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In addition to the co-investigators and research coordinators listed above, undergraduate and professional research assistants play an active role in the recruiting and data collection phase of this study. These persons have completed CITI training, and have been trained by the lead

Professional Research Assistant and project manager regarding responsible conduct in research and study specific procedures/guidelines.

I. OBJECTIVES

Please note that the grant supporting this project was recently funded through NIDA. The grant and protocol follow **many of the same procedures as four IRB-approved studies**: 1) our pilot study that has been approved for almost three years, "An Observational Study of Cannabidiol, Neurocognition, and Mood," (protocol 14-0087), where participants purchase a cannabis strain from a local dispensary with a range of very common THC and CBD ratios assigned our two follow up studies, 2) "Cognition, Mood, and Cannabis" (protocol 15-0797) and 3) "Acute Effects of Dabbing Concentrated Marijuana Products" (protocol 16-0768), where participants purchase a similar range of products (as in this study), or a very high THC products with no CBD, and 4) our more recent study in a pain population "Observational Study of Cannabis and Pain" (protocol 17-0268), where participants choose their edible cannabis product of choice. The current protocol overlaps heavily with the 1st, 2nd, and 4th studies above, so that our overall research endpoints can inform future medical and recreational questions regarding common current and real-world cannabis use.

Marijuana is approved for medical use in over half the states and is gaining traction for use as an "off-label" add-on therapy for treatment-resistant anxiety and stress-related disorders. Paradoxically, however, while data suggest that marijuana, in particular Δ9-tetrahydrocannabinol (THC), increases anxiety acutely, cross sectional and longitudinal data suggest associations between marijuana use and *lower* risk for anxiety disorders. Such findings imply biological and/or behavioral changes in the post-intoxicated state that impact anxiety related processes. In light of considerable evidence that inflammation plays a pivotal role in the etiology of anxiety disorders and the putative anti-inflammatory properties of marijuana, we propose that the anti-inflammatory properties of marijuana are linked with its anxiolytic effects. Importantly, prior work has not considered that the anxiolytic effects of marijuana are the compound action of different cannabinoids, which vary in their pharmacology and effects. Specifically, cannabidiol (CBD), a non-psychotomimetic component of marijuana (doesn't produce a "high"), is thought to have anxiolytic properties and may mitigate some of the harmful effects of THC. Further, preliminary data, including our own, suggest that THC and CBD render differential effects on anxiety-related processes, such as effects on inflammatory and stress responses. Differences in inflammation likely impact processes such as the degree of stress-reactivity and acute and/or chronic variability in anxiety. Therefore, the degree to which different types of marijuana influence stress-response and inflammation may have a large and clinically-relevant impact on the effects of marijuana on anxiety. We propose to systematically examine the effects of cannabinoids on anxiety, inflammation, and stress reactivity in mild to moderately anxious (Generalized Anxiety Disorder (GAD-7) ≥ 5) marijuana users**.**

Our global hypothesis is that the anxiolytic and inflammatory effects of marijuana vary as a function of the ratio of THC to CBD, and that these effects may shed light on the marijuana use/anxiety paradox. The goal of this study is to test the effects of real-world, commercially-available, common marijuana products, that differ markedly in their ratio of THC to CBD [3 flower THC:CBD ratios (1:0, 1:1, or 0:1) and 3 edible THC:CBD ratios (1:0, 1:1, or 0:1) currently available]. To that end, we will test the effects of the following **marijuana ratios: 1) +THC/-CBD (1:0), 2) +THC/+CBD (1:1), or 3) -THC/+CBD (0:1)** on anxiolytic,

inflammatory, and stress-reactivity processes. The first and last strains test the effects of THC and CBD in isolation, respectively, while the middle strain (+THC/+CBD) provide additional data on the effects of THC when CBD is also in blood, a critical comparison given our pilot data, suggesting that CBD mitigates the effects of THC.

We will employ a design with marijuana users assigned to one of three (flower users) or one of three (edible users) marijuana strains (differing by ratio) for 4 weeks of ad-libitum cannabis use. We will compare marijuana users to non-users (a matched control group), who are not assigned and do not use marijuana, and who do not have a desire to use marijuana to cope with their anxiety. Users, with a desire to self-administer marijuana to cope with their anxiety, will have the option to self-administer a flower (one of three ratios) or an edible (one of three ratios) cannabis product, however, each subject (regardless of their type of administration chosen) will be assigned to a product with +THC alone (1:0), a combinations of +THC and +CBD (1:1), or +CBD alone (0:1), and will use their assigned product ad libitum (using as much or as little cannabis as they would like).

Aim 1: To **assess anxiety** in mild to moderately anxious marijuana users exposed to one of three marijuana strains: 1) +THC/-CBD, 2)+THC/+CBD, or 3)-THC/+CBD compared to matched (non-using) controls.

Hypothesis 1. Based on numerous studies, including our own pilot data, that suggest CBD may be anxiolytic and mitigate the harmful effects of THC, we hypothesize a step wise effect of strain such that the –THC/+CBD group will demonstrate the lowest anxiety, as compared to the +THC/+CBD group(s), which will have lower anxiety levels than the +THC/-CBD and non-using control groups. *In addition to strain assignment, CBD and THC blood levels will also be tested in relation to anxiety, with greater CBD levels associated with lower levels of anxiety.*

Aim 2: Based on the notion that CBD decreases inflammation and acute stress, the study will **examine: 1) circulating inflammatory markers and 2) behavioral and biological markers of stress reactivity** in mild to moderately anxious marijuana users exposed to one of three marijuana strains: 1) +THC/-CBD, 2) +THC/+CBD, or 3) -THC/+CBD versus a matched (non-using) group.

Hypothesis 2A. There will be a main effect of strain such that the –THC/+CBD group will have the lowest levels of circulating peripheral inflammatory markers, as compared to the +THC/+CBD group(s), which will have lower levels than the +THC/-CBD and non-using control groups. *In addition to strain assignment, CBD and THC blood levels will also be tested in relation to inflammation, with CBD levels associated with lower levels of inflammation.*

Hypothesis 2B. There will be a main effect of strain such that the –THC/+CBD strain group will have the lowest levels of stress-reactivity to a laboratory stressor (measured via self-report, physiological, and inflammatory stress responses), as compared to the +THC/+CBD group(s), which will have lower levels of reactivity than the +THC/-CBD and non-using control groups. *In addition to strain assignment, CBD and THC blood levels will also be tested in relation to stress-reactivity outcomes, with CBD levels associated with less stress-reactivity.*

Aim 3: Based on the notion that the effects of CBD on inflammation or stress may mediate the effects of marijuana on anxiety, the study will **examine the mechanisms by which CBD influences anxiety**.

Hypothesis 3. We will estimate a mediational model that tests whether the effect of marijuana strain on anxiety is mediated by changes in circulating inflammatory markers, stress-reactivity, or both.

II. BACKGROUND AND SIGNIFICANCE

Marijuana use is on the rise with the number of adults reporting medical and recreational use doubling in the past decade[1]. Among adult medical marijuana users, 39% report using marijuana for the purposes of self-treating or coping with anxiety[2]. Despite limited scientific data on the effects of marijuana on anxiety, public perception is growing regarding a role for marijuana in anxiety treatment.

Mixed data on marijuana and anxiety. There is some evidence demonstrating that marijuana use is associated with increases in acute anxiety and anxiety disorders[3]. However, other data (including our own recent, prospective work in Colorado where various marijuana strains are widely available) suggests that marijuana use may be protective for adolescents at-risk for anxiety and decrease the chances of developing an anxiety disorder during college[4]. This finding is consistent with a growing body of evidence from animal models suggesting that marijuana has anxiolytic and anti-inflammatory properties[5]. Clarifying the anxiolytic effects of specific strains that differ in their cannabinoid composition may explain these discrepant findings. Thus, regardless of whether our results support or refute the anxiolytic properties of marijuana, findings from this study fill a critical void and can inform public perception.

Marijuana contains different cannabinoids that may have differential effects on anxiety. Advertising materials provided by marijuana organizations go so far as to recommend particular marijuana strains to treat specific anxiety conditions. The data the scientific community has provided thus far is strikingly limited and disconnected from public perceptions and industry messages. Overall, research studies have largely ignored the fact that marijuana exists in different forms and have not characterized the effects of marijuana as the compound action of different cannabinoids that vary in terms of their pharmacological effects. Two of the primary cannabinoids, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), have opposing effects, and the ratio of THC to CBD varies dramatically among different strains of marijuana, with some strains in Colorado testing at greater than 20 to 1 CBD to THC, while many strains have a 1:1 THC to CBD ratio or have negligible CBD. Importantly, THC is thought to be acutely anxiogenic[6], while treatment with CBD, a non-psychotomimetic cannabinoid, induces anxiolytic effects without the experience of a "high" and may mitigate some of the harmful effects of THC[7]. However, the impact of CBD *level* and its *ratio* to THC on the anxiolytic effects of marijuana is largely unknown. Here, we propose to test the hypothesis that the anxiolytic effects of marijuana vary as a function of the ratio of CBD to THC in order to address this major gap in understanding of the effects of cannabinoids on anxiety.

Does CBD alter the effects of THC on anxiety? In addition to epidemiological data, one of the primary methods used to understand the effects of marijuana on anxiety in humans has been standardized smoking of marijuana in a laboratory setting. With respect to self-report measures, a number of studies have found that marijuana acutely increases positive affect as well as anxiety[8-10] _ENREF_8. These effects also appear to follow a dose dependent function based on the THC content of the marijuana (e.g.,[11]). Studies have also compared the effects of smoked marijuana to the effects of THC in pill form, suggesting that both forms produce similar effects

on subjective mood ratings (e.g.,[12,13]). However, almost all of this research was conducted with low potency marijuana provided by the government and almost all of this research was focused on the effect of one particular cannabinoid, namely THC. More recent analyses suggest that CBD may have very different effects on mood and attenuate the anxiogenic effects of THC[14-17]. Importantly, there are no current data that can speak to the longer term effects of either THC or CBD, or their combination, on anxiety processes. Clearly, the evidence suggests that acute marijuana use is associated with mood alterations and that marijuana use is likely related to long-term anxiety outcomes, although the data are mixed as to the direction of the effect. Thus, a key issue and a focal point of the present proposal is that the anxiolytic effects of CBD differ considerably from those of THC, an issue critical to understanding the spectrum of post-intoxication effects of marijuana strains on anxiety. Given that CBD is thought to be anxiolytic and non-psychotomimetic (doesn't produce a "high"), and mitigates some effects of THC, the use of strains containing CBD will likely result in different anxiety outcomes. Importantly, our design includes tests of the effects of a THC based strain (1:0), a CBD based strain (0:1), or a +THC/+CBD combination strain (1:1). All three are critical comparisons given emerging data supporting potentially differential effects of each of these cannabinoids on their own, and when tested in different combinations (See Preliminary Studies).

The role of inflammation in anxiety. Rodent and human studies support the role of inflammatory processes in the development of anxiety and stress-related disorders. Several laboratories have shown that neural IL-1β and the subsequent pro-inflammatory cascade is both necessary and sufficient to induce anxiety-like behaviors in rodents[18,19] ENREF 19[20]. Although the data is more equivocal, human studies also suggest that pro-inflammatory cytokines are involved in the generation of anxiety-related symptoms, such as anxiety, fear, and rumination, putatively via interactions with the stress system[21]. Research suggests that acute stress such as rumination on traumatic experiences or exposure to emotional visual images (e.g. during the Rumination Induction or Dot Probe task) triggers cytokine signaling (e.g. IL-6, TNF- α, IL-2, IL-1, CRP, etc.) and oxidative stress, which have downstream effects on inflammatory responses (Boyle et al 2017, Cooper et al 2017, Rood et al 2012, Hannibal et al 2014). Importantly, inflammation is associated with a number of negative consequences including risk for anxiety and stress-related disorders, immune-suppression, and deleterious neurocognitive effects, and has important implications for overall health and well-being [24,25]. Several studies have reported significantly higher concentrations of various pro-inflammatory cytokines in serum in cerebrospinal fluid (CSF) of individuals with anxiety and stress-related disorders[26,27]. Further a considerable number of studies have shown that acute psychological stress in human subjects also increases pro-inflammatory cytokines (IL-1β) in serum[18].

Marijuana has anti-inflammatory properties. Cannabinoids have profound effects on immune system function and inflammation, both peripherally and centrally (for review see Klein[28]). A number of studies have suggested that cannabinoids, both THC and CBD, modulate pro-inflammatory cytokines, including TNF, IL-2, IL-6, IL-12, IL-1β (see review Klein[28]). Importantly, although both THC and CBD exert inhibitory effects on the production of inflammatory cytokines, their activities seem to involve distinct intracellular pathways which remain somewhat elusive and appear to involve non-CB1 or CB2 related mechanisms[29]. Rodent models suggest that this may be particularly true for CBD[30,31], where treatment with CBD or a synthetic derivative blocked the LPS-induced increase in serum TNF and other cytokine immune responses[32]. Martin-Moreno et al.[33] found that CBD treatment in mice reduced the

neuroinflammatory effects of amyloid beta, specifically cortical levels of the pro-inflammatory cytokine IL-6. In a similar set of studies, CBD attenuated several facets of the neuroinflammatory response to amyloid beta in the hippocampus including IL-1 and GFAP expression[34] _ENREF_32 and inducible nitric oxide synthase, NF-kB activation and astrocytic reactive gliosis[35]. Anti-inflammatory effects of CBD in brain were also observed in models of multiple sclerosis (e.g. decreased IL-1)[36] and meningitis[37].

A unique role for CBD in anxiety through inflammatory pathways. These data, consistent with our pilot data, suggest that CBD has profound anti-inflammatory effects on peripheral and neuro- inflammatory markers (for review see [38]), which play a pivotal role in the etiology of psychiatric disorders including anxiety disorders, PTSD, and depression[39]. These are exactly the questions our proposed design is ideal to answer. Given that pro-inflammatory shifts are thought to underlie stress-responses and anxiety behaviors, marijuana's anti-inflammatory effects may be key players in explaining associations among marijuana use and anxiety disorders. While THC also has some anti-inflammatory properties, possibly via its effects on CB2 receptors, the totality of data (including our own pilot work) suggest that CBD may be a highly potent anti-inflammatory component of marijuana[40] that could potentially mitigate harmful effects of THC or create a synergy whereby a balance of these cannabinoids have the strongest anxiolytic effects.

Significance. In order to unpack associations among marijuana use and the biological and behavioral processes underlying anxiety, studies that consider multiple cannabinoids and then quantitatively measure cannabinoid levels in blood are critically needed. This is becoming increasingly important, because the types of marijuana commercially available have increasingly high levels of THC (typical products widely commercially available in Colorado dispensaries are 18% to 25% THC) and there is huge variation in the ratio of THC to CBD in these products. While some products have a near 1:1 ratio of THC to CBD (e.g., Jamaica Lion), others are closer to a 1:2 ratio of THC to CBD (e.g., Gumbi). On the other hand, many have almost no CBD at all (e.g., a 1:0 ratio with Starfighter is 27% THC, 0% CBD) and others have virtually no THC and are entirely CBD (e.g., a ratio of 0:1 with Charlotte's Web). It is difficult to describe the enormous variation in the products that are currently available in dispensaries throughout Colorado and other states that have legalized marijuana (see http://thefarmco.com/ marijuana-strains-descriptions-all/ for an example). In contrast, the human laboratory work on the effects of marijuana use on mood, health behavior, etc. have nearly all been conducted with marijuana grown by a single government source in Mississippi, with a THC potency of approximately 3-6% and 0% CBD. This is no fault of investigators, as to date this was the only legal avenue for pursuing this work. The critical point from a scientific perspective is that as a result of the constraints on prior research, we know virtually nothing about the effects of different ratios of cannabinoids in marijuana on biological and behavioral processes relevant to anxiety. This is critical information from a public health perspective, and we simply cannot gather relevant data without conducting externally valid research with everyday users of these products.

THC is well known for having immediate negative effects both acutely and potentially even over the longer term on anxiety, suggesting that higher potency THC strains could have negative consequences that exacerbate anxiety. CBD, on the other hand, is non-psychoactive, has high tolerability in humans, and may counteract some of the negative cognitive effects of THC, at least in the short term (see Preliminary Studies). Further, protective effects of CBD have been widely shown in preclinical models of inflammation and anxiety[40]. In order to determine: 1) whether marijuana has effects on anxiolytic and inflammatory processes, 2) whether these effects

are more likely to be due to THC, CBD, or some combination of the two, and 3) to provide data on the minimization of harm due to marijuana use with respect to anxiety symptoms, the current study will quantitatively assess THC and CBD blood levels in mildly to moderately anxious marijuana users who have a desire to use cannabis to cope with their anxiety and will use one of three different strains of marijuana (that vary in their levels of THC and CBD). These participants (marijuana users) will be compared to a matched control group that does not currently use or have a desire to use marijuana to cope with their anxiety. We propose that the opposing pharmacology effects of THC and CBD may result in differing inflammation and stress-reactivity profiles in anxious marijuana users as compared to anxious non-users.

Limitations of the Extant Literature. All—or nearly all—published data linking marijuana use to anxiety are taken from either: 1) large surveys or 2) from studies of acute effects on mood that rely on low potency THC marijuana. Thus, it is not possible to know whether there is any causal (direct or indirect) effects of marijuana use on biological or behavioral mechanisms relevant to anxiety. Legal impediments have to date prevented human laboratory human studies from examining the effects of marijuana on anxiety processes using strains that are currently available and widely used. Further, studies have largely not characterized the effects of marijuana as the compound action of different cannabinoids that vary in terms of their medical and pharmacological effects. Two of the primary cannabinoids, THC and CBD, although both classified as partial agonists and antagonists, appear to have opposing effects. However as discussed, the ratio of THC to CBD varies dramatically among different strains of marijuana. Thus, studies that examine marijuana strains, as they are commercially available and used in the real world, are critically needed in order to understand the potential health impact of increasing marijuana use. Notably, data from Colorado and other states with legal marijuana suggest that medical users are more likely than recreational users to use edible marijuana, which is a form of marijuana which remains psychoactive much longer than smoked marijuana (Pacula et al 2016). The most recent data from Colorado suggest that over 650,000 edible products were sold per month in Colorado dispensaries in 2015. Importantly, there have been no reported studies of the mechanistic effects of *edible* marijuana products on anxiety, inflammatory, and cognitive processes, despite the fact that consumption of edible marijuana is prevalent and rapidly increasing in individuals with anxiety. Therefore, we will observe our participants self-dosed cannabis use (flower or edible) and measure neurocognitive and inflammatory mechanisms underlying potential changes in anxiety.

Summary. Our goal is to understand the anxiolytic effects of cannabinoids, in particular the effects of THC-based strains vs. CBD-based strains vs a combination of THC to CBD (1:1) strains on inflammation, stress-reactivity, and anxiety. This design (including some experimental elements) will capitalize on the novel opportunity to examine the effects of real world marijuana strains (selected for their THC and CBD ratios) on anxiety, stress-reactivity, and peripheral inflammation. Thus, the proposed set of studies directly characterize the effects of specific cannabinoids on anxiety, stress-reactivity, and inflammatory pathways, and have the capacity to identify the potentially less harmful components of marijuana. These studies will provide the public, physicians, policy makers, and the scientific community valuable data regarding the effects of marijuana strains at commonly commercially available potencies and ratios in individuals with anxiety.

III. PRELIMINARY STUDIES

Prior experience and expertise of the team. The CU Change lab has been conducting behavioral and imaging research on marijuana[41,42] as well as research on the relationship of marijuana use to broader health and risk behavior[43-45] for many years. For example, the PI Bidwell conducted a longitudinal study of adolescent and young adult marijuana use and found that higher levels of marijuana use were protective against anxiety for at-risk adolescents and decreased the chances of developing an anxiety disorder during college, even after controlling for baseline levels of psychopathology and the use of other substances[4]. More broadly, her research focused on the overlap of substance use and mental health outcomes. Co-I Hutchison has conducted prior research on the acute effects of cannabis (Schacht, Selly, & Hutchison, 2009) as well as research on cannabis withdrawal and cue-elicited craving for cannabis (Haughey et al., 2008; Filbey et al., 2009; 2010) and the association between brain structure and cannabis use (Schacht et al, 2011; Weiland et al., 2015). More broadly, his research focuses on the study of neurocognitive and genetic factors underlying response to interventions to decrease substance-use and related risk behavior, and increase health behaviors. The co-PI (Dr. Bryan) has also studied the association of cannabis use and risk behavior (Bryan et al., 2012) and has extensive experience in research related to health behaviors and interventions. Thus, they are uniquely qualified to head this research project.

Previous and Current Marijuana Studies. More recently, the lab has focused on the development of cutting edge study designs to examine the effects of various types of marijuana. One current pilot study utilizes an extensive experimental design compared to the one proposed in this application, but with many of the same outcome measures. In the pilot study, regular marijuana users (n=22) were asked to switch strains for three days after a washout period. Participants used either +THC/CBD (~14% THC, <1% CBD) or a +THC/++CBD (7% THC, 14% CBD) *smoked* strain that is acquired from a local dispensary. Both the researchers and participants are blinded to strain condition, and the blind is maintained by the dispensary and one senior member of the lab. After a washout period of no marijuana use, participants use the assigned marijuana strain daily for three days, including the last use on the third day. Blood draws and assessment of cognitive responses are collected before the 3-Day use period at the Baseline Appointment (i.e., after washout), immediately after and 1 after the last self-administration use, at the 3-Day Appointment (participants come to the lab by taxi within 15 minutes of last marijuana use). In our ongoing studies, participants complete similar measures in our Mobile pharmacology and phlebotomy lab, such as the currently proposed motor battery, the at-home microbiome collection kit, and use cannabis ratios identical to the ones in this proposal (i.e., all THC with no CBD (+THC/-CBD, 1:0, equal THC to CBD (+THC/+CBD, 1:1), or all CBD (-THC/+CBD, 0:1).

This pilot work provides proof of concept and hypothesis-consistent data on several levels. 1) It confirms that we can work with our IRB, legal team, and local dispensaries to recruit participants and complete the proposed research. 2) Our pilot data suggest that CBD blood levels are associated with mitigating THC-associated verbal recall deficits. These effects further support our hypothesis that the harmful effects of marijuana vary by THC vs CBD composition across different strains. 3) It shows that we have measured peripheral inflammatory markers (e.g. TNF-a, IL-1B, IL-6) in our participants prior to strain assignment (Before 3-Day Self-Administration), immediately after last use (Immediately After 3-Day Self-Administration), and 2-hours post use (2 Hours After last 3-Day Self-Administration), and that these markers vary

based on cannabinoid blood levels. We have successfully been collecting and storing gut flora from the at-home microbiome kit (fecal sample) for subsequent analysis but are still in the early stages of analyzing these pilot samples. However, the cytokine associations among marijuana strains are in the hypothesized direction (e.g., use of +THC/+CBD marijuana is associated with the lowest cytokine levels and the strongest anti-inflammatory responses, while the +THC/-CBD strain is associated with comparatively higher levels of inflammation. Thus, effects on peripheral inflammatory markers suggest that strains that include both +THC and +CBD may have more positive effects on inflammatory responses than +THC alone and we suspect that diversity of gut flora may be altered based on drug use and immune function (Lowry et al 2016, Skosnik & Briones 2016, Mu et al 2016, DiPatrizio 2016, Cani et al 2016). Given the limited scope of the pilot study, we were not able to test a longer duration of cannabis use, the range of products (i.e., CBD only), complete a microbiome analysis on gut flora, or collect measures of sleep and physical activity (subjective and objective) that potentially mediate inflammatory and cognitive effects, but will in the current proposal.

IV. RESEARCH STUDY DESIGN

Overview and Design of the Proposed Study. Consistent with our preliminary study, we will recruit (from the Denver and Boulder areas): 450 participants who report a desire to use marijuana to cope with their anxiety, have had previous experience with marijuana, and report a Generalized Anxiety Disorder-7 (GAD-7) score of ≥ 5 (at least mild to moderate anxiety) and 75 non-using cannabis controls (matched for anxiety via GAD-7 ≥ 5) who do not report an intention to use marijuana to cope with their anxiety nor any marijuana use over the prior six months. A summary of the Assessment schedule and the additional inclusion/exclusion criteria are detailed below in Table 1 and Section VI (respectively).

All participants will come to the CUChange Lab for a Baseline Appointment (Session 1) involving extensive measures of anxiety (e.g., Depression, anxiety, and stress scale, a rumination induction task, a dot probe task, rumination self-report scale, etc.) and other psychiatric symptoms, health behavior (e.g., stress, quality of life, sleep, alcohol use), the cognitive testing battery utilized in our preliminary studies, a blood draw for biomarkers of inflammation, and a motor battery (**Baseline Appointment, Session 1**). During this Baseline Appointment, a research assistant will provide information on three at-home aspects of the study: 1) the range of assigned cannabis products (e.g., differing THC to CBD ratios possible) and potential prices, 2) the ActiGraph wearable watch device that participants will be loaned to measure physical activity and sleep (during the final 2 weeks of the four week study), and 3) a microbiome collection kit that they will be given to complete (once before and once after 4 weeks of *ad libitum* use of their cannabis product). The experimental aspect of this study will be the assigned cannabis ratio, while the other aspects of this study are ad libitum based, with regard to the dosing schedule and choice of product type (flower or edible), by participants that would already like to use cannabis to cope with their anxiety.

We are recruiting individuals who have indicated that they are unsure of what marijuana product to try and therefore want to try any of the ratios assigned in the study, would like to continue their typical strain use, or would like to change their strain to one of the ratios assigned in this study. We will ask that all subject inform of us of their subsequent strain purchased (assigned either strain C, D, or E), and the amount of cannabis purchased (not assigned) via an online short daily message (RedCap, provided as a CCTSI resource). Participants will then self-

administer the product as they see fit, without any instructions from study staff, for 4 weeks. Marijuana users (who according to our preliminary data, typically use marijuana with a THC potency of ~18%) will be assigned to use one of three strains with differing levels of THC and CBD. Considering the wide range of potencies, ratios, and types of cannabis products used, **we will assign one of three common flower cannabis ratios** [(THC:CBD = 1:0 (18/0%), 1:1 (12/12%), and 0:1 (0/18%)] and **one of three common edible cannabis ratios** [THC:CBD = 1:0 (10 mg), 1:1 (10/10 mg), or 0:1 (0/10 mg)]. Therefore, both flower and edible users will be identically assigned to +THC alone (1:0), a combination of +THC and +CBD (1:1), or +CBD alone (0:1), and will use their assigned product ad libitum (using as much or as little cannabis as they would like). Users will be advised to use their assigned cannabis product as they choose (i.e., their typical behavior), while non-users (control group) will be advised not to change their behavior and all groups will be instructed to report any change to us.

For cannabis user groups, we must recruit participants that have previously or currently used marijuana products and have a desire to continue or begin using any of the ratios and potencies potentially assigned during the study period. Thus, assignment to the all THC strain (+THC/-CBD, 1:0) serves as a naturalistic control group and our primary hypotheses surround effects of switching anxious marijuana users to either a strain with THC and CBD (e.g., +THC/+CBD, 1:1) or to a CBD-based strain without THC (i.e., -THC/+CBD, 0:1,), which are both likely to reduce anxiety symptoms over 4 weeks of use. Assessments occur at 2 and 4 weeks of ad libitum use, the typical time frame that symptom change would be detected from pharmacological treatment (e.g. SSRI). Details regarding marijuana strain assignment procedures, sample ascertainment, timing of assessments, power analyses, and the analytic plan follow.

As an exploratory endpoint, we will track participant's self-reported anxiety level, marijuana use, and sleep pattern with *daily follow-up messages* (online via email and the RedCap tool) over the course of the 4-week study period. This aspect will provide important data on patient choice, patient behavior, and edible impact on anxiety.

After the first two weeks (of four), all participants will return to the CINC (Creativity for Innovation & Creativity) laboratory (**2-Week Appointment, Session 2**) to complete the full set of biological measures and nearly all of the self-report measures and exercises with the elimination of one acute stressor (Rumination Induction) and the addition of one short survey on symptom change (Patient Global Impression of Change). After two more weeks (four weeks after the beginning of the study period), a final set of measures will be collected in our Mobile Van Pharmacology and Phlebotomy Laboratory (**4-Week Mobile Appointment: Pre & Post self-administration, Sessions 3 & 4**), to assess the longer/chronic (4-week, pre-administration, Session 3) and shorter/acute (0-1 hour, post-administration, Session 4) effects of cannabis use on all measures. Two additional measures will be added at this point to report the participant's current feelings after cannabis self-administration (Addiction Research Center Inventory (ARCI) and Drugs Effects Questionnaire (DEQ)).

While the main portion of the study will be complete after this 4-Week Appointment, we will continue to track participant's self-reported anxiety level, sleep pattern, and marijuana use with a *monthly follow-up survey* (online via email and the RedCap tool), over the course of a five-month follow-up, in keeping with the exploratory nature of the design. This aspect will provide important data on patient choice, patient behavior, and the effects of other marijuana products on anxiety.

In sum, the study will involve a 4 (marijuana-user groups: +THC/-CBD, , +THC/+CBD,, -THC/+CBD, and non-users) x 4 (Session: 1, 2, 3, and 4) mixed factorial design. The complete

schedule of assessments and measures taken at each assessment are outlined in Table 1 and described below.

Table 1. Schedule of Assessments.

Visit	Duration	Includes
1. Orientation @ CINC Lab (Session 1) ASAP after phone screen [Part of Baseline Appointment]	0.5 hrs	- Description of study procedures and measures - Informed consent; Eligibility re-screening - Strain assignment for marijuana users.
2. Baseline Assessment @ CINC Lab (Session 1 continued) ASAP after phone screen [Part of Baseline Appointment]	1.5 hrs	- Pregnancy test, Blood Alcohol Content, Toxicology drug screen - Self-report of anxiety, stress and depression (e.g., GAD-7, BAI, DASS) - Rumination Induction & Dot Probe Task - Questionnaires on health behavior, substance use, Psychiatric interview, and psychological measures - Inflammation & cannabinoid blood draw - Motor & Cognitive battery
3. *Daily follow-up assessment for all participants* 1x/day for 4 weeks (~31 days) [Between Baseline, 2-, & 4-Week Appointments]	~2 mins /day	- Daily online message report on anxiety/stress management, *ad libitum* marijuana use (if used: amount, strain, method), and sleep; individually tailored links
4. 2-Week Assessment @ CINC Lab (Session 2) 2-weeks after Baseline Appointment	1hr	- All Baseline measures (exception: rumination induction task removed and participant global change index added) - Microbiome kit & Actigraph distributed
5. 4-Week Assessment @ Mobile Lab (Sessions 3 & 4) Pre (Immediately) & Post (Immediately for flower or 1 hour for edible) self-administration [4-Weeks after Baseline Appointment]	2 hrs	- All Baseline measures/tasks - Microbiome kit & Actigraph wearable collected
3. *Monthly follow-up assessment for all participants* 1x/month for 5 additional months [After 4-Week Appointment]	~12 mins /month	- Monthly online survey; individually tailored links
Total time (6 months)	**8 hrs**	**Participants paid $335**

Marijuana Administration at Home. Unlike other studies done in the United States to date, the proposed work will utilize a design that allows participants to choose their type of administration and ad libitum dosing schedule, but contains an experimental element (i.e., strain assignment). Specifically, at the end of the baseline session, participants will be given instructions to purchase assigned strain, differing by THC to CBD ratio (n=75/flower strain; n=50/edible strain): 1) a THC-based strain akin to a typically used strain with normal levels of THC and little to no CBD (+THC/-CBD), 2) a 1:1 strain with equivalent THC and CBD levels (+THC/+CBD), or 3) a CBD-enriched strain with little to no THC (-THC/+CBD) at a good clinical practice dispensary in Boulder or Denver, Colorado.

The market for flower versus edible cannabis products are similar, but differ slightly in their servings, typical doses, and labeling system (potency listed by percent with flower products rather than by mg with edibles) making it currently impossible to assign identical flower and

edible potency groups, or ratios. However, Colorado requires all strains to be tested by a state licensed lab, which allows us to have a precise measure of potency to operationalize our strain assignments. Therefore, the flower groups will be assigned either the +THC/-CBD strain (containing 18% THC and <1% CBD), a combination of THC and CBD strain (a 1:1 ratio +THC/+CBD containing 12% THC and 12% CBD), or the -THC/+CBD strain (containing <1% THC and 18% CBD). Like the flower group, the edible groups will be assigned to one of the same ratios (an all THC strain, a combination of THC to CBD strain with a 1:1 ratio, or an all CBD strain) and they will choose when and how much they would like to use this assigned edible over the next four weeks. However, the participants that choose to be in the edible group (rather than the flower group), will be assigned an edible product with 10 mg of THC, CBD, or a combination of THC and CBD per edible piece (comparable to the flower products with 6, 12, or 18% THC, CBD, or a combination of THC and CBD).

The dispensary will set aside a specific lot of each flower (to be smoked or vaporized) and edible form of the assigned strain, which will be packaged in childproof bottles and labeled as "Strain C", "Strain D" or "Strain E", such that participants and dispensary staff will be blind to the ratio assignment until the product is purchased (THC and CBD content will be printed on the label, as required by state law) , and the research staff will remain blind to which strain was assigned until after data analysis. Dr. Hutchison (co-investigator) will maintain the blind during the study period. The instruction to purchase "C", "D" or "E" will be randomized across participants such that equal numbers of male and female participants are included across the ratio conditions. The design involves the instruction that participants can use as little or as much of the marijuana as they wish over the course of the four weeks of the study, and are asked not to use any other strain during that time (but if so, to just report what was used). Therefore, a research assistant will give participants that are marijuana users, a card with directions to a local dispensary. The card will have instructions to purchase a particular strain of cannabis (e.g., "strain C") but participants will choose the amount of cannabis that they expect to use during the study (i.e., we will not assign a specific amount for them to purchase or to use during the 4-week study period). While the potency may vary slightly by the strain assignment and the amount used may vary by each person, we expect approximately one gram of flower cannabis to cost $12 and approximately 75mg of edible cannabis to cost $15 (comparable across typical serving amount). However, this will depend in part on what cannabis type and how much cannabis each participant chooses to use. Study staff will not provide any directions regarding dosing and administration.

Marijuana-using subjects are prompted by email to report how much was used each day using an online reporting system (RedCap). We will also provide patients with safety information that will contain warnings regarding driving or operation of machinery, consistent with the warnings used with other over the counter and prescription drugs that may impact cognitive and motor functions (e.g., Benadryl, robitussin, benzodiazepines, narcotics, etc.). The important marijuana safety warnings will include: Do not drive, operate machinery, or perform other hazardous activities while using cannabis. It may cause dizziness, drowsiness, and impaired judgment. Do not drink alcohol while using cannabis. Alcohol will increase dizziness, drowsiness, and impaired judgment. Cannabis may increase the effects of other drugs that cause drowsiness, including antidepressants, alcohol, antihistamines, sedatives (used to treat insomnia), pain relievers, anxiety medicines, seizure medicines, and muscle relaxants. More information can be found on the National Institute of Health (NIH) DrugFacts page "What is marijuana?" (https://www.drugabuse.gov/publications/drugfacts/marijuana).

Rationale for Marijuana Administration Procedure. With respect to the naturalistic aspects of the design, it is important to note that we carefully considered alternative designs. For example, we previously conducted a study that involved a highly controlled laboratory administration of low THC and zero CBD marijuana obtained from the NIDA supplier in Mississippi[42]. However, external validity of the study was compromised because participants informed research staff that the marijuana was of significantly poorer quality than they had ever experienced. Further, although NIDA currently supplies a greater variety of marijuana strains that include some quantity of CBD, the NIDA-supplied marijuana does not reflect the levels of THC and CBD available through Colorado dispensaries and used by Colorado residents and patients (www.drugabuse.gov). Thus, those procedures simply did not, and cannot reflect the strains and administration of marijuana in our and other states. In addition, the federally sourced marijuana supplied through NIDA does not currently offer any edible marijuana products (www.drugabuse.gov), although these are widely available through Colorado dispensaries and used in great numbers by Colorado residents and patients to provide relief from anxiety. Thus, we are not able to acquire marijuana edibles via NIDA-supply and administer them directly to participants via a tightly controlled randomized clinical trial. Given the dearth of data available on why so many people are turning to marijuana as a treatment for anxiety and on the effects of marijuana edibles more broadly, we developed our design that naturalistically reflects common routes and methods of administration of marijuana in patients, particularly in states with legalized marijuana.

Because the long-term goal of this work is to better understand the effect of different levels of cannabinoids in anxiety patients, as they are used real world, we decided that it was critically important to emphasize external validity in both the flower and edible forms of cannabis use. The disadvantage of our design is that we have little control over how the participants use the marijuana or how much they use. To address this limitation, both in our pilot study and in this application, we rely on blood quantitation of cannabinoids (from both flower and edible users) to determine the level of THC and CBD in each subject. Thus, regardless of how the participant uses the marijuana, we have an objective measure of the dose received for both THC and CBD for each subject, which is the sine qua non of pharmacological research. This aspect is also critical for our analytic approach, which allows for the analysis of THC and CBD blood levels as continuous measures. In this way, we also capitalize on the greater variability in blood levels expected in the design by analyzing overall levels of CBD and THC as well as by strain, which by design (in flower and edible forms) will have different THC/CBD ratios. This further allows for a direct test of whether any differences in outcomes for participants randomly assigned to the –THC/+CBD strain are due to an increase in CBD or a decrease in THC as compared to their Baseline. This strain is a critical comparison as there are data to suggest that the effects of THC differ when CBD is also in the blood.

Power Analysis and Sample Size Requirements. Sample size was selected to permit analysis of the primary research questions at two-tailed alpha of .05 and power level of .80. Estimates of effect size follow Cohen[46] and were conducted in G*Power 3[47]. The primary analyses in **Aims 1-3** broadly is the test of the group X time interaction effects across repeated assessments of THC/CBD and anxiety, inflammatory markers, and stress reactivity. For the current data analysis plan, we will only focus on the ratios and time points that we currently have supporting preliminary data for and hypothesize to have the greatest impact on anxiety. Hence, in this data analysis plan we will focus on the 3 main time points (out of 4) for a chronic use endpoint [Baseline, 2 Week, and 4-Week Pre-administration (Sessions 1-3)] and the 3 main cannabis product so that there will be

an all THC, all CBD, and a combination of THC to CBD group [(+THC/-CBD, +THC/+CBD, and -THC/+CBD, ratios: 1:0, 1:1, and 0:1)].

To be conservative, we thus estimated an ICC of .50. Using a mixed model ANOVA design, with four groups, three assessment points, and an average ICC = .5 between assessment points, a total *n*=450 (75 per flower group and the control, and 50 per edible group) will allow us to detect a groupXtime interaction effect as small as *f*=.09. In **Aim 2B,** the test of the effects of group on stress-reactivity will again involve four groups (3 cannabis and 1 control) but only one assessment point.. Note that our power analysis is conservative, as our actual analysis will involve more powerful tests (e.g., focused interaction contrasts as opposed to overall *F* tests). For **Aim 3**, the estimation of the mediational model via path analysis (Hypothesis 3**;** see Figures 3a and 3b below), Monte Carlo simulation strategies formed the basis of our power analysis[48]. Given that the model in Figure 3b is the more complex of the two, we utilized the model structure with the three planned contrasts as exogenous variables in our simulations. The key test of Hypothesis 3 will involve the use of structural equation modeling techniques[49]. Because we have almost no empirical data on which to base estimates of effect size, we conservatively utilized small to moderate effect sizes depending upon hypothesized relationships. For example, we expect small coefficients (.20) from each contrast to stress reactivity, whereas we expect a slightly larger path (.40) between the contrast comparing the –THC/+CBD strain to control and inflammation. Power analyses were conducted in Mplus and then in SAS following procedures for estimating the power of the likelihood ratio test of the significance of parameters in structural equation models[48]. We utilized Monte Carlo simulation to generate a population covariance matrix based on the hypothesized parameters in the model. For the smallest path in the model (which requires the most power to test), assuming two-tailed alpha of .05, our estimates of power at various sample sizes appear in Table 3. To assure .80 power or better to detect small associations between all groups, we thus require data from 450 participants (75 in the control and each flower group as well as 50 in each edible group) for our mediational analyses.

The impact of attrition. It is anticipated that some attrition will occur. Based on our current pilot study and previous marijuana studies, we anticipate a 10-15% attrition rate between the baseline session and the final four-week follow up session, though we have not experienced and do not anticipate differential attrition by condition. Thus, to allow for attrition, we assume an approximate 85% retention rate over the course of data collection. To adjust for this attrition rate in all groups, we will recruit n≈520 participants (*n*=86/flower or control group as well as 58/edible group). Our approach to power analysis and accounting for attrition is also quite conservative, in that the techniques we will utilize in data analysis use state of the art recommendations for the handling of missing data in longitudinal studies[50,51].

Statistical Analysis Plan. Analyses will be conducted primarily on the SAS system for Windows Version 9.4[52] and Mplus Version 7.3[53], which include capabilities to test multilevel models that appropriately model both normally and non-normally distributed data as well as missing data. To test the success of random assignment across the marijuana strain groups the equivalence of the three marijuana user and control groups across all pretest measures will be assessed via *t*-tests on continuous items (e.g., BAI) and χ^2 tests of categorical items (e.g., race). The Bonferroni approach [54] will be used to correct for alpha inflation (familywise alpha of .05). The distributional properties of continuous variables will be examined to determine appropriate analytic techniques or normalizing transformations prior any analyses.

Specific Aim 1 – To compare changes in anxiety in mild to moderately anxious marijuana users exposed to 4 weeks of use of one of three different marijuana strains (+THC/-CBD vs. +THC/+CBD vs. -THC/+CBD) to a group of non-using controls matched on anxiety. *Hypothesis 1* predicts a main effect of strain such that –THC/+CBD marijuana users will have lower levels of anxiety (BAI) compared to other marijuana groups and to non-using controls. To examine the main effects of group on these outcomes, we will estimate a random coefficient regression (RCR) models in SAS Proc Mixed wherein the change in BAI scores will be regressed on marijuana user group, time, and the groupXtime interaction. *Hypothesis 1* also predicts that higher CBD levels in blood among marijuana users will be associated with lower BAI. The analysis for this hypothesis will again be a RCR model, but instead of group membership, the independent variables in these models will be level of CBD in blood, time, and the CBDxtime interaction. We will conduct additional models to explore whether there may also be main effects of THC in blood or a THCXCBD interaction effect on BAI. Secondary models will test the effects of strain and /or cannabinoid blood levels on the daily and monthly assessments of anxiety using a multi-level modeling approach. These additional analyses will appropriately control for Type I error, depending upon the number of tests run. Co-I Bryan has used such models extensively in prior work.[55,56]

Specific Aim 2 - To assess inflammation and stress reactivity [biological and behavioral stress change scores during a stress induction (TSST)] in mild to moderately anxious marijuana users exposed to 4 weeks of use of one of three different marijuana strains compared to a group of non-using controls matched on anxiety. *Hypothesis 2A* predicts a main effect of strain such that –THC/+CBD marijuana users will have lower levels of circulating peripheral inflammatory markers compared to other marijuana groups and to non-using controls. To examine the main effects of group on these outcomes, we will estimate a random coefficient regression (RCR) models in SAS Proc Mixed wherein the summary values of the panel of inflammatory markers utilized in our preliminary study will be regressed on marijuana user group, time, and the groupXtime interaction. *Hypothesis 2A* also predicts that higher CBD levels in blood among marijuana users will be associated with lower levels of circulating peripheral inflammatory markers. The analysis for this hypothesis will again be a RCR model, but instead of group membership, the independent variables in these models will be level of CBD in blood, time, and the CBDxtime interaction. We will conduct additional models to explore whether there may also be main effects of THC in blood or a THCXCBD interaction effect on inflammatory markers. *Hypothesis 2B* predicts a main effect of strain on reduced stress reactivity. RCR models in SAS will again be utilized, wherein stress reactivity (pre-rumination induction–post rumination induction change scores of self-report, state anxiety, and inflammatory markers) will be regressed on marijuana user group. *Hypothesis 2B* also predicts that higher CBD levels in blood among marijuana users will be associated with even lower stress reactivity. As in the test of Hypothesis 2A, we use RCR wherein the independent variables in these models will be level of CBD in blood. As with Aim 1, we will conduct additional models to explore whether there may also be main effects of THC in blood or a THCXCBD interaction effect on stress reactivity, with appropriate Type 1 error corrections.

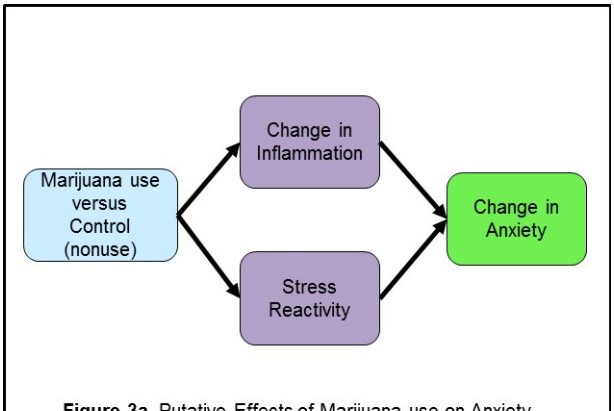

Figure 3a. Putative Effects of Marijuana use on Anxiety

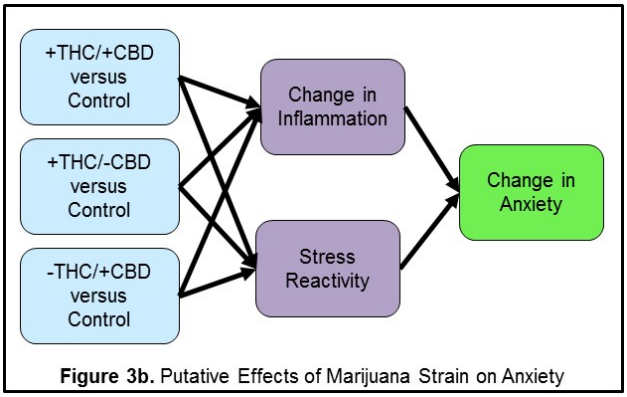

Figure 3b. Putative Effects of Marijuana Strain on Anxiety

Specific Aim 3 - To test indirect effects of marijuana use on anxiety (BAI) via inflammatory markers and stress reactivity in anxious marijuana users exposed to 4 weeks of use of one of three different marijuana strains compared to a group of non-using controls matched on anxiety. Hypothesis 3 will test mediational models that ask whether the effect of marijuana use and strain on anxiety is mediated by changes in inflammation, stress reactivity, or both. To this end, we will estimate the mediational models in Figures 3a and 3b via structural equation modeling[57]. To test the overall effect of use of marijuana versus non use, we will estimate model 3a wherein the sole exogenous variable is marijuana users (coded as 1) versus non-users (coded as 0).The mediators will be change in inflammation from Session 1 to Session 3 and stress reactivity at Session 3. The outcome variable is change in anxiety from Session 1 to Session 3. This model will be estimated and both the fit of the model and the significance of the path coefficients will be examined. Importantly, we will compare the path coefficients from user status to inflammation versus stress reactivity to determine whether use is associated with changes in inflammation, stress reactivity, or both. Assuming that the paths from inflammation and stress reactivity to anxiety are significant, we can test whether there are indirect effects of user status on anxiety that are mediated by inflammation, stress reactivity, or both. A test for completeness of mediation is employed through a 1 degree of freedom χ^2 test where a path directly from user status to anxiety is added to the model. A nonsignificant direct path and a nonsignificant change in χ^2 suggest that user group effects on the anxiety were mediated through the theoretical mediational constructs. A secondary test of mediation will utilize bootstrap methods to test the significance of, and confidence limits around, the mediated effect[58,59]. Similar mediational analyses have been widely used by Co-I Bryan[56,60,61]. The model depicted in Figure 3b will test the more focused hypothesis that the ratio of cannabinoids is critical for the biological and behavioral changes that drive anxiety. The analysis will follow identical procedures to those described to test Model 3a. Finally, it is also possible to use continuous values of THC and CBD in blood as the exogenous predictors in the model.

Amendment update Spring 2023 - We are reaching out to participants who have completed the study and have usable data in order to collect a) updated sexual orientation and gender identitiy (SO/GI) demographics including sex assigned at birth, and b) the Perceived Discrimination Scale (PDS). The rationale for this change is that the PDS was added late in the study and thus not all participations had the opportunity to complete the measure, and the current SO/GI-related items are not aligned with our current approach to collecting these data, which was also finalized very late in this study's course.

V. FUNDING

This study is expected to be funded by a grant from NIDA.

VI. ABOUT THE SUBJECTS

Ethnic diversity of this sample is expected to be representative of the greater Boulder-Denver area at large. A trained research assistant will screen prospective participants who call, email, or complete an online survey, according to the inclusion/exclusion criteria listed below. Given established associations with inflammatory markers (e.g. IL6, TNFa), we will exclude indviduals who report an immune-related disease (e.g. HIV), use of maximal doses of steriods, or use of psychotropic medications (other than anti-depressants or ADHD medications). Use of opiates for pain, anti-depressants, ADHD medications, menstrual cycles, and birth control medications will be tracked during the study. Specific criteria for study participants are listed below.

Criteria for inclusion in the study are:

1. Must be between the ages of 21 and 70 and provide informed consent;
2. Must report at least moderate anxiety (≥5 on GAD-7);
3. Users (cannabis group) must have used marijuana at least once;
4. Users (cannabis group) must have a desire to use marijuana to cope with anxiety;
5. Non-users (non-cannabis, control group) must not have used marijuana for prior 6 months;
6. Non-users (non-cannabis, control group) must not have a desire to use marijuana to cope with anxiety;
7. Must report not using other drugs (cocaine, methamphetamine) in the past 72 hours and must not test positive on a urine toxicology test for drugs of abuse at the Baseline Appointment;
8. Must not be using psychotropic, steroid-based medications, or maximal doses of non-steroidal anti-inflammatories (NSAIDs), however anti-depressants and ADHD medications are ok;
9. Must not have immune-relevant disease (e.g. HIV) or be using anti-viral medications;
10. Must not be a regular tobacco user (≤4 days per week; cigarette, E-cigs, or smokeless);
11. Must have a breath alcohol level of 0 at screening (to sign consent form);
12. Must not be actively seeking or in treatment for any substance use disorder (drug use levels will be carefully monitored via Timeline Follow Back (TLFB) throughout the study to assess any confounding influences of drug or alcohol use;
13. Female subjects must not be or trying to become pregnant (as indicated by a Pregnancy test & Screening form administered at Baseline)
14. Must not be in treatment for psychotic disorder, bipolar disorder, or major depression disorder with suicidal ideation; or a history with these disorders.

VII. VULNERABLE POPULATIONS

This study does not include any vulnerable populations.

VIII. RECRUITMENT METHODS

Recruitment will be via a number of sources that have been used successfully by our research team. First, as in our pilot project, we will recruit using flyers posted in and ads on the webpages and social media pages of dispensaries in the Denver and Boulder areas. Second, with assistance from local health care providers and physicians, we will advertise our study with flyers (see attached flyer) in and referrals from local mental health clinics. Third, we will utilize targeted mailings, advertising the opportunity to participate in our study. We will obtain a list of names and addresses of individuals who fit our target demographics and geographical area. These names/addresses are obtained from publicly available records purchased from a marketing firm (see http://www.alescodata.com/reseller-programs.html). Recruitment materials are mailed to each address on the list. We can narrow the list based on age, gender, geographic location, and other criteria. Fourth, we will use the NIH-funded free ResearchMatch (see https://www.researchmatch.org/) tool to link health criteria from potential participants to our specific research criteria. ResearchMatch has a large population of volunteers who have consented to be contacted by researchers about health studies for which they may be eligible. We will also advertise on free platforms like online patient groups and pay for platforms (e.g., Facebook/Twitter advertising) that often allow for targeting advertisements based on age, geographic location, and interests (e.g., following or "liking" posts related to anxiety).

In summary, we will recruit subjects locally and primarily through: target ads on anxiety groups in Facebook/Twitter, mailing flyers w/purchased names & addresses from Alesco and NIH-based ResearchMatch tool, flyers and referrals from providers' offices (e.g., Clinical Psychologists), recreational and alternative health centers (e.g., yoga studios, acupuncture and massage specialists, and gymnasiums, etc.), as well as in local restaurants and shops in Boulder. The combination of these varied recruitment methods should provide ample and steady potential participants, as in previous studies similar in size. All interested participants will be directed to complete an online screening survey (via RedCap) or to contact the CU Change Lab by phone or email (to be screened over the phone).

All of these advertisements will describe the opportunity to contribute to research regarding cannabis use. Specifically, the posts on social media will include the following wording "The University of Colorado is conducting research on how levels of CBD and THC are related to anxiety and stress." All social media pages will provide additional information about the study (see attached word flyer). The wording used on the social media page may also be used in flyers or other advertisements.

IX. COMPENSATION

Participants will receive $335 at the end of the study. Given the amount of time required in the study and the travel to and from the lab for the first and second session, this is a reasonable amount of money to compensate participants for their time and effort. Thus, there is no question of coercion. Participants will receive $80 in cash and for the Baseline Appointment (1st in-person Appointment at CINC), $60 for the 2-Week Appointment (2nd in-person appointment at CINC), and $80 for the 4-Week Appointment (mobile in-person appointment). Participants will also receive $1/day for each of the *daily follow-up messages* that they complete during the *ad libitum* 4-Week period, with a possible bonus of $10 for completing at least 26 of the 30 daily messages, for a maximum of $40 extra paid in cash. This will be split between the 2-Week Appointment

($20 for daily messages 1-15, $60 for Session 2, totaling $80) and 4-Week Appointment ($20 for daily messages 16-30, $80 for Sessions 3 & 4, totaling $100). Participants will receive a $6 Grocery Store/Amazon.com gift card for the 1st part (questionnaires) of the 5 subsequent and brief *Monthly follow-up surveys* completed and a $5 Grocery Store/Amazon.com gift card for the 2nd part (TLFB), with a bonus $20 Grocery Store/Amazon.com gift card if the 1st (questionnaires) and 2nd part (TLFB) of all 5 *Monthly follow-up surveys* are completed ($75 total, delivered online via email). If the 1st and 2nd part of only 4 out of 5 *Monthly follow-up surveys* are completed the participant will receive a $15 bonus gift card instead. If a participant does not complete the study, payment will be pro-rated for the Appointments/online follow-up assessments that they do complete, in-person, and in cash. At request, participants may also receive complementary RTD/Bus transit passes to help with transportation to and from our facilities.

X. CONSENT PROCESS

When a participant arrives for their Baseline Appointment (Session 1) at the Center for Innovation and Creativity (CINC), a member of the research team will greet him or her in the first-floor lobby. The research assistant will take the participant to a private room and provide the participant with a copy of the informed consent document. Prior to asking the participant to sign the consent form, the trained research assistant and the participant will have a discussion regarding the research study. Additionally, the research assistant will be available to answer any questions he or she may have about the study. We will also explain that participation includes an appointment in our Mobile Van Pharmacology and Phlebotomy Laboratory (4-Week Appointment), which will be seen outside their home, possibly by their neighbors, and that privacy may not be maintained. Participation will be clearly stated as voluntary, with the option to withdraw at any time. There will be no deception involved with this study. After discussing the study and going over the consent form with the researcher, the participant will be included in the study if they choose to initial and sign the informed consent document.

XI. PROCESS TO DOCUMENT CONSENT IN WRITING

In accordance with 45 CFR 46.117, a printed copy of the written informed consent document will be signed by the participant, and an unsigned copy of the form used to document consent will also be given to the study participant electronically.

XII. PROCEDURES

Participants will contact the researchers with their interest by phone, e-mail, or through an online screening survey (RedCap, provided as a CCTSI resource). In the case that a potential participant requests more information via e-mail or voicemail message, the research assistant responding to the message will ask the participant to call the lab phone number for more information (same phone number provided on all study advertisements), e-mail the study team a phone number at which the potential participant could be reached at and a desirable time for the research staff to call, or to complete the online survey. The first page of the online screening

survey will give a brief description and the estimated time involved in completing the screening process and the study (in the case that they are eligible and elect to participate) and instructed to click the *Next Page* button if they wish to be screened further. The online screening that follows will be identical to the phone screening. All subjects that complete the screening will be asked to leave their preferred contact information and the best time to contact them so that a research assistant can inform them of eligibility (according to the inclusion/exclusion criteria list in Section VI above) and answer any questions they may have. For participants that prefer to complete a phone screening, a trained research assistant will provide an overview of the study, answer any questions that the potential participant has, and then screen them for preliminary eligibility according to the inclusion/exclusion criteria list in Section IV above. This process will help to ensure participants have ample opportunity to be informed of the main study components and maintain their privacy as much as possible. If the participant meets the study criteria after the initial online screening survey or phone interview and the follow-up contact, they will be invited to come to the CU Change Laboratory at the CINC for their Baseline Appointment. If any subject is questionable for inclusion, our PI (Dr. Bidwell, a licensed clinical psychologist) and Co-I team will make the final determination of eligibility.

COVID-19: Before interacting with research assistants, eligible participants will be asked additional questions related to COVID-19. These questions will be administered prior to any in-person appointment and by a research assistant over the phone (or at a distance of at least 6-feet if the participant is unable to complete the questions over the phone). These questions will be administered to protect staff and participants from the spread of COVID-19. The questions are a precaution based on current university and Centers for Disease Control and Prevention guidelines and may need to be updated as the understanding of COVID-19 evolves. These procedural questions are temporary in nature and will be lifted once university guidelines deem it unnecessary.

Baseline Appointment (Session 1) Procedures. Subjects who meet inclusion criteria will be scheduled for a Baseline Appointment and given instructions for the assessments, including not consuming alcohol or marijuana for 24 hours, or caffeine for 4 hours prior to coming into the lab for their sessions. After arriving at the CUChange Lab, each subject will go through the consent process and be breathalyzed to ensure a breath alcohol concentration of zero. A urine toxicology screen (and a pregancy test for females) will also be administered to insure that subjects have not recently taken illicit drugs (and are not pregnant). Menstral cycles and birth control use will be reported. A baseline blood draw (see details below) will be taken to quantify inflammatory biomarkers and cannabinoids. A member of our research team who has completed a certified training in phlebotomy will collect a blood sample. All blood draw procedures performed during this study will involve collecting venous blood (40 ml total/session) venipuncture of a peripheral arm vein using standard, sterile phlebotomy techniques. Subjects will then be administered the Beck Anxiety Inventory (BAI), the State Trait Anxiety Inventory (STAI), the Depression, Anxiety, and Stress Scale (DASS), and complete measures related to alcohol/drug use history, mood, stress, and quality of life and will complete cognitive testing (see Table 2 below for measures to be completed).

After completing the final blood draw, subjects will receive training in the use of online daily assessments (see details below) and the three user groups will then be assigned a marijuana strain to use for the next four weeks instead of their typical strain. Marijuana using participants will be

randomly assigned to one of three marijuana strains (a THC-based strain akin to a typically used strain with normal levels of THC and little to no CBD, a THC and CBD strain in combination, with a 1:1 ratio between THC and CBD levels, or a CBD-enriched strain with little to no THC, and then given instructions to purchase their assigned strain (labeled either C, D, or E) at a good clinical practice local dispensary. Marijuana users will be asked to use their assigned strain (and only that strain) as they wish over the course of the next four weeks. Non-users will be asked to continue to abstain from marijuana use over the next four weeks. After completing the measures and procedures, research study staff will answer any questions subjects have on their daily messages (see details below), debrief participants, and compensate them with $80 in cash for their time and effort during the Baseline Appointment. Lastly, each participant will be scheduled for their 2-Week (Session 2) Apppintment at the CINC for two-weeks later.

Blood Levels of Inflammation. As noted in the background and in our description of our preliminary studies, we expect that CBD and THC will reduce biomarkers related to inflammation during the course of the study. Blood samples drawn at each Session will be assayed for IFNg, IL-1a, IL-1b, IL-6, IL-2, IL-4, IL-8, IL-10, IL-12, and TNF. Previous studies in animals (Mayfield et al., 2013) and humans (Bala et al., 2014), as well as our own preliminary data, suggest that cytokine activation is associated with the pathophysiology of anxiety disorders. This is the same assay used in our preliminary studies. We also expect CBD and THC to impact the responses of blood monocyte cells to a lipopolysaccharide (LPS) challenge (see details below). This may represent a more accurate test of the effects of the CBD and THC, since LPS stimulation of peripheral blood mononuclear cells (PBMCs) simulates the inflammation cascade that putatively mediates the neuroinflammatory effects of pain. To that end, 40 mL of whole blood will be drawn at each session (1-4). We will use 22 ml of blood to perform the inflammatory analysis, 10 ml of that blood for DNA extraction, and 8 ml of blood for cannabinoid analysis. There will be four blood draws total, with one during the Baseline Appointment (Session 1), one during the 2-Week Appointment (Session 2) and two during the 4-Week Appointment (Session 3 & 4 in the Mobile Van), separated by ~15 min (flower users) to one hour (edible users) for appropriate absorption. We will measure changes in cannabinoid and inflammatory levels after the long (2- and 4-week pre) and short/acute (4-week post) time course of cannabis consumption.

DNA collection. DNA will be extracted from 10 ml of blood (out of the total 40 ml collected) at each visit. After extraction, DNA will be quantified and stored at -80° for future analysis. DNA will be collected to look at differences in epigenetic and single nucleotide polymorphisms (SNPs) in participants with varying levels of cannabinoid content after choosing to use cannabis with high versus low THC and CBD content. The blood DNA samples collected in this study are primarily for bio-banking purposes at this time, as the analysis of these samples are expected to be performed at a future date and therefore may contribute to the current study and/or future studies.

Lipopolysaccharide (LPS)-PBMC challenge. Cannabis has been shown to mediate the pro-inflammatory effects of LPS, therefore, this LPS challenge may represent an accurate test of the effects of the CBD and THC, as LPS stimulation of peripheral blood mononuclear cells (PBMCs) simulates the inflammation cascade that putatively mediates the neuroinflammatory effects of anxiety. To that end, PBMCs will be separated using density gradient centrifugation. Cells will be counted and viability assessed using trypan blue exclusion. PBMCs will be exposed to LPS (0, 0.1, 1, 10 and 100 ng/ml) for 20h in a 96 well v-bottom plate. Protein levels of

cytokines and chemokines (IFNg, IL-1a, IL-1b, IL-6, IL-2, IL-4, IL-8, IL-10, IL-12, and TNF) will be measured in supernatant using a multiplex ELISA assay (Aushon Biosystems, Billerica MA). Messenger RNA (mRNA) levels of the same cytokines and chemokines will be measured in cell lysates using real-time PCR. We have successfully performed the PMBC isolation and LPS challenge in other projects.

Gut Microbiome Sample Collection. Participants will be instructed on how to collect and return microbiome samples at the Baseline Appointment. Gut flora (from fecal samples) will be collected from participants using at-home kits and then stored at -80°. Subjects will be given one kit during their 2-Week Appointment and will return the kit to research staff at their 4-Week Mobile Lab Appointment (in the Mobile Van). A short dietary survey (EATS) will be conducted at the 2-Week and 4-Week Pre-administration Appointment to complement the microbiome kit and blood data collected. The microbial DNA collected in this study are primarily for bio-banking purposes at this time, as the analysis of these samples are expected to be performed at a future date and therefore may contribute to the current study and/or future studies.

We expect to see changes in abundance and diversity of microbiota populations found in the gut as a result of cannabis use. Because substance use, inflammation, and the gut microbiome, as well as pain and anxiety, and inflammation and the gut microbiome are all heavily intertwined (Fung et al 2017, Gorky & Schwaber 2016, Leclerq et al 2014, Mayer et al 2015, Peterson et al 2017), we will examine the relationship between changes in the gut microbiome with changes in anxiety and inflammatory biomarkers in the blood (cytokines and blood monocyte cell response to LPS challenge) as a result of cannabis use. Microbial DNA will be extracted from fecal samples and then analyzed to determine how many and which bacterial species are present in the gut. Gut microbiome data will be collected at two time-points during this study, once before and one after study cannabis use (i.e., with assigned cannabis ratios).

Daily (Online-based message) follow-up Assessments. Ecological momentary assessment of marijuana use via messaging has been shown to be feasible in a variety of marijuana users[62,63]. During the 4 week study, marijuana users will answer online survey assessments via email with individually tailored links hosted by our university's licensed survey tool RedCap. During the 4-Week study period, participants will report *daily* on the anxiety, their marijuana use, other stress management strategies, and their sleep pattern. This brief message report will provide a daily repeated measure of our primary constructs (anxiety and marijuana use) and potential mediators or moderators over the course of the 4-Week study. All subjects will be given extensive instructions on completing the daily message at the Baseline Appointment and will practice answering the questions in the lab using their cell phone or a lab computer to ensure they understand the process and time commitment. To enhance the response rate, participation on daily message follow-ups will be monitored and participants will be contacted if they do not respond for two consecutive days of daily messages. Participants will then be compensated for their time and effort during the 2-Week and 4-Week Appointments, receiving a $1/day with a $5 bonus for completing 13 of 15 online follow up message reports for each two-week period ($40 in total possible).

2-Week Appointment (Session 2) & 4-Week Appointment (Sessions 3 & 4). All procedures in the baseline assessment (blood draws for cannabinoids and inflammation markers, anxiety and health self-report measures, cognitive testing, as well as past two week measures of health behavior and substance use will be completed two weeks after the baseline session (Session 2) and finally at four weeks after the baseline session (Sessions 3 & 4).

The 2-Week Appointment (Session 2) will take place at our lab in the CINC and includes all session 1 measures; peripheral inflammation, and cannabinoid levels. However, we will not conduct the Rumination Induction task, but will add the Patient Global Impression of Change scale (Guy, 1976), which asks participants to rate their improvement over the prior two weeks on a 7-point scale. Subjects will also be given one at-home micriobiome collecton kit at this visit with instuctions on completing and returning the completed kit as near as possible to their 4-Week Appointment (after using their product). Participants will be given an ActiGraph wearable device as well on this Appointment, to use to track their physical activity and sleep during the final 2-Week study period (http://actigraphcorp.com/). They will be instructed on how to wear and use the device at this 2-Week Appointment. Participants will be instructed to wear the device as long as they feel comfortable during the last 2-4 Week period and to return the device at the 4-Week Mobile Appointment (3rd Appointment). Up to three attempts will be made to contact the participant and collect any wearable watch device that is not recovered (for any reason). The research staff will not make further attempts to collect the device, and will consider the device forfeited and irretrievable after this point. At the end of the 2-Week Appointment, research study staff will debrief participants and compensate them with up to $80 ($60 for the 2-Week visit and an additional $20 for the first 15 or 30 daily message reports) in cash.

For the 4-Week Appointment (Sessions 3 & 4), we will travel to the participant in our Mobile lab in order to ease subject burden and to get a more accurate measurement of acute cannabis effects. We will collect the microbiome kit. We will also complete the Rumination Induction Exercise for the 2nd and 3rd time (pre and post-cannabis use), and measure stress induction (via the Visual Analog Scale 1-100) both before and after Rumination Induction. We will also complete two new surveys (Drugs Effect Questionnaire and Addiction Research Center Inventory) to document the current effects and feelings that the subject has before and after cannabis use. After completing the acute Post-administration (Immediate or 1-hour) assessments, participants will hand in their ActiGraph device, they will be debriefed, and the RA will escort them back to their home. Participants will then be compensated up to $100 ($80 for the 4-Week visit and an additional $20 for the last 15 of 30 daily message reports) in cash. See additional details for each measure in Table 2.

Monthly (Online-based survey) Follow-up Assessments. After the 4-Week *ad libitum* period of cannabis use with detailed online and in-person tracking, participants will be asked to provide monthly follow-up information. This will help track participant behavior and potential effects naturalistically, with a monthly self-report of marijuana use, sleep, anxiety ratings/stress management, and cognitive effects over each month, for the five additional months. All subjects will be given extensive instructions on completing the monthly message report at the Baseline Appointment and will practice answering the questions in the lab using their cell phone or a lab computer to ensure they understand the process and time commitment. They will answer online survey assessments with individually tailored links hosted by our university's licensed survey tool RedCap. To enhance the response rate, participation on monthly follow-ups will be monitored and participants will be contacted if they if they do not respond to one monthly survey. Participants will receive a $6 gift card by email for every questionnaire that they complete (1st part of *Monthly follow-up survey*) a $5 gift card by email for every TLFB that they complete (2nd part of *Monthly follow-up survey*), plus a bonus gift card of $20 or $15 for 5/5 or 4/5 months (1st and 2nd part) completed ($75 or $70 in total possible, respectively).

Table 2. Description of procedures by appointment.

Name of Instrument/Procedure	***Data collected***	
Eligibility Screening (ES)	Phone Interview or Online Survey questionnaire used to determine subject eligibility (based on inclusion/exclusion criteria, listed above).	
Baseline Appointment (only)		
Informed Consent (IC)	In-person discussion and review of consent document detailing all measures and any questions.	
Medical History (MH)	General assessment of medical history for any major disease or illness (11-items).	
Toxicology Drug Screening (TDS)	A urine sample will test for recent substance use (e.g., marijuana, cocaine, benzodiazepines, MDMA, sedatives, or methamphetamine) other than pain medications.	
Pregnancy Screening (PS)	All female participants will take a urine pregnancy test and report their last menstrual cycle and any birth control methods.	
Demographics (D)	Age, sex, sexual orientation, marital status, race/ethnicity, SES, occupation, income, education, and religious affiliation.	
Neuropathic Pain Survey	3-item self-report rating (0 no pain to 100 unimaginable pain) of any neuropathic pain.	
Alcohol Use History Questionnaire (AUH)	Targets the frequency of lifetime and recent use for alcohol.	
Childhood/Recent Trauma Scale (C/RTS)	A self-report questionnaire identifying potential traumatic events experienced, specifying the intensity of the trauma (1 = not at all traumatic, 7 = extremely traumatic) and the approximate age that it occurred.	
Alcohol Use Disorders Identification Test (AUDIT)	The AUDIT (Babor et al., 2001) will be used to examine the extent of co-morbid alcohol use and problems related to alcohol use.	
The Beck Depression Inventory-II (BDI-II)	Consists of 21 scaled statements designed to assess symptoms of depression over the past 2 weeks and will be administered to examine comorbid depression and covary baseline differences if necessary (Beck, Steer, Ball, & Ranieri, 1996).	
Marijuana Withdrawal Checklist (MWC)	A 16-item scale used to collect information on withdrawal symptoms that one may have experienced the last time they stopped smoking marijuana (Budney et al., 2003).	
Cannabis Questionnaire/Marijuana Users Health Cohort/ (MUHC)	Details the primary reasons, frequency, and type of cannabis use patterns in occasional and regular users.	
Marijuana Consumption Questionnaire (MCQ)	Ask participants to reflect on the frequency and quantity of their cannabis use, age of first use, and perceived availability of cannabis (Heishman, Singleton, & Liguori, 2001).	
Self-Trait Anxiety Inventory (STAI)	The Speilberger Description Inventory, (State-Trait Anxiety Inventory, STAI; (Spielberger 1983), a commonly used measure of trait and state anxiety, for presence and severity of distress symptoms and anxiety based on participants feelings at the current moment. Participants respond to statements of current feelings on a 1 to 4 scale.	
ADHD Self-Report Scale (ASRS)	ADHD Self-Report Scale *(ASRS; Kessler et al., 2005),* will be used to assess ADHD symptoms in participants, given the high rates of ADHD among marijuana users. This measure consists	

	of 18 items that correspond to the DSM-V ADHD symptoms and are rated over the past six months on a 1 (never) to 5 (very often) scale.	
Baseline, 2- & 4-Week (Pre-administration) Appointments		
Perceived Stress Scale (PSS)	The PSS (Cohen, Kamarck, & Mermelstein, 1983) is a 14-item scale that measures the degree to which situations in someone's life are perceived as stressful. Stress is a known correlate of substance use and anxiety and it will be crucial for us to obtain a measure of our participants' perceived stress levels while they are in the study.	
Rumination Self-Report Scale (RSRS)	A brief self-report/assessment of typical rumination behavior (two sub-sets: brooding and reflection) and change in rumination (Nolen-Hoeksema & Morrow, 1993).	
Generalized Anxiety Disorder-7 Scale (GAD-7)	Self-report, 7-item measure to assess generalized anxiety disorder, a diagnostic tool commonly used to assess symptoms in the primary care setting (Spitzer et al 2006, Kroenke et al 2010).	
The Beck Anxiety Inventory (BAI)	Consists of 21 items, each describing a common symptom of anxiety over the past week and will be administered to examine comorbid anxiety and covary baseline differences if necessary. (BAI; Beck, Epstein, Brown, & Steer, 1988).	
Marijuana Dependence Scale (MDS)	The MDS based on DSM-V criteria that were converted to a self-report measure. Individuals respond 'yes' or 'no' to each dependence item (e.g., When I used marijuana, I often used more than I intended). The items are then summed to form the scale. This scale has been previously used in the cannabis literature. The internal consistency of the MDS (based on the DSM IV) was good in our pilot study (α=.73) and even better in previously published reports (α=.85; see Stephens, Roffman, & Curtin, 2000).	
Impact of Marijuana (IMP)	Short self-report questionnaire on the impact and expected impact of marijuana (e.g. negative effects or benefits on sleep, mood, health, and anxiety) compared to other types of pain and anxiety management strategies (e.g., drugs, opioids, exercise).	
Health Related Quality of Life (HRQL, SF-12)	This is a Short Form 12 Health Survey, which consists of 12 questions across eight health domains and is sensitive in detecting changes in time (one month) in health-related quality of life.	
Timeline Follow-Back (TLFB)	TLFB is used to assess daily substance use (for the 2 weeks) prior to the Baseline Appointment, 2-Week Appointment, and the 4-Week Mobile Appointment. Our modified TLFB procedure will estimate both frequency of marijuana (and other drug) use and amount used per day, using visual stimuli as well as the method of administration. (Sobell & Sobell, 1992) (Sobell, Sobell, & VanderSpek, 1979).	
Blood Alcohol Content (BAC)	Breathalyzer test for recent alcohol consumption.	

Pittsburg Sleep Quality Index (PSQI)	Measurement of the quality and patterns of sleep from poor to good measuring seven domains (e.g., latency, duration, disturbances) over the last 2 weeks.	
Depression Anxiety Stress Scale (DASS)	The DASS is a 21-item short-form self-report instrument for measuring the three related negative emotional states of depression, anxiety, and tension/stress.	
Self-Rated Diet (SRD)	1-item self-rated assessment of overall diet quality and amount of fruit and vegetables consumed daily, on average.	
Functional Assessment of Cancer Therapy-Cognitive Function (FACT-Cog)	A subjective measure of perceived cognitive impairments, ability, as it impacts others, and quality or life.	
Marijuana Purchase Task (MPT)	The marijuana purchase task (MPT, Collins et al., 2014) is a valid measure of the relative economic value of marijuana is needed to characterize individual variation in the drug's reinforcing value. This asks participants to estimate the number of marijuana joints they would purchase at increasing prices and can be used to examine the associations between marijuana use and MPT demand indices.	
Stanford Leisure Time Activity Categorical item (L-CAT)	Single-item question on exercise/physical activity, comprised of six descriptive categories ranging from inactive to very active (Kiernan et al 2013).	
2- & 4-Week (Pre & Post-Administration) Appointments		
Patient Global Impression of Change (PGIC) Scale	Ask participants to rate their level of improvement on a 1-7 scale (e.g., from completely gone to much worse) scale over the course of the 4-week study period.	
4-Week (Pre & Post-Administration) Appointments		
The Drug Effects Questionnaire (DEQ)	The Drug Effects Questionnaire (DEQ) is a 5 items visual analog scale used to measure the strength of marijuana as well as the desirable effects (de Wit & Phillips, 2012).	
The Addiction Research Center Inventory (ARCI/M-Scale)	The Addiction Research Center Inventory (ARCI; Martin, Sloan, Sapira, & Jasinski, 1971), including the ARCI—Marijuana (M) scale (Chait, Fischman, & Schuster, 1985) will be used to measure subjective effects of marijuana in addition to drug-induced euphoria, stimulant-like effects, intellectual efficiency and energy, sedation, dysphoria, and other somatic effects.	
Baseline, 2- & 4-Week (Pre & Post-Administration) Appointments		
Dot Probe Task	Measure of attention bias to threatening and negative valance stimuli (MacLeod et al 2007).	
Physiological Measures	Heart Rate, Blood Pressure, and Weight/Height (baseline only) will be measured using a basic fingertip pulse oximeter, automatic blood pressure wrist cuff, and scale/stadiometer.	
Venous Blood Draw (BD)	A 21-23g needle and vacutainer will be used to obtain 40 mL of blood through a peripheral arm venipuncture using standard, sterile, phlebotomy techniques.	

Pain Intensity-current (PITc)	Consists of 1-item asking about the participant's level of pain currently. Participants are asked to rate their pain on a scale from 0 (no pain) to 10 (worst imaginable pain).	
Neurocognitive Toolbox Battery (NCB)	From the NIH toolbox, this cognitive battery will include the Flanker Inhibitory Control and Attention task, and the List Sorting Working Memory Test. The battery covers the domains found to be sensitive to the effects of marijuana (for review see Ranganathan & D'Souza, 2006).	
International Shopping List Test (ISLT)	The International Shopping List Task (ISLT; Thompson et al 2011) is a computer software program, consisting of a 12-item shopping list that is read out loud to the participant. The participant is asked to recall as many words as they remember. The list of words is presented again in the same order two additional times to facilitate memorization. After 30 minutes, a delayed recall trial is administered and participants are asked to recall the list again. At each session, participants are read a different shopping list.	
Marijuana Craving Questionnaire (MCQ)	A Marijuana Craving Questionnaire will be used to assess craving at each time point during the Mobile session. The MCQ was adapted from a valid tobacco craving questionnaire (Tiffany & Drobes, 1991) and has proven to be useful in cannabis studies (Budney et al., 2003). In our pilot study, internal consistency was very high (α= 0.90).	
Alcohol Craving Question (ACQ)	Asks participants to rate their desire to consume an alcoholic beverage at the current moment, as a self-rated measure of co-administration potential.	
Motor/Movement Battery (MB)	Physical function assessment of dynamic sway (balance) and finger tapping. A standard smartphone (iPod Touch) will be attached to participant with a simple elastic/Velcro strap. An App on the device will record their fine-grained movements while they are asked to stand as still as possible for 30-60 seconds, with eyes open or closed, while the iPod is attached to their hip. They will also be asked to tap the smartphone repeatedly for no more than 60 seconds.	
Baseline & 4-Week (Pre-Administration) Appointments		
Eating at Americas Table (EATS)	Extensive self-report of typical fruits and vegetables eaten daily over the last month (Subar et al 2001).	
Baseline & 4-Week (Pre- & Post-Administration) Appointments		
Rumination Induction (RI) Task	Focused breathing and rumination induction exercise to experimentally examine the active occurrence of rumination thought and how it affects stress and cognitive function,, intermixed with a visual analog scale of perceived anxiety and stress used to measure changes in intensity of negative emotional responses (0-10; Rood et al 2012, Arch & Craske 2006).	

Perceived Discrimination Scale (PDS)	A 20-item scale to measure how often people feel that others treat them badly or unfairly based race, ethnicity, gender, age, religion, physical appearance, sexual orientation, or other characteristics separated into two subscales: The Lifetime Discrimination Scale and Daily Discrimination Scale.	
Positive and Negative Affect Schedule (PANAS)	Self-rated measurement of positive and negative affect with two 10-item scales from 1 (not at all) to 5 (very much; Watson 1988).	
Profile of Mood States (POMS)	The Profile of Mood States (POMS) will be used to collect baseline information on mood as well as information on mood changes throughout the study. (Johanson & Uhlenhuth, 1980; McNair, Lorr, & Droppleman, 1971).	
4-Week (Post-Administration) Appointment		
Assessment of Marijuana Potency	At the end of the experimental session, participants will be asked to report which product they received including the ratio and potency of THC and CBD in that strain.	
Appointments/Time points	***Instruments/Procedures***	***Time to complete***
Baseline Appointment (CINC Lab) **Session 1**	Informed consent & study orientation; BAC test; Urine toxicology & Pregnancy test; Physiological measures; Anxiety & Stress ratings; 1st Blood draw; Questionnaires; Cognitive & Motor Battery; Microbiome distributed; Appointment 2 scheduling; Monetary Compensation.	120 minutes
Daily Follow-Up Messages ***(Days 1-15)***	Respond to brief questions (online) about marijuana use, sleep quality, and anxiety/stress management 1x/per day for 2 weeks.	~2 mins/day x 15 days (~30 mins total)
2-Week Appointment (CINC Lab) **Session 2**	BAC test; Physiological measures; Anxiety/Stress ratings; 2nd Blood draw; Questionnaires; Cognitive & Motor Battery; Microbiome & Wearable distributed; Appointment 3 scheduling; Monetary Compensation.	60 minutes
Daily Follow-Up Messages ***(Days 16-30)***	Respond to brief questions (online) about cannabis use, sleep quality, and anxiety/stress management 1x/per day for 2 weeks after self-dosed cannabis use.	~2 mins/day x 15 days (~30 mins total)
4-Week Appointments (Mobile Lab) **-Pre-administration Session 3**	Immediately before cannabis use (type and dose self-selected); Participant comes out to Mobile laboratory; Return completed microbiome kit, Physiological measures; Anxiety & Stress ratings; 3rd Blood draw; Questionnaires; Cognitive & Motor Battery; Return home to self-administer edible.	60 mins
-Post-administration Session 4	Immediately *(flower)* or 1-hour *(edible)* after self-dosing cannabis (time rage based on type of product chosen and similar drug onset time); Return to Mobile laboratory; Physiological measures; Anxiety & Stress ratings; 4th Blood draw; Questionnaires; Cognitive & Motor Battery; Return wearable; Debriefing; Monetary Compensation; Return home.	60 mins
Monthly Follow-up Surveys ***(Months 2-6)***	Respond to brief questions (online) about major behavioral changes (e.g., marijuana use, sleep quality, and anxiety/stress management) 1x/month for 5 months, Monetary compensation (each month).	~12 mins/month x 5 months (~60 mins total)

TOTAL TIME (6 months)		**~8 hours**

XIII. SPECIMEN MANAGEMENT

Blood and microbiome samples collected in the mobile lab will be kept in an insulated biohazard transport bag. All blood and microbiome samples collected during the study will be stored in locked freezers within the PI's laboratory designed specifically for storing biological specimens. Samples will be coded with a randomly generated participant ID number and all data collected will be stored on a password protected server and separate from the master list linking the ID numbers to participants' contact information, also stored on a password protected server and only accessible by a research team member. At study closure, all links between participant name and number will be destroyed, at which point the specimen will be considered de-identified. After all analyses are complete, these specimens will be destroyed.

XIV. DATA MANAGEMENT

Signed consent forms will be stored in a locked filing cabinet in the PI's lab at the CINC. All data from self-report and interview measures will be stored on password protected computers and on the PI's password protected server in the CINC, both of which are only accessible to research staff. All stored data will be recorded from secure survey software. Any identifying information and biological samples will be destroyed after all analyses are complete. After this, there will be no way to connect participant's names with participant data, at which point they will be considered de-identified.

XV. WITHDRAWAL OF PARTICIPANTS

Situations in which the entire study may be terminated early include the following: If the Principal Investigator or other governing official discovers serious concerns about subjects' safety, inadequate performance, or rate of enrollment (this includes a missed study session); because study objectives have been obtained according to pre-established statistical guidelines; or in the unlikely event that the Principal Investigator retires and no other additional investigators are able to succeed her role within the research project. Though highly unlikely, the circumstances under which a participant would be withdrawn without his or her consent include: obviously not following instructions or displaying behavior that is verbally or physically abusive towards research staff. Those who experience early withdrawal will receive prorated payment based on the number of sessions they completed.

XVI. RISKS TO PARTICIPANTS

Risks Pertaining to the Legality of Cannabis. The possession and use of cannabis is legal at the state level but illegal at the federal level. Any risk associated with this study is not greater than risks experienced by participants normally, since a participant in the cannabis groups must be regular cannabis users to be in the study**.** We will comply with all NIH guidelines, and a

Certificate of Confidentiality will be deemed applicable for our study, decreasing the risk for participants.

Risks Associated with Venipuncture. There is a small risk of local hematoma, infection, and syncope associated with phlebotomy. Any risk associated with undergoing four blood draws for a total of 160 ml over ~ five hours in three separate days will be minimized with certified and experienced venipuncture staff, collecting information on the subjects last meal and by offering a standard snack before the last blood draw.

Psychological Risks and Discomforts. While participants who use cannabis in the context of the research must already be experienced cannabis users in order to be in the study, it is still possible that some participants might experience some adverse effects from the cannabis such as changes in mood/affect, sleepiness, paranoia, and increased heart rate. There is a slight risk that using cannabis may be associated with a psychotic episode. However, participants will be monitored during the study period by the PI Dr. Bidwell who is a licensed clinical psychologist (Colorado license #4116) to make sure that there are no clinically significant events that occur. Increases in anxiety or discomfort will be monitored and should clinically severe or impairing anxiety be present, the participants will be removed from the study and referred to appropriate mental health treatment. This practice has been used frequently by the PI in other IRB approved studies and the risks for clinically meaningful increases in anxiety are not expected to be greater than what would be experienced in daily living. Information regarding anxiety treatment resources and appropriate referrals will be made to each participant at the end of participation, if requested.

Risks Pertaining to Loss of Confidentiality and Privacy. Confidentiality of participants is a priority for research staff and must be maintained unless the investigator obtains the express permission of the participant to do otherwise. Risks from breach of confidentiality include invasion of privacy, as well as social and economic risks. Economic risks include alterations in relationships with others that are to the disadvantage of the subject, and may involve embarrassment, loss of respect of others, labeling with negative consequences, or diminishing the subject's opportunities and status in relation to others. These risks include payment by subjects for procedures, loss of wages or income, and/or damage to employability or insurability.

Participants will be asked about illegal activities that they may have been involved in (i.e. illicit drug use). Participants will also be warned that there are some things that they might tell us that we CANNOT promise to keep confidential, however all NIH funded research involving human subjects and identifiable data, bio-specimens or genomic data, will be issued a Certificate of Confidentiality for all research that is commenced or ongoing after December 1st 2017. Updates to our associated documents (e.g., consent form, see attached) and IRB approval is underway and will be followed and managed, as directed by NIH. Participants will be informed that we are required to report information like child abuse or neglect, crimes that they tell us they or others plan to commit, or harm planned against themselves or others.

Unanticipated risks. Any experiment may involve risks that cannot be anticipated. If unanticipated risks occur, the investigators will follow the IRB guidelines for adverse event reporting.

XVII. MANAGEMENT OF RISKS

Risks Pertaining to the Legality of Cannabis. As mentioned previously, the risk is minimal given the legality of cannabis in Colorado and does not represent a significant increase in risk for non-cannabis or cannabis participants (individuals who are already planning to be or are regular cannabis users).

Protection against risks associated with Venipuncture. The risks of hematoma and infection are minimized by having trained personnel perform the procedures using sterile techniques. Any additional risks are decreased by using the participants preferred arm for venipuncture (in case prior injury or surgery has decreased function) and by reminding participants to adequately hydrate prior to their appointment. In addition, we provide snacks and complete supervision (with two trained research assistants) in a seated and protected position to reduce any risk of dizziness or falling.

Psychological Risks and Discomforts. In the unlikely event that a cannabis user has an adverse reaction to any cannabis or any participant has a negative experience and needs assistance, the research assistant will immediately notify Dr. Bidwell who will make herself immediately available to evaluate the condition of the participant and intervene if necessary. Dr. Bidwell (clinical psychologist), Dr. Bryan (social psychologist), and/or Dr. Hutchison (clinical psychologist) will be on call/reachable during all scheduled session. Although we do not anticipate adverse reactions or any greater risk then daily life, we have developed a plan should a cannabis or non-cannabis related negative event occur. First, we always have two trained members of the research staff in the mobile laboratory any time they are with a participant. Should a concerning event arise, if it is an emergency, staff will be instructed to immediately call 911. Second, or in a non-emergency case, staff will call the PI or Co-Is Bryan or Hutchison (one of them will be on call) to resolved the situation. First, it will be determined if the situation can be resolved over the phone. If not, the PI or Co-I will drive out to the mobile laboratory for further assessment of the participants and situation. The participant will be given the option of withdrawing from the study and medical or mental health referrals will be made as appropriate and as determined by the clinically trained Senior Investigators. Importantly, mobile lab staff are first aid trained and the PI Bidwell and Co-I Hutchison are both licensed clinical psychologists.

Risks Pertaining to Loss of Confidentiality and Privacy. We intend to mitigate risks as much as possible by collecting the minimum amount of identifying information from participants necessary to conduct our study. Participants' information will be coded with a randomly generated number, and the document linking their number with their contact information will be stored on a password protected server that is only accessible by members of the research staff.

All study computers are password protected and housed in the PI's lab space at the CINC, which are kept locked unless researchers or students are currently using the space. Further, there is no identifying information contained on the laptops. All identifying information (e.g., consent forms, contact information) is kept separate and secure from the data files and never on the same laptop.

XVIII. POTENTIAL BENEFITS

There is no direct benefit to participants for their participation; however, all cannabis user participants will have the opportunity to examine their own cannabis use in the context of completing the measures. The minimal costs associated with participation in this research seem reasonable in relation to the scientific importance of gaining insight into the health-related

implications of cannabis use, particularly given the timely nature of this study and the recent legislation regarding cannabis.

XIX. PROVISIONS TO MONITOR THE DATA FOR THE SAFETY OF PARTICIPANTS

The project manager will monitor and report to the PI on adherence to the protocol. He/she will assess adherence via periodic observation of the sessions, visual inspection of the completeness of data collection, and verification of follow-up information collected from participants (to ensure they have agreed to be contacted). He/she will give bi-weekly reports to the PI. The project manager will use the Reportable Event eForm and/or the Deviation eForm in eRA to report all adverse events consistent with those listed under points 19.1 (adverse events) and 19.2 (deviations) in the CUB IRB policy procedures document: (http://www.colorado.edu/VCResearch/integrity/humanresearch/SOP_TOC.html). Consistent with IRB policy, the reporting will occur: Immediately (within 24 hours) upon learning of a study-related death, study personnel will notify the IRB via e-mail by providing a brief summary of the event. Then, within ten business days, the PI or designee will submit a Reportable Event in eRA. For any other problem or event requiring reporting to the IRB, the PI or designee will submit to the IRB a Reportable Event or Deviation in eRA as soon as possible, but no later than 10 working days from notification of event. The PI will be in daily contact with the research assistants running the study and will be informed immediately of any adverse event.

XX. PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

To ensure participants' confidentiality, all data will be identified with a unique research subject identifier in a randomized, confidential manner. This system is operated by the research team member who can only access the program by using a login name and password. The list linking the numerical identifier to the participant's identifying information will be maintained separate and secure from the data and will be destroyed at study closure. The data files themselves will be maintained in the CINC at the University of Colorado, and will be identified only by the numeric identifier. Only staff cleared on a specific project can view data collected on that given project.

Fully informed consent will be sought to ensure that participants are aware of any possible risks. Participation in the research is completely voluntary, as is answering each particular question in all of the measures and providing each physiological measure.

XXI. MEDICAL CARE AND COMPENSATION FOR INJURY

Participants will be informed to contact Dr. Bidwell immediately by phone (303-735-5383) should they feel that they have been harmed while participating in this study. They will be told that the cost for any treatment will be billed to them or their medical or hospital insurance. Information regarding compensation for injury is included in the informed consent document.

XXII. COST TO PARTICIPANTS

Participants will be responsible for paying for the cannabis that they choose to buy. We estimate the cost of 1 grams of flower cannabis to be approximately $12 and 75mg of edible cannabis to be approximately $15 depending in part on what type and how much each participant chooses to use. Parking is free at the CINC.

XXIII. DRUG ADMINISTRATION

Not Applicable. All drug use will be self-directed.

XXIV. INVESTIGATIONAL DEVICES

Not applicable.

XXV. MULTI-SITE STUDIES

Not applicable.

XXVI. SHARING OF RESULTS WITH PARTICIPANTS

There are no plans to share results of the study with participants.

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