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Individual Differences in Cannabis Use Disorder with Implications for Endocannabinoid Modulation in Therapeutics Development

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Individual Differences in Cannabis Use Disorder with Implications for Endocannabinoid Modulation in Therapeutics Development

By

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To my mentors, to my dear friends, to Stephan Hughes, and to my past selves. Thank you for never giving up on me.

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KEY TO ABBREVIATIONS

Abbreviation	*Meaning*
ACE	Adverse Childhood Experience
AEA	*N*-arachidonoylethanolamide
AIDS	Acquired Immunodeficiency Syndrome
CBD	Cannabidiol
CB1R	Cannabinoid type 1 receptor
CB2R	Cannabinoid type 2 receptor
CCK	Cholecystokinin
CI	Confidence interval
CNS	Central nervous system
COX-2	Cyclooxygenase-2
C-SSRS	Columbia – Suicide Severity Rating Scale
CUD	Cannabis use disorder
CWS	Cannabis Withdrawal Scale
DAGL	Diacylglycerol lipase
DEA	Docosatetraenylethanolamide
DSE	Depolarization-induced suppression of excitation
DSI	Depolarization-induced suppression of inhibition
DSM	Diagnostic and Statistical Manual of Mental Disorders
eCB	Endocannabinoid
ELISA	Enzyme-linked immunosorbent assay
FAAH	Fatty acid amide hydrolase
FDA	United States Food and Drug Administration

GABA	γ-aminobutyric acid
GLMM	Generalized linear mixed effects regression model
HAM-D	Hamilton Depression Rating Scale
HPA	Hypothalamic-pituitary-adrenal
L	Liter
LC-MS	Liquid chromatography-mass spectrometry
LEA	Linoleylethanolamide
MAGL	Monoacylglycerol lipase
MDD	Major Depressive Disorder
mg	Milligrams
MINI	Mini-International Neuropsychiatric Interview
mL	Milliliter
mol	Moles
NA	Negative affect
NAE	*N*-acylethanolamine
NAPE-PLD	*N*-acyl phosphatidylethanolamine-specific phospholipase D
NESARC	National Epidemiologic Survey on Alcohol and Related Conditions
ng	Nanograms
NSDUH	National Survey on Drug Use and Health
OEA	Oleoylethanolamine
OR	Odds ratio
PA	Positive affect
PAG	Periaqueductal gray

PANAS	Positive and Negative Affect Schedule
PEA	Palmitoylethanolamide
PET	Positron emission tomography
SD	Standard deviation
SE	Standard error
SEA	Stearoylethanolamide
SHAPS	Snaith-Hamilton Pleasure Scale
SNRI	Serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonergic reuptake inhibitor
STAI	State-Trait Anxiety Inventory
THC	Δ^9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
TLFB	Timeline Follow-Back
TSST	Trier Social Stress Test
2-AG	2-arachidonoylglycerol

ABSTRACT

ERIN LINDSEY MARTIN. Individual Differences in Cannabis Use Disorder with Implications for Endocannabinoid Modulation in Therapeutics Development. (Under the direction of AIMEE L. MCRAE-CLARK).

Cannabis use disorder (CUD) is increasingly prevalent in the United States, but there is no effective pharmacological means to treat it. The endocannabinoid (eCB) system has emerged as a candidate therapeutic target demonstrating some evidence of efficacy in treating CUD. However, clinical trials evaluating eCB-modulating therapeutics have historically undervalued individual differences that could contribute to variation in treatment outcome (e.g. sex, comorbid psychiatric illness). To address this gap in the literature, the present set of studies (a) compared plasma eCB tone in groups underrepresented in treatment trials for CUD (females, individuals with comorbid major depressive disorder; MDD/CUD) with males or otherwise healthy people with CUD, (b) examined group differences in behavioral predictors of relapse (withdrawal symptoms, stress response), and (c) related plasma eCB tone to these behavioral predictors. We found that, as hypothesized, women or individuals with MDD/CUD self-reported more severe cannabis withdrawal symptoms compared to men or individuals with CUD alone, respectively. Self-reported withdrawal was moderately positively associated with eCB tone across studies, with the strongest associations observed in women with CUD. In MDD/CUD, however, self-reported withdrawal appeared largely uncoupled from objective withdrawal measures and abstinence from cannabis. With respect to stress, MDD/CUD was associated with a prolonged stress response relative to CUD alone, suggesting individuals with MDD/CUD may be at a greater risk for stress-induced relapse. Individuals with MDD/CUD also presented differently from those with CUD alone in stress-associated

eCB levels, raising questions as to the mechanistic role of peripheral eCBs in stress responding. Taken together, these studies demonstrate that exploration into individual differences in the eCB system, particularly in the periphery, is still in its infancy. The utility of eCB-modulating pharmacotherapeutics likely differs significantly across subpopulations of people with CUD. Greater mechanistic understanding of the eCB system across subpopulations is warranted.

CHAPTER 1: Background and Significance

Sections from this chapter have been published at the following citation:
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Cannabis Use Disorder

Cannabis is the most commonly used federally-illicit drug in the United States, with nearly 47% of individuals aged 12 and above reporting at least some use during their lifetime in 2022. [1] While evidence of cannabis use can be traced back to ancient times, [2] use prevalence has rapidly increased in recent history in concert with an evolving legal landscape. [3] According to the annual National Survey on Drug Use and Health (NSDUH), 4 million more people aged 12+ endorsed lifetime cannabis use in 2022 relative to 2021. [1] Fifteen percent of individuals in this age group also reported past-month use, up from 13% in 2021.

Notably, the incidence rate of frequent cannabis use seems to be most affected by its legal status, with the greatest incidence reported in states with legalized "recreational" use. Per NSDUH data, self-reported cannabis use on at least 20 days in the past month is highest in states with recreational cannabis laws, and prevalence of this frequent use is increasing with time. [4] Results from the International Cannabis Policy Study, which included responses from individuals ages 16-65 in the United States and Canada, also showed significantly higher daily, weekly, and monthly use in states with legalized non-medical cannabis use. [5] Simultaneously, however, risk perceptions of cannabis have substantially decreased with time, with the prevalence of perceiving cannabis use as "low risk" or "no risk" more than doubling from 23% to 47% between 2002 to 2018. [6] This

change in perception has also coincided with increased perceived availability of cannabis. [6] Taken together, increased use, increased availability, and decreased caution due to decreased risk perception may coincide to increase individual risk for the development of cannabis use disorder (CUD).

CUD is a syndrome in which cannabis use perseverates despite intrapersonal, interpersonal, and/or physiological consequences. [7] Consistent with the hypothesis that increased use and decreased perceived risk may contribute to increased incidence of CUD, prevalence of CUD has increased with time: 6.7% of individuals aged 12 and above endorsed past-year CUD in 2022, up from 6% in 2021. [1] As 22% of individuals in this age group reported any past-year cannabis use in 2022, [1] this means nearly a staggering one-third of individuals endorsing past-year cannabis use met CUD criteria. Importantly, treatment demand is high among individuals with CUD, with service utilization only third behind individuals seeking treatment for alcohol or opiate use. [8] Treatment demand also increases exponentially with CUD severity. [9,10] Yet, treatment options for CUD are extremely limited. Psychotherapeutic methods, such as motivational enhancement therapy and contingency management, are at best moderately efficacious and show the most robust effects when combined during treatment. [11] Unfortunately, however, combining methods has also been associated with reduced durability of treatment effects relative to psychotherapy without contingency management, calling into question the long-term utility of a combined psychotherapeutic strategy. [12] There is currently no pharmacotherapeutic intervention for CUD that has been approved by the U.S. Food and Drug Administration (FDA). [11]

While several of the CUD diagnostic criteria are shared with other substance use disorders (e.g. difficulty controlling use, craving, development of tolerance, presence of

withdrawal symptoms during abstinence), cannabis is associated with its own specific set of withdrawal symptoms. [7] Symptoms typically last up to two weeks following cessation of use and include: irritability, anger, or aggression; anxiety; sleep difficulty, including strange dreams; appetite or weight loss; restlessness; depressed mood; and physical symptoms such as stomach discomfort, shakes, sweating, hot flashes, chills, and/or headaches. [7,13] Critically, cannabis withdrawal has been shown to interfere with daily living and contribute to return to use, making it both clinically relevant and viable as a treatment target. [14] Alleviation of cannabis withdrawal as a treatment strategy has been assessed thus far most consistently via pharmacological targeting of the endocannabinoid (eCB) system.

The Endocannabinoid System

The plant *Cannabis sativa* L. is comprised of over 150 isoprenylated resorcinyl polyketides, commonly referred to as cannabinoids. [15] Most well-known of the cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), which each produce a unique constellation of pharmacological effects. THC is the constituent associated with the prototypic effects of cannabis, i.e. feeling “high” in human subjects [16] or “tetrad” behavior in rodents (antinociception, hypothermia, hypolocomotion, catalepsy). [17] THC administration also induces striatal dopamine release, [18] a pharmacological property commonly associated with addictive drugs. [19]

THC produces its myriad effects via partial agonism of the cannabinoid type 1 receptor (CB1R) in the central nervous system (CNS). [20] CB1R is part of an endogenous cannabinoid, or endocannabinoid (eCB), system that extends from the CNS to the periphery. [21] This eCB system is composed of two G protein-coupled receptors (CB1R

and CB2R); their primary endogenous ligands, *N*-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG); and the numerous enzymes implicated in the synthesis, degradation, and transport of these ligands. [22] While the eCB system is expansive, discussion of its components and activities will be limited in this work to retain focus on those processes most likely implicated in CUD. As such, the background provided herein will be primarily centered around CB1R and how the eCBs interact with it. With this in mind, the enzymes of greatest current relevance in CUD research are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), which are the principal degradative enzymes for AEA and 2-AG, respectively. [22] Cyclooxygenase-2 (COX-2), a minor degradative enzyme of both AEA and 2-AG, [23] is also briefly mentioned in Chapter 1, as it has received some recent, limited attention as a potential treatment target for CUD. Other components of the eCB system with a currently undefined role in CUD, but with potential for future exploration, will be discussed in Chapter 5.

The neurobiological distribution of CB1R provides several clues into its function. CB1R is expressed ubiquitously in the human brain, with greatest expression on the gross structural level in the dorsal striatum and prefrontal cortex. [24,25] At the cell-type level, CB1Rs are most highly expressed in GABAergic interneurons, particularly those at also express cholecystokinin (CCK), [26] although they are also expressed more sparsely in glutamatergic neurons. [27,28] Finally, at the subcellular level, CB1Rs are localized primarily to axon terminals. [26,29] Activation of CB1R inhibits both adenylyl cyclase activity [30] and the opening of voltage-gated calcium channels, [31] leading to hyperpolarization in the neuron and arrest of its activity.

The endogenous ligands of CB1R, AEA and 2-AG, are synthesized primarily by *N*-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and diacylglycerol

lipase (DAGL), respectively. [32,33] This synthesis is primarily localized to the post-synaptic neuron [34] and occurs “on-demand”, i.e. following increased calcium influx and subsequent neuronal depolarization. [35,36] ECBs then move through the post-synaptic cell membrane via an as-yet unconfirmed mechanism [37,38] and travel retrograde across the synaptic cleft to bind pre-synaptic CB1Rs; a simple schematic of retrograde eCB signaling is shown in **Figure 1.1**. Upon binding CB1R, AEA behaves as a partial agonist, like THC, [39,40] whereas 2-AG acts as a full agonist. [41] As agonists, binding of either of these molecules can produce the above-mentioned suppression of pre-synaptic neuronal activity. This short-term synaptic plasticity is referred to as depolarization-induced suppression of inhibition (DSI) or excitation (DSE) depending on whether the pre-synaptic neuron is primarily GABAergic or glutamatergic, respectively. [42]

ECB-mediated DSI/DSE is a basic homeostatic mechanism implicated in the modulation of neuronal dopamine, [43,44] serotonin, [45] and norepinephrine, [46] as well as the hypothalamic-pituitary-adrenal axis. [47] The ubiquity of this mechanism explains the breadth of behavioral effects associated with the eCB system including feeding, [48–51] sleep, [52–54] emotional regulation, [55–59] and modulation of the stress response. [60–63] Mimicking eCBs, commonly-reported subjective effects of acute cannabis intoxication in humans are positive mood, increased appetite, relaxation, and sleepiness, [64] consistent with the shared pharmacological properties between eCBs and THC. Conversely, cannabis withdrawal is marked by loss of appetite, sleep disturbance, and negative mood; [13] stress-coping is also a commonly cited justification for both initiation and maintenance of cannabis use. [65,66] This suggests that there may be adaptations to the eCB system with

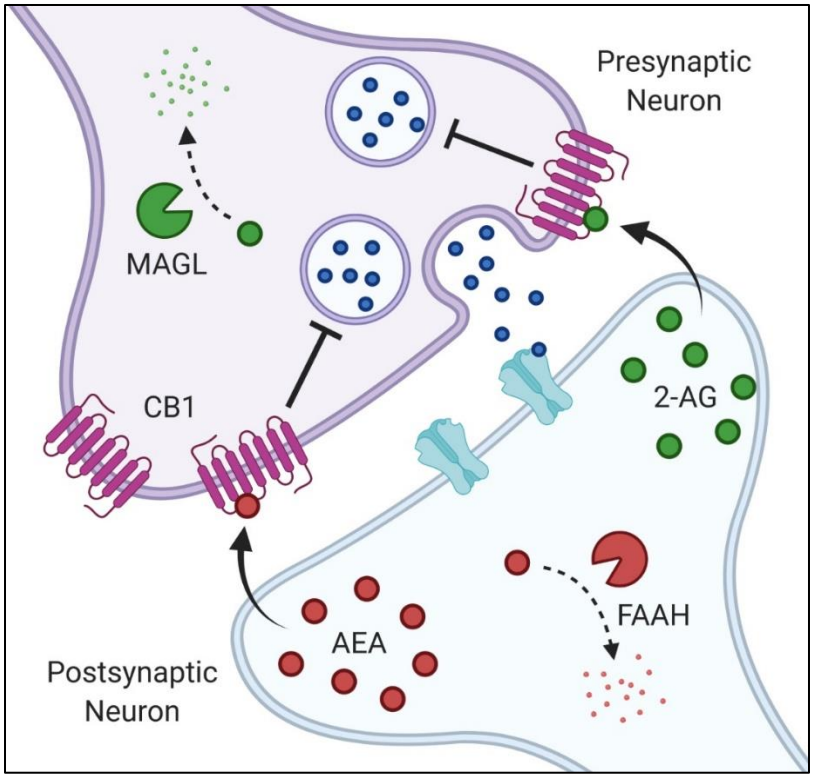

Figure 1.1. A simplified view of the endocannabinoid system at the synapse. The endocannabinoids (eCBs) N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) are synthesized on-demand (i.e. following calcium influx) in post-synaptic neurons. ECBs then travel retrograde across the synaptic cleft to bind CB1Rs on pre-synaptic neurons, inhibiting further neurotransmitter release from these neurons (depolarization-induced suppression of inhibition or excitation; DSI/DSE). AEA and 2-AG are then degraded by the enzymes fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), respectively.

repeated cannabis use that underlie clinically significant behavioral manifestations in CUD.

At the molecular level, heavy cannabis use is associated with neural eCB system dysregulation that includes CB1R downregulation and desensitization [67–69] and reduced FAAH [70,71] in both humans and animals. The effects of heavy cannabis use on AEA and 2-AG, particularly in humans, are presently unclear, though one study found modestly decreased cerebrospinal fluid AEA and significantly increased cerebrospinal fluid 2-AG in

individuals that used cannabis at least 10 times per month relative to non-using controls. [72] Even fewer studies have examined how the eCB system is impacted in CUD specifically. Notably, one such study found that CB1R availability rapidly increases in men with cannabis dependence during the first two days of abstinence, [73] and CB1R availability has been negatively associated with withdrawal symptom expression in men and women with CUD, [73,74] directly linking normalization of the eCB system to improved clinical outcome in individuals with CUD.

The Endocannabinoid System as a Therapeutic Target in CUD: Evidence from Human Subjects Research

Normalizing eCB signaling that has been disrupted by heavy cannabis use could serve as an effective and specific therapeutic target for CUD. This treatment strategy follows precedent established in the treatment of opioid and nicotine use disorders in the form of agonist replacement therapy. In addition to direct stimulation of cannabinoid receptors, indirect modulation of the eCB system is also achievable via pharmacological manipulation of biosynthetic and degradative enzymes.

CB1R Antagonists

While there are many unique pharmacotherapeutic targets available within the context of the eCB system, a more conventional approach, the exogenous antagonist, is presently nonviable. The CB1R inverse agonist rimonabant showed preliminary efficacy in attenuating cannabis use preclinically and in humans, [75–77] but research efforts have halted following demonstration of adverse psychiatric side effects. [78] Neutral antagonists,

which merely block activity at a given receptor rather than produce effects opposite to those of an agonist, may present as equally effective treatment options with a more favorable side effect profile. [77,79–81] However, research in humans is limited and a direct effect on CUD has yet to be assessed. Further, it is possible that this class of drugs would still have some degree of negative psychiatric effects, as chronic antagonism would still preclude eCB signaling necessary for mood regulation. The presence of such side effects might further disincentivize treatment adherence in this already difficult to treat population, even if they are less severe than those produced by inverse agonists.

CB1R Agonists: Dronabinol

Dronabinol, an orally bioavailable formulation of THC, is FDA-approved for the prophylaxis of chemotherapy-induced nausea and vomiting and for use in the stimulation of appetite and prevention of weight loss in patients with Acquired Immunodeficiency Syndrome (AIDS). [82] As a direct CB1R agonist, there is a substantial theoretical basis to support its utility as a treatment of CUD, and multiple studies have explored its potential as an intervention for cannabis withdrawal specifically.

Dronabinol has been shown to attenuate cannabis withdrawal symptoms in both inpatient and outpatient laboratory settings. [83,84] Dronabinol given at a dose of 10 mg five times daily in a laboratory environment decreased cannabis craving and withdrawal symptoms while producing no intoxication. [83] An outpatient evaluation in non-treatment seeking, daily cannabis users also showed that dronabinol at doses of 10 or 30 mg three times daily produced a reduction in withdrawal symptoms compared to placebo treatment. [84] A greater reduction in withdrawal symptoms was noted with the 90 mg/day dose

compared to the 30 mg/day dose, however, some signs of cannabis-like intoxication were associated with the higher dose, as were some drug effects such as euphoria and drug-liking. A within-subject crossover study of short-term dronabinol (0, 30, 60, and 120 mg/day for five consecutive days) also found a dose-dependent suppression of cannabis withdrawal, without decrements in cognitive performance. [85]

Laboratory studies investigating whether dronabinol alters cannabis self-administration have had mixed results. Hart and colleagues found no effect of dronabinol 40-80 mg/day on cannabis self-administration. [86] In a subsequent trial, dronabinol (60 mg/day) did not decrease cannabis self-administration alone, though a reduction was noted when dronabinol was administered in combination with the adrenergic agonist lofexidine. [87] In contrast, a recent trial comparing 12 days of high-dose dronabinol (180-240 mg/day), 120 mg/day dronabinol, and placebo found reduced cannabis self-administration in both dronabinol conditions. [88] These results suggest higher dronabinol doses may be needed to impact cannabis use behavior, perhaps due to its limited bioavailability.

Based on the promising outcomes with respect to cannabis withdrawal and the established utility of agonist substitution therapy in other substance use disorders, dronabinol was evaluated in a large randomized, placebo-controlled trial. [89] Cannabis-dependent adults received either dronabinol (40 mg/day) or placebo over a 12-week period with concomitant psychosocial treatment. Dronabinol improved treatment retention and reduced withdrawal symptoms relative to placebo, however, no difference was observed between treatment groups in cannabis use. Negative findings were also reported in a large, placebo-controlled trial of concurrent dronabinol (60 mg/day) and lofexidine (1.8 mg/day), with no treatment group differences observed in cannabis abstinence, withdrawal

symptoms, or treatment retention. [90] Together, these trials suggest limited potential of dronabinol for cannabis abstinence promotion.

CB1R Agonists: Nabilone

Nabilone (Cesamet™) is a synthetic cannabinoid that is FDA-approved to treat nausea associated with cancer chemotherapy. [91] Like dronabinol, nabilone is an oral medication that acts as an agonist at CB1R and CB2R [92] and produces similar interoceptive effects to THC in individuals that regularly use cannabis. [93,94] As such, also like dronabinol, nabilone represents a potential agonist replacement therapy for CUD. Notably, nabilone appears to have a lower misuse liability relative to smoked cannabis, [95,96] though this may be dose-dependent, [97] and may be attributable to the difference in route of administration.

Given other similarities to dronabinol, it is unsurprising that nabilone produces similar effects in the context of CUD, i.e. reduces withdrawal symptoms in the human laboratory [98] without promoting abstinence in an outpatient setting. [99] It is important to note that the dose used in the aforementioned outpatient treatment trial (2 mg) [99] was substantially lower than that used by Haney and colleagues in the human laboratory to attenuate withdrawal symptoms. [98] However, higher doses in previous laboratory studies were associated with substantial increases in “Good Drug Effects”, “Drug Liking”, and “Take Again”. [93,94,97] Thus, it is difficult to reconcile increasing the dose of nabilone given in an outpatient setting with these apparent increases in misuse liability. This is further substantiated by the lack of efficacy of dronabinol in promoting abstinence from cannabis, given the similarities between these medications.

A more suitable role for nabilone in the treatment of CUD may be as an adjunctive pharmacotherapy. A laboratory study found that daily nabilone and nightly zolpidem improved sleep and reduced anxiety and irritability during a withdrawal period. [100] This combination did not produce significant increases in “Drug Liking” or “Take Again” relative to placebo. Unfortunately, the combination was not directly compared with nabilone alone, and the addition of zolpidem did not significantly attenuate sleep-related withdrawal symptoms moreso than nabilone alone did in a prior study, [98] although the doses of nabilone used herein were slightly lower. It is therefore difficult to ascertain where these findings fall in a broader therapeutic context. Similarly, combined nabilone and varenicline attenuated withdrawal symptoms in individuals that use both cannabis and tobacco without appreciable effects on a laboratory model of relapse. [101] While these treatment combinations did not produce appreciably superior effects to nabilone alone, the lack of considerable drug interactions and continuing attenuation of withdrawal symptoms may be indicative of a more nuanced role for nabilone in the treatment of CUD moving forward.

Cannabidiol (CBD)

CBD has a broad and complex pharmacological profile, interacting with many classes of receptors, enzymes, and other targets. Although similar in structure to THC, CBD binds poorly to CB1R and CB2R. [102] However, CBD may still have pharmacological activity within the eCB system by acting as a negative allosteric modulator of CB1R and inhibiting the reuptake and hydrolysis of AEA. [103–105]

Outcomes from some human laboratory studies suggest that CBD can block acute adverse pharmacodynamic effects of THC such as anxiety [106] and memory impairment,

[107] leading to speculation that CBD may mitigate the effects of THC; this "dampening" effect is consistent with activity as a CB1R negative allosteric modulator. Further, anxiolytic effects may be attributable to inhibition of AEA hydrolysis, as this is also a known quality of FAAH inhibitors. [63] However, a study comparing acute doses of oral CBD (200, 400, and 800 mg) and placebo in the context of smoked cannabis among regular cannabis users reported no impact of CBD on cannabis self-administration, subjective effects, or physiologic responses. [108] Solowij et al. evaluated the impact of vaporized low (4 mg) and high (400 mg) CBD given in conjunction with THC. [109] Low doses of CBD enhanced the intoxicating effects of THC, particularly in infrequent cannabis users, while high doses of CBD were associated with a reduction of intoxicating effects.

Three trials to date have evaluated longer-term oral CBD administration in cannabis users. An open-label trial evaluated 200 mg daily CBD administration for 10 weeks among 20 frequent cannabis users. [110] Compared to baseline, participants reported fewer depressive and psychotic symptoms after CBD treatment and demonstrated improvement in cognitive measures. Increased euphoria when smoking cannabis was also reported. Results have also been published from a four-week adaptive trial in which three doses (200, 400, and 800 mg) of oral CBD were compared to placebo during a cannabis cessation attempt. [111] Following an initial treatment phase (*n*=48), the 200 mg dose was deemed inefficacious and the trial continued with the 400 mg, 800 mg, and placebo arms (*n*=34). At end of treatment, both doses of CBD were associated with lower 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH):creatinine ratios and modest reductions in self-report days per week of cannabis use relative to placebo; however, treatment effects were not found at follow-up timepoints. Of note, there was some indication of an inverted-U dose-response curve, with the 200 mg dose deemed inefficacious and marginal

indication that the 400 mg dose was superior to the 800 mg dose. No serious adverse events were noted, although lower sleep quality was reported among individuals in the 400 mg group. Limitations of the study include brief treatment period and insufficient sample sizes to robustly estimate effect sizes, making it difficult to fully ascertain the impact of CBD in promoting abstinence from cannabis. Most recently, a small, open-label 12-week trial was conducted evaluating the efficacy of vaporized CBD in attenuating cannabis use in individuals with CUD. [112] Participants in this study were able to self-titrate their daily dose of CBD, and the average amount consumed per day was 215.8 mg. Six of the 20 enrolled participants reported that their daily cannabis use reduced by at least 50% at end-of-treatment, somewhat supporting the potential for CBD in the treatment of CUD. However, this study is severely limited by its small sample size, attrition, and reliance on self-reported substance use. All participants in this study also used nicotine, impacting generalizability.

Nabiximols

Nabiximols is an oromucosal spray composed of THC (2.7 mg/spray), CBD (2.5 mg/spray), and various terpenoids. It is approved in the United Kingdom, Canada, and other countries primarily for the treatment of spasticity related to multiple sclerosis; it is not currently FDA-approved in the United States, although registry trials are ongoing.

With respect to CUD, an initial study evaluated a six-day course of nabiximols (maximum daily dose 86.4 mg THC and 80 mg CBD) compared to placebo among 51 treatment-seeking cannabis-dependent individuals during an inpatient admission. [113] Nabiximols reduced cannabis withdrawal symptoms and improved retention in treatment,

but no medication effect was observed on time to cannabis relapse or reduction in cannabis use following medication cessation. Trigo et al. evaluated fixed versus self-titrated doses of nabiximols and placebo for cannabis withdrawal and craving during one-week abstinence periods in an outpatient trial. [114] High fixed doses of nabiximols (108 mg THC/100 mg CBD daily) reduced cannabis withdrawal compared to placebo, but did not reduce cannabis craving; limited efficacy was noted with the lower self-titrated doses.

Two randomized clinical trials have evaluated nabiximols as a potential treatment for CUD. One twelve-week trial compared a flexible dose of nabiximols (up to 113.4 mg THC/105 mg CBD daily) with placebo in conjunction with motivational enhancement and cognitive behavioral therapy in 50 individuals. [115] Nabiximols reduced cannabis craving compared to placebo; however, no significant differences in cannabis withdrawal or cannabis use were observed. Recently, a larger trial (*n*=128) reported a reduction in self-reported cannabis using days among individuals receiving nabiximols relative to placebo both during treatment and at a three-month follow-up assessment. [116,117] No between-group differences were found in cannabis withdrawal, craving, or periods of abstinence, nor in health or psychosocial outcomes. In both trials, nabiximols was well-tolerated, but treatment retention was low. Nabiximols may have some promise for the treatment of CUD if findings related to cannabis use can be replicated.

FAAH Inhibitors

FAAH inhibitors increase levels of AEA through selective inhibition of its primary catabolic enzyme, [118] and increased AEA is associated with anxiolytic and antidepressant effects. [118,119] Like exogenous CB1R agonists, FAAH inhibitors have been shown to

alleviate symptoms of cannabis withdrawal in mice, [120] but are distinguished by their lack of readily apparent abuse liability. [121–123] FAAH inhibitors may also have reduced potential for the development of tolerance or physical dependence relative to direct CB1R agonists. [122] Only one trial examining the use of FAAH inhibitors has been published thus far in men with CUD, with promising outcomes. [124] Men that received the FAAH inhibitor PF-04457845 not only exhibited attenuated withdrawal symptoms, but also self-reported reduced cannabis use, later confirmed by urine toxicology. [124] Though limited by inclusion of only men and a relatively brief treatment period to truly assess risk of relapse, these have been some of the most promising CUD pharmacotherapeutic outcomes to-date.

A follow-up multi-site Phase IIB study using PF-04457845 recently completed in 2023 (NCT03386487). While the results for this study are currently unpublished, primary outcomes have since been posted to clinicaltrials.gov: PF-04457845 showed no effect on self-reported cannabis use with only marginal potential effects on urine THC-COOH. While these preliminarily null results are disappointing, formal analyses have not yet been conducted and it is not known if the inclusion of women in the follow-up study (comprising approximately 37% of the sample) affected results.

What is noteworthy from both of these studies is the lack of serious adverse events resulting from chronic treatment with PF-04457845. [124] Severe neurological side effects were previously associated with the FAAH inhibitor, BIA 10-2474. [125] However, the present studies corroborate other previous work indicating such side effects are more likely attributable to BIA 10-2474 or to questionable trial design than to the class of FAAH inhibitors as a whole. [126] Thus, while application of FAAH inhibitors may be limited to specific subpopulations of individuals with CUD or specific treatment scenarios, they are, at least, relatively safe.

COX-2 Inhibitors

Like FAAH, inhibition of cyclooxygenase-2 (COX-2) has been shown to increase levels of AEA and, to a lesser extent, 2-AG in animal models. [23] Moreover, COX-2 has been implicated in deleterious neurobiological effects of repeated THC exposure observed in animals. [127] Taken together, it seems likely that COX-2 inhibition could have some utility in CUD treatment. Haney and colleagues examined the effects of 200 mg celecoxib, a COX-2 inhibitor, relative to placebo on measures of cannabis intoxication, withdrawal symptoms, and simulated relapse in an inpatient laboratory study. [128] COX-2 inhibition had no effect on cannabis withdrawal symptoms or simulated relapse and increased cannabis craving relative to placebo. Furthermore, COX-2 inhibition showed no apparent impact on the distribution of circulating eCBs, also called peripheral eCB tone, in contrast with its described mechanism, its activity in the animal literature, and unlike FAAH inhibitors, as mentioned above. As such, COX-2 inhibitors, at least in the present dose and formulation, appear to be unlikely candidate medications for CUD.

Conclusions

Despite the high prevalence of CUD, [1] current treatment options are at best only moderately effective and there is no FDA-approved pharmacotherapy for its treatment. [11] The eCB system presents an attractive pharmacotherapeutic target, given its specific dysregulation by heavy cannabis use and the clinical success of agonist replacement therapy for opioid and nicotine use disorders. The most effective method for targeting the eCB system, however, remains unclear. While CB1R antagonism may effectively reduce cannabis use, severe psychiatric side effects preclude its use in a treatment setting,

especially given the high rate of psychiatric comorbidity already prevalent among individuals with CUD. [129] In contrast, synthetic CB1R agonists, such as dronabinol and nabilone, attenuate withdrawal symptoms during an abstinence period, but have no apparent impact on cannabis use in an outpatient setting and bear the additional burden of potential abuse liability. CBD- and FAAH inhibitor-based treatments show evidence of efficacy in both reducing cannabis use and curtailing associated withdrawal symptoms, but research is limited, and efficacy may be limited to specific subpopulations of individuals with CUD.

Future research should aim to expand on these preliminary positive findings. Surprisingly, there are currently no randomized clinical trials in progress assessing CBD for CUD, although one such trial is scheduled to begin later this year (NCT06107062). The potential role of sex differences in the effects of PF-04457845 should also be analyzed in the context of the multi-site FAAH inhibitor study. Finally, though results from the COX-2 study were negative, other alternative metabolic pathways within the eCB system may benefit from future exploration into their role in the treatment of CUD.

Individual Differences in CUD

Healthy men are overrepresented in the clinical studies for CUD listed above: study samples were 81% male on average and only 8 studies (of 30) included individuals with Axis I psychiatric diagnoses if they were stable and on medication. In the United States, 8% of males and 5.5% of females over the age of 12 met criteria for past-year CUD in 2022. [1] To match this distribution in research, study samples would need to be at least one-third female. Similarly, about 17% of U.S. citizens over the age of 18 with any mental

illness meet criteria for CUD, [1] and nearly one-third of people with CUD also meet diagnostic criteria for major depressive disorder (MDD). [130] Given the high prevalence of MDD in individuals with CUD and that over one-third of individuals with CUD are female, there is a pressing need to understand how these individual differences may translate to differences in CUD treatment outcome.

Regarding sex, prevalence of CUD is increasing among females. [1] Behaviorally, women progress more quickly from first use to the development of CUD—a phenomenon known as “telescoping”. [130,131] Women also report greater cannabis withdrawal symptom severity and withdrawal-related functional impairment, [132,133] putting them at greater risk of withdrawal-precipitated relapse. Yet, women only constituted 19% of subjects on average in the trials referenced above, and 6 of these studies included no women at all. This discrepancy contributes to two issues: 1) samples used in CUD treatment studies are not representative of the general population of individuals with CUD, negatively impacting generalizability of outcomes; and 2) studies are underpowered to analyze potential sex or gender differences in outcomes. Importantly, inattention to sex differences in general medications development has been linked to an increased prevalence of adverse drug reactions in women. [134] Even when considering all classes of drugs assessed for efficacy in the treatment of CUD, not just those that target the eCB system listed above, a diminishingly small number of treatment trials included sex-specific analyses. [135] Of those that did, two of three observed worse treatment outcomes in women relative to men. Taken together, it is clear that inattention to sex and gender is a systemic problem in pharmacotherapeutic development that negatively impacts women. Before the evaluation of eCB-modulating drugs in CUD moves forward, potential sex differences in the eCB

system and how those might translate to differences in treatment outcome must be adequately assessed.

Regarding MDD, one study using data from the 2012-2013 National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) found that 20% of men and 36% of women with past-year CUD also met criteria for past-year MDD. [130] Another study using 2011 NSDUH data found that people with a recent major depressive episode were nearly three times more likely to meet criteria for a CUD compared to people without a recent episode. [136] These general trends appear to have held with time: more than 26% of individuals aged 18 and above that reported a past-year major depressive episode met criteria for CUD in 2022, an increase from 23% in 2021. [1] Critically, even though the proportion of individuals with comorbid MDD/CUD compared to CUD alone is relatively stable, MDD/CUD represents an increasing proportion of the overall U.S. population, as rates of MDD and CUD are both increasing. [1] Yet, people with depression are often specifically excluded from clinical trials, despite having a greater desire to quit using cannabis than individuals without depression. [136] If they are included, the proportion of individuals with depression is rarely listed, and comparisons are not made between these and neurotypical study participants, making it difficult to determine how comorbid MDD impacts CUD treatment outcomes. A clinical trial limited to individuals with comorbid MDD/CUD found that cannabis withdrawal symptoms were commonly reported in this population and were frequently cited as a contributor to relapse, [137] however, it is unclear if this differs from what is already known about individuals with CUD alone. [14]

It is understandable that one might initially hesitate to include participants with comorbid MDD/CUD in medication trials for CUD. If these individuals are prescribed antidepressants outside of the study, that medication might interact with a study drug in

unanticipated ways. Individuals with depression might also be more likely to present with adverse events associated with having poorer mental health at baseline, particularly if a medication is known for psychiatric side effects. However, while reasons like these might justify past hesitancy, it is time to move forward. Women have historically been excluded from clinical trials due to concerns related to hormonal variation or potential effects of study medications on a fetus. Likewise, we must acknowledge that there are biological differences and unique risks associated with MDD. Yet, like women, that does not mean these individuals should be systematically excluded from science; rather, these individual differences should be uncovered and used to inform pharmacotherapeutic development.

Endocannabinoid Manipulation as a Therapeutic Strategy in Specific Sub-Populations of Individuals with CUD

There is only one study specifically examining the eCB system in women or females with CUD, [74] and there are no studies examining the eCB system in people with comorbid MDD/CUD. The studies detailed in the following chapters were designed to address these gaps in the literature, to inform potential best practices for the use of eCB-modulating drugs in these sub-populations. However, in the absence of explicit study, one can infer the therapeutic potential and possible pitfalls of eCB-modulating drugs in women/females or people with depression by examining the eCB system in people without CUD. Studies examining the subjective effects of THC in these populations might also provide useful insight into eCB function.

Women and Females

When evaluating a drug as a potential pharmacotherapeutic, one must consider potential for adverse effects. Moreover, if a drug has a comparable mechanism (i.e. direct or indirect CB1R agonism) to a substance with known misuse liability (i.e. THC), this must be considered to establish safe dosing practices. Women that do not use cannabis report a greater positive subjective response to acute THC administration relative to men. [138] In women that use cannabis heavily, sex differences in subjective responses to THC are inconsistent, and may depend on route of administration: one study using oral THC found no sex difference in subjective response to THC, [139] whereas another study using smoked cannabis replicated results observed in non-cannabis-using women. [140] These findings suggest that eCB-modulating drugs that may have direct (CB1R agonist) or indirect (FAAH or MAGL inhibitor) cannabimimetic effects may need to be dose-adjusted by sex to minimize misuse liability. At odds with this conclusion, however, is the finding that subjective responses to THC are uncorrelated with CB1R expression in women with CUD. [74] It is therefore possible that there is a non-eCB targeted effect of THC, or a specific interaction between THC and gonadal hormones not shared by eCBs [141] that is responsible for sex differences in its subjective effects. The mechanism underlying the sex differences in the subjective effects of THC, and potentially similar compounds, should be uncovered to determine the relative safety of eCB-modulating compounds in females with CUD.

Another important consideration when potentially administering eCB-modulating drugs to females is the influence of the menstrual cycle. THC has been shown to produce greater antinociceptive and motor effects preclinically in estrus, when estrogen is highest, relative to diestrus, when progesterone is highest. [142] In humans, effects of menstrual cycle

on the subjective effects of THC are more limited, [143] though self-reported cannabis use among daily cannabis users is highest in the premenstrual/late luteal phase. [144] With respect to the eCB system, CB1R density in the hypothalamus and CB1R ligand affinity in the limbic forebrain fluctuate over the menstrual cycle in animal models, peaking during diestrus. [145] Hypothalamic AEA was found to be highest during diestrus in another study, [146] with other cycle-dependent effects of both AEA and 2-AG observed in the pituitary, thalamus, hippocampus, and midbrain. While there is no available data on brain eCB expression across the menstrual cycle in humans, peripheral AEA has been associated with peripheral levels of estradiol, peaking during ovulation. [147] Interestingly, this pattern of human peripheral AEA matches onto the rodent pituitary AEA, albeit shifted slightly later in the menstrual cycle. These data suggest that eCB-modulating drugs may have differential effects by menstrual cycle phase due to cyclic variation in eCB tone. However, absence of data collected from individuals with CUD limit application to this population.

Finally, when considering therapeutic potential of eCB-modulating substances, it should be noted that both men [73] and women [74] with CUD show downregulated brain CB1R expression compared to healthy controls. In men, CB1R expression has been shown to rapidly upregulate during acute abstinence, [73] and CB1R expression is negatively associated with self-reported cannabis withdrawal symptoms during acute abstinence in both sexes. [73,74] Unfortunately, CB1R expression has not yet been compared between men and women with CUD, though women that do not use cannabis have greater CB1R expression than men throughout the brain. [74] Sex differences in brain CB1R expression may predict differences in CUD treatment response for eCB-modulating drugs, particularly with respect to withdrawal symptoms.

People with Depression

Individuals with depression are increasingly using medicinal cannabis to self-medicate, even though it is not approved for this use in any state. [148,149] Results from open-label retrospective studies generally support this practice, showing a significant and sustained effect of medicinal cannabis use on depression symptoms. [149–151] However, results from one of these retrospective works [149] and acute dosing studies [152,153] suggest that antidepressant effects of medicinal cannabis products are driven by CBD, rather than THC. In fact, THC may worsen depression symptoms, particularly with sustained exposure, [152–154] and heavy cannabis use, relative to non-use, is associated with reduced striatal activation to reward in individuals with mood disorders. [155] Taken together, these findings suggest interplay between depression and the eCB system that may be exacerbated by repeated or heavy cannabis use, as in comorbid MDD/CUD.

Incredibly, there are no published or ongoing (per clinicaltrials.gov) studies examining brain CB1R or FAAH expression in individuals with MDD using PET, even though CB1R-deficient mice were proposed as a model for depression in 2012 [156] and the pro-depressive effects of CB1R antagonists are well-known. [157] One master's thesis from 2022 reported that brain FAAH did not differ between individuals that were or were not experiencing a major depressive episode, but that FAAH expression in the prefrontal cortex, amygdala, ventral striatum, and substantia nigra were positively correlated with apathy in individuals with major depressive episode. [158] This might relate to the antidepressant, albeit not rewarding, effects of FAAH inhibition in rodents. [159] In contrast, FAAH is significantly lower throughout the brain in chronic cannabis users during early abstinence. [70] However, given the rapid recovery of CB1R during early abstinence observed in men with CUD, [73] it may be that FAAH reduction is specifically an effect of

abstinence, and potentially a recovery mechanism. Were this the case, FAAH inhibition could have considerable therapeutic utility in individuals with comorbid MDD/CUD.

In contrast to neuroimaging work, a handful of studies have examined peripheral eCB tone in individuals with MDD. Two seminal studies by Hill and colleagues reported consistently reduced serum 2-AG, and inconsistently reduced serum AEA, in women with MDD relative to healthy controls. [55,57] Two further studies found that antidepressant effects of transcranial magnetic stimulation [160] and SSRIs [161] were associated with increased peripheral 2-AG. These findings are consistent with preclinical work showing an antidepressant effect of pharmacological MAGL inhibition. [162,163] However, a final study examining the antidepressant effects of exercise found that 2-AG was reduced following exercise exposure, and that this change was negatively associated with self-reported depression. [164] Thus, while it generally seems like increasing 2-AG signaling (e.g. via MAGL inhibition) may have an antidepressant effect in people with MDD, this effect may be context-dependent. This synergizes well with CUD data in a treatment development context: greater MAGL expression has been associated with greater cortical thinning in postmortem brains obtained from people with CUD. [165] Yet, one must recall that prolonged CB1R activation, as would be the case with long-term MAGL inhibitor treatment, may worsen depression symptoms. [153] Studies evaluating the use of MAGL inhibitors in individuals with comorbid MDD/CUD must consider this possible adverse outcome in study design.

Summary

CUD is increasingly prevalent in the United States. However, this is still no effective pharmacological means to treat it. The eCB system, so named because of its direct activation by cannabis, has garnered interest as a therapeutic target for CUD. Research conducted thus far suggests that direct agonism or antagonism of CB1Rs may have limited utility due to psychiatric side effects (misuse liability or pro-depressive effects, respectively), but more subtle manipulation of the eCB system, e.g. through inhibition of eCB-regulating enzymes, may have a role in CUD treatment. Importantly, there is limited information about the eCB system in individuals other than healthy men with CUD, making estimating the utility of eCB-modulating drugs in other subpopulations difficult. The following studies sought to address this gap in the literature by measuring and comparing eCB tone in men and women with CUD during use and abstinence, as well as comparing eCB tone in people with CUD with or without comorbid MDD. ECB tone was evaluated for associations with withdrawal symptoms in all groups and for associations with stress response in individuals with or without comorbid MDD. As withdrawal and stress have been shown to each contribute to relapse in CUD, these data may provide important insights into the potential clinical utility of eCB-modulating therapeutics in CUD.

CHAPTER 2: Sex differences in endocannabinoid tone in a pilot study of cannabis use disorder and acute cannabis abstinence

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Introduction

One in twenty United States adults met DSM-5 criteria for CUD in 2020, [166] a condition for which there is no effective pharmacological intervention. [167] Like other substance use disorders, CUD presents with a distinct withdrawal syndrome which includes sleep disturbance, reduced appetite/gastrointestinal distress, and increased negative affect and anxiety. [14] As cannabis withdrawal has been shown to precipitate further cannabis use in individuals with CUD, alleviating withdrawal may serve as a viable therapeutic target. [14] Notably, however, cannabis withdrawal presents differently across sexes. When assessed retrospectively, women report more severe withdrawal symptoms overall relative to men during a quit attempt, with symptoms of negative affect and gastrointestinal distress heightened in particular. [132,133] Enhanced cannabis withdrawal severity in women may contribute to observed sex differences in treatment outcomes, [135,168] and pharmacotherapeutic interventions for CUD which target cannabis withdrawal may produce differential effects by sex.

The eCB system has emerged as a candidate pharmacotherapeutic target for CUD, as it is directly modulated by the primary psychoactive constituent of cannabis, THC. [169] The eCB system is composed of two cannabinoid receptor subtypes (CB1R, CB2R); two signaling ligands (AEA, 2-AG); and enzymes implicated in the degradation, transport, and synthesis of these ligands. [22] In addition to the canonical eCB system are eCB

congeners that share synthetic and degradative pathways with AEA (i.e. the other *N*-acylethanolamines [NAEs]: palmitoylethanolamide [PEA], stearoylethanolamide [SEA], linoleylethanolamide [LEA], oleoylethanolamine [OEA], docosatetraenylethanolamide [DEA]), and their associated enzymes and receptor subtypes that comprise an "endocannabinoidome". [170] The endocannabinoidome shows evidence of reactivity to acute cannabis use [171,172] and dysregulation during heavy use. [72,173,174] More recently, work has emerged suggesting that the eCB system may also undergo changes during acute cannabis abstinence in individuals with CUD. [73,128,175] This is consistent with the known role of the eCB system in reward, [176] sleep, [177] and feeding, [178] all of which are disrupted in cannabis withdrawal. [14]

Normalizing eCB signaling during early abstinence might serve as a viable pharmacotherapeutic strategy to reduce cannabis withdrawal symptoms and subsequently prevent relapse in people with CUD. Indeed, normalizing eCB tone via pharmacological inhibition of FAAH, a degradative enzyme for AEA, attenuated withdrawal symptoms and reduced cannabis use in a placebo-controlled study of men with CUD. [175] The same compound was also assessed in a recently completed multi-site clinical trial of men and women with CUD (NCT03386487), which, surprisingly, produced null outcomes. Notably, work evaluating eCB tone during early abstinence and the efficacy of eCB-modulating pharmacotherapeutics beyond direct CB1R agonism has been limited almost entirely to male subjects, and sex may be associated with differences in treatment outcome. [73,128,167,175] Despite known sex differences in the eCB system [74,146,179,180] and in the presentation of cannabis withdrawal, [132,133,181] sex differences in eCB tone in individuals with CUD during use and withdrawal have not been evaluated.

The present pilot trial sought to assess the effects of abstinence, relative to regular cannabis use, on peripheral eCB and eCB congener tone in men and women with CUD, and to examine associations between eCB and eCB congener tone and subjective experiences of withdrawal. We did so with the goal of evaluating potential sex differences during early abstinence in eCB dysregulation and prospective reporting of withdrawal symptoms. We hypothesized that acute abstinence would be associated with reduced AEA in both sexes, as has previously been observed in men, [175] and with dysregulation of the typical daytime rhythm of 2-AG, as has been observed following sleep restriction in both sexes. [182] We further hypothesized that changes in eCB and eCB congener tone would be correlated with the real-time subjective experience of withdrawal symptoms, as the eCB system has been implicated in processes specifically impacted by withdrawal. [176–178] Finally, as withdrawal symptoms are enhanced in women, we expected that women would present with a greater degree of eCB dysregulation during abstinence relative to men.

Methods

Participants

Ten adults between the ages of 18-45 (5 male, 5 female) with moderate or severe CUD were recruited for this study. This age range was selected to minimize incidence of age-related health conditions and to minimize age-related variation in the eCB system. [183] All participants were determined to be cisgender based on concordant responses at screening to the questions “What was your sex at birth?” and “What is your self-identified gender?”. Inclusion criteria were (1) English language proficiency; (2) self-reported

cannabis use ≥5 days per week in the month prior to screening; (3) body mass index between 18-30kg/m^2, as peripheral eCBs have show evidence of dysregulation in obese individuals; [184] and (4) access to a mobile phone capable of operating an ambulatory assessment battery (i.e. with access to internet). Female participants were required to have a regular menstrual cycle and be willing to use non-hormonal birth control for the duration of study participation. Exclusion criteria were (1) meeting DSM-5 criteria for any current major psychiatric disorder other than tobacco, caffeine, or cannabis use disorder as assessed by the Mini-International Neuropsychiatric Interview (MINI), [185] or only meeting DSM-5 criteria for mild CUD (i.e., <4 symptoms in the past year); (2) current use of psychotropic medications or supplements, including hormonal birth control; (3) evidence of anemia or other blood or clotting-related disorders; (4) urinalysis results indicative of recent substance use other than cannabis (amphetamines, benzodiazepines, cocaine, opioids, and THC were assessed via dip card test; One Step Multi-Drug Urine Test Panel); (5) current treatment-seeking for CUD or other substance use disorders; (6) blood pressure in excess of 150 (systolic) or 90 (diastolic); and (7) nursing, pregnancy, or plans to become pregnant in female participants. Criteria related to anemia and blood pressure were used to reduce likelihood of adverse events related to repeated blood draws or increased blood pressure during acute cannabis abstinence. [186] All participants provided written informed consent prior to study participation.

Procedures

Participants were recruited via media advertisements and through word-of-mouth. Interested individuals were first screened by phone to determine eligibility for a full screening visit at which written informed consent was obtained. Individuals that met all

eligibility requirements following the full screening visit were enrolled in a two-week study. Participants were allowed to use cannabis as usual during the first study week and were asked to abstain from use during the second week. Study weeks were separated by approximately one month; each week was scheduled to align with the early follicular phase of the menstrual cycle in females to control for changes in peripheral eCB tone associated with cyclical changes in the sex hormone, estradiol. [147] Cycle phase was estimated using a menstrual history diary of the 90 days prior to in-person screening. Substance use for the 90 days prior to screening, between screening and the use week, and between the end of the use week and the start of the abstinent week were assessed using retrospective Timeline Follow-Back (TLFB). [187]

On Days 1, 3, and 7 of each study week, participants attended five-hour in-person laboratory visits at the Medical University of South Carolina. The morning of each visit, participants were instructed to not eat breakfast or use cannabis but were allowed their usual amount of caffeine and/or nicotine. Upon arrival for each visit (approximately 0830), participants completed a breathalyzer test and provided a urine sample to assess recent drug use and to ensure female participants were not pregnant. Participants were not allowed to use nicotine during study visits due to known interactions between nicotine and the eCB system [188] that may impact peripheral eCB and eCB congener tone. Individuals that endorsed any nicotine use at screening were offered a nicotine patch upon arrival for each visit, but all participants declined this offer. During the abstinent week, participants also completed a supervised saliva drug test upon arrival for each visit (SalivaConfirm®; cutoffs: 12ng/mL THC-COOH, 75 ng/mL THC). Saliva drug tests were sensitive to cannabis use within the past 6-12 hours and participants presenting with a positive saliva drug test on Day 1 of the abstinent week were excluded from further study participation.

Abstinence was later verified through assessment of urine THC-COOH levels obtained at each visit during the abstinent week analyzed using liquid chromatography-mass spectrometry (LC-MS). Levels were normalized using simultaneous urine creatinine to control for dilution. [189] Urinalysis was not conducted for the first participant enrolled in the study (female), as the laboratory did not retain these samples for follow-up analyses.

Following arrival procedures, an indwelling catheter was placed for each participant by trained nursing staff. Blood was collected for eCB and eCB congener analyses at 0900, 1200, and 1300. After completing the 0900 and 1200 time points, participants were able to select food and drink of their choice from the hospital cafeteria. Blood collection time points were selected so as to be sensitive to possible effects of use vs. abstinence on diurnal variation in eCB and eCB congener tone, which is disrupted following sleep restriction, [182] and eCB and eCB congener response to eating. [190] Participants were discharged following completion of the 1300 time point.

Every day of each study week, participants completed an assessment battery at 0900, 1200, 1700, and 2100 on their personal mobile device. On laboratory days, assessments were completed following each blood draw and an additional assessment battery was completed at 1300. At each time point, participants received a text message with a link to a secure survey portal (RedCap®) and were given one hour to complete assessments. During the abstinent week, participants were also prompted to complete at-home saliva drug tests at 0900 and 2100. Participants recorded themselves completing each drug test and submitted recordings through the same survey portal. To maximize compliance with drug test self-administration, participants were allowed six hours from the time each video request was sent. Participants were compensated for each mobile assessment battery completed, for each video submitted, and were given bonus

compensation for each video in which the saliva drug test result was negative. Study procedures were approved by the Institutional Review Board of the Medical University of South Carolina and were conducted in accordance with the Declaration of Helsinki.

Mobile Assessment Battery

The mobile assessment battery took approximately five minutes to complete and was comprised of surveys probing cannabis withdrawal symptoms and recent drug, alcohol, and nicotine/tobacco use. Cannabis withdrawal symptoms were assessed at all time points via the Cannabis Withdrawal Scale (CWS). [191] Substance use was assessed via TLFB at all time points except those that only encompassed time in the laboratory (i.e. 1200 and 1300 on Days 1, 3, and 7). Past-night's sleep was assessed as part of each 0900 battery using the Consensus Sleep Diary, [192] and sleep efficiency was calculated using self-reported time asleep/time in bed. The State-Trait Anxiety Inventory (STAI-S) [193] and Positive and Negative Affect Schedule (PANAS) [194] were added to the assessment battery during laboratory visits to further probe participant affect and anxiety. Evening mobile assessments were not sent out following completion of either Day 7 laboratory visit.

Plasma eCBs and eCB Congeners

Blood specimens (4mL) for eCB and eCB congener analyses were collected using 10mL glass tubes containing sodium heparin. Following collection, samples were immediately placed on ice and were centrifuged to separate plasma within 1 hour. Formic acid in water (5%) was added to plasma in a 1:9 ratio as a stabilizer during storage.

Samples were stored in glass amber vials at -80°C until the study was completed, at which point all samples were shipped on dry ice overnight to the iC42 Clinical Research and Development Clinical Mass Spectrometry Service Center at the University of Colorado (Anschutz Medical Campus, Aurora, CO). Samples were analyzed using a validated high-performance liquid chromatography-tandem mass spectrometry as previously described. [195] ECBs (AEA, 2-AG) and related eCB congeners, the other NAEs (PEA, SEA, LEA, OEA, DEA) were quantified in said plasma samples.

Statistical Analyses

Baseline measures of demographic, clinical, and cannabis use history data were summarized using means and associated standard deviations for continuous variables and frequencies for categorical data; sex differences were assessed using *t*-tests or Fisher's exact tests as appropriate. Sex differences in at-home mobile assessment adherence during the study was assessed using a chi-squared test. To assess cannabis use (any use) during the use-as-usual week, generalized linear mixed effects regression models (GLMM) for binary outcomes (logit) were developed with primary factors for time of report and participant sex; data are presented as odds ratios (ORs) and associated 95% confidence intervals (CIs). These analyses were conducted using *R.*

Plasma eCB and eCB congener measurements were categorized as AM (0900) and PM (1200, 1300) based on examination of patterns over time. No significant differences were observed between 1200 and 1300 eCB or eCB congener values despite participants eating between these time points, in contrast to what has been previously observed in healthy volunteers. [190] To assess the association between use condition (use-

as-usual/abstinent weeks), measure time (AM/PM), and participant sex with eCB and eCB congener concentrations, GLMMs were developed. Prior to analysis, eCB and eCB congener concentrations were natural logarithm-transformed to achieve normality of model residuals. Models were adjusted for study day, time of measure, and use condition (week) as necessary and included sex-stratified analysis. Further, to measure the association of eCB and eCB congener levels with subjective measures of withdrawal, affect, anxiety, and sleep, concurrently measured CWS, PANAS, STAI, and subjective sleep efficiency measures were included in GLMMs. Prior to this analysis, both subjective measures and eCB and eCB congener concentrations were standardized such that the association would be equivalent to a 1 standard deviation change from the mean of the measurements. As an exploratory pilot study, statistical significance at $p<.05$ is noted with no correction for multiple comparisons. These analyses were conducted using SAS.

Results

Study Sample

Participant demographics, baseline subjective outcomes, and substance use reported at screening are listed in **Table 2.1**. No significant sex differences were evident in baseline characteristics and all participants reported previous experience with cannabis withdrawal (assessed via MINI). All enrolled participants completed the study. One participant (female) did not complete the Week 1 Day 7 0900 blood draw, and one participant (male) did not complete the Week 5 Day 3 1300 mobile assessment battery; all other laboratory visit data was complete. At-home completion of the mobile assessment

Table 2.1. Participant demographics and baseline measurements

Data are presented as mean (standard deviation) or as frequency. All participants reported at least some college education. No comparisons across sexes met the p<.05 threshold for statistical significance. CUD=Cannabis Use Disorder, PANAS=Positive and Negative Affect Schedule, STAI=State-Trait Anxiety Inventory.

	Females (*n*=5)	Males (*n*=5)
Age (Years)	30 (10.4)	24 (6.32)
Race (White/Black)	5/0	4/1
Unmarried	4	4
Employed	1	3
CUD Severity (Moderate/Severe)	2/3	4/1
Substance Use (Past 90 Days)		
Cannabis Use Days	86.2 (4.60)	86.2 (4.09)
Cannabis Sessions/Day	5.29 (2.83)	3.52 (1.30)
Any Nicotine Use	1	2
Any Alcohol Use	3	4
Subjective Measures		
PANAS Positive Affect	28.4 (7.02)	33.2 (6.10)
PANAS Negative Affect	10.6 (0.89)	10.4 (0.89)
STAI Total Score	28.2 (5.72)	26.6 (5.98)

battery (of 40 total assessments) was 78%; 83% in females and 74% in males [X^2 (1, N=10) = 4.8, p<.05].

Cannabis Use

When analyzing mobile assessments, females did not differ from males in odds of reporting cannabis use since the previous assessment during the use-as-usual week [OR=0.77; CI=-1.99, 1.47; p=.74]. There was a significant effect of assessment time on reported use, in that participants were more likely to report cannabis use since the last assessment at the 2100 time point relative to 0900 [OR=2.73; CI=0.12, 1.93; p<.05]. Likelihood of reporting use did not differ from 0900 for the 1200 [OR=0.48; CI=-1.78, 0.25; p=.15] or 1700 [OR=1.55; CI=-0.45,1.35; p=.34] time points. There were no significant effects of time relative to 0900 [1200 OR=0.80; CI=-0.72, 0.25; p=.37; 1700 OR=1.11; CI=-

0.29, 0.50; *p*=.59; 2100 OR=1.18; CI=-0.21, 0.54; *p*=.38] or sex [OR=0.68; CI=-1.10, 0.28; *p*=.21] on reported number of cannabis use sessions during the use week. All participants reported consistent cannabis use during the period between study weeks and all participants reported using cannabis the day prior to Day 1 of the abstinent week.

Three total instances of cannabis use were reported across two male participants during the abstinent week. No female participants reported cannabis use during the abstinent week. All submitted saliva drug test videos showed negative results, however, 8/100 expected videos were missing (7/50 male, 1/50 female). Videos that would have captured those self-reported cannabis use instances constituted 3 of the 8 missing videos. All saliva drug tests completed in the laboratory were negative for recent cannabis use. Of the participants for which LC-MS urinalysis was conducted (9/10), 8 of these showed a reduction in 11-nor-9-carboxy-THC:creatinine ratio between Day 1 and Day 7 of the abstinent week. The participant that did not show this pattern self-reported two use instances during the abstinent week.

Subjective Measures

Cannabis Withdrawal Scale (CWS) total scores were significantly greater during the abstinent week relative to use-as-usual [12.6 (SE=2.1) vs. 7.3 (SE=2.1)] (**Table 2.2**). Additionally, males overall had lower CWS scores relative to females [4.5 (SE=3.0) vs. 15.4 (SE=3.0)]. This sex difference was modified by a use week [interaction $F_{1,8}$=26.6; *p*<.01]; males had a lower increase in withdrawal symptoms from the use week to the abstinent week [Δ=1.2 (SE=1.2); *p*=.35] as compared to females [Δ=9.4 (SE=1.1); *p*<.01]. Further, in females, greater withdrawal was reported at 0900 compared to later

Table 2.2. Differences in withdrawal, affect, and sleep as a function of sex and week

Data are shown as model-based means and standard errors from generalized linear mixed effects models. CWS=Cannabis Withdrawal Scale, PANAS=Positive and Negative Affect Schedule (-PA=positive affect, -NA=negative affect), STAI=State-Trait Anxiety Inventory.

	Week		
	Abstinent	**Use**	**Statistic**
CWS	12.6 (2.1)	7.3 (2.1)	t_8=6.5; p<.01
PANAS-PA	22.3 (2.4)	25.1 (2.4)	t_8=-5.3; p<.01
PANAS-NA	11.6 (0.6)	11.4 (0.6)	t_8=0.7; p=.51
STAI	33.9 (2.7)	31.8 (2.7)	t_8=2.7; p=.03
Sleep Efficiency	77.9 (3.8)	83.1 (3.7)	t_9=-1.7; p=.13
	Sex		
	Male	**Female**	**Statistic**
CWS	4.5 (3.0)	15.4 (3.0)	t_8=-2.6; p=.03
PANAS-PA	26.5 (3.4)	21.0 (3.4)	t_8=1.2; p=.28
PANAS-NA	10.6 (0.9)	12.4 (0.9)	t_8=-1.5; p=.18
STAI	29.2 (3.7)	36.5 (3.7)	t_8=-1.4; p=.20
Sleep Efficiency	86.6 (4.9)	74.4 (4.7)	t_8=1.8; p=.11

measurements [1200, 1300, 1700, 2100; p<.05], though a statistically significant sex by time interaction was not observed.

State anxiety (STAI) and positive and negative affect (PANAS) were also collected during laboratory visits during both use and abstinent weeks. Like the CWS, anxiety scores were increased during the abstinent week relative to use [33.9 (SE=2.7) vs. 31.8 (SE=2.7)]. This difference shows some evidence of modification by participant sex [interaction $F_{1,8}$=5.1; p=.05], noting that males had a lesser increase in anxiety from the use week to the abstinent week [Δ=0.3 (SE=1.1); p=.77] as compared to females [Δ=3.9 (SE=1.1); p<.05]. Regarding affect, participants reported lower positive affect [PA: 22.3 (SE=2.4) vs. 25.1 (SE=2.4)] but no change in negative affect [NA: 11.6 (SE=0.6) vs. 11.4

(SE=0.6)] during the abstinent week relative to use. There were no sex differences or modification of PANAS scores by sex [PA: p=.76; NA: p=.35]. Sleep efficiency measured each morning was not significantly different between abstinent and use-as-usual weeks [p=.13] or between males and females [p=.11]. Sex did not modify the relationship between abstinence and sleep efficiency [p=.88].

Plasma eCB and eCB Congener Concentrations

Almost all NAEs (AEA, PEA, DEA, LEA, OEA) showed a significant reduction in plasma levels between the AM and PM time points that did not differ across sex or cannabis use condition (**Figure 2.1**, **Table 2.3**). SEA showed a significant reduction between AM and PM in both use conditions in females [Use t_8=5.1; p<.01; Abstinence t_8=4.2; p<.01], and between AM and PM in men during abstinence [t_8=2.4; p<.05], but not use [t_8=2.2; p=.05]. No effects of time of day were seen for 2-AG, regardless of sex or use condition. During the abstinent period, women had significantly greater AEA, SEA and LEA levels as compared to men at the AM time point [AEA: t_8=3.1; p<.05 SEA: t_8=2.9; p<.05 LEA: t_8=2.9; p<.05] (**Figure 2.1**). Sex differences were driven by numerical increases in NAE tone observed in females, but not males, during abstinence relative to use-as-usual. A significant sex difference was also seen for OEA at the PM time point during use [t_8=-3.1; p<.05]. No significant sex or use period effects were seen for PEA, 2-AG, or DEA.

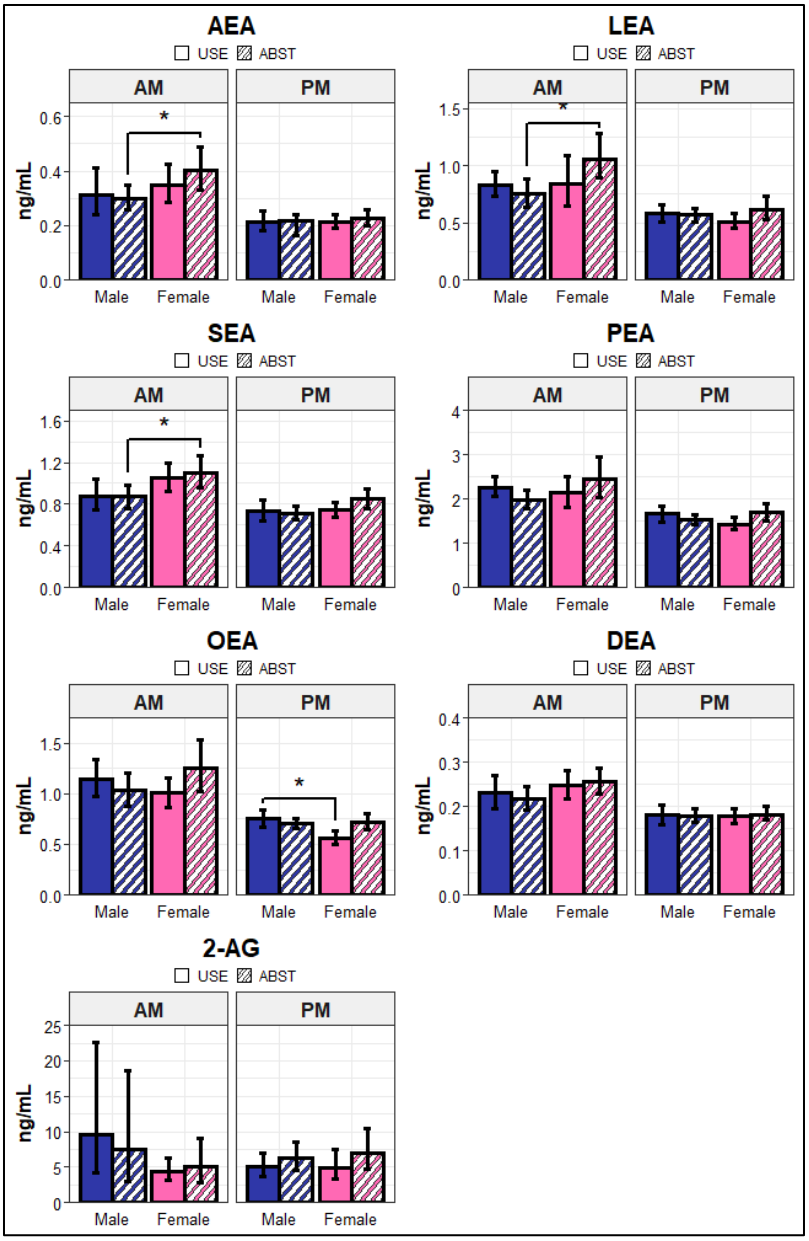

Figure 2.1. Plasma concentrations of eCBs and eCB congeners across sexes during cannabis use-as-usual and one week of abstinence. Significant time of day effects were seen for all *N*-acylethanolamines (NAEs); levels were reduced at the PM time points relative to the AM time point regardless of use condition, except for SEA during use-as-usual in men. No time of day effects were seen for 2-AG, independent of sex and use condition. Significant sex differences were seen for multiple NAEs (AEA, LEA, SEA) at the AM time point during abstinence. Data are presented as geometric means collapsed across study days and 95% confidence intervals, *indicates a significant sex difference, $p<0.05$. AEA=*N*-arachidonoylethanolamide, 2-AG=2-arachidonoylglycerol, DEA=docosatetraenylethanolamide, PEA=palmitoylethanolamide, SEA=stearoylethanolamide, LEA=linoleylethanolamide, OEA=oleoylethanolamine.

Table 2.3. Geometric mean and associated 95% confidence interval of plasma eCB and eCB congener concentrations collected during AM and PM measurements

Data are presented stratified by sex. ***p<0.05** as compared to early measurements; **łp<0.05** as compared men.

		Use-as-Usual Period		Abstinence Period	
		AM	**PM**	**AM**	**PM**
AEA	Male	0.31 (0.24,0.41)	0.21 (0.18,0.25)*	0.30 (0.26,0.35)	0.22 (0.17,0.24)*
	Female	0.35 (0.28,0.42)	0.22 (0.19,0.24)*	0.40 (0.33,0.49)ł	0.23 (0.20,0.26)*
2-AG	Male	9.63 (4.11,22.6)	5.04 (3.61,7.05)	7.50 (3.01,18.7)	6.26 (4.55,8.60)
	Female	4.37 (3.08,6.20)	4.95 (3.30,7.42)	5.02 (2.79,9.05)	7.04 (4.77,10.4)
DEA	Male	0.23 (0.20,0.27)	0.18 (0.16,0.20)*	0.22 (0.19,0.25)	0.18 (0.16,0.19)*
	Female	0.25 (0.22,0.28)	0.18 (0.16,0.19)*	0.26 (0.23,0.29)	0.18 (0.17,0.20)*
PEA	Male	2.26 (2.05,2.51)	1.66 (1.49,1.84)*	1.97 (1.77,2.20)	1.54 (1.43,1.65)*
	Female	2.13 (1.81,2.50)	1.43 (1.30,1.58)*	2.44 (2.02,2.95)	1.69 (1.51,1.89)*
SEA	Male	0.88 (0.75,1.04)	0.73 (0.64,0.84)	0.87 (0.76,0.98)	0.71 (0.65,0.78)*
	Female	1.05 (0.92,1.19)	0.75 (0.68,0.82)*	1.10 (0.96,1.27)ł	0.85 (0.76,0.95)*
LEA	Male	0.83 (0.73,0.95)	0.58 (0.51,0.66)*	0.75 (0.64,0.88)	0.57 (0.51,0.63)*
	Female	0.84 (0.65,1.09)	0.51 (0.45,0.58)*	1.06 (0.90,1.28)ł	0.62 (0.53,0.73)*
OEA	Male	1.14 (0.97,1.34)	0.75 (0.67,0.84)*	1.03 (0.88,1.21)	0.71 (0.66,0.76)*
	Female	1.01 (0.87,1.16)	0.56 (0.50,0.63)*ł	1.25 (1.02,1.53)	0.72 (0.64,0.80)*

Associations Between Plasma eCB and eCB Congener Concentrations and Subjective Measures

CWS, PANAS, STAI, and sleep efficiency scores were assessed for associations with concurrently measured plasma eCBs and eCB congeners and are noted in **Tables 2.4-2.8**. The overall association of peripheral eCB and eCB congener content with concurrently measured CWS total score was moderate and positive for LEA [β=0.16 (SE=0.09)]. In female participants, significant and positive associations between LEA [β=0.30 (SE=0.13)], PEA [β=0.22 (SE=0.13)], and 2-AG [β=0.32 (SE=0.15)] with CWS total score were stronger than in male participants [LEA β=-0.17 (SE=0.14); PEA β=-.01 (SE=.14); 2-AG β=-0.06 (SE=0.35)]. Additionally, female and male participants showed differing associations between OEA and CWS total score, where values were positively correlated in females and negatively correlated in males [Female β=0.17 (SE=0.11) vs.

Table 2.4. Correlation between Cannabis Withdrawal Scale (CWS) total score and eCB and congener tone

Note for Tables 2.4-2.8: Data are shown as the standardized beta and associated standard errors. Values represent the proportion of a 1 SD change in lipid level with a 1 SD change in subjective outcome measure score. Measures are adjusted for study period (use-as-usual/abstinence), measure timing (AM/PM) and participant sex where appropriate. **Bolded values** represent a significant association (p<.05); *indicates sex interaction (p<.05). AEA=*N*-arachidonoylethanolamide, 2-AG=2-arachidonoylglycerol, DEA=docosatetraenylethanolamide, PEA=palmitoylethanolamide, SEA=stearoylethanolamide, LEA=linoleylethanolamide, OEA=oleoylethanolamine.

	AEA	2-AG	DEA	PEA	SEA	LEA	OEA
Overall	.10 (.09)	.11 (.13)	.14 (.10)	.13 (.09)	.05 (.10)	***.16 (.09)***	.00 (.09)
AM	.17 (.12)	.13 (.21)	.11 (.12)	.05 (.12)	.11 (.12)	.15 (.11)	-.02 (.11)
PM	.20 (.13)	.22 (.17)	***.27 (.15)***	***.24 (.14)***	.02 (.14)	***.25 (.14)***	.03 (.13)
Abstinence	.13 (.13)	.08 (.20)	.13 (.13)	.19 (.14)	.02 (.15)	.12 (.14)	.06 (.12)
Use-As-Usual	.08 (.13)	.13 (.18)	.15 (.14)	.01 (.13)	-.13 (.16)	.13 (.12)	-.05 (.12)
Male	.09 (.16)	-.06 (.34)	.18 (.18)	-.01 (.14)	-.13 (.16)	-.17 (.14)	-.20 (.14)
Female	.04 (.12)	***.32 (.15)***	.05 (.12)	***.22 (.13)***	.01 (.12)	***.30 (.13)****	.17 (.11)*

Table 2.5. Correlation between PANAS PA score and eCB and eCB congener levels

	AEA	2-AG	DEA	PEA	SEA	LEA	OEA
Overall	***-.26 (.09)***	-.06 (.10)	-.17 (.09)	***-.16 (.08)***	***-.23 (.09)***	-.16 (.09)	-.10 (.08)
AM	-.24 (.14)	-.21 (.19)	-.11 (.14)	-.09 (.13)	***-.29 (.13)***	-.25 (.13)	-.03 (.11)
PM	***-.27 (.10)***	-.07 (.12)	***-.24 (.11)***	***-.21 (.10)***	-.12 (.11)	-.12 (.11)	-.13 (.10)
Abstinence	***-.24 (.10)***	-.15 (.14)	-.11 (.11)	-.16 (.11)	-.20 (.12)	-.15 (.12)	-.06 (.09)
Use-As-Usual	***-.32 (.15)***	.05 (.15)	-.28 (.16)	-.18 (0.13)	***-.30 (.14)***	-.21 (.13)	-.15 (.13)
Male	-.30 (.17)	***-.52 (.17)***	-.22 (.20)	-.20 (.13)	***-.52 (.16)***	-.07 (.13)	-.06 (.13)
Female	***-.26 (.10)***	.11 (.13)*	-.15 (.11)	-.18 (.10)	-.06 (.11)*	-.17 (.12)	***-.21 (.09)***

Table 2.6. Correlation between PANAS NA score and eCB and eCB congener levels

	AEA	2-AG	DEA	PEA	SEA	LEA	OEA
Overall	-.02 (.08)	-.02 (.11)	.02 (.08)	.09 (.08)	.01 (.08)	.13 (.08)	.01 (.07)
AM	.08 (.12)	-.03 (.21)	.04 (.11)	.02 (.12)	.18 (.11)	.12 (.11)	-.02 (.10)
PM	.06 (.09)	.04 (.13)	.08 (.11)	.14 (.10)	.01 (.09)	***.19 (.09)***	.04 (.09)
Abstinence	.04 (.09)	.01 (.14)	.01 (.10)	.09 (.10)	.04 (.10)	.19 (.10)	.03 (.08)
Use-As-Usual	-.13 (.12)	-.11 (.18)	.05 (.13)	.04 (.12)	-.02 (.13)	.02 (.12)	-.06 (.11)
Male	-.26 (.15)	.31 (.38)	-.20 (.17)	-.09 (.15)	-.21 (.16)	.03 (.15)	-.12 (.15)
Female	.01 (.09)	-.01 (.12)	-.04 (.09)	.10 (.10)	.02 (.09)	.07 (.10)	.04 (.09)

Table 2.7. Correlation between STAI score and eCB and eCB congener levels

	AEA	2-AG	DEA	PEA	SEA	LEA	OEA
Overall	.15 (.08)	.09 (.11)	.05 (.09)	.14 (.08)	***.19 (.09)***	.11 (.08)	.04 (.07)
AM	.16 (.13)	.12 (.22)	.07 (.13)	.01 (.13)	.24 (.12)	.16 (.12)	-.03 (.11)
PM	.19 (.10)	.11 (.13)	.18 (.11)	***.23 (.10)***	.18 (.11)	.13 (.11)	.07 (.10)
Abstinence	.16 (.09)	.15 (.14)	.06 (.11)	.13 (.10)	.14 (.11)	.09 (.11)	.01 (.08)
Use-As-Usual	.12 (.14)	-.03 (.16)	.07 (.15)	.14 (.13)	.27 (.14)	.14 (.13)	.07 (.13)
Male	.26 (.15)	***.52 (.19)***	.14 (.17)	.21 (.12)	***.61 (.13)***	-.01 (.12)	.01 (.12)
Female	.06 (.10)	-.05 (.13)*	-.03 (.11)	.11 (.11)	-.01 (.11)*	.10 (.13)	.13 (.09)

Table 2.8. Correlation between past-night sleep efficiency and eCB and eCB congener levels

	AEA	2-AG	DEA	PEA	SEA	LEA	OEA
Overall	***-.22 (.09)***	.04 (.10)	***-.20 (.09)***	-.06 (.08)	***-.28 (.09)***	.01 (.09)	-.06 (.07)
AM	***-.33 (.11)***	-.13 (.14)	-.22 (.11)	-.13 (.12)	***-.37 (.11)***	-.04 (.12)	-.11 (.11)
PM	-.08 (.10)	.06 (.11)	-.15 (.10)	-.02 (.10)	-.19 (.10)	.05 (.10)	-.04 (.09)
Abstinence	-.16 (.11)	.12 (.14)	-.16 (.12)	-.10 (.11)	-.24 (.13)	.02 (.13)	-.08 (.09)
Use-As-Usual	-.28 (.16)	-.01 (.14)	-.27 (.15)	.02 (.12)	***-.30 (.15)***	.06 (.14)	-.01 (.13)
Male	-.17 (.23)	-.16 (.26)	.09 (.25)	-.15 (.16)	-.27 (.23)	.20 (.16)	.08 (.16)
Female	***-.19 (.09)***	.08 (.11)	***-.23 (.09)***	.01 (.10)	-.18 (.10)	-.03 (.12)	-.12 (.08)

alone. When considering variation due to time of day in eCB and eCB congener tone, DEA [β=0.27 (SE=0.15)], PEA [β=0.24 (SE=0.14)], and LEA [β=0.25 (SE=0.14)] all showed moderate numeric associations with CWS total score at the PM time points, with no associations seen between values and CWS score at the AM time point.

In contrast to overall withdrawal, associations between positive affect (PA) and peripheral eCB and eCB congener levels were predominately negative, specifically for AEA [β=-0.24 (SE=0.09)], PEA [β=-0.16 (SE=0.08)], and SEA [β=-0.23 (SE=0.09)]. The association between AEA and PA persisted in both use-as-usual and abstinent weeks and showed no significant difference between male and female participants. Interestingly, the relationship of PA with SEA and 2-AG varied between male and female participants [SEA Male β=-0.52 (SE=0.16) vs. Female β=-0.06 (SE=0.11); $p<.05$; 2-AG Male β=-0.52 (SE=0.17) vs. Female β=0.11 (SE=0.13); $p<.01$] (**Figure 2.2**). Associations between

negative affect (NA) and eCB and eCB congener levels were largely insignificant. Like PA, the relationship of STAI with SEA and 2-AG varied between male and female participants, but in the opposite direction [SEA Male: β=0.61 (SE=0.13) vs. Female: β=-0.01 (SE=0.11); p=.01; 2-AG Male: β=0.52 (SE=0.19) vs. Female: β=-0.05 (SE=0.13); p<.05]. Past-night sleep efficiency was negatively associated with AEA [β=-0.22 (SE=0.09)], DEA [β=-0.20 (SE=0.09)], and SEA [β=-0.28 (SE=0.09)]. Associations with AEA and SEA were primarily driven by AM measurements [β=-0.33 (SE=0.11), β=-0.37 (SE=0.11)]. The relationships between sleep efficiency and eCB and eCB congener levels did not significantly vary between male and female participants [p>.05].

Discussion

In this study, we sought to assess 1) the impact of acute cannabis abstinence on peripheral eCB and eCB congener tone, 2) the association between eCB and eCB congener tone and cannabis withdrawal symptoms, and 3) if sex differences were present in the effects of abstinence on eCBs and eCB congeners. We found that, while abstinence did not have a significant effect on eCB or eCB congener tone overall, significant sex differences were present during abstinence for multiple NAEs (AEA, SEA, LEA) when accounting for variation due to time of day. Sex differences were driven by increased eCB and eCB congener tone during abstinence in females relative to use-as-usual. Relatedly, overall cannabis withdrawal symptoms were associated with eCB and eCB congener tone in both sexes (LEA) and then in females alone (2-AG, LEA, PEA), but not males alone. Significant sex differences in the directionality of withdrawal symptom-eCB congener associations were observed for both OEA and LEA, in which associations were positive in

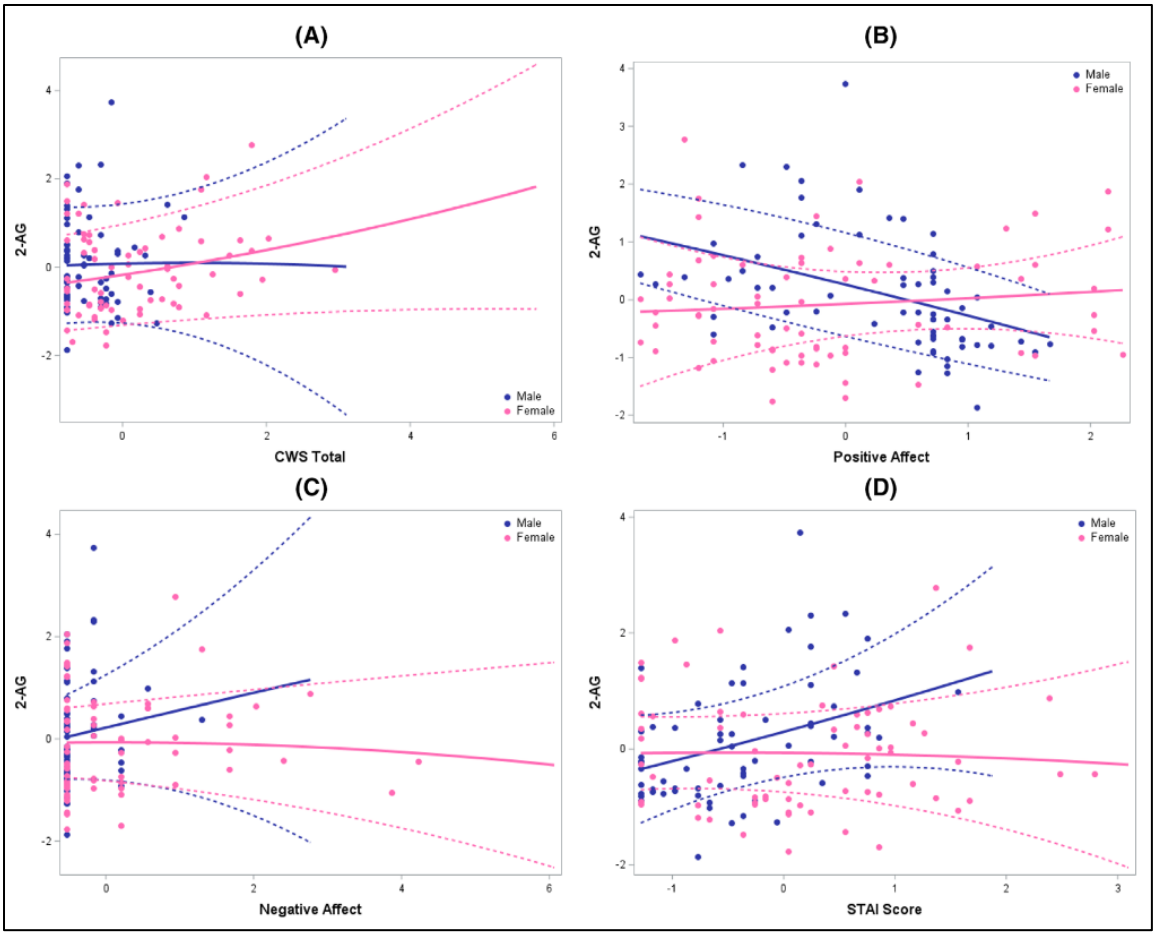

Figure 2.2. Association of 2-arachidonoylglycerol (2-AG) and subjective measures of withdrawal (CWS), affect (PANAS) and anxiety (STAI) stratified by participant sex. Scatter plot data represents individual measurements normalized (natural logarithm, 2-AG) and standardized (mean=0, Std Dev=1). Fitted regression line and 95% upper and lower confidence intervals derived from generalized linear mixed effects regression models, adjusted for use period (use as usual, abstinent) and time of measurement (0900, 1200, 1300). CWS=Cannabis Withdrawal Scale, PANAS=Positive and Negative Affect Schedule, STAI=State-Trait Anxiety Inventory.

females and negative in males. In contrast with the extant literature, [132] no interaction between sex and use period was observed for negative affect, and negative affect showed no association with peripheral eCB or eCB congener levels in either sex. Instead, positive affect was negatively associated with SEA and 2-AG in men, but not women, and state anxiety was positively associated with SEA and 2-AG in men, but not women. Self-

reported sleep efficiency was negatively associated with AEA, DEA, and SEA in both sexes. Taken together, results from this study suggest sex differences in eCB and eCB congener tone in individuals with CUD, as well as sex differences in how these eCB and eCB congener levels relate to mood and cannabis withdrawal symptoms.

The eCBs and eCB congeners in our study showing a strong positive association with cannabis withdrawal symptoms in women (LEA, PEA, 2-AG) were negatively associated with withdrawal symptoms in men. Further, though within-sex associations with CWS score were not as strong, a sex difference in the eCB congener tone-withdrawal association was also evident for OEA with comparable directionality in each sex. A negative association in men between eCB and eCB congener tone and subjective withdrawal appears consistent with the observation by D'Souza and colleagues that CB1R expression in brain (assessed via PET) was negatively associated with withdrawal symptom reporting in men with CUD during early abstinence. [73] In women, the association between changes in brain CB1R availability and withdrawal symptoms during an abstinence period has not been directly assessed. One study did find a negative association between amygdala CB1R availability and subjective ratings of anger/hostility in women with CUD during abstinence, however, other regional associations with measures of mood disturbance were not statistically significant, images were not obtained during cannabis use to assess changes in CB1R availability, and other withdrawal symptoms (e.g. gastrointestinal distress) were not assessed. [74] Notably, we did not observe increased negative affect (assessed via PANAS) in either sex during abstinence in the present study. This may be in part due to the measure used; while there is some overlap in the negative affective constructs assessed by the PANAS and typical cannabis withdrawal symptoms (e.g. hostile, irritable, nervous), some withdrawal symptoms are not

included in the PANAS (e.g. mood swings, depressed) and some constructs in the PANAS are not typical withdrawal symptoms (e.g. scared, ashamed). [14,194] It is therefore unclear if the difference in observed outcomes between ours and the aforementioned study is due to methodological limitations or due to phenotypic differences in withdrawal expression across two small cohorts of women. Future work examining how withdrawal symptom expression relates to eCB dysregulation in women is warranted. Also noteworthy is the discrepancy between our finding that AEA was negatively associated with self-reported sleep efficiency and the known role of AEA in sleep induction. [196] It is unclear if our finding is due to limitations of self-report sleep data (highlighted previously by Girschik and colleagues [197]) or a true reflection of dysregulated eCB signaling associated with sleep disturbance. Future studies examining this phenomenon should include use of validated objective sleep measures to confirm our findings.

Limitations of the present study include its sample size and reliance on participant adherence to abstinence protocols. Regarding sample size, results from this study were obtained from a small pilot project and should be interpreted as exploratory rather than confirmatory. Larger, more comprehensive studies are needed to truly ascertain the effects of cannabis abstinence on circadian rhythmicity in eCB and eCB congener tone, eCB and eCB congener response to food, and how these can relate to specific withdrawal symptoms, such as sleep disturbance or appetite loss. Additionally, while significant effects were observed for some eCBs and eCB congeners, others may have been lost due to individual variation in eCB and eCB congener tone (e.g. variation associated with race or ethnicity [198]), due to uncontrolled factors shown to influence eCB and eCB congener levels (e.g. routine exercise [199]), or due to other substance use behaviors (e.g. nicotine, alcohol use). For example, the variation observed in 2-AG levels in males in our

study was largely driven by a difference between the only male endorsing Black race and the remaining White males in the sample. While we cannot definitively attribute this difference to race, it is consistent with previously described racial differences in peripheral 2-AG [198] and highlights the importance of considering individual factors when conducting research related to the eCB system. Similarly, both eCB and eCB congener levels and subjective outcomes may have been impacted by differences in individual adherence to abstinence procedures, which were laxer than those previously described in the literature (i.e. inpatient observation). [73,128,175] Indeed, it is possible that missing saliva drug test videos in this study corresponded with instances of use, which may have impacted results. However, only one participant of those assessed did not show a reduction in urine cannabinoid levels during the abstinent week, and this participant self-reported cannabis use. Further, given that most treatment for CUD occurs in an outpatient setting, the present paradigm may have produced results that are more generalizable to real-world behavior and therefore uniquely able to inform clinical practice. Finally, outcomes were not adjusted for multiple comparisons due to the small, exploratory nature of the study. It is possible that the significant observations reported herein may not persist with statistical adjustment.

In addition to these limitations, the relationship between peripheral eCB and eCB congener tone and CNS dysfunction, as is present in CUD, remains poorly understood. [200] Recent evidence has emerged of potential crosstalk between the peripheral eCB system and the central nervous system through the use of peripherally-restricted CB1R antagonists, [201,202] however, direct associations between central and peripheral eCB levels have not been shown to-date. [200] Further work is needed to understand what, if any, effect peripheral eCB and eCB congener tone has on brain function, and if there is clinical efficacy in peripherally restricted psychiatric interventions. Finally, this study provides

limited insight into mechanistic explanations for our outcomes. Sex differences observed in this study may be attributable to interactions between chronic THC exposure, the endocannabinoidome, and sex hormones, a hypothesis preliminarily supported by the extant literature. [147,203–205] Future preclinical work exploring these interactions, particularly within the central nervous system, is warranted.

In sum, effects of cannabis abstinence on peripheral eCB and eCB congener tone can be observed in a sex- and time of day-dependent manner. Peripheral eCB and eCB congener tone is associated with the subjective experience of cannabis withdrawal, particularly in women. Though larger, more diverse studies are needed to confirm our findings, present results suggest that future pharmacological interventions for CUD that target the eCB system should be developed and assessed in a sex-conscious manner. Results may contextualize recently observed null outcomes in a large, multi-site trial examining the use of FAAH inhibitors in men and women with CUD (NCT03386487).

CHAPTER 3: Differences in cannabis withdrawal in individuals with cannabis use disorder alone and comorbid cannabis use disorder and major depressive disorder

Introduction

Cannabis use disorder (CUD) is increasingly prevalent in the United States, but there is no effective pharmacological means to treat it. [1,167] Like other substance use disorders, CUD is associated with a specific constellation of symptoms during acute cannabis abstinence, and this withdrawal syndrome has been shown to contribute to relapse. [14] While solely alleviating withdrawal has historically proven to be an ineffective treatment strategy for CUD, [169] it is still a vital component of treatment development that may have particular salience in specific subpopulations of individuals with CUD.

Nearly one-third of people with CUD also meet diagnostic criteria for major depressive disorder (MDD), more than double the prevalence of MDD in the general population. [130,136,206] Though individuals with comorbid MDD/CUD are more likely to seek treatment for CUD, [207] they are less likely to achieve abstinence relative to individuals with CUD alone. [208] A potential explanation for this discrepancy is that individuals with comorbid MDD/CUD present with a more severe cannabis withdrawal syndrome relative to individuals with CUD alone, which in turn contributes to an increased risk of relapse. Several symptoms are shared across MDD and cannabis withdrawal, including mood or sleep disturbance, appetite loss, and restlessness, [7] and presence of comorbid CUD has been associated with greater prevalence of these shared symptoms relative to MDD alone. [209] Moreover, a clinical trial assessing the use of fluoxetine in the treatment of adolescents with comorbid MDD/CUD found that withdrawal was both highly prevalent and specifically contributed to relapse in this population. [137]

Treating shared symptoms in individuals with comorbid MDD/CUD, rather than treating either syndrome individually, has historical precedent. While only three clinical trials have been conducted thus far attempting to treat specifically individuals with comorbid MDD/CUD, all have incorporated serotonergic antidepressants (e.g. SSRIs/SNRIs) and all have been unsuccessful in promoting abstinence from cannabis use, [210–212] mirroring outcomes seen in individuals with CUD alone. [213–215] Importantly, in addition to null outcomes associated with cannabis use, antidepressant trials in individuals with MDD/CUD showed no efficacy in treating MDD symptoms. This suggests that one syndrome may not be able to be effectively treated without also addressing the other. Supporting a dual treatment approach, an intensive psychosocial intervention (motivational interviewing + cognitive behavioral therapy) targeting both cannabis use and MDD symptoms was associated with improved clinical outcomes in both domains relative to a brief intervention. [216]

The endocannabinoid (eCB) system has recently emerged as relevant in both MDD and cannabis withdrawal. Briefly, the eCB system is characterized by two receptor subtypes (CB1R, CB2R), their two primary endogenous ligands (AEA, 2-AG), and the enzymes involved in the synthesis, transport, and degradation of these ligands. [22] With respect to MDD, women with MDD have been shown to have reduced peripheral eCB expression relative to healthy women, [55,57] and repeated CB1R antagonism produces pro-depressive effects. [78] With respect to cannabis withdrawal, neural CB1R expression is downregulated during heavy cannabis use and rapidly recovers during acute abstinence; CB1R expression during acute abstinence has been shown to be negatively associated with withdrawal symptom expression in men. [73] More recently, peripheral eCB and related lipid tone has also been shown to correlate with withdrawal symptom expression in both

men and women, albeit differentially by sex (Chapter 2). [217] Taken together, the eCB system may be the "missing link" that ties together MDD and cannabis withdrawal, and as such may serve as an effective pharmacotherapeutic target in individuals with comorbid MDD/CUD.

The goal of the present study was to compare real-time expression of withdrawal symptoms in individuals with comorbid MDD/CUD to individuals with CUD alone, as previous research has been limited to retrospective analyses. [137,209] Further, peripheral eCB tone and its association with withdrawal symptom expression was assessed to determine its potential as a candidate for pharmacotherapeutic intervention. We hypothesized that individuals with comorbid MDD/CUD would present with more severe withdrawal symptoms relative to individuals with CUD alone, that individuals with comorbid MDD/CUD would show greater eCB dysregulation during acute abstinence relative to CUD alone, and that peripheral eCB tone would be associated with withdrawal symptom expression in both groups, as has been demonstrated in previous work (Chapter 2). [217]

Methods

Participants

Seventeen adults (*n*=11 CUD, *n*=6 MDD/CUD) were recruited for this study. Inclusion criteria were: (1) age 18-45, to minimize incidence of age-related health conditions and age-related variation in eCB tone [183]; (2) body mass index between 18-30kg/m^2, to minimize variation in peripheral eCB content associated with obesity [184]; (3) self-reported cannabis use ≥5 days per week in the month prior to screening; (4) current moderate or severe CUD, per DSM-5 criteria [7]; (5) providing a THC-positive urine sample

at screening; and (6) access to a mobile phone capable of operating an ambulatory assessment battery (i.e. with access to internet). Depressed participants were required to either meet DSM-5 criteria for current major depressive episode at time of study enrollment or otherwise provide significant evidence of current depression per clinician judgement. Female participants were required to have a regular menstrual cycle and be willing to use non-hormonal birth control for the duration of the study. Exclusion criteria were: (1) meeting DSM-5 criteria for any current major psychiatric disorder other than MDD, tobacco, caffeine, or cannabis use disorder; (2) current use of psychotropic medications or supplements that may impact primary study outcomes, including antidepressants and hormonal birth control; (3) evidence of anemia or other blood or clotting-related disorders; (4) urinalysis results indicative of recent substance use other than cannabis; (5) current treatment-seeking for CUD or other substance use disorders; (6) blood pressure in excess of 150 (systolic) or 90 (diastolic); and (7) nursing, pregnancy, or plans to become pregnant in female participants. To reduce the risk of psychiatric adverse events associated with stopping cannabis use, individuals with severe depression (scores ≥24 on the Hamilton Depression Rating Scale; HAM-D) [218,219] or with suicidal behavior or suicidal ideation with intent in the past 6 months (assessed via the Columbia – Suicide Severity Rating Scale; C-SSRS) [220] were also excluded from study participation. Exclusion criteria related to anemia and blood pressure were incorporated to reduce likelihood of adverse events related to repeated blood draws or increased blood pressure during acute cannabis abstinence. [186] All participants provided written informed consent prior to study participation.

Procedures

General study procedures were adapted from a previously published pilot study (Chapter 2). [217] Participants were primarily recruited via posted flyers and social media advertisements. Written informed consent to participate in the study was obtained at an in-person screening visit assessing eligibility criteria. Participants that met eligibility criteria were enrolled in a 6-day study protocol. In females, this 6-day period was scheduled to begin within a week of onset of menstruation to align with the follicular phase of the menstrual cycle and control for cyclical changes in peripheral eCB content. [147] Participants were allowed to use cannabis as usual (except when in the laboratory) on Days 1 and 2 of the study, were instructed to stop use by 1830 on Day 3, and were asked to remain abstinent through the remainder of the study period. Monetary incentive was given for providing saliva drug tests negative for THC during the abstinent period; saliva drug testing procedures are described in further detail below.

Participants attended in-person laboratory visits on Days 1, 4, and 6 of the study period. These visits were always scheduled for a Friday, the following Monday, and then Wednesday, respectively, to maximize consistency in assessment timing while operating within limitations of study staff availability. For each laboratory visit, participants arrived at the Medical University of South Carolina at 0830. Participants were instructed to not eat breakfast or use cannabis the morning of each visit, but were allowed their usual amount of caffeine and/or nicotine. Upon arrival for each visit, participants completed a breathalyzer test and provided a urine sample to assess recent drug use and to ensure female participants were not pregnant. On Study Day 1, participants were each provided with an Actiwatch Spectrum to objectively measure sleep during the study period and self-reported substance use was recorded for days since the screening visit (TLFB). On Study

Days 4 and 6, participants completed a supervised saliva drug test (SalivaConfirm®; cutoffs: 12ng/mL THC-COOH, 75 ng/mL THC) before beginning other study procedures. Saliva drug tests were sensitive to cannabis use within the past 6-12 hours and participants presenting with a positive saliva drug test on Day 4 were excluded from further study participation. Participants were not allowed to use nicotine during study visits due to known interactions between nicotine and the eCB system that may have affected study outcomes. [188] As such, all participants that endorsed regular nicotine use were offered a nicotine patch for use during each visit; no participants accepted this offer.

Blood for lipidomics and cortisol analyses was drawn at 0900 and 1200 by trained nursing staff. On Day 6, an indwelling catheter for drawing blood was placed prior to the 0900 time point. Participants completed an assessment battery following each time point (described below) and were provided with food and drink of their choice from the hospital cafeteria following completion of the 0900 time point procedures. On Days 1 and 4, participants were discharged following completion of the 1200 time point. On Day 6, participants completed additional procedures (Chapter 4) before being discharged at approximately 1400. Study procedures were approved by the Institutional Review Board of the Medical University of South Carolina and were conducted in accordance with the Declaration of Helsinki.

Assessments

Participants completed a brief (approx. 5 minutes) mobile assessment battery at 0900, 1200, 1700, and 2100 on Days 1-5 of the study period, and at 0900 and 1200 on Day 6. The battery was distributed via text message as a link to a secure survey portal

(RedCap®), and participants were allotted one hour to respond to all questions. The battery comprised measures of cannabis withdrawal (Cannabis Withdrawal Scale; CWS), [191] substance use (TLFB), and, at the 0900 time point, past-night's sleep (Consensus Sleep Diary). [192] At study visits, the State-Trait Anxiety Inventory (STAI-S) [193] and Positive and Negative Affect Schedule (PANAS) [194] were also administered with each mobile assessment battery. Withdrawal and substance use questions were framed as a participant's experience since the previous scheduled assessment time (e.g. "Since 0900 this morning…"). On Days 1 and 6, participants completed the HAM-D and Snaith-Hamilton Pleasure Scale (SHAPS) [221] following mobile assessments to assess depression symptomology during cannabis use and abstinence. Adverse Childhood Experiences (ACEs) [222] were assessed at baseline, as they are associated with increased odds of developing depression or a substance use disorder independently, [222,223] and cumulative number of ACEs may be predictive of relapse to substance use following treatment. [224]

At 2100 on Day 4 and at 0900 and 2100 on Day 5, participants were prompted to submit a video recording of themselves completing a saliva drug test. Participants were allotted up to 6 hours to submit these videos following each prompt to maximize adherence. Participants were compensated for each mobile assessment battery and saliva drug test video completed at home, with bonus compensation provided for each saliva drug test that was negative for THC.

Plasma Lipidomics and Cortisol Analysis

Blood specimens (10mL) were collected using glass tubes containing sodium heparin. Samples were placed on ice immediately following collection and were

centrifuged to separate plasma within 30 minutes. Plasma cortisol was determined using enzyme-linked immunosorbent assay (ELISA). Samples for lipidomics analyses were stored in glass amber vials at -80°C and were periodically shipped on dry ice overnight to Indiana University, Bloomington for analysis. Lipidomics analyses were conducted using high-performance liquid chromatography-tandem mass spectrometry and included quantification of eCB (AEA, 2-AG) and related *N*-acylethanolamine (NAE; PEA, SEA, LEA, OEA, DEA) concentrations in plasma samples.

Statistical Analyses

Baseline demographic and clinical characteristics were summarized as means and associated standard deviations or as frequency. CUD and MDD/CUD groups were compared across baseline factors using Welch's *t*-tests or Fisher's exact tests as appropriate. Fisher's exact tests were also used to assess at-home mobile assessment and saliva drug test adherence by group.

Subjective, actigraphy, lipidomic, and cortisol outcomes were assessed across groups (CUD vs. MDD/CUD) and use condition (use vs. abstinence) and for group by use condition interactions using mixed effects models; GLMMs were used for subjective outcomes and linear mixed effects models for other outcomes due to differences in outcome residual distributions. Mixed effects models were also constructed to test associations between lipid and cortisol concentrations with concurrently measured cannabis withdrawal symptoms. For these analyses, withdrawal symptom measures and cortisol and lipid outcomes were standardized such that the association between outcomes would be equivalent to a 1 standard deviation change from the mean of the

measurements. Models in which lipids or cortisol were the primary outcome measure were adjusted for time of sample collection, as time has previously been shown to influence these outcomes. [217,225] Prior to analyses, lipid and cortisol concentrations were natural log- and log10-transformed, respectively, to approximate normality of model residuals. All outcome values reported in the text are model-based means and associated standard errors unless otherwise specified, with lipid and cortisol outcomes back-transformed following modeling. Significance was set at p=.05 (two-sided) with no correction made for multiple comparisons. Analyses were conducted in *R* version 4.3.2.

Results

Study Sample

Participant demographics and baseline clinical characteristics are reported in **Table 3.1**. Groups did not differ with respect to demographic features or self-reported substance use at baseline, but MDD/CUD participants reported significantly greater depression, anxiety, and childhood adversity (ACE). Interestingly, there were no differences between groups in baseline positive or negative affect [p's>.05]. All enrolled participants completed the 6-day protocol. Of the 16 at-home mobile assessments, CUD participants completed 89% on average, and MDD/CUD participants completed 85% on average [p=.99]. All saliva drug tests completed in the laboratory and all submitted saliva drug test videos were negative, however, 6/51 expected videos were missing (5 missing videos across 4 CUD participants, 1 MDD/CUD). One additional participant (MDD/CUD) self-reported one instance of cannabis use on Study Day 5.

Table 3.1. Participant demographics and baseline clinical characteristics

Data are presented as mean (standard deviation) or as frequency. CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder, ACEs=Adverse Childhood Experiences, PANAS=Positive and Negative Affect Schedule, STAI=State-Trait Anxiety Inventory. *Significant difference between groups, $p<.05$. [t]Data not reported for 2 participants (1 CUD, 1 MDD/CUD), as these participants primarily used cannabis oils rather than flower products.

	CUD (*n*=11)	MDD/CUD (*n*=6)
Age (Years)	26 (6.3)	31 (8.7)
Sex (Female/Male)	4/7	3/3
Race (White/Black/Mixed Race)	6/2/3	4/1/1
CUD Severity (Moderate/Severe)	4/7	0/6
Years of Cannabis Use	7.2 (5.9)	12.8 (10.0)
Substance Use (Past 90 Days)		
Cannabis Use Days	76 (17.0)	84 (5.9)
Cannabis Sessions/Day	2.2 (1.6)	2.4 (1.2)
Cannabis Grams/Day[t]	1.4 (1.6)	1.6 (1.0)
Any Nicotine Use	4	2
Any Alcohol Use	8	2
Subjective Measures		
HAM-D Total Score*	7.0 (3.9)	21.3 (2.4)
HAM-A Total Score*	6.9 (3.3)	18.5 (3.6)
ACEs*	2.7 (2.2)	5.3 (1.5)
PANAS Positive Affect	32 (8.2)	30 (8.2)
PANAS Negative Affect	12 (3.1)	14 (2.9)
STAI Total Score	28 (6.3)	38 (8.9)

Subjective Measures

Cannabis Withdrawal Scale (CWS) total score over the course of the study differed by group [β=1.01, SE=0.33, $p<.01$] and by use condition [β=0.35, SE=0.12, $p<.01$] with a significant group by use condition interaction [β=-0.40, SE=0.19, $p<.05$] (**Figure 3.1A**). Individuals with CUD alone had lower CWS scores during both use [6.8 (SE=1.4) vs. 18.8 (SE=5.0)] and abstinence [9.7 (SE=1.9) vs. 17.9 (SE=4.8)] relative to individuals with MDD/CUD. However, abstinence was associated with an increase in CWS score in individuals with CUD [$p<.01$], but not MDD/CUD [$p=.73$]. The negative impact of cannabis withdrawal symptoms on daily activities also differed by group [β=0.92, SE=0.40, $p<.05$]

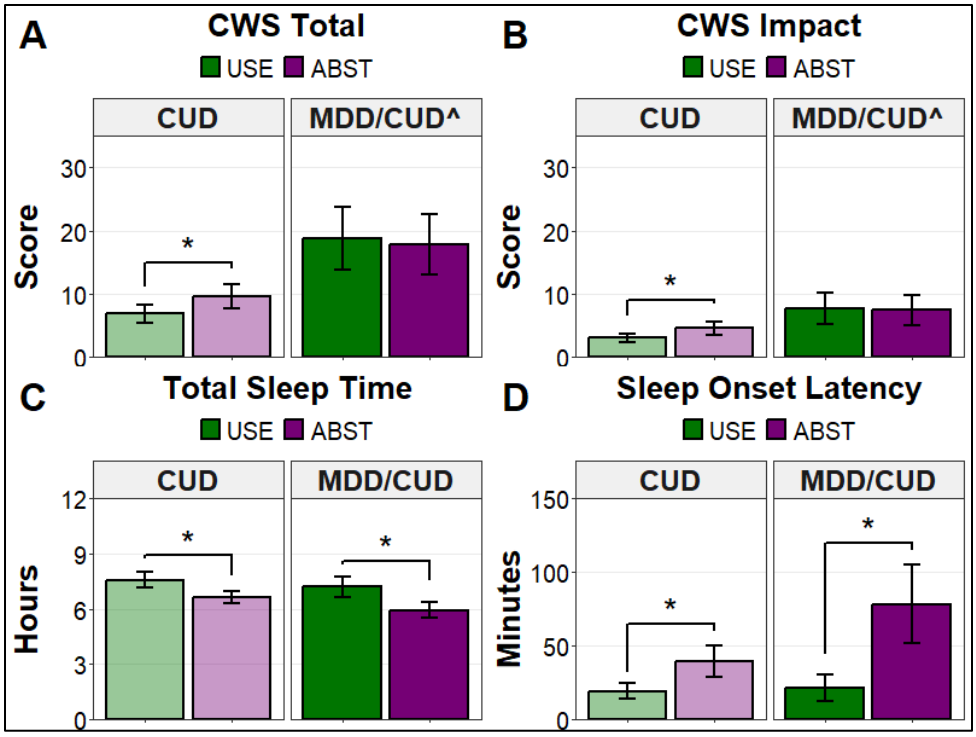

Figure 3.1. Cannabis withdrawal symptoms during cannabis use and acute abstinence in individuals with CUD alone and comorbid MDD/CUD. Individuals with CUD alone self-reported increased cannabis withdrawal symptoms and negative impact of cannabis withdrawal symptoms during abstinence relative to use, a pattern not observed in individuals with MDD/CUD (A, B). However, both groups showed a significant decrease in objectively-measured total sleep time (C) and increase in sleep onset latency (D) during abstinence relative to use, consistent with cannabis withdrawal. Data are presented as model-based means and associated standard errors collapsed across study days and time points. *indicates a significant effect of use condition, $p<.05$. ^indicates a significant difference by group, $p<.05$. CWS=Cannabis Withdrawal Scale, CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder.

and use condition [β=0.41, SE=0.17, $p<.05$], but without a significant group by use condition interaction [β=-0.44, SE=0.28, p=.11] (**Figure 3.1B)**. Relative to MDD/CUD participants, CUD participants reported lower withdrawal symptom impact during use [3.0 (SE=0.72) vs. 7.7 (SE=2.4)], but not abstinence [4.6 (SE=1.1) vs. 7.4 (SE=2.3)], and CUD participants reported an increased impact of withdrawal symptoms during abstinence vs. use [$p<.05$] that was not seen in MDD/CUD participants [p=.88].

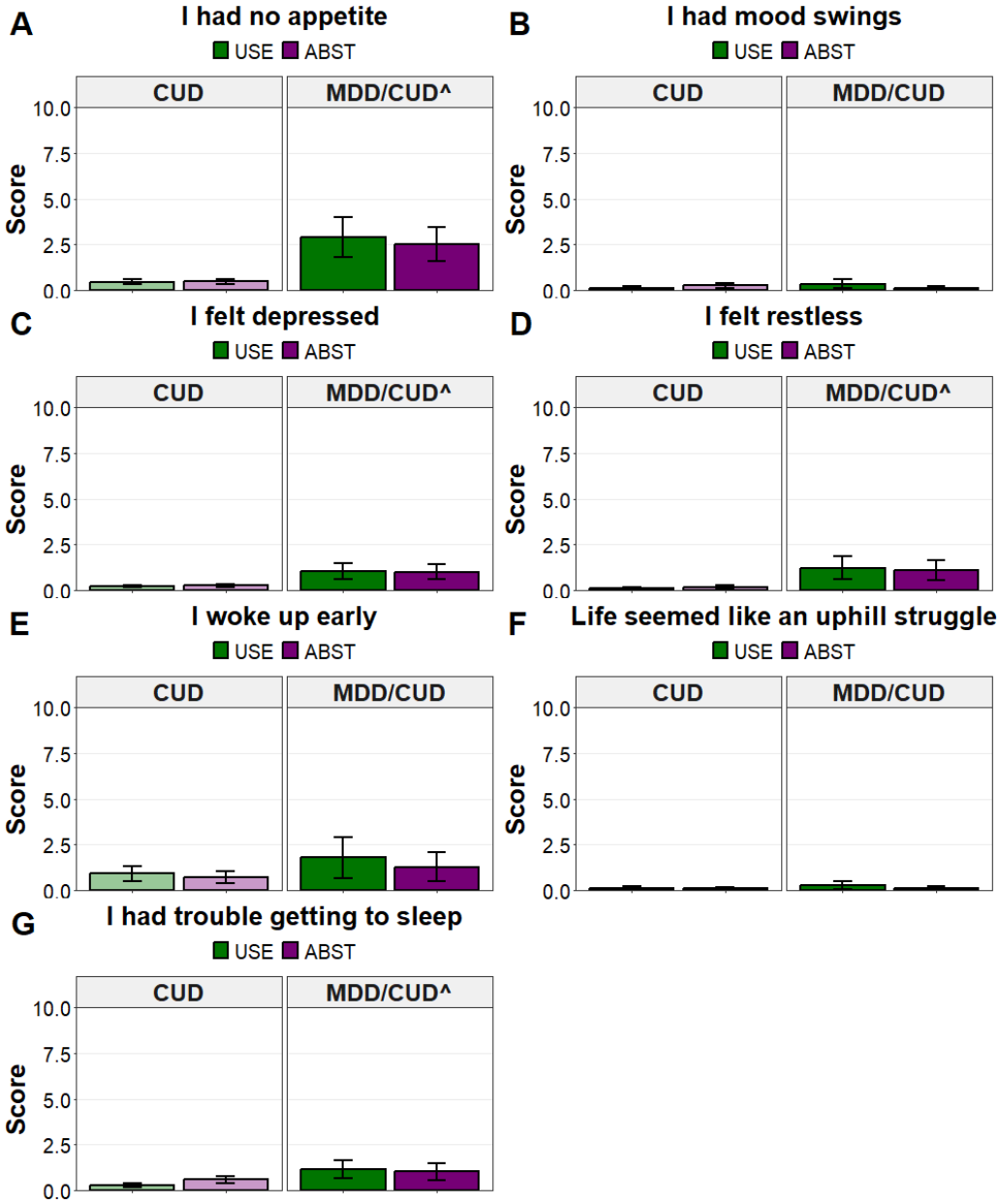

Figure 3.2. Individual cannabis withdrawal symptoms that overlap with depression symptoms across cannabis use and abstinence in individuals with CUD alone and comorbid MDD/CUD. Data are presented as model-based means and associated standard errors, collapsed across study days and time points. ^indicates a significant difference by group, $p<.05$. CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder.

In addition to total withdrawal scores, CWS items that overlap with known depression symptoms ("I had no appetite", "I had mood swings", "I felt depressed", "I felt restless", "I woke up early", "Life seemed like an uphill struggle", "I had trouble getting to sleep at night") were examined individually to determine if these symptoms might drive group differences in withdrawal expression (**Figure 3.2**). "I had no appetite", "I felt depressed", "I felt restless", and "I had trouble getting to sleep at night" all showed a significant effect of group [all *p*'s<.05], but not condition or a group by condition interaction [all *p*'s>.05]. All symptoms showing a significant group effect had greater expression in MDD/CUD participants relative to participants with CUD alone. "I had mood swings" showed a significant group by use condition interaction [β=-1.73, SE=0.76, *p*<.05], though neither group nor use condition showed a significant effect on mood swings alone [*p*'s>.05]. Mood swings marginally increased during abstinence relative to use in individuals with CUD alone [0.3 (SE=0.1) vs. 0.1 (SE=0.1)], but marginally decreased during abstinence relative to use in individuals with MDD/CUD [0.1 (SE=0.1) vs. 0.3 (SE=0.2)]. "I woke up early" and "Life seemed like an uphill struggle" showed no effect of group, use condition, or their interaction [all *p*'s>.05].

Overall depression symptoms, anhedonia, and affect were assessed using the HAM-D, SHAPS, and PANAS, respectively. As expected, overall depression symptoms differed significantly across groups [β=0.74, SE=0.24, *p*<.01] (**Figure 3.3A**), and this pattern was mirrored when examining anhedonia specifically [β=2.51, SE=1.01, *p*<.05] (**Figure 3.3B**). However, neither overall depression nor specific anhedonia showed an effect of use condition [HAM-D: *p*=.62; SHAPS: *p*=.21] or a group by use condition interaction [HAM-D: *p*=.54; SHAPS: *p*=.39]. Neither positive nor negative affect showed an effect of group, use condition, or an interaction between these factors [all *p*'s>.05]

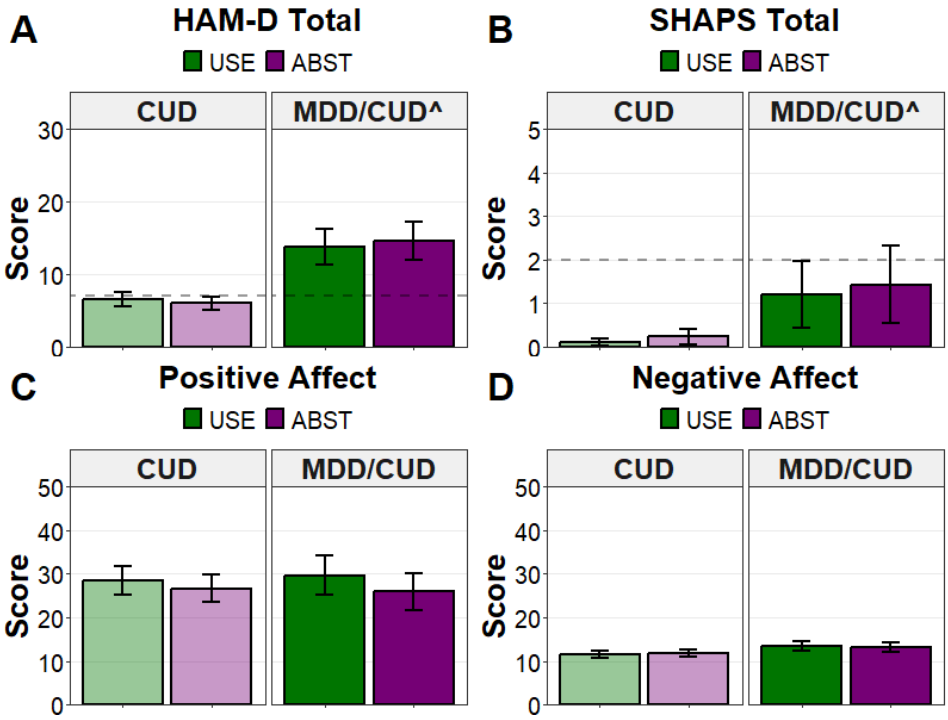

Figure 3.3. Overall depression symptoms, anhedonia, and positive and negative affect across cannabis use and abstinence in individuals with CUD alone and comorbid MDD/CUD. Data are presented as model-based means and associated standard errors collapsed across study days and time points. Dashed line in panel A represents the cutoff for "mild" clinical depression symptoms, [219] and dashed line in panel B represents cutoff for clinically significant anhedonia. [221] ^indicates a significant difference by group, $p<.05$. CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder, HAM-D=Hamilton Depression Rating Scale, SHAPS=Snaith-Hamilton Pleasure Scale.

(**Figure 3.3C-D**).

Actigraphy

Total sleep time, sleep efficiency, sleep onset latency, and wake after sleep onset were assessed via actigraphy; data were limited to n=15 participants (n=10 CUD, n=5 MDD/CUD) due to equipment issues. Sleep efficiency and wake after sleep onset were not significantly affected by group, use condition, or their interaction [all p's>.05]. Total

sleep time, however, was significantly impacted by use condition [*β*=-0.13, SE=0.06, *p*<.05] (**Figure 3.1C**), but not by group [*β*=-0.05 SE=0.10, *p*=.61] or a group by use condition interaction [*β*=-0.07, SE=0.10, *p*=.52]. Total sleep time (in hours) was reduced in abstinence relative to use both in individuals with CUD alone [6.6 (SE=0.3) vs. 7.6 (SE=0.4)] and with comorbid MDD/CUD [5.9 (SE=0.4) vs. 7.2 (SE=0.6)]. Likewise, sleep onset latency was significantly impacted by use condition [*β*=0.72, SE=0.35, *p*<.05] (**Figure 3.1D**). Both participants with CUD alone [39.5 (SE=10.4) vs. 19.3 (SE=5.5)] and MDD/CUD [78.3 (SE=26.5) vs. 21.7 (SE=8.9)] showed a marked increase in sleep onset latency during abstinence relative to use, consistent with expected effects of cannabis withdrawal. There was no significant effect of group [*β*=0.12, SE=0.49, *p*=.81] or group by use condition interaction [*β*=0.57, SE=0.60, *p*=.34] on sleep onset latency.

Lipids, Cortisol, and Their Associations with Cannabis Withdrawal Symptoms

Lipidomics data are limited to *n*=7 participants (5 CUD, 2 MDD/CUD) and cortisol data are limited to *n*=16 participants (11 CUD, 5 MDD/CUD). Consistent with previous work, [217,225] a significant effect of time was observed for AEA [*β*=-0.20, SE=0.09, *p*<.05] and cortisol [*β*=-0.14, SE=0.05, *p*<.01], in which plasma concentrations were lower at the 1200 time point relative to the 0900 time point (**Figure 3.4A, 3.4H**). No lipidomic or cortisol outcomes showed a significant effect of group, condition, or a group by condition interaction [all *p*'s>.05].

CWS total scores were tested for associations with concurrently measured plasma lipid and cortisol concentrations; model results are listed in **Table 3.2**. OEA concentration was significantly positively associated with CWS score [*β*=0.43, SE=0.19, *p*<.05], but all

Table 3.2. Associations between lipid and cortisol concentrations and concurrently measured cannabis withdrawal symptoms

Data are presented as standardized betas, associated standard errors, and *p*-values from linear mixed effects models. Values represent the proportion of a 1 standard deviation change in lipid or cortisol plasma concentration with a 1 standard deviation change in withdrawal measure. Models were adjusted for group (CUD vs. MDD/CUD), use condition (use vs. abstinence), interaction between group and use condition, and time (0900 vs. 1200). Total sleep time is measured in hours, sleep onset latency is measured in minutes. **Bolded values** indicate a significant association ($p<.05$). AEA=*N*-arachidonoylethanolamide, OEA=oleoylethanolamine, PEA=palmitoylethanolamide, SEA=stearoylethanolamide, LEA=linoleylethanolamide, DEA=docosatetraenylethanolamide, 2-AG=2-arachidonoylglycerol, CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder.

	CWS			**Total Sleep Time**			**Sleep Onset Latency**		
	β	**SE**	***p***	***β***	**SE**	***p***	***β***	**SE**	***p***
AEA	.24	.15	.11	-.27	.18	.16	-.74	.47	.15
OEA	**.43**	**.19**	**.03**	-.09	.35	.79	-.15	.91	.87
PEA	.26	.21	.22	.11	.38	.79	.30	1.01	.78
SEA	.25	.24	.32	-.02	.37	.96	-.12	.98	.91
LEA	.32	.18	.09	-.35	.26	.19	-.84	.69	.25
DEA	.16	.14	.27	-.25	.28	.38	-.57	.73	.45
2-AG	.05	.24	.85	.19	.30	.55	.32	.80	.69
Cortisol	-.06	.08	.50	.07	.13	.60	-.25	.23	.29

other lipid and cortisol associations with CWS scores were statistically insignificant [all *p*'s>.05]. Similarly, all associations between sleep measures that showed a significant abstinence effect (total sleep time, sleep onset latency) and plasma lipid and cortisol concentrations were insignificant [all *p*'s>.05].

Discussion

The present study sought to examine real-time cannabis withdrawal in individuals with comorbid MDD/CUD, to compare that to individuals with CUD alone, and to determine if withdrawal was associated with changes in the peripheral eCB system, as this may indicate relevance of the eCB system as a pharmacotherapeutic target in MDD/CUD.

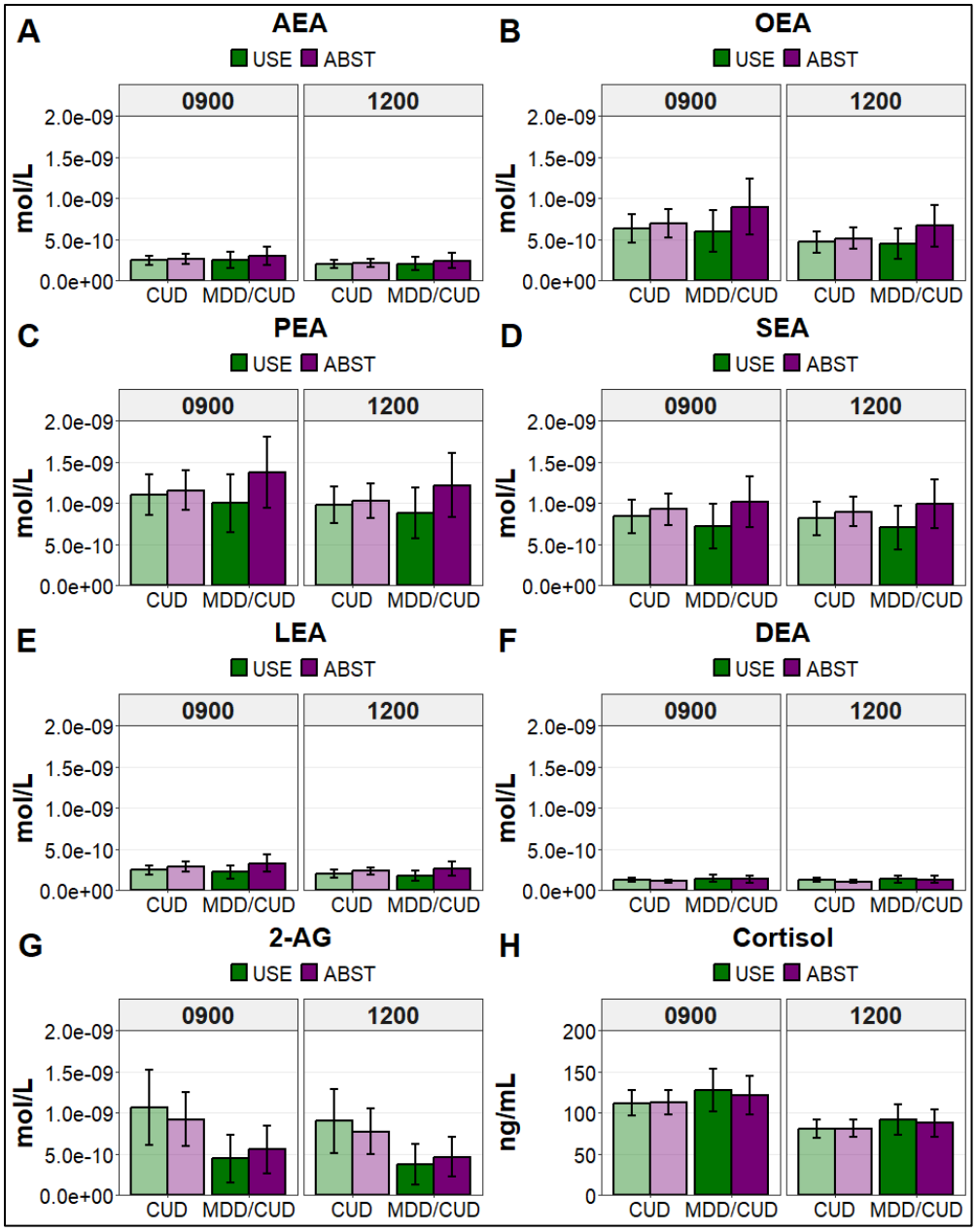

Figure 3.4. Plasma lipid and cortisol concentrations during use and abstinence in individuals with CUD alone and comorbid MDD/CUD stratified by time of sample collection. No significant effects of group or use condition were observed for any lipid or cortisol outcomes, but a significant effect of time of sample collection was seen for AEA (A) and cortisol (H). Data are presented as model-based means and associated standard errors, collapsed across study days. AEA=*N*-arachidonoylethanolamide, OEA=oleoylethanolamine, PEA=palmitoylethanolamide, SEA=stearoylethanolamide, LEA=linoleylethanolamide, DEA=docosatetraenylethanolamide, 2-AG=2-arachidonoylglycerol, CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder.

symptoms" relative to individuals with CUD alone, as hypothesized, these self-reported symptoms did not appear linked to cannabis abstinence as they were in individuals with CUD alone. In contrast, withdrawal symptoms related to sleep disturbance, measured objectively via actigraphy, showed a significant effect of abstinence without a difference by group. Finally, while underpowered, moderate, positive associations between self-reported cannabis withdrawal symptoms and peripheral eCB and related lipid concentrations were evident, in line with previous work (Chapter 2). [217] Taken together, results from this study suggest that at least some symptoms of cannabis withdrawal are comparable between individuals with comorbid MDD/CUD and CUD alone, that self-report may not be a valid measure of withdrawal symptoms in individuals with comorbid MDD/CUD, and that the eCB system may be a relevant treatment target in both individuals with comorbid MDD/CUD and CUD alone.

Based on the extant retrospective literature comparing individuals with MDD alone to those with MDD/CUD, we initially hypothesized that cannabis withdrawal symptoms and shared MDD symptoms would have an additive effect during abstinence in individuals with MDD/CUD that would not be observed during regular cannabis use. [209] Instead, participants with MDD/CUD self-reported more severe withdrawal symptoms during use and abstinence relative to individuals with CUD alone, without an effect of use condition. This self-report contrasted with objectively observed withdrawal, which showed a significant effect of abstinence without a difference by group. Rather than an additive effect of MDD on cannabis withdrawal symptoms, these outcomes may reflect inaccurate self-report as a product of impaired self-assessment in individuals with MDD. This is consistent with previous work indicating high prevalence of alexithymia in individuals with MDD, [226,227] as well as the tendency for individuals with MDD to ruminate on their own depressive

symptoms. [228] Especially because the change in withdrawal symptoms between use and abstinence in the CUD alone group was numerically modest, despite being statistically significant, individuals with comorbid MDD/CUD may have difficulty perceiving such a modest change if they are already substantially impaired at baseline. These data support the need for objective, rather than subjective, assessment of withdrawal symptoms in individuals with comorbid MDD/CUD when assessing potential treatment efficacy.

Peripheral eCB tone being positively associated with subjective withdrawal symptoms in the whole study sample was consistent with previous work (Chapter 2), [217] and supports the potential utility of pharmacotherapeutics modulating the eCB system in the treatment of CUD. However, given the aforementioned discrepancy between subjective and the objective sleep-related withdrawal symptoms assessed in this study, it is important to note that eCB tone was not significantly associated with sleep-related outcomes. This is contradictory with previous work indicating an association between eCB tone and sleep, measured both subjectively [217] and objectively. [53] It is possible that the lack of significant association observed herein is merely a reflection of an underpowered analysis, as lipidomics data were only available for 7 participants and the directionality of effect appears similar to the association seen previously with subjective outcomes. [217] Associations between other withdrawal symptoms and peripheral lipid profile may also be of interest in future work (e.g. affect, food intake), as well as an overall assessment of differences in lipidomic profile between individuals with MDD/CUD and CUD alone across cannabis use and abstinence.

This study is limited by its sample size, particularly with respect to lipidomics outcomes, and its reliance on participant adherence to study protocols. In addition to concerns regarding statistical power for primary outcomes, including inability to analyze

differences in associations between withdrawal symptoms and eCBs by group, sample size limitations make it difficult to determine if differences between the CUD and MDD/CUD groups herein (e.g. in CUD severity, alcohol use) have clinical relevance despite their statistical insignificance. Likewise, beyond controlling for hormonal variation as part of the study protocol, consideration of potential sex differences is limited due to sample size; this should be addressed in future work, as the relevance of sex in a similar paradigm has clearly been previously (Chapter 2). [217] While a larger sample was included in the initial study proposal (*n*=34), sustained difficulty with recruiting such a specific population of individuals with MDD (mild/moderate illness, not currently taking antidepressant medication, no additional comorbidities) suggest that meeting the initial recruitment goal was not feasible without a significant change in available resources. Future studies examining individuals with comorbid MDD/CUD, particularly in a highly controlled laboratory setting with stringent inclusion/exclusion criteria, must consider this limitation during study development. Regarding reliance on participant adherence to protocols, completion of at-home mobile assessments and saliva drug tests was generally good. While it is possible that missing saliva drug test videos correspond with use instances during the proposed "abstinent" period, actigraphy data indicate that both groups were in objectively-measured cannabis withdrawal, which is what the present study sought to target. Future work using a similar paradigm should analyze concurrently-collected urine THC-COOH levels to corroborate participants' self-reported abstinence.

In summary, this study found that individuals with comorbid MDD/CUD and CUD alone experience similar levels of sleep disturbance during cannabis withdrawal, and that self-reported and objectively-measured withdrawal symptoms are uncoupled in individuals with comorbid MDD/CUD. The eCB system may serve as a relevant pharmacotherapeutic

target in both individuals with MDD/CUD and CUD alone, but future research is needed to determine the effects of eCB-modulating drugs in these populations, including potential differences in therapeutic efficacy across groups.

CHAPTER 4: Differences in stress response in individuals with cannabis use disorder alone and comorbid cannabis use disorder and major depressive disorder

Introduction

Stress can precipitate relapse in cannabis use disorder (CUD), [65] but attempts to target stress response in pharmacotherapeutic development for CUD have shown limited evidence of efficacy thus far. [229] Notably, pharmacotherapeutic efforts to specifically address stress-induced relapse have not yet targeted the endocannabinoid (eCB) system, despite its well-documented role in the regulation of stress responding. [60,61] The eCB system therefore presents a prime candidate for potential drug development for the treatment of stress-induced relapse in CUD.

The eCB system is composed of two receptor subtypes (CB1R, CB2R), their endogenous ligands (AEA, 2-AG), and various enzymes implicated in the synthesis, transport, and degradation of these ligands. [22] Stress exposure is consistently associated with increased peripheral AEA in healthy people, with more divergent patterns seen in 2-AG. [55,198] This increased peripheral AEA has historically been interpreted as CB1R-dependent regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to stress, mirroring what is observed in the CNS. [47] Supporting the link between the eCB system and the HPA axis in the periphery, acute administration of THC, which has a comparable pharmacological profile to AEA, [22] is associated with increased peripheral cortisol in healthy individuals that do not use cannabis. [230]

Importantly, heavy cannabis use is associated with CB1R downregulation in the CNS, [67] and CB1R-mediated stress regulation may be impaired in individuals with reduced CB1R availability. Consistent with this hypothesis, acute THC administration produces a

blunted cortisol response in heavy cannabis users relative to non-users. [230] Drugs that increase concentrations of AEA or 2-AG via inhibition of their respective primary metabolic enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), demonstrate stress-reducing properties, but have not yet been evaluated for these effects in individuals with CUD. [231,232] As such, there is an increasing need to understand the interplay between stress and the eCB system in individuals with CUD in order to preliminarily gauge the effectiveness of these drugs in stress-induced relapse.

Major depressive disorder (MDD) is highly comorbid with CUD [206] and individuals with comorbidity present with worse addiction treatment outcomes than individuals with CUD alone. [208] Notably, MDD is associated with dysregulated stress responding, including heightened negative affective response, [233] and a blunted, but prolonged, cortisol response relative to healthy controls. [234] In comparison, heavy cannabis use is associated with both an attenuated subjective stress and cortisol response relative to non-use. [235,236] Interestingly, both MDD and CUD are associated with impairments in the eCB system which may contribute to dysregulated stress response; reduced cerebrospinal fluid AEA has been observed in heavy cannabis users relative to controls [72] and reduced serum AEA and 2-AG have also been seen in women with MDD. [55,57] As eCB tone is downregulated in both MDD and CUD, there may be additive in individuals with comorbid MDD/CUD. Individuals with comorbid MDD/CUD may therefore be at a particularly heightened risk of stress-induced relapse given the role of the eCB system in terminating stress responding.

The present study sought to assess stress responding in individuals with CUD alone and with MDD/CUD as measured by subjective, cortisol, and peripheral eCB responses to a social stressor. We hypothesized that individuals with MDD/CUD would report a heightened negative affective response to the stressor, in line with prior work, [233]

but an attenuated cortisol response relative to individuals with CUD alone. We further hypothesized that individuals with MDD/CUD would have a lower eCB, particularly AEA, response to the stressor, consistent with theorized additive impairment of the eCB system in comorbidity. Finally, we hypothesized that this additive dysregulation in stress responding in individuals with MDD/CUD would be associated with increases in subjective predictors of stress-induced relapse, such as cannabis craving or wanting, in line with worse treatment outcomes observed in this population. [208]

Methods

Study Procedures

The present study utilizes the same participant sample described in Chapter 3. Following procedures described therein (i.e. after completion of the Day 6 12:00 time point), participants completed the Trier Social Stress Test (TSST). [237] Participants had been abstinent from cannabis for approximately 3 days at time of testing. Single-item subjective measures of anxiety ("How anxious are you?"), stress ("How stressed are you?"), and desire to use cannabis ("How badly do you want to use cannabis?", "Do you feel like you need to use cannabis?", "Do you crave cannabis?", "How hard would it be to resist using cannabis if it were offered to you right now?"); systolic and diastolic blood pressure; and heart rate were collected at 12:10 (baseline) and at approximately 0, 10, 30, and 60 minutes following completion of the TSST. Responses to subjective items were given on a 0-10 scale (0=Not at all, 10=Extremely). Blood samples for plasma cortisol and lipidomic analyses were collected at the 0-, 10-, 30-, and 60-minute time points; the Day 6 12:00 sample was used as the baseline sample for plasma analyses. Study participation

concluded following completion of the 13:45 time point. All blood samples were processed, stored, and analyzed as described in Chapter 3. All participants provided written informed consent and all procedures were in accordance with the Declaration of Helsinki.

Trier Social Stress Test

The TSST is a social stressor which has been shown to consistently evoke an HPA axis response in people with CUD or MDD individually. [238,239] For this procedure, participants were given 5 minutes to prepare a 5-minute speech as to why they should be hired for their "dream job". Participants were informed that the presentation would be observed by 3 individuals, that the presentation would be recorded, and that one of the individuals was specifically instructed to observe their behavior. At the end of the 5-minute preparation period, 3 staff members unfamiliar to the participant and dressed in white lab coats entered the room. Staff members did not emote during the speech period and the participant was encouraged to continue presenting if they paused for any period of time. After 5 minutes, one of the staff members instructed the participant to perform a mental serial subtraction task; if the participant made a mistake, they were instructed to start again at the beginning. Staff members left the room after 5 minutes, at approximately 12:45. Post-TSST data collection began as soon as staff members left the room.

Statistical Analyses

Baseline sample characteristics were summarized as means and standard deviations or as frequencies and compared across groups using *t*-tests or Fisher's exact tests as appropriate. Stress response (subjective, blood pressure, heart rate, plasma lipids

and cortisol) was assessed over time by group (CUD vs. MDD/CUD) using mixed effects models. Models were constructed testing associations between subjective outcomes and lipid or cortisol concentrations while controlling for time and group effects, and associations between lipid and cortisol concentrations were also examined. Lipid and cortisol values were natural log- and log10-transformed, respectively, prior to inclusion in models and values included in the text were back-transformed from model-derived estimated marginal means. For association analyses, subjective measures and cortisol and lipid outcomes were standardized such that the association between outcomes would be equivalent to a 1 standard deviation change from the mean of the measurements. Statistical significance was indicated at $p<.05$ with no corrections made for multiple comparisons. Analyses were conducted in *R* version 4.3.2.

Results

Participants

Participant characteristics are listed in **Table 4.1**. Groups did not differ with respect to demographic or substance use characteristics, but participants with MDD/CUD reported greater depression, anxiety, and adverse childhood experiences (ACEs) compared to participants with CUD alone. All enrolled participants completed the TSST.

Subjective Measures

No effect of time, group, or their interaction was observed for “How badly do you want to use cannabis?”, “Do you crave cannabis?”, or “How hard would it be to resist using cannabis if it were offered to you right now?” [all *p*’s>.05] (**Figure 4.1**). “Do you feel like

Table 4.1. Baseline sample characteristics

Data are presented as mean (standard deviation) or as frequency. Demographic information and baseline assessments were collected at screening. CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder, ACEs=Adverse Childhood Experiences, PANAS=Positive and Negative Affect Schedule, STAI=State-Trait Anxiety Inventory. *Significant difference between groups, $p<.05$. [t]Data not reported for 2 participants (1 CUD, 1 MDD/CUD), as these participants primarily used cannabis oils rather than flower products.

	CUD (*n*=11)	MDD/CUD (*n*=6)
Age (Years)	26 (6.3)	31 (8.7)
Sex (Female/Male)	4/7	3/3
Race (White/Black/Mixed Race)	6/2/3	4/1/1
CUD Severity (Moderate/Severe)	4/7	0/6
Years of Cannabis Use	7.2 (5.9)	12.8 (10.0)
Substance Use (Past 90 Days)		
Cannabis Use Days	76 (17.0)	84 (5.9)
Cannabis Sessions/Day	2.2 (1.6)	2.4 (1.2)
Cannabis Grams/Day[t]	1.4 (1.6)	1.6 (1.0)
Any Nicotine Use	4	2
Any Alcohol Use	8	2
Subjective Measures		
HAM-D Total Score*	7.0 (3.9)	21.3 (2.4)
HAM-A Total Score*	6.9 (3.3)	18.5 (3.6)
ACEs*	2.7 (2.2)	5.3 (1.5)
PANAS Positive Affect	32 (8.2)	30 (8.2)
PANAS Negative Affect	12 (3.1)	14 (2.9)
STAI Total Score	28 (6.3)	38 (8.9)

you need to use marijuana?" was increased significantly from baseline at the 0-minute [β=1.10, SE=0.41, $p<.01$] and 10-minute [β=1.00, SE=0.42, $p<.05$] time points following the TSST. Despite the absence of a significant group by time interaction, this time effect was driven by participants with CUD alone, who showed significant increases in response to this measure [0-minute $p<.01$, 10-minute $p<.05$], an effect not seen in participants with MDD/CUD [0-minute $p=.49$, 10-minute $p=.86$]. Significant time effects were also observed in response to "How anxious are you?" and "How stressed are you?" (**Figure 4.2A-B**), specifically at the 0- [Anxious: β=0.81, SE=0.25, $p<.01$; Stressed: β=0.98, SE=0.26, $p<.01$] and 10-minute [Anxious: β=0.62, SE=0.26, $p<.05$; Stressed: β=0.75, SE=0.27, $p<.01$] time

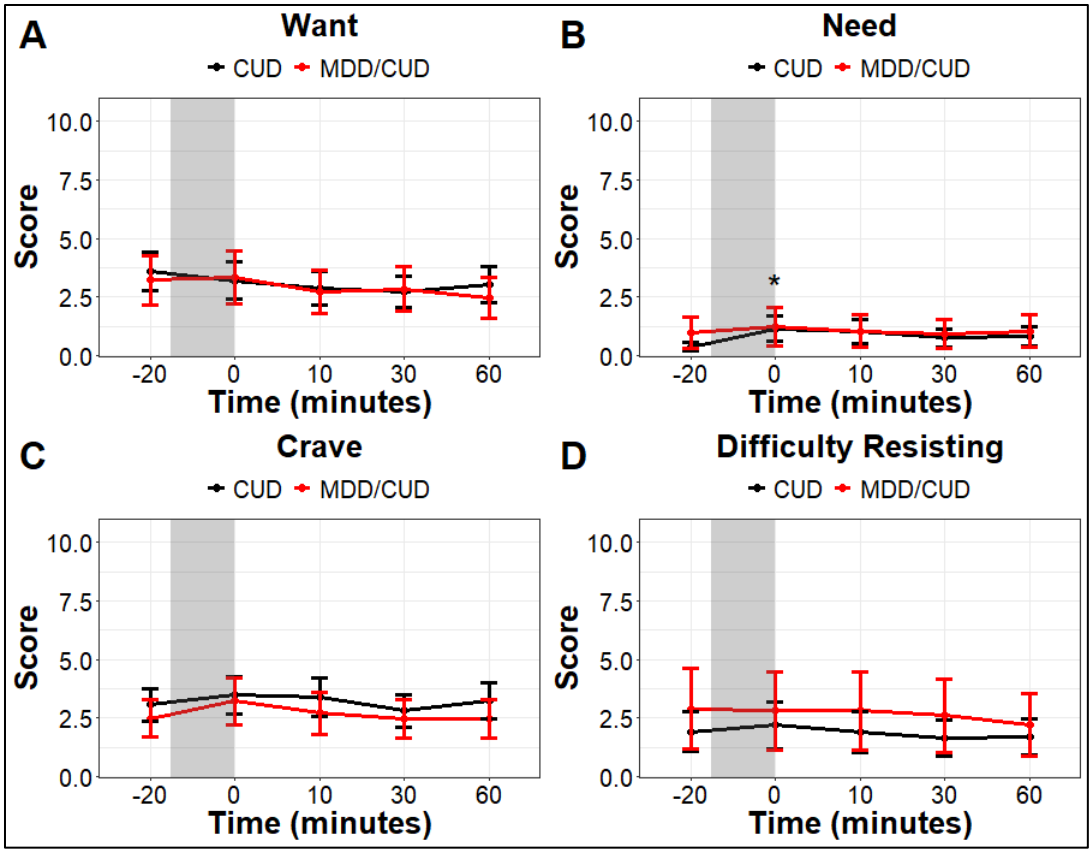

Figure 4.1. Subjective desire to use cannabis following stress exposure in individuals with CUD alone and comorbid MDD/CUD. A limited effect of stress on desire to use was observed for most outcomes. Data are presented as model-based means and associated standard errors. Only "Need" to use cannabis showed a significant increase from baseline following stress exposure, and this was driven by individuals with CUD alone (B). The shaded region denotes the period during which participants were exposed to the stressor. Black * indicates a significant increase from baseline in individuals with CUD alone, $p<.05$.

points. These significant effects of time on stress and anxiety were evident when examining just individuals with CUD or just individuals with MDD/CUD, except the 10-minute time point for anxiety in participants with MDD/CUD which lost statistical significance [$p=.19$]. Interestingly, stress scores also remained significantly elevated from baseline at the 30-minute time point in participants with MDD/CUD [$p<.05$], but not CUD alone [$p=.20$].

Blood Pressure and Heart Rate

Systolic blood pressure was increased significantly at the 0-minute time point relative to baseline [β=14.6, SE=4.76, p<.01], and this was driven by participants with CUD alone [p<.01; MDD/CUD: p=.70] (**Figure 4.2C**). No effect of group or group by time interactions were seen for systolic blood pressure [all p's>.05]. This pattern was mirrored by diastolic blood pressure [0-minute time point effect in CUD alone: β=8.09, SE=2.45, p<.01] (**Figure 4.2D**). Conversely, heart rate showed a significant effect of group [β=-10.7, SE=4.83, p<.05], but not time or any group by time interactions [all p's>.05]. Participants with MDD/CUD had numerically lower heart rate than participants with CUD alone at all time points, including at baseline (**Figure 4.2E**).

Plasma Cortisol and Lipids

Cortisol was significantly elevated at the 0-minute [β=0.17, SE=0.04, p<.01], 10-minute [β=0.21, SE=0.04, p<.01], and 30-minute [β=0.13, SE=0.04, p<.01] time points relative to baseline when assessing both groups combined (**Figure 4.3A**). While there was no significant effect of group [β=14.6, SE=4.76, p<.01], group by time interactions were evident at all time points following stress exposure [all p's<.01]. The increase in cortisol was driven by participants with CUD alone, as cortisol was highest at baseline in individuals with MDD/CUD, and in fact dipped significantly below baseline 60-minutes post-stress [p<.01].

AEA, PEA, OEA, LEA, and DEA showed no significant effect of group, time, or their interactions when assessing the whole sample or just participants with CUD or just participants with MDD/CUD [all p's>.05]. 2-AG showed no effect of group or time, but a

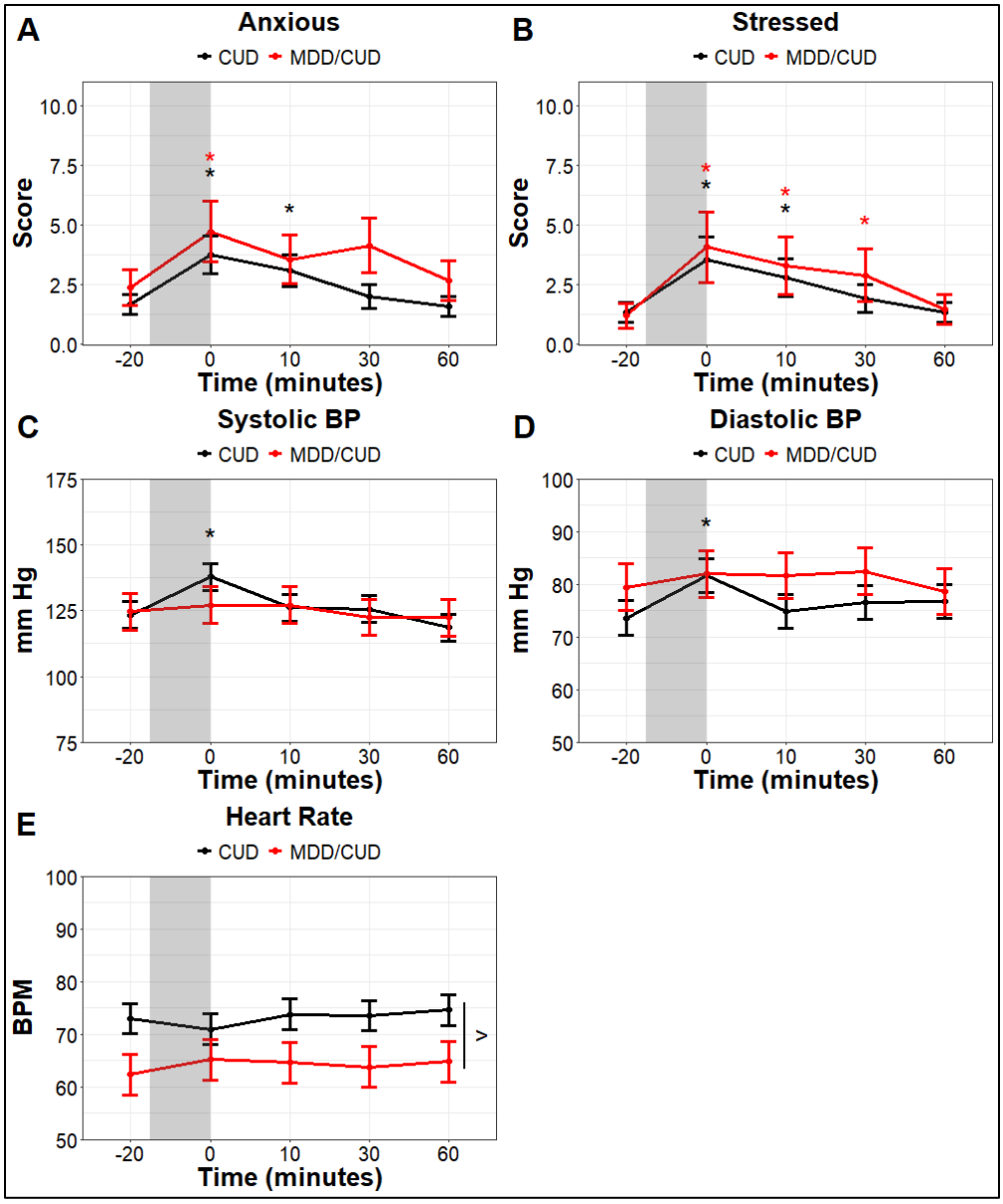

Figure 4.2. Subjective and cardiovascular responses to stress in individuals with CUD alone and comorbid MDD/CUD. All participants self-reported increased stress and anxiety immediately following social stress exposure (A,B). Increased systolic and diastolic blood pressure following stress exposure was observed in participants with CUD alone, but not MDD/CUD (C,D). Heart rate was significantly lower in participants with MDD/CUD relative to participants with CUD alone at all time points (E). Data are presented as model-based means and associated standard errors. The shaded region denotes the period during which participants were exposed to the stressor. Statistical significance was indicated at $p<.05$; black * indicates a significant change from baseline in individuals with CUD alone, red * indicates a significant change from baseline in individuals with MDD/CUD, ^ indicates a significant main effect of group.

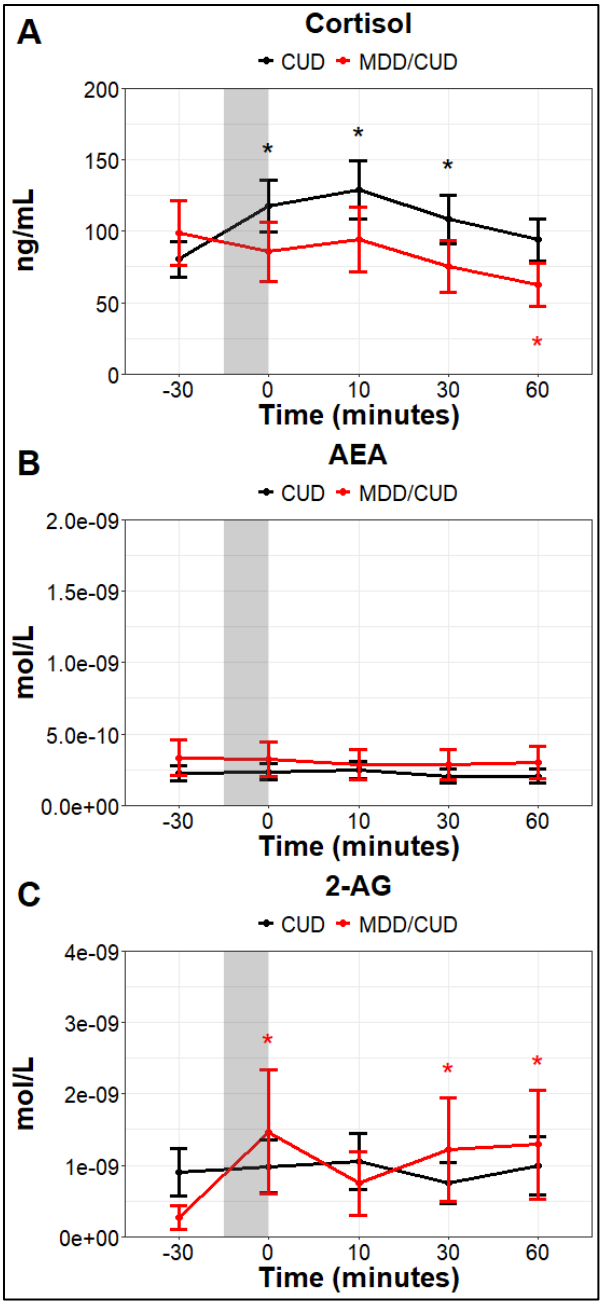

Figure 4.3. Plasma cortisol and endocannabinoid responses to stress in individuals with CUD alone and comorbid MDD/CUD. Cortisol increased in response to the stressor in participants with CUD alone, but not MDD/CUD (A). AEA showed no effect of stress in either group (B). 2-AG was elevated in response to the stressor in individuals with MDD/CUD, but not CUD alone (C). Data are presented as model-based means and associated standard errors. The shaded region denotes the period during which participants were exposed to the stressor. Statistical significance was indicated at $p<.05$; black * indicates a significant change from baseline in individuals with CUD alone, red * indicates a significant change from baseline in individuals with MDD/CUD.

Table 4.2. Associations between lipid and cortisol concentrations and concurrently measured subjective outcomes

Data are presented as standardized betas and associated standard errors from linear mixed effects models. Values represent the proportion of a 1 standard deviation change in lipid or cortisol plasma concentration with a 1 standard deviation change in subjective measure score or cortisol concentration. Models were adjusted for group (CUD vs. MDD/CUD), time, and an interaction between group and time. **Bolded values** indicate a significant association (p<.05). AEA=*N*-arachidonoylethanolamide, OEA=oleoylethanolamine, PEA=palmitoylethanolamide, SEA=stearoylethanolamide, LEA=linoleylethanolamide, DEA=docosatetraenylethanolamide, 2-AG=2-arachidonoylglycerol, CUD=Cannabis Use Disorder, MDD=Major Depressive Disorder.

	AEA	**OEA**	**PEA**	**SEA**
Want	**-.48 (.19)**	**-.54 (.19)**	**-.48 (.21)**	-.39 (.22)
Need	-.36 (.22)	-.38 (.20)	-.45 (.19)	-.31 (.19)
Crave	-.30 (.18)	-.34 (.17)	-.35 (.19)	-.37 (.19)
Hard to Resist	-.42 (.22)	-.33 (.23)	-.31 (.25)	-.18 (.23)
Anxious	-.17 (.15)	-.29 (.14)	-.38 (.18)*	-.36 (.20)
Stressed	-.12 (.21)	-.24 (.20)	-.30 (.24)	-.31 (.25)
Cortisol	.09 (.24)	.17 (.24)	.32 (.27)	.11 (.27)
	LEA	**DEA**	**2-AG**	**Cortisol**
Want	-.31 (.18)	-.29 (.21)	-.09 (.23)	.09 (.11)
Need	-.31 (.19)	-.20 (.24)	.22 (.23)	.22 (.11)
Crave	-.22 (.15)	-.18 (.20)	.20 (.22)	**.26 (.10)**
Hard to Resist	-.10 (.22)	-.42 (.23)	-.03 (.26)	**.32 (.13)**
Anxious	-.18 (.11)	-.26 (.18)	-.15 (.20)	.08 (.08)
Stressed	-.22 (.16)	-.45 (.23)	-.02 (.09)	.00 (.10)
Cortisol	.07 (.20)	.07 (.28)	.44 (.29)	--

significant interaction was present at the 30-minute time point [p<.05]. Further, in participants with MDD/CUD, but not CUD alone, 2-AG was elevated relative to baseline at the 0-minute, 30-minute, and 60-minute time points [all p's<.05]. SEA showed a significant effect of time, but not group or their interactions, at the 10-minute [β=-0.85, SE=0.36, p<.05], 30-minute [β=-0.80, SE=0.36, p<.05], and 60-minute [β=-0.85, SE=0.36, p<.05] time points, and this was driven by participants with CUD alone. SEA was significantly decreased at these time points relative to baseline in individuals with only CUD.

Associations Between Lipids, Cortisol, and Subjective Outcomes

Associations between lipid and cortisol concentrations and subjective outcomes are listed in **Table 4.2**. Significant negative associations were observed between cannabis wanting and levels of AEA, OEA, and PEA [all p's<.05]. PEA was also negatively associated with self-reported anxiety [p<.05]. Conversely, cortisol levels were positively associated with craving [p<.01] and difficulty resisting cannabis if it were offered [p<.05].

Discussion

The goal of this study was to examine stress responding in individuals with comorbid MDD/CUD and to compare that responding to individuals with CUD alone. We hypothesized that individuals with comorbid MDD/CUD would present with a highly dysregulated stress response, and that this would correlate with increased subjective predictors of stress-induced relapse (i.e. desire to use cannabis after stress exposure). A robust stress response was observed in participants with CUD alone, evidenced by increases in subjective stress and anxiety, blood pressure, plasma cortisol, and perceived need to use cannabis. However, of these, only subjective measures of stress and anxiety increased in individuals with MDD/CUD. Instead, participants with MDD/CUD showed a significant 2-AG response to the stressor that was not seen in individuals with CUD alone. Finally, when examining both groups together, peripheral AEA, OEA, and PEA tone was negatively associated desire to use cannabis, and this relationship was statistically significant for cannabis wanting. PEA was also negatively associated with subjective anxiety.

Consistent with our initial hypotheses and with previous work, [233–236] MDD/CUD was associated with a more blunted cortisol response to stress compared to CUD alone. Further, while initial negative affective response to stress was similar between groups, participants with MDD/CUD showed a prolonged negative affective response relative to participants with CUD alone. It is possible that the blunted cortisol response in individuals with MDD/CUD in part explains this prolonged negative affective response: reduced cortisol may lead to diminished negative feedback within the HPA axis and a subsequently prolonged stress response. While the exact mechanisms implicated in our observed group differences warrant further exploration, these findings support the need to develop interventions for stress-induced relapse in individuals with comorbid MDD/CUD, as they may be at higher risk relative to individuals with CUD alone.

Interestingly, participants with MDD/CUD exhibited impairment in the sympathetic nervous system in addition to the HPA axis, here measured by heart rate and blood pressure. Compared to participants with CUD alone, participants with MDD/CUD presented with an overall lower heart rate and showed no cardiovascular response to the TSST. This contrasts previous work in individuals without CUD, in which MDD is associated with a higher resting heart rate [240,241] and no difference in cardiovascular stress response compared to healthy controls. [242] Moreover, previous work in individuals with CUD alone showed no difference from healthy controls in cardiovascular stress response. [243] Taken together, it is unclear from our current data why our groups would differ with respect to sympathetic nervous system response to stress. Preclinical data suggests a role for eCB signaling in the periaqueductal gray (PAG) in the modulation of heart rate and blood pressure. [244,245] Similarly, chronic stress-induced depression-like behavior in animals is mediated by reduced glutamatergic transmission in the PAG, [246,247] though it is

not currently known if this plasticity is governed by the eCB system. Future preclinical research should explore eCB tone within the PAG for its potential role in linking depression and cardiovascular tone.

With respect to the eCBs measured in our study, observed outcomes differed substantially from our initial hypotheses in some respects, but not others. We observed no effect of stress on peripheral AEA, inconsistent with previous work in healthy individuals and those with MDD. [55,198] This may be because AEA is attenuated in both MDD and CUD, [55,72] reducing availability for appropriate stress responding. We also observed a stress-induced increase in 2-AG in individuals with MDD/CUD, but not CUD. In the CNS, 2-AG release stimulated by glucocorticoid receptor activation contributes to appropriate termination of the stress response. [61] These mechanisms appear more disassociated in the periphery, with a lack of stress-induced increase in 2-AG in participants with CUD alone being consistent with what is observed in healthy volunteers in some, [198] but not other, [55] work. However, stress has previously been associated with increased peripheral 2-AG in women with MDD. [55] Of potential relevance, preclinical experiments involving peripherally-restricted CB1R antagonists suggest peripheral eCB regulation of adrenergic and noradrenergic signaling related to stress, [202,248] and peripheral norepinephrine has been shown to be increased at least in certain subtypes of MDD. [249] Increased eCB signaling in response to stress may therefore be compensatory for increased noradrenergic signaling in individuals with MDD/CUD. Finally, we found that, though AEA, OEA, and PEA were not directly affected by stress, they were negatively associated with cannabis wanting, and PEA was negatively associated with anxiety. Increasing systemic concentrations of these lipids, e.g. via inhibition of their shared primary metabolic enzyme,

fatty acid amide hydrolase (FAAH), may have utility in the treatment of CUD in individuals with or without comorbid MDD outside of a stress context.

Limitations of this study include a small sample size, particularly with respect to lipidomic outcomes, and only moderate translation to a clinical setting. With respect to sample size, it is possible that study outcomes with large standard errors, such as those related to 2-AG, may change with a larger sample. A limited sample also means present analyses did not examine the likely significant [229] role of sex differences in outcomes—this should be explored in future work. With respect to translation, the present study only considered subjective cannabis desire as a proxy for potential for stress-induced relapse, participants in this study were not seeking treatment for CUD, and the abstinence period was relatively short (3 days). It is possible that a longer abstinence period might evoke a greater desire to use following stress exposure, or that recruiting a different population of individuals with CUD or using a laboratory model of simulated relapse (such as that used by Haney and colleagues [128]) might produce outcomes more aligned with those observed in clinical settings. Future studies evaluating stress-induced relapse should consider these variations in study design.

To conclude, we found that stress responding was highly dysregulated in individuals with MDD/CUD relative to individuals with CUD alone, and 2-AG was differentially affected by stress across groups. Peripheral AEA, OEA, and PEA appeared to be linked with desire to use cannabis unrelated to stress. Taken together, eCB-modulating treatment for stress-induced relapse in CUD likely differs between individuals with or without comorbid MDD. Pharmacotherapeutics that increase AEA and related compounds, such as FAAH inhibitors, may have general application in the treatment of

CUD with or without comorbidity, but could have limited utility in a stress context in this population.

CHAPTER 5: Conclusions and Future Directions

Conclusions

Cannabis use is increasing in the United States, and nearly one-third of individuals with past-year cannabis use meet DSM-5 criteria for CUD. [1] Yet, there is still no FDA-approved pharmacotherapeutic intervention for CUD. [11] Drugs that target the eCB system show preliminary evidence of utility in CUD, particularly in alleviating cannabis withdrawal symptoms. [169] The eCB system also has a demonstrable role in healthy stress responding, [60,61] and eCB-modulating drugs show stress-reducing properties. [231,232] As both stress and withdrawal are common precipitants of relapse in CUD, there is clear potential for eCB-modulating drugs in CUD treatment. However, clinical study samples for eCB-modulating drugs have historically not been representative of the overall population of individuals with CUD, including limited representation of females and exclusion of those with comorbid psychiatric illnesses. [169] It is therefore important to understand how the eCB system may differ in these subpopulations and how individual differences in the eCB system may relate to predictors of relapse, such as withdrawal symptoms and stress response, before further clinical trials for CUD are initiated involving the use of these drugs.

In Chapter 2, males and females with CUD were compared over two weeks in the dimensions of cannabis use, cannabis withdrawal symptoms, and peripheral eCB tone. During the first week, participants were allowed to use cannabis as desired, and during the second week, participants were asked to abstain from cannabis use. Females in this study reported significantly greater cannabis withdrawal relative to males during the abstinent week, despite comparable rates of cannabis use during the use week. Moreover, eCB response to cannabis abstinence differed across sexes: females showed increased

concentrations of AEA and related congeners (SEA, LEA) during abstinence relative to use, and this pattern was not observed in males. Self-reported cannabis withdrawal was also positively associated with 2-AG and PEA in females, but not males, and LEA was associated with cannabis withdrawal in the whole sample.

Results from Chapter 2 are consistent with prior work showing a more severe withdrawal symptom phenotype in females with CUD relative to males. [132] However, the findings that eCBs are differentially expressed by sex and, in turn, differentially associated with withdrawal are novel. Importantly, a clinical trial has recently concluded evaluating the effects of a FAAH inhibitor in the treatment of CUD (NCT03386487), with the final study sample including 37% female participants. The trial was formulated following demonstration of reduced cannabis use and attenuated cannabis withdrawal in men with CUD following repeated treatment with a FAAH inhibitor, [175] the working theory being that increased AEA via FAAH inhibition might compensate for reduced eCB signaling associated with heavy cannabis use. [73] However, results from Chapter 2 bring into question the relevance of increased AEA and related congeners in females with CUD. Results for NCT03386487 currently posted to clinicaltrials.gov support this notion, as impressive abstinence-promoting effects of the FAAH inhibitor observed in an all-male sample were lost when the study sample included a significant proportion of females. Sex differences in treatment outcome for this trial have not yet been explicitly examined, however.

Cannabinoids that act on the CB1R, like THC, demonstrate biphasic properties across multiple domains. For example, in the context of anxiety, low doses of a CB1R agonist are anxiolytic, but high doses are anxiogenic. [250] AEA, like THC, is a CB1R agonist. [39,40] In Chapter 2, females demonstrated an increase in peripheral AEA content during

acute withdrawal, rather than a decrease that could theoretically benefit from pharmacological supplementation. It is therefore possible that further augmenting AEA tone in women during acute withdrawal might worsen cannabis withdrawal symptoms, rather than ameliorate them, due to the biphasic properties of CB1R agonists. Future studies administering eCB-modulating drugs to females with CUD must consider fundamental sex differences in eCB expression, and how these can relate to treatment efficacy and risk for adverse effects.

In Chapter 3, individuals with comorbid MDD/CUD were compared to individuals with CUD alone across subjective and objective measures of withdrawal and peripheral eCB tone. Prior work has shown that MDD/CUD is associated with increased self-reporting of symptoms shared across MDD and cannabis withdrawal relative to MDD alone. [209] As such, we hypothesized that individuals with MDD/CUD would experience greater withdrawal relative to individuals with CUD alone. We also hypothesized that withdrawal would be associated with eCB tone, as it was in Chapter 2, and thus individuals with comorbid MDD/CUD would present with a more dramatic change in eCB expression during abstinence relative to use compared to individuals with CUD alone.

While participants in this study with MDD/CUD did self-report more severe withdrawal symptoms relative to participants with CUD alone and this difference was largely driven by symptoms shared across MDD and cannabis withdrawal, self-reported withdrawal symptoms in individuals with MDD/CUD seemed largely disassociated from objectively measured withdrawal symptoms and eCB tone. There was also no difference between self-reported withdrawal symptoms in individuals with MDD/CUD across the cannabis use and abstinence conditions, suggesting self-reported outcomes in individuals with MDD/CUD may not reflect withdrawal at all. Difficulty with self-assessment of

emotions, called alexithymia, is prevalent in individuals with MDD. [226,227] We suggest that the inability to perceive differences in withdrawal symptoms between use and abstinence in individuals with comorbid MDD/CUD may in part be due to alexithymia or related pathology. Further, given the high level of baseline symptoms reported by individuals with MDD/CUD, self-reported outcomes may be impacted by a ceiling effect. This is especially likely when considering the only modest increase in withdrawal symptoms reported by individuals with CUD alone; it is possible that such a modest increase is imperceptible with such high baseline symptom expression. Regardless, self-report may not be a viable method of assessing withdrawal symptoms in individuals with comorbid MDD/CUD.

Consistent with Chapter 2, peripheral eCB concentrations reported in Chapter 3 were positively associated with withdrawal symptoms when both CUD and MDD/CUD groups were combined, and this association was statistically significant for OEA. However, eCB measurements were limited to only a small subset of study participants (*n*=7; 5 CUD, 2 MDD/CUD). It is therefore unclear whether these associations would hold when analyzing a larger study sample, or if associations would differ when examining groups individually, as they did between males and females in Chapter 2. Given the group differences observed in eCB responses to stress reported in Chapter 4, it seems possible that differences in eCB tone as well as differential associations between eCBs and withdrawal symptoms would emerge across groups. As in Chapter 2, such an observation would suggest a need for distinct treatment regimens across subpopulations when using eCB-modulating drugs for CUD.

Chapter 4 compared stress responding between individuals with comorbid MDD/CUD and CUD alone. Stress response was assessed using subjective single-item measures, plasma cortisol, and peripheral eCB content. We hypothesized that individuals

with MDD/CUD would present with a more dysregulated stress response relative to individuals with CUD alone, evidenced by heightened negative affect, reduced circulating cortisol, and reduced eCB content following exposure to the stressor. We also hypothesized that this highly dysregulated stress responding would be associated with predictors of stress-induced relapse, such as cannabis craving or perceived need to use cannabis.

As anticipated, we did observe a blunted cortisol response and heightened negative affective response to stress in individuals with MDD/CUD relative to individuals with CUD alone. This is consistent with prior work comparing healthy people with those with MDD. [233,234] Unexpectedly, we also saw impaired sympathetic nervous system responding to the stressor in individuals with MDD/CUD compared to individuals with CUD alone. Heart rate was significantly depressed in MDD/CUD and no change in blood pressure was seen in response to the stressor, in contrast to what has been reported previously in studies of healthy people and people with MDD alone [240–242] and in contrast to what we saw in people with CUD alone. While eCB regulation of the cardiovascular system is well-documented (reviewed by Maccarrone et al. [251]), it is not immediately clear why the above pattern in cardiovascular outcomes would emerge; in fact, one would hypothesize that comorbid MDD would be associated with increased heart rate and blood pressure response to a stressor given the well-documented link between depression and cardiovascular disease. [252] We suggest preclinical mechanistic examination of the PAG as a jumping-off point for exploring this relationship, as the PAG is implicated in eCB-regulated modulation of heart rate and blood pressure, [244,245] as well as chronic stress-induced depression-like behavior. [246,247]

Regarding eCBs, stress was associated with an increase in peripheral 2-AG in individuals with MDD/CUD, but not CUD alone, and no effect was seen on AEA in either group. We theorize that the lack of stress effect on AEA is due to significant dampening of AEA by both MDD [55] and CUD, [72] limiting availability for stress responding. Stress-induced increases in 2-AG in participants with MDD/CUD are consistent with what has been observed in individuals with MDD alone. [55] However, this same study also showed a stress-induced increase in 2-AG in healthy volunteers, drawing into question why participants with CUD alone did not show this increase in our study. While bearing in mind that the samples used herein are extremely small (*n*=7; 5 CUD, 2 MDD/CUD), we hypothesize that stress-induced increases in 2-AG in individuals with MDD/CUD may reflect an increased need for peripheral eCB-governed noradrenergic regulation in this group. [249] Studies utilizing peripherally-restricted CB1R antagonists (e.g. TM38837 [253]) in human laboratory stress paradigms might further interrogate this relationship. Finally, while no association was seen between stress exposure and AEA, OEA, or PEA, nor did stress appear to increase subjective measures of cannabis desire in either group, AEA, OEA, and PEA were significantly negatively associated with cannabis wanting in the whole sample, and PEA was negatively associated with anxiety. These findings suggest that FAAH inhibition, which would functionally increase concentrations of these compounds, may have some utility in attenuating cannabis wanting in individuals with CUD with our without comorbid MDD, but likely does not have a role in curbing stress-induced relapse in these populations. Moreover, effects of FAAH inhibitors may differ by sex, as suggested by results reported in Chapter 2.

Taken together, these studies show distinct patterns of eCB tone in different subpopulations of individuals with CUD (males vs. females, individuals with vs. without

comorbid MDD) and across different predictors of relapse (withdrawal, stress). Given these prevailing differences, it appears unlikely that individuals with CUD can be treated as a monolith using eCB-modulating drugs. It is also apparent that different drugs may be necessary for different use cases; for example, FAAH inhibitors may generally reduce cannabis wanting, but may not alleviate stress in individuals with CUD. Further mechanistic evaluation of the eCB system in these subpopulations, including direct assessment of the effects of eCB-modulating drugs in these groups under controlled conditions, is warranted before clinical trials incorporating eCB-based therapeutics can be recommended.

Limitations

All studies included herein are limited by their sample size (Chapter 2 *n*=10, Chapters 3 and 4 *n*=17). As such, these studies should all be considered as pilot studies evaluating potential differences across groups examined, rather than definitive assessments of differences between these groups. Small sample sizes not only affect the power of final analyses, but limit exploration into additional factors that may modulate outcomes. This is particularly significant in the context of the eCB system, as eCB tone can be impacted by many external factors (time of day, recent eating, lifestyle, substance use, medications, menstrual cycle phase, race/ethnicity, etc.). While several of these factors were accounted for in study designs (time of day, eating, medications, menstrual cycle phase), others, such as physical fitness, fat distribution, alcohol use, or general diet, were not. Future studies could address this limitation through either the enrollment of more study participants and subsequent statistical correction for factors known to influence eCB

tone, or through more stringent study design (i.e. inpatient observation, matching participants across groups on relevant factors). Future analyses should also include statistical correction for multiple comparisons.

Next, while the intention of these studies was to be more inclusive of groups historically underrepresented in clinical trials, many people were still excluded to limit the influence of the above factors on primary outcomes. Women taking hormonal birth control, individuals with comorbid MDD/CUD taking antidepressants, or people with other comorbid psychiatric conditions (particularly anxiety) constitute a large fraction of their respective populations, but were not included here. As interest in personalized medicine continues to increase, [254] studies specifically evaluating the effects of, for example, hormonal birth control on the eCB system in females with CUD are expected and encouraged.

The final limitation shared across all of the studies included here was a reliance on participant adherence to the study protocol, particularly with respect to abstinence procedures. Participants were asked to remain abstinent during a specific period, were given twice-daily saliva drug tests during that period (and were provided additional compensation if the test result was negative for THC), and provided urine samples over the course of each study protocol that could be tested for changes in the primary metabolite of THC, THC-COOH, during the use and abstinence periods. While these procedures seem comprehensive, participants could have used small amounts of cannabis during the “abstinent” period while avoiding detection: saliva drug tests were only sensitive for the 6-12 hours prior to administration, and urine THC-COOH only provides an imperfect estimate of recent use given differences in metabolism due to sex, genetics, or route of cannabis administration and the high levels of baseline metabolite in

individuals with CUD. [255,256] In addition to these technical constraints, participants were only excluded from further study participation if the first saliva drug test collected in the laboratory was positive, as this would demonstrate a fundamental inability to adhere to study guidelines. Thus, a small number of participants openly endorsed use later during the abstinent period or simply did not complete saliva drug tests. Ideally, sensitivity analyses excluding time points surrounding estimated use periods would be conducted, with these results compared to those obtained from the whole sample. Unfortunately, due to the sample size limitations mentioned above, these analyses could not be performed.

Future Directions

Functional Relevance of the Peripheral Endocannabinoid System and Endocannabinoid Congeners in Psychiatry

Nearly all of the background provided in this dissertation on the psychiatric relevance of eCBs and the eCB system is based on observations in the CNS, even though all of the eCB outcomes reported herein are derived from the periphery; this is not due to an oversight on the part of the writer, but rather, a reflection on the state of the field. This state is perhaps most aptly summarized in the title of a 2018 review by Dr. Cecilia Hillard: "Circulating endocannabinoids, from whence do they come and where are they going?" [200]

The nature of human subjects research means that examination of central eCB tone is limited by the invasiveness of the necessary procedures (PET imaging, lumbar puncture). As such, preference in clinical studies has been given to less intrusive methods, like blood collection, as the eCB system is distributed throughout the body. [21] Indeed, since

eCBs are lipophilic and could therefore cross the blood-brain barrier, it has even been theorized that peripheral eCB concentrations represent “spillover” from the CNS (referenced by Hillard [200]). While this may be true in specific situations or in limited quantities, it is more likely that peripheral eCBs are produced in a localized manner on-demand, much like they are in the CNS, again given that the eCB system is distributed throughout the body. Supporting this, a preclinical study incorporating a chronic unpredictable stress model of depression found no association between brain and plasma AEA or 2-AG content. [56] Yet, there appears to be some behavioral effect of peripherally-restricted CB1R antagonists [201,202,248] and evidence of a neurobiological response to peripherally-restricted FAAH inhibitors in other preclinical studies. [257]

It is possible that these drugs are not as peripherally-restricted as suggested; a study characterizing a peripherally-restricted MAGL inhibitor found no evidence of THC-like “tetrad” effects in rodents, although other behavioral effects of the drug were not assessed. [258] Further, one of the aforementioned studies found that the stress-potentiating effects of the CB1R antagonist used (AM6545) were not, in fact, CB1R-dependent, calling into question the mechanism responsible for the behavioral effects of this compound. [201] Finally, and perhaps most importantly, this latter study also highlights the fundamentally interconnected nature of the body; even a peripherally-restricted drug can have some type of indirect feedback onto the brain, and vice versa. Understanding the nature of these holistic interactions is critical to both understanding the consequences of heavy cannabis use on brain and bodily systems, and to the development of eCB-modulating drugs, particularly if one is looking to avoid psychiatric side effects typically associated with CB1R agonists (i.e. THC-like effects [20]) or CB1R antagonists (i.e. anxiety, depression [78]).

Much like the discrepancy between what is known about the eCB system in the CNS compared to the periphery, there is also a lack of understanding regarding the role of the eCB congeners in psychiatry (e.g. the other NAEs). While these are often grouped in with eCBs due to structural similarity and shared metabolic pathways, they have distinct pharmacological properties, including lack of activity at CB1R. [259] Thus, the behavioral consequences of these congeners are not yet known. Of greatest interest is likely PEA, which has shown evidence of antidepressant effects in humans while maintaining a favorable safety profile. [260] PEA is currently being evaluated in a placebo-controlled trial for its effects in bipolar depression (NCT06229977). Interestingly, PEA was also negatively associated with anxiety and cannabis wanting in individuals with CUD with or without comorbid MDD, reported in Chapter 4. PEA may have specific psychiatric utility in one or both of these populations.

Behavioral Effects of Drugs That Modulate the Endocannabinoid System in Humans

As eCB-modulating drugs show consistent therapeutic effects in animals, there is a need to evaluate the effects of these drugs in humans sooner rather than later. This is particularly important when considering the history of the CB1R antagonist, rimonabant: decades of preclinical research showed a clear therapeutic effect, only for the emergence of its psychiatric side effects to make it nonviable in humans. [261] While there is a clear need to continue preclinical lines of research to elucidate the specific mechanisms involved in a drug action or disease state, this preclinical work should be conducted concurrently with basic human laboratory studies as soon as a compound has been shown to be safe for human consumption.

That said, one cannot overstate the need for clinical vigilance. FAAH inhibitors, like rimonabant, have shown evidence of therapeutic potential in preclinical models (for a review, see [262]). Yet, poor trial design and worse clinical supervision precipitated severe neurological adverse events, including death, in an ascending dose study of the FAAH inhibitor, BIA 10-2474. [263] Beyond needless tragedy, this carelessness has made it much more difficult to obtain regulatory approval to test the effects of FAAH inhibitors in humans, slowing lines of research that may improve the quality of life for countless people. It is critical that research into eCB-modulating compounds be conducted with the necessary care to avoid these outcomes.

To conclude, a slew of compounds show potential therapeutic efficacy preclinically, but have limited or no data obtained from human subjects: peripherally-restricted FAAH inhibitors [264] and CB1R antagonists, [201,202,248] MAGL inhibitors (both peripherally- [258] and centrally-mediated [265]), and CB1R neutral antagonists [266] complement the set of thus far unevaluated eCB congeners. None of these compounds have been assessed in individuals with CUD, who present with a specifically dysregulated eCB system. Examining effects of eCB-modulating drugs in individuals with CUD will not only provide insight into their potential therapeutic efficacy in this population, but also into the function of the eCB system in humans.

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