PROTOCOL TITLE:

MUSC Specialized Center of Research Excellence (SCORE) on Sex Differences: Stress-Reactivity and Cannabis Use in Cannabis-Using Older Adults

PRINCIPAL INVESTIGATOR:

- Andreana Benitez, Ph.D.

1.0 OBJECTIVES / SPECIFIC AIMS

This study is funded through an administrative supplement to the parent SCORE grant (U54 DA016511, Pro# 00081360; PI: McRae-Clark), hereafter referred to as the "parent study."

The primary objective of this supplement study is to collect pilot data to expand the relevance of Cannabis use disorder (CUD) to Alzheimer's disease and related dementias (ADRD). We will recruit older adults ages 50-80 with CUD to undergo the proposed assessments in addition to ADRD-focused biomarkers (from MRI, blood, and neurocognitive testing) of ADRD risk.

Specific Aim: Evaluate the associations between cognition, stress, endogenous progesterone, cannabis use, and biomarkers of ADRD risk, and whether sex plays a moderating role. Consistent with the CUD literature in adulthood, we hypothesize that older adults with CUD will demonstrate normatively worse cognitive function. We will conduct preliminary analyses to examine whether this effect is greater in women than in men, hypothetically due to post-menopausal loss of progesterone and greater levels of stress driving cannabis use. We will examine the extent to which cognitive decrements are associated with ADRD biomarkers to generate subsequent hypotheses regarding the potential impact of cannabis use on ADRD risk.

2.0 BACKGROUND

Cannabis use in aging and risk for cognitive decline. Cannabis use among persons ages 50+ is rising. About 9.0% of adults ages 50-64 and 2.9% of adults ages 65+ have used cannabis in the past year (Han et al., 2018). Despite this trend, there is limited data on the impact of cannabis use in older adults (Briscoe et al., 2018). One particular risk of cannabis use for which older adults are especially vulnerable is cognitive decline. By mid-life an individual's lifetime risk for Alzheimer's disease and related dementias (ADRD) is 10-20% (Chene et al. 2015) and rises to where 1 in 3 older adults die of ADRD (Alzh Assn, 2019). Recent or heavy cannabis use may also increase risk for medical conditions such as vascular disease (Pacher et al., 2018), which, in turn, increase risk for ADRD (Kloppenborg et al., 2008) Thus, cannabis use may increase the incidence of cognitive decline and ADRD in older adulthood.

Sex differences in ADRD and depression/stress. While the incidence of ADRD is comparable between sexes, sex differences in risk factors may disproportionately elevate risk for women. Women are twice as likely to experience depression than men and mid-life depression confers as much as a 70% increased risk for ADRD. Thus, women may be at greater risk via a stress-mediated pathway (Mielke, 2018). This pathway may be especially relevant when investigating risk for ADRD within the context of cannabis use, as approximately one-third of older current cannabis users are women (Benitez et al., 2020).

Stress in older cannabis-using women. No prior research has verified whether sex differences in the role of stress on cannabis use motivations and craving extend to older adulthood. There is reason to believe that this stress-mediated pathway may differ in aging. The prevalence of depression decreases over the lifespan, and in older adulthood depression differs in risk factors, presentation, etiology, and prognosis depending on its onset (Fiske et al., 2009). That is, "late onset" depression is more likely to have vascular disease or incipient ADRD, whereas "early onset" depression is associated with significant family mental health history or predisposing traits. These variations may correspond to different patterns in cannabis use, motivations, or craving, and potentially to how cannabis ultimately exerts its effects.

Progesterone and substance abuse. The association between progesterone and substance abuse in post-menopausal women has not been studied, although these interactions are currently of great funding interest (Gipson & Bimonte-Nelson, 2020). Post-menopausal women may not have the benefit of

experiencing the stress-reducing effects of progesterone as this attenuates substance use, thereby potentially making them at disproportionately greater risk for substance use-related outcomes and ADRD.

3.0 STUDY ENDPOINTS

Primary Outcome: Global Cognitive Ability (GCA). Consistent with the CUD literature in adulthood (Sagar & Gruber, 2018), we hypothesize that older adults with CUD will demonstrate normatively worse cognitive function. The primary measure is the GCA factor (Kiselica et al., 2020). This is a psychometrically robust, factor analytically-derived (using 12 neurocognitive test scores), and demographically-normed (for age, sex, and years of education) index that yields a z-score representing global cognitive function.

Planned analysis: We will first conduct one-sample t-tests to determine whether this CUD sample's z-score mean is significantly below the normative population mean of z-score=0.We will then statistically test whether GCA is lower in women than in men, hypothetically due to post-menopausal loss of progesterone and greater levels of stress driving cannabis use. Next, we will assess the associations between GCA and progesterone measurements and stress (i.e. averaged pre- and post-cue CREMA session data and TSST cortisol and subjective ratings), by sex. Finally, we will examine the associations between GCA and ADRD biomarkers, by sex, to obtain preliminary estimates of potential associations between cognition and ADRD risk in the setting of CUD.

5.0 INCLUSION AND EXCLUSION CRITERIA/ STUDY POPULATION

Inclusion Criteria

i. Age 50-80.
ii. English as a first/primary language.
iii. Functional visual and auditory acuity (aided or unaided) to complete tests.
iv. Capacity to independently provide informed consent and function at an intellectual level sufficient to allow completion of all instruments.
v. Currently meets DSM-5 criteria for CUD or uses cannabis at least 4 days per week.
vi. Consent to abstain from alcohol and cannabis use for >12 hours prior to Study Visit (Day 0), and TSST (Day 8).
vii. Consent to abstain from all drugs other than cannabis or nicotine for the duration of the study.

Exclusion Criteria

i. Meet DSM-5 criteria for moderate or severe alcohol or substance use disorder (other than nicotine or cannabis) within the last 12 months.
ii. History of major neurocognitive disorder or developmental disorder per DSM-5.
iii. A Telephone Interview for Cognitive Status (TICS) score of $\leq$22.
iv. Significant or unstable medical condition/s that impact cognition as deemed by study investigators, such as active significant cardiac, cerebrovascular, neoplastic, infectious, or metabolic disease, or longstanding and intractable severe mental illness (e.g. schizophrenia spectrum disorder, bipolar disorder).
v. Daily use of medications that adversely impact cognition in aging (i.e. anticholinergics and sedatives).
vi. Current suicidal or homicidal ideation/risk.
vii. Unable to complete/comply with procedures or pose threat to research staff.
viii. Standard MRI contraindications (e.g., implants, claustrophobia).
ix. Women who are pregnant, nursing or of childbearing potential and not practicing an effective means of birth control.

6.0 NUMBER OF SUBJECTS

Target completion is 50 participants, comprising men (n=25) and women (n=25).

7.0 SETTING

Procedures will take place at the SCTR Research Nexus, Addiction Sciences research space at 125 Doughty Street, and the research-dedicated Center for Biomedical Imaging 3T Siemens Prisma MRI at 30 Bee Street. Certain procedures, such as consenting and questionnaire completion may be completed virtually through telehealth using an MUSC approved electronic platform (e.g. musc.doxy.me or REDCap).

8.0 RECRUITMENT METHODS

Potential Participants will be recruited via:

- Ongoing research studies by the study PI/Co-Investigators in which participants have consented to be contacted for other studies
- Direct referrals through MUSC Research staff
- Community recruitment by a member of the study team that is qualified and is delegated by the PI. Recruitment will be in both written and verbal format including but not limited to IRB approved printed and electronic materials. Participants will be recruited at community events as well as events marketed toward health and disease awareness.

Recruitment materials may include flyers, handouts, electronic and physical bulletin board postings, social media/message boards (i.e., Craigslist, MUSC Broadcast Research studies section of Yammer, Instagram, Facebook), web-based recruitment tools (e.g., ResearchMatch, Trial Match), newsletter/newspaper/media advertisements, and recruitment talks at local community events/organizations and surrounding community. Approvals will be obtained prior to displaying flyers in other community locations if/as needed.

Pre-Screening and Screening

Individuals interested in participating will be screened either by telephone or in person by research staff and the study and its requirements will be briefly explained to them. If subjects verbalize interest in participating in the study, staff will explain the screening process and ask if they would be willing participate in the screening.

If the potential Participant agrees to be screened, research staff will ask questions that focus 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. Pre-screening will include the administration of the Telephone Interview for Cognitive Status (TICS). TICS is a brief telephone-based cognitive assessment frequently used in clinical trials, where a score of ≤ 22 is a validated cut-off to exclude potential participants with possible dementia (Manly et al., 2011).

If the potential Participant meets all inclusion/no exclusion criteria that can be assessed via subject report and TICS, he/she will be asked to consent to the full study.

9.0 CONSENT PROCESS

Following recruitment and screening, the potential participant will be given a copy of the informed consent form (ICF) in-person or via postal mail or e-mail for review. There is no required timeline between their receipt of the ICF and deciding whether or not to participate. Participants will be encouraged to read the consent and take their time to decide.

Informed consent will take place on the MUSC campus or through telehealth (eConsent) by trained research staff in a private area. If consenting through telehealth using an IRB-approved electronic/online platform (i.e. REDCap or musc.doxy.me; all future references to an "electronic/ online platform" hereafter refer to these), the research staff will first confirm that participants have the appropriate technology to complete the electronic consent process. In the case of eConsent, the participant will be asked to locate a private and interruption-free environment in order to complete the appointment.

The study PI, a Co-I, or other qualified research staff will obtain informed consent. The research staff member will ensure that the Participant comprehends the study purpose and procedures, is provided with ample opportunity to ask questions. Participants will verbalize their consent to participate, sign the informed consent and HIPAA documents, and will be given a copy for their records. If consenting through an electronic/online platform, research staff will use a secure system for electronic or digital signature and will provide subjects with a copy for their records.

10.0 STUDY DESIGN / METHODS

Participants will complete 4 sets of procedures: Eligibility Assessment, Study Visit (Day 0), Cue Reactivity Ecological Momentary Assessment (CREMA; Days 1-7), and the Trier Social Stress Task (TSST; Day 8). *With the exception of the procedures within Study Visit 0, all of the procedures described here are IRB-approved for the parent study (Pro# 00081360):*

Procedure: **ELIGIBILITY ASSESSMENT**
Setting: Either or a combination of electronic/online platform or in-person delivery (at the Addiction Sciences research space at 125 Doughty Street), depending on participant preference
Personnel: Research staff
Duration: 1.5 hours
Description: Following consenting, trained research staff will administer the following semi-structured interviews and questionnaires to ascertain that he/she meets the clinical eligibility criteria using measures that are already approved and being administered in the parent study.
Measures: Clinical Assessments and Substance-Related Questionnaires

- Mini-International Neuropsychiatric Interview (MINI)
- Marijuana Use Questionnaire
- Time Line Follow-back (TLFB)
- Inventory of Drug-Taking Situations (IDTS)
- Perceived Stress Scale (PSS)
- Adverse Childhood Experiences (ACE) Questionnaire

Personal History (May be done at Eligibility Assessment or Day 0: Study Visit)

- Subject Demographics, Medications, and Health History: All participants will complete 3 brief questionnaires from the National Alzheimer's Coordinating Centers Uniform Dataset (NACC-UDS) to capture all relevant personal and medical history variables.
- Geriatric Depression Scale: A 30-item self-report measure of depression severity.
- Menstrual, Reproductive, and Hormone Therapy History: Female participants will complete a brief questionnaire based on the PhenX Toolkit. These measures were administered in NIH-funded lifespan studies including the Women's Interview Study of Health and the Study of Women Across the Nation.
- Mobile Device Proficiency Questionnaire-16 (Roque & Boot, 2018): This brief measure will index participants' facility with mobile technology. Researchers will use this information to triage participants in need for more assistance with CREMA.

Procedure: **DAY 0: STUDY VISIT**
Setting: Center for Biomedical Imaging (CBI; 30 Bee Street) and Research Nexus

Personnel: Research staff
Duration: 1 hour (at CBI) and 2 hours (at Nexus)
Description: Female participants who are of childbearing potential will first undergo a pregnancy test. A negative pregnancy test is required to proceed with all other study procedures. The breathalyzer test [alcohol] and saliva drug test [THC, cocaine, amphetamines, opioids, benzodiazepines] will be administered to ensure sobriety prior to initiation of all procedures. If breathalyzer or saliva test result is inconclusive, test will be repeated. If second test is also inconclusive but patient self-reports abstinence and does not exhibit signs of impairment, patient may proceed with study visit. Participants will also provide a urine sample, which will be tested for the presence of cocaine, opiates, barbiturates, benzodiazepines, and stimulants. The urine sample will also be quantitatively analyzed for cannabinoids and creatinine to characterize past week cannabis use. If results of the breathalyzer, saliva drug test are clear, then brain MRI, vitals, fasting blood collection, personal history questionnaires, and neurocognitive testing will be conducted ideally within the same day to collect the data for the following outcome variables:

- ADRD risk based on imaging and blood-based biomarkers (see below)
- American College of Cardiology/American Heart Association (ACC/AHA) 10-year risk of heart disease/stroke (Goff et al. 2013) to quantify vascular disease risk.
- Global Cognitive Ability (GCA) derived from neurocognitive testing.

Paper-and-pen and computerized cognitive tests will be administered to each participant by trained research staff. Testing will include ample time for breaks. Verbal responses to some tests will be recorded using a digital voice recorder to facilitate scoring accuracy. Audio files will be uploaded onto a secure server and labeled with the participant ID. The files on the digital voice recorder will be deleted after uploading.

Participants will also receive a study iPhone (if they do not have or wish to use their own) and will receive training on how to use it for the CREMA sessions. Female participants will receive a kit and instructions for providing saliva samples in conjunction with the CREMA sessions.

Measures:

<u>ADRD MRI-based biomarkers</u>

Medial temporal/hippocampal atrophy and leukoaraiosis volume: These metrics will be obtained using established image analysis pipelines in the PI's laboratory. Structural and functional MRI sequences will be acquired to obtain these and other preliminary data for future studies.

<u>Health Variables</u>

Vitals (blood pressure, pulse, oxygenation rate, height, weight)
Total lipid panel (from blood plasma)
High-sensitivity C-reactive protein (from blood plasma)

<u>ADRD blood-based biomarkers</u>

AB40, AB42, pTau-181, Glial fibrillary acidic protein, Neurofilament light: Measurements will be obtained from blood plasma and assayed through the vendor Quanterix which uses Simoa® technology for sensitive biomarker detection.

<u>Neurocognitive testing</u>

Version 3 of the NACC-UDS neuropsychological battery (Weintraub et al., 2018): This is the 40-minute test battery that produces the scores needed the generate the Global Cognitive Ability factor.

NIH Toolbox – Cognition Battery: We will additionally administer this 20-minute computerized battery to obtain complementary data on cognitive functioning for preliminary data/future research.

Procedure: **DAYS 1-7: CREMA**
Setting: At home, with study-issued or own iPhone
Personnel: Research staff will be available should participants need assistance

Duration: 3-4 minutes per session, 3 sessions per day

Description: The Cue Reactivity Ecological Momentary Assessment (CREMA) is a cue reactivity assessment in which photographic stimuli are presented multiple times per day on a mobile electronic device. Auditory alarms will alert participants to complete three, randomly scheduled CREMA sessions per day (session = 3-4 mins). Alarms will be distributed over a participant-selected 12-hour period, divided into three, 4-hour blocks with a minimum time of 30 minutes between alarms/CREMA sessions.

Measures: Female participants will be asked to give a saliva sample every morning they complete CREMA. Female participants will be given instructions on how to collect and store a saliva sample on Day 0. They will complete the sample within 30 minutes of waking up and before eating or drinking. All participants will complete the pre- and post-cue subjective measures of craving and stress, obtained as follows. At the start of each CREMA session, participants will be asked to provide ratings on craving, mood, and stress using a modified version of the Within Session Rating Scale (WSRS; Childress et al., 1986). This visual analog scale is anchored with adjectival modifiers. The scale includes items assessing anxiety, stress, irritability, and craving. 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 WSRS 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 WSRS the participants will be asked to record data on cannabis use since the last assessment session.

Procedure: **DAY 8: TSST**

Setting: Addiction Science research space at 125 Doughty Street

Personnel: Research staff

Duration: 3 hours

Description: On day 8, 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. Upon arrival, female participants will provide study staff with their saliva samples from the past 7 days. Women of childbearing potential will undergo a pregnancy test. All participants will be breathalyzed and undergo a saliva drug test to ensure sobriety prior to initiation of TSST. If breathalyzer or saliva test result is inconclusive, test will be repeated. If second test is also inconclusive but patient self-reports abstinence and does not exhibit signs of impairment, patient may proceed with study visit. Participants will also provide a urine sample, which will be tested for the presence of cocaine, opiates, barbiturates, benzodiazepines, and stimulants. The urine sample will also be quantitatively analyzed for cannabinoids and creatinine to characterize past week cannabis use.

Research staff will administer the Time Line Follow-back (TLFB) interview to assess marijuana use, with the time window being from the last TLFB (during the Eligibility visit) to today/Day 8.

The participant will provide a saliva sample to test for progesterone levels.

At 11:30am a blood pressure cuff will be placed on the participant's arm. Questionnaires (Marijuana Craving Questionnaire [MCQ], Mood, State-Trait Anxiety Inventory [STAI] questionnaires), hormonal (cortisol), and physiologic measures (blood pressure and heart rate) will be collected twice, once at 11:40am and again at 11:55am.

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 then starts the countdown clock (placed in view of the

individual). The experimenter leaves the room to allow the participant to prepare. Five minutes later, 3 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 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 third saliva sample will be collected (for cortisol) and physiological (blood pressure and heart rate) and questionnaires (MCQ, Mood, STAI) obtained. Measurements will also be obtained at 5-, 30-, and 60-minutes post-task.

Measures: Physiological (progesterone [1 sample], cortisol [6 samples], blood pressure, heart rate) measures and subjective (craving, stress) measures (MCQ, Mood, and STAI).

DAY 8: TSST

11:00	▪ SCREEN: Breathalyzer, Saliva drug screen, Urine drug screen ▪ SAMPLE: Collected urine will also be tested for cannabinoids and creatinine (quantitative) ▪ INTERVIEW: TLFB update
11:30	▪ PHYSIO: Place blood pressure cuff on arm ▪ SAMPLE: Collect salivary progesterone (1 of 1 sample, label “<studyid>_prog”)
11:40	1st of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, label “<studyid>_11:40”, store in fridge
11:55	▪ Bring supplies for Trier into the room: pen, paper, timer, clipboard 2nd of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, label “<studyid>_11:55”
12:00	▪ Read participants the script and set a 5-minute countdown clock. ▪ Leave the room and place cortisol <studyid>_11:55 sample in the fridge. ▪ Prepare the audience
12:05	▪ **When timer goes off, enter the room** ▪ Collect the participant’s pen and paper and tell the participant “Members of the research team will be with you shortly” ▪ 3 audience members enter the room, and 1 tells the participant to begin ▪ The speech must be for 5 minutes; If the participant pauses they will be prompted to continue
12:10	▪ Ask the participant to serially subtract 13 from 1,022 as quickly and accurately as possible for 5 minutes ▪ Then participant will be asked to be seated

12:15	▪ Audience will leave the room and you will immediately go in for the next two data collections ▪ Bring 2 saliva tubes, iPad, data collection sheet and bp cuff 3rd of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, then label "<studyid>_12:15"
12:20	4th of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, label "<studyid>_12:20", and store both <studyid>_12:15 and <studyid>_12:20 in fridge)
12:45	5th of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, label "<studyid>_12:45", store in fridge)
1:15	6th of 6 measurements: ▪ PHYSIO: Measure HB/BP ▪ QUESTIONNAIRES: MCQ, Mood, STAI on iPad ▪ SAMPLE: Collect salivary cortisol, label "<studyid>_1:15", store in fridge)
DEBRIEF	▪ Let the participant know that the task is meant to be stressful and that the people were not actually judging them
DISCHARGE	▪ **If patient's craving is significantly elevated** (20% higher than baseline), find qualified staff to talk to them prior to discharge. Have the clinician write a progress note to document their intervention. ▪ **If the patient's craving not elevated:** thank participant and discharge

Participant Compensation: Participants will be compensated as follows:

Eligib. Assessment:	$50
Study Visit (Day 0):	$55 (for brain MRI)
	$40 (for vitals and provision of blood and saliva samples)
	$50 (for neurocognitive testing)
CREMA (Days 1-7):	$105 (max, at a rate of $5/completed CREMA session [21 total sessions])
	$70 (max, at a rate of $10/completed saliva sample [7 total samples] – females only)
TSST (Day 8):	$50
Total:	$350 per participant upon the completion of all procedures (males only)
Total:	$420 per participant upon the completion of all procedures (females only)

Participants will receive payments after completion of each procedure as outlined above. Payments will be made using ClinCards. In the event that the Eligibility Assessment is initiated via telehealth, the ClinCard will be mailed to the participant after completing the session.

11.0 SPECIMEN COLLECTION AND BANKING

Urine, saliva, and blood samples will be collected and analyzed at the SCTR Research Nexus Laboratory, Clinical Neurobiology Lab at MUSC Institute of Psychiatry or an external lab. Unused/unanalyzed saliva, blood plasma, and urine samples will be stored at the SCTR Research Nexus Laboratory and maintained for future, undesignated research. Only the PI will have permission to request sample retrieval for IRB-approved research on which the PI is a principal or co-investigator. Only de-identified data will be shared along with these samples. Samples will not be used for genetic analysis. Participants can request to withdraw samples from banking by contacting Research Staff or the PI.

12.0 DATA MANAGEMENT

Specific Aim: Evaluate the associations between cognition, stress, endogenous progesterone, cannabis use, and biomarkers of ADRD risk, and whether sex plays a moderating role.

Consistent with the CUD literature in adulthood (Sagar & Gruber, 2018), we hypothesize that older adults with CUD will demonstrate normatively worse cognitive function. The primary measure is the Global Cognitive Abilities factor (Kiselica et al., 2020). This is a psychometrically robust, factor analytically-derived (using 12 neurocognitive test scores), and demographically-normed (for age, sex, and years of education) index that yields a z-score representing global cognitive function. We will conduct one-sample t-tests to determine whether this CUD sample's z-score mean is significantly below the normative population mean of z-score=0.

We will conduct preliminary analyses to examine whether this effect is greater in women than in men, hypothetically due to post-menopausal loss of progesterone and greater levels of stress driving cannabis use. The measures of interest are the Global Cognitive Abilities factor z-score, baseline progesterone measurements, and stress (i.e. averaged pre- and post-cue CREMA session data and TSST cortisol and subjective ratings). First, we will conduct independent samples t-tests to determine whether the Global Cognitive Abilities factor z-score is different between sexes. Second, we will run analyses of covariance (covariate: age) to test differences in baseline progesterone between men and post-menopausal women (which should be absent per Oettel & Mukhopadhyay, 2004). Next, we will run Spearman's rank correlations to investigate associations between stress and Global Cognitive Abilities by sex. Should stress be a more salient feature among women with CUD, we would find an inverse correlation between CREMA session data and Global Cognitive Abilities in women.

We will examine the extent to which cognitive decrements are associated with ADRD biomarkers to generate subsequent hypotheses regarding the potential impact of cannabis use on ADRD risk. We will conduct Spearman's rank correlations between the Global Cognitive Abilities factor z-scores and ADRD biomarkers (with transformations, as appropriate), by sex, to obtain preliminary estimates of potential associations between cognition and ADRD risk in the setting of CUD.

Power and Sample Size. This is a pilot study for the purposes of generating preliminary data to establish feasibility and power analyses for subsequent grants. The proposed analyses will provide effect sizes with which to generate sample size estimates. It will also provide us preliminary data for the acceptability of "real world" assessment of stress via CREMA in a population for whom proficiency with mobile technologies is diminished. Importantly, with our detailed assessment of menstrual, reproductive, and menopausal history, we will be able to meaningfully characterize hormonal influences on the hypothesized effects among women.

13.0 PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

To ensure data integrity and participant safety, the PI and all research staff involved with the project will discuss study-related concerns during monthly meetings throughout the study. In each meeting, updates on study visits, data collection, and entry will be tracked for each subject.

Quality Control. QC will include regular data verification of documentation (Integrity of the Consent and HIPPA, scores on the assessments, MRI scanning information), study progress and participant status, adverse events, and protocol deviations. Events determined by the PI to be unanticipated problems involving risks to subjects or others (UPIRTSOs) will be reported by the PI to the IRB as soon as possible and no more than 10 working days per policy.

Storage of Collected Data. All paper-based data will be stored in a locked laboratory suite. Data will only be removed from the file cabinet during data entry or audit. The PI and trained research staff will be responsible for the secure storage of all data. A file will be maintained that associates the participants' personal information with their respective alphanumeric identification numbers. This file will be in a file cabinet separate from the paper-based study data. Each participant will be assigned a non-identifiable study ID. All study charts will be stored in a locked office suite, and all study electronic files will be saved in a secure server. All data will be stored in a secure database using MUSC REDCap. Audio recordings will initially be captured on an end-user device and transferred to a secure server at our earliest convenience and then removed from the end-user device.

Data Entry Requirements. All paper-based data will be reviewed by research staff prior to data entry into REDCap. Research staff will maintain a log to ensure that all problems encountered will be addressed identically in the event of repeat occurrences. The data entry system will require a login identification and password to access the data. Validation and range rules will be set to entry fields.

Audit/Verification of Entered Data. All data collected and entered into REDCap will be subjected to a 100% cross-referencing procedure with the original paper copy. This audit must have an error rate less than 1%. If verification fails the audit, all data will be re-entered, the original computer files discarded, and the newly re-entered data audited. This process will continue until the audit no longer exceeds the maximum allowable error rate. All audits will be supervised and documented by the PI.

Final Storage of Paper Data. Once data have been coded, entered, and have passed audit verification, paper copies of the data will be housed at a facility that specializes in the storage of medical/research information. Only the subject's study identification number will be present on the forms. Any indication of the subject's name will be removed from the questionnaires prior to its archiving. The destruction date of these files will be at least 7 years from the termination of the study and will be authorized by the PI.

Monitoring Adverse Events. The PIs and Co-Investigators will be responsible for monitoring the safety of the study and complying with the reporting requirements. Continuous, close monitoring of participant safety will include prompt reporting of safety data (i.e., adverse/serious adverse events) to the MUSC IRB. Serious adverse events will be reported to IRB within 48 hours of the time project staff become aware of the incident. The review of data and procedures may result in amendment to the protocol, or changes to the data collection plan. Should the protocol or data collection plans be amended as a result of data review, the IRB will be notified and the amendment approved prior to study amendment implementation. In addition, the participants will be notified of any significant new findings that develop during the course of research (e.g., other potential risks) that may affect their wish to continue participation in the study.

14.0 WITHDRAWAL OF SUBJECTS

The PI reserves the right to withdraw a Participant from the study if it is in their best interest. The Participant may withdraw from the study at any point during their participation, at which point no other study procedures will be carried out. Study data will be retained unless otherwise requested by the Participant.

If a Participant wishes to have his/her data removed from the research study, he/she will submit a written request to the PI. The request will be filed in the Participants' study file and their data will be removed from the study database.

15.0 RISKS TO SUBJECTS

Risk of physical discomfort associated with venipuncture. Minimization: Participants may experience a slight pinch in their arm when the blood sample is taken, and subsequent slight bruising at the venipuncture site. While these effects commonly occur during this fairly routine procedure, these are transient and unlikely to cause persistent discomfort. Participants will be informed of this risk prior to the venipuncture to enhance their preparedness.

Risk of psychological discomfort while completing substance use and psychiatric assessments. Minimization: These are all non-invasive, should add no risk, and have been used without difficulty or any adverse events in prior studies. Some participants may feel uncomfortable disclosing personal thoughts and feelings. 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. A referral to a mental health provider will be given if the participant requests it.

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.

Risk of psychological discomfort associated with neurocognitive testing. Minimization: Neurocognitive testing is safe and non-invasive. It is possible, though unlikely, that participants may experience anxiety or distress during testing. If any of the tasks or questions elicit undue discomfort, participants will be encouraged to take breaks and will be reminded that they can discontinue testing or the study at any time. A referral to a mental health provider will be given if the participant requests it.

Risk inherent to undergoing an MRI especially with regard to metal implants. Minimization: Research staff will ensure that participants will undergo a thorough multi-step screen to determine MRI safety, wherein questions about metal exposure over the course of their lifetime through occupational experiences, medical interventions, etc. will be asked. Research staff trained in MRI safety will conduct the first screening interview during recruitment/screening, and again during the informed consent process. This process will ensure that individuals are not scheduled for an MRI, and then deemed ineligible later in time. Prior to scanning, the MRI technologist will again conduct a brief safety screen. All participants will be fully informed of the potential risks associated with MRI use.

Risk of discomfort from loud noises and the small space inside the MRI scanner. Minimization: Participants will be given ear plugs that significantly minimize the noise heard in the scanner. If this is unacceptable to the patient, and if the patient reports anxiety from confined spaces, the participant will be excluded from the study. No sedation will be administered during this time period. During the MRI scan, the participant

will be periodically monitored through a voice box and visually observed using multiple CCD cameras. If any problems arise, the study will be immediately terminated.

Risks of loss of confidentiality. Minimization: As with all research, there is a risk that participant information could be stolen. However, given that IRB requires that sensitive data be stored in locked filing cabinets in locked offices, and only accessible to the PI and very limited other research personnel, this risk is minimal. To ensure confidentiality, all participant data will be anonymized with alphanumeric codes, and only the investigators will have access to the master lists of codes. 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.

16.0 POTENTIAL BENEFITS TO SUBJECTS OR OTHERS

There will be no direct benefit to participants for participating in this study.

17.0 SHARING OF RESULTS WITH SUBJECTS

Participants may request a copy of their MRI. If an incidental finding is identified on MRI during data collection, the subject will be notified via telephone and will be given a CD copy of his/her MRI images (sent by mail or given in-person) to assist with his/her clinical follow-up.

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