A. SIGNIFICANCE

A.1. Prevalence of cannabis use disorder (CUD) and need for effective treatments. Current data suggest that 2.5% of adults (Hasin et al., 2016) and 3.0% of adolescents (Han et al., 2017) in the United States meet Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for CUD (DSM-IV or DSM 5). Though estimates vary, several studies have suggested that approximately 8-10% of individuals who have experimented with cannabis at least once in their lifetime develop DSM-IV cannabis dependence (Lopez-Quintero et al. 2011; Wagner and Anthony 2002). In 2014, over one million Americans received treatment for cannabis related problems (SAMHSA, 2015). Although a high demand for effective interventions exists, few specific treatments have been developed for CUD. Further, the treatments that have been examined have had limited efficacy, with few individuals achieving abstinence (Sherman & McRae-Clark, 2016). As such, it is critical to explore new strategies to improve treatment outcomes.

A.2. Sex differences in CUD. Preclinical studies have demonstrated important sex differences in regards to cannabis. Females are more sensitive to the behavioral, psychological, and reinforcing effects of cannabinoids, and demonstrate faster acquisition of self-administration, higher rates of responding, and increased rates of drug-induced reinstatement than males (Craft et al., 2013; Fattore et al., 2009; Fattore et al., 2007). Clinical investigations have also revealed cannabis-related sex differences. Across substances of abuse, including cannabis, men are at greater risk for lifetime use disorder diagnosis (Becker & Hu, 2008). However, cannabis using women show greater abuse liability (Cooper & Haney, 2014), more rapid progression from use to disorder (Khan et al., 2013; Kerridge et al., 2018), more severe withdrawal symptoms (Copersino et al., 2010; Herrmann et al., 2015; Sherman et al., 2017), and greater barriers to care (Becker et al., 2017). Further, there is emerging evidence that women may not respond as well to CUD treatment as men (McRae-Clark et al., 2015a; McRae-Clark et al., 2015b; Sherman et al., 2016).

A.3. Stress reduction as a target intervention for cannabis using women. Among chronic users of cannabis, stress has been shown to be a significant factor in initiation and maintenance of use (see Hyman & Sinha 2009 for review). For example, in a treatment seeking sample, Copeland and colleagues (2001) found stress relief to be the most commonly reported benefit from, and reason for continuing, cannabis use. Our group demonstrated that completion of a social stress task increased craving in individuals with CUD (McRae-Clark et al., 2011). Studies have also shown that cannabis users expect tension reduction from use (Galen & Henderson, 1999) and that coping motives are associated with levels of cannabis use (Bonn-Miller et al., 2007) and cannabis-related problems (Simons et al., 2005; Lee et al., 2007). Further, stress-related factors such as negative life events (Wills et al., 2001; Windle & Wiesner, 2004) and traumatic stress (Lipschitz et al., 2003; Bremner et al., 1996; Vlahov et al., 2004; Schiff et al., 2007) have demonstrated associations with cannabis use. It has therefore been suggested that interventions specifically focused on reducing, tolerating, and coping appropriately with stressors could improve treatment outcomes in cannabis users (Hyman & Sinha, 2009), and such an approach may have particular relevance for women.

Women report more severe negative affect symptoms and greater related functional impairment during cannabis withdrawal than men (Copersino et al., 2010; Hermann et al., 2015, Sherman et al., 2017). Studies in adolescents and young adults have indicated that females are more likely to use cannabis as a coping strategy for tension and stress (Simons et al., 1998; Terry-McElrath et al., 2009; Cuttler et al., 2016), whereas males often identify enhancing positive affect as a motive for cannabis use (Chabrol et al., 2005; Newcomb et al., 1998). Congruent findings have been demonstrated in human laboratory paradigms, with cannabis-using women reporting increased craving following stress tasks as compared to men (Buckner et al., 2011). Of note, exposure to trauma has been shown to impact stress responding in cannabis using women, but not men, suggesting that consideration of past traumatic experiences may be important in sex-specific treatment development (Chao et al., 2018).

A.4. Progesterone and substance abuse. A growing literature suggests that the ovarian hormone progesterone, which fluctuate naturally during the menstrual cycle, may play a key role in sex differences in substance abuse (Moran-Santa Maria et al., 2014). Preclinical research from our SCOR demonstrated that when progesterone levels are low (during the estrous phase in rats) females evidence greater cocaine seeking-behavior (Feltenstein & See, 2007). Vallee and colleagues (Vallee et al., 2014) examined the effects of pregnenolone, a precursor to progesterone, on THC administration in rats and reported that it suppressed THC-induced effects including hypothermia, locomotor suppression, and analgesia, as well as dampened neurobiological effects in reward-related brain regions (VTA, NAc). Numerous preclinical studies have also

shown progesterone administration attenuates the reinforcing effects of cocaine (Frye, 2007; Evans & Foltin, 2010) and reduces drug-reinstatement and self-administration of cocaine (Anker et al., 2007; Larson et al., 2007) and nicotine (Mello, 2010).

Clinical studies in tobacco, amphetamine, and cocaine users examining **endogenous ovarian** hormones support these findings. Women in the luteal phase of the menstrual cycle, when progesterone is high, showed decreased subjective cocaine (Evans, Haney, & Foltin, 2002; Sofuoglu et al., 1999) and amphetamine (Justice et al., 1999) effects compared to women in the follicular phase, while female smokers randomly assigned to quit during the luteal phase had lower relapse rates than those assigned to quit during the follicular phase (Allen et al., 2008). Research from one of our previous SCOR projects found that increasing levels of endogenous progesterone were associated with a 37% increase in the odds of smoking abstinence in women undergoing a relatively brief 4-week course of nicotine replacement therapy (Saladin et al., 2015). Of special note, our most recent SCOR project (Gray & Saladin, PIs) examined the association between daily salivary progesterone levels and cigarettes smoked per day over a 2-week period; preliminary findings indicated that increased levels of daily progesterone were associated with decreased cigarettes smoked per day in non-abstaining women (β=-0.32; SE=0.14; $p<0.05$). Further, we recently demonstrated that high levels of endogenous progesterone attenuate subjective responses of anxiety and craving to drug-cues that are preceded by a stressor, suggesting a role of progesterone in reducing stress-induced relapse (Moran-Santa Maria, 2018).

Recent clinical studies have investigated **exogenous progesterone** as a potential pharmacologic intervention for substance use disorders. Compared to placebo, progesterone has been shown to attenuate subjective and physiological effects of cocaine in female cocaine users (Evans & Foltin, 2006; Sofuoglu et al., 2002; 2004), as well as decrease cue-induced craving and cortisol response in early abstinent cocaine-dependent women (Fox et al., 2013). In a 12-week trial, progesterone reduced weekly cocaine use in post-partum cocaine-dependent women compared to placebo (Yonkers et al., 2014). Exogenous progesterone, compared to placebo, has also been shown to attenuate craving and subjective effects of smoking (Sofuoglu et al. 2001; 2011), and improve cognitive performance in women but not men (Sofuoglu et al., 2011).

A.5. Progesterone may address sex differences in negative affect and stress reactivity and improve outcomes for female cannabis users. Preclinical work has demonstrated that allopregnanolone (Allo), a key metabolite of progesterone, decreases indices of anxiety and reduces stress-induced elevations of adrenocorticotropic hormone (ACTH) and corticosterone (Patchev et al., 1996; Bitran et al., 1995; Biggio et al., 2014). Animal studies have also reported that decreased levels of Allo in select neurons in the medial prefrontal cortex, hippocampus and basolateral amygdala are associated with augmented fear responses (Pibiri, Nelson, Guidotti, Costa, & Pinna, 2008) and that changes in Allo levels are inversely associated with anxiety-related behaviors (Frye & Walf, 2002, 2004; Frye, Walf, Rhodes, & Harney, 2004; Gulinello, Orman, & Smith, 2003; Rhodes & Frye, 2001; Walf, Sumida, & Frye, 2006). Other preclinical data indicate that progesterone dampens corticotropin-releasing factor (CRF)-enhanced startle responding (Toufexis, Davis, Hammond, & Davis, 2004). Studies in humans suggest that progesterone has sedative properties (deWit et al., 2001; Solderpam et al., 2004) and can dampen cortisol activity and decrease stress-induced alertness, arousal and negative mood (Childs et al., 2010). There is also a small but growing body of animal and human findings that suggests progesterone is implicated in the pathophysiology of several anxiety disorders, notably PTSD and panic disorder (Nillni, Toufexis, & Rohan, 2011; Rasmusson et al., 2006). These studies show progesterone (and/or Allo) may have the ability to reduce anxiety related symptomatology. Lastly, two studies have reported on the effects of progesterone on stress responding and drug craving in cocaine dependent individuals. In the first study, Sinha et al., (2007) reported that women with high serum levels of progesterone showed significantly lower stress-induced and drug cue-induced cocaine craving, and trends towards reduced drug cue-induced anxiety level and lower drug cue-induced systolic and diastolic blood pressure levels compared to women with low serum progesterone. Fox and colleagues (2013) reported that cocaine-dependent women but not men receiving progesterone reported lower ratings of negative emotion and higher ratings of relaxed mood following a stress exposure. Collectively, these findings, coupled with the preclinical and clinical (exogenous/endogenous) progesterone data discussed above, suggest that progesterone has anxiolytic-like properties that may diminish the effects of stress, particularly in drug using women, and be protective against relapse.

A.6. Ambulatory assessment of stress cue reactivity. To date, the majority of cue reactivity assessment in cannabis users has occurred in the laboratory. While such a setting provides significant experimental control, it lacks the ecological validity that defines the rich context of cannabis users' day-to-day natural environment. Ambulatory assessment refers to the collection of self-report, physiological, or other data from individuals in their natural environment, and allows for examination of real-world outcomes that may not be measurable by other

traditional research methodologies. In our previous SCOR, we utilized ambulatory assessment to conduct the first evaluation of sex influences on smoking- and stress cue-elicited craving in response to standardized cues presented in the natural environment of cigarette smokers (Wray et al., 2015; Tomko et al., 2017). Participants received pictorial smoking, stress, and neutral cues on a mobile device four times per day for two weeks using Cue Reactivity Ecological Momentary Assessment (CREMA; Warthen & Tiffany, 2009; Wray et al., 2015). We found that female smokers endorsed greater craving, stress, and negative affect than male smokers when exposed to stress cues, relative to neutral imagery (Wray et al., 2015). This real-world methodology also provides the unique opportunity to examine sex differences in the influence of stress/negative affect on real-world substance use behavior in daily life.

A.7.**Endocannabinoids.** Surprisingly little is known about how heavy cannabis use or cannabis dependence can affect the endocannabinoid (eCB) system, particularly in women. Preclinical research has shown that anandamide (AEA) and 2-Arachidonoylglycerol (2-AG) levels in different regions of the brain fluctuate throughout the menstrual cycle, and there is evidence of a bidirectional interaction between hormones, predominantly estrogens, and eCBs [1]. Additionally, repeated THC administration has been shown to lead to decreased levels of AEA and 2-AG in the rat striatum, and increased AEA in the limbic forebrain [2].

In humans, levels of AEA in plasma have been shown to shift over the menstrual cycle, peaking during ovulation, followed by the follicular phase, and with levels lowest during the luteal phase [3,4]. As with animal models, AEA was highly correlated with levels of estradiol and luteinizing hormone [4]. In one study, heavy cannabis users were observed to have higher serum 2-AG after 24 hours abstinence relative to controls, without any difference seen in AEA, OEA, or PEA [5], but this is contradicted by another study, which found significantly reduced AEA and OEA in individuals with cannabis abuse or dependence [6]. Finally, women have been shown to have a different eCB response to exogenous THC administration than men, and change-from-baseline OEA concentrations were found to be related to THC concentration [7].

Notably, no studies of heavy or dependent cannabis users have controlled for menstrual cycle phase when measuring eCB levels. Further, it is unclear how eCBs are affected by heavy cannabis use due to limited, contradicting results. No research has been conducted on how a brief period of abstinence might affect eCBs in this population, nor have other molecules implicated in the eCB system been assayed (e.g. SEA, NADA). To address these research gaps, we look to assess blood eCB levels in cannabis-dependent women in the follicular phase of the menstrual cycle that are both currently using or one week abstinent, and in the luteal phase after presumed resumption of use. We will also examine the effects of exogenous progesterone versus placebo taken during the abstinence period on blood eCB levels. To determine if there are gender differences in eCB levels, we will also collect samples in male participants, and compare across groups.

A.8. Summary. Numerous sex differences in behavioral, biological, and clinical correlates of substance use disorders have been identified (Becker et al., 2017), yet there exists a dearth of sex-informed treatment options. Women are more likely to use cannabis for stress reduction (Simons et al., 1998; Terry-McElrath et al., 2009; Cuttler et al., 2016) and demonstrate increased cannabis craving after stress compared to men (Buckner et al., 2011); as such, interventions targeting stress response may have particular salience for cannabis using women. Ovarian hormones have been identified as potential mechanisms of these stress-related disparities (Moran-Santa Maria et al., 2014), and recent clinical trials have begun to examine progesterone's utility as a possible pharmacotherapeutic agent (Evans & Foltin, 2006; Fox et al., 2013; Sofuoglu et al., 2001; 2004; 2011). While progesterone has shown promise as a treatment for women with cocaine and tobacco use disorders, it has not yet been tested in cannabis users. Progesterone may effectively target stress-related craving and cannabis withdrawal symptoms in women, thus reducing relapse rates and improving daily functioning.

B. INNOVATION

The proposed study will test truly novel hypotheses. Specifically, it will build on our previous SCOR work identifying progesterone as an important target in stress-related craving and relapse, while expanding the focus to a previously unstudied addictive disorder. To our knowledge, there are no previous or ongoing studies investigating this promising approach to treating CUD. Additionally, we plan to examine the potentially important effect that progesterone may have on stress, one of the most important contributors to continued cannabis use

in women. We adopt a novel dual-level approach by examining progesterone's effects on stress provoked in a laboratory stress paradigm and on stress occurring during a 3-week period of naturalistic observation. In the latter case, we will use innovative mobile technology to measure stress repeatedly during and after progesterone treatment. This will allow assessment of craving and stress reactivity in the "day to day" natural environment of cannabis users. These approaches differ significantly from other research teams investigating progesterone in other drugs of abuse (i.e., Sofuoglu et al. focused on acute non-stress-provoked laboratory effects in cigarette smokers and cocaine users, and Allen et al. focused on cessation via potential effects of progesterone on impulsivity in cigarette smokers) and represent a highly innovative "next step" in sex-specific treatment development for CUD.

APPROACH

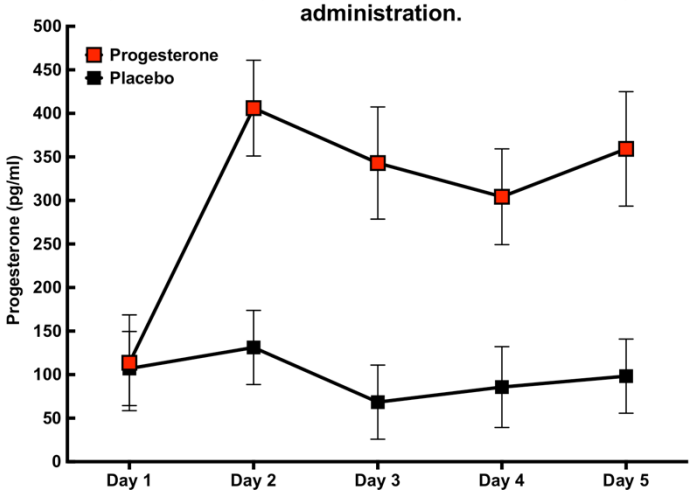
C.1. Preliminary Studies.

C.1.a. Capacity of Research Team. Completion of the proposed research will require experience with 1.) recruitment, retention, and assessment of cannabis use disordered individuals, 2.) administration of exogenous progesterone, 3.) ambulatory assessments, 4.) stress induction procedures, and 5.) collection and interpretation of progesterone levels. As detailed below, the research team has the expertise to successfully conduct the proposed work and a proven track-record in similar research areas. The proposed project will be co-directed by Drs. Aimee McRae-Clark and Michael Saladin. Dr. McRae-Clark is a Professor in the Department of Psychiatry and a productive clinical researcher with NIH-funding focused on clinical trials and human laboratory work with cannabis using individuals. Dr. Michael Saladin is a Professor in the Department of Health Sciences and Research and clinical psychologist with expertise in craving, ovarian hormone measurement, and the interface between stress and substance use disorders. Drs. McRae-Clark and Saladin have both been investigators with the MUSC SCOR since its inception. Co-investigators will also contribute meaningfully to the conceptualization, conduct, and reporting of this research. Dr. Kevin Gray is a board-certified psychiatrist with significant experience in conducting CUD clinical trials; he will provide the necessary medical expertise to monitor for participant safety throughout the study. Drs. Brian Sherman and Rachel Tomko, clinical psychologists, have experience with human laboratory studies involving cannabis using participants and ambulatory assessment, respectively. Of note, all members of the investigative team have a history of successful collaborations.

C.1.b. Experience with Recruitment, Retention, and Assessment of Cannabis Use Disordered Individuals. Dr. McRae-Clark has successfully conducted multiple NIH-funded studies involving cannabis using individuals (K23DA15540, PI: McRae-Clark; R21DA018221, PI: McRae-Clark; R21DA022424; PI: McRae-Clark; R01DA026782, PI: McRae-Clark; R21DA034089, PI: McRae-Clark; UG3DA04323, MPIs McRae-Clark/Gray). We have an active recruitment network in place. As such, we do not anticipate any issues with successful recruitment of the proposed study sample.

C.1.c. Experience with Administration of Exogenous Progesterone. We recently completed a small pilot trial (n=8) comparing progesterone to placebo administration in cannabis using women during cannabis withdrawal. Conduct of this trial allowed us to validate the research procedures that will be utilized in the proposed study, including (1) pharmacy compounding procedures for micronized progesterone and matching placebo capsules, (2) ability to recruit and retain cannabis using individuals willing to abstain from cannabis use for the required study period, (3) feasibility of using ambulatory assessment to verify cannabis abstinence and medication adherence, and (4) ability to increase endogenous progesterone levels with twice daily exogenous progesterone administration (see **Figure 1;** p=0.027). Supporting our study rationale, preliminary data also provided suggestive evidence of a potential treatment by time interaction (p=0.071) in regards to cannabis craving, with women receiving progesterone reporting reduced cannabis craving compared to women receiving placebo over the five-day treatment period.

Figure 1: Salivary progesterone levels after 200 mg BID administration.

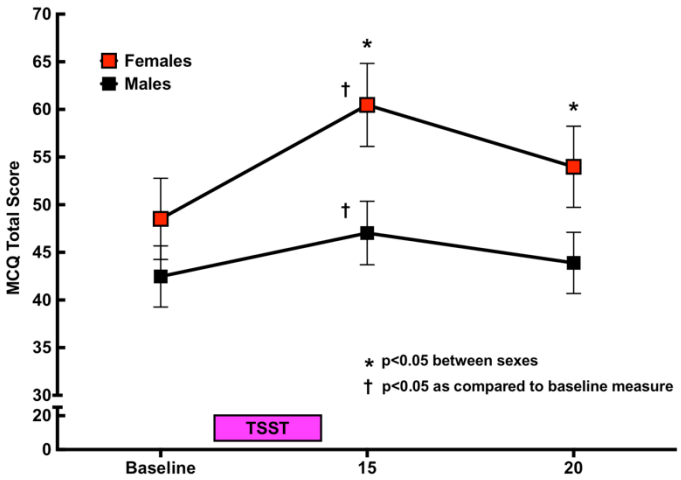


C.1.d. Experience with Ambulatory Assessments. Our research group has developed ambulatory assessment tools to assess stress reactivity and drug craving, collect data on drug use and withdrawal

symptoms, validate abstinence, and measure and encourage medication adherence. As discussed above, in our previous SCOR, we modified the Cue Reactivity Ecological Momentary Assessment (CREMA) paradigm to assess cue- and stress-related nicotine craving (Wray et al., 2015). Over 80% of CREMA sessions were successfully completed (Tomko et al., 2017), supporting the feasibility of remote collection of stress reactivity and craving data. Our previous work involved four CREMA sessions per day; in the current proposal, three sessions per day will be utilized, which will likely improve compliance with session completion. We have also capitalized on the capabilities of the REDCap data system to remotely collect self-report and video confirmation of drug use biomarkers and medication adherence (Tomko et al., 2018). In a subsequent smoking cessation clinical trial, we found high compliance with video completion (73.8%) and self-reports (74.8%). Further, all participants agreed that the surveys and videos were easy to use, and 78.9% of participants preferred the REDCap assessments to traditional, paper measures. In an ongoing clinical pharmacotherapy trial for CUD (UG3DA04323), we have also found high compliance with video uploads for medication adherence, with a median of 80% of medication doses verified.

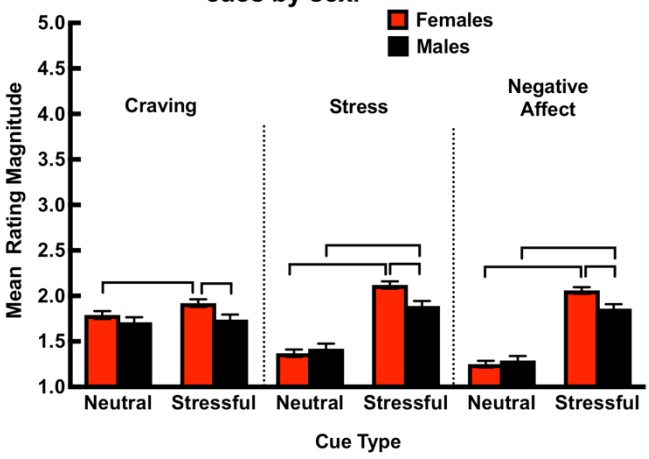
C.1.e. Experience with Stress Induction Procedures. Our research team has extensive experience with the use of stress induction paradigms in cannabis using individuals. We administered the Trier Social Stress Task (TSST), which will be used in the proposed study, to 87 cannabis-dependent individuals. Cannabis craving (as measured by the Marijuana Craving Questionnaire, MCQ) was significantly elevated following the TSST (p=0.029) (McRae-Clark et al., 2011). Of interest, female participants reported increased craving following the TSST as compared to male participants (**Figure 2**; p=0.027), congruent with the notion that acute social stressors may be more closely related to relapse in cannabis using women vs. men. This work also underscores a need for further evaluation of (a) sex differences in response to stressors, and (b) therapeutic interventions focused on stress reduction.

Figure 2: MCQ total score as a function of time and sex.



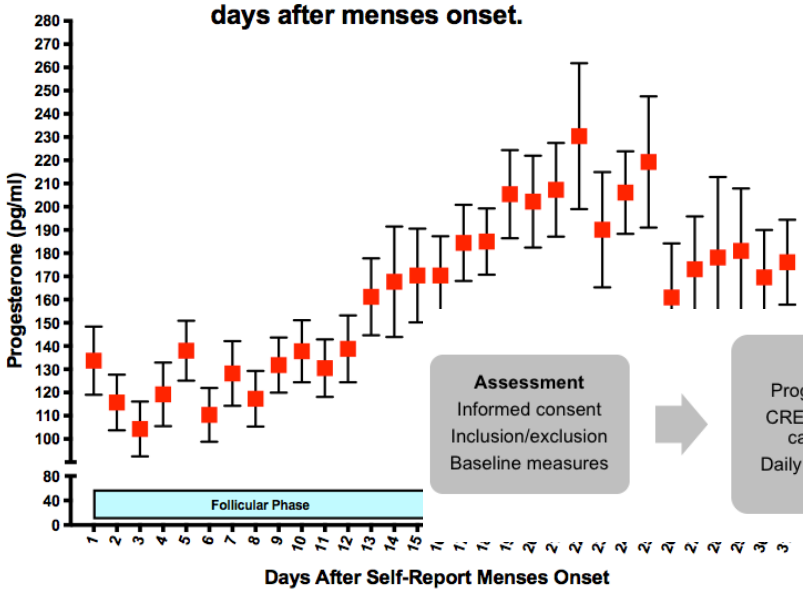
As discussed above in Significance, our previous SCOR utilized the CREMA platform to expose cigarette smokers to stress and neutral images from the International Affective Picture System. Consistent with our hypotheses in cannabis users, we found (**Figure 3)** significant sex by cue type interactions indicating that females vs. males endorsed greater craving (b=0.10, SE=0.03, p<0.01), stress (b=0.27, SE=0.04, p<0.01), and negative affect (b=0.24, SE=0.03, p<0.01) following the presentation of stressful vs. neutral cues. These analyses take into account time since last cigarette, pre-cue stress and craving, and day in study have replicated these findings (In figure, bars denote significant differences between post-cue model-based means).

Figure 3. Model-adjusted mean subjective responses to stressful vs. neutral cues by sex.

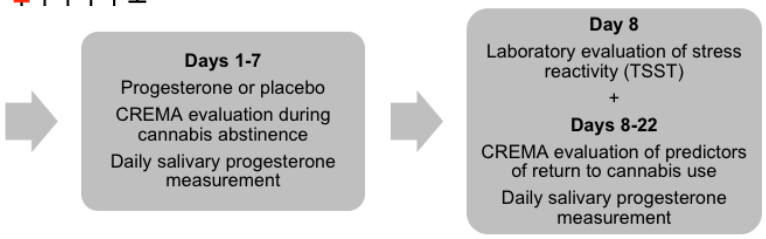


C.1.f. Experience with the Collection and Interpretation of Ovarian Hormone Levels. The previous three iterations of our SCOR project have focused primarily on advancing knowledge about the role of both menstrual cycle phase and ovarian hormones in sex differences in cigarette smoking behavior and cessation (Carpenter et al., 2006; Carpenter et al., 2008; Gray et al., 2010; Saladin et al., 2015a; Saladin et al., 2015b; Schiller et al., 2012; Weinberger et al., 2015; Allen et al., 2016). Our publication record on this topic attests to our experience collecting and interpreting ovarian hormone data. One major goal of our most recent SCOR component (Saladin & Gray PIs) was to evaluate relationships between daily ovarian hormone levels and subjective responses to smoking and stress cues presented in the natural environment of smokers (via an iPhone app). This goal was achieved by having male and female smokers collect **daily saliva samples** (half hour after awakening) over a 14-day period. These samples were then assayed, using commercially available immunoassay kits (Salimetrics[tm]), by qualified laboratory technicians at MUSC's Clinical and Translational Research Center (CTRC). This study has permitted our research group to become highly proficient/experienced at the effective collection of daily biological samples and has yielded a rich data set. Preliminary analyses of the data indicate high levels of compliance with daily sample collection. Specifically, more than 90% of the participants who completed the study protocol provided ≥ 80% of the daily saliva samples. This high level of compliance resulted in stable and meaningful estimates of daily progesterone levels during the collection period, as indicated in **Figure 4**. Briefly, the figure shows the mean daily salivary progesterone levels (and SEs) of female smokers as a function of days (self-report) since last menses; it shows that progesterone levels are low and stable during most of the first half (follicular phase) of the menstrual cycle, followed by a steep rise during the early- and mid-portions of the second half of the cycle (luteal phase), followed by a decline as the next menses approach. The depicted pattern of time-dependent progesterone variation closely resembles that which has been reported in greater scientific literature (Schiller et al., 2012; Wira et al., 2015; Balen, 2007; Laycock & Meeran, 2012; Starr & McMillan, 2007). We believe these findings attest to our group's ability to reliably collect and assay large volumes of daily saliva samples for the purposes of estimating daily fluctuations in progesterone hormone levels.

Figure 4: Salivary progesterone level as a function of days after menses onset.



C.2. Research Design and Methods. In this project, the impact of progesterone on stress-related cannabis craving will be investigated in cannabis use disordered men and women. Cue Reactivity Ecological Momentary Assessment (CREMA), involving the assessment of reactivity to stressful cues, will be conducted three times daily during a one-week period of cannabis cessation; participants will be randomized to receive either progesterone or placebo during the abstinence period. Participants will be followed for two weeks after the cessation period to track return to cannabis use. Naturalistically collected stress reactivity data will be complemented by administration of a validated laboratory stress procedure (TSST) immediately after the cessation period, and daily salivary sampling will be used to track changes in progesterone levels. A schematic of study procedures is shown.



C.2.a. Participants. A total of 160 non-treatment seeking men (n=90) and women (n=70) with CUD, aged 18 to 45 years of age, will be randomized over a 54-month period. Additional inclusion criteria include consent to random assignment and ability to read and provide informed consent. Exclusion criteria include women who are pregnant, nursing, or planning to become pregnant during the course of the study; having a history of or current major psychiatric or medical disorder; and meeting criteria for moderate or severe use disorder for another substance with the exception of nicotine. A detailed list of inclusion/exclusion criteria can be found below.

C.2.b. Recruitment. Participants will be recruited primarily through media advertisements. Participants may also be recruited through ResearchMatch.org. As noted above in C.1.b., we have had good results using this method in previous studies.

C.2.c. Procedures.

C.2.c.1. Strategies to Ensure a Robust and Unbiased Approach and Rigor and Transparency. As detailed throughout this section, the proposed study will achieve robust and unbiased results via several design features including: explicit inclusion/exclusion criteria; randomization of treatment condition; placebo control; blinding; use of validated laboratory and interview/self-report measures and methods; explicit hypotheses with a priori/targeted data analytic plan; precision sample size estimation; a priori/strategic management of retention/attrition and missing data; and careful consideration of potential confounds. All experimental details are reported in a detailed and fully transparent manner to support replication.

C.2.c.2. Screening and Eligibility Assessment. Individuals interested in participating will be screened either by telephone or in person by the research study intake coordinator. A quick screen will be used to initially determine study eligibility. This questionnaire is focused on inclusion/exclusion psychiatric diagnoses, medical status, current medication regimen, and ability and willingness to fill out the necessary assessments and commit to completion of study procedures. Potential participants will be given a full description of the study procedures and asked to read and sign an IRB-approved Informed Consent Form if they choose to participate. Inclusion/exclusion criteria are detailed below.

Inclusion Criteria: 1.) Able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments. 2.) Meet DSM-5 criteria for mild, moderate or severe cannabis use disorder (within the past three months) and report using cannabis at least five times weekly over the past month. While individuals may also meet criteria for mild use disorders of other substances, they must identify cannabis as their primary substance of abuse. 3.) Age 18-45. 4.) For women, regular menses (every 25-35 days). 5.) Consent to remain abstinent from alcohol for 12 hours prior to study visits, and all other drugs other than cannabis or nicotine for the duration of the study. 6.) Women of childbearing potential must agree to utilize an effective means of birth control. 7.) Must consent to random assignment.

Exclusion Criteria: 1.) Women who are pregnant, nursing or of childbearing potential and not practicing an effective means of birth control. 2.) Women who are amennorheic or using progesterone-based contraceptives. 3.) Evidence or history of major medical illnesses, including liver diseases, abnormal vaginal bleeding, suspected or known malignancy, thrombophlebitis, deep vein thrombosis, pulmonary embolus, clotting or bleeding disorders, heart disease, diabetes, history of stroke or other medical conditions that the investigator deems as contraindicated for the individual to be in the study. 4.) Subjects taking antipsychotics (unless for indication other than psychosis), or regularly taking opiates or benzodiazepines 5.) History of or current psychotic disorder or bipolar affective disorder. 6.) Current suicidal or homicidal ideation/risk. 7.) Known allergy to progesterone or peanuts (vehicle for micronized progesterone). 8.) Meet DSM-5 criteria for moderate or severe substance use disorder (other than nicotine or cannabis) within the past 60 days. 9.) Unable to comply with study procedures or pose threat to study staff.

***a. Screening and Diagnostic Instruments*. Quick Screen:** This assessment will quickly determine whether an individual meets study inclusion or exclusion criteria. The instrument is designed to assess for substance dependence and obvious psychiatric, medical, and logistic exclusions. **Mini-International Neuropsychiatric Interview (M.I.N.I.):** The M.I.N.I. is a brief structured interview that was designed to assess DSM-5 diagnoses using a series of questions in dichotomous format (yes/no). Earlier studies have found that the M.I.N.I. is similar in sensitivity, specificity, and inter-rater reliability to other lengthier diagnostic interviews, such as the SCID-I/P (Sheehan et al., 1998). The alcohol and drug use disorder modules will be used to thoroughly assess both current and lifetime diagnostic status for abuse and dependence. It has excellent inter-rater and test-retest reliability (First et al., 2002). Participants will also have a physical exam. **Menstrual History Diary:** Participants will be asked to estimate the timing of their cycle for the 90-days prior to study entry and to track their cycle during study participation. **Perceived Stress Scale (PSS):** The Perceived Stress Scale is a 10-item assessment of daily stress (Cohen et al., 1983) that has previously been used in substance use research (Fox et al., 2009; Hyman et al., 2007). **Adverse Childhood Experiences (ACE) Questionnaire:** The ACE questionnaire assesses childhood maltreatment and exposure to household dysfunction. It has been utilized to examine the relationship between adverse childhood experiences and health risk behavior in adulthood (Felitti et al., 1998). *In addition to providing data to assess the impact of daily stress and early childhood trauma on cannabis use and stress reactivity, utilization of the PSS and ACE will likely allow for potential cross-SCORE collaborations as these scales have been previously recommended as components of the core assessment battery utilized across*

centers. **Coronavirus Impact Scale:** 12 item questionnaire assesses life changes that have occurred as a result of the coronavirus (Stoddard & Kaufman, 2020). We will also ask how substance use has been impacted

b. Substance-Related Instruments. **Time Line Follow-back:** The Time Line Follow-back (TLFB; Sobell & Sobell, 1992) is a calendar-based instrument designed to assess daily substance consumption. Cannabis use will be recorded in times used per day as well as quantity (e.g., grams, number of blunts/joints) in order to standardize for different types of cannabis use. **Urine Drug Screening:** Qualitative drug screens will be performed using the One Step Multi-Drug Test Dip Card (Drugconfirm™), a lateral flow chromatographic immunoassay for the qualitative detection of drug or drug metabolite in the urine at the following cutoffs (ng/ml): cocaine (300), amphetamines (1000), THC (50), opiates (2000), and benzodiazepines (300). In addition, quantitative urine cannabinoid drug screens will be performed using the AXSSYM® system from Abbott Laboratories. This assay is semi-quantitative with a detection cut-off value of 30.00 ng/ml (Abbott AXSSYM® System package insert). Urine creatinine will also be obtained, as creatinine normalization has been proposed as a method to differentiate new cannabis use from residual drug excretion (Huestis & Cone, 1998; Schwilke et al, 2011). Results will be used to ascertain abstinence prior to study procedures, cannabinoid levels, and to substantiate self-reports of all substance use. **Breathalyzer** (AlcoSensor III, Intoximeters, Inc., St. Louis): To ascertain abstinence from alcohol prior to study visits, participants will have their breath sampled for the presence of alcohol (Alco-Sensor III, Intoximeters Inc., St. Louis, MO). **Inventory of Drug-Taking Situations (IDTS)** (Annis et al., 1997): This instrument measures typical drug using situations based on Marlatt and Gordon's (1985) eight category taxonomy. This instrument contains eight subscales mapping frequency of use in eight distinct types of situations including use during negative situations (unpleasant emotions) and positive situations (social situations). Although initially developed to assess alcohol use, this scale has been modified for use with drug abusers and has demonstrated sensitivity to sex differences (Ross et al., 1994).

c. Remote screening visit alternative. Patients may also complete the initial visit remotely if warranted. In this case, subjects will be electronically consented through a secure platform such as Doxy.me or Redcap. The informed consent will be emailed to the participant prior to the video call. The patient will have the opportunity to ask questions on the call and will electronically sign the document. A signed copy will be emailed to the participant. The M.I.N.I interview and medical history then be conducted, also via Doxy.me. If clinically indicated, patients may have a physical exam the UDS visit. To date, no patients have been excluded due to physical exam findings. Patients will be advised to find a private location during these procedures to protect privacy and confidentiality. Patients will be sent survey links to complete self-report questionnaires.

d. Blood draws: In order to investigate the impact of moderate to heavy cannabis use and menstrual cycle on the endocannabinoid system, blood will be taken at Days 1, 8 and 22.

C.2.c.3. Progesterone Administration Procedures (Study Days 1-7). Following completion of screening procedures, participants will be scheduled to come to the clinic for training on CREMA and saliva (progesterone) collection procedures. The TLFB calendar will be completed. For female participants, menstrual history data will be used to schedule this session in the early follicular phase to standardize progesterone (or placebo) administration. During the early follicular phase, defined as the first 7 days after the onset of menses, ovarian hormones are low and largely invariant (approximately <1.1 ng/ml for progesterone and <85 pg/ml for estradiol; Chabbert-Buffet et al., 1998). This is critical for two reasons; first, to minimize the interaction between endogenous and exogenous sex hormones, and second, to achieve consistency between males and females as progesterone levels in men are steady and similar to levels observed in women during most of the follicular phase (Zumoff et al., 1990). At this visit, a urine sample will be collected to assess baseline cannabinoid level for purposes of cannabis abstinence verification (described below). Participants will be instructed to refrain from cannabis use during this study period.

a. Medication Randomization. Randomization will be done by the MUSC Investigational Drug Service, who will keep a record of the blind and be available should unblinding be required. Randomization will be stratified on the presence/absence of nicotine use as well as the presence/absence of comorbid anxiety disorders (as these disorders can influence stress reactivity).

b. Micronized progesterone (Prometrium; 200mg)**:** Progesterone is a hormone released mainly during the luteal phase of the menstrual cycle and pregnancy, and has been used for over 50 years in the treatment of various disorders including ovarian failure, premenstrual symptoms, amenorrhea, dysfunctional uterine bleeding, menopausal symptoms and for contraception (de Lignieres, 1999; Simon, 1995). Because it is poorly absorbed and has extensive first pass metabolism, several synthetic progesterone derivatives have been synthesized and marketed. However, these synthetic progesterone derivatives have additional androgenic, corticosteroid and

anabolic effects and have been associated with unfavorable side effects including fluid retention, androgenic effects (e.g., unwanted hair growth and acne) and alterations in lipid profile (Goodman, Gilman, Hardman, Gilman, & Limbird, 1996). Consequently, micronized progesterone formulations that can be taken orally have been developed to overcome the poor absorption of progesterone and the unfavorable side effects of synthetic progesterones (McAuley, Kroboth, & Kroboth, 1996; Simon, 1995). The safety, tolerability and efficacy of micronized progesterone have been demonstrated in multiple studies. Acute effects of micronized progesterone on physiological, performance and subjective measures have been investigated over a wide dose range, from 200 to 2,000 mg/day (Freeman, Rickels, Sondheimer, & Polansky, 1995; Gron, Friess, Herpers, & Rupprecht, 1997; Schweizer, Case, Garcia-Espana, Greenblatt, & Rickels, 1995). Micronized progesterone provides a specific pharmacological tool to investigate the effects of this hormone, devoid of the additional pharmacological effects of synthetic progesterone derivatives.

Participants will be provided with a seven-day supply of progesterone or placebo. Randomization to treatment will be done by the MUSC Investigational Drug Service (IDS). Dosing will begin in the evening on study day 1. On study days 2-7 participants will take medication at home in the morning and evening, and on day 8 the final dose will be taken at in the morning prior to completion of the laboratory stress task (described below). Progesterone and matching placebo capsules will be prepared and dispensed by the MUSC IDS. Previous studies (Fox et al., 2013; Sofuoglu et al., 2001; 2011) have used twice daily administration of 200mg progesterone during the early follicular phase to achieve stable levels of progesterone comparable to normal luteal phase levels (2-20 ng/ml). Safety and tolerability of micronized progesterone have been established in numerous studies with nicotine (Sofuoglu et al., 2001; 2011) and cocaine (Evans & Foltin, 2006; Fox et al., 2013; Reed et al., 2011 Sofuoglu et al., 2002; 2004) dependent individuals.

c. Cannabis Abstinence Verification. Cannabis abstinence will be assessed using self-report, saliva drug testing, and cannabinoid levels. Self-report will be collected at study visits using the TLFB as well as collected daily through mobile surveys or “morning reports” completed via mobile electronic device. Participants will be trained to self-administer saliva drug screening (Oratect FDA approved saliva test.). This test is able to detect THC in saliva for up to 14 hours. Participants will be instructed to complete two saliva drug tests daily using the RedCap video upload procedure described below under *Medication Adherence/Drug Test Monitoring*. A test will be completed in the office on Day 1 so patients can practice the procedure. Cannabinoid levels will also be assessed using urine toxicology collected on Study Days 8 and 22. Creatinine-corrected cannabinoid levels will be compared from Day 1 to Day 8 to confirm abstinence during the study period. Our group has recently published on the need for utilization of multiple methods to determine cannabis abstinence (Baker et al., in press). Contingency management (monetary compensation) procedures will be incorporated to promote abstinence (described below).

d. Medication Adherence/Drug Test Monitoring. Participants will record themselves taking their morning and evening medication doses as well as completing the twice daily saliva drug test swab procedure. Photos of the completed saliva test will be uploaded.A survey link will be sent to the participant’s (or study provided) cell phone via CREMA app alert twice daily. Video capture will occur as part of a RedCap survey. As discussed in the Preliminary Data section, we have previously utilized this technology successfully for both medication adherence monitoring and to remotely assess substance use. To encourage compliance with study procedures, participants will receive $10 for completion of each medication adherence video. Participants will receive an additional $30 for each saliva drug screen that is negative for all drugs of abuse. A similar compensation schedule was effective in our pilot progesterone study. Study staff will call participants twice during this week to see if they are having any difficulties with the procedures or side effects from the medication. Study staff will also text abstinence reminders to patients whose saliva tests positive for drugs of abuse.

e. CREMA Assessment. Cue Reactivity Ecological Momentary Assessment (CREMA; Warthen & Tiffany, 2009; Wray, Godleski, & Tiffany, 2011; Wray et al., 2015) is a cue reactivity assessment in which photographic stimuli are presented multiple times per day on a mobile electronic device (i.e iPhone). In cases where participants do not have a smart phone or do not wish to use their own device for the purposes of the study, a phone will be provided. Participants will be instructed to keep their phone with them at all times. Phones will be password protected to prevent access to the study assessments and surveys by anyone other than the participant. Auditory alarms will alert participants to complete <u>three, randomly scheduled CREMA sessions per day</u> (session duration = 3-4 minutes). Alarms will be distributed over a 12-hour period, divided into three, 4-hour blocks with a minimum time of 30 minutes between alarms/CREMA sessions. In order to accommodate varying participant wake-sleep schedules, participants will be able to choose the 12-hour period during which they will be prompted and this may vary from weekdays to weekends. There will be two types of picture cues that differ based on content:

stress-related (i.e., a child in distress) or neutral (i.e., sunglasses). Both stress and neutral picture cues will be derived from the International Affective Picture System or IAPS (Lang, Bradley, & Cuthbert, 1998). The IAPS is a set of over 1000 pictures which have been normed across 18 separate studies on three key features of emotion: (1) valence (feeling happy vs. unhappy), (2) arousal (feeling excited vs. calm) and (3) dominance (feeling in control vs. controlled). The photos that will be employed in the proposed study will be matched for valence and arousal using normative values provided with the IAPS. Specifically, stress and neutral pictures will be drawn from the mid-range of quantitative valence and arousal ratings; this should serve to minimize widely disparate responding from one trial to the next. Of the three CREMA sessions presented each day, one will contain a single neutral picture, one will contain a stress-related picture and one will not show picture cues, but will collect data on ambient (non-cue elicited) stress and craving, as well as cannabis use since last assessment (detailed below). We chose this frequency of stress cue presentation (once daily for 21 days) based on our previous CREMA work, which showed that stress responsiveness was maintained across 21 CREMA-based stressful picture presentations.

At the start of each CREMA session, participants will be asked to stop using cannabis (if they are at the time), and provide ratings on craving, mood, and stress using a modified version of the Within Session Rating Scale (Childress et al., 1986). This visual analog scale is anchored with adjectival modifiers ("not at all, mildly, moderately, and extremely"). The scale includes items assessing anxiety, stress, irritability, and craving, and we have utilized it successfully in numerous previous studies. If the session is one of the two daily picture assessments, next a photograph (either stress-related or neutral) will be presented and the participant will be instructed to look at it carefully for 10 seconds. Each picture cue will appear only once during the study. An alarm will sound at the end of each trial, after which participants will complete a post-cue Within Session Rating Scale assessment, ratings of how carefully they looked at the photographs and their distraction level during each trial, and data on their cannabis use since last assessment. If the session is only collecting ambient stress and craving, immediately after the Within Session Rating Scale the participants will be asked to record data on cannabis use since the last assessment session. To encourage CREMA session completion, participants will be compensated $5.00 for each completed session. Previous studies employing lower incentive strategies have reported 80-90% CREMA completion rates (Gass et al., 2012; Warthen et al., 2009), including in our previous SCOR (Wray et al., 2015); we have slightly increased compensation to maximize potential data collection over the 3-week period. CREMA sessions will begin on Day 2.

f. Progesterone Salivary Sample Collection. Participants will be instructed to collect a saliva sample each morning (1/2 hour after awakening). Samples will be dispensed into containers supplied by study staff and stored in participants' home freezers until bringing the samples to a subsequent study visit for analysis of progesterone levels. These daily home collection and storage procedures have been used in numerous previous studies with excellent participant adherence and sample quality (Edler et al., 2007; Klump et al., 2008). As discussed above in Preliminary Data, we successfully utilized a similar procedure in our last SCOR project. Participants will receive $10 daily for collection of ovarian hormone salivary samples.

g. Cannabis Withdrawal Assessment. The Cannabis Withdrawal Scale (CWS; Allsop et al., 2011) will be used to assess cannabis withdrawal symptoms, including negative affect and craving. CWS is a 19-item self-report measure that assesses withdrawal symptoms in the past 24 hours. The CWS will be administered twice daily during the progesterone administration phase as part of the RedCap medication adherence survey that will be sent via text message. Participants will be asked to respond with reference to the past 12 hours.

In order to assist participants with completing instruments and assessments at home, we will provide them with a link to an instructional video on YouTube and an FAQ sheet. These items will be emailed to participants after their Day 1 visit.

h. Cognitive Testing. Cognitive functioning will be assessed at BL and Study Day 8 to examine whether cognitive functioning is improved following progesterone administration and cannabis abstinence or whether changes in cognitive functioning mediate stress reactivity. Implicit cognitive function will be assessed using the Cannabis Approach-Avoidance Task (Jacobus et al., 2018; Sherman et al., 2018) which assesses the automatic tendency to approach rather than avoid drug cues, a cannabis adaptation of the dot-probe task that has shown sensitivity across substances of abuse (Field et al., 2006; Garland et al., 2013) which assesses cannabis attentional bias, and a cannabis adaptation of the Implicit Association Task (Wiers et al., 2011) which assesses cannabis memory bias. Explicit cognitive function shown to be impacted by SUDs will be assessed, including working memory – verbal (WAIS-IV Digit Span Subscale) and nonverbal (N-Back) (McClernon et al., 2016) tasks, and the Symbol Digit Modalities Test (SDMT) (Smith, 1982) which measures sustained attention, response speed, and visuomotor coordination.

As cognitive testing is not a primary outcome, it will not be completed at visit 1 or 8 when exposure needs to be limited for health and safety.

C.2.c.4. Laboratory Stress Induction Session (Day 8). Following the one-week period of progesterone or placebo administration, participants will return to the study clinic to complete a laboratory stress task (Trier Social Stress Test, TSST). Data from this task will be used to complement the "real world" stress reactivity data collected via CREMA. If participants become unable to attend the session on Day 8, they may be re-scheduled for the following day. However, participants will not be re-scheduled after Day 9.

a. Session Preparation. Participants will be instructed to arrive at 11:00am on the day of the TSST, and to avoid caffeinated beverages on the test day since caffeine can inflate reactivity to the stressor. If the individual is nicotine-dependent, (s)he will be provided with a nicotine patch throughout their study visit. Upon arrival, the participant will be breathalyzed and will provide urine and saliva samples, which will be tested for the presence of marijuana, cocaine, opiates, barbiturates, benzodiazepines, and stimulants. The urine sample will also be quantitatively analyzed for cannabinoids and creatinine to confirm past week cannabis abstinence. At 10:30am, saliva (progesterone) will be collected, and a blood pressure cuff will be placed on the participant's arm. Two baseline assessments of subjective, hormonal (cortisol; see Assessments below for details), and physiologic measures (blood pressure and heart rate) will be collected, one at 11:40am and the second at 11:55am.

b. TSST. The TSST is a standardized psychological stress challenge which has been used extensively in research studies. A meta-analysis supports its utility for provoking a HPA axis stress response in a laboratory setting (Dickerson & Kemeny, 2004). We have used it in our previous work to induce a robust and reliable physiological stress response in cocaine, alcohol, and cannabis-dependent individuals (McRae et al., 2006; McRae-Clark et al., 2011; Waldrop et al., 2010). At 12:00pm, the participant will be told that (s)he will give a speech and perform an arithmetic task. The topic of the speech will be why (s)he should be hired for a particular job (the individual's "dream job"). The participant will deliver the speech as though speaking to a group of potential employers. The experimenter then tells the participant that (s)he has 5 minutes to prepare the speech and starts the countdown clock (placed in view of the individual). The experimenter leaves the room to allow the participant to prepare. Five minutes later, three individuals unfamiliar to the participant (the audience) enter the room and are seated; the individual will be instructed by one audience member to stand and begin his/her prepared speech (without notes). The speech will be delivered for 5 minutes. If the individual pauses, (s)he will be instructed to continue. At the end of the speech task, the individual will be instructed to serially subtract 13 from 1,022 as quickly and accurately as possible. The mental math recitation will continue for 5 minutes, at the end of which time, the spokesperson will instruct the individual to stop and be seated, and the audience leaves the room. At this point, a saliva sample will be collected (for cortisol) and physiological (blood pressure and heart rate) and subjective measures (Within Session Rating Scale, Marijuana Craving Questionnaire, STAI) obtained. Measurements will also be obtained at 5-, 30-, and 60-minutes post-task.

Modifications may be made to TSST procedures if necessary for patient and staff safety, ie fewer confederates, remote procedures.

c. Assessments. Self-Report Measures. *Within Session Rating Scale*: As described above and used with the CREMA sessions, to allow for cross-task comparisons. *State-Trait Inventory (STAI):* The STAI is a 20-item self-report scale (Spielberger, 1983) employing a Likert-scale format with four responses per item (1-4). Ten of the STAI items measure feelings of stress and anxiety, while the remaining ten items measure feelings of relaxation. The STAI has good psychometric properties. *Marijuana Craving Questionnaire (MCQ):* The MCQ (Heishman et al., 2001) is a Likert-based self-assessment of cannabis craving shown to be a valid and reliable instrument for measuring cannabis craving. The 12-item MCQ will be used, which has been constructed by selecting the three items from each factor of the full 47-item MCQ that exhibited the most within-factor reliability. Physiological Measures. Heart rate and blood pressure will be measured using an intermittently inflatable cuff as indices of physiological arousal during the test session. Hormonal Measure. *Cortisol:* Cortisol will also be measured in unstimulated passive saliva using the Salimetrics expanded range, high sensitivity salivary cortisol enzyme immunoassay kit. This kit has a lower sensitivity level of <0.003 μg/dL. The correlation between salivary and serum cortisol has been shown to be high ($r=0.91$, $p<0.0001$). Cortisol is commonly measured as an indication of stress response in human laboratory studies.

d. Discharge. Following the 60-minute assessment, participants will be given instructions regarding monitoring of stress and cannabis use over the next seven days. A member of the research staff will be available to discuss management of any cravings if they remain elevated at the end of study procedures.

C.2.c.5. Follow-up Assessment (Days 8-21). During the follow-up period, participants will continue to be

queried three times daily regarding stress and craving levels (as assessed by the Within Session Rating Scale), as well as cannabis use in order to determine precipitants of, and sex differences in, return to use. As detailed above, two of these sessions will also include picture presentations (one neutral picture session and one stress-related picture session) to continue to assess stress reactivity during this period. Daily saliva samples will also be collected by participants as above. This monitoring will continue for two weeks following the laboratory stress session. At the end of the two weeks, on Day 22, participants will return to the clinic. At this visit, participants will return collected salivary hormone samples, provide a urine drug screen to validate self-reports of drug use, turn in any clinic-provided phones, complete a questionnaire regarding their experience with the CREMA and REDCap surveys, and be provided referrals to treatment if interested.

Participant Compensation: Participants will be compensated $40 for completing the screening visit ($20 for physical, $20 for interview) and $20 for Day 1. Participants will receive $5 for each completed CREMA session, for a total possible compensation of $300. Participants will receive a $5 bonus for each day they complete all 3 sessions for a possible total of $100. During the cannabis abstinence and progesterone administration period, participants can earn a total of $120 for completing saliva tests $360 for negative salivary drug tests and $140 for completing medication adherence video uploads. Participants will receive $190 for daily collection of salivary samples for ovarian hormone measurements. Participants will also be compensated $100 for Study Visit 8 ($50 if they re-schedule for Day 9) and $100 for Study Visit 22 , for a total potential compensation of $1470. If they borrowed an iPhone and do not return it they will be paid $50 for Visit 22. ClinCards may be mailed to participants or obtained at their first clinic visit. Participants will complete a w-9 which may be done in person or via Doxy.me.

Confidentiality: Confidentiality of all research data will be maintained by keeping all data in a locked file, limiting access to the computer database to only study personnel, and by using patient code numbers as opposed to names on all paperwork.

Data Management and Reduction: All paper-based assessments (other than laboratory reports) will be entered into REDCap, a secure, web-based application designed exclusively to support data capture for research studies. REDCap provides an intuitive interface for data entry (with data validation), audit trails for tracking data manipulation, and automated export procedures for data downloading to statistical packages such as SPSS and SAS. Quarterly database management and data integrity audits will be conducted.

C.2.d. Statistical Considerations

C.2.d.1. Statistics and Data Analysis (complete data analytic plan is provided in Biostatistics Resource Core)

Aim 1: Examine the impact of exogenous progesterone on stress cue reactivity and cannabis craving and withdrawal symptoms during a period of cannabis abstinence in the natural environment.

The primary measures of interest are stress and craving ratings in response to stress-related cues using Cue Reactivity Ecological Momentary Assessment (CREMA) in heavy cannabis using women and men. Hypothesis 1: Compared to placebo, progesterone will attenuate reactivity to stress cues presented in the natural environment among women vs. men with CUD. Hypothesis 2: Compared to placebo, progesterone will attenuate cannabis craving and withdrawal symptoms among women vs. men with CUD. To assess these hypotheses multilevel generalized linear mixed effects models with repeated measures (GLMMs) will be developed using CREMA session response data from the 1-week period during active medication administration. Pre-cue stress and craving rating will be centered and used as a time varying covariate in all analysis. Least squares means and associated standard errors will be used for post-hoc comparisons of cue response and CWS scores between treatment group assignments globally as well as time-specific (AM, PM) treatment group differences. Least squares means and associated standard errors will also be used for post-hoc comparisons of craving and stress response between women and men over time. Relevant interactions will be assessed in the models.

Aim 2: Examine the impact of exogenous progesterone on stress cue reactivity during a laboratory stressor task.

Following the initial 7-day active exogenous progesterone treatment period, participants will return for a laboratory-based stressor a session. The primary study outcomes of interest in Aim 2 are the differential craving, stress and anxiety responses of progesterone- vs. placebo-treated participants in response to the psychosocial stressor (TSST). *Hypothesis 1: Compared to placebo, progesterone will attenuate stress cue reactivity during a human laboratory stress paradigm among women but not men with CUD.* Immediately prior to and following the TSST (baseline, +0, +5, +30, and +60 minutes), self-reported measures of stress and craving will be collected. To assess the effect of exogenous progesterone administration on stress and craving response to the TSST, linear mixed effects models will be developed to assess differential treatment responses over time. The primary independent variable of interest will be randomized treatment assignment (progesterone vs. placebo). In addition to overall treatment differences, least squares means and associated standard errors will be used for post-hoc

comparisons between treatment groups at each post-cue time point. Additionally, sex will be added to the model and differential effect of progesterone will be assessed using an interaction term. When significant, stratified treatment effects will be compared and presented across females and males.

Aim 3: Evaluate stress, sex, and endogenous progesterone influences on cannabis craving and use. Endogenous progesterone levels (via saliva samples) will be obtained daily for two weeks following the completion of the active medication portion of the study. Daily progesterone levels will be assessed for associations with stress and craving responses to stress vs. neutral CREMA cues. *Hypothesis 1: Among women, higher daily levels or increasing endogenous progesterone will be associated with decreased cannabis use.* To evaluate this hypothesis, a multilevel generalized linear mixed effects models with repeated measures (GLMMs) will be developed using CREMA session response data from the 2-week follow up period. Statistical models will include day and subject level data, measured longitudinally. Daily salivary progesterone levels will be included as the primary independent predictor cannabis use. Relevant interactions will be assessed in all models. Additionally, increases in progesterone during the early luteal phase of each female participant's cycle will be evaluated; models will include dynamic changes in progesterone (between day rates of change) as a predictor of attenuated cannabis use. *Exploratory Hypothesis 2: High stress will be associated with cannabis craving and return to cannabis use in women more than men.* Time from the laboratory session to return to cannabis use (latency, in days) will be computed for all study participants. Kaplan-Meier estimates and log-rank tests will be used to assess initial relationships between measures of stress reactivity during the TSST (e.g., subjective stress, anxiety, BP, HR, cortisol) and relapse/latency while Cox proportional hazards models will be developed to assess the differential latency to use across sex for each stress reactivity measure during the TSST (using an appropriate interaction term; e.g. Peak TSST stress response x sex). Additionally, daily stress and craving responses to CREMA picture cues will serve as time-dependent covariates in latency models to assess the influence of recent stress and craving responses on relapse/latency.

C.2.d.2. Missing Data and Attrition. Missing data in longitudinal studies can be a problematic feature but can be mitigated through study design considerations. In order to minimize missing data and study attrition, study simplification and enhanced communication between study coordinators and participants will be emphasized. We will make every effort to prevent attrition, e.g., telephone reminders prior to visits, participation compensation, flexible scheduling, and reinforcing adherence at each visit. However, these methods do not ensure that all data will be collected and appropriate analytic methods will be employed to accommodate missing data (Refer to Biostatistics Resource Core for a more detailed discussion).

C.2.d.3. Power and Sample Size (complete sample size estimation is provided in Biostatistics Resource Core). In the proposed study, cue response and withdrawal data will be tested using GLMMs (assuming a Gaussian distribution) and the study will be powered to sufficiently address the smallest effect sizes determined to be clinically meaningful for the study hypothesis. Fox and colleagues (2013) examined exogenous progesterone and placebo administration on cue response in 42 cocaine dependent participants. Results indicated that female participants receiving placebo experienced larger negative emotion scores in response to stress-related cues than male participants on placebo (Δ=4.7; SD_P=3.4; between sex Cohen's d >1.0, p<0.01). Fox and colleagues also found that administration of exogenous progesterone reduced stress-cue related negative emotions significantly in women (Δ=2.4; SD_P=3.4, d=0.71) but not in men (Δ=-0.7; SD_P=3.3 d=-0.2; treatment x sex interaction Δ=3.1; SD_P=4.7, d=0.67). Assuming a similar response profile between treatment assignments and sex as above, a sample size of 64 participants (32 women, 32 men to each treatment assignment) randomized to the progesterone vs. placebo groups would guarantee 80% power with a type 1 error rate of 5% to detect the least effect size of d=0.67 in the stress reduction treatment x sex interaction (total n=128) during the 7-day, active treatment CREMA assessment period. Although the proposed study is of relatively short duration, we do anticipate 10% attrition between randomization and the end of the 1-week active treatment period and laboratory session. Thus, inflating the necessary sample size to 70 participants (35 men and 35 women), a total of n=140 randomized participants will be needed. Due to app issues at the beginning of the study, some initial data is unusable, so we will randomize 90 men. Additionally, data from our recently completed SCOR project, which examined daily salivary progesterone levels and cigarette smoking behavior for 14 consecutive days in untreated female participants, indicated that increased levels of daily progesterone within participant was associated with decreased cigarettes smoked per day (β=-0.32; SE=0.14; p<0.05). To detect a similar correlation (β=±0.32; SD=1.0) between daily progesterone levels and associated daily use metrics in the proposed study, the randomized sample of 70 women (62 with available follow up data), with 14 days of follow up use data, and with an autocorrelation of 0.9, would provide 80% power. The proposed sample size of **n=160** randomized

participants would provide 80% power or better to detect the proposed between sex and treatment comparisons noted in the primary study aims.

C.2.e. Design Considerations. i) Choice of between vs. within participant design. We considered implementation of a within-subject crossover design but opted instead for a between-subjects parallel-group comparison study for multiple reasons. Most notably, a within-subjects approach risks potential carryover effects between conditions, as well as potential dampening of cue reactivity and participant response fatigue over extended periods of testing across conditions. Additionally, withdrawal symptoms may not present consistently or reliably across multiple conditions in series without a lengthy/sustained return to *ad libitum* cannabis use between conditions. **ii)** Choice of administration of progesterone during cannabis withdrawal. Cannabis withdrawal is characterized by increased anxiety, irritability, craving, and physiological symptoms, which peak within the first 2-3 days of abstinence (Budney et al., 2008). Acute cannabis withdrawal is therefore an ideal period to investigate potential therapeutic effects of progesterone on stress cue reactivity and craving. **iii)** Focus on progesterone. The proposed study focuses on progesterone because evidence suggests that estradiol may enhance drug reinforcement and cue-reactivity. In preclinical studies, estradiol has been shown to increase dopamine release in the ventral striatum in response to nicotine administration (Thompson et al., 1994), and facilitate methamphetamine-induced conditioned place preference (Chen et al., 2003) and cocaine self-administration (Lynch, 2008; Lynch et al., 2001). Clinical research on menstrual cycle phase has demonstrated greater smoking cue-elicited neural activity and craving response during the follicular phase (where estradiol dominates) compared to the luteal phase (Franklin et al., 2015; Mendrek et al., 2014). Likewise, women with a higher estradiol to progesterone ratio took more cigarette puffs and smoked more of a cigarette in a smoking topography study (Schiller et al., 2012). Furthermore, compared to men, women smokers in the follicular phase showed increased stress response and arousal while women in the luteal phase showed increased craving (Saladin et al., 2015b). Together, evidence suggests that estradiol enhances drug reinforcement and cue-reactivity, and hence may contribute to the more deleterious outcomes observed in substance using women. Accordingly, we have focused this project on expanding knowledge about the treatment potential of progesterone (as characterized in the Significance above) by examining its effects on stress reactivity and substance use in persons with CUD. **iv)** Male comparison group. Apart from the obvious relevance of a male comparison group in studies of sex differences, there are empirical grounds for including men in proposed study. Sofuoglu et al., (2004) found that 2 oral doses of 200 mg progesterone significantly dampened cardiovascular responsiveness (diastolic BP) to iv cocaine in male, not female, cocaine users. Likewise, Fox et al., (2014) reported that exogenous progesterone vs. placebo attenuated cue-induced cocaine craving and cortisol responses in both men and women, suggesting that progesterone may also have some treatment potential for male cocaine users. Based on these and similar findings (Childs et al., 2010), we believe the proposed study's inclusion of both women and men with CUD will optimize our ability to identify therapeutic effects of progesterone that are either unique to one sex, or present in both.

C.2.f. Operational Plan and Research Timetable. Funding for five years is requested. The first three months will be used for hiring and training personnel and preparing for study initiation. As we have previously established standard operating procedures, we anticipate a rapid study start-up. 54 months will be needed for participant recruitment and data collection. The final three months will be used for data analysis and manuscript preparation.

PROTECTION OF HUMAN SUBJECTS

1. RISKS TO THE SUBJECTS

a. Human Subjects Involvement and Characteristics

Admission into the study is open to men and women and to all racial and ethnic groups, age 18-45. 160 cannabis use disordered individuals (90 men and 70 women) will be recruited primarily through internet and newspaper advertisements. Inclusion/exclusion criteria that apply to all participants are listed below:

General Inclusion / Exclusion Criteria

Inclusion Criteria

1. Able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments.

2. Meet DSM-5 criteria for mild, moderate or severe cannabis use disorder (within the past three months) and report using cannabis at least five times weekly over the past month. While individuals may also meet criteria for mild use disorders of other substances, they must identify cannabis as their primary substance of abuse and must not meet criteria for any other moderate or severe substance use disorder (except tobacco) within the last 60 days.

3. Age 18-45.

4. For women, regular menses (every 25-35 days).

5. Consent to remain abstinent from alcohol for 12 hours prior to study visits, and all other drugs other than cannabis or nicotine for the duration of the study.

6. Women of childbearing potential must agree to utilize an effective means of birth control.

7. Must consent to random assignment.

Exclusion Criteria

1. Women who are pregnant, nursing or of childbearing potential and not practicing an effective means of birth control.

2. Women who are amennorheic or using progesterone-based contraceptives.

3. Evidence or history of major medical illnesses, including liver diseases, abnormal vaginal bleeding, suspected or known malignancy, thrombophlebitis, deep vein thrombosis, pulmonary embolus, clotting or bleeding disorders, heart disease, diabetes, history of stroke or other medical conditions that the investigator deems as contraindicated for the individual to be in the study.

4. Subjects taking antipsychotics (unless for indication other than psychosis), or regularly taking opiates or benzodiazepines.

5. History of or current psychotic disorder or bipolar affective disorder.

6. Current suicidal or homicidal ideation/risk.

7. Known allergy to progesterone or peanuts (vehicle for micronized progesterone).

8. Unwilling or unable to maintain abstinence from alcohol 12 hours prior to study visits and all other drugs other than cannabis or nicotine for the duration of the study.

9. Meet DSM-5 criteria for moderate or severe substance use disorder (other than nicotine or cannabis) within the past 60 days.

10. Unable to comply with study procedures or pose threat to study staff.

b. Sources of Materials

Research material obtained from individual participants includes questionnaires and interviews with study personnel, ambulatory assessment data, and saliva and urine samples. To ensure confidentiality, all participant data will be letter/number coded, and only the investigators will have access to the master lists of codes. The research material will be obtained specifically for research purposes. Written research material obtained will be stored in the Addiction Sciences Division, in an office that is locked when not in use. Urine samples will be stored and processed in the Clinical Neurobiology Laboratory; saliva samples will be stored and processed in the Medical University of South Carolina Research Nexus Laboratory.

c. Potential Risks

1. Risks due to progesterone: Micronized progesterone is generally well tolerated. The most common adverse effect is headache. Other adverse effects that occurred at rates of 10% or more and at greater rates than placebo include headache, dizziness, abdominal cramps, breast pain, breast tenderness, joint pain, depression, dizziness, abdominal bloating, hot flashes, urinary problems, and vaginal discharge.

2. Risks due to study procedures: Participants will be asked not to smoke cannabis for 7 days. During this cannabis abstinence period, participants may experience symptoms of cannabis withdrawal such as craving cannabis, mild anxiety, insomnia, irritability, restlessness, stomachache, headache, nausea, loss of energy or appetite, chills, and diaphoresis. These symptoms are generally transient.

3. Potential risks of rating scales and questionnaires: These are all non-invasive, should add no risk, and have been used without difficulty or any adverse events in our previous studies. The only minor inconvenience could be the time taken to complete them. Some participants may feel uncomfortable disclosing personal thoughts and feelings.

4. Risks of stress induction procedures: There is a small risk of increased stress or cannabis craving as a result of the TSST and CREMA procedures; however, it will likely not differ substantially from the reactivity elicited by stimuli commonly encountered in the day-to-day environment of study participants. Participants that report sustained significant stress or craving after the laboratory session will be provided a debriefing to address symptoms.

5. Risks of blood draw: The risks of drawing blood include temporary discomfort from the needle stick, bruising, and infection. Fainting could occur.

6. Risks of loss of confidentiality: Careful efforts aimed at maintaining confidentiality have been effective in previous research, and only participants' code numbers will be recorded on the forms themselves to protect confidentiality. Phones provided by study staff to participants will be password protected to limit access to study assessments and pictures; participants utilizing their own phones will be asked to password protect their device for the duration of the study.

2. ADEQUACY OF PROTECTION AGAINSTS RISKS

a. Recruitment and Informed Consent

Potential study participants will primarily be recruited through the use of advertisements (internet, newspaper). Medical records will not be reviewed to identify potential study participants. The study PI, a Co-I, or other qualified study staff will obtain informed consent. The informed consent form includes a detailed description of the study procedures, along with statements regarding participants' rights to withdraw from the procedure at any time without consequences. The informed consent form will be explained to participants in easy-to-understand language, and subjects will be instructed to read the form carefully prior to signing it. Consent will be documented by the signature of the participant on the informed consent agreement, accompanied by the signature of the individual obtaining the consent.

b. Protections Against Risks

All study participants will be closely monitored for psychiatric and medical stability. All sessions will be conducted under the supervision of experienced personnel. If crisis intervention is necessary, senior staff will be available to evaluate the subject and provide an intervention or referral. If hospitalization is indicated, the patient will be hospitalized through the Center for Drug and Alcohol Programs at MUSC or an appropriate referral will be made. All participants will be fully informed that they may withdraw from the study at any time without penalty.

To ensure confidentiality, all participant data will be coded by letters and/or numbers, and only the investigators will have access to the master lists of codes. All participant records will be kept in a locked cabinet in an office that will be locked at times when not in use. The research staff understands the importance of maintaining confidentiality, and this method of maintaining confidentiality has been used for several years by our research group and has been effective. All electronic databases are stored on HIPAA-compliant servers with restricted access. All co-investigators and study personnel have completed (or will complete upon hiring) training in Good Clinical Practices as mandated by NIH and the MUSC IRB.

Participants will be taught about potential side effects of progesterone, and will be closely followed by psychiatrists, a PharmD, and other members of the research team. Pregnancy tests will be performed prior to progesterone administration. Adverse events will be monitored throughout the study. A member of the research staff will be available to discuss management of any craving urges if craving remains elevated at the end of any study procedures.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECT AND OTHERS

Possible risks to study participants include adverse reactions to progesterone administration or stress induction procedures. Potential benefits include detailed assessment of substance use and referral for treatment. Participants in the progesterone condition may experience a reduction in withdrawal symptoms. The minimal risks are reasonable in relation to the potential benefits to be gained from the study.

4. DATA AND SAFETY MONITORING PLAN

Trial Management.

The study will be managed from the Addiction Sciences Division within the Department of Psychiatry and Behavioral Sciences at the Medical University of South Carolina. The target population is described above in the inclusion/exclusion criteria.

Data Management and Analysis.

Data will be entered by research assistants directly into a computer using standard database software using REDCap. The data analysis plan is outlined in the Data Analysis Plan section.

Quality Assurance.

Quarterly data audits will be conducted. Confidentiality protections are outlined above.

Regulatory Issues.

Potential conflicts of interest will be reported using the upcoming NIH rules for disclosure. Adverse Events (AEs)/Serious Adverse Events (SAEs) occurring during the course of the project will be collected, documented, and reported in accordance with protocol and IRB reporting requirements. All research staff involved with adverse event reporting will receive general and protocol specific AE/SAE training including identification, assessment and evaluation, and documentation and reporting. A research assistant will identify any potential adverse events during the course of the study from participant self-report and administration of the visit assessments and procedures. The research assistant will provide information to a study physician, who will be responsible for AE/SAE assessment and evaluation including a determination of seriousness and study relatedness. Any significant actions taken by the local IRB and protocol changes will be relayed to ORWH/NIDA.

Definition of AE and SAE.

An Adverse Event (AE) is defined as any untoward medical occurrence in a study subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment (ICH GCP). Any unwanted change, physically, psychologically or behaviorally, that occurs in a study participant during the

course of the trial is an adverse event. A Serious Adverse Event (SAE) is defined as an adverse event that has one of the following outcomes:

• Results in death,

• Is life-threatening,

• Requires inpatient hospitalization or prolongation of existing hospitalization,

• Results in persistent or significant disability/incapacity,

• Is a congenital anomaly/birth defect OR

• Requires intervention to prevent one of the above outcomes.

Documentation and Reporting.

AEs/SAEs are documented and reported as per protocol and IRB requirements. Research staff will identify adverse events and obtain all available information to assess severity, seriousness, study relatedness, expectedness, outcome and the need for change or discontinuation in the study intervention. Adverse events are generally documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information if available should be documented in a progress note in the research record as appropriate to allow monitoring and evaluating of the AE. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms are completed and disseminated to the appropriate persons and within the designated timeframes as indicated above. For each AE/SAE recorded, the research staff will follow the AE/SAE until resolution, stabilization or until the participant is no longer in the study as stated in the protocol. When a reportable SAE is identified, the research staff will notify the MUSC Institutional Review Board (IRB) within 24 hours and complete the AE report form in conjunction with the PI. The MUSC IRB meets monthly and is located at 165 Cannon Street, Rm. 501, Charleston, SC 29425. Communication with the IRB is through email, memos, official IRB forms, and online reporting. A report will also be sent to the NIH program officer assigned to the project.

If complete information is not available when the initial 24-hour SAE report is disseminated, follow-up information will be gathered to enable a complete assessment and outcome of the event. This information may include hospital discharge records, autopsy reports, clinic records, etc. The research staff will attach copies of source documents to the SAE report for review by the PI and for forwarding to the NIH program officer as appropriate within 2 weeks of the initial SAE report. In addition, the PI will provide a signed, dated SAE summary report, which will be sent to the ORWH/NIDA Medical Safety Officer within two weeks of the initial SAE report.

We will report adverse events to the Medical University of South Carolina (MUSC) Institutional Review Board (IRB) online as soon as possible, but no later than 10 working days after the investigator first learns of the event. The MUSC IRB AE reporting requirements are as follows: All deaths that occur during the study or 30 days post termination from the study are required to be reported as adverse events even if they are expected or unrelated. Other adverse events are reportable to the MUSC IRB if the AE is unexpected AND related or possibly related AND serious or more prevalent than expected. All three criteria must be met for an AE to be reported to the MUSC IRB. The IRB definition of unexpected is that the AE is not identified in nature, severity or frequency in the current protocol, informed consent, investigator brochure or with other current risk information. The definition of related is that there is a reasonable possibility that the adverse event may have been caused by the drug, device or intervention. Reportable AEs are reviewed by the IRB Chair and reported to the IRB Board at the next meeting.

Trial Safety.

The potential risks and benefits and methods to minimize these risks are outlined above. The research staff will report any unexpected AEs or any scores of “severe” on the side-effect symptom rating form or any FDA-defined serious AEs to the PI within 24 hrs so that the PI can decide on the appropriate action. All unexpected AEs will be monitored while they are active to determine if treatment is needed. Study procedures will follow the FDA’s Good Clinical Practice Guidelines (www.fda.gov/oc/gcp). Any outside requests for information or any breaches in confidentiality will be reported to Dr. McRae-Clark.

Trial Efficacy.

An interim analysis is not planned at this time; however, an analysis will be performed if requested by ORWH/ NIDA or the DSMB. DSM Plan Administration.

Drs. McRae-Clark and Saladin will be responsible for monitoring the study, and will participate in weekly study meetings. A DSM report will be filed with the IRB and ORWH/NIDA on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of study participants, quality assurance issues and reports of AEs, significant/unexpected AEs and serious AEs. We will report outcomes at the end of the trial.

DSM Board.

A Data Safety and Monitoring Board will be formed to monitor both the rate and severity of adverse events. This panel will include 3 clinicians with expertise in substance use disorders and a statistician.

Risk Benefit Ratio.

The assessments and questionnaires are non-invasive and have inherently minimal risks. Potential risks of concern are loss of confidentiality and adverse events to progesterone and stress induction procedures. As discussed above, our research team will attempt to minimize these risks. Knowledge gained by the proposed study would help fill an important void in development of a potential gender-specific treatment for cannabis use disorders.

5. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

This study may provide important information that can improve treatment for future patients with cannabis and other drug use disorders. The moderate risks of the investigation are considered reasonable in relation to the expected knowledge to be gained.

6. CLINICALTRIALS.GOV REQUIREMENTS

In accordance with Public Law 110-85, this project will be registered at the ClinicalTrials.gov Protocol Registration System Information Website prior to study initiation.