Subconscious threat processing and cannabidiol: A randomized controlled trial.

by

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Abstract

Over the past two decades, neuroimaging researchers have produced strong evidence in support of a network of regions in the human brain which are responsive to socially salient threat signals in the form of fearful facial expressions even when such signals are presented outside the bounds of conscious awareness. Independent of the exploration of the neural substrate of non-conscious threat processing, research into the psychopharmacology of cannabis has revealed a that the major, non-psychoactive cannabinoid found in cannabis – cannabidiol (CBD) – attenuates the normal neural response to consciously presented fearful faces. However, no study to date has examined the effect of cannabidiol on the neural processing of fearful faces presented below the level of conscious awareness. In the current study, I planned to examine the impact of a single, orally administered dose of cannabidiol on the neural response to fearful faces presented below the normal threshold for conscious awareness in the context of a double-blind, placebo-controlled randomized controlled crossover trial in a sample of normal, healthy participants. Based on the literature reviewed in Chapters 1, 2, 3, and introduction of Chapter 4, I hypothesized that CBD would attenuate the activity of three distinct brain regions previously implicated in the processing of fearful faces – the amygdala, anterior cingulate cortex, and superior temporal sulcus – while participants view subliminally presented fearful faces. Potential implications are discussed at the conclusion of the manuscript.

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Chapter 1: Brief Overview and Introduction to the Current Research

Few naturally occurring molecules have garnered the simultaneous adoration and demonization of the human species quite like those from the flower of the cannabis plant. Over the past few decades in the United States, however, cannabis and its constituent cannabinoids have slowly begun to lose the stigma which was, in my view, inappropriately attributed. The cultural shift in perceptions on cannabis can surely be attributed to several things: the counterculture movements of the 1960's, the music of the 1970's, the pioneering research of people such as Bulgarian chemist, and professor of Medicinal Chemistry at Hebrew University of Jerusalem, Dr. Raphael Mechoulam – a topic deserving of a much deeper dive than will be offered in the text which follows. All this to say, the rich history that surrounds this mysterious plant has led us to a moment of great public interest and burgeoning scientific progress in the discovery of the unique molecular and psychopharmacological properties it has to offer. Though there are endless directions one may take in this line of research, one area serves as the motivation for the current project – cannabidiol (CBD) and fear processing.

In an effort to study the apparent differential effects of the two major cannabinoids of cannabis, CBD and tetrahydrocannabinol (THC), Bhattacharyya et al. (2010) found that subjects dosed with CBD before viewing fearful faces in a functional magnetic resonance imaging (fMRI) procedure had distinct, and moreover, opposite physiological responses than those that were dosed with THC. Specifically, the authors found that pretreatment with 600mg of CBD attenuated the natural response of the left amygdala to the (consciously attended) visual presentation of fearful faces. Subjects that were pretreated with 10mg of THC showed similar, but slightly altered responsivity in the left amygdala when compared to placebo. Moreover, the attenuating effect of the CBD was also correlated with a physiological index of anxiety/arousal – namely skin

conductance response (SCR). Though the evidence (which I will review in more detail in Chapters 2 and 4) for CBD as an anxiolytic has not been thoroughly demonstrated from a randomized control trial perspective, the finding here by Bhattacharyya et al. (2010) does offer some insight into a potential neurophysiological mechanism for CBD as an anxiolytic.

The second source of motivation for the current project comes from research by Liddell et al. (2005) on subconscious fear. In their classic study, Liddell and colleagues (2005) demonstrated that consciously undetected presentations of fearful faces reliably elicit activity in what has been termed a subcortical 'alarm system', involving a complex set of pathways between the superior colliculus, pulvinar nucleus of the thalamus, amygdala, and locus coeruleus, among others. Specifically, fearful faces elicit this subcortical threat response system when fearful faces are subconsciously presented via masked presentations below 20 milliseconds (ms) – in this case, 16.7 ms. This finding is quite important and for several reasons. Principally, as it relates to the present research, the finding by Liddell and colleagues (2005) demonstrates that humans need not be consciously aware of threats in their environment for their brains to begin a cascade of activity which ultimately result in some semblance of fight or flight behavior.

The two empirical findings in the preceding paragraphs are the principal sources of motivation for the project which I outline in Chapter 4. More specifically, to date, no research has tested the potential effect of CBD on subconscious threat/fear processing in healthy human participants. To adequately explicate the nature of both CBD and anxiety/fear, I provide a review of the literature for each in the sections which follow. In Chapter 2, I review the historical and contemporary literature on CBD through the exploration of its pharmacology, comparisons with the psychoactive THC, documented behavioral/therapeutic effects, and discuss potential barriers in the research on CBD as it relates to the issues surrounding dosing. In Chapter 3, I briefly review

the historical literature on anxiety, clarify its nature and manifestations amid conceptual confusions, modern conceptual frameworks of fear and anxiety, and finally discuss experimental procedures which have been designed to manipulate it. Finally, in Chapter 4, I present the current research project outlined in the introduction and discuss the major findings and implications, limitations, and future directions for the original research.

Chapter 2: Historical and Contemporary Accounts of Cannabidiol

Introduction

On December 20th, 2018, the President of the United States signed the Agriculture Improvement Act of 2018 which, among other programs and changes to the agricultural industry, reclassified hemp and derivatives of cannabis with less than 0.3% of the psychoactive delta-9-tetrahydrocannabinol (THC) from a controlled substance made illegal under the 1970 Controlled Substances Act to a highly-regulated, but legal agricultural product (Abernethy, 2019; "Agriculture Improvement Act of 2018," 2018). Though the bill is marred with regulatory legalese that make the exact legal status of cannabidiol (CBD) and other non-psychoactive phytocannabinoids somewhat unclear (Grinspoon, 2018), stores across the country are selling CBD products to hopeful consumers who have likely heard anecdotal claims of its anti-inflammatory, anxiolytic, analgesic, and sleep-promoting properties. In a recent survey conducted by Gallup, one in seven U.S. adults claim to use CBD products – 40% of which use it for pain (non-specific), 20% for anxiety, and 11% for sleep/insomnia (Brenan, 2019). Given the recent legal status change, broad therapeutic claims behind the substance, and apparently growing interest among the American public (Leas et al., 2019), much research remains to be done.

Used for thousands of years as a medicine, cannabis is one of the oldest known plants to be used for therapeutic properties (Mechoulam, 1986; Pacher, Bátkai, & Kunos, 2006) and today remains the most widely trafficked and abused drug on earth, constituting half of all illicit drug seizures worldwide according to the World Health Organization (2020). Despite being the most widely used psychotropic substance in the United States behind alcohol (NIDA, 2020) a complex and tumultuous medical and legal history surrounds cannabis. Before the 1930's, cannabis was

widely used as a patent medicine dating as far back as the mid-1800's (Bridgeman & Abazia, 2017). Outside of various minor forms of regulation which required proper labeling by pharmacies and drug stores before sale, cannabis was largely legal for sale and use in the United States until political pressure from alarmist groups – who worried about the spread of the drug by immigrants from Mexico – spurred *The 1937 Marijuana Tax Act* (Musto, 1972) – a law which heavily regulated importation and prohibited the use of cannabis for recreational purposes.

Early Research on Cannabis

Compared to other psychoactive drugs like morphine and cocaine, modern research on cannabis was rather slow, in large part due to the ignorance surrounding its chemical makeup (Mechoulam & Parker, 2013). Though pharmacological research on the constituent cannabinoids of the *Cannabis sativa* plant had begun in the early 1940's (Pertwee, 2009), the first reported isolation of the pure, principle psychoactive component delta-9-tetrahydrocannabinol (THC) was not until roughly two decades later in 1964 (Gaoni & Mechoulam, 1964), a year after the chemical structure of the non-psychoactive CBD was discovered (Mechoulam & Shvo, 1963). Prior to the late 1980's, very little was known about the precise mechanism of action by cannabinoids in the central nervous system and it was assumed that they acted through non-specific membranous activity (Mechoulam & Parker, 2013). The discovery of cannabinoid receptors in the rat brain (Devane et al., 1988) was closely followed by the finding that the distribution of these receptor sites were consistent with the known pharmacological properties of the psychoactive THC using radiolabeled synthetic THC (Herkenham et al., 1990). The clear evidence for the existence of the G-protein coupled cannabinoid receptor activated by cannabinoids by Howlett et al. (2002) however, opened the door for extensive research into the endocannabinoid system.

The Endocannabinoid System

Spread throughout the central and peripheral nervous systems, the endocannabinoid system is comprised of endogenous cannabinoids (i.e., Anandamide and 2-Arachidonoylglycerol), enzymes which degrade and synthesize these endogenous cannabinoids, and the receptor sites at which they bind (i.e., cannabinoid receptor type 1 (CB_1) and type 2 (CB_2)) (Freund et al., 2003; Howlett, 2005; Howlett et al., 2002). Much work has been done on determination of cannabinoid receptor sites (as well as receptor density at those sites). Utilizing an *in vitro* autoradiographic technique leveraging a radiolabeled synthetic cannabinoid to determine the location of receptors to which the specific ligand bind, Herkenham et al. (1991) found CB_1 receptors throughout many areas of the central nervous system, densely populated in motor and cognitive areas which elicit the well-known behavioral/functional alterations produced by THC exposure, including the basal ganglia, cerebellum, hippocampus, and cerebral cortex. Additionally, CB_1 receptors have been located in the peripheral nervous system (Mackie, 2005) and tissues, including the liver (Osei-Hyiaman et al., 2005), skeletal muscle (Cavuoto et al., 2007), and endothelial tissue (Liu et al., 2000). These findings provide some preliminary support the anecdotal observations of CBD's diverse therapeutic effects.

Early research on CB_2 receptors suggested that they were not expressed to a significant degree in the central nervous system (Zhang et al., 2014), but largely expressed in the immune system (Galiègue et al., 1995; Kaminski et al., 1992; Munro et al., 1993; Schatz et al., 1997), showing 10-100 fold higher levels of expression in immune cells than the more centrally situated CB_1 (Clayton et al., 2002). More recent research, however, has found CB_2 receptor expression in the central nervous system, specifically within the perivascular microglia (Núñez et al., 2004), and small populations of cells in the cerebellum (Ashton et al., 2006), brainstem (Van Sickle et

al., 2005), and prefrontal pyramidal neurons (Den Boon et al., 2012). The location of CB_2 receptors coupled with the numerous findings which show these receptors mediating a large number of immunosuppressive effects to limit damage caused by inflammatory response in the brain (e.g., (Benito et al., 2005; Chung et al., 2016; Croxford & Yamamura, 2005; Ramírez et al., 2005; Wu et al., 2013), indicate a central role in immune response regardless of their location. In fact, in a comprehensive review of lipid signaling through CB_2 receptors, Pacher and Mechoulam (2011) suggest that this system of receptors may serve a role as part of a larger biological protective system within the mammalian body.

Perhaps the clearest indication of the distinct roles of the two established endocannabinoid receptors comes from studies utilizing the gene knockout technique developed by geneticist Capecchi (1989) which involves the development of organisms (e.g., mice) with a selectively disrupted gene known to express in a specific manner (e.g., encoding certain receptors). Using knockout mice with invalidated CB_1 receptors, Ledent et al. (1999) demonstrated that mutant mice are virtually unresponsive to cannabinoids in typical behavioral assessments of cannabinoid exposure in wild-type mice. Further, using CB_2 knockout mice, Buckley et al. (2000) provided evidence that THC exposure to these mutant mice disrupted immunoregulatory mechanisms (i.e., helper T cell activation) while the typical central nervous system effects of THC remained the same. Based on the evidence to date, it is fair to say that CB_1 receptors of the endocannabinoid system mediate the majority of the effects which the public attribute to the recreational use of cannabis.

Major Exogenous Cannabinoids

THC: Major Findings and Potential Therapeutic Effects

THC and Memory. Of the over 105 known cannabinoids (Ahmed et al., 2015), its principle psychoactive component, THC, has been the central focus of the majority of cannabis research (Russo, 2011). Among the most robust findings, research on the effects of THC exposure has clearly demonstrated impairment of various aspects of memory performance (Bolla et al., 2002; Darley et al., 1974; Fadda et al., 2004; Hunault et al., 2009; Ilan et al., 2004; Varvel et al., 2001). These memory effects appear to be mediated by the relatively high density of CB_1 receptors in the hippocampus (Katona et al., 2000; Matsuda et al., 1993). Elegant examples which lend credence to this notion come from use of rodent models of memory. Electrophysiological recordings of the rat hippocampus show suppression of long-term potentiation following injection of THC, but these effects were blocked in rats which were dosed with a cannabinoid antagonist prior to THC injection (Hoffman et al., 2007). Similarly, rats dosed with THC into the dorsal (CA1) region of the hippocampus show impaired performance in the radial arm maze task, but this effect was fully attenuated in rats who were first injected with a CB_1 antagonist (Wise et al., 2009). Further, evidence also suggests that the memory deficits brought about by exposure of hippocampal neurons to THC may actually be a consequence of THC-driven neurotoxicity (Chan et al., 1998).

THC and Psychosis. Of course, the story of THC and memory is only a small segment of a much larger picture. Other lines of research have focused on another particularly alarming symptom associated with the use of cannabis: induction of psychosis or transient psychotic states. For instance, data from a double-blind intravenous THC administration in 22 healthy controls (i.e., screened for risk factors associated with psychosis) indicate that this potent psychoactive molecule is capable of transiently inducing cognitive and behavioral symptoms typically associated with schizophrenia and other psychoses such as blunted affect, anxiety,

perceptual (e.g., time, body perception) alterations, feelings of unreality, and unusual thought content (D'Souza et al., 2004). The research on THC, however, goes well beyond associations with psychosis.

A quite similar comprehensive study of THC and its effect on psychotic symptoms as well as cognitive function and subjective-rated affect was conducted by researchers at King's College London. For the experiment, 22 psychiatrically screened healthy males (28 ± 6 years old) were recruited for a placebo-controlled, double-blind study whereby synthetic THC (Dronabinol, 2.5mg) or a placebo would be injected shortly before a battery of tests. The researchers found that, in support of the previously discussed work, the THC-administered group scored significantly higher on a clinical positive psychotic symptom measure than baseline at the 30-minute, post injection mark but scores were no different than placebo by the 80-minute mark. Moreover, those who had reported previous exposure to THC were less likely to experience the psychotic symptoms. Further, the THC group experienced transient feelings of tense arousal and feelings of dysphoria above that of the placebo group. THC also transiently impaired free recall, digit span, and reasoning ability while producing no significant differences in verbal fluency (Morrison et al., 2009). Interestingly, the authors note that there was no relationship between performance on the digit span and reasoning tasks, dissociating the working memory impairment from the reasoning ability impairment. Given both the transience of the symptoms and the finding that participants with prior experience with THC were less likely to experience psychotic symptoms than their cannabis-naïve peers, it seems reasonable to suggest that more research is necessary to make strong statements about the causal link between cannabis use and psychotic symptoms (Gage et al., 2016).

The relationship between psychotic states/symptoms and THC is, frankly, quite unclear in terms of causality. Although epidemiological and cohort studies have consistently shown a link between cannabis use and psychotic symptoms (Arseneault et al., 2002; Tien & Anthony, 1990; Zammit et al., 2002) only a small percentage of cannabis users develop these symptoms and it is likely that gene-environment mechanisms play a large role in the relationship (Henquet et al., 2008). A longitudinal study following individuals from birth through adulthood found evidence for such a gene-environment interaction, where those with preexisting genetic vulnerabilities were more likely to develop psychoses if they used cannabis compared to their peers (Caspi et al., 2005). Furthermore, somewhat recent work has also suggested that higher potency (i.e., higher concentrations of THC) cannabis carries a higher risk of psychosis (Di Forti et al., 2015) – a rather concerning point, given that the typical cannabis strains of the past are far lower in THC concentration than they are today, on average with a reported 212% increase in THC content between 1995 and 2015 (Stuyt, 2018). Finally, although some researchers have suggested that, in review of the evidence, cannabis does not seem to cause psychotic disorders (Ksir & Hart, 2016), the strong epidemiologic evidence of the link is sufficient to warrant public health messages on the matter.

THC: Therapeutic Evidence. Despite the abundance of literature providing evidence for the negative effects of THC exposure, its potential as a therapeutic for various diseases and ailments ought not be ignored. In addition to appetite enhancing effects which have been leveraged to treat AIDS related wasting disease (Beal et al., 1997), some evidence indicates that THC may be efficacious as a therapeutic treatment in human cancers via promotion of autophagic death of cancer cells (Salazar et al., 2009; Vara et al., 2011). Another, more brain-centric, avenue for therapeutic use of this molecule comes from research on the devastating, yet

sadly pervasive, neurodegenerative disorder, Alzheimer's disease (AD). Evidence from various methodological approaches ranging from cell cultures, to computational modeling, to clinical trials has shown THC or the synthetic version, dronabinol (brand name *Marinol*), to be useful in treating AD (Cao et al., 2014; Defrancesco & Hofer, 2020; Eubanks et al., 2006; Volicer et al., 1997; Walther et al., 2006). THC and other cannabinoids have been demonstrated to be neuroprotective, shielding neurons from damage produced by glutamate toxicity (Hampson et al., 1998). Though THC may hold yet unknown therapeutic potential on its own, combining it with its naturally co-occurring phytocannabinoids could perhaps prove more promising as suggested by Mechoulam and Ben-Shabat (1999).

CBD: Comparisons to THC, Major Findings, and Potential Therapeutic Effects

CBD Versus THC. Although THC's psychotropic properties are primarily mediated through CB_1 receptors in the central nervous system, the other heavily studied exogenous cannabinoid CBD exerts is pharmacological impact beyond the CB_1 and CB_2 receptors (Bridgeman & Abazia, 2017). Further, CBD appears to show very low affinity for both CB_1 and CB_2 receptors (Mechoulam et al., 2007), even exhibiting high potency antagonistic properties at both receptor sites (Thomas et al., 2009). In fact, relatively large doses of CBD (up to 100mg oral and 30mg intravenous) produced no discernable effects typically associated with cannabis (Hollister, 1973). Furthermore, early studies of interactions between these molecules indicated that, when taken simultaneously, CBD attenuates the anxiety provoked by ingestion of THC (Zuardi et al., 1982). This synergistic mechanism widens the scope of possible therapeutic applications for THC, given the potent psychoactive properties of THC in its pure form.

CBD/THC Combination. The combination of THC and CBD has shown promising evidence as a potential therapeutic route for cannabinoid administration. For instance, a

THC/CBD combination oromucosal spray has demonstrated efficacy in treating muscle spasticity, improving gait-related issues (Izquierdo, 2017), and improved aspects of daily living (e.g., improvement in ability to stand up; Mallada Frechín, 2018) in patients with multiple sclerosis in clinical trials. Although the mechanism for these improvements has likely yet to be fully elucidated, very recent evidence in mice models suggest that these patients may see symptom improvement as at least a partial consequence of suppression of neuroinflammation by THC/CBD combination – an effect not found through use of either cannabinoid on its own (Al-Ghezi et al., 2019). Additional therapeutic evidence for combination of THC and CBD have been found in mice models of both AD (Aso et al., 2015) and Huntington's Disease (Valdeolivas et al., 2012). THC and CBD combination has also shown efficacy in the treatment of seizures. Thus, this combination holds promise as a therapeutic agent for several disorders.

In a recent study using a preclinical rat model of Focal Impaired Awareness Seizures, the most common seizures in human adults and previously thought to be treatment resistant, pure THC, pure CBD, and a CBD:THC combination were tested for therapeutic value. To induce seizure, the rats in this model are implanted with electrodes into the right basolateral amygdala which are then stimulated suprathreshold after a short dosing window following injection. Both THC and CBD on their own were sufficient to reduce seizure activity with one important caveat: the THC dose required to reduce seizures is also sufficient to induce the well-known and therapeutically undesirable psychoactive effects. In step with the notion that these drugs are perhaps better therapeutics for certain pathologies when combined, when the dose of CBD was administered with a small, non-psychoactive quantity of THC it lowered the effective dose required to attenuate both generalized and focal seizures (Fallah & Burnham, 2019), buffering against the more harmful psychoactive effects.

Whole Plant CBD/THC Combination. Though much less controlled than many of the studies discussed above, observational, longitudinal, and cross-sectional studies on whole-plant cannabis use and symptom relief offer another avenue to explore synergistic effects of CBD and THC – though it should also be noted that the data in the remaining paragraphs of this section are clearly subject to confounding influence by the relative concentrations of THC/CBD as well as synergistic effects produced by combinations of other phytocannabinoids within the plant material. One such study which examined chronic pain patients found that after enrollment into New Mexico's Medical Cannabis program, chronic pain patients were more likely to stop/reduce their prescription opioid intake and reported improved quality of life (Vigil et al., 2017). Another observational study using a web-based survey to assess Parkinson's diseases and multiple sclerosis patients on a number of dimensions found that cannabis users within the sample reported reductions in prescription medication use, lower levels of disability across dimensions of mood, memory, and fatigue, and were less likely to be obese than their cannabis-abstinent cohort (Kindred et al., 2017).

Interestingly, despite the well-established finding that the activity of THC at CB_1 receptors mediates psychoses-like symptoms reviewed above, cannabis use (and abuse) among people suffering from schizophrenia is much higher than that of the general population (Volkow, 2009). Data from functional magnetic resonance imaging (fMRI) indicate more pronounced grey matter volume loss in schizophrenic patients who used cannabis than patients who had not used cannabis after a five year follow-up (Rais et al., 2008), though extrapolation from these findings should be carried out with caution due to limited sample size (i.e., N = 19 for cannabis-using patients) and the fact that direct causation cannot be addressed in such a study. Still, despite the reported increase in positive psychotic symptoms in cannabis-using schizophrenic patients

(Dubertret et al., 2006; Grech et al., 2005), other data show that cannabis use in schizophrenic patients has apparent benefits. For instance, a clinical evaluation of cannabis-using and non-using schizophrenic patients found that cannabis showed lower overall scores for negative symptoms associated with the disorder, indicating possible cannabis-related attenuation of these symptoms (Peralta & Cuesta, 1992) – a finding supported in subsequent work (Bersani et al., 2002; Compton et al., 2004). Data from observational study of schizophrenic patients also found patients who used cannabis reported improved cognitive function, among other improvements including increased energy levels and control of symptoms (Costain, 2008). Data from cross-sectional and longitudinal study of schizophrenic patients agree with these findings, indicating cannabis-using patients showed better attention and executive function than did their non-using counterparts (Rodríguez-Sánchez et al., 2010).

Recent evidence points to potential therapeutic use for cannabis in post-traumatic stress disorder (PTSD). First, patients with higher scores on a PTSD measure were more likely to self-medicate with cannabis at a higher frequency than low scorers. Additionally, those with high PTSD scores indicated *sleep* and *coping* as motives significantly more than those with lower scores (Bonn-Miller et al., 2014). Although these data do not speak precisely to efficacy for these symptoms, they do indicate a possible therapeutic route for cannabis use in PTSD. Cross-sectional work examining 80 PTSD patients using the Clinician Administered Posttraumatic Scale for the DSM-IV (CAPS) found an over 75% reduction in PTSD symptoms in patients who were using cannabis in comparison to non-user patients (Greer et al., 2014). Beyond PTSD, a review by Ashton and colleagues (2005) suggested, based on the available data, that in terms of their pharmacological profiles, both THC and CBD may be helpful in bipolar disorder. A retrospective study in this domain found that although bipolar patients with a history of cannabis

use disorder (CUD) also had higher incidences of psychosis than their peers without a CUD history, they also performed significantly better on neurocognitive measures of attention, processing speed, and working memory (Braga et al., 2012). Despite the limited, but somewhat positive results discussed above, there have yet to be any clinical trials assessing the treatment of PTSD via cannabinoids (Sarris et al., 2020).

The observational evidence of cannabis use is far from only positive. Although research in this domain has been systematically hampered by legal issues surrounding cannabis, meta-analytic work on longitudinal cannabis use and subsequent psychotic outcomes suggests that, despite the transient nature of the effects upon acute administration, cannabis use may increase the risk the likelihood of a psychotic illness later in life (Moore et al., 2007). As indicated by the authors, however, it should be noted that several of the studies used in the meta-analysis did not adequately control for potential confounding factors (e.g., alcohol use), making the causal connection between psychotic illness and cannabis use more difficult to establish. Furthermore, while cannabis use among healthy individuals may be linked to psychosis in later-life, the relationship between cannabis use and subsequent psychotic illness was much stronger for those with an established vulnerability to psychotic illness (Van Os et al., 2002). In addition to predisposition toward psychotic illness, age of use onset may be another important risk factor for the development of schizophrenia (Arseneault et al., 2002; McGrath et al., 2010).

Although THC and CBD have fairly well-documented neuroprotective effects in animal models as well as potentially positive efficacy in treating symptoms of psychological disorders, repeated use of THC can lead to the development of tolerance at CB_1 receptors which mitigates its neuroprotective properties unlike CBD, which exerts no significant influence over the known cannabinoid receptors (Hayakawa et al., 2007). Given the relatively limited therapeutic profile of

THC in its pure form as a consequence of its induction of psychosis-like symptoms (D'Souza et al., 2004; Favrat et al., 2005) and the ability of CBD to attenuate those effects (Russo & Guy, 2006; Zuardi et al., 1982), it is quite possible that CBD may prove to hold the better therapeutic index overall.

Therapeutic Mechanisms of CBD

The evidence reviewed above demonstrate the discrepant pharmacodynamic behavior of CBD in comparison to the psychoactive THC. Consequently, and somewhat counterintuitively, the natural implication from these findings is that the effects of CBD, like its purported anti-inflammatory properties, may be exerted through non-endocannabinoid-system-exclusive mechanisms. For instance, consider evidence related to the neurotransmitter adenosine. Adenosine is released in response to cellular injury or stress and acts as an anti-inflammatory immunosuppressive, preventing further tissue injury as a result of the natural inflammatory response (Haskó & Cronstein, 2004). CBD has been shown to inhibit the reuptake of adenosine (via inhibition of equilibrative nucleoside transporters, specifically), thereby enhancing its anti-inflammatory effect (Carrier et al., 2006; Izzo et al., 2009; Ribeiro et al., 2012). Another example which demonstrates the effectiveness of CBD in regulating inflammatory response is evidence from mice studies of pneumococcal meningitis – a bacterial driven inflammation of the meninges surrounding the brain. Prolonged treatment with CBD was shown to reduce the proinflammatory cytokine in the frontal cortex called tumor necrosis factor-alpha (TNF-α) (Liu et al., 1994), which resulted in the reduction of inflammation and prevented memory impairment associated with the disease (Barichello et al., 2012). Additionally, CBD has also been shown to be protective against factors associated with demyelinating pathologies (e.g., multiple sclerosis) (Kozela et al., 2011; Mecha et al., 2012).

Pure CBD has also been shown efficacious in treating symptoms related to AD through a diverse set of mechanisms. For instance, CBD may limit the damage on cells caused by exposure to beta-amyloid (Aβ) peptide – a key marker of Alzheimer's disease pathology. In a study by Iuvone and colleagues (2004), cultured rat cells which were treated with CBD before exposure to the beta-amyloid peptide showed decreased apoptotic cell death in comparison to untreated cells. The authors suggest that the neuroprotection offered by CBD comes by way of modulation of enzymatic activity (i.e., enzyme caspase 3) involved at the execution phase of the apoptosis cascade (Iuvone et al., 2004). Another mechanism through which CBD may ameliorate AD pathology is via modulation of pro-inflammatory cytokine activity, many of which are known to play a role in AD (Swardfager et al., 2010). An *in vivo* study of Alzheimer's related pathology whereby mice were injected with the same Aβ peptide in the right dorsal hippocampus found that CBD dose-dependently inhibited inflammatory markers – in this case, a cytokine protein known as interleukin 1 beta (IL-1β) and an inflammatory enzyme called inducible nitric oxide synthase (iNOS)) – induced by Aβ peptide exposure, further supporting its therapeutic potential in (AD) (Esposito et al., 2007). CBD has also been shown to modulate the function of microglia in rats in vitro and prevent cognitive impairments seen in the Morris Water Maze task for untreated rats injected with Aβ peptide (Martín-Moreno et al., 2011). It is clear from the evidence reviewed here that the purported pathophysiological mechanisms subserving CBD's therapeutic effects is quite diverse across several disease models.

CBD and Anxiety. Anxiety has been defined as "apprehension, tension, or uneasiness from the anticipation of danger, the source of which is largely unknown or unrecognized" (Allen et al., 1995, p. 1). Although anxiety is a shared human experience it can become pathological when it begins to interfere with quality of life (Yudofsky & Hales, 1992). CBD has been shown

to exhibit anxiolytic properties, and therefore may become a viable therapeutic for the treatment of various anxiety disorders. As with much of the evidence reviewed above, some of the best available data on CBD and anxiety come from administration studies in animal models. In the Vogel test, rats are deprived of water for a variable time interval and then presented with an opportunity to lick a waterspout at the cost of receiving a mild shock. When the rodents are administered a benzodiazepine drug, the number of punished licks goes up – demonstrating anxiolytic properties of the compounds (Vogel et al., 1971). Early work on potential anxiolytic properties of CBD showed that rats who were dosed with the molecule in the Vogel paradigm also increased their number of punished responses (Musty et al., 1985) – suggesting that CBD may mimic the anxiolytic effect of benzodiazepines. Further, in another study utilizing another experimental model called the elevated plus maze (discussed in greater detail below) of anxiety, CBD produced an anxiolytic effect. It was also demonstrated that this effect could be blocked by the use of a benzodiazepine receptor antagonist (Onaivi et al., 1990), in support of the notion that CBD may potentially mimic benzodiazepines. However, a subsequent study using the Vogel test found that CBD did in fact produce anxiolytic effects as demonstrated by increased punished responding, but that this effect was not blocked by a benzodiazepine receptor antagonist (Moreira et al.,2006) – leaving open the door for the precise anxiolytic mechanism of CBD for future exploration of this compound.

More recent studies of anxiety using rats have also utilized the elevated plus maze to explore the effects of various compounds. To briefly summarize, the elevated plus maze involves the use of a raised maze-like apparatus in the shape of a cross, with two wall-enclosed arms and two open arms, meant to exploit the drive to avoid open/exposed areas and induce anxiety in rodents (Commissaris, 1993). In one study involving the use of this paradigm, rats were injected

with CBD into the central nucleus of the amygdala. Compared to control rats, those with the CBD injections spent more time in the open arms and less time in the enclosed arms, suggesting an anxiolytic effect of the injected CBD. Interestingly, the CBD appeared to show an inverted U-shaped dose effect, whereby low dose (0.5μg) attenuated the anxiety and the high dose (1.0μg) showed an effect similar to baseline (Hsiao et al., 2012). This study confirmed the findings of a previous study using the same paradigm (Guimarães et al., 1990), but extended this work by demonstrating that one of the mechanisms whereby CBD attenuated anxiety-like behavior was directly through the amygdalae.

The prediction made by Hsiao et al. (2012) that CBD may provide therapeutic value for anxiety stemmed from previous work from these (and other) authors on the value of CBD in sleep regulation via the amygdala. Specifically, CBD was found to alter the sleep cycle via reduction of slow wave sleep and increased wakefulness. Though the precise mechanism was not fully elucidated, the authors suggest that this alteration of the slow wave sleep came partially by modulation of the serotonergic neurons in the amygdala. Specifically, the authors propose that CBD antagonizes the CB_1 receptors situated on the presynaptic terminals of serotonin neurons in the amygdala – evidenced by the finding that buspirone, which exhibits anxiolytic properties by agonizing the presynaptic 5-HT_{1A} serotonin receptor (Tunnicliff, 1991) – blocked the slow wave sleep alteration of CBD dose-dependently. The anxiolytic effect of CBD was also blocked by a 5-HT_2 receptor antagonist on the post-synaptic serotonin neuron, further evidencing the serotonergic modulatory properties of CBD (Yi et al., 2008).

The anxiolytic properties of CBD, however, may well be mediated by other structures beyond the amygdala. For instance, other research using rats in the same elevated plus paradigm discussed previously have targeted the midbrain periaqueductal grey (PAG) – a structure which

is involved in the initiation of passive or active emotional coping behaviors (Bandler et al., 2000) (e.g., freeze or flight behavior in the rat). In this study, rats were injected with CBD at different doses (i.e., 15, 30, and 60 nanomoles (nmol)) within the dorsolateral PAG (dlPAG) - one group was given the 30nmol dose outside of the dlPAG, and one final group was given only the vehicle. In the 30nmol dose group the both the number of entries into the open arms of the maze and the time spend in the maze were significantly increased over baseline (i.e., vehicle only). As with the previously discussed work exploring CBD an anxiety by Hsiao et al. (2012), an inverted U-shaped dose response curve was shown, with both the 15nmol and 60nmol doses showing no significant differences in open arm-entry or time spent in open arms over vehicle only. Further, in support of the anxiolytic effect of CBD and mediation of this effect through the dlPAG, the effect of the 30nmol dose was blocked by a $5HT_{1A}$ receptor antagonist and injection of the 30nmol dose outside of the dlPAG did not significantly change behavior over rats in the vehicle-only group (Campos & Guimarães, 2008), in line with previous findings of the agonistic effect of CBD at serotonin neurons. It should be noted, however, that the authors did not indicate the location of the injection for rats given the dose outside the dlPAG.

Finally, although limited, there is some direct evidence of the anxiolytic effect of CBD in humans. For instance, in a double-blind placebo-controlled study of healthy volunteers, CBD, a benzodiazepine, a partial $5HT_{1A}$ agonist, or a placebo were administered prior to a simulated public speaking test. Although CBD did reduce anxiety, it only did so *after* the simulated public speaking test had occurred (Zuardi et al.,1993) – a finding which was later replicated by these and other researchers in a real life public speaking experiment (Zuardi et al., 2017). More recent work using a simulated public speaking test with a similar design found that pretreatment with CBD resulted in reduced anxiety *during* the speech, highlighting the small therapeutic window of

CBD (Linares et al., 2019). Another study which explored the potential therapeutic use of CBD in teenagers with social anxiety disorder using a double-blind design found that anxiety – as assessed by two self-report scales – was significantly reduced after 4 weeks of 300mg daily treatment with CBD compared to the placebo group (Masataka, 2019).

Investigations of the neural underpinnings of the anxiolytic mechanisms of CBD in humans are somewhat limited. The first functional neuroimaging study on the acute effects of CBD in humans utilized event-based fMRI and skin conductance response electrodes, scanning participants while they were presented with neutral, mildly fearful, and intensely fearful faces after receiving 10mg of THC, 600mg of CBD, or a placebo. The results showed that CBD attenuated the BOLD responsivity in the left amygdala, left anterior cingulate cortex (ACC), and right posterior cingulate cortex, all of which correlated with a reduced number of skin conductance responses in response to the intensely fearful faces (THC had the opposite effect, increasing anxiety) (Fusar-Poli et al., 2009). The findings presented are consistent with the evidence showing preferential processing of fearful faces by the left amygdala (Morris et al., 1996) and coactivation of the left amygdala and ACC during fear-related processing, reflecting increased attention to the salient fear stimulus (Pissiota et al., 2003). More recent research has also found support for the reduction in amygdala and ACC activation after CBD administration, finding a more specific mechanism of disrupted connectivity between the two regions as a possible explanation of the anxiolytic properties of CBD (Fusar-Poli et al., 2010). This seminal research served as a cornerstone in the development of the current research project.

Obstacles in the Study of CBD

Although the evidence of the therapeutic potential of CBD is quite strong and building, there are many challenges in the study of the substance. Unfortunately, there is a noticeable lack

in research into effective administration of the drug with respect to dosing in human subjects (Millar et al., 2018), leaving many more questions than answers. One critically important challenge in CBD research is route of administration, particularly in studies of acute effects. In terms of invasiveness, oral or sublingual may be preferred to intravenous administration. However, the bioavailability of orally administered CBD is quite low – with early estimations ranging from 13% to 19% (Mechoulam et al., 2002) to clinical trial findings with evidence that it may even be as low as 6% (Zhornitsky & Potvin, 2012). The low levels of bioavailability through oral administration can likely be attributed to the fact that CBD (and THC) is lost due to a first-pass effect (Huestis, 2007), a metabolic phenomenon whereby the concentration is diminished at a specific site within the body (e.g., liver) before the drug can reach widespread circulation (Herman & Santos, 2019). More recently, cannabinoids have been coupled with dietary or pharmaceutical grade lipids which take advantage of a mechanism called intestinal lymphatic transport (Zgair et al., 2016) – allowing the drug to enter systemic circulation in the body by avoiding the metabolizing effect of liver (Yáñez et al.,2011).

Another important obstacle in the study of CBD in humans is the problem of dose. Recall the previously mentioned studies by Campos and Guimarães (2008) and Hsiao et al. (2012). In both studies, an inverted U-shaped dose response curve was found for CBD in its therapeutic dose for the attenuation of anxiety symptoms in rodent models. In both cases, CBD was administered via a cannula, avoiding the metabolization issues that may arise in orally administered CBD. However, the CBD was injected into two distinct areas of the brain – suggesting that this biphasic dose response is characteristic of CBD. Indeed, this effect has been found in other studies of CBD in animal models. The first study which uncovered this dose response using intraperitoneal injections of CBD in 2.5, 5.0, 10.0, and 20.0mg/kg found that

although the 2.5-10.0mg/kg doses increased the number of entries into the open area in the elevated plus maze, the anxiolytic effect was strongest at the 5.0mg/kg dose and the effect did not differ from controls at the highest dose (Guimarães et al., 1990). Further, this dose response relationship has also recently been found to occur in zebrafish (Nazario et al., 2015) as well as two recent human studies on the anxiolytic effect of CBD in both real life and simulated public speaking (Linares et al., 2019; Zuardi et al., 2017).

In conclusion, cannabidiol is a deeply complex molecule in terms of its pharmacodynamics and pharmacokinetics, showing a wide variety of therapeutic applications. Although exploration of its effects on humans has yielded promising results with relatively minimal drawbacks, much research remains to be done to elucidate the short-term and long-term impacts of use as well as the mechanisms through which they are produced. Future research should focus on understanding the precise neuropharmacological mechanisms of action of cannabidiol as well as its subsequent effects on neural activation and functional connectivity. Finally, the use of animals in research on cannabidiol has undoubtedly advanced our understanding of the molecule, especially considering the legal issues surrounding cannabis which have systematically hampered large-scale research efforts in humans. As the legal landscape changes, we will likely see much more advancement in our understanding as we move into more human-centric research.

Chapter 3: What is Anxiety?

Introduction

Anxiety in and of itself is a completely functional, normal part of the human experience – it increases our awareness of our surroundings and aids in readying our bodies in the event that a real threat emerges (Calhoon & Tye, 2015). Anxiety has been described as a future-oriented mood state, catalyzed by immediate or possible threat – a lasting state of apprehension that is normal until it becomes extreme (Davis et al., 2010). In extreme cases, this normal, preparatory anxiety can evolve into one of many pathological conditions that can cause severe debilitation. Within the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), these anxiety disorders are described as pathological forms of anxiety and fear, often developed during childhood and persist if untreated. The disorders are categorized into six distinct but highly comorbid subcategories – generalized anxiety disorder, panic disorder, phobias/specific phobias, agoraphobia, social anxiety disorder, and separation anxiety disorder (APA, 2013). Anxiety disorders are quite common in the general population, with lifetime prevalence rate estimates around 29%, making them the most prevalent class of disorders, and are also comorbid with other serious psychopathologies such as major depression and personality disorders according to a fairly recent epidemiological report (Kessler et al., 2005). Further highlighting the disruptive nature of these disorders, data from a large longitudinal study has also identified anxiety disorders as an independent risk factor for suicidal ideation and suicide attempts (Sareen et al., 2005).

State and Trait Anxiety

Based on the factor analytic work of Cattell and Scheier (1958; 1961), Spielberger (1966) elaborated on the nature of anxiety, suggesting it can be broken down into two distinct forms:

state anxiety and trait anxiety. In his description, Spielberger offers an example to elucidate the difference between these two ideas with the following statement: "Mr. Smith is anxious" (1966; p. 12). Although quite simple, this statement may imply that Mr. Smith is experiencing anxiety in the present moment which, assuming one had the appropriate tools, could be measured by self-report or by some physiological indicator (e.g., elevated heart rate). State anxiety, therefore, describes the temporally transient psychophysiological/emotional response provoked by a stressful situation. On the other hand, Mr. Smith may just be a *generally anxious* person. In that sense, anxiety can be described as a feature of personality – a persistent characteristic that might predispose someone particularly high in *trait anxiety* toward *state anxiety* in response to a stressful stimulus (Kennedy et al., 2001).

Experimental studies have employed various strategies to investigate differences in the so-called *trait* anxiety. In an early work on anxiety, Malmo (1957) examined a number of anxiety experiments of the previous years, ultimately leading him to the suggestion that psychiatric patients with chronic anxiety tended to have higher levels of physiological reactivity in the presence of a stressor. Subsequent work confirmed this notion, finding that individuals who score higher on an anxiety trait measure (i.e., the Manifest Anxiety Scale) show higher levels of state anxiety in the presence of a stressor but are similar to controls when not in the presence of a stressor (Spielberger & Smith, 1966). Extrapolating from these findings, trait anxiety may be seen as a predisposing factor for the magnitude of the anxiety state produced by a given stimulus – perhaps a consequence of and more deeply explained by genetic variabilities.

Indeed, work in behavioral genetics has manipulated trait anxiety on a genetic level, allowing the breeding of both high and low-trait anxiety breeds of rats for use in animal models of anxiety (Landgraf & Wigger, 2002). Studies using these strains of rats could demonstrate just how

influential trait anxiety levels, high or low, might be on subsequent behavior, providing a unique (albeit somewhat extreme) model of human anxiety and behavior. For example, Frank et al. (2006) subjected both the high and low-trait anxiety strains to a "social defeat" task. In this task, the high or low-trait anxiety rats are placed in a dominant male rat's enclosure which provokes attack from the dominant male which has been previously trained to attack intruders. The authors found unique patterns in the coping behaviors between the high and low-trait anxiety strains, whereby high-trait anxiety rats froze position longer and produced more ultrasonic vocalizations (USV) than did the low-trait anxiety rats. Another intriguing example comes from a high and low-trait anxiety mice study which found that these selective trait anxiety breeding patterns again produced strong phenotypic differences in behavior, whereby mice with high trait anxiety exhibited higher levels of maternal behavior than the low trait anxiety mice. These studies provide a unique lens through which to model the possible behavioral consequences of the latent personality variable of trait anxiety.

Early Theoretical Measurement: Disentangling Anxiety and Depression

The breakdown of anxiety into a unidimensional construct whereby high trait anxiety might lead to higher state anxiety in the presence of a sufficient stressor was presented by Spielberger (1970), leading to his development of an instrument for assessment of clinical anxiety – the State-Trait Anxiety Inventory (STAI). Others have argued, however, that both trait and state anxiety are multidimensional constructs. For instance, Endler and colleagues (1989) proposed an alternative measure of anxiety that takes into account individual differences which vary across aspects of the situational context. Although this conceptualization may have merit, it seems that the unidimensional approach may be more practically useful, given its widespread use in empirical study (Rossi & Pourtois, 2012).

Early research on the reliability and validity of anxiety measures reported strong correlations between anxiety and depression on a number of rating scales, calling into question the idea that the two concepts can be meaningfully distinguished in an empirical manner (Dobson, 1985b; Mountjoy & Roth, 1982; Riskind et al., 1987). Indeed, the early lack of empirical distinction between to two constructs fit quite well within the popular emotion framework of the time – the *circumplex model of affect* described by Russell (1980). In his model, Russell conceptualizes emotions as falling within a circular model represented by two spatial dimensions: valence (the *X* axis) and arousal (the *Y* axis). In this model, the *X* axis serves as a metaphorical representation of the pleasure-displeasure of affective states while the *Y* axis serves as the relative level of arousal associated with each. Using this conceptualization, anxiety and depression would be quite similar in terms of their displeasing valence but different in terms of the level of arousal/excitement (i.e., anxiety would be characterized as a higher arousal emotion). Given the issues surrounding identification and measurement of discrete emotions and the need for a valid, reliable, and distinct measure of anxiety, Beck and colleagues (1988) developed the Beck Anxiety Inventory (BAI). Despite the efforts made to discriminate the anxiety measure from existing measures of depression, the correlation between the BAI and the Beck Depression Inventory (BDI) remained moderately high (r = .48). Subsequent study of the psychometric properties of the instrument confirmed the high correlation, but did provide evidence that the BAI showed better discriminant validity than did the STAI (and the other widely used anxiety measurements of the time) (Fydrich et al., 1992).

Several studies on anxiety and depression have indeed found strong evidence for a high degree of comorbidity (e.g., Dobson, 1985a; Gorman, 1996; Gotlib & Meyer, 1986) . For instance, in their analysis of the National Comorbidity Survey conducted in 8098 healthy

American adults between 1990 and 1992 by the Survey Research Center at the University of Michigan, the authors report a lifetime comorbidity of (any) anxiety disorders and major depressive disorder (MDD) at 58% and the 12-month comorbidity at 51.2% – the highest reported comorbidity. The authors additionally report that anxiety disorders are both the most common primary disorders associated with a secondary diagnosis of MDD and the most common secondary diagnosis after a primary diagnosis of MDD. In a review of epidemiological studies in children and adolescents, Angold and Costello (1993) report comorbidities between anxiety and depression ranging from 30-75%.

The evidence referenced above has led some researchers to propose that anxiety and depression might be best conceptualized as a unitary construct. In her analysis of relevant literature, Feldman (1993) suggested that distinguishing between self-report measures of depression and anxiety may be practically useless in nonclinical populations – that two-factor models were so highly correlated that anxiety nor depression offered much unique variance over the other. In her view, as far as nonclinical samples are concerned, self-report measures of anxiety and depression might be best considered as measures of general distress. Other work, in fact, has shown that in a homogenous sample of individuals presenting with *clinical* anxiety, separate factors for anxiety and depression emerged through factor analysis with very little overlap. In light of these findings, the proposal by Clark and Watson (1991) of a tripartite model of anxiety and depression seems quite useful. Essentially, in their evaluation of the relevant psychometric data, they conclude that anxiety and depression share a common factor of general affective distress but that we may distinguish them by two other means: physiological hyperarousal (specific to anxiety) and low positive affect (specific to depression). In a subsequent paper, Mineka et al., (1998) review supporting evidence from gene studies (e.g.,

Jardine et al.,1984) indicating that depression and anxiety share a single, common genetic factor along with trait neuroticism. Thus, a genetic explanation may account for the substantial shared variance between the two constructs. In my view, this makes sense of the discrepant psychometric findings between the clinical and nonclinical samples.

Conceptual Issues and a Path Forward: Anxiety, Fear, and Dissociations

Excessive fear is a hallmark of the anxiety disorders and although they are closely related, there has been disagreement in the conceptual boundaries between them (Sylvers et al., 2011). For instance, Beck et al., (2005) distinguish anxiety from fear by suggesting that anxiety is emotional response while fear is cognitive appraisal of the stimulus, stating, "anxiety is the unpleasant feeling state evoked when fear is stimulated" (p. 9). In an similar formulation from Horwitz (2013), "fear… is anxiety that is attached to a particular thing or circumstance" (p. 4) – after which, he proceeds to use the term anxiety interchangeably with fear. Barlow (2004), on the other hand, drew a slightly sharper distinction by framing anxiety as "future-oriented mood state" (p. 64), suggesting that anxiety is anticipatory in nature while fear is a reaction to an immediate/proximal threat.

More in line with the latter interpretation, research from studies on rats has indicated that fear and anxiety are dissociable mechanisms which may manifest in distinct neurobiological substrates. To precisely explicate how this understanding emerged, I'll provide a brief evidentiary background. In a method first described and tested using rats by Brown and others (1951), it was shown that the startle reflex in response to a sudden, loud auditory stimulus can be potentiated when it is presented with a stimulus (i.e., presentation of a light) which has been previously paired with an aversive stimulus (i.e., moderate shock) – thus, the aptly named *fear*-potentiated startle effect was born. It has subsequently been demonstrated that this effect is

dependent on the fear conditioning component and that the effect may also follow an inverted U-shaped curve, where both mild shocks and intense shocks produced a smaller effect than moderate shocks (Davis & Astrachan, 1978). It was also shown that the effect is highly temporally specific, as its magnitude appears to peak at the time when the paired shock would typically occur (Davis et al.,1989). Furthermore, anxiolytic drugs block the *increase* in the startle effect in the presence of the light but do not actually decrease the baseline level of startle in general (Kehne et al., 1988). Taken together, these data suggest the fear-potentiated startle effect is "a sensitive measure of anticipatory *fear* or anxiety" (Davis et al.,1993, p. 177). To be clear, this fear-potentiated startle effect, I would argue, reflects a stimulus-induced state of fear as opposed to a general anxiety state which may not have an immediate or proximal stimulus. Extensions from this line of research have found alternative procedures which may allow researchers to explore a more general, more sustained state of anxiety.

Based on previous work which suggested that sustained exposure to bright lights may be anxiogenic for rodents (e.g., McLearn, 1960), Walker and Davis (1997a) conducted a series of experiments to test if the exposure to bright lights could enhance startle effects. The experimenters randomly divided twenty-four rats into three groups which were each subjected to extended periods of exposure to white fluorescent bulbs of varying intensities (e.g., low, medium, high). In the first experiment, it was confirmed that the level of illumination reliably increased the amplitude of the startle response – as measured by an accelerometer attached to the bottom of the cage – to an acoustic stimulus. In the second experiment, sixteen rats were tested across various conditions, manipulating the illumination (i.e., on or off) and testing the effect of an anxiolytic drug. Specifically, the rats were tested under four conditions: (*i*) dark cage to illuminated cage, injected with saline, (*ii*) dark cage to illuminated cage, injected with buspirone,

(*iii*) dark cage to dark cage, injected with saline, (*iv*) dark cage to dark cage, injected with buspirone. The data from the second experiment indicated that the increase in magnitude of the startle response in the presence of an anxiogenic light could be attenuated by a classic anxiolytic drug. In the general discussion, the authors argue that the influence of the light on the acoustic startle effect reflects an *anxiety* experience by the rats, perhaps as an evolutionary defense mechanism given that rats are nocturnal and exposure to bright lights increases vulnerability to predators. Furthermore, the experiment produced the so-called light-enhanced startle effect which occurs through apparently different mechanisms, given that the effect produced by the presence of the light in this procedure does not depend on prior conditioning unlike the fear-potentiated startle effect.

Evidence that these two mechanisms operate through fundamentally different neurobiological substrates was produced by Walker and Davis (1997b). Specifically, in a set of experiments, the authors examined the effect of intracranial infusions of an AMPA receptor antagonist drug (i.e. 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(F)-quinoxaline, (NBQX)) into three distinct regions known to play various roles in fear/fear conditioning. In each of these cases, the AMPA receptor antagonist drug blocks the influence of glutamate, thereby preventing normal excitation of these regions. The regions tested were: (1) the basolateral nucleus of the amygdala – well known to play a central role in fear conditioning (e.g., LeDoux, et al.,1990); (2) the central nucleus of the amygdala – also involved in fear conditioning (e.g., Falls & Davis, 1995); and (3) the bed nucleus of the stria terminalis (BNST) (a cluster of twelve nuclei which surround the caudal portion of the anterior commissure (Dumont, 2009)) – shown to receive input from the amygdala and play a role in fear response (Sullivan et al., 2004). The fear-potentiated startle effect was blocked by the NBQX infusion into the central nucleus of the

amygdala while the light-enhanced startle effect was blocked by the NBQX infusion into the BNST. Further, both the fear-potentiated startle and light-enhanced startle effects were blocked by the NBQX infusion into the basolateral nucleus of the amygdala. These results provide evidence for a double dissociation between "fear" (i.e., disruption of the central nucleus of the amygdala) and "anxiety" (i.e., disruption of the BNST), at least insofar as they have been conceptualized in terms of phenomenology.

Research on the fear-potentiated startle effect in humans has shown analogous findings to those found in rodent models. For instance, Grillon and colleagues (1993) conducted a study on the potentiation of the acoustic startle reflex on 20 participants trained to anticipate mild electric shocks delivered via electrodes on their wrists. The startle reflex was recorded under both the threat condition and the non-threat conditions which were signaled by a blue light and red light, respectively (the blue light and red light were reversed for half the participants). The subjects were told that the conditions would last 50 seconds, and the shock could only be delivered in the last 10 seconds of the threat condition. Further, they were told that they may receive between one and three shocks and that the second and third shocks, if delivered, would be more intense than the first so that a consistent level of fear could be maintained through the duration of the current and remaining threat conditions – only one shock would ever be delivered, however. Two quite relevant findings emerged from the results. First, the authors found that, just as in the rodent studies discussed above, the anticipation of the shock during the threat condition reliably facilitated the startle effect. Here, again, we find a consistent behavioral effect driven the fear of an aversive, proximal stimulus. The second relevant finding from this study comes from the added manipulation of the temporal dimension (i.e., 50 second condition in which the shock(s) would occur during the last 10 seconds). The authors report that the fear-potentiated startle effect

reached a peak at the 45-second mark – the time at which the shocks were delivered during the threat condition. The temporal specificity of this startle effect is analogous with the temporal specificity found in the rat model discussed above (i.e., Davis et al.,1989). At minimum, this finding would seem to further enhance the generalizability of the rodent model of anxiety to humans. It is also of note that this fear-potentiation effect has also been shown in the context of psychophysiological study, where a slow cortical potential (a distinct, slow event-related signal in EEG (Strehl et al., 2006)) called Stimulus-Preceding Negativity was identified as an indicator of the fear induction. Here, the data indicated that this fear induction signal may have been generated by the anterior cingulate cortex (Böcker et al., 2001).

Research which seems to parallel work discussed above on the anxiogenic effect of light exposure in rats (i.e., Walker and Davis, 1997a) has shown that humans exhibit a similar enhancement of startle effects with a manipulation of light. In humans, however, the potentiation of the acoustic startle effect is produced in the presence of darkness (Grillon et al.,1997). In this study, twenty-five participants were asked to sit in a reclining chair and keep their eyes open for the entire experiment. Each participant was affixed with an EMG electrode underneath their left eye (i.e., at the orbicularis oculi muscle) to assess startle reactivity via eyeblink strength. After the experiment began, participants went through an adaptation period where they were presented with the startle probe – a 102 decibel burst of white noise – six times so that the initial startle reactivity due to surprise might be reduced during the experimental blocks. Next, the participants went through a light and dark phase (the two blocks) while they were randomly presented with different orders of stimulus presentations (a mix of a quieter, 65 decibel white noise "prepulses" and the startle probe). The authors report that darkness did reliably and significantly enhance the acoustic startle effect in humans. In this case, darkness elicits an anxiogenic state in humans as

the presence of light produced an anxiogenic effect in rats. Further supporting this notion, the authors report the data from a subjective anxiety questionnaire given at the end of the study in which it was found that participants who reported greater fear of the dark as children showed increased startle responsivity during the experiment. The authors also offer an explanation invoking evolutionary mechanisms to explain the anxiogenic effect of light exposure in rats and dark exposure in humans, suggesting that relative vulnerability to predation may account for this heightened responsivity. The finding of startle magnitude increases in the dark as opposed to the light was confirmed in a subsequent study of civilians and combat veterans with and without post-traumatic stress disorder (PTSD) (Grillon et al., 1998). The findings show that the significant effect persisted across groups, but the difference in startle magnitude between light and dark contexts were significantly greater in non-PTSD and PTSD veterans than in civilians, suggesting that learning (i.e., combat experience) can contribute to the abnormal startle reactivity.

Assuming the fear-potentiated startle effect and the light (dark)-enhanced startle effects are analogous to the previously discussed conceptualizations of fear and anxiety, respectively, one might also argue that these two effects can be distinguished by their temporality. Specifically, the fear-potentiated startle effect might be seen as a phasic startle potentiation while the light-enhanced startle effect represents a more sustained form of fear potentiation – quite in line with the previously discussed notion that fear is the immediate response to a proximal threat while anxiety is a future-oriented mood state. Pharmacological research has also provided evidence for a dissociation between these two phenomena in humans. The common benzodiazepine alprazolam was used to test the hypothesis that these two states are indeed distinguishable. Consistent with this hypothesis, it was found that alprazolam did not affect the (phasic) fear-

potentiated startle effect but did reduce the effect of the sustained, contextual anxiety on startle magnitude (Grillon et al., 2006). Here, the authors provide relatively strong evidence for a dissociation of fear and anxiety in terms of neurobiological substrate. More specifically, if these two phenomena were not distinct, they should be influenced (or not) systematically by the same drug.

Regardless of the granular mechanism, the fear conditioning research clearly identifies the amygdala as a central site for both fear and anxiety states. Strong evidence for in support of this notion also comes from lesion studies of the amygdala and subsequent interference of fear acquisition and behavioral response. For instance, using the same fear-potentiated startle paradigm in rats whereby presentation of a light previously paired with a shock increases magnitude of the startle response to an acoustic stimulus, bilateral lesions of the central nucleus of the amygdala blocked the conditioned fear response (Hitchcock & Davis, 1986).

Beyond the Amygdala: Hippocampal Contributions to Stress and Fear

Although the amygdala has received ample attention for its role in fear and anxiety-related processes, the hippocampus has also been identified as a major region of interest in this domain – particularly for its apparent role in stress. Stress has been shown to play a major role in the development of mood and anxiety disorders (Young et al., 1997) and there is evidence that reduction of stress through the mindfulness-based stress reduction technique has sustained beneficial effects on anxiety disorders and anxiety symptomology, broadly (Miller et al., 1995; Vøllestad et al.,2011). In fact, a well-established line of literature has elucidated the mechanisms through which stress impacts the hippocampus – the ventral hippocampus – in particular. For instance, Henke (1990) showed that lesions of the ventral hippocampus altered stress responses (i.e., the development of ulcers in response to a stressful environment) in rats, suggesting that the

ventral hippocampus may play a role in coping. Quite in line with this finding, Sapolsky and colleagues (1990) showed direct evidence that prolonged exposure to the stress hormone cortisol produced significant tissue damage in the ventral hippocampus (i.e., CA3/CA2) of the vervet monkey. These and similar data led to the review by Moser and Moser (1998) where the authors summarize the data indicating that hippocampus did not act as a unitary structure, but was functionally heterogenous consisting of at least two functional units – a dorsal and ventral gradient where the former may support spatial learning while the latter seems to be involved in emotion-related behaviors, given its connections with the hypothalamus in addition to amygdala. The functional segmentation of the hippocampus in humans has also received support from more recent neuroimaging work (Robinson et al., 2015).

Consistent with the functional segmentation data, the dorsal hippocampus appears to support other processes related to anxiety beyond stress reactivity. In a fear conditioning study of rats, Phillips and LeDoux (1992) found that electrolytic lesions of the dorsal hippocampus selectively interfered with the acquisition of the freezing response to contextual stimuli (i.e., the location of the tone-shock pairing) but not to the conditioned stimulus (i.e., the tone), whereas amygdalae-lesioned rats showed interference with the conditioned stimulus but not the contextual stimuli – This selective role of the dorsal hippocampus in fear conditioning has received support from additional lesion studies (Kim & Fanselow, 1992; Maren & Fanselow, 1997; Selden et al.,1991), suggestive of a central role for the hippocampus in contextual coding of fear stimuli. More recent work using optogenetic targeting of cells in the rat dorsal and ventral hippocampus, the dorsal hippocampal cells contributed to spatial and contextual learning. Surprisingly, the authors also report that excitation of these cells significantly increased exploratory behavior in a novel environment, producing anxiolytic effects similar to those also shown in stimulation of the

ventral hippocampus (Kheirbek et al., 2013). Nonetheless, it is clear from the evidence reviewed above that the hippocampus is intricately involved in processes related to anxiety.

The work reviewed above represent an attempt to define and conceptualize the states of anxiety, fear, and stress while providing evidence for dissociable neurobiological substrates which may underlie each. Our understanding of these emotional states, however, has clearly been heavily reliant on animal models. Animal models are clearly important for identifying the precise mechanisms in the brain which generate these states, and some analogous mechanisms between these models and actual human behavior have been identified, such as the startle potentiation effect discussed above. While great progress has been made through use of these models, some in the field have begun to call into question the validity and generalizability of these findings to how we understand these states in humans (LeDoux, 2012).

New Directions in Anxiety and Fear Research: Two-System Approach

Though the research discussed above makes clearer the phenomenological, behavioral, and neurobiological differences between fear and anxiety, recent calls have been made to rethink the way we conceive of these states. Although there are several reasons for these calls for reconceptualization, one quite relevant issue is the apparent stagnation in the development of novel anxiolytic drugs for the treatment of pathological anxiety. For instance, Griebel and Holmes (2013) point out in their review of anxiolytic drug development that the current well-established anxiolytic drugs (i.e., benzodiazepines and selective serotonin reuptake inhibitors (SSRIs)) are quite non-selective in their mechanisms of action which necessarily limits the ability to hone in on the identified neurobiological targets identified through the study of anxiety. Furthermore, it has been suggested that the study of fear and anxiety has also failed to significantly advance due to an overreliance on specific paradigms such as fear conditioning

tasks – attractive, given the high degree of experimental control they allow, but narrow in scope (Paré & Quirk, 2017). Further clouding the issue is the question of face validity regarding these animal models. For instance, Garner (2014) points out that pathological anxiety is "fundamentally different" (p. 443) than what normal anxiety measures assess, making the generalizations offered from these measures highly questionable.

In what may be one of the more controversial challenges to the field of anxiety and fear research, LeDoux (2012) suggests that, upon examination of the relevant literature, it isn't at all clear what is meant by the term *emotion* in the first place. He further points out that our conceptual issues surrounding what we mean by emotion essentially center around the conflation of the physiological survival mechanisms with the conscious, experiential feeling states which coincide with them. Consistent with this notion, LeDoux and Pine (2016) put forth a two-system framework for understanding fear and anxiety to alleviate the conceptual barriers surrounding these phenomena. Under this framework, we may distinguish between survival circuits – the innate survival mechanisms which are phylogenetically conserved across mammalian species– and conscious feelings generated in concert with them; that they are emergent from distinct neurobiological mechanisms. The authors suggest that the conscious feelings occur within us when our consciousness detects the activation of a survival circuit, and we subsequently evaluate and consciously label that state. Quite obviously, as the authors point out, we cannot know what conscious feelings are associated with activations of survival circuits in, for example, rodents subjected to shock in a fear conditioning paradigm might be. Further, LeDoux and Hofmann (2018) argue that scientific use of the term 'emotion' be restricted entirely to subjective experiences – that we use other terminology when describing the objective, behavioral responses that tend to co-occur with the subjective experience. Thus, this reconceptualization allows us the

opportunity to avoid conscious feelings altogether in the exploration of specific, generalizable survival-based mechanisms explored throughout the behavioral neuroscientific literature but leaving open the door to study the nature of conscious emotional experience.

The proposal by LeDoux and colleagues (2012; 2016; 2018) is not without opposition. One notable example of theoretical opposition comes from Fanselow and colleagues. In direct response to LeDoux and Pine (2016), Fanselow and Pennington (2017) challenge the notion that the subjective experience should be so sharply distinguished, arguing that the behavioral and physiological responses along with the subjective experience represent an integrated response. Further, they suggest that if it is indeed the case that subjective experience of fear (or anxiety) and behavioral/physiological responses emerge from independent brain mechanisms, that we must necessarily return to reliance on subjective self-report; that behavioral and physiological responses would have predictive value for subjective experiences. In a previous work, Perusini and Fanselow (2015) also challenge the reconceptualization effort by arguing that one aim of science is to replace "replace inaccurate subjective explanations of our feelings and actions with more precise and scientifically grounded explanations of these phenomena" (p. 418), thereby avoiding entirely the subject of subjective experience and instead focusing exclusively on the activity of innate fear systems centering around amygdalae function. While this approach most certainly sidesteps the potentially muddy business of unravelling the mysteries surrounding human consciousness, it does so while ignoring the incremental progress being made in that domain (LeDoux et al., 2018) and is "indispensable in the effort to understand human nature" (LeDoux & Brown, 2017, p. 1).

If we are to accept the two system model reformulation, the terms 'anxiety' and 'fear' should be limited to their primary meanings – that is, descriptions of mental states only (at present)

capable of being understood through means of self-report (LeDoux & Pine, 2016). What then of the innate circuitry conserved across species which governs the response to threats in the environment discussed above (i.e., hippocampus, central/basolateral nucleus of the amygdala, BNST)? LeDoux and Pine (2016) suggest that these regions fall under 'defensive circuits' and the subsequent responses they produce would be referred to as 'defensive behaviors'. Under this model, the behavioral neuroscientific literature discussed above (e.g., fear conditioning studies) remain useful in describing the subcortical system of circuits which have been conserved in humans and still clearly govern our threat detection and response behaviors. The remaining theoretical system is the system which governs our conscious experience driven through cortical circuits. The authors suggest that very same regions which have typically been implicated in conscious experiences in humans constitute this system, such as those that govern working memory, attention, and regions implicated in interoception and sensory integration regions like the insula and posterior parietal cortices, respectively. In summary, if we wish to fully account for both the subjective experience of anxiety (and fear) as well as the defensive physiological mechanisms and behaviors which coincide with our experience of these emotional states, the two-system approach seems well-suited for the task.

Experimental Manipulation of Anxiety

Several experimental paradigms have been used to induce anxiety in both clinical and nonclinical populations. The paradigms are widely variable, ranging from the use of carbon dioxide (CO_2), mood induction, and threat of shock. Here, I provide a brief overview of various procedures, with a particular emphasis on procedures which induce state anxiety as opposed to paradigms which highlight differences in individuals with trait anxiety.

Carbon Dioxide Induced Anxiety

Based on largely anecdotal reports and some limited data indicating that hyperventilation may play a role in the pathogenesis of anxiety disorder, Gorman et al. (1984) tested the psychological effects of acute exposure to CO_2 (5% mixture), sodium lactate by infusion, and normal air hyperventilation on sixteen subjects (12 with diagnosed panic disorder, 4 healthy volunteers). Contrary to their hypothesis, the authors found the 5% CO_2 mixture was just as reliable as the established effect of sodium lactate in provoking panic attacks in subjects with panic disorder. Subsequent work using CO_2 to induce anxiety symptoms has confirmed the findings in both clinical and nonclinical samples (Papadopoulos et al.,2010; Schmidt & Zvolensky, 2007; Van den Hout & Griez, 1984; Woods et al.,1988), lending strong support to the anxiogenic effect of hypercapnic gas.

In an exploration of the mechanisms which might underlie this anxiogenic effect, Argyropoulos et al. (2002) subjected fourteen healthy male volunteers to a single inhalation of 35% CO_2 while simultaneous measures of plasma cortisol and blood pressure were taken. The results show that the acute inhalation produced significant activation of the hypothalamic-pituitary-adrenal (HPA) axis (as assessed by blood cortisol level increase after exposure) in addition to elevated blood pressure and subjective reports of a transient fear state reported by the volunteers. A more recent study found that exposure to 7.5% CO_2 over a twenty-minute session reliably and significantly increased blood pressure and heart rate as well as a number of subjective indices of anxiety including the previously mentioned Spielberger STAI (Bailey et al.,2005). The authors suggest that this procedure may in fact be a better model for generalized anxiety disorder than panic disorder and is relatively safe for most participants.

A follow up study using this procedure found that the anxiogenic effect produced by the prolonged exposure appears to be attenuated by two drugs with demonstrated efficacy in treating

GAD, lorazepam (benzodiazepine) and paroxetine (selective serotonin reuptake inhibitor (SSRI)) to a lesser extent, providing additional support for the potential of this model in the study of GAD (Bailey et al.,2007). Based on these and other data, Bailey and colleagues (2009) suggest that the anxiogenic effect of CO_2 is likely mediated to some extent by GABAergic neurons. Though a strong neurobiological explanation for this effect has yet to be established, a recent pilot functional magnetic resonance imaging (fMRI) study explore the association between negative affective valence networks and the prolonged exposure to CO_2 model found increased connectivity between the ventromedial prefrontal cortex and right amygdala and decreased connectivity between the midcingulate cortex and left amygdala when subjects viewed angry faces. Further, these connectivity differences were associated with a stronger anxiogenic response (Huneke et al.,2020).

Mood Induction

Following the Velten (1968) depression/elation induction procedure, Orton and others (1983) produced a list of 50 self-referent statements centering around feelings of anxiety (e.g., "I'm feeling more and more jittery", "This is awful", "What if I lose control of my feelings?") in addition to a depression and neutral list. Sixty female participants were randomly assigned into each of the three conditions. The authors report that the anxiety-inducing self-referent statements produced significant increases in tonic heart rate and self-reported state anxiety in comparison to the depression and neutral groups. Using a similar reformulation of Velten's (1968) procedure, Eysenck (1984) reports increased worry in participants in the anxiety-induction procedure, but that this effect was temporally transient in individuals with low trait-anxiety. Overall, the mood induction procedures are not well documented in the literature and likely offer little utility in inducing anxiety in the laboratory.

Situation Specific/Social Anxiety

In a study of the language anxiety phenomenon experienced by individuals learning a second language, Macintyre and Gardner (1994) significantly induced state anxiety by subjecting seventy-two first year students enrolled in French courses simply by introducing a video camera during a vocabulary learning task. Similarly, Leite and colleagues (1999) effectively induced anxiety through introduction of a video camera while participants performed the Stroop Color-Word Test. The anxiogenic effect of the camera introduction was blocked by a standard anxiolytic benzodiazepine drug, further supporting the notion that this procedure did in fact provoke anxiety. Successful state-anxiety induction was also achieved in studies exploring alcohol use and aggression/stress where participants in the anxiety induction groups were told they would have to deliver a speech regarding what they liked and disliked about their body in front of a video camera (Phillips & Giancola, 2008; Sayette et al., 2001). Finally, several studies have successfully used public speaking (either simulated or real) as a specific anxiety induction procedure (Linares et al., 2019; McNair et al., 1982; Wieser et al., 2010; Zuardi et al., 2017). In sum, the situation specific anxiety induction procedures appear to be quite reliable and relatively easy to employ in a laboratory setting.

Threat of Shock

In what likely amounts to be one of the most ubiquitously employed anxiety induction procedures in the literature with respect animal models, the threat of shock has been shown to be a consistent and well-controlled induction of state anxiety (Robinson et al., 2013). In addition to being highly translatable across human and rodent models (Davis et al., 2010), paradigms utilizing threat of expected or unexpected aversive events such as shock are quite well-validated across pharmacological and clinical studies in both children and adults (Schmitz & Grillon,

2012). Although these procedures necessarily involve some level of harm or deceit, they are well-documented to reliably induce anxiety-like states.

Conclusion

In sum, anxiety is a deeply complex concept which deserves attention if for nothing else than the harm that can result from its pathological forms. For better or worse, anxiety is a central part of the human experience. It quite obviously serves an adaptive evolutionary function given the ubiquity of anxiety-like states throughout the animal kingdom. Future studies on anxiety should aim to rigorously clarify their terminology as a first step before embarking on whichever paradigm will be employed to avoid the conceptual confusion that may result. Finally, the aforementioned two-system framework by LeDoux and colleagues (2012; 2016; 2018) may be the best theoretical framework for a clear understanding of past research and on which to conduct future research in anxiety as well as fear and stress. In order to adequately address pathological human anxiety, we must move forward in our understanding of the cortical, conscious state as well as the subcortical defensive circuitry which govern it.

Chapter 4: Subconscious Threat Processing and Cannabidiol

Introduction

Cannabidiol (CBD), a non-psychoactive cannabinoid found in the cannabis plant, appears to be increasing in popularity across the United States. A recent Gallup poll shows that 14% of Americans claim to use CBD products (Brenan, 2019). The very same Gallup poll shows that Americans are using CBD for a variety of reasons, including pain (40%), anxiety (20%), and sleep (11%). The scientific evidence to support the use of CBD for these health issues, however, does not appear to be substantial – specifically in the case of anxiety. According to the 2017 National Academies of Sciences, Engineering, & Medicine report on the current state of research on the health effects of cannabis and cannabinoids, the evidence for therapeutic efficacy of CBD in the treatment of anxiety in humans appears to be rather limited. Moreover, the report points out that the positive evidence reviewed therein is derived from studies with methodological/design weaknesses, further limiting the evidentiary basis for anxiolytic efficacy (National Academies of Sciences & Medicine, 2017). However, it should be noted that the aforementioned report was quite restrictive in their inclusion criteria, only considering a single systematic review by Whiting et al. (2015) – a review which itself only includes a single randomized control trial (RCT) in patients with social anxiety disorder during a public speaking task (Bergamaschi et al., 2011).

Despite the dearth of RCT evidence in normal human participants, research on CBD in clinical samples offers some insight into the therapeutic potential for the cannabinoid in reducing anxiety. In a retrospective study of adult psychiatric patients who were treated with CBD as an adjunct to their typical regimen, mean anxiety scores (as assessed by the Hamilton Anxiety Rating Scale) were shown to decrease in a sustained fashion over the 3-month treatment period (Shannon, 2019). Further, in a study of Japanese teenagers with social anxiety disorder and avoidant

personality disorder, a daily 300mg dose of CBD over the course of a month significantly decreased anxiety when compared to a placebo (Masataka, 2019). In another study of individuals with social anxiety disorder, pretreatment with 400mg of CBD significantly decreased subjective anxiety over several timepoints during an anxiety-provoking, single photon emission computed tomography (SPECT) session (Crippa et al., 2011). Nonetheless, direct evidence in support of an anxiolytic effect is sparse – at least as it relates to anxiogenic paradigms, specifically. Other work with designs more peripherally related to anxiety and fear, however, have shown promising results.

Cannabidiol and Fearful Faces

A series of similar studies focused on the distinct effects of the two most heavily studied sister molecules in cannabis research: THC and CBD. The thrust of the research sought to explore the relationship between these major cannabinoids and the apparent discrepancy in cannabis-induced modulation of anxiety. More specifically, it is well-documented throughout the psychopharmacology literature that ingestion of THC, particularly pure THC, can produce acute anxiety in otherwise healthy human subjects (Martin-Santos et al., 2012; Morrison et al., 2009; Zuardi et al.1982). The relevant research also shows that CBD may reduce or attenuate the anxiogenic effect of THC (Freeman et al., 2019; Zuardi et al., 1982). Extending this work, Fusar-Poli et al., (2009) used event-related fMRI to assess the effects of THC, CBD, or a placebo during an emotional-face viewing procedure. In agreement with previous work, THC increased anxiety during the presentation of fearful faces. CBD, on the other hand, produced a trend-level decrease overall across the measured indices of anxiety. Importantly, when compared to the neural activation in the left amygdala in response to fearful faces in subjects who received the placebo, activity of the left amygdala in the CBD-group was significantly diminished. This attenuation of amygdala responsivity was also correlated with the number of SCR fluctuations during the fearful

face trials (i.e., CBD reduced the fluctuations in SCR). In an extension of this work, Bhattacharyya et al. (2010) again found that subjects dosed with CBD before viewing fearful faces in a functional magnetic resonance imaging (fMRI) procedure had opposite physiological responses than those subjects dosed with THC. Finally, in an effective connectivity study, Fusar-Poli et al. (2010) found that CBD, but not THC, disrupted connectivity between the amygdala and anterior cingulate cortex during the presentation of fearful faces to participants – offering a potential neurofunctional mechanism behind the anxiolytic effect of CBD.

Anxiety and Fear

While the above research provides a nice account of the effect of CBD in a specific paradigm, it could be argued that the paradigm itself conflates "anxiety" with fear. Fear is certainly (and perhaps inextricably) tied to anxiety. And while excessive fear is a signature of the anxiety disorders, generally, the conceptual and definitional boundaries between the two emotional states are a subject of debate (Sylvers et al., 2011). Beck et al. (2005), for instance, suggests that fear describes the cognitive appraisal of a threatening stimulus, while anxiety is the unpleasant emotional state elicited by the cognitive appraisal. Similarly, Horwitz (2013) states, "fear… is anxiety that is attached to a particular thing or circumstance" (p. 4). Barlow (2004) draws a slightly sharper distinction, framing anxiety as "future-oriented mood state" (p. 64), characterizing anxiety as anticipatory in nature while fear is a reaction to an immediate or proximal threat. In line with Barlow's conceptualization, a wealth of data from rodent and human fear conditioning research indicates that fear and anxiety are dissociable in terms of their respective neurobiological substrate (for a review see Davis, et al., 2010). Working from this understanding of fear and anxiety, the effect of CBD in the fearful faces paradigm may be more precisely described as an attenuation of what LeDoux & Pine (2016) would call "defensive responses" in response to threatening stimuli.

In an effort to help catalyze new research in the development of anxiolytic drugs in the wake of conceptual confusion and remarkably little progress over the last few decades, LeDoux and Pine (2016) outline a new framework for understanding fear and anxiety – the "Two-System Model". In the Two-System Model, it is suggested that "fear" is a product of cortical, cognitive circuits (e.g., circuits which are more akin to executive functions like working memory or inhibitory control) while subcortical circuits govern the defensive behavioral and physiological repertoire elicited in response to threats in the environment. Alternatively, the traditional "Fear Center" model makes no such distinction between the cognitive and behavioral consequents of fear. If I accept the two-system formulation, the lack of progress in the production of novel anxiolytic drugs over the last 50 or so years (see Griebel & Holmes, 2013) is largely a result of a failure to understand the nature of fear and anxiety themselves. Further, LeDoux and Hofmann (2018) argue that scientific use of the term 'emotion' ought to be restricted to subjective experiences – that I ought to use other terminology when describing the objective, behavioral responses that tend to co-occur with the subjective experience. Thus, the reconceptualization effort allows us the ability to avoid conscious feelings altogether in the exploration of specific, generalizable survival-based mechanisms explored throughout the behavioral neuroscientific literature, but also leaves open the door to study the nature of conscious emotional experience.

Subconscious Fear Processing

There is strong and intriguing evidence consistent with the two-system framework which supports the idea that fear behavior can be elicited by stimuli detected below the level of conscious awareness. For instance, Morris and colleagues (1999) found support that a subcortical pathway involving the amygdala, thalamus, and superior colliculus work in concert to process indiscriminate, but behaviorally relevant visual stimuli using a positron emission tomography

(PET) procedure. This subcortical route for fear processing was further confirmed by Liddell et al. (2005), who outlined a procedure to test whether subjects' processing of such salient behavioral stimuli would still be elicited by consciously undetected emotional faces. That is, previous work (e.g., Morris et al.,1999) used emotional faces stimuli that were presented quickly enough to blind the subjects from the affect/valence of the facial stimuli, the face stimuli itself could be nonetheless detected above chance (Liddell et al., 2005; Williams et al., 2004). Drawing on signal detection analysis work from Williams et al., 2004 which found that stimulus presentations ranging from 30-50ms were detected above chance, Liddell et al. (2005) presented their emotional faces stimuli below 20ms (16.7ms). Consistent with the notion of a fast-route for processing of fear-related stimuli, Liddell and colleagues (2005) found that the subliminal presentation of such stimuli was sufficient to produce activation in subcortical structures and eventually the amygdala and cortex without being processed by the visual cortex.

Work by Luo and others (2007) using magnetoencephalography (MEG) further supported this notion and confirmed a prediction made by LeDoux's two-system hypothesis for a fast route for fear processing by mapping the event-related synchronizations in response to fear-provoking stimuli (i.e., fearful faces). Specifically, Luo and colleagues (2007) found that fear expressions were processed sequentially by the hypothalamus/thalamus around 10-20ms post-stimulus followed by the amygdala at the 20-30ms mark, well before this information was processed by the visual cortex at the 40-50ms mark. Moreover, evidence from Tamietto et al. (2009) indicates that such passive exposure to unseen facial expressions prompts higher levels of arousal and facial reactions on behalf of the participants than evoked by facial expressions which were consciously processed. Together, these data provide strong evidence for a system of brain regions capable of

detecting socially salient signals from conspecifics even when such information falls outside the bounds of normal conscious awareness.

Present Study

Despite the well-established findings of a subcortical processing route for unseen emotional facial expressions and strong evidence that CBD attenuates autonomic arousal evoked by fearful facial expressions, no research to date has explored the effect of CBD on subconscious fear processing. The present research seeks to address this gap and extend the research on the anxiolytic nature of CBD by assessing its effect on the processing of non-consciously detected fearful faces. Accordingly, I utilized fMRI to examine functional changes in the left ACC and left amygdala—regions previously shown (e.g., Fusar-Poli et al., 2009) to exhibit attenuated responsivity in fearful faces tasks in participants who consume CBD. Finally, I will also examine functional changes in a region of the temporal cortex embedded within the right superior temporal sulcus (STS) previously found to be preferentially responsive to masked fearful faces in a highly correlated manner with the amygdala (Jiang & He, 2006). In fact, a recent combined theta burst transcranial magnetic stimulation (TBS) and fMRI study indicates a causal connection between the STS and the amygdala during a face perception task (Pitcher et al., 2017). More specifically, disrupting the activity of a face selective region in the posterior STS reduced activation in the amygdala during face perception, but not body or object perception. Regarding the proposed work, the primary outcome of interest is the threat response to subliminally presented fearful faces in placebo vs. active (CBD) groups. Specifically, I hypothesize the following:

1. Hypothesis 1: The mean blood-oxygen-level-dependent (BOLD) activation of the left amygdala in the active group will be significantly lower than the placebo group. Given that previous work has demonstrated that CBD attenuates the activity of the amygdala during

consciously perceived fearful faces relative to consciously perceived neutral faces, and that the amygdala has been shown to activate in response to subliminally presented fearful faces, I expect the attenuation of the amygdala via CBD will maintain during subliminally presented fearful faces.

2. Hypothesis 2: The mean BOLD activation of the left ACC in the active group will be significantly lower than the placebo group. Given that previous work has demonstrated that CBD attenuates the activity of the ACC during consciously perceived fearful faces relative to consciously perceived neutral faces, and that the ACC has been shown to activate in response to subliminally presented fearful faces, I expect this ACC attenuation via CBD will maintain during subliminally presented fearful faces.
3. Hypothesis 3: The mean activation level of the right STS in the active group will be significantly lower than the placebo group. Previous work has demonstrated that the STS is preferentially activate in response to visually suppressed fearful faces and that this STS activation is highly correlated with amygdala activation – indicative of a functional relationship between the two regions (Jiang & He, 2006; Pitcher et al., 2017). Despite no reported findings of STS attenuation by CBD specifically, I expect the activity of the STS will be attenuated as a consequence of the apparent functional connectivity between the STS and amygdala.

Methods

Based on the literature reviewed above, I hypothesized that, on average, the active dose group would show a blunted fear response, as assessed by amygdala reactivity (i.e., BOLD response magnitude), compared to the placebo group during the fearful face condition. To assess the differential activation in the hypothesized ROIs, I used a predefined ROI over the left amygdala

(Bhattacharyya et al., 2010), left ACC (Fusar-Poli et al., 2009), and right STS (Jiang & He, 2006). Neuroimaging data collection was carried out on the Siemens 7T MAGNETOM at the Auburn University MRI Research Center. The scanner is outfitted with a 32-channel head coil provided by Nova Medical (Wilmington, MA). All methods and specific hypotheses for the study are preregistered at the following web address: https://clinicaltrials.gov/ct2/show/NCT04831294. Additionally, Auburn University IRB approved documents (#20-107) are included in the appendices, including the prescreen (Appendix A), pre-scan questionnaire (Appendix B), post-scan questionnaire (Appendix C), recruitment documents (Appendix D), and informed consent forms signed by the participants (Appendix E).

Recruitment and Participants

I recruited 15 healthy right-handed participants between the ages of 21-50 years old for the experiment. However, one participant experienced discomfort in the MR environment and opted out of the second part of the study and, as such, this data was removed from the analysis. A technical issue with the experimental software also prevented the data from one subject from being recorded during their second scanning session, which is accounted for in the analyses below. The final analysis includes 14 participants (mean age = 26.14 years, SD = 6.15; 7 males and 7 females; 2 Black/African American, 1 Asian/Asian American, 11 White (5 Hispanic/Latino)). Exclusion criteria for the study included: 1) contraindications to the MR environment (e.g., implanted cardiac pacemakers, embedded metal objects/fragments, claustrophobia), 2) use of psychotropic or neurological/neuropsychiatric medication, 3) history of heart disease or stroke, 4) diabetes or other metabolic conditions, 5) self-reported high blood pressure, 6) history of concussions, 7) any diagnosed psychiatric or neurological condition, 8) have consumed alcohol in the 24-hour period prior to a scan, 9) consumed pain relievers in the 12-hours prior to a scan, 10) consumed food or

drinks (except water) and/or nicotine/caffeine an hour prior to any scanning, 11) have used or take THC/CBD, or 12) exercised within an hour of a scan. To be included in the experiment, participants had to meet the following criteria: 1) right-handed, 2) between 21-50 years of age, 3) no current diagnosis of psychiatric or neurological conditions, 4) no history of heart disease or stroke, 5) generally healthy, and 6) pass a screening test for the MR environment. Participants were recruited from within the Auburn University Department of Psychological Sciences through an electronic recruitment system (Sona Systems) and outside the department with flyers and social media posts.

For Auburn University students, compensation was received in the form of extra credit for their psychology courses in addition to the monetary compensation which all participants received upon completion of the study. There were three timepoints for data collection, including the online screening form (timepoint 1), the first fMRI scan (timepoint 2), and the final fMRI scan (timepoint 3). All participants received monetary compensation for their completion of timepoint 2 ($75.00) and timepoint 3 ($125.00).

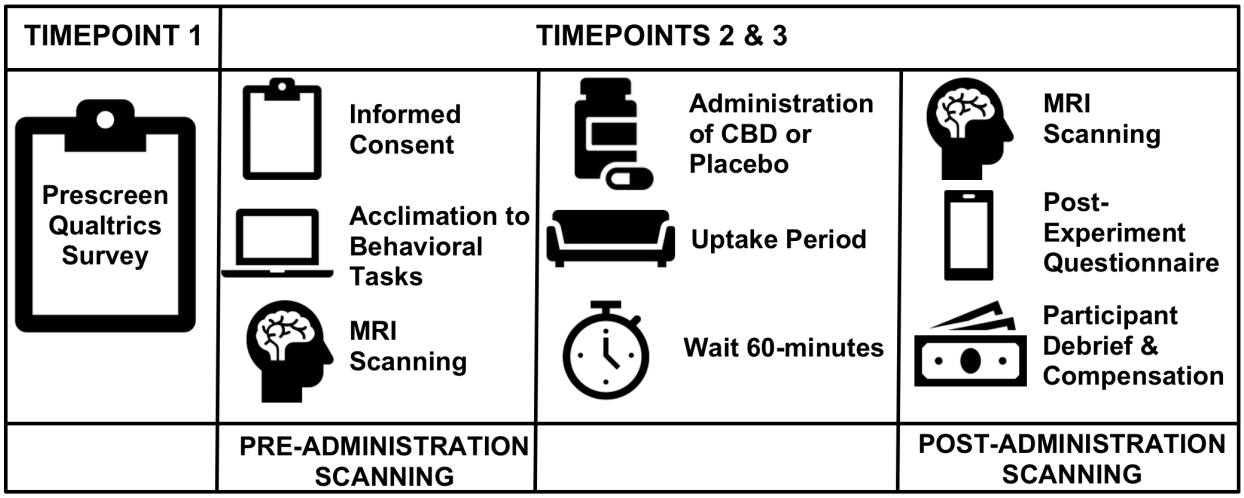
Study Design

The study design (Figure 1) was a randomized, double-blind, placebo-controlled, crossover trial. Each participant experienced the active condition at one timepoint and the placebo condition at the other timepoint. Further, to minimize any carryover effects of the drug, participants were brought back for the second scanning period (i.e., timepoint 3, Figure 1) after a washout period of at least 72 hours. On the two scanning days, participants were given a formalized procedure which included informed consent and a short acclimation to the behavioral tasks. After the first scans, participants were administered the treatment in a tincture via dropper underneath the tongue which they were asked to hold for 60 seconds before swallowing. After the tincture was consumed,

participants were asked to relax in the waiting room for 60 minutes to allow the drug to take effect. Following the 60-minute wait period, participants began the second task scanning period. Finally, participants were given a post-experiment questionnaire and compensated/debriefed.

Figure 1

Study Design Overview



Note. The above figure shows an overview of the study design.

Stimuli and Procedure

The experimental protocol was developed in the Millisecond software (version 6.3.5) platform and the code for the experiment will be published on an open access online repository (e.g., Open Science Framework (OSF)) upon publication. Following the basic design presented by Liddell et al. (2005), I utilized a backwards masking procedure (illustrated in Figures 2 and 3) which has been shown to prevent the masked stimulus from being consciously perceived while still provoking a threat response in the amygdala. In this experiment, the fearful and neutral faces were chosen from a set from a validated set of pictures from the Max Planck FACES Database (Ebner et al., 2010). Along with the face images expressing discrete emotions, the FACES Database also provides information with the accuracy with which participants in the validation study rated the faces correctly. I chose a subset of the images with fear and neutral expressions

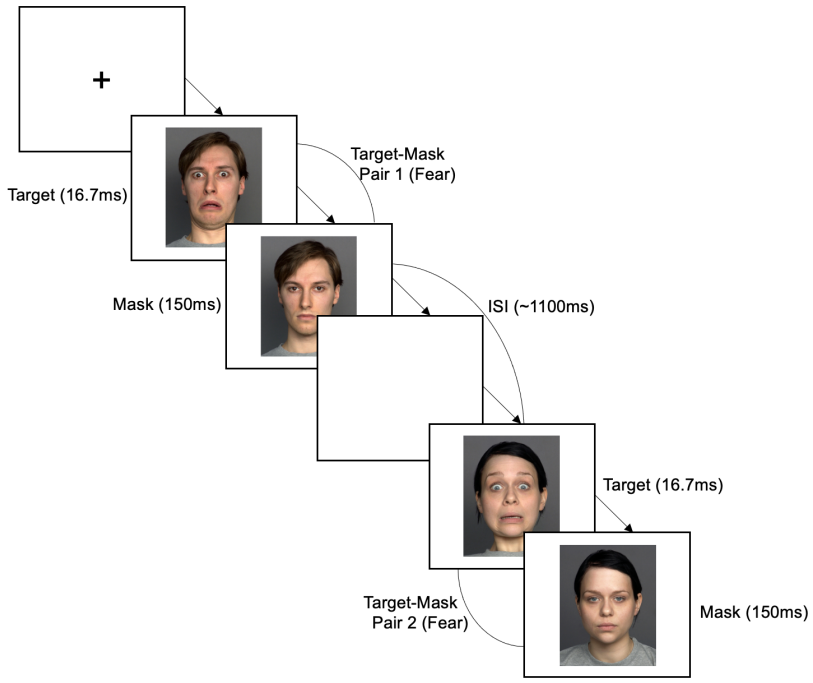
that were the highest rated of each category and contained an equal distribution of male and female faces. Each face chosen for the stimulus set had an accuracy rating ≥ 90%.

The experiment involved presenting participants with 8 total experimental blocks, including 4 "fear" blocks (Figure 2) and 4 "neutral" blocks (Figure 3) in a randomized order without replacement. A total of 10 unique target-mask pairs were presented in each block, and each block contained a random selection (without replacement) of target-mask pairs from a pool of 40 possible target-mask pairs. Each target-mask pair contained images of the same person with a total of 40 unique individuals depicted in the stimulus set. Additionally, the mask images were offset by 1 degree (on the four diagonals) to the right to reduce apparent motion artifacts, in line with previous work (Williams et al., 2004; Liddell et al., 2005). Consistent across the experimental protocol, the target was presented for 16.7ms (or 1 frame in a 60hz monitor) immediately followed by the mask, presented for 150ms (or 9 frames in a 60hz monitor) with an interstimulus interval (ISI) of 1,100ms between each target-mask pair. Finally, between each block, participants were asked to choose which emotion they believe they saw during the previous block, asked to rate their confidence in their guess, and were given a 30 second break before the next block.

During the fMRI data acquisition, participants were asked to observe the screen presented to them on the projector screen inside the scanner. Participants were instructed to carefully observe the stimuli during the experimental blocks. To maintain participant attention during the procedure, participants were told that despite the target image being difficult to see, they should pay attention to answer questions about the stimuli after the experiment concludes. After the instruction screen was read to the participant, the first block begins after the presentation of a fixation cross for three seconds. Fixation crosses were presented at the begging of each subsequent block. The total running time of the experiment was eight minutes and eleven seconds.

Figure 2

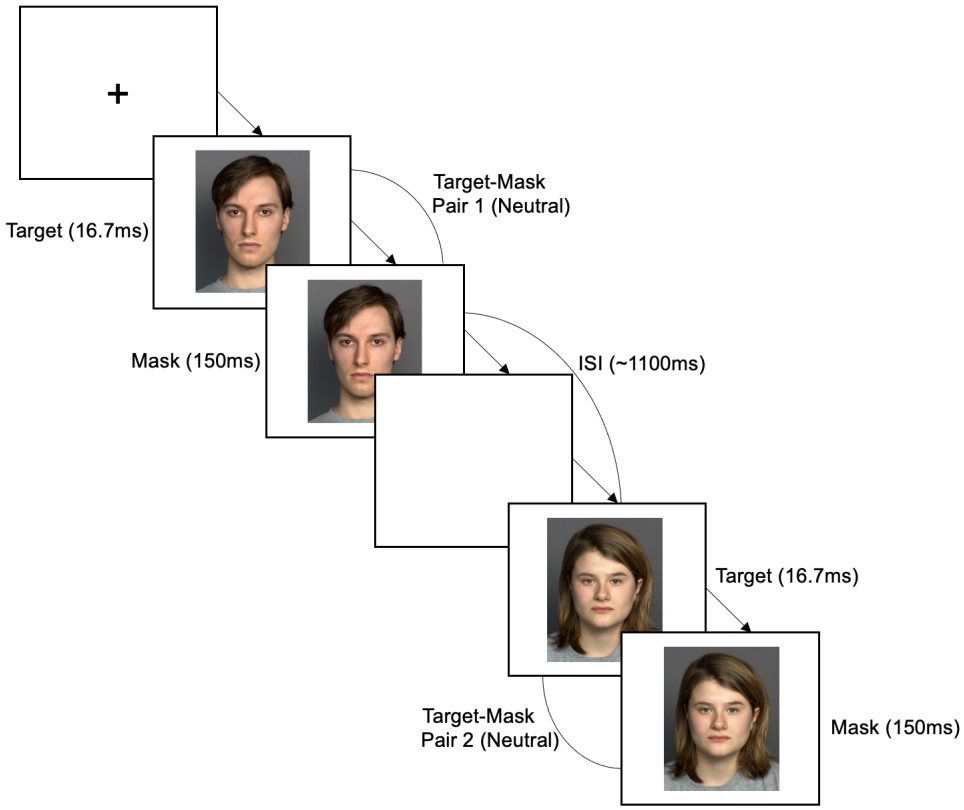
Fear Block Stimulus Example



Note. This figure is an example of the fear block stimulus presentation procedure given to each participant. A total of four neutral blocks were presented to each participant.

Figure 3

Neutral Block Stimulus Example



Note. This figure is an example of the neutral block stimulus presentation procedure given to each participant. A total of four neutral blocks were presented to each participant.

Cannabidiol and Placebo

Both the placebo and active material formulations are detailed in Tables 1 and 2. The tinctures prepared for this study were prepared and provided by Folium Biosciences. For the active dose, the broad-spectrum CBD oil contains 125mg of CBD, 100mg of which was expected to remain after metabolism. More specifically, due to a metabolic process referred to as the first-pass effect, cannabinoids are metabolized rapidly by the liver upon oral administration (Huestis, 2007). Therefore, to achieve an active dose of 100mg of CBD after the first-pass effect with the distinctive material formulation provided by Folium Biosciences, each tincture contained 125mg of CBD. Additionally, participants were instructed to place the tincture under their tongue with the provided dropper and to hold the material in their mouths for 60 seconds to maximize absorption, as oromucosal administration allows substances to avoid first-pass metabolism effects. Participants were also asked to arrive at the site of the experiment in a fasted state to minimize any controllable metabolic effect which may affect the efficacy or effect-onset of the tincture. Unfortunately, data for the bioavailability of CBD in human as well as animal studies are scarce and there is little available information regarding peak serum concentration/time (C_{max}/T_{max}) for different administration procedures, but recent meta analytic work suggests that oromucosal administration is common and, further, co-administration with a fat increases efficacy (Millar et al., 2018). As such, my formulation included additional lipids for co-administration (see Tables 1 and 2). Finally, as is standard procedure with randomized control trials (RCT), the researchers as well as the participants were blinded to which tincture, active or placebo, was taken by the participant. Participant treatment-order was determined in R, using the package 'randomizeBE' (package source and accompanying documentation can be found at: https://www.r-

pkg.org/pkg/randomizeBE) which creates a random treatment sequence list for crossover studies based on number of treatments and number of participants.

Table 1

Placebo Material Formulation

Ingredient	Dose (mg)	Concentration
Sunflower Lecithin	149	8%
Peppermint Oil	56	3%
Hempseed Oil	1661	89%
Total (2ml)	1867	100%

Note. List of ingredients, the dosing, and concentration information of the placebo material given to each subject; *mg* = milligrams.

Table 2

Active (CBD) Material Formulation

Ingredient	Dose (mg)	Concentration
Broad Spectrum CBD Oil	125	6.7%
Sunflower Lecithin	24	1.3%
Peppermint Oil	56	3%
Hempseed Oil	1661	89%
Total (2ml)	1867	100%

Note. List of ingredients, the dosing, and concentration information of the active material given to each subject; *mg* = milligrams.

Neuroimaging

Data were acquired on the Auburn University MRI Research Center Siemens 7T MAGNETOM outfitted with a 32-channel head coil by Nova Medical (Wilmington, MA). A whole-brain high-resolution 3D MPRAGE image (256 slices, 0.70mm^3, TR/TE: 2200/3.05, 7° flip angle, base/phase resolution 384/100%, collected in an ascending fashion, acquisition time = 7:39) was acquired for registration purposes. I utilized an EPI sequence, optimized in-house, for data acquisition (37 slices acquired parallel to the AC-PC line and emsuring amygdala coverage,

0.85mmx0.85mmx1.5mm voxels, TR/TE: 3000/28ms, 70° flip angle, base/phase resolution 234/100, A>P phase encode direction, iPAT GRAPPA acceleration factor = 3, interleaved acquisition, 110 time points, total acquisition time 9:16:21).

Analyses

I used field-standard analysis packages to preprocess and analyze the participant data. Preprocessing of data was carried out using fMRIprep (Esteban et al., 2019) following standard procedure (Poldrack et al.,2011) including brain extraction, motion correction, band-pass filtering, and normalization to a standard brain space (i.e., Montreal Neurological Institute (MNI)). Results included in this manuscript come from preprocessing performed using fMRIPrep 21.0.1 (Esteban et al., (2018); Esteban et al., (2018); RRID:SCR_016216), which is based on Nipype 1.6.1 (Gorgolewski et al., 2011; Gorgolewski et al., 2018; RRID:SCR_002502).

Anatomical data preprocessing

T1-weighted (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, RRID:SCR_004757). The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 6.0.5.1:57b01774, RRID:SCR_002823; Zhang et al.,2001). A T1w-reference map was computed after registration of the 4 T1w images (after INU-correction) using mri_robust_template (FreeSurfer 6.0.1; Reuter et al., 2010). Volume-based spatial normalization to one standard space (MNI152NLin2009cAsym) was performed through nonlinear registration with antsRegistration (ANTs 2.3.3), using brain-extracted versions of both T1w reference and the T1w template. The following template was selected for spatial

normalization: ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al., (2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym).

Functional data preprocessing

For each of the BOLD runs found per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) were estimated before any spatiotemporal filtering using mcflirt (FSL 6.0.5.1:57b01774; Jenkinson et al., 2002). BOLD runs were slice-time corrected to 1.47s (0.5 of slice acquisition range 0s-2.93s) using 3dTshift from AFNI (Cox & Hyde, 1997; RRID:SCR_005927). The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying the transforms to correct for head-motion. These resampled BOLD time-series is referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD reference was then co-registered to the T1w reference using mri_coreg (FreeSurfer) followed by flirt (FSL 6.0.5.1:57b01774, Jenkinson & Smith, 2001) with the boundary-based registration (Greve & Fischl, 2009) cost-function. Co-registration was configured with six degrees of freedom. Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions; Power et al., 2014) and Jenkinson (relative root mean square displacement between affines; Jenkinson et al., 2002). FD and DVARS were calculated for each functional run, both using their implementations in Nipype (following the definitions by Power et al., 2014). The three global signals were extracted within the CSF, the WM, and the whole-brain masks. Additionally,

a set of physiological regressors were extracted to allow for component-based noise correction (CompCor; Behzadi et al., 2007). Principal components were estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM and combined CSF+WM) were generated in anatomical space. This implementation differs from that of Behzadi et al. (2007) in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted a mask of pixels that likely contain a volume fraction of GM. This mask was obtained by thresholding the corresponding partial volume map at 0.05, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks were resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components were also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values were retained, such that the retained components' time series were sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components were dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al. 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 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 antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos, 1964). Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer). Many internal operations of fMRIPrep use Nilearn 0.8.1 (Abraham et al., 2014; RRID:SCR_001362), mostly within the functional processing workflow. For more details of the pipeline, see the section corresponding to workflows in fMRIPrep's documentation.

After the data was passed through the fMRIprep pipeline, I conducted a first-level analysis of each individual participant's functional neuroimaging data in FSL FMRI Expert Analysis Tool (FEAT; Woolrich et al., 2001) which included 5mm FWHM smoothing, high pass temporal filtering, and an interleaved slice timing correction. For the first-level analysis, data were registered to a standardized space (MNI 152 T1 2mm brain, standard input of FSL), and contrasts of interest were specified for each subject (e.g., fear, neutral, fear>neutral) based on their participant-specific order. Data were thresholded at $Z = 2.3$, and a cluster p threshold 0.05. Next, ROI analyses were conducted using FSL utilities (i.e., fslmeants via featquery; Woolrich et al., 2001) with the predefined ROI masks mentioned in the hypotheses. To make these masks, peak activation coordinates reported for each ROI were extracted from the papers discussed in the introduction (left amygdala ROI from Bhattacharyya et al., 2010; left ACC ROI from Fusar-Poli et al., 2009; and right STS ROI from Jiang & He, 2006), and converted from Talaraich to MNI space when necessary. Binarized spherical masks, centered around the coordinates (5mm for the left amygdala and right STS ROIs, 10mm for the ACC) were then created using FSLeyes

(Woolrich et al., 2001). These spherical masks were then input into featquery for the time-series BOLD extraction. For the left ACC ROI, however, there was not a reported peak activation coordinate so I elected to proceed with a 10mm mask over the approximate location reported in the Fusar-Poli et al., (2009) study.

Specifically, featquery examines the contrast of parameter estimates (COPE) from the first-level analysis, and calculates the BOLD percent signal change for each condition (i.e., fear, neutral, fear > neutral). Thus, this allowed for the assessment of the anxiolytic effect of CBD. Since the proposed study involves three distinct ROIs, I extracted data from the predefined ROI masks and analyzed it using analysis of variance (ANOVA) in both SPSS and R. The design of this study is a balanced full factorial design. As such, I analyzed the data with three separate 2x2 repeated measures ANOVA, testing for the simple main effect of treatment (i.e., placebo > CBD), the simple main effect of condition (fear > neutral), and finally – the main outcome of interest – the interaction of treatment*condition. This analysis was repeated for each of the three hypothesized ROIs to detect any significant change in BOLD signal brought about by the CBD during the presentation of fearful faces. Prior to running the ANOVAs, data was inspected for normality. Values which fell below Q1-2 of the interquartile range (IQR) or above Q3+2 IQR (i.e., Tukey's fences; Tukey, 1977) were judged as outliers. Outliers were inspected on a case-by-case basis to determine possible issues such as measurement error. In total, three outliers were determined unlikely to be a result of measurement error but still exceeded the upper and lower bounds established by Tukey's fences and were replaced by upper (median + 2 IQR) or lower limits (median – 2 IQR) (Tukey, 1977).

Results

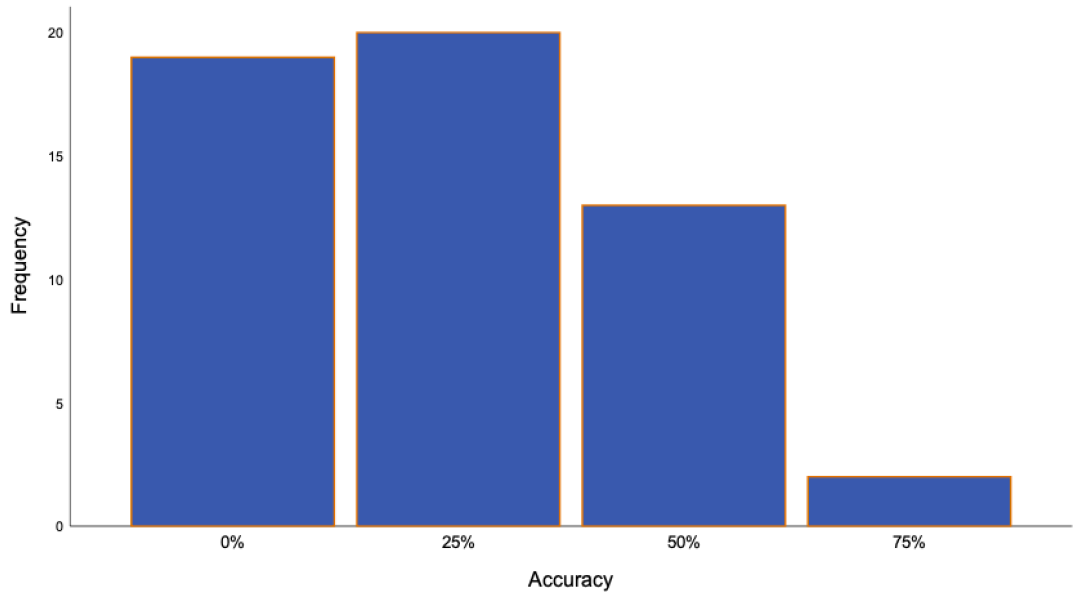
Region of Interest Analyses

Emotion Discrimination Check

As the task unfolded, each participant was asked to identify which emotion they believed they saw on the previous block. The primary reason for recording these scores was to ensure that the participants were not consciously aware of the emotion they were being presented. Mean accuracy scores for discrimination of the fear faces were below chance at 24% accuracy (M = 0.2407; SD = 0.217), signifying that the task was working as intended and the subjects were not able to accurately discriminate the fear targets. The emotion discrimination accuracy scores for all participants during the fear condition are illustrated in Figure 4.

Figure 4

Fear Discrimination Accuracy



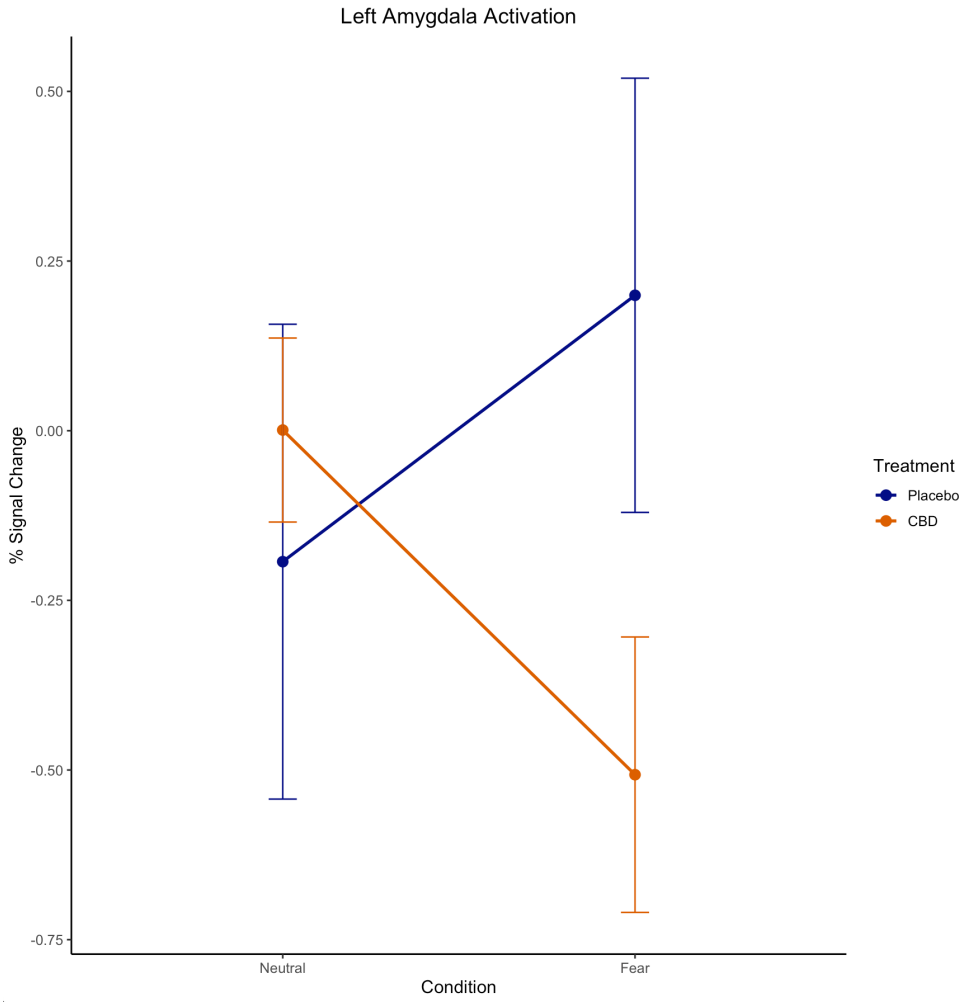
Note. The mean accuracy scores (illustrated as a frequency distribution) on the emotion discrimination for each participant broken during the fear presentation blocks. By the end of the study, each participant will have experienced 16 experimental blocks of the fear condition (4 blocks per scan, 4 scans). To find the accuracy scores, I divided the number of hits (i.e., correct responses) per block by the total number of possible hits (4) per block. The numbers along the *x*-axis represent bins of the percent correct (e.g., .50 = 50% accuracy) per block and the *y*-axis shows the number of experiment-blocks completed by the participants which fall within each bin.

Left Amygdala

To assess Hypothesis 1, I performed a two-way repeated measures ANOVA on the mean BOLD activation (e.g., percent signal change) during the time series data extracted from the left amygdala ROI during the fear and neutral conditions at each level of treatment (i.e., placebo and active) for each participant. Means and standard deviations for each of the ROI measurements for each condition/treatment can be found in Table 3. Analysis of variance showed a statistically significant difference in mean activation in the left amygdala ROI between treatments, $F_{(1,12)} = 6.252$, $p = .028$, $\eta_p^2 = .343$. No significant differences were observed in mean activation between the conditions, $F_{(1, 12)} = 0.003$, $p = .958$, $\eta_p^2 = .000$ and there was no significant interaction effect, $F_{(1, 12)} = 1.897$, $p = .194$, $\eta_p^2 = .136$. Pairwise comparisons of 'treatment' (placebo versus CBD) revealed that the mean activation was significantly higher in the placebo group than the CBD group. Although the interaction was not significant, the plotted estimated marginal means (Figure 5) indicate that the treatment produced the hypothesized effect. Specifically, CBD attenuated the activation in the amygdala during the fear condition relative to placebo. Moreover, a *t*-test revealed a significant difference in activation of the left amygdala during the fear condition between the CBD ($M = -0.507$; $SD = 0.731$) and placebo groups ($M = 0.351$; $SD = 1.096$), $t_{(12)} = 2.392$, $p = .034$, $d = 0.663$.

Figure 5

Means Plot for the Left Amygdala



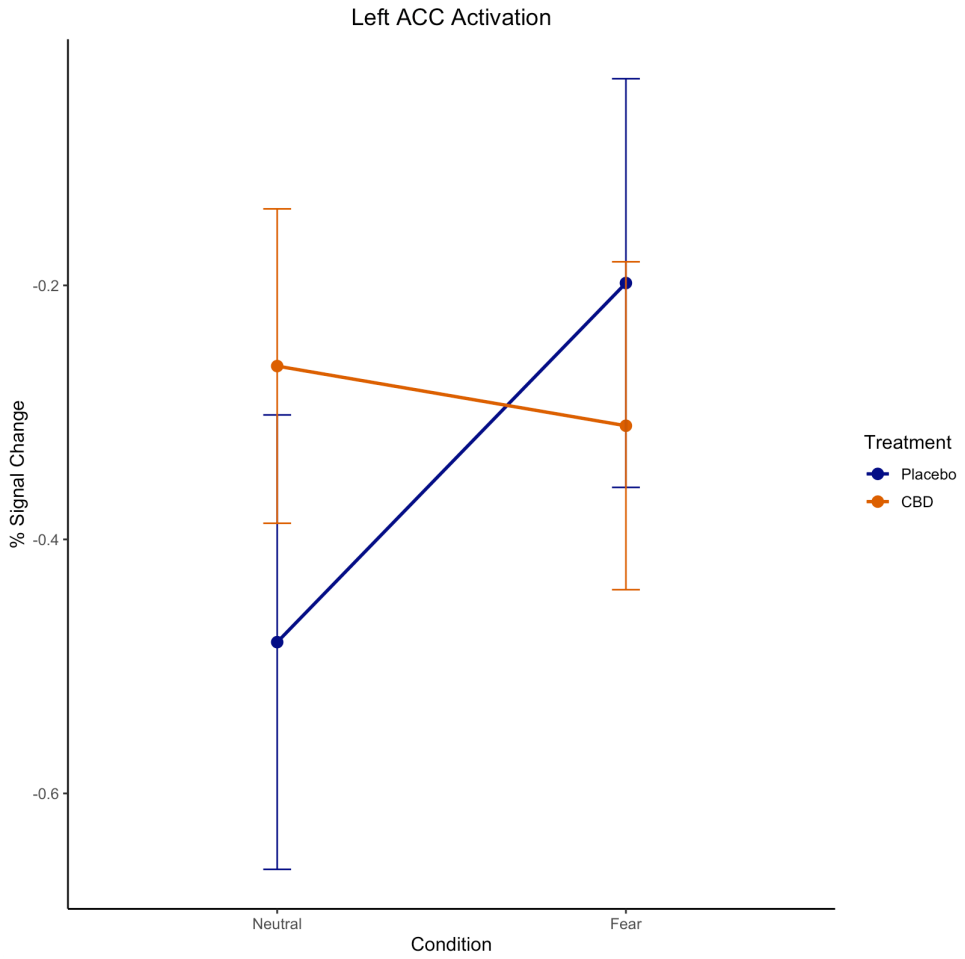
Note. The mean activation (i.e., percent signal change) of the left amygdala during each treatment and condition of the experimental procedure. Error bars represent the 95% confidence interval.

Left Anterior Cingulate Cortex

The repeated measures ANOVA for the left anterior cingulate cortex (ACC) revealed no statistically significant differences in mean activation between conditions, $F_{(1, 12)} = 0.163$, $p = .694$, $\eta_p^2 = .013$, treatment, $F_{(1, 12)} = 3.161$, $p = .101$, $\eta_p^2 = .208$, and no statistically significant interaction, $F_{(1, 12)} = 2.723$, $p = .125$, $\eta_p^2 = .185$ (Hypothesis 2). For repeated measures ANOVAs, we also performed post-hoc comparisons, which were to be interpreted conservatively, regardless of the significance of the main effects (Chen et al., 2018; Howell, 2010). Pairwise comparisons of the interaction of treatment and condition revealed that there was a significant difference between means of condition within the placebo group. A post-hoc *t*-test showed that the mean activation in the left ACC of the placebo group during the fear condition ($M = -0.198$; $SD = 0.602$) was significantly higher that the left ACC activation of the placebo group during the neutral condition, ($M = -.481$; $SD = 0.669$), $t_{(13)} = 2.489$, $p = .027$, $d = 0.665$. This activation pattern across conditions and treatments closely approximates the prediction in the hypotheses, illustrated by the plot of estimated marginal means (Figure 6). Although there was no significant interaction, the CBD does appear to blunt/attenuate the activity of the left ACC during the fear condition relative to placebo and the left ACC does appear to be sensitive to the effect of condition in the hypothesized direction.

Figure 6

Means Plot for the Left ACC



Note. The mean activation (i.e., percent signal change) of the left ACC during each treatment and condition of the experimental procedure. Error bars represent the 95% confidence interval.

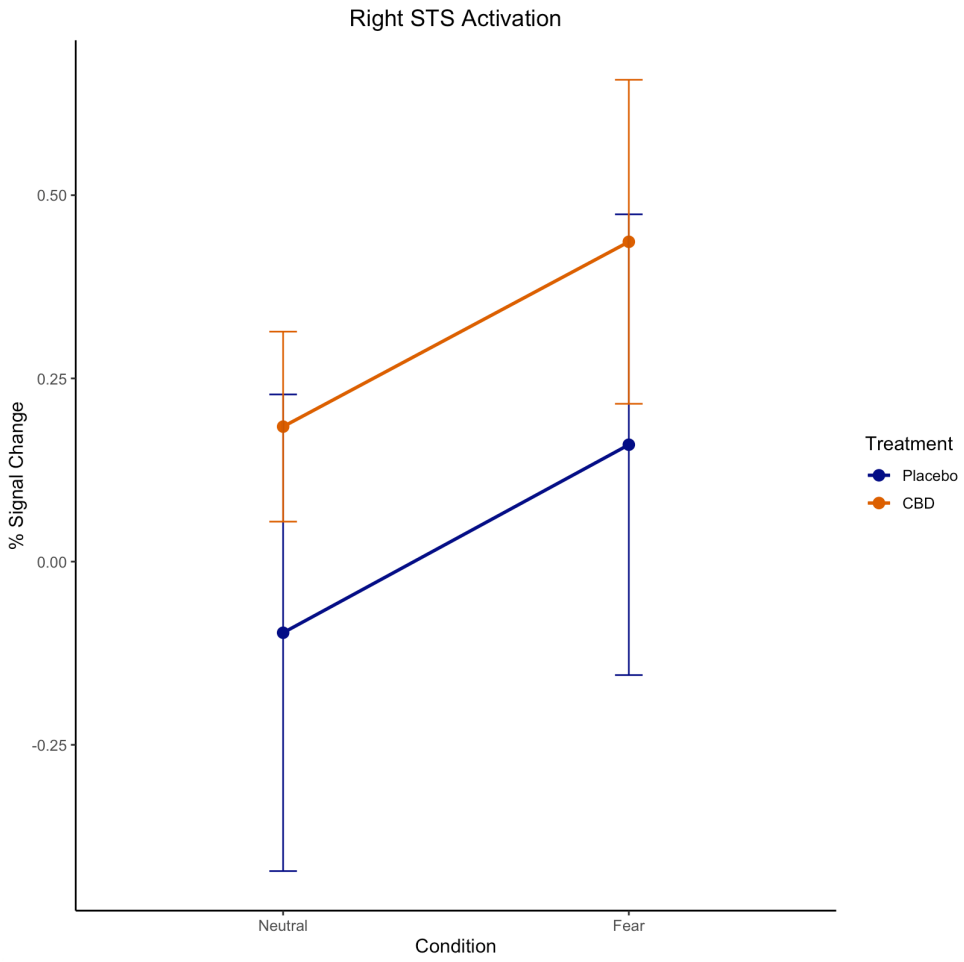
Right Superior Temporal Sulcus

The repeated measures ANOVA for the right superior temporal sulcus ROI revealed a statistically significant simple main effect of condition, $F_{(1,12)} = 4.889$, $p = .047$, $\eta_p^2 = .289$ (Hypothesis 3), but no significant main effect of treatment, $F_{(1, 12)} = .125$, $p = .730$, $\eta_p^2 = .010$, nor a significant interaction , $F_{(1, 12)} = 0.004$, $p = .951$, $\eta_p^2 = .000$. Pairwise comparisons of the

conditions revealed that the mean activation was greater in the fear condition than the neutral condition for both CBD and placebo groups. There was no significant difference in activation between CBD and placebo groups across the conditions. The plotted estimated marginal means (see Figure 6) indicate that the effect of condition followed the pattern predicted in the hypotheses (i.e., fear > neutral).

Figure 7

Means Plot for the Right STS



Note. The mean activation (i.e., percent signal change) of the right STS during each treatment and condition of the experimental procedure. Error bars represent the 95% confidence interval.

Table 3

Descriptive Statistics

	Left Amygdala Neutral Placebo	**Left Amygdala** Fear Placebo	**Left Amygdala** Neutral CBD	**Left Amygdala** Fear CBD	**Left ACC** Neutral Placebo	**Left ACC** Fear Placebo	**Left ACC** Neutral CBD	**Left ACC** Fear CBD	**Right STS** Neutral Placebo	**Right STS** Fear Placebo	**Right STS** Neutral CBD	**Right STS** Fear CBD
M	-.04	.43	.00	-.51	-.50	-.21	-.26	-.31	.12	.39	.18	.44
SD	1.23	1.31	.49	.73	.69	.62	.45	.47	.93	.84	.47	.80
n	13	13	13	13	13	13	13	13	13	13	13	13

Notes. ACC = Anterior Cingulate Cortex. STS = Superior Temporal Sulcus.

Discussion

My findings indicate that cannabidiol (CBD) appears to attenuate or blunt the activity of the left amygdala—the main region of interest. While the two-way repeated measures ANOVA omnibus test for the left ACC revealed no significant main effect of condition or treatment, post-hoc *t*-tests revealed that the left ACC was significantly more active during the fear condition than the neutral condition within the placebo group. Finally, the two-way repeated measures ANOVA of the right superior temporal sulcus ROI revealed a significant main effect of condition (i.e., fear > neutral) for both treatments.

Taken together, the results indicate that CBD can produce significant attenuation of the left amygdala, in line with the results from previous work (Bhattacharyya et al., 2010; Fusar-Poli et al., 2009). Furthermore, in line with the work by Liddell et al. (2005), each of the ROIs appear to be particularly sensitive to the subliminally presented fear faces relative to the neutral faces, apart from the left amygdala and left ACC under the attenuating effect exerted by cannabidiol. Although the findings depart somewhat from the predictions laid out in the hypotheses (e.g., effect of treatment on the STS ROI), the general thrust of the results are quite in line with the notion that CBD reduces the automatic response to fearful faces even when presented below the threshold for normal conscious visual processing.

While the mechanism(s) of action by which CBD might exert its attenuating influence of the amygdala are not well-understood, research on the endocannabinoid system have offered some clues. The amygdala (particularly the basolateral amygdala) is moderately- to densely-populated with CB_1 receptors (Mailleux & Vanderhaeghen, 1992), a major class of receptors within the endocannabinoid system. Fusar-Poli et al. (2009) pointed to prior work which implicated the CB_1 receptors in the extinction of aversive memories via anandamide-mediated inhibitory effects on

cells within the amygdala (Marsicano et al., 2002). Anandamide is a central neurotransmitter of the endocannabinoid system, binding to CB_1 receptors with high affinity (Devane et al., 1992), but is rapidly broken down under normal circumstances through intracellular hydrolytic degradation, or hydrolysis (Di Marzo et al., 1994). Work in mouse models has shown that CBD can inhibit the hydrolysis (inactivation) of anandamide (Bisogno et al., 2001; Watanabe et al., 1996) as well as reuptake of anandamide (Rakhshan et al., 2000), thereby increasing the availability of the molecule at the synapses of the CB_1 receptors of the amygdala. Recent work provides additional support for the hypothesis that CBD upregulation of anandamide may be the key mechanism of action for its anxiolytic effect by demonstrating that increased anandamide signaling (via inhibition of fatty acid amide hydrolase (the catabolic enzyme of anandamide)) reduces fear-related behaviors and indices of stress and anxiety (Mayo et al., 2020; Morena et al., 2019). The results from the present study are congruent with this psychopharmacological account of CBD-amygdala attenuation—at least at the level of neurophysiology.

The analyses of the left ACC did not reveal any significant main or interaction effects. Prior research using a similarly designed backward masking procedure has demonstrated a functional link between the ACC and the amygdala (Killgore & Yurgelun-Todd, 2004), but these results were uncovered using a fundamentally different analytic approach which involved the use of anatomical ROIs rather than predefined masks based on previous work. It is possible that the mask selection in the present study may have limited the ability to capture the effects of the cingulate cortex sensitivity to emotional stimuli. Other work in the domain of fear-related processing has also shown that there may be functional specialization for subliminal and supraliminal presentations of fear stimuli, where the former was associated with a more ventral region of the ACC and the latter a more dorsal region, similar to the ROI in the present study

(Williams et al., 2006). A wealth of prior research suggests that a more ventrally situated portion of the ACC may reflect greater coupling with the activity of the amygdala (Etkin et al.,2011). Additionally, still other research has shown that situational (i.e., expectation), individual factors (i.e., temperament) (Clauss, Cowan, & Blackford, 2011), and manipulations of attention (Klumpp et al.,2012) may influence the activation or lack thereof in the dorsal anterior cingulate cortex responses to fear faces.

However, consistent with the findings of Fusar-Poli et al. (2009), the post-hoc *t*-test and means plot (see Figure 6) appear to be indicate that the left ACC is both differentially sensitive to fear vs. neutral faces and potentially attenuated by CBD relative to placebo in the fear condition, though the present study was likely too underpowered to adequately capture an effect of the treatment. Nonetheless, the results from the present study give some indication that the well-known functional and structural connections between the ACC and amygdala (Etkin et al., 2006; Felmingham et al., 2007; Killgore & Yurgelun-Todd, 2004; Wang et al., 2009) are likely to be influenced in the presence of CBD. Increasing the size of the ROI via an anatomical mask may be one way to enhance the ability to detect the functional relationship. Future research should focus on this and other ways to parse which portion of the ACC might best capture the relationship, perhaps through and exploratory pilot study utilizing a variation of my task and other statistical techniques such as functional connectivity analyses and multivoxel pattern analysis.

A particularly interesting result was the main effect of condition (fear > neutral) coupled with the absence of any impact of the treatment within the STS ROI. Within the ROI mask used for the analysis lies the region referred to as the (right) tempoparietal junction (rTPJ). The rTPJ is situated at the posterior end of the STS, inferior parietal lobule, and the lateral edge of the occipital cortex and is generally thought to be part of the dorsal attention network. This region has been

implicated in a number of higher order cognitive processes, but primarily associated with the reorientation of attention and social cognitive processes (e.g., Theory of Mind/mentalizing; Mars et al., 2012). Given the nature of the task used in the present experiment, social cognition and reorientation of attention may plausibly contribute to the functional changes instigated by the experimental condition. More precisely, it may be the case that the STS ROI increase in the fear relative to neutral condition reflects an emotional valence/salience signal concomitant with the perception of the fearful faces. In line with this notion, recent work provides evidence that anodal stimulation of the rTPJ improves reaction time and accuracy in discrimination of fearful faces from surprise faces (Donaldson et al., 2019).

The STS ROI was chosen specifically for its apparent functional connectivity with the amygdala evidenced by the work of Jiang and He (2006) and Pitcher et al. (2017). Briefly, in each of those studies, the STS region was found to be functionally linked to the amygdala in the processing of fearful face stimuli and, in the case of the Pitcher et al. (2017) study, suppression of the STS via transcranial direct current stimulation (tDCS) also diminished the activity of the amygdala while processing dynamic faces, providing evidence for a causal link between the two regions and, additionally, providing support for the existence of a cortico-amygdala pathway from the STS to the amygdala. The results from the present study suggest that if this specialized pathway does in fact exist, the functional relationship between these two regions may be unidirectional since the suppression/attenuation of the amygdala by CBD did not coincide with an attenuation of the STS. It should be noted that the Pitcher et al. (2017) study reported this apparent causal functional relationship between the *right* amygdala and STS, but post-hoc analyses of the right amygdala within the present sample supports the same inference as above—the impact of CBD on

the amygdala does not appear to influence the activation of the STS with respect to its response to fearful faces.

The results from the present study provide some evidence for a possible neurophysiological mechanism by which CBD can plausibly reduce anxiety. As previously noted, the amygdala is populated with CB_1 receptors which can be inhibited through the binding of the body's own endogenous cannabinoid, anandamide. Under normal circumstances, anandamide is quickly removed from the synapse, but CBD as well as other cannabinoids such as tetrahydrocannabinol (THC) prevent the breakdown/reuptake of anandamide, prolonging the inhibitory effect it exerts on the amygdala. It is plausible that it is precisely this effect which mediates the attenuation of the amygdala activity within the current sample, but the precise pharmacological mechanism is beyond the scope of the present work. Nonetheless, this finding provides some promising evidence for the role of CBD as an anxiolytic drug without the transient, but potentially harmful effects of the major psychoactive cannabinoid, THC.

Limitations

The above findings should be considered preliminary considering some of the potential limitations. First, the sample size for the study is small. The randomized, crossover design offsets some of the issues with the sample size by limiting concerns about within-subject variability, and recent research has highlighted the advantages of small, robust designs over larger group-based studies in capturing significant individual variability (Laumann et al., 2015). Nonetheless, future work should focus on replicating these findings in a larger sample. Next, the dose of CBD given to participants was relatively small by comparison to other similar studies. It is possible that the effects in the ACC, for example, were simply too small to be captured sufficiently and that a higher dose of CBD may be necessary to elicit them. Along these same lines, no measurements were

taken to assess the levels of CBD present in the blood of the participants at the time of scanning. Therefore, future research should focus on achieving a larger sample size, a higher dose of CBD (perhaps administered intravenously), and the utilization of biometric screening methods to ensure that peak absorption levels are attained before conducting the scanning/experimental sessions. Finally, I did not include any emotional expressions in the task beyond fear and neutral. It is possible that other emotional expressions such as surprise or happiness may also elicit similar activation from the regions selected for the present study. Future research should focus on assessing differences in activation with face stimuli of a range of emotional expressions beyond fear.

Conclusions

Overall, these data provide additional insight into two individual lines of experimental inquiry via a unique blend of a RCT of CBD and a subliminal fear elicitation task. Specifically, I found support for the elicitation of a fear/threat response in emotional and face processing regions of the brain which can be produced even in the absence of conscious awareness of the fear stimuli. Additionally, these data provide additional support to the notion that CBD can attenuate the activation of the amygdala. The present work also adds to the growing body of literature suggesting potentially significant therapeutic value of CBD in the treatment of the disorders of anxiety and fear, such as generalized anxiety disorder and PTSD.

Given the predictable inevitability of cannabis decriminalization/legalization, it is increasingly important to understand the impact of major and minor cannabinoids on the function of neurotypical brains in addition to clinical populations. To my knowledge, this is the first study to assess the impact of CBD on the processing of subconsciously presented fear stimuli, with results providing insight into the nature of the innate, automatic threat-response system of the

brain. Future research is needed to precisely delineate the bounds of this automatic processing, the extent to which CBD and other cannabinoids may affect them, and the potential clinical value in the suppression of amygdala activation via CBD.

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Appendix A: Prescreen/Information Letter

AUBURN UNIVERSITY

Information Letter

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COLLEGE OF LIBERAL ARTS

Information Letter for a Research Study entitled:
"Exploring Brain Health Supplements"

You are invited to participate in a research study examining the effects of cannabidiol (CBD) supplements on the brain. CBD has been shown to have effects on brain function. In this research study, we will be examining if CBD effects cognitive function, and how this may be supported physiologically. The CBD used in this study is >99.97% THC-free. Although the exact preparation of the material used in this study is not sold commercially, all of the individual ingredients are. This research study is being conducted by Dr. Jennifer Robinson in the Department of Psychological Sciences at Auburn University.

What will be involved if you participate? If you decide to participate in Part 1 of this research study, you will be asked to complete online questionnaires. The questionnaires will relate to mental and physical health. Completing these questionnaires should take approximately 30 minutes. Based on your responses to specific questions, some participants may be eligible to participate in Parts 2 & 3 of this research study, which involves magnetic resonance imaging (MRI) sessions.

Are there risks or discomforts? The risks associate with participating in Part 1 of this research study are that you could experience emotional discomfort from answering questions related to your mental or physical health. If you find yourself experiencing distress, you may discontinue by exiting out of the

survey. Should you decide to discontinue, you will receive research hours via Sona Systems that correspond to the time spent completing the questionnaires.

There are also risks associated with confidentiality breaches. To minimize this risk, only investigators have access to the data obtained in connection with the research study that can be identified as belonging to you. If you decide to withdraw from the study at any time, you may withdraw any dats that has been collected as long as it is still identifiable. You will be assigned a participant number so that your name and other pieces of identifying information are not directly associated with data collected. All data, including your responses to these questionnaires, will be associated with that participant number. Following completion of data collection, all links to identifiable information will be destroyed. The results of this study may be presented in professional venues, such as within a journal or at a conference. However, in such events, group data or completely anonymized data will be presented.

Are there benefits to yourself or others? If you participate in Part 1 of this research study, you can expect to receive no direct personal benefits.

Will you receive compensation? During Part 1, you will be compensated for participation with 0.5 research hours via Sona Systems. Your instructors should assign specific values of course credit to these hours. Please check with your instructors for more information. During Part 2, you will be compensated for participation with 4.5 hours of Sona Systems credit and $75. During Part 3, you will be compensated for participation with an additional 4.5 Sona Systems credit, and $125.

Are there costs? If you decide to participate in this research study, you will not incur any costs. If you require medical attention, you will be responsible for all costs for medical attention/treatment.

If you change your mind about participating, you can withdraw from the research study at any time. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate will not jeopardize your relationship with Auburn University, or any associated/affiliated department, center, or office.

If you have any questions about this study, please contact Dr. Jennifer Robinson (jrobinson@auburn.edu).

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board by phone (334-844-5966), or by email (hsubjec@auburn.edu, IRBadmin@auburn.edu, or IRBChair@auburn.edu).

NOTE: DO NOT AGREE TO PARTICIPATE UNLESS IRB APPROVAL INFORMATION WITH CURRENT DATES HAS BEEN ADDED TO THIS DOCUMENT (SEE NEXT SENTENCE).

The Auburn University Institutional Review Board has approved this document for use from March XX, 2020 to March XX, 2021. Protocol #XX-XXX EP XXXX, Robinson.

If you would like to print this document, please click the "Print" button below:

Print

Having read the information provided, you must decide whether or not you wish to participate in this research study. Your endorsement indicates your willingness to participate.

- ○ Yes, I wish to participate in this research study.
- ○ No, I do not wish to participate in this research study.

Email

Before completing Part 1 of this research study, you will be asked to provide your email address. We will use the provided email address to contact you about Parts 2 & 3 of this study, if you are eligible to participate. Your privacy will be protected, and email addresses will not be shared with any individual outside of the research team. After you have completed the study, your email address will be deleted from the records.

Please provide your email address in the space below so that we may contact you about Parts 2 & 3 of the research study.

Demographics

How old are you?

What is your sex?

- ○ Male
- ○ Female

Are you right or left handed?

- ○ Right handed
- ○ Left handed
- ○ Ambidextrous

How would you describe yourself? Please select one that best describes you.

- ○ American Indian or Alaskan Native
- ○ Asian or Asian American
- ○ Black of African American
- ○ Hawaiian or Pacific Islander
- ○ White

How would you describe yourself? Please select one that best describes you.

- ○ Hispanic or Latino
- ○ Non-Hispanic or Non-Latino

What is the highest level of education that you've achieved?

- ○ Never attended school or only attended kindergarten
- ○ 1st-8th grade (i.e., elementary school)
- ○ 9th-11th grade (i.e., some high school)
- ○ 12th grade or GED (i.e., high school graduate)
- ○ Currently enrolled in an undergraduate program
- ○ Completed an undergraduate degree
- ○ Currently enrolled in graduate program
- ○ Completed a graduate program

What is the primary language that you speak at home?

- ○ English
- ○ Spanish
- ○ Other

Please indicate "yes", "no", or "unsure" to the following questions.

	Yes	No	Unsure
Have you ever been diagnosed with a psychiatric condition? Examples include, but are not limited to ADHD, depression, or bipolar disorder.	○	○	○
Have you ever been diagnosed with a neurological condition? Example include, but are not limited to, epilepsy, stroke, or Parkinson's disease.	○	○	○
Have you ever had a concussion?	○	○	○
Have you ever been diagnosed with any cardiovascular or pulmonary diseases or disorders?	○	○	○
Do you have high blood pressure?	○	○	○
Do you consider yourself to be generally healthy?	○	○	○

Have you ever taken medication for a psychological/psychiatric condition?

- ○ Yes
- ○ No

Are you currently taking a medication for a psychological/psychiatric condition?

- ○ Yes
- ○ No

How long has it been since you have taken the medication prescribed for your psychological/psychiatric condition?

- ○ Less than a day
- ○ Less than a week
- ○ Less than 2 weeks
- ○ Less than a month
- ○ More than a month

Have you ever been hospitalized for psychological/psychiatric reasons?

- ○ Yes
- ○ No

Do you take any medications for any neurological conditions (i.e., epilepsy, multiple sclerosis, etc.)?

- ○ Yes
- ○ No

Please indicate "yes", "no", or "unsure" to the following questions.

	Yes	No	Unsure
Have you ever had an MRI before?	○	○	○
Do you have a permanent retainer?	○	○	○
Do you have any implanted metal in your body?	○	○	○
Have you ever been a welder?	○	○	○

	Yes	No	Unsure
Have you ever had any accidents involving metal (i.e., shrapnel)?	○	○	○
Are you claustrophobic?	○	○	○

Do you currently take any dietary supplements or vitamins?

- ○ Yes
- ○ No

What vitamins/supplements do you take?

Pittsburgh Sleep Quality Index (PSQI)

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

When have you usually gone to bed?

How long (in minutes) has it taken you to fall asleep each night?

What time have you usually gotten up in the morning?

How many hours of actual sleep did you get at night?

How many hours were you in bed?

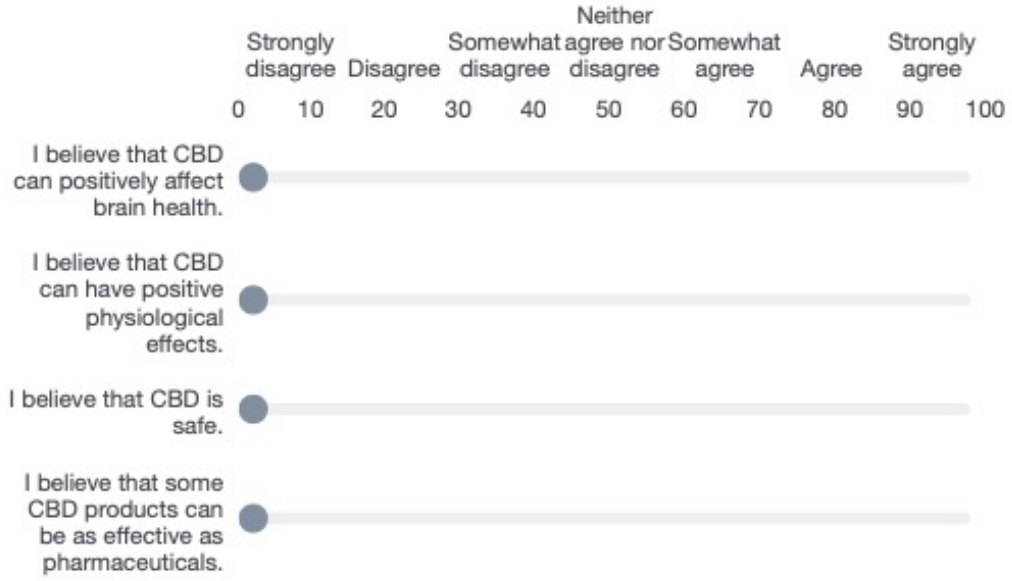
During the past month, how often have you had trouble sleeping because you....

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
Cannot get to sleep within 30 minutes	○	○	○	○
Wake up in the middle of the night or early morning	○	○	○	○
Have to get up to use the bathroom	○	○	○	○
Cannot breathe comfortably	○	○	○	○
Cough or snore loudly	○	○	○	○
Feel too cold	○	○	○	○
Feel too hot	○	○	○	○
Have bad dreams	○	○	○	○
Have pain	○	○	○	○
Other reason(s), please describe, including how often you have had trouble sleeping because of this/these reason(s):	○	○	○	○
During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?	○	○	○	○
During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	○	○	○	○

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?	○	○	○	○
During the past month, how would you rate your sleep quality overall?	○	○	○	○

Nutraceuticals

On a scale of 1-100, with 1 being "not at all" and 100 being "definitely", please answer the following:



Would you be willing to undergo brain scans before and after taking a CBD supplement?

○ Yes

○ No

Would you be willing to complete 2 brain scanning sessions, each requiring approximately 4.5 hours of your time? You would be compensated $75 for the first 4.5 hours, and $125 for the second 4.5 hours. You would not be in the scanner the entire time. For each session, you would: 1) be scanned for approximately 1 hour, 2) be asked to consume an encapsulated CBD product (or placebo), 3) be asked to wait 1 hour in a lounge area, and 4) be scanned for 1 hour.

- ○ Yes
- ○ No

Would you be willing to refrain from alcohol for 24-hours prior to an MRI scan?

- ○ Yes
- ○ No

Would you be willing to refrain from taking pain relievers (i.e., ibuprofen, aspirin, etc.) for 12-hours prior to an MRI scan?

- ○ Yes
- ○ No

Would you be willing to fast for an hour prior to an MRI scan? Fasting would include no food or drink, other than water.

- ○ Yes
- ○ No

To participate in the second part of the study, you must **not**:

1. have contraindications to the MRI environment (this includes having metal implants or a permanent retainer),
2. use any psychotropic or neurological medication,
3. have history of heart disease or stroke,
4. have diabetes or other metabolic conditions,
5. have high blood pressure,

6. have a history of concussions,
7. have any diagnosed psychiatric or neurological condition,
8. have used or take CBD or THC.

Do you meet these requirements?

- ○ Yes
- ○ No

Appendix B: Pre-Scan Questionnaire

AUBURN UNIVERSITY

State of Being

Before you get scanned, we would like to ask you a few questions about how you feel today. Please answer each question honestly, and to the best of your ability.

Over the last 3 days, on average,....

	Always	Most of the time	About half the time	Sometimes	Never
...how alert have you felt?	○	○	○	○	○
...how focused have you felt?	○	○	○	○	○
...how attentive have you felt?	○	○	○	○	○
...how in-control have you felt?	○	○	○	○	○
...how sleepy have you felt?	○	○	○	○	○
...how determined have you felt?	○	○	○	○	○
...how motivated have you felt?	○	○	○	○	○
...how happy have you felt?	○	○	○	○	○
...how upset have you felt?	○	○	○	○	○
...how "sharp" have you felt?	○	○	○	○	○
...how often have you been in pain?	○	○	○	○	○

	Always	Most of the time	About half the time	Sometimes	Never
...how often you have felt anxious?	○	○	○	○	○

Over the past 3 days, how often have you forgotten something (i.e., keys)?

- ○ More than once a day
- ○ Once a day
- ○ I haven't forgotten anything over the last 3 days.

How would you describe your sleep last night?

- ○ Excellent
- ○ Good
- ○ Average
- ○ Poor
- ○ Terrible

Please indicate how focused you have felt over the last 3 days.

Not focused at all Somewhat focused Very focused

0 10 20 30 40 50 60 70 80 90 100

.

Please indicate how alert you have felt over the last 3 days.

Not alert at all Somewhat alert Very alert

0 10 20 30 40 50 60 70 80 90 100

.

Please indicate how attentive you have felt over the last 3 days.

Not attentive at all Somewhat attentive Very attentive

0 10 20 30 40 50 60 70 80 90 100

Not attentive at all Somewhat attentive Very attentive

0 10 20 30 40 50 60 70 80 90 100

.

Please indicate how much pain you have been in over the last 3 days.

No pain at all Some pain A lot of pain

0 10 20 30 40 50 60 70 80 90 100

.

Please indicate how anxious you have felt over the last 3 days.

Not anxious at all Somewhat anxious Very anxious

0 10 20 30 40 50 60 70 80 90 100

.

Have you consumed any alcohol or drugs in the past 24 hours?

- ○ Yes
- ○ No

Have you exercised within the last hour?

- ○ Yes
- ○ No

Have you consumed anything other than water in the last hour?

- ○ Yes
- ○ No

Have you taken any pain relievers in the last 12 hours?

- ○ Yes
- ○ No

Please read each statement and indicate how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend much time on any statement.

	Did not apply to me at all	Applied to me to some degree, or some of the time	Applied to me to a considerable degree, or a good part of the time	Applied to me very much, or most of the time
I found myself getting upset by quite trivial things	○	○	○	○
I was aware of dryness of my mouth	○	○	○	○
I couldn't seem to experience any positive feeling at all	○	○	○	○
I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)	○	○	○	○
I just couldn't seem to get going	○	○	○	○
I tended to over-react to situations	○	○	○	○
I had a feeling of shakiness (e.g., legs going to give way)	○	○	○	○
I found it difficult to relax	○	○	○	○
I found myself in situations that made me so anxious I was most relieved when they ended	○	○	○	○
I felt that I had nothing to look forward to	○	○	○	○
I found myself getting upset rather easily	○	○	○	○
I felt that I was using a lot of nervous energy	○	○	○	○
I felt sad and depressed	○	○	○	○

	Did not apply to me at all	Applied to me to some degree, or some of the time	Applied to me to a considerable degree, or a good part of the time	Applied to me very much, or most of the time
I found it hard to calm down after something upset me	○	○	○	○
I feared that I would be "thrown" by some trivial but unfamiliar task	○	○	○	○
I was unable to become enthusiastic about anything	○	○	○	○
I found it difficult to tolerate interruptions to what I was doing	○	○	○	○
I was in a state of nervous tension	○	○	○	○
I felt I was pretty worthless	○	○	○	○
I was intolerant of anything that kept me from getting on with what I was doing	○	○	○	○
I felt terrified	○	○	○	○
I could see nothing in the future to be hopeful about	○	○	○	○
I felt that life was meaningless	○	○	○	○
I found myself getting agitated	○	○	○	○
I was worried about situations in which I might panic and make a fool of myself	○	○	○	○
I experienced trembling (e.g., in the hands)	○	○	○	○
I found it difficult to work up the initiative to do things	○	○	○	○

Appendix C: Post-Scan Questionnaire

AUBURN UNIVERSITY

Post Scan Questionnaire

Thank you for completing the MRI scan. We would like for you to complete some post-scan questions to help us better understand your data.

To begin, please provide your participant number.

Was this your first, second, or third visit to the MRI center for this study?

- ○ 1st
- ○ 2nd

If you had to guess, do you think the supplement that you took today was the placebo?

- ○ Yes
- ○ No

Did you feel a difference after taking the supplement?

- ○ Yes
- ○ No

Describe the differences that you felt.

Please characterize any changes in the following areas after taking the supplement today:

	Much better	Moderately better	Slightly better	About the same	Slightly worse	Moderately worse	Much worse
Alertness	O	O	O	O	O	O	O
Focus	O	O	O	O	O	O	O
Concentration	O	O	O	O	O	O	O
Memory	O	O	O	O	O	O	O
Emotional Reactivity	O	O	O	O	O	O	O
Calmness	O	O	O	O	O	O	O
Jittery	O	O	O	O	O	O	O
Relaxed	O	O	O	O	O	O	O
Motivated	O	O	O	O	O	O	O
Sharp	O	O	O	O	O	O	O
Vigilent	O	O	O	O	O	O	O
Attentive	O	O	O	O	O	O	O
Anxiety	O	O	O	O	O	O	O
Pain	O	O	O	O	O	O	O

We are interested in the thoughts and feelings that you experienced during the scans, particularly when you weren't engaged in a task (e.g., structural scans, or "resting" scans). Please indicate the extent to which each of the following statements characterized your thoughts and feelings during the scans.

	Strongly agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
I thought about my feelings.	O	O	O	O	O	O	O
I felt restless.	O	O	O	O	O	O	O
I felt anxious.	O	O	O	O	O	O	O

	Strongly agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
I felt tired/sleepy.	○	○	○	○	○	○	○
I felt bored.	○	○	○	○	○	○	○
I felt uncomfortable.	○	○	○	○	○	○	○
I felt relaxed.	○	○	○	○	○	○	○
I felt happy.	○	○	○	○	○	○	○
I enjoyed the session.	○	○	○	○	○	○	○
I was worried.	○	○	○	○	○	○	○

St. Mary's Sleep Questionnaire

The next set of questions refers to your sleep over the past 24 hours. Please try to answer each question to the best of your ability.

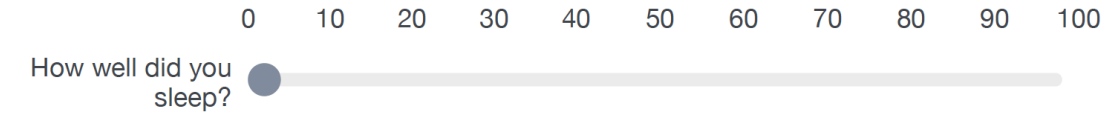
Last night, at what time did you settle down?

Last night, at what time did you finally fall asleep? It's ok to estimate.

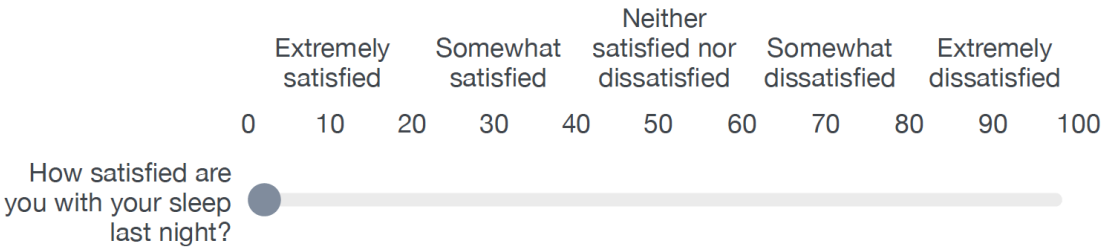
What time did you wake up this morning?

Please use the slider to describe how light/deep your sleep was, where 0 = "very light" and 100 = "very deep".

Please use the slider to describe how well you slept, where 0 = "very badly" and 100 = "very well".



Please use the slider to describe how satisfied you were with your sleep, where 0 = "unsatisfied" and 100 = "very satisfied".



Approximately how many times did you wake up last night?

Diet Questions

Do you follow a specific diet plan (i.e., paleo, keto, vegetarian, Whole30, etc.)?

- ○ Yes
- ○ No

Can you briefly describe your diet?

Have your eating habits been consistent the past 72 hours?

- ○ Yes
- ○ No

Have your eating habits been consistent the past 3 weeks?

- ○ Yes
- ○ No

Briefly describe how your eating habits have been different.

Appendix D: Study Recruiting Advertisement

Social Media Posts
Have you ever wondered what CBD does to your brain?

Researchers in the Department of Psychology are testing 100% THC-free cannabidiol (CBD) supplements to better understand how they may impact brain function. You are invited to participate in the study if:

- You are right-handed.
- You are between 21-50 years of age
- You are generally healthy and do not have a history of psychiatric, neurological, or cardiovascular conditions

The study will involve taking a pre-screen survey. If you are eligible to participate, then you may be contacted for 2 brain scanning sessions. The brain scanning sessions will involve approximately 4 hours of your time. You would be compensated $75 for the first brain scan and $125 for the second.

If you are interested in participating, please email aucanlab@gmail.com, or take the pre-screening questionnaire by scanning the QR code below:

In-Class Script
Researchers in the Department of Psychology are testing cannabidiol (CBD) supplements to better understand how they may impact brain function. You are invited to participate in the study if:

- You are right-handed.
- You are between 21-50 years of age
- You are generally healthy and do not have a history of psychiatric, neurological, or cardiovascular conditions

The study will involve taking a pre-screen survey. If you are eligible to participate, you may be contacted for 2 brain scanning sessions. The brain scanning sessions will involve approximately 4 to 5 hours of your time. You would be compensated $75 for the first brain scan and $125 for the second. Additionally, you will receive SONA credit for your participation in both the survey and the brain imaging sessions. To sign up, please go to SONA and look for the study titled, “Effects of cannabidiol (CBD) on the brain”.

Email Invitation to Parts 2 & 3
Dear <insert name>,
Thank you for filling out our pre-screen survey for the study, “Effects of cannabidiol (CBD) on the brain”. We have determined that you are eligible to participate, and that you have expressed an interest in having your brain scanned before and after taking a CBD supplement. If you are still interested in participating, we kindly ask that you reply to this email with 3-4 good times for us to

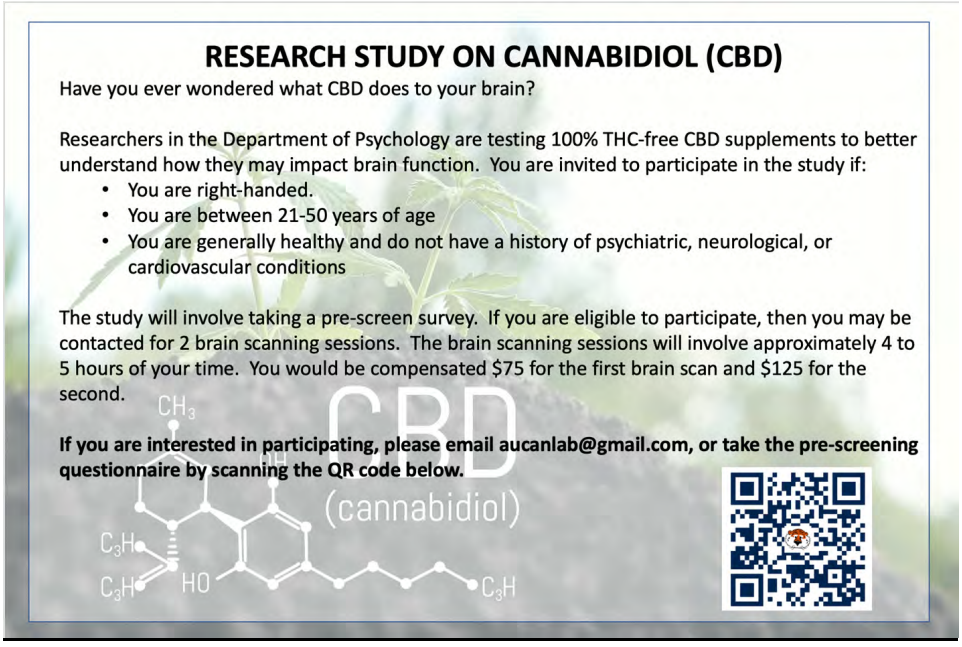
call you and discuss the next portion of the study. Please also provide the best phone number to contact you.

As a reminder, the next part of the study involves completing 2 brain imaging sessions. Each of these sessions will take approximately 4 hours of your time, and will occur in the morning (the start time will be 7am or 8am). You will be compensated with SONA credit (4.5 hours per session, if applicable), as well as $75 for the first brain scanning session, and $125 for the second.

We look forward to hearing back from you,

Dr. Jennifer Robinson

Community Flyer



Appendix E: Informed Consent Document

AUBURN
UNIVERSITY

COLLEGE OF LIBERAL ARTS

DEPARTMENT OF PSYCHOLOGICAL SCIENCES

(NOTE: DO NOT SIGN THIS DOCUMENT UNLESS AN IRB APPROVAL STAMP WITH CURRENT DATES HAS BEEN APPLIED TO THIS DOCUMENT.)

Department of Psychological Sciences /Auburn University MRI Research Center
INFORMED CONSENT
for a Research Study entitled

"Effects of cannabidiol (CBD) on the brain"

Regarding COVD-19: Due to the need for your physical presence at the research site, face to face interactions with the researcher or others, etc., there is a risk that you may be exposed to COVID-19 and the possibility that you may contract the virus. For most people, COVID-19 causes only mild or moderate symptoms. For some, especially older adults and people with existing health problems, it can cause more severe illness. Current information suggests that about 1-3% of people who are infected with COVID-19 might die as a result. You will need to review the Information on COVID-19 for Research Participants that is attached to this consent document. To minimize your risk of exposure we will screen you for symptoms of COVID-19 or risk factors for COVID-19 prior to your arrival at the Auburn University MRI Research Center and again prior to admitting you to the MRI suite. Anyone with symptoms of COVID-19 or risk factors for COVID-19 will be (i) informed that they cannot complete a scanning session if detected prior to arrival or (ii) excused from the scanning session and escorted out of the MRI suite if not detected prior to arrival. Upon arrival at the Auburn University MRI Research Center, but before admitting you to the MRI suite, we will take your temperature with a touchless forehead thermometer. If your temperature is 99.0 degrees or higher, we will inform you that you cannot complete your scanning session. At that time, we may reschedule the scanning session for another date no less than 14 days from the initial scanning session. Researchers will wear a surgical mask, gloves, eye protection, and lab coats at all times. Researchers will remain, at minimum, 6 feet away from you and other protocol personnel when possible. We will provide you with a surgical mask. Also, we will ask you to wear the surgical mask at all times while in the MRI suite including the time you spend inside the scanner. You may keep the face mask provided to you by the study team. However, it is important to note that this face mask is primarily to protect others from you and does not protect you from others that may be infected with the virus. We will adhere to these procedures for all participants/visitors.

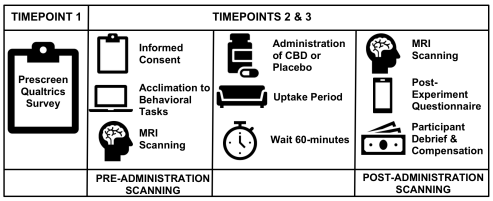
You are invited to participate in a research study to characterize the changes in the brain associated with cannabidiol (CBD), a naturally derived, >99.97% THC free product that has promising health effects. Previous studies have demonstrated that CBD supplements may alter brain function. However, very little is known about the physiological effects of CBD in the brain. In this study, you will be asked to complete 2 research sessions. Both sessions are identical, except that in one session you will receive a CBD supplement, and in the other session, you will receive a placebo. The CBD supplement was specifically prepared for this study, but the individual ingredients of the supplement are commercially available. There is approximately 125mg of CBD oil in the tincture, along with other naturally occurring ingredients. A tincture is a concentration of an herbal or plant extract made by soaking the herb or plant in alcohol or vinegar. The alcohol or vinegar pulls out the active ingredients from the plants, concentrating them in liquid. You will place the liquid in your mouth for 45 seconds before swallowing it. The placebo will also be a tincture of naturally occurring oils. During the sessions, we will ask to look at your brain function using a non-invasive imaging technique.

 Participant Initials _____

Your participation in this study is voluntary. It is important that you read what is written below, and ask questions about anything you do not understand. You may want to talk with your family, friends, or others to help you decide if you want to be part of this study. When you feel that your questions have been answered, you will be asked if you agree to be part of the study or not. If you agree, you will be asked to sign this consent form. You will be given a copy of this form to keep.

Why is this research study being done?
The main objective of this study is to identify and measure differences in brain function associated with CBD. By having a better understanding of how CBD effects the brain, we may be able to better understand how it works and what it does.

What will be involved if you participate?
If you decide to participate in this research study, you will be asked to undergo 4 functional magnetic resonance imaging (fMRI) scans. Your total time commitment will be approximately 8.5 total hours (4 hours total for each of two sessions, plus the 0.5-hour online screening). Below, we provide a diagram to help you understand the design of the study.



fMRI Scanning
For the fMRI scans, you will first be asked screening questions to make sure it is safe for you to undergo an MRI scan. You will then be asked to lie on a bed that slides into the long tube of the scanner and place your head in a cylindrical head coil. The scanner is a magnet with a small, enclosed space. Radio waves and strong, changing magnetic fields are used to make images of your head and brain. You will be given earplugs to protect your ears since these changing magnetic fields cause loud knocking, thumping, and pinging noises. You will be asked to remain very still during the scan. To help keep your head as still as possible, we will put cushioning around your head. We will also place a pillow under your knees if it is more comfortable for you. The investigator will check in with you periodically throughout the scan. If you have any discomfort, please notify the investigator during these check-in points.

Multiple scans will be performed in a single session with approximately one minute of rest between scans. The longest time you will be in the scanner will be about 50 minutes at a time. Seven scans will be performed during your session. You will look at images on a video screen, and be instructed about a specific task, which will ask you to respond by pushing a button.

There will be two "tasks" that we will ask you to complete. In one task, we will ask you to look at shapes and try to remember if the shape you are looking at is the same or different from the shape you saw 2 trials previously. In another task, we will ask you to respond to the letters x and y as they alternate, but to not respond if you see two x's or two y's in a row. We will practice these tasks outside of the scanner. When you are in the scanner, we will check in with you to give you instructions, and check on your comfort throughout the brain scan. You may discontinue the scan at any time.

There will be times where you will be asked to just lie still. This will allow us to collect very detailed images of your brain.

 Participant Initials _____

None of the scans that are done during this study are appropriate for clinical interpretation. This means that they are not designed to assess any medical condition that you may have. They are not designed to reveal any existing disease or pathology. Rather, they are intended solely for research purposes.

Are there any risks or discomforts?
The risks associated with participating in this study are:

1. The most obvious personal risk from having an MRI is blunt trauma due to metallic objects being brought into the magnetic field. As such, all necessary steps will be taken to make sure neither you nor anyone else who enters the MRI scanner room is in possession of an unrestrained metal object and no unauthorized person will be allowed to enter the MRI scanner room.
2. Participants who have iron or steel implants or clips from surgery within their body or metallic objects such as shrapnel or metal slivers in their body may be pulled by the magnet and cause injury.
3. The MRI machine produces an intermittent loud noise, which some people find annoying.
4. Some participants may feel uncomfortable being in an enclosed place (claustrophobia) and others find it difficult to remain still.
5. Some people experience dizziness or a metallic taste in their mouth if they move their head rapidly in the magnet.
6. Some people experience brief nausea when being put into or taken out of the scanner.
7. One of the potential risks to be considered in this study includes the risk of revealing personal and sensitive information on the part of the participant. Participants will be asked personal questions regarding their mental health status and engagement in risky behaviors including alcohol use.
8. Although long-term risk of exposure to the magnet is not known, the possibility of any long-term risk is extremely low based on information accumulated over the past 30 years.
9. There is a small risk of exposure to COVID-19.

To minimize these risks, we will:

1. Have you fill out a screening form to determine if you have iron or steel implants, clips from surgery, or other metallic objects in your body. If you have implants, clips, or objects in your body, you will not be able to undergo an MRI scan. An exception can be made to allow you to participate with implanted devices as long as MRI Center personnel verify that the device is compatible at 3T. The list the MRI Research Center will use is located at http://mrisafety.com/list.asp.
2. Ask you to change into surgical scrubs supplied by the center and remove any watches, rings, earrings, or other jewelry or metallic objects. You will be provided a private place to change and you may retain your undergarments. If you are female, you will be asked to remove your bra if it has an underwire or metal fasteners.
3. Scan you with a handheld metal detector to detect any unknown metallic objects.
4. Provide you with either earplugs or a set of headphones specifically designed to work in an MRI scanner.
5. Maintain visual and verbal contact with you during the scan and check with you frequently to determine if you are having any negative feelings or sensations.
6. If some unknown risk becomes a safety issue, the research team will immediately stop the scan and remove you from the scanner.
7. You can stop the scan at any time and be immediately removed from the scanner.
8. In order to protect the confidentiality of all information, consent forms attained will be immediately collected and placed in a locked cabinet. All data files will be stored on password protected, encrypted, computers with limited access to investigators of this study. All data will be stripped of identifiable information and provided with a participant ID.
9. If you experience discomfort you can stop your participation at any time. You may skip the task or cease participation in the study, at any time.
10. In order to minimize the risk of discomfort by revealing personal information, participants will be given the option to refuse to answer any questions without penalty or exclusion from the study.

 Participant Initials _____

11. In order to minimize the risk of COVID-19 exposure, we will enforce social distancing and ask you to wear a face mask. All research personnel will wear a face mask, gloves, lab coat, and eye protection. Additionally, all research personnel and participants must have a temperature < 99.0 degrees to be in the MRI suite. Finally, all research personnel will wash their regularly and disinfect the MRI scanner between participants.

Are there any benefits to yourself or others?
You may not directly benefit from participating in this study. This study is not designed to diagnose or treat any illness. The information that we learn in this study may help us understand how CBD affects the brain.

Will you receive compensation for participating?
You will receive $75 for completing the first session, and $125 for completing the second session, for a total of $200. If applicable, you will also receive Sona Systems credit. Specifically, you will receive 4.5 hours of credit for completing the first set of scans, and an addition 4.5 hours of credit for the second set of scans.

What happens if I am injured as a result of taking part in this research study?
No compensation is available for research-related injuries. You are not waiving any legal rights. If you believe you have sustained a research-related injury, please contact the PI. If you have questions, please contact Dr. Jennifer Robinson at jrobinson@auburn.edu.

If you change your mind about participating, you can withdraw at any time during the study. You may remove yourself from the study at any time, for any reason, without prejudice or loss of benefits to which you are entitled. You may also be removed from the study by a study investigator should your continued participation be injurious to your health and well-being, or that of others, at any time, or if you fail to comply with the study in a way that endangers the integrity of the study data.

Failure to follow instructions or comply with procedural requirements for orderly and safe conduct of the study may result in termination of your role in the study and your dismissal following consultation with the PI.

You may be withdrawn due to temporary failures of instrumentation or data recording systems. You will still be compensated if this occurs.

If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate or to stop participating will not jeopardize your future relations with Auburn University or the Auburn University MRI Research Center.

Your privacy will be protected. Any information or data obtained in connection with this study will remain confidential. Information obtained through your participation may be presented at professional meetings, or written about in peer-reviewed journals. However, there will not be any identifiable information in these outlets. Access to source data will be restricted. Only individuals immediately involved in data collection will have access to any of the data files. Specifically, individuals with access to source data and documents will include the study investigators. Federal agencies may have access to study data as part of their duties and responsibilities to protect human subjects in research.

FUTURE USE OF DATA

We would like to store your data for future research related to brain function. We will label your data with a code instead of your name, and only include demographic information that is important for brain imaging data, such as age and sex. Your data will be stripped of any identifying information to the greatest extent possible (i.e., we will remove any non-brain material so that facial reconstruction cannot be performed).

Participant Initials _____

Sending data to research collaborators outside partners.
Your anonymized data may be shared with researchers outside of Auburn University. This means that the data will be impossible to trace back to you. Data will be transferred between our collaborators using secure transfer protocols. Furthermore, with your permission, your anonymized data could be distributed to publicly available databases. Please initial below whether you agree to allow your anonymized data to be shared in public databases, and with collaborators outside of the scope of this project:

_____ **I give permission for my anonymized data to be shared.**
_____ **I do not give permission for my anonymized data to be shared.**

Incidental findings. All study procedures are carried out purely for experimental purposes. The MRI scans that are acquired in this study are not the same as those acquired during a clinical examination as requested by a medical doctor. Therefore, they are not useful to investigate any abnormalities or medical conditions. Furthermore, the investigators who will analyze these images are not medical doctors and are not trained to evaluate these scans. It is possible, however, that an abnormality may be noticed. In the case of an MRI incidental finding, the Auburn investigators will follow protocol and a physician will contact you.

If you have questions about this study, please ask them now or contact Dr. Jennifer Robinson at jrobinson@auburn.edu. A copy of this document will be given to you to keep.

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board by phone (334)-844-5966 or e-mail at hsubjec@auburn.edu or IRBChair@auburn.edu.

I have read the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction.

_____	_____
Participant's Signature Date	Investigator's Signature Date
_____	_____
Printed Name	Printed Name

Participant Initials _____