IRB Protocol Title: Psychophysiology, Neurosteroids, and Stress in Premenstrual Dysphoric Disorder
IRB Continuing Review Approval Date: 2/1/22
NCT Number: NCT02777372

Study Protocol

The study will include up to four visits: a Baseline visit (eligibility screening for all participants), Test Day 1 (acoustic startle for all participants, occurring in the follicular phase for half of female participants and luteal phase for half of female participants), Test Day 2 (identical to Test Day 1; in a crossover manner for menstrual cycle phase for female participants), and Test Day 3 (identical to Test Day 2, occurring in the luteal phase for female participants). These are described in detail below.

Screening

Potential participants will contact the study coordinator. The coordinator will read a brief description of the study and answer any questions. Potential participants who are still interested will provide verbal consent 1) to complete a phone screen to determine initial eligibility, and 2) for female participants, to complete the Daily Record of Severity of Symptoms (DRSP).

Screening: Female Participants
The screening is performed over the phone.
The participant will:

1. Hear a description of the study
2. Provide verbal consent to complete phone screen, DRSP
3. Complete phone screen
4. Receive DRSP

Baseline Visit

At the baseline appointment the participant will sign informed consent, and will then undergo screening to ensure adherence to the inclusion and exclusion criteria, including a SCID to assess psychiatric history. Participants will be asked to submit a urine sample for toxicology screening. The urine analysis will be completed at the Women's Mood Disorders Center, therefore, the results of the screen will not be entered into the subject's medical record. Participants will undergo the "workup" and "habituation" portions of the acoustic startle procedure (see p. 17) to determine the appropriate level of stimulation for the startle protocol at the subsequent Test Day, and to determine whether they can tolerate the acoustic startle procedure. Participants will be asked to prospectively track symptoms associated with her menstrual cycle for 1 month with the DRSP. The baseline visit will last approximately 2 hours. Individuals who appear to meet criteria for PMDs will meet with the study medical professional to discuss the

use of sertraline and to review potential side effects as well as other options for the treatment of PMDs.

Baseline Visit:
The baseline visit includes signing informed consent and additional screening.
The participant will:

1. Sign informed consent
2. Complete clinician-administered rating scales
    a. SCID
3. Complete self-report rating scales:
    a. Participant Demographics Form
    b. Timeline Followback Calendar: Alcohol
    c. Timeline Followback Calendar: Cigarette Smoking
4. Acoustic startle protocol workup, habituation
5. Urine toxicology screen
6. Female participants will be reminded to continue to track symptoms daily on the Daily Record of Severity of Problems (DRSP), and will be provided a urine LH kit to determine ovulation.

Test Day 1

All participants will participate in this visit. This visit will be scheduled between Day 5 and Day 11 of the menstrual cycle (follicular) or at least 5 days after suspected ovulation or when progesterone levels are ≥3 ng/ML (luteal). At Test Day 1, participants will complete the self-report measures above, and will undergo the acoustic startle procedure. Participants will be asked not to use caffeine for 8 hours prior to the visit or nicotine for 1 hour before this visit, as this could affect ASR results. The acoustic startle protocol is described below. These measurements will be collected to the best of study staff's ability, however due to time constraints resulting from the detailed nature of the study visit schedule, some data points may not get captured. Participants will be asked to prospectively track symptoms associated with her menstrual cycle for 1 month with the DRSP. Test Day 1 will last about 3 hours.

Test Day 1:
The participant will:

1. Complete self-report rating scales:
    a. Timeline Followback Calendar: Alcohol
    b. Timeline Followback Calendar: Cigarette Smoking
2. Height and weight measurements
3. Complete the acoustic startle protocol
4. Undergo blood draw
5. Female participants will be reminded to continue to track symptoms daily on the Daily Record of Severity of Problems (DRSP) and will be provided a urine LH kit.

Test Day 2

All participants will participate in this visit. If Test Day 1 occurred during the follicular phase, Test Day 2 will be scheduled at least 5 days after suspected ovulation or when progesterone levels are $\geq$ 3 ng/ML. If Test Day 1 occurred during the luteal phase, the Test Day 2 will be scheduled between Day 5 and Day 11 of the menstrual cycle (follicular). If scheduled for the luteal phase, participants will be asked to come to the lab 1-2 days prior to the Test Day for a blood draw to confirm her progesterone levels. At Test Day 2, participants will complete the same self-report measures as above, and will undergo the acoustic startle procedure and hormone measures as described below. Participants in the PMD groups will be provided sertraline, described below. Test Day 2 will last about 3 hours.

Test Day 2: Female Participants with PMDs

The participant will:

1. Complete self-report rating scales:
    a. Timeline Followback Calendar: Alcohol
    b. Timeline Followback Calendar: Cigarette Smoking
2. Height and weight measurements
3. Complete the acoustic startle protocol
4. Undergo blood draw
5. Female participants will be reminded to continue to track symptoms daily on the Daily Record of Severity of Problems (DRSP) and will be provided a urine LH kit to determine ovulation if needed.
6. Receive sertraline

Test Day 2: Female Participants who are Controls

The participant will:

1. Complete self-report rating scales:
    a. Timeline Followback Calendar: Alcohol
    b. Timeline Followback Calendar: Cigarette Smoking
2. Height and weight measurements
3. Complete the acoustic startle protocol
4. Undergo blood draw
5. Female participants will be reminded to continue to track symptoms daily on the Daily Record of Severity of Problems (DRSP) and will be provided a urine LH kit to determine ovulation if needed.

Test Day 3

All participants will participate in this visit. Test Day 3 will be scheduled at least 5 days after suspected ovulation or when progesterone levels are >/ 3 ng/ML (luteal) of the menstrual cycle. At this visit, the PMD participants will be taking sertraline 50 mg/d. Based on the participants' menstrual tracking, she will be scheduled for Test Day 3 to coincide with her next luteal phase.

At Test Day 3, participants will complete the same self-report measures as above, and will undergo the acoustic startle procedure as described above. Test Day 3 will last about 3 hours.

Test Day 3: Female Participants

The participant will:

1. Complete self-report rating scales:
    a. Timeline Followback Calendar: Alcohol
    b. Timeline Followback Calendar: Cigarette Smoking
2. Height and weight measurements
3. Complete the acoustic startle protocol
4. Undergo blood draw
5. Female participants will be reminded to continue to track symptoms daily on the Daily Record of Severity of Problems (DRSP)

Detailed Description of Study Visit Components

Questionnaires:

Structured Clinical Interview for DSM-5 (SCID-5): The SCID (First, Spitzer, Gibbon, & Williams, 2002) is a structured interview to assess for the presence of other DSM-5 Axis I disorders, including major depression, dysthymia, bipolar disorder, schizophrenia, panic disorder, phobic disorders, generalized anxiety disorder, obsessive-compulsive disorder, somatoform disorders, and substance abuse and dependence. The widely used SCID-5 is a reliable and valid diagnostic interview.

Daily Record of Severity of Problems (DRSP): The DRSP (Endicott, Nee, & Harrison, 2006) is a well-validated daily symptom chart that was developed to aid in the diagnosis of PMS and PMDD. It includes 11 domains that address the criterion symptoms of premenstrual dysphoric disorder (depression, anxiety, mood lability, anger, interest in activities, concentration, lethargy, appetite, sleep, control, and physical symptoms). Participants rate each item daily, on a scale of 1 ("not at all") to 6 ("extreme").

Timeline Followback Calendar: Alcohol, Cigarette Smoking: These calendars provide a measure of alcohol and cigarette use in the past month.

Acoustic Startle Protocol:

The acoustic startle protocol will employ an NPU-threat task, which is designed to evoke both short-duration (fear-potentiated; amygdala-mediated; in the P condition) and long-duration (anxiety-potentiated; BNST-mediated; in the U condition) startle response. Participants will be asked not to smoke for at least one hour prior to the acoustic startle protocol, as nicotine may affect results.

The startle response in this project will be measured using the eyeblink reflex. The eyeblink reflex will be measured by recording activity from the orbicularis oculi muscle with two surface disk electrodes (Ag-AgCl) applied underneath the left eye with medical tape. A ground electrode will be placed on the forearm. Electrode impedance level will be below 5 Kohms.

Startle stimuli will be 50ms white noise bursts at 95 to 105dB with zero rise time presented through circumaural earphones. Warning or prestimuli will be a 60 to 75dB 1000 Hz tones with a 4-ms rise and fall time.

Instructions: The participant will be provided a description of the experiment using visual aids depicting the stimuli that will be presented during the NPU trials (images and script included in supplemental material). Briefly, the experiment will consist of three conditions: no shock (N), predictable shock (P), and unpredictable shock (U). In the N condition (green circle) no shocks will be delivered. In the P condition (red square), shocks will be administered only in the presence of a cue. In the U condition (blue triangle), shocks will be administered at any time, without cue. Participants will be provided the following information on the computer monitor throughout the testing procedure: (N) "no shock", (P) "shock only during red screen", and (U) "shock at any time". The participant will be asked to remain still, with their eyes open and focused on the computer screen during the trials.

Workup: Participants will undergo an initial shock workup procedure to determine a level of shock that is unpleasant but not painful. Given that this threshold may vary from person to person, the intensity of the shock will be increased until the participant feels that it is uncomfortable but not painful and this intensity will then be used for the duration of the test. Shock may range from 1-5 mA. The workup consists of administration of eight sample shocks, delivered in a graded manner. Each shock is rated by the participant, on a scale of 1 (barely felt) to 5 (very unpleasant / uncomfortable). The desired rating is 4 (quite unpleasant / uncomfortable). Based on the rating, the subsequent shock intensity is adjusted. The shock intensity will be adjusted in steps of 0.5 mA until the rating of 4 is achieved. If the rating of 4 is not reached after eight shocks, the maximum intensity of 5 mA will be used. The workup will be performed at the Baseline visit, and may be repeated at Test Days if the participant, during the "ratings" portion of the acoustic startle protocol, rates the startle protocol as less than moderately anxiety provoking (less than 5 on Questionnaire #2, Item 2).

Setup: Participants will be seated in a comfortable chair in a quiet room. The startle response will be measured using the eyeblink reflex, measured by recording activity from the orbicularis oculi muscle. Recording will be performed via two surface disk electrodes (Ag-AgCl) applied underneath the left eye; one in line with the pupil and one 1-2 cm lateral to the first one. The skin will be cleaned with alcohol and NuPrep prior to electrode application. Startle probes will be 50ms white noise bursts at 103dB with zero rise time presented through circumaural earphones. Aversive events will be a mild shock, 1-5 mA, lasting 100 ms, produced by a constant current stimulator and applied to the wrist. Once all of the electrodes have been applied, the participants will be told that they will wear a set of earphones and that occasionally they will hear different sounds through the earphones. They will also be instructed to sit still and quiet and to try to remain awake. Participants will be asked to orient to the screen and to passively view each screen during the startle session. Upon session completion, participants are asked to view the pictures one more time to rate them with respect to intensity of valence and arousal.

Habituation: The startle probe will be presented via earphones to the participant, who will confirm that s/he can hear the white noise. Participants will then undergo a habituation trial. This reduces excessive initial startle reactivity, prior to the threat of shock protocol. The habituation trial consists of nine startle probes delivered every 18-25 seconds. No shocks are administered at any time during the habituation trial.

NPU Threat Task: The task consists of two P, two U, and three N conditions. The participant will undergo two "blocks" of these N/P/U conditions (e.g. Block 1: P N U N U N P, Block 2: U N P N P N U), separated by a five minute break. Each N/P/U condition will last approximately two and a half minutes (120 s), so that each block of seven N/P/Us is approximately 17.5 minutes. Thus, total task time including the five minute break is 40 minutes.
Cues: Each N, P, U condition includes three cues (an 8-second presentation of a colored shape). The cue signals the possibility of a shock in the P condition only, and has no stimulus value in the N or U conditions.

Startle Probes*:* In each N, P, and U, six acoustic startle probes will be presented. The startle probe (50ms white noise burst at 103dB with zero rise time) is administered once during each cue presentation and once during each interval between cues. Startle probes are separated by a minimum interval of 20 s.
Shocks: During P and U, shocks will be administered. Shocks will be applied once or twice per P (to coincide with the cue, e.g. 0.5 s before the end of the cue) or U condition (randomized during absence of the cue, e.g. occurring 7-10 seconds after cue offset), resulting in 6 shocks per block and 12 shocks overall during the experiment.
Break: The participant will have a 5-10 minute break between the two blocks, at which time s/he may drink water, stretch her legs, and will complete ratings.

Ratings: After each block, the participant will be asked to rate her anxiety level retrospectively using a rating form (included in supplemental material).

Debriefing: After the startle protocol has been completed, study staff will meet with the participant to discuss the protocol and to review his/her experience and distress level.

Blood Draw: At each Test Day, blood will be drawn to assess the following.

Sex Steroid Hormones:
We will draw blood for sex steroid hormones.

SSRI Treatment:
At Test Day 2, PMD participants will receive SSRI antidepressant medication in the form of sertraline 50 mg capsule/tablet with instructions on how and when to take the medication. Medication will be taken only during the luteal phase after ovulation has been confirmed by urine LH kit. Participants will be asked to contact the study coordinator to let them know when the urine LH kit has indicated ovulation and to document the date of onset of sertraline treatment. This is known as intermittent dosing, which has shown efficacy in treating PMDD

(Yonkers et al., 2005, Steiner et al., 1997, Freeman et al., 2004). Women will remain on sertraline until onset of their next menstrual period at which time they will stop taking the medication. Participants in the healthy control group will not receive sertraline.

a. Study duration and number of study visits required of research participants.

The study will include up to four visits and a participant may be enrolled in the study for up to 6 months: a Baseline visit (eligibility screening for all participants), Test Day 1 (acoustic startle for all participants, occurring in the follicular phase for half of female participants and luteal phase for half of female participants), Test Day 2 (identical to Test Day 1; in a crossover manner for menstrual cycle phase for female participants), and Test Day 3 (identical to Test Day 2, occurring in the luteal phase for female participants). These are described in detail above.

b. Blinding, including justification for blinding or not blinding the trial, if applicable.

N/A; this is an open-label study.

c. Justification of why participants will not receive routine care or will have current therapy stopped.

All participants will receive routine clinical care and will continue to be monitored by their outpatient provider. Anyone who is decompensating clinically will be referred to the study PI in the Johns Hopkins University's Women's Mood Disorders Center for a clinical evaluation to determine next steps for treatment.

d. Justification for inclusion of a placebo or non-treatment group.

Only participants who meet criteria for Premenstrual Dysphoric Disorder will receive treatment in order to determine whether SSRI treatment is effective.

e. Definition of treatment failure or participant removal criteria.

Participants will be removed if they are lost to follow-up or are noncompliant with the study protocol.

f. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants will return for clinical care to their outpatient provider at the end of the study or if their participation ends prematurely.

1. Inclusion/Exclusion Criteria

Inclusion Criteria

Participants must:

1. Be aged 18 - 50 years per self-report
2. Be Able to give written informed consent per self report
3. Be Fluent in written and spoken English
4. Have normal or corrected to normal hearing and vision per self report
5. Must be experiencing regular menstrual cycles (24-35 days) per self-report
6. Have a negative urine drug screen

Exclusion Criteria

Participants cannot have:

1. Use of a psychotropic medication anytime in the past 2 months per self report, except benzodiazapeines PRN. Benzodiazapeines may not be taken for 24 hours prior to test days.
2. Drug or alcohol abuse history within previous 2 years
3. Lifetime history of psychotic disorder including, schizophrenia, schizoaffective disorder, major depression with psychotic features and bipolar disorder per self report
4. Currently homeless per self report
5. History of any Axis I disorder other then specific phobia within the past 12 months per SCID interview
6. Active suicidal ideation (suicide plan or suicide attempt) per SCID interview within the previous 6 months
7. Steroid hormone or hormonal contraceptive use in the past 6 months per self report, except use of levonorgestrel as an emergency contraceptive or temporary hormone use for oocyte cytopreservation, if it has not affected menstrual cycle regularity.
8. Pregnancy in the past year per self report if it has continued to affect current menstrual cycle regularity. Pregnancy during the study is also exclusionary. Participants must use a reliable, non-hormonal form of birth control during the study. If a participant becomes pregnant, she must inform study staff.
9. Sensitive hearing, per self-report.

Subject Selection and Withdrawal

Women ages 18 - 50 will be recruited via fliers, paid advertising, the Women's Mood Disorders website, MyChart recruitment through the ICTR and through clinician referrals. We will recruit healthy female controls and PMD participants. We intend to enroll 64 participants (27 per group, plus 5 per group predicted attrition) similar to previous studies by our group (Epperson 2007). Over the course of 4 years, this translates to recruiting 1-2 participants per month.

Participants may enroll in 1 of 2 study groups: the PMS/PMDD group or the healthy control group.

A presumptive diagnosis of PMDD, based on retrospective report during phone screening, will be further assessed with prospective daily ratings on the Daily Record of Severity of Problems (DRSP) as well as the clinician administered interview.

To meet criteria for the PMDD group based on the DRSP, women must have a 30% increase in mean DRSP score for five symptoms (listed below) comparing the mean score for each symptom from the follicular phase with the mean symptom score from the premenstrual/luteal phase, with at least one of the symptoms being irritability, mood lability, depressed mood, and/or anxiety/tension. She must have at least moderate symptom scores (≥4) for at least two days on 5 total symptoms in the luteal phase, with at least one of the symptoms being irritability, mood lability, depressed mood, and/or anxiety/tension. She must not have greater than mild (>3) symptom scores in the follicular phase. The Structured Clinical Interview for Diagnosis (SCID) for DSM-V will be used to confirm DRSP diagnosis and determine past and present Axis I disorders in all participants.

Women classified as control participants are asymptomatic during the premenstrual week and do not exhibit any Axis I disorder, past or present.

<u>Menstrual Cycle Phases:</u>
Day 1: First day of menstrual flow
Day 5 – 11: Follicular Phase
Day 10-14 post ovulation or when Progesterone levels are >/ 3: Luteal Phase

<u>DRSP Menstrual Symptoms:</u>

1. Felt depressed, sad, down, or blue or felt hopeless; or felt worthless or guilty
2. Felt anxious, tense, keyed up or on edge
3. Had mood swings (i.e. suddenly feeling sad or tearful)
4. Felt angry or irritable
5. Had less interest in usual activities (work, school, friends, hobbies)
6. Had difficulty concentrating
7. Felt lethargic, tired, or fatigued, or had lack of energy
8. Had increased appetite or overate, or had cravings for specific foods
9. Slept more, took naps, found it hard to get up when intended, or had trouble falling / staying asleep
10. Felt overwhelmed, unable to cope, or out of control
11. Had breast tenderness / swelling, bloated sensation, weight gain, headache, muscle / joint pain, other physical symptoms

Subject Recruitment and Screening

This research study will be conducted at the Johns Hopkins University's Women's Mood Disorders Center (WMDC). The potential participant may call the WMDC after seeing a flier or the website, or after being given a study flier or card by study personnel or their provider, or receiving an email (via MyChart).

All women who contact WMDC regarding study participation will have the study explained to them using the IRB approved phone script. Following the phone script all women who express interest in the study will have the option to complete a phone interview to determine initial eligibility. Prior to completing the phone interview, women will be read a verbal consent document and then choose to give or deny verbal consent to go forward with the phone screening. We have requested a waiver of documentation of informed consent to complete this interview. If participants are eligible based on the phone screen and 1 month of DRSP data, she will be scheduled for a baseline visit.

Early Withdrawal of Subjects

When and How to Withdraw Subjects

Early withdrawal of subjects may occur for the following reasons:

1. The subject fails to return for visits or fails to adhere to protocol requirements
2. Failure to locate subject
3. The subject withdraws her consent for participation
4. The PI determines that the subject is no longer eligible for participation in the study.

Potential subjects will be informed that they do not have to participate in this study, and that refusal to participate will involve no penalty or loss of rights to which they are otherwise entitled. Subjects will be made aware that they may withdraw from the study at any time without penalty or loss of benefits. Subjects who wish to withdraw are asked to provide written notice to the study coordinator. The subject's decision to withdraw and disposition upon withdrawing from the study will be recorded in her research chart. Subjects may be withdrawn from the study if they fail to adhere to protocol requirements or fail to return for study visits. There is minimal risk of adverse symptoms when abruptly discontinuing sertraline at 50-100 mg/d.

Data Collection and Follow-up for Withdrawn Subjects

All attempts to retain individuals in the study will be made, including calling the participant to remind them of their appointment, calling participants 2 times after a missed appointment and then sending a letter.

2. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Drug name: sertraline
Pharmacological class: selective serotonin reuptake inhibitor (SSRI)
Structural formula (if known): $C_{17}H_{17}NCl_2 \bullet HCl$
Formulation and dose: Tablets 25 mg, 50 mg, and 100 mg
Route of Administration: Oral

Sertraline hydrochloride, (chemical name: (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine hydrochloride), is a selective serotonin reuptake inhibitor; its mechanism of action is inhibition of CNS neuronal uptake of serotonin. Sertraline hydrochloride is a white crystalline powder that is slightly soluble in water and isopropyl alcohol, and sparingly soluble in ethanol. It is supplied for oral administration in scored tablets containing sertraline hydrochloride, dibasic calcium phosphate dehydrate, coloring, hydroxypropyl cellulose, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide. Absorption: T_{max}=4.5-8.4 hrs. Distribution: Plasma protein binding (98%). Metabolism: Liver (extensive); N-demethylation, oxidative deamination, reduction, hydroxylation, glucuronide conjugation. Elimination: Feces (12-14% unchanged), urine (minor); $T_{1/2}$=26 hrs. In humans following oral once-daily dosing of 50 to 200 mg for 14 days, mean peak plasma concentrations of sertraline occurred between 4.5 to 8.4 hours post-dosing. The average terminal elimination half-life of plasma sertraline is approximately 26 hours.

This dose was selected based on the recommended dosing in the Physicians' Desk Reference for Premenstrual Dysphoric Disorder. The PDR recommends the following dosing for PMDD, via oral route: Initial: 50mg daily continuous or limited to luteal phase of cycle. The dose may be titrated up to 150 mg/d if taking the medication continuously or to 100 mg/d for luteal phase dosing. As women in this study will be given one month of luteal phase dosing, we have chosen to use the 50 mg/d dosage. Previous studies using intermittent dosing have used a similar dosage (50-100 mg) and dosage period as proposed in the present study (Yonkers 2005, Freeman 2004). Side effects of sertraline may include dry mouth, increased sweating, somnolence, tremor, anorexia, dizziness, headache, diarrhea, dyspepsia, nausea/vomiting, agitation, insomnia, nervousness. Potential benefits include reduction of PMD symptoms and improved knowledge about the psychophysiology of premenstrual mood disorders (PMDs).

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Description

This medication is supplied in a 50 mg scored tablet. The tablets are blue and film-coated.

Treatment Regimen

Sertraline will be provided at a dose of 50 mg daily for up to 3 weeks, depending on the length of a woman's luteal phase. Medication will be taken only during the luteal phase. Women will initiate sertraline treatment upon determining that they have ovulated (using a urine LH Kit) and remain on sertraline until onset of their next menstrual period at which time they will stop taking the medication.

Method for Assigning Subjects to Treatment Groups

After completing Test Days 1 and 2, female participants with PMDs will receive sertraline (50 mg QD) in an open-label manner for up to three weeks.

Preparation and Administration of Study Drug

The Investigational Drug Service (IDS) will purchase the sertraline (generic). The capsule will contain 50 mg sertraline. IDS has been selected on the HS ERA application.

Subject Compliance Monitoring

Participants will be reminded to bring their pill bottles with them to each appointment so that pill usage can be more closely monitored.

Prior and Concomitant Therapy

We will exclude individuals who require the use of any antidepressant or other psychotropic medication with the exception of occasional benzodiazepine use. No other psychotropic medication will be allowed during the sertraline treatment phase of the study.

Packaging

Sertraline is designed as a tablet for oral administration. It is commercially available from the manufacturer. All study drug used in this study will be obtained directly from the manufacturer and dispensed by the Investigational Drug Service (IDS).

Receiving, Storage, Dispensing and Return

Receipt of Drug Supplies

Drug will be sent from the manufacturer to the Investigational Drug Service (IDS). Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

Storage

Medication will be stored at the study pharmacy (IDS) using standard methods.

Dispensing of Study Drug

The drug used for this study will be placed in a bottle labeled with the participant's name, instructing them to take the 50mg capsule(s) by mouth. The capsules will be ordered through the IDP at Johns Hopkins Medical Institute with the help of an assigned Lead Research Nurse and picked up the WMDC research staff and given to the participant. Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug

remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

c. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Not applicable.

a. Early stopping rules.

N/A -This is not a high risk study, and is not examining primary safety endpoints.

3. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Potential Study Risks:

The risks associated with this study are minor. While there are several assessments conducted, including questionnaires, a clinical interview, blood draws and saliva samples, they are all within the scope of routine clinical practice.

Questionnaires, Interview: Subjects may experience mild psychological distress while answering questions about their mood. If any subject reports significant distress or suicidality, a trained psychologist will evaluate the subject and a decision about emergency treatment versus referral will be made.

Blood Draws: The risks of the blood draw procedure are local site pain, hematoma, bleeding, fainting and infection all of which are minimal and the same as with any standard blood draw. The amount of blood drawn will be far less than a standard blood donation. All research staff members dealing with blood draw are highly experienced in phlebotomy.

Startle Procedure: There are no risks of physical or psychological injury associated with this procedure. The electric shocks, while unpleasant, are not intense enough to cause physical injury. The test is non-invasive and does not require the administration of any needles, drug, or dyes. The only direct contact with the subject is the placement of the electrodes to record the physiological measures. These electrodes are connected to amplifiers and recording devices

that have been specifically designed for human studies. The possibility of skin irritation from contact with the saline electrode paste exists. However, this is unlikely as the salt concentration of the paste is very similar to that of human sweat.

Startle Probe: The acoustic white noise delivered through the earphones may prove to be unpleasant to some participants and rarely may cause discomfort during or after study procedures

SSRI Treatment: The most common side effects from sertraline include diarrhea, nausea, insomnia, dry mouth, and fatigue, per sertraline prescribing information. A risk of this study is that the treatment will be ineffective. Patients who do not respond to the treatment may be referred for treatment outside the study. Another risk is symptom worsening

Nonspecific risks: As a result of their participation in these studies, participants may be inconvenienced by frequent study visits. There is also the remote possibility that confidentiality could be breached.

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b. Steps taken to minimize the risks.

Questionnaires, Interview: A trained research assistant will be available at all times while the participant is filling out questionnaires. The Principal Investigator will also be available by telephone, and WMDC clinicians (including a psychiatrist) will be onsite during the administration as well. Should the participant become distressed, the study staff member can provide reassurance and evaluate her psychiatric state. Participants will be informed during screening and consenting procedures that they do not have to answer or complete tasks that cause them distress. Each participant will be directed to inform the study team member if she wishes to skip a certain part. Research staff will evaluate the participant's scores on the mood scales and psychiatric interview (SCID, determines if the participant is currently psychiatrically ill based on DSM-V criteria). The study psychiatrist and/or research staff will also interview the patient regarding safety and if appropriate (the participant is thought to be in danger of harming herself or others) escort the participant to the emergency room (across the street from the location of the Women's Mood Disorders Center) or direct the patient to emergency care. Alternatively, if the participant is experiencing significant mood or anxiety symptoms but is not in acute danger, the research staff will provide a referral guide to the participant and offer to make an appointment for a consultation at the Women's Mood Disorders Center.

Blood Draws: All research staff members dealing with blood draw are highly experienced in phlebotomy.

Startle Procedure: Every effort will be made to help the participants feel comfortable with the initially new surroundings and with the wires and electrodes. Care will be taken in applying and removing the tape so as to avoid any skin irritation or discomfort. All subjects will be debriefed following the procedure. We encourage participants to let us know if they are in a state in which they cannot continue with study procedures due to any aspect of the test day.

SSRI Treatment: Subjects will be asked to contact study personnel if their symptoms worsen between visits. Monitoring during the study will permit therapies to be terminated rapidly, when clinically indicated, for patients who show signs of worsening or the onset of significant suicidality. If a suicidal crisis develops after the study is initiated, patients will be withdrawn from the protocol, hospitalized if necessary, and treated with whatever type of therapy seems most clinically indicated. Subjects will be asked to provide permission to be contacted if they miss sessions to help ensure close monitoring of their conditions.

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c. Plan for reporting unanticipated problems or study deviations.

Reports will be made to the IRB and necessary healthcare providers in the event of an unanticipated problems or study deviations. The study team will follow IRB protocol to determine if a deviation or problem needs to be reported immediately or if it can be reported at the yearly continuing review. All serious adverse events will be reported to the IRB immediately by the PI.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Confidentiality: Careful procedures will be employed to protect the confidentiality of all participants. Participants will be assigned unique ID identifiers not associated with personally identifying information. These will be used on all interview protocols and lab results. No identifying information other than the unique ID will appear on interview protocols. All data files and analyses will be performed on research computers using only code numbers to identify participants. Only summaries of group data will be reported in any publications or presentations, with no identification of individuals. All study personnel will maintain their certification in the Protection of Human Subjects training program prior to participating in the research. Only authorized persons will be granted access to enter and view study data. Subject IDs with subject names will be kept in CRMS and only designated personnel on the research team will have access. Charts containing subject data for active subjects will be kept in a locked filing cabinet or room and will be identified only by subject ID. All precautions will be taken to protect the confidentiality of the study participants. As with participation in any research study there is a slight risk of a breach in the security procedures that would affect the confidentiality of the research participant. Every effort will be employed to minimize this risk. If such a breach occurs, the research participant will be informed as well as the IRB.

e. Financial risks to the participants.

Not applicable.

4. Benefits

a. Description of the probable benefits for the participant and for society.

A potential benefit of this study is that the treatment may reduce symptoms, however, this is not guaranteed. This study will benefit society in general, by advancing knowledge of PMD psychophysiology, an understudied area. We believe that the risks associated with this study are small, compared to the benefits participants may receive from participating. Any treatment for PMD is associated with some chance of failure to respond or subsequent relapse or recurrence.

5. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will be compensated $25 at Baseline for the completion of patient and clinician ratings and $75 at each visit for completing Test Days 1-3.

Participants will receive $10 for each DRSP they submit. Then, at the end of their study participation, they will receive the remainder to amount to $100 total. Every participant who turns in all DRSP ratings on time and completes study participation will receive the same $100, but the speed at which she receives this will depend on the speed at which she turns in her DRSP. This system only applies to DRSPs received after study enrolment, not DRSPs used as a study screening tool prior to consent. Participants will only receive payments for DRSPs that are filled out according to study instructions. If it becomes apparent that a participant has been filling out her DRSP retrospectively and not in real time, or she turns in DRSPs with missing dates, she will not receive compensation for that month's DRSP and will receive $10 less at the of her study participation.

Participants who complete the entire study (Baseline – Test Day 3) with all data complete will receive a total of $350.

6. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

N/A

Statistical Analysis Plan

Study Statistics

b. Primary outcome variable.

- ASR magnitude:

- During the luteal phase vs the follicular phase of the menstrual cycle in women with PMDs, compared to luteal and follicular ASR in healthy female controls.
- While anticipating a mild shock during the luteal phase vs the follicular phase of the menstrual cycle in women with PMDs, compared to luteal and follicular ASR in healthy female controls.
- During luteal phase treatment with an SSRI vs the follicular phase of the menstrual cycle in women with PMDs, compared to luteal and follicular ASR in healthy female controls.

c. Secondary outcome variables.

- Hormone levels (estrogen, progesterone)

d. Statistical plan including sample size justification and interim data analysis.

Sample Size Determination

Based upon preliminary data from 12 women, we have estimated our main outcome, difference in anxiety-potentiated ASR in both controls and women with PMDD. We observed an *SD* 11.7 for change in follicular to luteal anxiety-potentiated ASR in controls, and an *SD* 13.6 increase in anxiety-potentiated ASR from follicular to luteal in PMDD participants. Assuming the larger of the observed *SD*, a 15% attrition rate, 80% power, type I error of 5%, equal group sizes, and a detectible effect size is 0.77 *SD*, we will recruit 32 women per group, to have 27 per group with complete data. We hypothesize that change in startle response between follicular and luteal phases of the menstrual cycle will be unchanged in controls, and represents an increase of 10.5 µV in women with PMDD. This increase would be consistent with that seen in other populations that have participated in the NPU paradigm[55]. Regarding the exploratory aim to examine effect of childhood adversity on ASR, if 30% (consistent with our sample to date) of the sample are in the high ACE (ACE score ≥2) group, we will be able to detect an effect size of 0.85 *SD.*

Statistical Methods

Descriptive statistics will be used to characterize the demographic characteristics of the group and provide measures of central tendency for all outcome measures. Correlations among outcome variables will be assessed. For each outcome measure, T-tests will be used to compare pre-treatment to post-treatment scores. Linear regression and logistic regression will be used to examine relationships between outcome variables.

The main data analysis will focus on the amplitude of the eye-blink. For **Aims 1 & 2**, the data will be compared across conditions and groups. For the primary outcome of baseline ASR magnitude over the menstrual cycle, peak amplitude of the blink reflex will be determined in the 20–120-ms time frame following stimulus onset relative to baseline (baseline is the average baseline EMG level for the 50 ms immediately preceding auditory stimulus onset). Cued fear is measured as the difference between startle magnitude during cues and startle magnitude during intertrial intervals (ITI; when no cues are given), and contextual anxiety is measured as

startle magnitude during ITI (no cues). Initially, data will be examined descriptively using means, standard deviations and graphs. Each outcome will be tested for normality using Kolmogorov–Smirnov test statistics and normal probability plots. Mixed models will be used to evaluate the magnitude of baseline ASR and ASR during threat of shock. The model will include group (healthy females and females with PMD) as a between-participant factor, and phase (follicular and luteal) as a within-participant factor. The interaction between group and phase will also be modeled.

For **Aim 3**, we will examine the effect of SSRI treatment among PMD participants. We will use mixed models to compare luteal phase baseline ASR pre and post treatment.

Subject Population(s) for Analysis

Analyses may be performed on the following subgroups within the sample:

- All Enrolled: Baseline symptom, demographic, and diagnostic data from all enrolled participants will be evaluated.
- Intent to Treat: Any subject who completed at least one study visit.
- Protocol completers: Any subject who completed the full protocol