University of Colorado Boulder

TITLE: Cognition, Mood, and Cannabis

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In addition to the co-investigators listed above, undergraduate and professional research assistants will 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 this project is supported by a grant funded by the National Institute on Drug Abuse. The grant and protocol follow many the same procedures as our pilot study that has been approved for almost two years; "An Observational Study of Cannabidiol, Neurocognition, and Mood," and the protocol number is 14-0087 (e.g., having the participants purchase a strain from a local dispensary with some subjects being assigned

to less harmful strains that are lower in THC or higher in CBD). IRB approval of the protocol is needed to respond to the just-in-time request by January 2016.

Over the last several years, the United States has witnessed enormous changes concerning the acceptance of marijuana. Colorado, Washington, *Oregon, Alaska, and D.C*. have legalized recreational use of marijuana with several other states likely to follow in 2016. In the first four months of 2014 in Colorado, sales of marijuana exceeded $200,000,000. In 2015, state officials expect demand in the legal market to exceed 130 metric tons. Considering the rapidly changing cultural, political, and legal landscape, the scientific literature that ***should*** inform public policy and harm reduction approaches is inadequate and outdated.

There are a number of critical limitations in the extant literature that must be addressed if scientists are to influence the regulation of marijuana. First and foremost, scientists need to understand the effects of commonly used marijuana strains, **as they are used in every day life**, as opposed to relying solely on testing the effects of government grown marijuana in controlled laboratory experiments. This information is critical for making recommendation about how to reduce the potential for harmful use of marijuana. For example, cannabidiol (CBD) appears to mitigate some of the effects of tetrahydrocannabinol (THC), and the ratio of CBD to THC may have a large impact on the effects of the marijuana, which in turn may have important implications for harm reduction. To that end, the overarching objective of this program of research is to advance a more nuanced understanding of the potential harm associated with different strains of marijuana, using a naturalistic design with high external validity, thereby avoiding many of the pitfalls and limitations of previous research. **To that end, regular marijuana users who normally use marijuana with a THC potency of ~18% will be asked to either continue to smoke or ingest normal marijuana or switch to a strain that is theoretically less harmful (i.e., has less THC and/or more CBD).** The information gained from this research will inform the public about the potential harms of different cannabinoids and strains. It may also have a translational application with respect to identifying THC levels or CBD:THC ratios that could be the focus of future regulatory actions.

1. The first specific aim will examine cannabis products that differ markedly on THC potency (low, medium, or high: i.e. 6%, 12%, 18% THC for smoked flower and low, medium, and high: i.e. ranging from less than 2.5mg to greater than 10mg THC for edibles) but do not differ on the potency of other major cannabinoids (< .5%) to determine whether the level of THC in the product is associated with increased intoxication and greater harmful effects using a naturalistic design.

- **Hypothesis 1.** Assuming that greater THC potency leads to greater levels of intoxication, it is hypothesized that individuals who receive the higher THC strains will demonstrate significantly greater blood THC levels. *Alternatively, it may be that individuals titrate their dose based on potency so that they achieve the same blood levels, in which case blood levels of THC should not differ between the strains.*
- **Hypothesis 2.** It is hypothesized that blood levels of THC will be positively associated with greater harm on a number of variables previously shown to be associated with THC, such as diminished working memory and increased drug reward.

II. BACKGROUND AND SIGNIFICANCE

Background

Over the last several years, the United States has witnessed enormous changes concerning the public acceptance of marijuana. As of July of 2014, 23 states and the District of Columbia have legalized the medical use of marijuana. In addition, Colorado and Washington have now legalized recreational use of marijuana.

Since the original grant was submitted, Oregon, Alaska, and D.C. have also legalized recreational use. Several other states (e.g., CA, AZ, NV, VT, NH, ME) are likely to follow in 2016. In the first four months of 2014 in Colorado, sales of recreational marijuana totaled approximately $70,000,000, while sales of medical marijuana were close to $133,000,000. In addition, officials with the State of Colorado estimate the demand for legal marijuana will exceed 130 metric tons in 2015. This estimate does not include consumption in the black market. Considering how much has already changed on the cultural, political and legal front regarding marijuana use and given how rapidly these changes have taken place, the scientific literature that **should** inform public policy and consumer decisions, especially with respect to harm reduction, is inadequate and outdated.

There are a number of critical limitations in the extant literature that must be addressed if NIDA scientists are to influence the regulation of marijuana. First and foremost, scientists need to understand the effects of commonly used marijuana strains, **as they are used in every day life**, as opposed to relying solely on testing the effects of government grown, low potency marijuana in controlled laboratory experiments. These studies are devoid of external reliability because commonly available strains of marijuana have 3-5x greater potency as compared to strains used in previous laboratory studies and because commonly used methods of administration are very different than the methods used in laboratory studies. It is entirely possible that laboratory based studies underestimate the effects of more potent strains that are widely available. A second limitation is that scientists have focused on the effects of tetrahydrocannabinol (THC) while mostly ignoring the other major cannabinoids found in marijuana. Scientists need to conceptualize the effects of marijuana as the compound action of a number of different cannabinoids. Many cannabinoids may modulate the effects of others and some cannabinoids are more inherently harmful than others (see Huestis, 2007; see our preliminary studies). For example, THC and cannabidiol (CBD), two of the major cannabinoids in marijuana, have different effects. CBD may mitigate some of the negative effects of THC and even have some beneficial effects. In fact, **one major premise of this application, reviewed in detail below, is that the ratio of CBD to THC may be a primary determinant of many of the effects of marijuana, including some of the harmful effects.** The review below will summarize the extant literature on the effects of marijuana, specifically what is known about the effects of the two major cannabinoids, THC and CBD, before discussing the limitations of the literature and significance of addressing these limitations in the context of the current application.

Acute Effects on Mood, Reward, and Cognition

Literature on the acute effects of marijuana dates back to the 1970s. One of the primary methods used to understand the harmful effects of marijuana has been standardized smoking of marijuana in a laboratory setting. Measures related to cognition and drug reward are collected before and after administration. With respect to self-report measures, a number of studies have found that marijuana acutely increases positive mood and measures of reward (Heishman et al., 1990; Chait & Zacny, 1992; Chait et al., 1988). These effects also appear to follow a dose dependent function based on the THC content of the marijuana (e.g., Hart et al., 2001). 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 ratings of mood and reward (e.g., Hart et al., 2002; Wachtel et al., 2002). More recent studies have found similar results: that acute marijuana use increases subjective effects such as 'high' and 'liking,' (Cooper & Haney, 2008; Schacht, Selling, & Hutchison, 2009) even when using a "balanced placebo" design (see Metrik et al., 2012). Thus, there is a long history of studies in the literature documenting that acute marijuana use produces changes in measures related to positive mood and reward.

Other studies have focused on the harmful cognitive effects of marijuana. Studies suggest that marijuana produces acute cognitive impairment, especially relating to memory and attention during intoxication (for review see Ranganathan & D'Souza, 2006; Lundqvist, 2005). For example, studies that were published as early as the 1970's suggest that marijuana disrupts immediate and delayed free recall of information (e.g. Abel, 1971; Darley et al., 1974). Recall of words from a list is one of the most common approaches to demonstrating the effect of THC on recall performance (e.g., Heishman et al., 1989). Studies have also suggested that acute

marijuana may interfere with working memory, as evidenced by the effect of marijuana on the digit symbol substitution task (DSST), which some studies suggest may be the working memory measure most sensitive to the acute effects of marijuana (see Wilson et al., 1994). In sum, the literature suggests that acute administration of marijuana impairs the encoding and retrieval of information, albeit to a modest degree. However, it is important to note that one of the limitations of this literature is that most, if not all of these studies, have utilized a laboratory administration of low potency marijuana that may not accurately reflect the effects experienced in the real world.

Is Greater THC Potency Associated with Greater Harm?

Recent reviews have suggested that the greater potency of today's marijuana, compared to earlier decades, may lead to significantly greater levels of intoxication and harm (e.g., Volkow et al., 2014). However, only a few previous studies have actually examined the effect of marijuana potency on smoking topography and subjective and cognitive measures, and these studies are outdated. For example, an early study that compared the different potencies of marijuana (.36%, .73%, 1.45% THC) found that individuals who were given the lower potency marijuana smoked significantly more than individuals who smoked the high potency marijuana, suggesting that individuals may compensate for lower potency by simply smoking more (Cappell & Pliner, 1974). However, overall levels of subjective intoxication were still greater in the high potency group, indicating that individuals may smoke less but still experience greater harm with the higher potency marijuana. A subsequent study found no effect of potency on smoking topography (Perez-Reyes et al., 1982). A study published in the late 1980s used a more sophisticated approach to compare marijuana with three different potencies (0, 1.3%, 2.7% THC). Heishman et al. (1989) suggested that individuals significantly changed their smoking topography when receiving the higher potency marijuana, as evidenced by lower puffs and inhalation volumes. Others have also noted changes in topography, such that individuals who receive higher potency marijuana smoke less, even though there were still dose response effects (Kelly, Foltin, & Fischman, 1993). In sum, a handful of studies prior to 1993 suggest that individuals smoke less when they are smoking a higher potency strain, but may still experience greater intoxication and marijuana effects. As noted by Volkow et al. (2014), this literature is seriously limited by the potency of the marijuana used (usually 0 to 3.5%). The THC potency of marijuana in Colorado often exceeds 18%. Studies are desperately needed to examine these higher potency strains.

Does CBD Mitigate the Harmful Effects of THC?

Clearly, the evidence suggests that acute marijuana use is associated with cognitive impairment, mood alterations, and reward responses. 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. The effects of other cannabinoids, such as CBD, are important to consider when interpreting the harmful effects of marijuana use, especially given that marijuana includes more than 80 additional cannabinoids (Izzo et al., 2009; Huestis, 2007). Strains commonly available in states like Colorado can differ dramatically on the potency of these cannabinoids. As a concrete example regarding the importance of cannabinoids other than THC, several studies have demonstrated that THC and CBD have very different effects on mood and cognition. As noted previously, results from both imaging and non-imaging studies suggest that THC is associated with memory impairment (for review see Ranganathan & D'Souza, 2006), anxiety/negative mood effects (Vann et al., 2008), and positive mood and reward (Bhattacharyya et al., 2012; Cooper & Haney, 2008). Conversely, analyses suggest that CBD may attenuate the negative effects of THC on mood, reward, and cognition (Englund et al., 2013; Hermann & Schneider, 2012; Fusar-Poli et al., 2010; Demirakca et al., 2011; Schubart et al., 2011; Vann et al., 2008; Bhattacharyya et al., 2012; Bhattacharyya et al., 2010; Morgan, Schafer, Freeman, & Curran, 2010). Most of the work on CBD has involved the administration of THC and CBD in pill form. Much less is known about the combined effects of THC and CBD when using

marijuana in the plant form. Given that oral CBD mitigates some effects of THC, this is a high priority area of research in terms of understanding how to reduce the potentially harmful effects of marijuana.

Limitations of the Extant Literature

There are important limitations to the extant literature that must be addressed given the changing landscape. The most important limitation of studies to date is the exclusive use of government produced, low potency marijuana administered in a tightly controlled laboratory environment, which compromises external validity. In all of the studies that used plant material cited above, the source of that material was the NIDA sponsored farm, which is the only legal source for laboratory administration of marijuana in the United States. Until very recently, this supply was derived from seed stock obtained from marijuana grown in Mexico in the 1960s. In effect, all of the studies published through 2006 used this low potency marijuana, while the potency of CBD in this research marijuana was close to zero. As a concrete example of compromised external validity, when we conducted a study with a controlled administration of marijuana in 2006, we were provided with marijuana with a THC potency of 3% (see Schacht et al., 2009). The participants in this study reported that the quality of the marijuana was poor and reported that they had a difficult time smoking it, which is not surprising given the average quality and potency of marijuana in Colorado in 2006 was considerably greater. Consequently, the external validity of that study was compromised. Currently, the THC potency of recreational marijuana in Colorado can exceed 20%, and the potency of CBD comes close to 20% in some strains. One of the often-cited potential harms of marijuana legalization is the availability of high potency marijuana, which may lead to greater levels of intoxication and greater harmful effects (Volkow et al., 2014). Unfortunately, the data on this issue are very limited, if not non-existent. We know nothing about the effects of high potency marijuana. Clearly, we need to develop a knowledge base regarding the effects of commonly used, high THC strains of marijuana in order to understand the harmful effects.

A related limitation is the exclusive reliance on laboratory administration approaches that do not reflect how marijuana is used outside the laboratory. While laboratory studies that emphasize internal validity certainly play an important role in the scientific literature, it is critical that the knowledge base also includes studies that emphasize external validity. Previous laboratory studies often combined the low potency marijuana cigarette with a controlled puffing procedure (see Heishman et al, 1989; Schacht, Selling, & Hutchison, 2009). While this method helps to control the dose across subjects, it may have little to do with how people actually use marijuana, especially given the huge discrepancy in THC potency between what has been traditionally available from NIDA (e.g., 6%) and what is available in a state like Colorado (e.g., 20+%). Thus, prior studies that have relied on government produced marijuana may be underestimating effects given the lower potency of government marijuana. Alternatively, prior studies may be overestimating the effects given that subjects are often asked to smoke an entire marijuana cigarette in 5 minutes (e.g., Schacht, Selling, & Hutchison, 2009). The fact that we know little about harmful effects of high THC marijuana, as it is commonly used in legal states like Colorado, is the primary justification for Specific Aim 1.

The other important limitation of previous studies on the harmful effects of acute marijuana use is that they often ignore other cannabinoids such as CBD, which may mitigate the effects of THC. In fact, the only studies to date that have examined CBD have used a synthetic, pill version. While these studies show that CBD mitigates the effects of THC, marijuana users do not use synthetic THC or synthetic CBD in pill form. Thus, the field desperately needs data regarding how cannabinoids, particularly CBD, may mitigate or exacerbate the harmful effects of THC in the real world, which is the primary justification for Aim 2.

Finally, CBD is just one of 80+ cannabinoids that may mitigate or exacerbate the harmful effects of marijuana. The genetic and chemical diversity of cultivated marijuana is enormous, and the diversity is readily evident in the dispensaries. An examination of dispensaries across Colorado suggests that there are several hundred distinct strains and the primary cannabinoids (THC, CBD, CBG, CBN, THCV) may vary more than 40 fold across these strains. To get a sense of this diversity, see http://tgscolorado.com/strains or

http://riverrockcolorado.com/mainmenu/flowers/. Some of these cannabinoids, like CBD, may mitigate the harmful effects of THC. Others may exacerbate the harmful effects. While it is beyond the scope of this application to examine all of these cannabinoids, we have included an exploratory aim and analyses to examine additional cannabinoids and to examine the genetic diversity underlying the chemical diversity.

Summary of Significance, Impact, and Innovation

With respect to public education regarding marijuana more generally, harm reduction efforts to date have focused on the message that all marijuana is harmful. This approach is potentially problematic because of numerous reports in the press (e.g. Sanjay Gupta's 2013 CNN report) that some forms of marijuana are showing efficacy at treating seizures in children and other disease states. It is becoming increasingly clear to the lay public that there are many different forms of marijuana. An evidence-based message clearly describing the potential harms of specific forms or compositions of marijuana would be more likely to have an impact in terms of harm reduction efforts. It is expected that the proposed research will generate information about which strains produce the most harmful effects and which strains produce the least harmful effects. Successful dissemination of this information to the public is likely to inform personal decisions about whether to use marijuana and which type of marijuana to use. It will also inform policy makers who seek to develop new regulations, perhaps mandating specific cannabinoid levels, to reduce the harm of recreational marijuana use.

To be more specific about the significance of this application, the proposed research is designed to address the limitations noted above, and in doing so, is expected to have a significant impact on the knowledge base regarding the acute effects of marijuana, as it is used in the real world, particularly with respect to the effect of different levels of THC. The existing literature that relied heavily on low potency marijuana may have little relevance to the situation we face today. Study 1 is significant because it will provide real world data on the degree to which the THC potency of the marijuana leads to greater harm, which others have noted is of paramount importance (Volkow et al., 2014). Study 1 also has direct translational implications. Data linking higher potency to greater harmful effects could be used to inform the public about which types of marijuana to avoid. This information may also be used to eventually identify THC potency targets for future regulatory efforts. Thus, we expect Study 1 to have an important translational impact in terms of harm reduction efforts.

III. PRELIMINARY STUDIES

As noted above, the PI (Hutchison) has conducted prior research on the acute effects of cannabis (Schacht, Selly, and Hutchison, 2009) as well as research on cannabis withdrawal and cue-elicitied 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. Suzanne Taborsky-Barba conducted a five year NIDA funded study (Ito, Hutchison, Bryan et al. 2009) which involved over 1000 personal interviews on detailed cannabis use. Thus, they are uniquely qualified to head this research project.

IV. RESEARCH STUDY DESIGN

Overview and Design of Study 1 (e.g., Aim 1, Hypotheses 1 and 2).

Study 1 is designed to test the hypothesis that a high THC strain of marijuana will be associated with greater blood levels of THC and greater harmful effects, as compared to a lower THC strain, when used in a

naturalistic environment. To that end, regular flower 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 no CBD but with different THC potencies (low, medium, or high: e.g. 6%, 12%, or 18% THC for smoked flower) and regular edible marijuana users who typically purchase 10 mg products will be allowed to use as they normally would (producing naturally occurring low, medium, and high groups: e.g. less than 2.5mg to greater than 10 mg THC n=33 per group) for five days prior to participating in the experimental session. Measures will be collected at Appointment 1: (Baseline/**Session 1**), after 5 days of using the assigned strain, but in a sober condition before use in our mobile laboratory at Appointment 2: (**Session 2**), they will then return to their home to use the assigned strain and return to the mobile laboratory (**Session 3**), and 60 minutes after their last use at home (**Session 4**). Details regarding sample ascertainment, marijuana administration, timing of measures to be collected, power analyses, and the analytic plan for Study 1 follow. Thus, Study 1 will involve a 3 Cannabis strains: high, medium, and low THC x 4 (Time: 1, 2, 3, 4) mixed factorial design.

Participant Recruitment and Selection.

Recruitment to date has primarily come from flyers, business cards, and announcements placed in dispensaries in Boulder. With our new approach that involves driving the mobile lab to the participants' location, we will be able to broaden our catchment area. Interested participants will be asked to call the lab. The study technician will then screen the participant according to the inclusion criteria below. If the participant meets inclusion criteria, he or she will be scheduled for the next available appointment to complete informed consent and baseline measures. Participants will be instructed not to drink alcohol within 24 hours of the appointment, or consume caffeine for one hour prior to the appointment (note: individuals who smoke tobacco daily, consume alcohol more than 2x per week (and 3 or more drinks per occasion), or use other illicit or prescription drugs are excluded). It is important to note that dispensaries in Boulder attract customers who are diverse in age, sociodemographic status, gender, and race. In addition, we exclude University of Colorado students and employees for legal reasons, which has the added benefit of resulting in a more diverse sample. Thus, men and women of all ethnic backgrounds will be recruited into the study. We expect that the sample will reflect the diversity of Boulder/Denver area of Boulder, such that approximately 35% of the final sample will represent Latino and non-Caucasian individuals.

Marijuana Administration at Home.

Unlike other studies done in the United States to date, the proposed work will utilize a design that is observational but contains some experimental elements. Specifically, at the end of Appointment 1 (Baseline/Session 1) participants will be given instructions to purchase 3 grams of either a normally high THC strain, a medium THC strain, or a low THC strain at a dispensary (The Farm) in Boulder, Colorado. The normally high THC smoked flower strain contains 18% THC and 0% CBD and edibles can range from a low of 2.5 mg to greater than 10 mg of THC and 0 mg of CBD.. **Please note the term "high" is relative given that the average potency in Colorado exceeds 18%and edibles are normally packaged in 10 mg THC doses.**. The medium flower strain contains 12% THC and 0% CBD. The low flower THC strain contains 6% THC and 0% CBD. Colorado requires all strains of flower and edibles to be tested by a state licensed lab, which allows us to have a precise measure of potency to operationalize our high, medium, and low THC strains. Furthermore, the dispensary that we will work with (The Farm - see letter of support) performs cannabinoid and terpene potency testing on each batch produced. The dispensary will set aside a specific lot of the strains which will be labeled as "Strain A", "Strain B", and "Strain C" such that participants, dispensary staff, and research staff will be blind to which strain was purchased. Thus, 3 grams of each marijuana strain will be packaged in childproof bottles labeled as "Strain A", "Strain B", and "Strain C." Our preliminary data suggests that 3 grams is sufficient for the study. Dr. Bryan (co-investigator) will maintain the blind. The instruction to purchase "A" or

"B" or "C" will be urn randomized across participants such that equal numbers of male and female participants are included in the high, medium, and low conditions. The observational aspect of the design involves the instruction that participants can use as little or as much of the marijuana as they wish during the five days prior to the experimental appointment. The five-day period allows each participant to become accustomed to the strain. Subjects are prompted by email or text message to report how much was used each day using an online reporting system. On the fifth day, the research assistant calls the participant one hour prior to their appointment to remind the participant about the upcoming appointment from the research team.

After consulting with CU legal counsel, we now plan to utilize a mobile lab, which will facilitate blood and data collection on site, rather than transporting the subjects to the lab for assessments as we did in our preliminary study. The mobile lab will be based on the Dodge/Mercedes Sprinter van platform (see pictures) with comfortable seating and space for blood draws and cognitive testing on laptops. This change addresses potential problems, like the variable (10 - 20 minute) delay between the time the individual uses the marijuana and the time we can collect the first blood sample, self-report, and cognitive data. This change also addresses concerns with how feasible it is to recruit a sufficient number of subjects within a short driving radius of the lab. There are other advantages; the mobile lab will be outfitted in a comfortable, lounge-like atmosphere with appropriate décor (i.e., glassware and artwork found in dispensaries). This is consistent with the reviewer's suggestion to create a more naturalistic environment for the assessments. Thus, rather than bring the subject to the lab as we did in the preliminary study, the RA and phlebotomist will travel to the location of each participant. The subject will use the strain in the privacy of their home, and then walk to the mobile lounge lab. The phlebotomist will immediately draw blood and the RA will begin assessments. At the end of the appointment, the subject will be compensated and escorted back to their home.

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 marijuana obtained from the NIDA supplier in Mississippi (Schacht, Selling, & Hutchison, 2009). As noted previously, the external validity of the study was compromised because the marijuana was of significantly poorer quality than subjects had ever experienced. Further, laboratory protocols are unlikely to mirror real-world marijuana use. Thus, those procedures simply did not, and cannot reflect the strains of marijuana and methods of administration of marijuana in states like Colorado. Because the long-term goal of this work is to better understand the effect of different levels of cannabinoids that result from marijuana use in order to develop more effective harm reduction strategies, we decided that it was more important to emphasize external validity. 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 to determine the level of THC and CBD in each subject in both Study 1 and Study 2. 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 analysis approach, which emphasizes the analysis of blood levels of THC and CBD in Study 2 as continuous measures. In this way, we also capitalize on the greater variability in blood levels expected in the observational design by analyzing overall levels of THC and/or CBD as well as the ratio of THC to CBD (in Study 2).

Assessment of Compliance.

We ask participants to bring the receipt from the dispensary to verify which strain was purchased, and the RA visually confirms the label (e.g., A, B, or C) at the experimental session.

Mobile Laboratory Experimental Session.

A member of our research team who has completed a certified training in phlebotomy will collect blood samples from participants (see preliminary studies above for cannabinoid blood level data). All blood draw procedures performed during this study will involve collecting venous blood through venipuncture of a peripheral arm vein using standard, sterile phlebotomy techniques. The amount of blood to be drawn will vary for each appointment. We will collect 32 ml of blood during Appointment 1 (Baseline/Session 1). We will use 10 ml of that blood for DNA extraction, 20 ml of blood to perform the inflammatory analysis, and 2 ml of blood for cannabinoid analysis. During Appointment 2 (Sessions 2-4), we will collect 22 ml of blood (20 ml for inflammatory analysis and 2 ml for cannabinoid analysis). The 2 ml of blood will be used to examine changes in levels of cannabinoids (i.e., THC, CBD) from pre- to post-five days of use of the randomly assigned strain.

Laboratory Post-Experimental Appointment.

DNA Collection.

DNA will be extracted from the 10 ml of blood collected during Appointment 1. After extraction, DNA will be quantified and stored at -80° for future analysis. DNA will be collected only at Appointment 1 in order to look at differences in single nucleotide polymorphisms (SNPs) in heavy cannabis users versus light cannabis users. The DNA samples collected in this study are for biobanking purposes only. Analysis of these samples are expected to be performed at a future date and therefore will not contribute to the current study at this time.

Blood levels of cytokines. Two tubes of blood (20 ml) will be drawn at Appointments 1 & 2 and used to observe changes in inflammatory markers. Blood samples will be assayed for IFNg, IL-1a, IL-1b, IL-6, IL-2, IL-4, IL-8, IL-10, IL-12, and TNF. This is the same assay used in our preliminary studies. The blood samples will be used to examine changes in inflammatory markers (i.e., THC, CBD) from pre- to post-five days of use of the randomly assigned strain.

Lipopolysaccharide (LPS)-PBMC challenge. We also expect different strains cannabis, with their varying cannabinoid content, to impact the responses of blood monocyte cells to an LPS challenge. LPS stimulation of PBMCs simulates the TLR4 inflammation cascade. Cannabis has been shown to mediate the pro-inflammatory effects of LPS. Peripheral blood mononuclear cells (PBMC) 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 (IL1,TNF, IL6, IL4, IL10, IFNg, MIP-1a) will be measured in supernatant using a multiplex ELISA assay (Aushon Biosystems, Billerica MA). 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. We expect to see changes in both the number and diversity of microbiota populations found in the gut as a result of cannabis use. Because inflammation and the gut microbiome are heavily intertwined, we will examine the relationship between changes in the gut microbiome with changes in inflammatory biomarkers in the blood (cytokines and blood monocyte cell response to LPS challenge) as a result of cannabis use. Fecal samples will be collected from participants using at-home kits and then stored at -80°. 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. Subjects will be given the at-home collection kits at their 1st appointment and will

return them to a research assistant at their 2nd appointment. The microbial DNA collected in this study are for biobanking purposes only. Analysis of these samples are expected to be performed at a future date and therefore will not contribute to the current study at this time.

Power Analysis and Sample Size Requirement for Hypotheses 1 and 2

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 (1988) and were conducted in G*Power 3 (Faul et al., 2007). The primary analysis is the test of the strain X time interaction effects across repeated assessments of THC in the blood as well as assessments of working memory and the rewarding effects of the marijuana. To be conservative, we thus estimated an ICC of .60. Using a mixed model ANOVA design, with three groups, four assessment points, and an average ICC = .6 between assessment points, a total n=99 (33 per group) will allow us to detect an interaction effect as small as f=.12.

The impact of attrition.

It is anticipated that some attrition will occur in this population. Based on our current pilot study and previous marijuana studies, we anticipate a 10% attrition rate between Appointment 1 baseline session and Appointment 2 the experimental session, though we have not experienced and do not anticipate differential attrition by condition. Thus, to allow for attrition, we assume a 90% retention rate over the course of data collection. To adjust for this attrition rate, we will recruit n≈111 participants (n=37 per group). Our approach to power analysis and accounting for attrition is extremely conservative, in that the techniques we will utilize in data analysis use state of the art recommendations in iterating the estimation of missing data (Schafer & Graham, 2002; Little, 1988). All analyses are intent-to-treat and all available data are utilized in analyses.

Analysis Plan for Hypotheses 1 and 2

Analyses will be conducted primarily on the SAS system for Windows Version 9.1, which includes capabilities to test multilevel models that include nonlinear effects and missing data (Cohen et al., 2013). Preliminary analyses will include checking the data for normality and reliability. To test the success of random assignment, the equivalence of conditions across all pretest measures will be assessed via t-tests on continuous items (e.g., depressive symptoms) and χ^2 tests of categorical items (e.g., race). We will use the Bonferroni approach (Keppel, 1991) to correct for alpha inflation with a familywise alpha of .05. Tests of the main hypotheses for effects of THC on primary outcomes will be conducted in a multilevel framework to account for the repeated observations within participants over time. Random coefficient regression (RCR; Cohen et al., 2013) models in SAS Proc Mixed will be utilized for continuous outcomes (e.g., working memory, drug reward).

Effects of THC Potency on THC Blood Levels, Working Memory, and Reward

The first aim is designed to test whether the "potency" of marijuana in terms of THC level in the plant translates directly to THC levels in the blood. To test **Hypothesis 1,** those individuals who receive the high THC strain will demonstrate significantly greater blood THC levels, we will utilize random coefficient regression (RCR; Cohen et al., 2013) via SAS Proc Mixed. The between subjects factor will be strain (high THC versus medium THC versus low THC) and the within subjects factor is time (Time 1, Time 2, Time 3, Time 4). Blood level of THC is the dependent measure. The test of the strain X time interaction will indicate whether the pattern of effects is such that blood levels increase to a greater degree and remain higher across the course of the study in the strains with higher THC. Thus, we expect both a significant interaction as well as a main effect of strain. Assuming a significant omnibus test of the interaction, we will decompose the interaction

via planned posthoc contrasts in order to understand at which time points the three strains are different. At Appointment 1, Time 1 (baseline) we do not expect differences in THC since none of the participants will have switched strains. Appointment 2, Time 2 is the sober, before use at Time 3. Thereafter, we expect a difference in THC at Appointment 2, Time 3 in the high THC versus the medium and low THC groups, and that while all groups will decrease in THC over the course of the laboratory appointment between Time 3 and 4, the high THC group will continue to show higher blood levels than the medium THC group, and the medium THC group will show higher blood levels than the low THC group. Note that we will include a quadratic effect in the multilevel model both to appropriately model the quadratic effect and whether that quadratic trend differs by condition. It is important to note that since we are not directly controlling administration, and have instructed participants to ingest the marijuana ad libitum, that they themselves may moderate their consumption to achieve "desired" THC levels, as our pilot data suggest. Thus, those in the low and medium THC group may ingest more in order to match their typically desired level, and thus the groups might converge. In this case, it is possible that we may see fewer difference in blood levels of THC, or non-significant differences at some timepoints across groups.

Hypothesis 2 tests whether blood levels of THC are positively associated with diminished working memory and greater rewarding effects of the marijuana, operationalized with the DEQ. If analyses of Hypothesis 1 show that there are main effects by group suggesting that the higher THC strain results in significantly higher THC levels in the blood than the medium or low THC strains, the between-groups variable for models testing Hypothesis 2 will be group and we will include all four time points. Since participants were randomly assigned to group, this test allows for the highest internal validity in terms of causal conclusions about the effects of THC. If, on the other hand, participants titrate their own use such that there are few reliable between-groups differences, then actual blood levels of THC will become the between-groups (continuous) independent variable in tests of Hypothesis 2 and we will utilize timepoints 3-4 as the within-subjects effect of time. Our preliminary studies confirm wide variability in THC levels even among individuals using the same strain, so there will be adequate between-participant variability for these analyses. In either case, we will again use the RCR approach described above, and standard approaches to the estimation of interactions in regression (Aiken & West, 1991) in the case of a continuous IV (i.e., mean-centering prior to creation of the interaction term). In each model, the predictors will include the group variable (or continuous THC blood levels), a time variable, and their interaction.

V. FUNDING

This study is 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. The researchers know this population has a common tendency to use either marijuana flowers or marijuana edibles, thus assigning them to the correct condition will not be difficult.

Criteria for inclusion in the study are:

1. Must be between the ages of 21 and 70 and provide informed consent;
2. Must have prior experience with edibles in order to be eligible to participate in the edible group;
3. Must have used marijuana at least 4x per month in the past month. This includes any form of marijuana consumption including flower, oil, wax, tinctures and edibles;

4. Must not report currently using a strain with greater than 1% CBD or less than 18% THC. This will ensure that there are no subjects who could be harmed by switching to a lower CBD/higher THC strain. All of the subjects in our preliminary study would have met this criterion.
5. Must not be a University of Colorado student or employee;
6. Must report not using other drugs (cocaine, opiates, methamphetamine) in the past 60 days or test positive on a urine test for those drugs of abuse at baseline;
7. Must not smoke greater than 5 cigarettes a day;
8. Must have a breath alcohol level of 0 at screening (to sign consent form) and must not report drinking greater than 3x per week and greater than 5 drinks on occasion for men and 4 drinks on occasion for women;
9. Female subjects must not be pregnant, as indicated by a pregnancy test which will be administered at baseline, or trying to become pregnant;
10. Must not donate plasma, blood, or platelets for 7 days prior to the 2 appointments and 7 days after;
11. Must not be in treatment for psychotic disorder, bipolar disorder or schizophrenia.

VII. VULNERABLE POPULATIONS

This study does not include any vulnerable populations.

VIII. RECRUITMENT METHODS

Subjects will be recruited from the front range area through the social media sites (e.g., facebook, twitter, etc.) or websites of cannabis dispensaries or patient advocacy groups. 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 mood and cognition." All social media pages will provide additional information about the study (see attached word file). The wording used on the social media page may also be used in flyers or other advertisements, or directly mailed to potential participants. We request specify age groups by zip code through a direct marketing company and they provide names and addresses.

IX. COMPENSATION

Participants will receive $150 at the end of the study. If a participant does not complete the study, payment will be pro-rated at $50 for Appointment 1 and at Appointment 2: $50 for session 2, and $25 for Session 3 and 4. Given the amount of time required in the study and the travel to and from the lab for Appointment 1, this is a reasonable amount of money to compensate participants for their time and effort. Thus, there is no question of coercion.

X. CONSENT PROCESS

When a participant arrives for his or her first appointment 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. 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 initial and sign the informed consent document.

XI. PROCESS TO DOCUMENT CONSENT IN WRITING

In accordance with 45 CFR 46.117, an unsigned copy of the form used to document consent will also be given to the study participant.

XII. PROCEDURES

Participants will contact the researchers with their interest by phone or by e-mail. The researcher will respond with an overview of the study and answer any questions the potential participant has before screening the caller for preliminary eligibility. In the case that a potential participant requests more information via e-mail, the research assistant responding to the e-mail will ask the participant to call the lab phone number (same phone number provided on all study advertisements) for more information, or e-mail the study team a phone number at which the potential participant could be reached and a desirable time for the research staff to call. This process will help to ensure participants have ample opportunity to be informed of the main study components, ask questions, and decide if they are willing to participate before they sign up for the study. If the participants meet the study criteria during the initial phone interview, they will be invited to come to the CINC for the Appointment 1, baseline session.

Appointment 1, Baseline Assessment Procedure (Session 1). Subjects who meet inclusion criteria will be scheduled for the first available appointment, baseline session. After arriving at the lab, subjects will be provided with a consent form. Each subject will be breathalyzed to insure that they have not been drinking alcohol prior to each appointment. A urine toxicology screen will also be administered to insure that subjects have not recently use illicit drugs other than cannabis. Additionally, female participants will be required to take a urine pregnancy test (provided by the study team at no cost to the participant). 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 via venipuncture of a peripheral arm vein using standard, sterile phlebotomy techniques. Subjects will then complete measures related to alcohol and drug use, cannabis withdrawal, cannabis craving, measures of mood, and neurocognitive measures (see section below for details of measures to be completed). After completing the measures, each participant will be scheduled for their 2nd appointment to include Sessions 2, 3, & 4. Before the particpant leaves this appointment they will be given instructions to purchase 3 grams of either a normally high THC strain, a medium THC strain, or a low THC strain at a dispensary (The Farm) in Boulder, Colorado to be used for 5 days as they normally would, they will also be given a gut micriobiome colleciton kit to take home with them and bring back at their 2nd appointment.

Appointment 2, Mobile Lab Experimental Procedure (Session 2, 3, 4). For Sessions 2, 3 & 4 the study coordinator will drive the mobile lab to a location close to the partipant's home. The participant will come to the mobile lab where they will complete a blood draw (22 ml), self-report measures, and the cognitive tests for Session 2. At the end of Session 2, participants will return to their home to use the assigned strain as they normally would.

Then they will return to the mobile laboratory for Session 3, where a blood draw self-report measures, and the cognitive battery will be collected in the mobile lab. The blood draw (22 ml), questionnaires and cognitive battery will be repeated at 1 hour after Session 3 at the mobile lab (Session 4). After the last assessment, the participant will be paid $150 to compensate them for their time and effort (Appointment 1: $50 for Session 1, Appointment 2: $50 for Session 2, $25 for Session 3, $25 for Session 4), debriefed, and escorted back to their home.

Name of instrument/tool/procedure	*Purpose (i.e. what data is being collected?*	*Time to Complete*
Appointment 1: Baseline/Session 1		
Urine Testing	• A urine sample will test for illicit drugs other than marijuana (e.g. cocaine, benzodiazepines, opiates, MDMA, sedatives, or methamphetamine).	
Pregnancy Screening	• All female participants will take a urine pregnancy test.	
Demographics Questionnaire	• Age, sex, marital status, race/ethnicity, SES, occupation, income, education, and religious affiliation.	
Alcohol Use History Questionnaire	• Targets the frequency of lifetime and recent use for alcohol.	
Pain Intensity	• Consists of one item asking about the participant's level of pain in the past seven days. Participants are asked to rate their pain on a scale from 0 (no pain) to 10 (worst imaginable pain).	
Promis Pain Interference CAT	• This asks participants about how their experience of pain interfered with or affected their enjoyment of various daily activities in the past seven days. The scale is computer-adapted and has a minimum of four questions and a maximum of 12 questions. The questions ask for a response on a 5-point scale. Some of the items are rated: Not at all; A little bit; Somewhat; Quite a bit; Very much; other items are rated: Never; Rarely; Sometimes; Often; Always.	
Health Related Quality of Life	• This is a Short Form 36 Health Survey, which consists of 36 questions across nine health domains and is sensitive in detecting changes over time in health-related quality of life.	

Self-Rated Question of Diet Quality	• In general, how healthy is your overall diet? Would you say (1) excellent, (2) very good, (3) good, (4) fair, or (5) poor? (Loftfield, Yi, Immerwahr, Eisenhower)	
Leisure-Time Categorical Item (L-Cat)	• A single item comprised of six descriptive categories ranging from inactive to very active (Kiernan, Schoffman, Lee, Brown, Fair, Perri, & Haskell, 2013). Each category consists of 1–2 sentences describing common activity patterns differing in frequency, intensity, duration, and types of activities, thus encompassing content validity (van Poppel, Chinapaw, Mokkink, van Mechelen, & Terwee, 2010).	
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)	
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 smoked marijuana, I often smoked more or for longer periods of time 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).	
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 (BD-II)	• Consists of 21 scaled statements designed to assess symptoms of depression and will be administered to examine comorbid depression and covary baseline differences if necessary. (Beck, Steer, Ball, & Ranieri, 1996)	
The Beck Anxiety Inventory (BAI)	• Consists of 21 items, each describing a common symptom of anxiety and will be administered to examine comorbid anxiety and covary baseline differences if necessary. (BAI; Beck, Epstein, Brown, & Steer, 1988)	

Marijuana Withdrawal Checklist (MWC)	• A 15-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)	
Physical Activity Participation	• This is one question that asks participants to list 3 sports/activities they engage in. (Bryan, A.D., & Rocheleau, C.A. (2002)	
Brief Sensation Seeking Scale (BSSS)	• The BSSS will be used to examine whether sensation seeking moderates the effect of cannabis consumption on exercise cognitions. (Hoyle, Stephenson, Palmgreen, Lorch, & Donohew, 2002)	
Expectancy Questions	• These 3 questions assess a participant's knowledge of cannabis and their health	
The Pittsburgh Sleep Quality Index (PSQI)	• The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep. It differentiates "poor" from "good" sleep by measuring seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month. The client self rates each of these seven areas of sleep	
Session 1 & 2		
Timeline Follow-Back (TLFB)	• TLFB is used to assess daily substance use for 30 days prior to the baseline session. Our modified TLFB procedure will estimate both frequency of marijuana use and amount used per day, using visual stimuli as well as the method of administration. (Sobell & Sobell, 1992) (Sobell, Sobell, & VanderSpek, 1979)	
Session 2		

The Pittsburgh Sleep Quality Index (PSQI)	• The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep. It differentiates "poor" from "good" sleep by measuring seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month. The client self rates each of these seven areas of sleep	
Cold Presser Task	• Participants immerse their hand in cold liquid and withdraw their hand from the liquid (pain tolerance) and the timing is recorded. There is a risk of discomfort.	
Session 2 & 3		
Exercise Cognitions	• This measure will be used to examine the acute cognitive effects of cannabis consumption on participants' exercise cognitions, specifically exercise intentions, motivation, and self-efficacy. These measures are slight variations of assessments of exercise cognitions used in our prior work (Bryan & Rocheleau, 2002).	
Session 3 & 4		
The Drugs 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	• 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.	
Session 4		
Cold Presser Task	• Participants immerse their hand in	

	cold liquid and withdraw their hand from the liquid (pain tolerance) and the timing is recorded. There is a risk of discomfort.	
Assessment of Blind and Potency of Marijuana	• At the end of the experimental session, participants will be asked to guess which strain they received and to guess the potency of THC and CBD in that strain.	

Sessions 1, 2, 3, 4

Venous Blood Draw	• A BD Vacutainer Safety-Lok needle and microtainer will be used to obtain blood through a peripheral intravenous line (PIV).	
Heart Rate	• Heart rate will be collected using a basic fingertip pulse oximeter.	
Neurocognitive Battery	• The cognitive battery will include the Flanker Inhibitory Control and Attention task, the Dimensional Change Card Sort Task, the Picture Sequence Memory Test, the Auditory Verbal Learning Test (Rey), 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).	
Marijuana Craving Questionnaire (MCQ)	• A Marijuana Craving Questionnaire will be used to assess craving at each time point during the experimental 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= .90).	
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)	

Appointments 1 & 2:	***Procedures/Tools***	***Time to complete***
Appointment 1 Baseline/Session 1	Informed consent & study orientation; BAC test; Urine drug test; Pregnancy test; Blood draw; Heart rate; Questionnaires; Cognitive battery; Appointment 2 scheduling	*2.5hours*

Appointment 2 Session 2	Heart rate; Questionnaires; Cognitive battery; Blood draw.	*1 hours*
Session 3	Heart rate; Questionnaires; Cognitive battery; Blood draw; 1 hour relaxation	*1 hours*
Session 4	Heart rate; Questionnaires; Cognitive battery; Blood draw; Debriefing; Monetary Compensation	*1 hour*
TOTAL TIME		*5.5hours*

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XIII. SPECIMEN MANAGEMENT

Blood samples collected from an arm vein through venipuncture in the mobile lab will be kept in an insulated biohazard transport bag. All blood 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.

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 Department of Psychology & Neuroscience in Muenzinger Hall, both of which are only accessible to research staff. All stored data will be recorded from secure survey software (e.g.). Any identifying information will be destroyed after the last session. 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 appointment); 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 his 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 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 must be a regular cannabis user to be in the study**.**

Risks Associated with Venipuncture.

There is a small risk of local hematoma, infection, and syncope associated with phlebotomy. Also, to avoid any minor health risks associated with multiple donations participants must not donate plasma, blood, or platelets for 7 days prior to the 2 appointments and 7 days after.

Risks Pertaining to the Collection of Genetic Material.

The collection of genetic material (i.e. from the blood) entails risks to confidentiality. Ethical guidelines for the collection and use of genetic material continue to develop; however, set guidelines for these purposes have yet to be officially set forth. The collection of genetic material has become commonplace within several research disciplines, and entails only minimal risk to study participants.

Psychological Risks and Discomforts.

While participants are experienced cannabis users to be in the study, it is still possible that some participants might experience some adverse effects from the cannabis such as increased anxiety, sleepiness, paranoia, and increased heart rate. There is a slight risk that the ingestion of cannabis may be associated with a psychotic episode.

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. 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 these individuals because they are already 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.

Risks Pertaining to the Collection of Genetic Material.

The collection of genetic material has become commonplace within several research disciplines, and entails only minimal risk to study participants. All genetic material will be coded and stored with a randomly generated number. The master list linking the numbers to participants' names will be stored on a password-protected server. At study closure, all links between participant name and URSI will be destroyed, at which point the specimen/data will be considered de-identified. Participants will be informed, both verbally and in writing upon initial consent, of the risks of genetic research.

Psychological Risks and Discomforts.

In the unlikely event that a highly experienced cannabis user has an adverse reaction to any cannabis they use and needs assistance, the research assistant will immediately notify Dr. Hutchison who will make himself immediately available to evaluate the condition of the participant and intervene if necessary. Dr. Bryan and Dr. Hutchison will be on call/reachable during all scheduled appointments.

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 either Muenzinger Hall or the CINC, which are both 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 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 appointments, 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.

(2) 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 Psychology Department 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. Hutchison immediately by phone (303-492-9549) 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 buy. We estimate the cost of 3 grams of cannabis to be $36. Parking is free at the CINC.

XXIII. DRUG ADMINISTRATION

Not Applicable.

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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