatter, Butane Hash Oil, Dabs)
- Other: _____

15. Which of the following best captures the average frequency you <u>currently</u> use cannabis **oil**?

- I do not use cannabis oil
- Less than once a year
- Once a year
- Once every 3-6 months (2-4 times/year)
- Once every 2 months (6 times/year)
- Once a month (12 times/year)
- 2-3 times a month
- Once a week
- Twice a week
- 3-4 times a week
- 5-6 times a week
- Once a day
- More than once a day

16. Which of the following best captures how long you have been using cannabis **oil** <u>at this frequency</u>?"

- less than 1 month
- 1-3 months
- 3-6 months
- 6-9 months
- 9-12 months
- 1-2 years
- 2-3 years
- 3-5 years
- 5-10 years
- 10-15 years
- 15-20 years
- More than 20 years

17. What is the average THC content of the cannabis you typically use?

- 0-4%
- 5-9%
- 10-14%
- 15-19%
- 20-24%
- 25-30%
- Greater than 30%
- I don't know

18. What is the average CBD content of the cannabis you typically use?

- 0-4%
- 5-9%
- 10-14%
- 15-19%
- 20-24%
- 25-30%
- Greater than 30%
- I don't know

Substance Use Calendar

DATE: _____   **SUBJECT ID**: _____

Today						
	6 am – 10 am	10 am – 2 pm	2 pm – 6 pm	6 pm – 10 pm	10 pm – 2 am	2 am – 6 am

CBD Belief Rating

DATE: _____   **SUBJECT ID**: _____

Please indicate how much you believe the following statements about the properties of CBD. There are no right or wrong answers.

		1	2	3	4	5	6	7	8	9	10	
Improves Mood	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Improves Memory/ Cognition	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Stress	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Anxiety	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Pain	Not at all	1	2	3	4	5	6	7	8	9	10	Completely

THC Belief Rating

DATE: _____   **SUBJECT ID**: _____

Please indicate how much you believe the following statements about the properties of Tetrahydrocannabinol (THC). There are no right or wrong answers.

		1	2	3	4	5	6	7	8	9	10	
Improves Mood	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Improves Memory/ Cognition	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Stress	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Anxiety	Not at all	1	2	3	4	5	6	7	8	9	10	Completely
Reduces Pain	Not at all	1	2	3	4	5	6	7	8	9	10	Completely

VAS

DATE: _____   **SUBJECT ID**: _____

ADMIN: 1   2   3   4   5   6

Please choose the number that best describes how you are feeling **RIGHT NOW.**

Stressed	Not at all	1	2	3	4	5	6	7	8	9	10	Extremely
Anxious	Not at all	1	2	3	4	5	6	7	8	9	10	Extremely
Sedated	Not at all	1	2	3	4	5	6	7	8	9	10	Extremely
Energized	Not at all	1	2	3	4	5	6	7	8	9	10	Extremely
Craving Cannabis	Not at all	1	2	3	4	5	6	7	8	9	10	Extremely

Task Difficulty VAS

DATE: _____   **SUBJECT ID**: _____

Please choose the number that describes how difficult you found the counting task.

Difficult   Not at all   1   2   3   4   5   6   7   8   9   10   Extremely

Concluding Questions

DATE: _____   **SUBJECT ID**: _____

A. What product were you given during the study session today?

   Please circle one of the following:

   1) CBD oil

   2) CBD-free/Hemp seed oil

   3) Not sure

B. Please choose the number that describes how much effort you put into the counting task you completed during the study session today.

   None   1   2   3   4   5   6   7   8   9   10   A lot

C. Do you have any comments or suggestions for the researcher or about the study? *Please describe:*

   _____

   _____

   _____

   _____

   _____

APPENDIX E: DEBRIEFING

CBD fMRI Study Debriefing Script

DATE: _____   **SUBJECT ID**: _____

Hello, I am ___ calling from the Dalhousie Substance Use Laboratory. I am calling about the cannabidiol (CBD) and brain activity study you participated in on (*date of participation*). Do you have some time now to go over the study with me? I will describe the study and tell you about the oil you were given during the study. Please ask any questions you may have about the study.

You were initially told that the goal of this study was to understand the impact of CBD oil on brain activity and thinking abilities. The true goal of this study was to look at how believing you received CBD oil would impact stress, anxiety and brain activity. Overall, we hoped to better understand the benefits of CBD.

In recent years, there has been more and more research suggesting that CBD may be helpful for a number of stress- and anxiety-related conditions. However, to our knowledge no research has looked at how beliefs about receiving CBD can impact stress, anxiety and brain activity. Because of this gap, it is not clear whether the benefits of CBD are caused by the drug itself, or by simply expecting that CBD will be helpful for these reasons.

During the study session, you received a syringe with oil and were told it either contained CBD oil or a CBD-free hemp seed oil. In reality however, all participants were given the CBD-free hemp seed oil during their study session. Research has shown that expectancies or beliefs about a drug can influence how you respond to it. Therefore, we wanted to see how expecting to receive CBD could influence your stress and anxiety levels, and your brain activity.

Do you have any questions about the study?

All of the information you provided during the study is strictly confidential. This means that nobody aside from the study researchers have access to any information that could be used to identify you. All of the study information is stored in a locked cabinet or on a password-protected computer in a locked laboratory at Dalhousie University. All brain imaging (i.e., MRI) data is stored on a password protected computer in a locked laboratory at the Queen Elizabeth II Hospital. If you do not feel comfortable about having your information included in this study, you can ask for all of your information to be removed from the study. If you choose to do this, the questionnaires you filled out, the brain imaging data and all of the other information you gave us will not be used in this study. Would you like to have your information removed from the study?

Circle one: Yes   No

Do you have any questions about your participation in the study? If you have any future questions, please contact us at dal.substance.use.lab@gmail.com or at 902-494-4596.

Thank you for participating in the study and for your time today.