Individual Differences in Response to THC
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With the current escalation in availability and use of *Cannabis* in the United States, there is an urgent need to understand the physiological and subjective effects of the drug. Understanding the sources of variation in responses to this drug is critical to both maximize its possible medical use as well as minimize public health risks. A recent review highlighted the need to study both acute and chronic effects of cannabis specifically in females. Females are increasingly using cannabis. They are more susceptible to developing cannabis abuse and dependence, have more severe withdrawal symptoms, and are more likely to relapse than males. Yet, females have been underrepresented in both preclinical and clinical research. In this project we focus on sources of variability to acute doses of the primary psychoactive ingredient of cannabis, delta-9-tetrahydrocannabinol (THC), in females. The effects of THC vary both across individuals and across occasions. It can increase heart rate and elicit paranoia, or produce calming effects and euphoria. At higher doses, and in certain individuals, THC can produce anxiety, eliciting a stress response, including activation of the autonomic nervous system (ANS) and the hypothalamus-pituitary-adrenal axis (HPA). Two variables known to modulate stress response in females are estradiol and circulating endogenous cannabinoids (eCBs). This raises the possibility that some of the variability in responses to THC may be related to baseline variations in estradiol and eCBs, which augment the effect of THC on the ANS and HPA axis. Here, we examine, in females, the idea that high estradiol levels and low circulating eCB levels predict larger increases in subjective anxiety after moderate oral doses of THC, with a stress response of increased heart rate, blood pressure and salivary cortisol. **My hypothesis is females tested during the late follicular phase, when estradiol levels are high, and females with lower circulating levels of endocannabinoids**, **will report greater increases in subjective anxiety and also exhibit a larger physiological stress response—a greater increase in heart rate, blood pressure, and salivary cortisol—after THC administration.**

Aim 1: To examine whether high levels of estradiol augment the effect of THC on subjective anxiety, heart rate, blood pressure and salivary cortisol in females. To test this, female occasional cannabis users will receive a single dose of THC (0, 15 mg) during either the early follicular phase (days 1-8) when estradiol levels are low, or during the late follicular phase (days 9-14) when estradiol levels are high. Participants will attend two experimental sessions separated by at least four days, to allow for clearance of the drug. Plasma levels of estradiol, progesterone, and endocannabinoids, as well as salivary cortisol, physiological measures and subjective mood ratings will be obtained at the beginning of all three sessions. During the sessions, physiological measures, subjective ratings of drug effects and mood, and salivary cortisol will be obtained at regular intervals after drug consumption. I hypothesize THC will produce a greater increase in subjective anxiety, heart rate, blood pressure and salivary cortisol during the late follicular phase, and that this will be correlated with higher circulating levels of estradiol.

Aim 2: To examine whether low levels of circulating endocannabinoids augment the effects of THC on subjective anxiety, heart rate, blood pressure and salivary cortisol in females. To test this, the plasma samples mentioned above will also be analyzed for endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels. Baseline AEA and 2-AG levels will be compared to THC-induced increases in subjective anxiety, heart rate, blood pressure and salivary cortisol. I hypothesize females with lower endocannabinoid levels will exhibit greater THC-induced increases in subjective anxiety, heart rate, blood pressure and salivary cortisol levels**.**

Aim 3: To evaluate determine whether subjective anxiety mediates the physiological stress response after THC. To test this, I will use a multilevel longitudinal mediation model to analyze the collected data. Our independent variables will include within-subjects drug dose and between subjects estradiol (EFG or LFG), AEA and 2-AG levels. Our mediator variable will be subjective anxiety across session time. Our dependent outcome measures include heart rate, blood pressure and salivary cortisol across session time. A multilevel

longitudinal mediation model will allow for the determination of how drug condition and baseline estradiol, AEA and 2-AG levels affects subjective anxiety across session time, which mediates changes in heart rate, blood pressure and salivary cortisol across session time. I hypothesize THC-induced increases in subjective anxiety mediate increases in heart rate, blood pressure and salivary cortisol across session time.

Significance.

The goal of this project is to determine the influence of estradiol and endocannabinoids on THC-induced increases in subjective anxiety and how this may mediate increases in heart rate, blood pressure and salivary cortisol. Cannabis and THC accessibility and consumption are increasing as medical and recreational use is being legalized across more states. Although there is evidence both low doses of cannabis and oral THC have some therapeutic effects, such as anxiolytic, analgesic and hunger stimulating effects, at moderate and higher doses the drugs can also produce serious unwanted effects including anxiety and paranoia (Zeiger et al., 2010; Crean et al., 2011). THC's effects vary across individuals, and may depend in part on body mass index, age, frequency of cannabis use and other variables such as hormone or endogenous cannabinoid levels (Wiley et al. 2014, D'Souza et al. 2008, Pope et al. 1996). Uninformed use of the substance could pose a public health risk, especially as recreational use is on the rise. A better understanding of individual differences in acute responses to THC will help to maximize the potential benefits and minimize adverse consequences of THC and cannabis-related products.

We will examine individual differences in response to THC, specifically caused by estradiol and endocannabinoid levels in healthy females. This research will provide a profile of the dose-related physiological and subjective effects of THC, including the influence of menstrual hormone levels, estradiol and progesterone, and circulating endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA). The proposed project will be carried out in a controlled environment, using double-blind drug administration, standardized and established physiological and subjective measures. The design will allow us to investigate the acute effect of THC on heart rate, blood pressure, state anxiety and salivary cortisol in females. We will test these effects in healthy female volunteers who have had some experience with cannabis, but are not chronic daily users. This proposed study will help identify risk factors for adverse responses to the drug. The study will also elucidate potential mechanisms underlying differences in subjective response to THC and why females demonstrate a more rapid progression from first use to cannabis use disorder (Khan et al. 2013, Ehlers et al. 2010, Hernandez-Avila et al. 2004, Westermeyer & Boedicker 2000).

To fully understand the effects of cannabis, as the drug becomes legal in more states and consumption increases, individual differences must be taken into account. If the proposed aims are achieved, this study will contribute significantly to the small literature on individual differences in response to THC. It will lay foundation for future individual-specific cannabis use guidelines and new individual-specific cannabis use disorder treatment options. Uncovering the individual differences in subjective response to THC will allow for more preventive action against THC-induced anxiety, paranoia, and psychosis. And a greater understanding of the physiological individual differences could prevent cases of THC-induced rapid heart rate (>100 bpm), drops in blood pressure and fainting, or nausea.

Approach

For this study we will use a mixed within- and between subject design to examine effects of THC (0 and 15 mg) in two groups of female occasional cannabis users: women tested in the early follicular phase of their menstrual cycle (EFG; N=30) and women tested in the late follicular phase (LFG; N=30). Occasional cannabis users will be defined as individuals who have used cannabis 10 or less occasions in the past thirty days. Females will be randomly assigned EFG or LFG groups. EFG women will be tested 1 to 8 days since the first day of menstruation and LFG women between days 9 and 14. Subjects will attend two four-hour experimental sessions in which they receive THC (15 mg THC) or placebo in counterbalanced order under double blind conditions. Plasma and saliva samples will be obtained before capsule consumption at the start of each session for baseline hormone, endocannabinoid and cortisol levels. The primary outcome measure is salivary cortisol, which will be measured both at baseline and periodically post-drug consumption. Secondary outcome measures include heart rate, blood pressure, body temperature, subjective drug effects (i.e. "feel drug effect"), and THC-induced anxiety. For Aim 1 we will examine THC-induced increases in cortisol in relation to baseline estradiol levels. For Aim 2 will examine THC-induced cortisol in relation to baseline endocannabinoid levels. For Aim 3 we will examine THC-induced increases in heart rate and subjective drug effects relative to THC-

induced increases in cortisol. The study will be approved by the Institutional Review Board at the University of Chicago, in accordance with the Code of Federal Regulations (Title 45, Part 46) adopted by the National Institutes of Health and the Office for Protection from Research Risks of the US Federal Government. The studies will be conducted in accordance with the Helsinki Declaration of 1964 (revised 1989) and the National Advisory Council on Drug Abuse Recommended Guidelines for the Administration of Drugs to Human Subjects.

Participants 60 healthy female volunteers who are occasional cannabis users (18-35 years, <11 uses of cannabis in past month) will be recruited by posters, advertisements, and word-of-mouth referrals, without regard to race or ethnicity. An undergraduate from the University of Chicago college will only be considered as a potential participant if she is within their 4th year of study. Potential participants will undergo a semi-structured clinical psychiatric interview (American Psychiatric Association, 2013) and provide information about current and lifetime history of drug use. Individuals taking any medications, or with serious psychiatric disorders such as psychosis or severe Post-Traumatic Stress Disorder or Obsessive Compulsive Disorder will be excluded. We will also exclude individuals with moderate or severe Substance Use Disorder, and those with BMI outside the normal range (less than 19 or more than 26), abnormal EKG, or pregnant or planning to be pregnant. Participants must have regular menstrual cycles and not be lactating or using hormonal contraceptives. Participant selection will be monitored throughout the study to ensure that the two groups, EFG and LFG, are matched on demographic characteristics, including age, race, education, SES, smoking, drinking, and drug use. Participants will be paid a $180 for completing all sessions. They will also have the opportunity to win an additional $8-$35 based on experimental task performance.

Orientation Session Participants will first participate in a 1-hour orientation session to provide informed consent, agree to the study conditions described above, complete the Trait Anxiety Inventory (Spielberger et al. 1971) and the Anxiety Sensitivity Inventory (Reiss et al. 1986), and practice questionnaires that will be administered throughout the sessions. Participants will be instructed to refrain from any use of drugs or alcohol for 24 before the experimental sessions. They will be advised that they must pass urine tests on the days of their experimental sessions for recent drug use including cannabis, and to pass these tests they should abstain from use for one week if they are light users (1-2 times per week) and for two weeks if they are heavier users (e.g., every other day). They will be told to have a normal night's sleep before the session and abstain from eating for at least 6 hours prior to their session. For blinding purposes, subjects will be told the capsules used in the study may contain a placebo, stimulant, cannabinoid-like drug, or sedative. The reason for blinding is we wish to study the pharmacological effects of this drug, without the possible confound of expectancies. We and others have shown that expectancies influence responses to drugs, in ways that might obscure true biological differences in the effects of the drug (see Mitchell et al. 1996; Kirk et al. 1998; Metrik et al. 2009; Heinz et al. 2013). Subjects will then practice the physiological measures and subjective questionnaires.

Experimental Session Timeline Participants will attend two experimental sessions lasting from 12:00 pm to 4:00 pm. Sessions are run from 12:00 pm to 4:00 pm to account for the diurnal rhythm of salivary cortisol—levels reach lowest levels at around midnight, start to rise at around 2:00 am to 3:00 am, reach a peak at around 8:30 am, an then slowly decrease back down to complete the cycle over 24 hours (Debono et al. 2009). Females in EFG will participate 1-8 days after first day of menstruation, at a time when both estradiol and progesterone levels are low (Barbieri et al. 2014). Females in the LFG will participate 9-14 days after first day of menstruation, at a time when estradiol levels are peaking or have just peaked, but progesterone levels remain low. The half-life of oral THC in infrequent users is about 1.3 days (Smith-Kielland et al. 1999). Therefore sessions will be scheduled with a minimum of 4 days to allow for washout.

Upon arrival at each session at 12 pm, subjects will complete breath, drug, and pregnancy screens to verify compliance (Alco-Sensor® III, CLIAwaived, Inc. Rapid Drug Test Cup, CLIAwaived, Inc. Pregnancy Urine (Dip-Strip)). Subjects who test positive will be dismissed. Then, pre-capsule heart rate, blood pressure and body temperature will be measured, and subjects will complete subjective questionnaires measuring mood and drug effects (see below). Then a trained and certified research assistant will collect a saliva sample and draw two 5 ml samples of blood to be analyzed for ovarian hormones, estradiol and progesterone, and endocannabinoids, anandamide (AEA) and 2- arachidonoylglycerol (2-AG) (see assay section below). Then they will consume a capsule containing placebo or THC (7.5, 15 mg; see drug section below). Sessions will be run from 12:00 pm to 4:00 pm. Every 30 or 60 min throughout the session, heart rate, blood pressure, and body temperature will

be measured, a saliva sample will be taken and subjective questionnaires assessing mood, the Profile of Mood States (POMS, McNair et al., 1971) and State Anxiety Inventory (STAI, Spielberger et al. 1971), and drug effects, the Addiction Research Center Inventory (ARCI, Haertzen et al. 1963) and the Drug Effects Questionnaire (DEQ, Johanson and Uhlenhuth, 1980; Morean et al. 2013), will be administered (see timeline below).

Measures *Physiological:* Blood pressure will be recorded at half hour intervals (see timeline below) using portable Omron 10 Series Upper Arm Blood Pressure Monitor to measure cardiovascular effects of the drug. Body temperature will also be measured using a Metene Medical Forehead and Ear Thermometer. An electrocardiogram and thoracic impedance will be measured to examine effects on heart rate, heart rate variability, and respiration. Seven disposable self-adhesive electrodes will be placed on the participant's chest and back to produce a standard lead II configuration for ECG and standard tetrapolar electrode configuration for thoracic impedance. ECG and thoracic impedance measures will be amplified and processed by an integrated Mindware Bionex system (Mindware, Gahanna, OH). These measures will be used to both track the cardiovascular effects of the drug, and ensure participant safety.

Subjective: Participants will complete standardized questionnaires.
1. *State-Trait Anxiety Inventory* (STAI, Spielberger et al. 1971): This scale measures both state and trait anxiety symptoms. For state anxiety symptoms, participants indicate the intensity of their state feelings on a 4-point scale from "not at all" (1) to "very much so" (4). For trait anxiety symptoms, responses for 20 anxiety symptom items are recorded on a 4-point scale from "almost never" (1) to "almost always" (4). Range of scores is 20–80, the higher score indicating greater anxiety.

2. *Anxiety Sensitivity Index* (ASI, Reiss et al. 1986): This scale contains 16-items designed to assess the construct of anxiety sensitivity: the dispositional tendency to fear the somatic and cognitive symptoms of anxiety due to a belief that these symptoms may be dangerous or harmful. Each item is rated on a five-point Likert scale ranging from "very little" (0) to "very much" (4). The ASI is the most widely used measure of anxiety sensitivity, and its predictive validity has been well established (for reviews, see Peterson & Reiss, 1992; Peterson & Plehn, 1999).This measure will be used to obtain baseline trait anxiety sensitivity during the orientation session.

3. *Addiction Research Center Inventory* (ARCI, Haertzen et al. 1963): This scale contains 53 statements commonly used to describe subjective effects of psychoactive drugs. The participant is to respond true or false when presented with a statement. The questionnaire is comprised of subscales for different substances: AMP-like drugs (A scale), morphine and benzedrine like drugs (MBG scale), lysergic acid-like drugs (LSD scale), benzedrine-like drugs (BG scale), pentobarbital-chlorpromazine and ALC-like drugs (PCAG scale), and cannabis-like drugs (M scale). We will focus on the M scale, as it represents the typical effects and symptoms of cannabis intoxication and it will provide a manipulation check to ensure the drug produced the typical drug-specific effects.

4. *The Drug Effects Questionnaire* (DEQ, Johanson and Uhlenhuth, 1980; Morean et al. 2013): This questionnaire contains visual analogue scales measuring subjective drug effects. It contains five questions, each associated with an anchored 100 mm line, on which participants indicate their response: "Do you feel any drug effect?" (rated from "none at all" to "a lot"), "Do you like the effects you are feeling now?" (rated from "not at all" to "very much"), "Do you dislike the effects you are feeling now?" (rated from "not at all" to "very much") "Are you high?" (rated from "not at all" to "very much") and "Would you like more of what you consumed, right now?" (rated from "not at all" to "very much"). This measure will be used another manipulation check to ensure the drug produced drug effects, but it will not be specific to a certain drug class.

5. *The Profile of Mood States* (POMS, McNair et al., 1971): This scale consists of 72 adjectives commonly used to describe momentary mood states. Participants indicate how they feel in relation to each of the 72 adjectives on a 5-point scale from "not at all" (0) to "extremely" (4). The questionnaire is comprised of eight subscales (Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, Elation). Two summary scales are derived from the other scales: Arousal = (Anxiety + Vigor) - (Fatigue + Confusion); Positive Mood = Elation – Depression. This measure will be used to examine changes in state anxiety pre- and post-drug consumption.

Time	Activities	Measures

12:00 pm	Arrive, urine, and breath tests	Drug and pregnancy tests, breathalyzer
12:15 pm	Time point (baseline), saliva sample and blood draw	Subjective measures, HR, BP, Temp., saliva sample and blood draw
12:30 pm	Capsule (0, 15 mg THC)	
1:00 pm	Time point	Set up ECG, subjective reports, HR, BP, Temp.
1:30 pm	Time point	Saliva sample, subjective reports, HR, BP, Temp.
2:00 pm	Time point	Saliva sample, subjective reports, HR, BP, Temp.
2:30 pm	Time point	Saliva sample, subjective reports, HR, BP, Temp., EEfRT
3:00 pm	Time point	Saliva sample, subjective reports, HR, BP, Temp.
4:00 pm	Departure	End of session questionnaire, removal of electrodes

Behavioral task The Effort Expenditure for Reward Task (EEfRT; Treadway et al. 2009) is a multitrial game in which participants will be asked to choose on each trial between a "hard" and "easy" task option to obtain varying monetary rewards. Briefly, each trial presents the subject with a choice between a "hard task", requiring 100 button presses with the nondominant pinky finger within 21 s, and an "easy task" requiring 30 button presses with the dominant index finger within 7 s. For easy-task choices, subjects will be eligible to win $1.00 for each successfully completed trial. For hard-task choices, subjects will be eligible to win higher amounts that varied per trial within a range of $1.24 –$4.30 ("reward magnitude"). Subjects are not guaranteed to win the reward if they complete the task; some trials are "win" trials, in which the subject will receive the reward amount, while others are "no win" trials, in which the subject will not receive money. To help subjects determine which trials are more likely to be win trials, subjects will be provided with accurate probability cues during the choice period. Trials will have three levels of probability: "high" 88% probability of a win trial, "medium" 50% and "low" 12%. Probability levels apply to both the hard and easy task, and there are equal proportions of each probability level across the experiment. Button presses will be completed on a standard keyboard.

Drug THC (Marinol® [dronabinol]; Solvay Pharmaceuticals) will be orally administered in a 15 mg dose, in opaque capsules with dextrose filler. Placebo capsules contain only dextrose. This dose of THC is known to produce performance impairments as well as subjective intoxication with little to no adverse reactions in experienced occasional, but non-daily cannabis users (Mntreyl et al., 2005; Issa et al. 2016).

Risks *Blood draw*: The risks involved in drawing blood from a vein may include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of lightheadedness when the blood is drawn, and rarely, an infection at the site of the blood draw.

Electrocardiogram: There are few, if any, risks related to an ECG. Some may experience a skin rash where electrodes were placed, but this is temporary and will clear without treatment.

Drugs:

Side effects: The drugs used in this study may produce a number of side effects, although most people do not experience any problems at the doses used in this study. Side effects may include abdominal pain, nausea, vomiting, dizziness, feelings of wellbeing, paranoia, sleepiness, abnormal thinking.

Driving or operating machinery: There may be risks to participants if they decided to drive, bike or operate machinery after the session. Therefore, they will not be allowed to drive or bike themselves to the session.

Confidentiality: There may be a risk of loss of confidentiality concerning some of the personal information participants provide to the investigators. As with any study, we will take many precautions to protect participant confidentiality.

Saliva Sample Protocol and Analysis Saliva samples for cortisol analysis will be obtained pre-drug consumption and 1, 1.5, 2, and 2.5 hours post-drug consumption by unstimulated passive drool. Participants will tilt their head forward, allowing the saliva to pool on the floor of their mouth, and then pass the saliva

through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Levels of cortisol in saliva will be measured using a salivary cortisol ELISA kit, a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol, at the University of Chicago Clinical Research Center.

Plasma Sample Protocol and Analysis

Ovarian Hormone Analysis 5ml blood draws will be obtained while participants are seated and collected in BDVacutainer Gold Top Serum tubes. Samples will be centrifuged at least 30 minutes after collection. The top layer of serum will be collected, aliquoted, and frozen at −80 ˚C until analyses for hormone levels occur. Plasma estradiol levels will be measured using an ELISA Estradiol (E2) assay. Plasma progesterone levels were measured using the Coat-a-Count Progesterone procedure (Diagnostic Products Corporation).

Endocannabinoid Analysis 5ml blood draws will be obtained while participants are seated and collected in ethylenediaminetetraacetic acid (EDTA) containing tubes (BDVacutainer, K3E EDTA K3). They will then be centrifuged (4° C at 3,500 RPM) within 5 minutes of collection, separated into aliquots, and frozen at −80 ˚C until analyses occur. Plasma samples will be assayed for Anandamide (AEA) and 2-Arachidonoylglycerol (2-AG) at the Medical College of Wisconsin, using the following methods: Plasma samples (0.5 mL each) will be thawed and made up to 15% ethanol, to which the internal standards, [2H8]-AEA (16.9 pmol) and [2H8]-2-AG (46.5 pmol) (Cayman Chemicals, Ann Arbor, MI) will be added. Samples will then be vortexed and centrifuged at 12,000 RPM for 4 min. The resulting supernatant will load onto Bond Elut C18 solid-phase extraction columns (1 mL: Varian Inc, Lake Forest, CA), which will be conditioned with 1 mL redistilled ethanol and 3 mL of double distilled water (ddH2O).The remaining pellet will be rinsed with 100 uL of 15% ethanol and recentrifuged at 12,000 RPM for 3 minutes. The resulting supernatant will also be loaded onto the C18 column. Columns will be washed with 5 mL ddH2O (eluate discarded) followed by1 mL of ethyl acetate (eluate collected). The ethyl acetate layer in the resulting eluate will be removed and dried under nitrogen. The samples will then be resuspended twice in ethyl acetate and dried. The final samples will then be resuspended in 30 uL of methanol and stored at −80 ˚C. Following preparation, the concentrations of eCBs (AEA and 2-AG), will be quantified in 5 µl of the methanol extract using stable isotope-dilution, electrospray ionization liquid chromatography/mass spectrometry of the daughter ions (LC-ESI-MS-MS). Standard curves will be generated for 2-AG, AEA and internal standards [2H8]-AEA and [2H8]-2-AG. Concentrations of the analytes will be determined from standard curves of the area ratios (standard/analyte) versus the concentration ratios (standard/analyte); [2H8]-AEA will be used as the standard for AEA, while [2H8]-2-AG will be used for 2-AG.

Statistical Analyses *Sample Size Calculation:* Sample size calculations were conducted using G*Power3.1 Software. Since there are no published data on effects of estradiol or eCB levels on response to THC a small effect size was assumed (d = 0.3). Recruiting 30 participants for each experimental group, 30 females in early follicular phase, 30 females in late follicular phase, for this study will allow for 80% power to detect an effect at alpha level p = 0.05.

A multilevel longitudinal mediation model will be used to analyze the collected data. Our independent variables include within-subjects drug dose and between subjects estradiol (EFG or LFG), AEA and 2-AG levels. Our mediator variable is subjective anxiety across session time. Our dependent outcome measures include heart rate, blood pressure and salivary cortisol across session time. A multilevel longitudinal mediation model will allow for the determination of how drug condition and baseline estradiol, AEA and 2-AG levels affects subjective anxiety across session time, which mediates changes in heart rate, blood pressure and salivary cortisol.

Expected Outcomes Based on several preclinical studies, I expect the LFG to have higher levels of estradiol and anandamide at baseline than the EFG, but no significant differences in baseline trait anxiety, anxiety sensitivity or salivary cortisol (Reed and Carr, 2000; Gorzalka and Dang, 2012; Monero-Lopez et al. 2018). Further, I expect that the effect of THC on subjective anxiety, heart rate and salivary cortisol will be greater in the LFG compared to the EFG, even after including any possible baseline differences in cortisol, trait anxiety or endocannabinoids as a covariate. Additionally, based on preliminary data and prior literature, I expect females with lower endocannabinoid levels, after controlling for cycle phase or estradiol levels, to experience larger increases in subjective anxiety, heart rate and report significantly greater subjective drug and mood effects of oral THC in a dose dependent manner (Hill et al. 2010). Finally, I expect THC-induced change in cortisol to mediate the increase in heart rate and greater subjective drug and mood effects. These results will contribute significantly to the small literature on individual differences in response to THC and lay a foundation for future

sex-specific cannabis use guidelines, to prevent against THC-induced anxiety, paranoia, and psychosis, and new sex-specific cannabis use disorder treatment options.

Potential problems, Alternative strategies, and Limitations Several problems could arise. First, it may be difficult to recruit qualifying participants in the planned time frame. This seems unlikely since similar inclusion criteria have been used in prior studies within the lab and there has not been significant difficulty recruiting these individuals. But if this is the case, I will increase venues for advertising and identify new online recruitment sources. Second, the differences in responses to THC in females at different phases of their menstrual cycle may be too small to detect with this sample. This is possible, even though we based the sample size on a small effect size. If the findings look promising we would consider increasing our sample size or utilize the data as a pilot for a future study with a larger sample. Third, the two groups of females may differ in pre-capsule measures, such as mood states, cortisol levels, eCB levels, blood pressure or in demographic characteristics such as prior drug use. I will include any of these significant differences between groups as a covariate in the final analyses.

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